NTP TECHNICAL REPORT

ON THE

MULTIGENERATIONAL REPRODUCTIVE TOXICOLOGY STUDY OF ETHINYL ESTRADIOL

(CAS NO. 57-63-6)

IN SPRAGUE-DAWLEY RATS

(FEED STUDIES)

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

August 2010

NTP TR 547

NIH Publication No. 10-5888

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

FOREWORD

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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

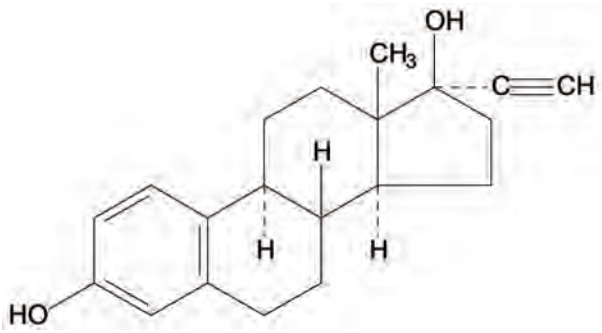
Background
Ethinyl estradiol is a potent synthetic estrogen that is widely prescribed in oral contraceptives and is also used in the treatment of breast and prostate cancer. Ethinyl estradiol is one of a class of chemicals known as "environmental estrogens" which can affect the hormone activities and 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 and their offspring were exposed to ethinyl estradiol over the course of four generations.

Methods
The continuous-breeding study began with groups of 35 Sprague-Dawley rats of each sex exposed to ethinyl estradiol in their feed at concentrations of 2, 10, or 50 parts per billion (ppb). Control animals received the same feed with no ethinyl estradiol added. Animals from the same dose treatment groups were paired and mated, and 25 litters of pups at each exposure concentration (culled to four males and four females each) were continued on study and given feed containing the same concentration of ethinyl estradiol. The process was repeated through a second and third generation, after which the pups were given control feed only, and two more generations were bred in the same manner and given control feed without ethinyl estradiol. Measures of fertility and reproduction were taken for each generation and tissues from the study animals were examined histopathologically.

Results
In all three offspring generations the time to vaginal opening (a measure of onset of puberty) was accelerated in females fed 50 ppb ethinyl estradiol. In the first two offspring generations the estrous cycles of the exposed females were prolonged or aberrant prior to mating. Male rats exposed to ethinyl estradiol had increased rates of mammary gland hyperplasia and mineralization of the kidney tubules.

Conclusions
We conclude that exposure to trace amounts of ethinyl estradiol in the feed showed clear biological activity in male and female rats, including reduced body weights in both sexes, perturbed estrous cycles in females, and induction of mammary gland hyperplasia and kidney tubule mineralization in males.

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, Anovlar, Diogyn-E, Dyloform, Ertonyl, Esteed, Estigyn, Estinyl, Eston-E, Estoral, Eticyclin, Eticyclol, Eticylol, Etinestrol, Etinestryl, Etinoestryl, Etistradiol, Feminone, Follicoral, Ginestrene, Inestra, Linoral, Lynoral, Menolyn, Neo-Estrone, Nogest-S, 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. Because of these concerns, ethinyl estradiol was selected as one of the compounds to be examined in a protocol utilizing Sprague-Dawley rats 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 (see Figure 1 of Overview). Results of short-term reproductive dose range-finding and mutigenerational reproductive toxicology studies are reported in this Technical Report, and results of the 2-year study are reported separately (NTP, 2010).

Reproductive Dose Range-Finding Study

A series of short-term studies with ethinyl estradiol was conducted with two goals: to obtain data necessary to establish exposure concentrations to be used in the subsequent multigenerational reproductive toxicology and chronic toxicity studies and to evaluate the effects of ethinyl estradiol on estrogen-sensitive endpoints outside the reproductive tract. Ethinyl estradiol was administered in a soy- and alfalfa-free diet at concentrations of 0, 0.1, 1, 5, 25, 100, or 200 ppb to pregnant Sprague-Dawley dams starting on gestation day 7 (GD 7) and continuing through pregnancy. These dietary exposure concentrations resulted in ingested doses of approximately 0.008, 0.08, 0.39, 1.77, 7.26, or 13.33 µg ethinyl estradiol/kg body weight per day to the dams. Dietary exposure of the dams continued through lactation, during which time ingested doses were approximately 0.03, 0.26, 1.37, 6.53, 29.68, or 51.93 µg/kg per day. Pups

from five litters, culled to eight per litter with an equal sex distribution on postnatal day (PND) 2 were maintained on the same dosed feed as their mother after weaning until sacrifice at PND 50. Ingested doses were approximately 0.02, 0.22, 1.14, 5.48, 21.00, or 45.24 µg/kg per day for male pups and 0.02, 0.22, 1.18, 5.60, 22.92, or 45.87 µg/kg per day for female pups.

Daily body weights of pregnant dams showed a negative exposure concentration-related trend with significantly decreased body weights in the 100 and 200 ppb groups relative to the controls on GDs 12 to 21 and 10 to 21, respectively. Daily feed consumption was also decreased in the 100 and 200 ppb groups on multiple days in the early period of treatment (within the period from GDs 8 to 14). Overall body weight gain and feed consumption during pregnancy also showed significant negative 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 parameters (gestation duration, proportion of vaginal plug-positive dams producing litters) or litter data (total pups per litter, proportion of stillborn pups, sex ratio, anogenital distance) did not show significant exposure concentration-related effects. Preputial separation, a marker of male puberty, was accelerated in the 5 and 25 ppb groups relative to the controls; however, the proportion of male pups showing preputial separation in the 200 ppb group by the time of scheduled sacrifice at PND 50 was less than that in the control group. Vaginal opening, a marker of female puberty, was accelerated in the 25, 100, and 200 ppb groups relative to the control group. The mean body weights of 200 ppb males and females were significantly less than those of controls from PND 42 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 using organ-weight-to-body-weight ratio or by using body weight as a covariable in an analysis of covariance. For 200 ppb males, ventral prostate gland (absolute and relative) and testis (all statistical models) weights were decreased relative to controls while the relative pituitary gland weight was increased. Regardless of the statistical model used, the dorsolateral prostate gland weight in the 5 ppb group was increased relative to the control group. In 200 ppb females, absolute and relative ovary weights were decreased while relative liver weight was increased.

Microscopic evaluation indicated exposure-induced changes in multiple organs of both sexes. Relative to the control group, incidences of ductal mammary gland hyperplasia were significantly increased in males exposed to 25 ppb or greater. In the testis, incidences of degeneration of pachytene spermatocytes and depletion of elongated spermatids in the 100 and 200 ppb groups and degeneration of round spermatids in the 200 ppb group were significantly increased compared to the control group. Testicular spermatid head counts were significantly less in the 200 ppb group. Relative to the control group, the seminal vesicle showed increased incidences of depletion of secretory material in the 100 and 200 ppb groups and atrophy in the 200 ppb group. The incidences of mild mineralization of renal tubules were increased in 100 and 200 ppb males. In females, significant disturbance of the estrous cycle occurred in animals in the 100 and 200 ppb groups, with the ovaries of 2 of 15 and 14 of 15 animals, respectively, diagnosed as anestrus. In the 200 ppb group, significantly increased incidences of uterine atrophy and vaginal mucocyte metaplasia and dystrophy occurred.

The severity of reproductive tract effects in 200 ppb male and female pups clearly eliminated this exposure concentration from consideration for the multigenerational reproductive toxicology study, while the effects of 100 ppb on dam body weight and feed consumption and reproductive tract effects in pups were primary reasons for concern for the use of this exposure concentration in the multigenerational reproductive toxicology study. The high exposure concentration for the multigenerational reproductive toxicology 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.

Multigenerational Reproductive Toxicology Study

The multigenerational reproductive toxicology study (F_0 through F_4, with F_5 litters terminated at weaning) 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. Exposure concentrations of 0, 2, 10, or 50 ppb resulted in ingested doses of approximately 0, 0.1, 0.7, or 4 µg ethinyl estradiol/kg body weight per day

to males and 0, 0.2, 1, or 6 µg/kg per day to females during the time that the rats were directly consuming dosed feed. Animals (140 of each sex) from the NCTR CD (Sprague-Dawley) rat colony were obtained at weaning. Thirty-five animals per sex were assigned to exposure groups by a weight-ranked randomization procedure prior to the start of dietary exposure of the parental (F_0) generation at 6 weeks of age. At the time of mating, males were paired with females from the same exposure group and they were housed together until evidence of successful mating was detected or for a maximum of 14 days. Litters were randomly standardized to four males and four females on PND 2, and 25 litters per exposure group and their associated sires and dams were randomly selected to continue on study to produce the next generation (through F_5) and then necropsied at termination at 20 weeks (F_0 through F_4) of age. Similar procedures were used to produce each generation. Dosed feed was removed from the F_3 pups at the time of weaning, and this generation and subsequent generations were maintained on control feed for the remainder of the study. The F_5 litters were terminated at weaning.

In the postweaning period, exposure to 50 ppb ethinyl estradiol reduced body weights of males and females of generations in which rats were ingesting the compound throughout adulthood (F_0 through F_2). Significantly decreased body weights were also observed in the 10 ppb F_0 female group and the 2 and 10 ppb F_2 male groups. The body weight decreases were not consistently linked to decreased feed consumption. While pup birth weights were not significantly affected by exposure in any generation, during the preweaning period, significantly decreased body weight gains were observed in the 50 ppb groups of the F_1, F_2, and F_3 generations.

Measures of fertility (mating, pregnancy, and fertility indices, time to mating, gestation length, litter size, pup birth weight) were not adversely affected by ethinyl estradiol exposure. The sex ratio of the litters was also not altered. Anogenital distance (AGD) of exposed male pups measured on PND 2 and covaried by body weight, was significantly less than that of controls in the F_3 generation. In exposed females, AGD covaried by body weight was significantly increased relative to controls in the F_2 generation, but decreased in the F_3 generation. In all cases, the AGD differences in exposed groups relative to controls were less than 10% and were of questionable biological significance. Females exposed to 50 ppb ethinyl estradiol showed an accelerated time of vaginal opening in the F_1, F_2, and F_3 generations. Body weight at vaginal opening was also decreased in the 50 ppb groups of the F_1, F_2, and F_3 generations and the 10 ppb group of the F_1 generation. When examined shortly after vaginal opening, the estrous cycles in all exposed groups of the F_1 generation and the 50 ppb group of the F_2 generation were significantly longer than those in their respective control groups and were approximately doubled in length in the 50 ppb groups. Compared to the control groups, the 50 ppb groups of the F_1 and F_2 generations also had significant increases in the percentage of time that they were in estrus and increased percentages of abnormal cycles. When the estrous cycles of older animals were examined after pregnancy and lactation and prior to termination, there were no significant treatment effects. No significant treatment-related effects on male sexual development were noted with the exception of an increased time of preputial separation (an indication of delayed puberty) in the 50 ppb F_2 group and increased or decreased time of testicular descent in the 2 ppb groups of the F_1 and F_4 generations, respectively. Sporadic statistically significant effects on ovarian follicle, epididymal sperm, and testicular spermatid head counts were not convincingly treatment-related as the magnitudes of the effects were generally within the variation seen in control animals and did not show a consistent pattern in the exposed generations. While multiple statistically significant effects on organ weights in both sexes were observed, these appeared for the most part to be secondary to body weight changes and/or were not consistent across exposed generations. In males, but not females, relative pituitary gland weights were significantly greater in the 50 ppb groups of the F_0 through F_2 generations than in the respective control groups. Relative spleen weights were similarly greater in these males, while relative spleen weights of females were greater in the 2 ppb group of the F_1 generation and in all exposed groups of the F_2 generation.

Biologically significant treatment-related microscopic lesions appeared to be confined to the male mammary gland and kidney. Relative to the controls, incidences of mammary gland alveolar/ductal hyperplasia were increased in the 50 ppb groups of the F_0, F_1, F_2, and F_3 generations, the 2 and 10 ppb groups of the F_1 generation, and the 10 ppb group of the F_2 generation. The effect of ethinyl estradiol on the occurrence of male mammary gland hyperplasia was more pronounced in the continuously exposed F_1 and F_2 generations compared to the late adolescent and adult exposure of the F_0 generation and the preweaning-only exposure of the F_3 generation, indicating that both developmental and

adult exposures contributed to the maintenance of this effect into adulthood. Although a slight increase in the incidence of mammary gland alveolar hyperplasia occurred in 50 ppb males in the unexposed F_4 generation, the increase was not statistically significant. Significant effects of ethinyl estradiol on the male kidney were limited to the 50 ppb group of the continuously exposed F_1 and F_2 generations, where incidences of mild mineralization of the renal tubules were increased relative to those in the controls.

Summary

Ethinyl estradiol administered at exposure concentrations of 2, 10, or 50 ppb in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats showed clear biological activity including potentially adverse effects. Both preweaning and postweaning body weights of males and females were decreased during periods of direct exposure to dosed feed. Ethinyl estradiol accelerated the attainment of puberty of females under continuous exposure conditions (F_1 and F_2) and of animals where dosing was terminated at weaning (F_3). Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, time in estrus) in young females after vaginal opening and prior to mating was observed in the F_1 and F_2 generations. In males, statistically significant inductions of male mammary gland hyperplasia (F_0 through F_3 generations) and mild mineralization of renal tubules (F_1 and F_2 generations) were observed. The majority of these effects were observed at 50 ppb, but significant effects on body weight reduction and male mammary gland hyperplasia were observed at the lowest exposure concentration (2 ppb). With the possible exception of a 1.5-day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations.

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

GD = gestation day; NA = not applicable; PND = postnatal day; ANCOVA = analysis of covariance; ↑ or ↓, significant increase or decrease relative to controls at the exposure concentration indicated in parentheses; "–", no exposed group significantly different from the control group in that generation in pairwise comparisons

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Body Weight						
Female						
Preweaning	NA	↓ (50)	↓ (50)	↓ (50)	-	NA
Postweaning	↓ (10, 50)	↓ (50)	↓ (50)	-	-	NA
Male						
Preweaning	NA	↓ (50)	↓ (50)	↓ (50)	-	NA
Postweaning	↓ (50)	↓ (50)	↓ (2, 10, 50)	-	-	NA
Feed Consumption						
Female	↓ (2)	-	↓ (50)	↑ (10, 50)	-	NA
Male	↓ (2, 10)	↓ (2)	↓ (2, 10, 50)	-	-	NA
Water Consumption						
Female During Lactation	-	-	-	-	-	NA
Pregnancy Index	-	-	-	-	-	NA
Mating Index	-	-	-	-	-	NA
Fertility Index	-	-	-	-	-	NA
Mating Time	-	-	-	-	-	NA
Gestation Length	-	-	-	-	-	NA
Litter Size	NA	-	-	-	-	-
Pup Birth Weight						
Male	NA	-	-	-	-	-
Female	NA	-	-	-	-	-
Sex Ratio (M:F)	NA	-	-	-	-	-

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Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Stillbirths	NA	-	-	-	-	-
Anogenital Distance						
Male PND 2						
ANCOVA	NA	-	-	↓(50)	-	-
Ratio	NA	-	-	-	-	-
Female PND 2						
ANCOVA	NA	-	↑(50)	↓(10, 50)	-	-
Ratio	NA	-	↑(50)	↓(50)	-	-
Vaginal Opening						
Age	NA	↓(50)	↓(50)	↓(50)	-	NA
Body Weight	NA	↓(10, 50)	↓(50)	↓(50)	-	NA
Preputial Separation						
Age	NA	-	↑(50)	-	-	NA
Body Weight	NA	-	-	-	-	NA
Testicular Descent						
Age	NA	↑(2)	-	-	↓(2)	NA
Vaginal Cytology After Vaginal Opening						
% Time Estrus	NA	↓(10) ↑(50)	↑(50)	-	-	NA
% Time Diestrus	NA	-	-	-	-	NA
% Time Proestrus	NA	↓(50)	↓(50)	-	-	NA
% Abnormal Cycles	NA	↑(50)	↑(50)	-	-	NA
Number Abnormal Cycles	NA	↑(50)	↑(50)	-	-	NA
Length of Cycle	NA	↑(2, 10, 50)	↑(50)	-	-	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Vaginal Cytology Before Termination						
% Time Estrus	-	-	-	-	-	NA
% Time Diestrus	-	-	-	-	-	NA
% Time Proestrus	-	-	-	-	-	NA
% Abnormal Cycles	-	-	-	-	-	NA
Number Abnormal Cycles	-	-	-	-	-	NA
Length of Cycle	-	-	-	-	-	NA
Estrous Cycle Synchrony in Reproductive Organs at Necropsy	-	-	-	-	-	NA
Terminal Body Weight						
Male	↓(50)	↓(50)	↓(2, 10, 50)	-	-	NA
Female	↓(10, 50)	↓(50)	↓(50)	-	↑(10)	NA
Male Organ Weights						
Adrenal Gland						
Absolute	-	-	-	-	-	NA
Relative	↑(50)	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Brain						
Absolute	-	-	-	-	-	NA
Relative	↑ (50)	↑ (50)	↑ (2, 10, 50)	-	-	NA
ANCOVA	↑ (2, 50)	-	↑ (2)	-	↑ (2)	NA
Epididymis						
Absolute	-	-	-	-	-	NA
Relative	-	-	↑ (50)	-	-	NA
ANCOVA	-	-	-	-	-	NA
Kidney						
Absolute	↓ (50)	↓ (50)	↓ (2, 10, 50)	-	-	NA
Relative	↑ (50)	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Liver						
Absolute	↓ (50)	-	↓ (2, 50)	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Pituitary Gland						
Absolute	-	↑ (10)	-	-	-	NA
Relative	↑ (50)	↑ (50)	↑ (50)	-	-	NA
ANCOVA	↑ (50)	↑ (50)	-	-	-	NA
Prostate Gland Dorsal Lobe						
Absolute	-	-	-	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Prostate Gland Lateral Lobe						
Absolute	-	-	-	-	↑ (10)	NA
Relative	-	-	↑ (50)	-	↑ (10)	NA
ANCOVA	-	-	-	-	↑ (10)	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Seminal Vesicle/Coagulating Gland						
Absolute	-	-	↑(10)	-	-	NA
Relative	↑(50)	-	↑(2, 10, 50)	-	-	NA
ANCOVA	-	-	↑(10)	-	-	NA
Spleen						
Absolute	-	-	-	-	-	NA
Relative	↑(50)	↑(50)	↑(50)	-	↑(2)	NA
ANCOVA	-	↑(2, 50)	-	-	↑(2)	NA
Testis						
Absolute	-	-	-	-	-	NA
Relative	↑(50)	-	↑(50)	-	-	NA
ANCOVA	-	-	-	-	-	NA
Thymus						
Absolute	-	-	-	↑(2)	-	NA
Relative	-	-	-	↑(2)	-	NA
ANCOVA	-	-	-	↑(2)	-	NA
Thyroid Gland						
Absolute	-	↓(10, 50)	-	-	-	NA
Relative	-	↓(10, 50)	-	-	-	NA
ANCOVA	-	↓(10, 50)	-	-	-	NA
Ventral Prostate Gland						
Absolute	-	-	-	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Female Organ Weights						
Adrenal Gland						
Absolute	-	-	-	-	-	NA
Relative	-	↑ (50)	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Brain						
Absolute	-	-	-	-	↑ (2)	NA
Relative	↑ (10, 50)	↑ (50)	↑ (50)	-	-	NA
ANCOVA	-	-	-	-	-	NA
Kidney						
Absolute	↓ (2, 10, 50)	↓ (50)	↓ (50)	-	↑ (10)	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Liver						
Absolute	↓ (50)	↓ (50)	↓ (50)	-	↑ (2, 10)	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Ovary						
Absolute	-	↓ (50)	-	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Pituitary Gland						
Absolute	-	-	-	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Spleen						
Absolute	↓ (50)	↓ (50)	↑ (2)	-	-	NA
Relative	-	↑ (2)	↑ (2, 10, 50)	-	-	NA
ANCOVA	-	↑ (2)	↑ (2, 10)	-	-	NA
Thymus						
Absolute	-	-	-	-	↑ (2, 50)	NA
Relative	-	↑ (2, 50)	↑ (50)	-	↑ (50)	NA
ANCOVA	-	-	↑ (50)	-	↑ (2, 50)	NA
Thyroid Gland						
Absolute	↑ (10)	-	-	-	↑ (10)	NA
Relative	↑ (2, 10)	-	-	-	-	NA
ANCOVA	↑ (10)	-	-	-	-	NA
Uterus						
Absolute	-	-	-	-	-	NA
Relative	-	-	-	-	-	NA
ANCOVA	-	-	-	-	-	NA
Ovarian Follicle Counts						
Small	↑ (50)	-	-	-	-	NA
Growing	-	-	-	-	↓ (50)	NA
Antral	-	↑ (10)	↑ (10)	-	-	NA
Epididymal Sperm Count	-	-	↑ (10, 50)	-	-	NA
Testicular Spermatid Head Count	-	↓ (50)	-	-	-	NA
Sperm Motility	-	-	-	-	-	NA
Sperm Morphology	-	-	-	-	-	NA

Summary of Observations From the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation					
	F_0 Exposed from PND 42 to PND 140	F_1 Exposed from GD 0 to PND 140	F_2 Exposed from GD 0 to PND 140	F_3 Exposed from GD 0 to PND 21 (Terminated on PND 140)	F_4 Not Exposed (Terminated on PND 140)	F_5 Not Exposed (Terminated on PND 21)
Histopathology						
Male						
Mammary Gland Alveolar/Ductal Hyperplasia	↑ (50)	↑ (2, 10, 50)	↑ (10, 50)	↑ (50)	-	NA
Renal Tubule Mineralization	-	↑ (50)	↑ (50)	-	-	NA
Female						
Mammary Gland Lobules, Hyperplasia	-	-	↑ (10, 50)	-	-	NA
Alveolar, Hyperplasia	-	-	-	-	-	NA
Renal Tubule Mineralization	-	↓ (50)	-	-	↓ (2, 50)	NA

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 multigenerational toxicology study 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 multigenerational reproductive toxicology study of ethinyl estradiol by providing background on the joint NIEHS-NCTR series of endocrine disruptors studies, which include both multigenerational studies to test for accumulative or persistent effects, as well as the standard 2-year carcinogenesis bioassays. Dr. Delclos discussed the uses, occurrence and metabolism of the synthetic estrogen ethinyl estradiol and the results of the reproductive dose range-finding study of ethinyl estradiol. He then described the exposure regimen for the various portions of the multigenerational reproductive toxicology study, the patterns of body weight and reproductive system measures on the different generations of the study, and the occurrence of nonneoplastic lesions in the male rats.

The proposed summary for the multigenerational reproductive toxicology study was: "Ethinyl estradiol administered at exposure concentrations of 2, 10, or 50 ppb in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats showed clear biological activity and potentially adverse effects. Ethinyl estradiol suppressed both preweaning and postweaning body weights of males and females during periods of direct exposure to dosed feed. Ethinyl estradiol accelerated the attainment of puberty of females under continuous exposure conditions (F_1 and F_2) and of animals where dosing was terminated at weaning (F_3). Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, increased time in estrus) in young females after vaginal opening and prior to mating was observed in the F_1 and F_2 generations. In males, statistically significant inductions of male mammary gland hyperplasia (F_0 through F_3 generations) and mild mineralization of renal tubules (F_1 and F_2 generations) were observed. Treatment-related effects may have carried over into the unexposed F_4 generation since there was a marginal increase in the incidences of alveolar hyperplasia in the male mammary gland in that generation. The majority of these effects were observed at 50 ppb, but significant effects (body weight reduction, prolonged estrous cycle time, and male mammary gland hyperplasia) were observed at the lowest exposure concentration (2 ppb). With the possible exception of a 1.5-day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations."

Dr. Walker, the first principal reviewer, felt the study was well reported. He expressed familiarity with the study design and did not have major scientific criticisms.

Dr. Crump, the second principal reviewer, noted the large volume of data and questioned whether in some cases the analytic techniques indicated significant differences in values that seemed nearly identical. He also queried whether it was appropriate to refer to reduced body weight as an adverse effect. He felt that the difference in estrous cycle length might not merit mention in the summary statement and that the final sentence of the conclusion may not be necessary.

Dr. Delclos replied that the analysis for the one table with similar values had been noticed and corrected, and agreed that while body weight was a measure of chemical response, it might not be called an adverse effect.

Dr. Cattley, the third principal reviewer, inquired whether it was possible to detect the difference between spontaneously occurring and chemical induced hyperplasia in the male mammary gland. Dr. P.W. Mellick, Pathology Associates, NCTR, replied that the feminization of the males by the chemical produced discernible differences in the mammary gland. Dr. Cattley asked for some statement about the consensus of the adequacy of dose selection.

Dr. Tammy Stoker, an ad hoc reviewer for this report, questioned whether there was indeed any direct or transplacental transfer of ethinyl estradiol to the fetuses or neonates from the gestational exposures. She suggested that the delay in preputial separation in males may have been a real effect, correlating well with a decrease in testosterone level and body weight. She queried the measurement procedure of taking smears to gauge estrous cycles 3 days after vaginal opening. Dr. Delclos replied that was a consequence of the several other requirements of the study protocol.

In discussion of the proposed summary, several statements were debated. Regarding the statement about mammary gland hyperplasia in the unexposed F_4 males, the statistical significance of the incidences and the possible distinction between feminized males were discussed. Dr. Kirkvliet called the question, and the panel voted unanimously with seven votes to remove that sentence. Regarding the mention of body weight changes, discussion centered on whether the statements of effects implied they were adverse. In the second sentence, the decrease in body weight was to be noted but not referred to as being "suppressed." That amendment was approved unanimously. Dr. Soper suggested that mention of prolonged estrous cycle time be deleted from the penultimate sentence. The motion to remove that phrase, and not include the other two effects as a parenthetical expression, was approved unanimously. Dr. Crump suggested removing mention of the preputial separation from the final sentence. Dr. Walker also thought that it was a singular point and questioned the use of the term "magnified." Dr. Delclos pointed out that this effect was seen in the F_2 generation but was not seen in the F_1 generation that received similar exposure. Dr. Kirkvliet noted that one of the major purposes of the study was to determine if there was any intergenerational accumulation of effects. Dr. Mirsalis felt the sentence was accurate and clear as written. Dr. Kirkvliet called the question, and by a vote of five to two the panel approved retaining the final sentence as written. Dr. Sikka suggested changing "and" to "including" in the first sentence, and the motion was approved unanimously. Dr. Cattley suggested deleting the word "increased" from the phrase "time in estrus" for the sentence about the perturbation of the estrous cycle; that motion was carried unanimously. Finally, Dr. Cattley moved, and Dr. Walker seconded, that the full summary be accepted as modified. That motion was 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, 1995) entitled "Estrogens in the Environment III," the NIEHS 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 NIEHS/NTP and 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 produced 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 is contributing 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 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 (see below). 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 dose range-finding study was used for the neurotoxicity studies. 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 (see below), 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 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 should not be used. As mentioned earlier, the

current studies utilized outbred female CD (Sprague-Dawley) rats from the NCTR breeding colony. This colony was established at NCTR in 1972 using Sprague-Dawley rats from the Charles River Laboratories. The NCTR colony at present is a distinct substrain of Sprague-Dawley rat that has been previously shown to differ substantially from the Charles River and other strains of SD rats 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 (CFSAN, 2000; OECD, 2004). 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 N) 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 by both sexes and the body weights of males fed PMI 5K96 were significantly lower than in rats fed NIH-31.

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

As in the short-term studies, the multigenerational reproductive toxicology and chronic studies were conducted with the NCTR CD 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 into subsequent unexposed generations. Standard toxicologic 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, which is reported separately (NTP, 2009), was also examined for two test compounds (ethinyl estradiol and genistein). Three exposure windows were examined in the chronic studies (Figure 1); continuous exposure from conception through 2 years (designated F_1 continuous, or F_1C), exposure from conception through PND 140 followed by control diet to 2 years (designated F_1 truncated at 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 exposure groups indicates that these animals were siblings of the F_3 animals from the current 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.

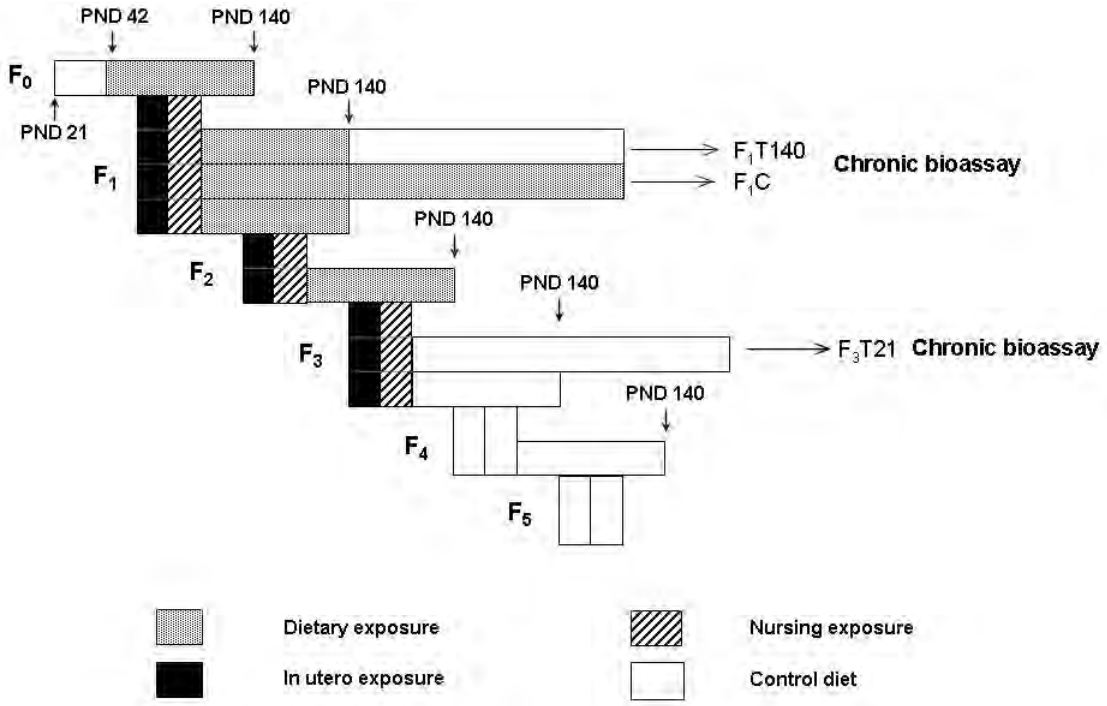
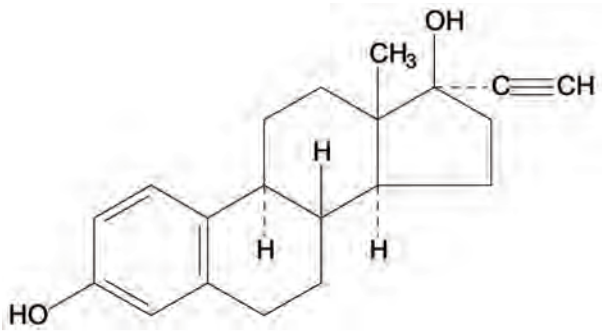


Figure 1
Dosing Schedule for the Multigenerational Reproductive Toxicology and Chronic Studies

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, Anovlar, Diogyn-E, Dyloform, Ertonyl, Esteed, Estigyn, Estinyl, Eston-E, Estoral, Eticyclin, Eticyclol, Eticylol, Etinestrol, Etinestryl, Etinoestryl, Etistradiol, Feminone, Follicoral, Ginestrene, Inestra, Linoral, Lynoral, Menolyn, Neo-Estrone, Nogest-S, 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 non-aqueous solvents such as ethanol, ether, acetone, dioxane, chloroform, and vegetable oil (*Merck*, 2006). Ethinyl estradiol is a potent synthetic estrogen first reported by Inhoffen and Hohlweg (1938). Now it 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 results 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-hydroxylations (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 alpha-fetoprotein) 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 intraindividual 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 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 adult rat 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}. Following an oral gavage dose of 1 mg/kg in that same study, 57% of the serum ethinyl estradiol was present as glucuronide and sulfate conjugates 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 maximal concentrations (C_{max}) 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 rats and humans needs to be considered when comparing the relative responsiveness of the species to ethinyl estradiol.

Estrogen Receptor Binding, Estrogenic Activity, and Oral Toxicity of Ethinyl Estradiol

Studies using uterine estrogen receptors, which are predominantly the classical estrogen receptor alpha (ER-α), have indicated similar binding affinities and gene expression profiles for estradiol and ethinyl estradiol (Anstead *et al.,* 1997; Hyder *et al.,* 1999). Studies comparing binding affinities and reporter gene induction utilizing recombinant human ERs-α and -β have indicated somewhat higher potency for ER-α (Barkhem *et al.,* 1998; Gutendorf and Westendorf, 2001). In the former study, there was a 35-fold preference of ethinyl estradiol for ER-α over ER-β in an *in vitro* reporter gene assay compared to a four-fold preference for estradiol. In an Organization for Economic Cooperation and Development (OECD)-sponsored validation study of the uterotrophic assay for detection of estrogenic activity in immature female Sprague-Dawley or Wistar rats, orally administered ethinyl estradiol was of lower potency than the subcutaneously administered compound (as expected) due to first-pass metabolism (Kanno *et al.,*

2001). In this validation study, which involved 16 laboratories, doses ranging from 0.03 to 10 µg/kg per day were tested. Eleven laboratories observed a statistically significant increase in uterine weight after 3 days of 1 µg/kg per day, while four laboratories reported a lowest observed effect level (LOEL) of 0.3 µg/kg per day, and the remaining laboratory reported a LOEL of 3 µg/kg per day (Kanno *et al.,* 2001). Several studies have reported dose-response evaluations of gene expression changes in response to ethinyl estradiol administered by subcutaneous injection or by gavage to rats or mice with the goal of defining a pattern of estrogen-regulated gene expression useful for the evaluation of putative estrogenic substances. Naciff *et al.* (2005) evaluated ethinyl estradiol using subcutaneous injections over a dose range of 0.001 to 10 µg/kg per day to pregnant Sprague-Dawley rat dams consuming a soy- and alfalfa-free diet (Purina 5K96) from gestational day (GD) 11 to GD 20 and evaluated gene expression in the combined testes and epididymides of male pups on GD 20. Changes in gene expression were noted at 0.1 µg/kg per day or greater. The only morphological effect in the male pups noted was the presence of prominent nipples and areolas at 10 µg/kg per day; no histological effects on the testes and epididymides were noted at any dose. The same group (Naciff *et al.,* 2002) reported a study of gene expression in the combined uteri and ovaries of female pups using a similar protocol with doses of 0.5, 1, or 10 µg/kg per day administered by subcutaneous injection to pregnant Sprague-Dawley dams consuming a standard chow diet (Purina 5001). Again, prominent nipples and areolas in the female pups at the highest dose were the only effects noted, although dose-responsive changes in gene expression were noted with some genes affected at the lowest dose tested. Subcutaneous injections of immature female rats with ethinyl estradiol elicited a uterotrophic response at 1 µg/kg per day, with some evidence of uterine histological changes at 0.1 µg/kg per day and clear evidence of gene expression changes at the 0.1 µg/kg per day dose, but not at the lower doses tested (Naciff *et al.,* 2003). In C57BL/6 mice dosed orally with 0.1 to 250 µg/kg per day, hepatic gene expression changes occurred with an ED50 less than 10 µg/kg per day, while uterotrophic effects, a classical *in vivo* assessment of estrogenic activity, have been reported at ED50s of 10 to 100 µg/kg per day (Boverhof *et al.,* 2004).

Reports in the open literature on the adverse effects of *in utero* and neonatal exposure to ethinyl estradiol are more limited than those on the effects of diethylstilbestrol. Diethylstilbestrol is an orally bioavailable synthetic estrogen that has an estrogen receptor binding affinity and transcriptional activating potency similar to that of ethinyl estradiol (Blair *et al.,* 2000; Gutendorf and Westendorf, 2001), although ethinyl estradiol has been reported to have a higher estrogen receptor α-selective potency than diethylstilbestrol in some transcriptional activation systems (Barkhem *et al.,*1998). The reports of the consequences of developmental exposure to ethinyl estradiol, as summarized below, are generally similar to those that have been reported for diethylstilbestrol, except that the carryover of effects across generations has not been evaluated with ethinyl estradiol as it has been with diethylstilbestrol (Newbold, 1995; Newbold *et al.,* 2006).

A series of studies in which pregnant female mice were exposed to oral doses ranging from 0.02 to 2.0 mg ethinyl estradiol/kg body weight and effects evaluated in the progeny have been reported. A significant rate of fetal mortality was observed at doses of 0.2 and 2.0 mg ethinyl estradiol/kg body weight administered by gavage in multiple doses from GDs 11 to 17 or in single doses on GDs 8 or 11 (Yasuda *et al.,* 1981). In the same study, a significant depression of body weight gain of the pups at all doses was observed, and hypertrophic nipples were induced in female pups exposed to the high dose (2.0 mg/kg). In 10- to 14-week-old female pups born to mothers exposed to 0.01 or 0.02 mg ethinyl estradiol/kg body weight by gavage on GDs 11 through 17, cystic glandular hyperplasia and epidermization were observed in the endometrium, and decreased numbers of primordial follicles and microcysts resulting from atretic follicles were observed in the ovaries (Yasuda *et al.,* 1977a). Hypertrophy of the ovarian interstitial tissue without corpora lutea was observed in 16-week-old animals exposed to the same *in utero* treatment (Yasuda *et al.,* 1977b). A significantly increased incidence of follicular cell hyperplasia was reported in mice on GD 18 after treatment with 0.2 mg/kg per day of ethinyl estradiol on GDs 11 through 17 (Yasuda *et al.,* 1987). Male pups were also affected by *in utero* exposure from GDs 11 to 17 to 0.02 to 0.2 mg/kg per day of ethinyl estradiol. Abnormal differentiation of gonocytes and fetal Sertoli cells, acceleration of prespermatogenesis, and decreased testicular testosterone were observed in male fetuses examined on GD 18 (Yasuda *et al.,* 1985a,b, 1986a,b). In 20- to 22-month-old males exposed *in utero* by the above exposure regimen (0.02 mg/kg per day), testicular testosterone was decreased, seminiferous tubules were atrophied, sperm were absent in epididymides, and Leydig

cell hyperplasia was observed (Yasuda *et al.,* 1988). More recently, Thayer *et al.* (2001) have reported that oral exposure (by pipetting into the mouth) of pregnant CF1 mice to ethinyl estradiol from GDs 0 through 17 to doses as low as 20 ng/kg per day produced a statistically significant increase in the prostate gland weight of male pups at 50 days and 5 months of age and a decrease in daily sperm production at the early, but not the later, time point. Similarly administered oral doses of 100 ng/kg per day to CD1 mice on GDs 14 through 18 were reported to produce a significant increase in the number of ducts in the dorsolateral prostate gland, an increase in dorsolateral prostate gland duct volume, and increased proliferation in the basal epithelial cells of these ducts in near term male fetuses (Timms *et al.,* 2005). In the same study, similar effects were produced by a low oral dose of diethylstilbestrol (100 ng/kg per day), while a high diethylstilbestrol dose (200 µg/kg per day) inhibited dorsolateral prostate gland duct development.

In utero through lactational exposure [GD 7 to postnatal day (PND) 18] of Sprague-Dawley rats to gavage doses of 0.5, 5, or 50 µg ethinyl estradiol/kg body weight per day had no adverse effects on the dams; clear treatment-related effects were confined to pups of the high dose group where reduced body weight gain was observed in both sexes, and cleft phallus was reported in the females (Sawaki *et al.,* 2003a). High-dose females had normal fertility at 15 to 17 weeks of age but showed ovarian dysfunction including abnormal cyclicity with persistent estrus, follicular cysts, and the absence of corpora lutea at 6 months of age (Sawaki *et al.,* 2003b). In a series of studies investigating perinatal (GD 15 through PND 9 to 11, depending on the study) dietary ethinyl estradiol administered at 0.02 to 0.5 ppm to Sprague-Dawley rats, effects at 0.5 ppm included reduced body weight gains in pups of both sexes, delayed onset of puberty in males and accelerated onset of puberty in females, decreased volume of the sexually dimorphic nucleus of the preoptic area in males, irregular estrous cycles, increased relative weights of the pituitary and adrenal glands in females, hyperplastic effects in the pituitary and mammary glands in females, hypertrophy of the endometrial epithelium, and increased atretic follicles and decreased corpora lutea in ovaries (Masutomi *et al.,* 2004a,b; Takagi *et al.,* 2004; Shibutani *et al.,* 2005). The effects on females were exacerbated when ethinyl estradiol was administered in a soy-containing diet compared to effects in a soy-free diet (Masutomi *et al.,* 2004a), and genistein was reported not to be responsible for the effect of the soy diet (Takagi *et al.,* 2004). This exposure regimen was also reported to increase the proportion of prolactin-secreting cells in the pituitary gland of females examined at postnatal week 3 but not at postnatal week 11 (Masutomi *et al.*, 2004b). Expression of the γ-aminobutyric acid transporter type 1, an estrogen responsive gene, was decreased in the hypothalamic preoptic area at 0.02 ppm and greater in females and at 0.5 ppm in males at the end of the exposure, while the expression of another estrogen-responsive gene, the antiapoptotic gene bcl-xL, was not changed in either sex (Shibutani *et al.,* 2005). Ethinyl estradiol at 0.5 ppm from GD 15 through PND 10 up-regulated the expression of steroid receptor coactivator-1 in the hypothalamic preoptic area of males and the expression of ER-β and progesterone receptor in females (Takagi *et al.,* 2005).

Effects of Ethinyl Estradiol on the Reproductive Tract, Fertility and Pregnancy in Mature Rodents

Chronic dietary administration of ethinyl estradiol at 0.15 or 1.5 ppm to female Sprague-Dawley rats caused exposure concentration-related luminal dilatation of uterine horns and endometrial glands, uterine inflammation, and squamous metaplasia of the endometrium and endometrial glands (Schardein, 1980). Estrogens, including ethinyl estradiol, have profound antifertility effects when administered prior to or immediately after conception. Administration of ethinyl estradiol by gavage to female Long-Evans rats during the mating period completely inhibited pregnancy at 0.05 mg/kg and significantly inhibited pregnancy at 0.005 mg/kg (Watnick *et al.,* 1964). When administered by gavage at a dose of 0.05 mg ethinyl estradiol/kg body weight after mating, no interference with ova transport or implantation was observed, but fetal resorption was induced, with the most significant effect observed when treatment was started on day 1 of pregnancy (Watnick *et al.,* 1964). In mice, ethinyl estradiol administered by gavage on the first day of pregnancy significantly inhibited the progress of pregnancy at a dose of 0.01 mg/mouse, with complete inhibition observed at 0.1 mg/mouse (Yanagimachi and Sato, 1968). At these doses, failure of pregnancy was attributed to abnormal development and transport of ova. This effect was reversible, in that a second pregnancy in these mice after cessation of treatment was normal. Administration of 25 µg ethinyl estradiol/kg body weight, but not 6.25 µg/kg, by gavage to pregnant CD

rats from GD 8 to GD 21 resulted in an apparent decrease of fertility in the female pups, although treatment groups were too small to draw a firm conclusion (Edgren and Clancy, 1968). Administration of daily gavage doses up to 25 µg ethinyl estradiol/kg body weight to lactating CD rats did not have an effect on the reproductive organ weights of the pups (Clancy and Edgren, 1968), consistent with limited transfer of ethinyl estradiol through the milk.

Ethinyl estradiol has also been shown to affect the reproductive tract and fertility of mature male rats, although at higher doses than those that affect female fertility. Schardein (1980) found that chronic administration of ethinyl estradiol to male Sprague-Dawley rats at 0.15 or 1.5 ppm caused an exposure concentration-related atrophy of the testicles, prostate gland, and seminal vesicles. Iwase *et al.* (1995) treated male Sprague-Dawley rats for 4 weeks prior to mating at doses ranging from 0.1 to 10 mg ethinyl estradiol/kg body weight. Dose-dependent decreases in body weight and feed consumption relative to controls were observed at all doses. Males in the highest dosed groups, 3 and 10 mg/kg, were completely infertile, while males treated with 0.1 or 0.3 mg/kg showed a decreased copulation index but normal fertility. Epididymal sperm counts were decreased, with sperm completely absent at the two higher doses. Testes, epididymides, seminal vesicle, and prostate gland weights were decreased and dose-dependent atrophy and degeneration of spermatocytes, spermatids, and Sertoli and Leydig cells were observed. These changes were largely reversible on removal of treatment.

Several studies have evaluated ethinyl estradiol using OECD Test Guideline No. 407, a 28-day repeated dose toxicity bioassay, with enhancements to detect endocrine activity (Andrews *et al.,* 2002; Yamasaki *et al.,* 2002a,b). Andrews *et al.* (2002) administered 0, 0.01, 0.05, or 0.2 mg ethinyl estradiol/kg body weight by gavage to male and female Wistar rats starting at 7 weeks of age. Reduced body weight gain and decreased relative weights of the male accessory reproductive organs, increased relative adrenal gland weight, degeneration of the germinal epithelium, and atrophy of the Leydig cells and male accessory glands were observed at the high dose, although sperm parameters (counts and percentage of abnormal sperm) were not affected. Feminization of the male mammary gland was detected at all doses. In females, relative liver weights were increased in the 0.05 and 0.2 mg/kg groups. Increased apoptotic early-stage follicles and corpora lutea were observed at 0.05 mg/kg and increased heights of the luminal and glandular epithelium of the uterus were seen at 0.01 mg/kg or greater. While females were sacrificed when vaginal cytology indicated that they were in diestrus, histopathology of the uterus and vagina indicated that some animals were in estrus or proestrus in all dosed groups, but not in controls. Yamasaki *et al.* (2002a) reported a similar 28-day study of ethinyl estradiol in 7-week-old Sprague-Dawley rats with daily gavage doses of 0, 0.01, 0.05, and 0.2 mg/kg per day. Effects in males observed in the 0.05 and 0.2 mg/kg groups included decreased relative weights of the prostate gland and seminal vesicles and increased relative liver and pituitary gland weights. In addition, increased relative testis and adrenal gland weights were observed in the high dose males, along with atrophy of the prostate gland, seminal vesicle, and mammary gland and cortical hypertrophy in the adrenal gland. In female rats, relative liver weight was increased in all dosed groups, while relative uterus and kidney weights increased and ovary weight decreased at 0.2 mg/kg. Abnormal cycles were seen in the 0.2 mg/kg group, and histological changes in the uterus (hypertrophy of the epithelial cells) and vagina (mucification) were also observed in this high dose group. A second study by Yamasaki *et al.* (2002b) focused on Sprague-Dawley male rats and used daily gavage doses of 0, 0.015, 0.075, or 0.375 mg/kg per day for 28 days. Alpha2u-globulin, an estrogen-regulated protein expressed primarily in adult male liver, was significantly reduced in the high dose group. Both absolute and relative dorsolateral prostate gland weights were reduced in the middle and high dose groups, and increases in abnormal sperm, degenerative changes in the testis, and atrophy of the prostate gland and seminal vesicles were observed in the high dose group. Treatment of adult male Sprague-Dawley rats with daily gavage doses of 1 or 10 mg ethinyl estradiol/kg body weight per day for up to 4 weeks significantly reduced testosterone, luteinizing hormone, follicle stimulating hormone, reproductive and accessory reproductive organ weights, testicular and epididymal sperm counts, and sperm motility, and caused atrophy of the seminiferous tubules (Kaneto *et al.,* 1999). The 10 mg/kg dose also severely impaired fertility of the treated males, and this reduction occurred before the reduction in testicular spermatids was evident. A later study (Shimomura *et al.,* 2005) found that coadministration of testosterone almost completely blocked the adverse reproductive tract effects

of a 10 mg/kg dose of ethinyl estradiol in male Sprague-Dawley rats, suggesting that the effects of ethinyl estradiol were secondary to its depression of testosterone levels. This is in agreement with the work of Rivas *et al.* (2003), which demonstrated reversal of the majority of the effects of neonatally administered diethylstilbestrol on the male reproductive tract by testosterone treatment.

Human Toxicity

Ethinyl estradiol is and has long been the predominant estrogen used in oral contraceptives, and there are extensive data on the dose-dependent adverse effects of oral contraceptives in women (Vessey, 1989; Rosenberg *et al.,* 1997; Chasan-Taber and Stampfer, 1998; Hannaford and Kay, 1998; Loose and Stancel, 2006). Adverse cardiovascular effects have been of particular concern and were important considerations in the gradual reduction of the estrogenic component of oral contraceptives since their original introduction (Vessey, 1989; Rosenberg *et al.,* 1997; Chasan-Taber and Stampfer, 1998). Steroidal estrogens, including ethinyl estradiol, have also been classified as known human carcinogens (IARC, 1987; NTP, 2004).

Pregnancies do occur in women who are taking oral contraceptives; various studies have reported postconception oral contraceptive use ranging from 0.4% to 2.5% of oral contraceptive users (Li *et al.,* 1995), while Potter (1996) estimated the mean pregnancy rate for oral contraceptive users to be between 4% and 8%. While these exposures are like those that occurred in the case of diethylstilbestrol in that they involve exposure to a potent estrogen, they differ significantly in dose, in the coadministration of a progestin, and in the likely timing of exposure. In addition, these *in utero* exposures to oral contraceptives are inadvertent, so that determination of the exact timing of exposure for the purposes of an epidemiologic study is difficult, if not impossible. In general, epidemiologic studies that have addressed the issue of the potential adverse effects of *in utero* exposure to oral contraceptives have focused on various defects detectable at birth and possible alterations in sex ratio. While there have been positive associations with various defects reported in some studies, the majority have not found an increased rate of defects or an alteration of sex ratio resulting from these exposures (Raman-Wilms *et al.,* 1995). Li *et al.* (1995) reported a significant increase in congenital urinary tract anomalies resulting from maternal exposure to oral contraceptives after, but not prior to, conception. Several studies found no association between exposure to oral contraceptives early in pregnancy and hypospadias in male infants (Storgaard *et al.,* 2006; Wogelius *et al.,* 2006). There have apparently been no studies that have focused on effects that may be expressed only later in life, such as anomalies of sperm production, fertility, and cancer. One study that involved deliberate exposure of pregnant women to a combination of norethindrone acetate (20 mg) and ethinyl estradiol (40 µg) prior to scheduled abortions found no effect on androgen synthesis in the fetal testes (Kellokumpu-Lehtinen *et al.,* 1991). Thus, the risks of obvious defects detectable at birth resulting from inadvertent exposure of the fetus to oral contraceptives appear to be low (USFDA, 2004; WHO, 2004). Potential subtle long-term consequences of such exposures have not been addressed.

Dose Selection for the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Many of the studies examining the toxicity of ethinyl estradiol mentioned above were reported after the multigenerational reproductive toxicology study reported here was begun in 2000, and none of those studies used the experimental system used here. To select exposure concentrations for the present multigenerational reproductive toxicology study, a reproductive dose range-finding study was conducted in the same test system used for the multigenerational reproductive toxicology and chronic studies, that is, the NCTR CD Sprague-Dawley rat with doses administered in the Purina 5K96 soy- and alfalfa-free diet. The results of the reproductive dose range-finding study are presented later in this Technical Report.

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 (Delclos and Weis, 2004). 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.

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 con-

tinued on these diets through PND 77 (Ferguson *et al.*, 2003). 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 consumption of sodium-flavored solution and regular water was seen in both sexes at 200 ppb as the only treatment-related behavioral effects. As in the reproductive dose range-finding study summarized above, treatment-related reductions of body weight gain and feed consumption were observed in dams, and mean pup birth weight was decreased 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.

An 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 (Guo *et al.,* 2005). Terminal body weights for the F_1 pups of both sexes were decreased at 200 ppb. The activity of natural killer (NK) cells was enhanced in 25 and 200 ppb F_0 and F_1 females. Splenocyte proliferation induced by anti-CD3 antibodies, a marker of cell-mediated immunity, was increased in 200 ppb F_1 males and females. Spleen cell numbers were decreased in 200 ppb F_1 males (B, T, and NK cells) and females (B cells). A significant decrease in bone marrow DNA synthesis was observed in 5 ppb F_1 males but not the 25 or 200 ppb groups, and decreased erythrocyte progenitors were observed in 5 and 25 ppb F_1 females but not in the 200 ppb group.

In summary, these results coupled with those of the reproductive dose range-finding study 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 multigenerational reproductive toxicology studies, 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 multigenerational reproductive toxicology studies. The high exposure concentration for the multigenerational reproductive toxicology studies 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. The calculated ingested doses of ethinyl estradiol by animals consuming these dietary levels of ethinyl estradiol in the multigenerational reproductive toxicology study are given in Table 1.

Table 1
Approximate Ingested Doses of Ethinyl Estradiol in Rats Exposed to 2, 10, or 50 ppb Ethinyl Estradiol in the Multigenerational Reproductive Toxicology Study of Ethinyl Estradiol [a]

Sex/Dosing period	Generation	Mean Dose (µg/kg Body Weight Per Day) ± Standard Error		
		2 ppb	10 ppb	50 ppb
Males, Entire Feeding Period	F_0	0.1 ± 0.01 (13)	0.7 ± 0.04 (13)	3.8 ± 0.3 (13)
	F_1	0.2 ± 0.02 (17)	0.8 ± 0.07 (17)	4.2 ± 0.5 (17)
	F_2	0.1 ± 0.01 (17)	0.7 ± 0.06 (17)	3.7 ± 0.3 (17)
	F_0 through F_2 inclusive	0.1 ± 0.01 (47)	0.7 ± 0.04 (47)	3.9 ± 0.2 (47)
Females, Entire Feeding Period	F_0	0.2 ± 0.02 (12)	1.1 ± 0.1 (12)	6.0 ± 0.6 (12)
	F_1	0.2 ± 0.01 (17)	1.1 ± 0.1 (17)	6.0 ± 0.4 (17)
	F_2	0.2 ± 0.02 (17)	1.1 ± 0.1 (17)	5.5 ± 0.5 (17)
	F_0 through F_2 inclusive	0.2 ± 0.01 (37)	1.1 ± 0.1 (46)	5.8 ± 0.3 (46)
Females, Non-lactating	F_0	0.2 ± 0.01 (9)	0.9 ± 0.1 (9)	5.2 ± 0.4 (9)
	F_1	0.2 ± 0.01 (14)	1.1 ± 0.1 (14)	5.6 ± 0.4 (14)
	F_2	0.2 ± 0.01 (14)	1.0 ± 0.1 (14)	5.0 ± 0.4 (14)
	F_0 through F_2 inclusive	0.2 ± 0.01 (37)	1.0 ± 0.0 (37)	5.2 ± 0.2 (37)
Females, Lactating	F_0	0.3 ± 0.04 (3)	1.6 ± 0.2 (3)	8.7 ± 1.2 (3)
	F_1	0.3 ± 0.02 (3)	1.6 ± 0.2 (3)	8.0 ± 1.1 (3)
	F_2	0.3 ± 0.04 (3)	1.5 ± 0.2 (3)	8.1 ± 1.2 (3)
	F_0 through F_2 inclusive	0.3 ± 0.02 (9)	1.6 ± 0.1 (9)	8.3 ± 0.6 (9)

[a] The mean ingested dose was calculated for each week by multiplying the dietary concentration of ethinyl estradiol (ppb, or ng/g feed) 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 seven to give the mean daily dose given in the table. The number in parentheses is the number of weeks for which data were available for the calculation. Mean doses for females were calculated for the entire feeding period, the period during which the dams were not lactating, 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. Only the F_0 through F_2 generations are shown since F_3 animals were removed from exposure at weaning (PND 21) and F_4 animals were not given dosed feed.

MATERIALS AND METHODS

Procurement and Characterization of Ethinyl Estradiol

Ethinyl estradiol was obtained from Sigma-Aldrich Corporation (St. Louis, MO) in one lot (57H1178) which was used in the reproductive dose range-finding study and the multigenerational reproductive toxicology study. 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 studies 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 (EI) 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 17 α-derivatives of estradiol (Dionne and Poirier, 1995).

Before, during, and after the studies, the purity of lot 57H1178 was determined using ^{1}H-NMR (based on –CH groups), GC-EI MS, and/or 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 multigenerational reproductive toxicology 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 studies by the study laboratory 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 studies 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 NIH-31 feed and contains casein in place of soy and alfalfa. 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 studies, 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 studies, 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 every 9 weeks or as needed 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 and 50 ppb dose formulations were analyzed using GC with electron capture (EC)

detection, and the 200 ppb dose formulation was analyzed by HPLC-fluorescence. Stability studies of the 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 6° 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-EI MS or GC-EC. Because of the very low exposure concentrations utilized in these studies, 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 studies. For the reproductive dose range-finding study, specifications for the dose formulations were set as being within 50% of the target concentration with a coefficient of variation of ± 20%. For the multigenerational reproductive toxicology study, these specifications were set as being within 30% ± 20% of the target concentrations. Prior to and during the reproductive dose range-finding study, the dose formulations were analyzed approximately monthly (Table C3); all five of the dose formulations analyzed met the study specifications. During the multigenerational reproductive toxicology study, the dose formulations were generally analyzed every 6 weeks (Table C4). All 51 of the dose formulations analyzed and used in the study were within the study specifications.

Reproductive Dose Range-Finding Study

Two weeks prior to breeding to untreated F_0 males, 70- to 91-day old F_0 female rats from the study laboratory's breeding colony were shifted from the standard NIH-31 pellet diet to the soy- and alfalfa-free Purina 5K96 meal diet. Vaginal plug-positive females were assigned to the study, marked by tail tattoo, and housed individually until allocation to the exposure groups.

On gestation day 6 (GD 6, plug date = day 0) 10 to 12 vaginal plug-positive dams were randomly assigned to each exposure group to ensure that five litters would be obtained for each exposure concentration. Administration of dosed feed (0, 0.1, 1, 5, 25, 100, or 200 ppb ethinyl estradiol) was started on GD 7, and dams were continued on the same diets through weaning of their litters on postnatal day 21 (PND 21). Pregnant females were observed twice daily from GD 7 until parturition, and any signs of abnormal appearance or behavior were recorded. During this period, feed consumption and body weights of nonsentinel dams were recorded daily; statistical analyses of these endpoints included all nonsentinel dams assigned to the study.

The day of birth was designated as PND 1, and gestation duration was calculated from this date. Data on litter production, length of gestation, and litter parameters were collected and were reported on all litters produced, but only five litters per exposure group were randomly selected for further evaluation. Pup anogenital distance (AGD) was measured on PND 2 on the subset of litters that could potentially have been selected for continuation on the study. At the midpoint and at the end of the study, two control dams were sent for microbiological surveillance according to the protocols of the study laboratory's Sentinel Animal Program (Appendix O). The sera were analyzed for antibody titers to rodent viruses and *Mycoplasma* organisms, and all sentinel animals were examined for ectoparasites, endoparasites, and bacterial pathogens. All results were negative. From parturition to weaning, feed consumption and body weights of dams were recorded weekly; statistical analyses of these data were limited to the dams producing the five litters kept on the study. At weaning, all nonsentinel dams were euthanized and not otherwise evaluated.

On PND 1, the number of live and dead pups, litter weight (live pups), sex ratio, and any gross malformations were recorded for the F_1 animals. On PND 2, the pups were weighed, litters were randomly standardized to four males and four females each, AGD was measured with an ocular micrometer, and the pups were identified by paw tattoo. During litter randomization, littermates were kept together. Pups were fostered within exposure groups when necessary; however, this was rare (a total of five pups, one female in the 0 ppb group and four males in the 1 ppb group), and none of the reported necropsy data are from fostered pups. A litter mean AGD for each sex was calculated from three measurements made on each pup by a reader blind to exposure group. Pups were monitored daily for developmental landmarks, including day of eye opening, incisor eruption, ear unfolding, fur development, and timed righting reflex.

On PNDs 4, 7, 14, and 21, body weights and number of pups alive and dead were recorded. At weaning on PND 21, the pups were individually identified by tail tattoos, housed in same sex pairs, and continued on the same dosed feed as their dams until the day prior to sacrifice on PND 50. Feed consumption and body weights of the pups were recorded weekly between PNDs 21 and 49. Starting on PND 21, female pups were monitored daily for vaginal opening, and male pups were examined for preputial separation and testicular descent. Feed and filtered tap water were provided *ad libitum* throughout the experiment. Additional details of the study design and animal maintenance are summarized in Table 2.

Necropsies were performed on three male and three female pups per litter; the fourth pup of each sex in each litter was removed and used for neuroanatomical studies (reports in support of these studies are on file in the NCTR archives). Animals to be necropsied were fasted overnight prior to weighing and euthanasia on the morning of PND 50. Three animals of each sex from each litter were examined for organ weights and histopathology. Of these, two were anesthetized with a mixture of carbon dioxide and air, bled by cardiac puncture, and then sacrificed with carbon dioxide. Hemoglobin concentration, hematocrit, red and white cell counts, platelet count, and red cell indices were determined in the blood samples using a Cobas Minos Vet hematology analyzer (Roche Diagnostics, Somerville, NJ). Serum chemical analytes were measured using Cobas Minos Plus (Roche Diagnostics) methodologies. Differential leukocyte counts were performed manually. The clinical pathology parameters measured are listed in Table 2.

At necropsy, carcasses were examined for gross lesions, and lesions and protocol-specified tissues were processed for microscopic evaluation. Reproductive organs, accessory reproductive organs, and mammary glands were examined in all exposure groups; all other protocol-specified tissues were examined in the 0 and 200 ppb groups. If an increase in incidence or severity of lesions was detected microscopically in any of the specified tissues of the 200 ppb animals, those tissues were examined in all the animals from the intermediate exposure concentration groups. For females, the ovary/oviduct, uterus, and vagina were weighed separately, fixed in Bouin's solution, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E) for histologic evaluation. For males, the testis and epididymis were weighed. The right testis and epididymis were used for determination of homogenization-resistant spermatids and for sperm analysis, respectively (Robb *et al.,* 1978). The left testis and epididymis were fixed in Bouin's solution and embedded in paraffin. The fixed and embedded testes were sectioned and stained with periodic acid-Schiff/hematoxylin to detect different stages of the seminiferous tubules. Seminal vesicles with coagulating and preputial glands were weighed and fixed in 10% neutral buffered formalin (NBF). The prostate gland was fixed in NBF, and the dorsolateral and ventral lobes were dissected and weighed separately. For both sexes, adrenal gland, bladder, heart, kidney, liver, lung, pituitary gland, spleen, thymus, thyroid gland, ureter, and urethra were collected. The liver, spleen, and thymus were weighed, fixed in NBF, embedded in paraffin, sectioned, and stained with H&E. Pituitary and thyroid glands were weighed after fixation in NBF and then processed for histopathologic evaluation. Adrenal gland, heart, kidney, lung, and urinary tract were fixed in NBF and processed for histopathologic evaluation. The third left abdominal mammary gland was prepared as a whole mount fixed in NBF and stained with alum carmine for qualitative assessment of terminal end buds, terminal ducts, alveolar buds, and lobules. The corresponding mammary gland from the right side was fixed in NBF, embedded in paraffin, and stained with H&E for histologic evaluation. The right femur was removed, measured, and fixed in NBF. After decalcification, a cross-section at exactly mid-shaft was stained with H&E. Bone marrow from the sternum was evaluated histologically. For all tissues, sectioning was conducted at 4 to 6 µm.

Microscopic evaluations were completed by the study laboratory pathologist, and the pathology data were entered into the study laboratory's Micropath Data Collection System. The slides, paraffin blocks, and residual wet tissues were sent to the study laboratory's Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit.

Multigenerational Reproductive Toxicology Study

Study Design

Groups of 35 (for the F_0, F_1, F_3, and F_4 generations) or 40 (for the F_2 generation) mated pairs of rats were fed diets containing 0, 2, 10, or 50 ppb ethinyl estradiol for 98 (F_0 generation), 161 (F_1 through F_4 generations), or

42 (F_5 generation) days. Exposure to dosed feed varied by generation, and the schedules for each generation are shown in Figure 1 and described in Table 2. Twenty-five rats per sex from each generation (F_0 through F_4) were randomly selected for in-life studies and scheduled for necropsy on PND 140.

Source and Specification of Animals

The Multigeneration Support System, which was developed by R.O.W. 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 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 through F_5 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 O).

Animal Breeding and Maintenance

Animals of 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. At this point, the singly housed animals were reidentified with a unique tail tattoo and began receiving 5K96 feed containing 0, 2, 10, or 50 ppb ethinyl estradiol. In order to determine whether major exposure-related cycle disturbances were related to any fertility problems detected in the F_0 matings, two vaginal smears were taken 2 days apart, with an option for a third if results were ambiguous, during the first week of exposure and again 7 to 10 days prior to mating. No exposure-related mating effects were observed in the F_0 mating, and these data were therefore not statistically evaluated and are not reported. Males were housed individually in wire breeding cages for acclimation on PND 56 to PND 60. 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. When a vaginal plug (*in situ* or in pan below cage) was detected, males and females were separated and housed individually for the remainder of the study. In cases where no vaginal plug was detected, animals were separated after 14 days of cohabitation. The date of plug detection was designated as the day of conception or gestation day 0 (GD 0). Only animals for which a vaginal plug was detected were used in the analysis of endpoints requiring knowledge of the conception day (e.g., time to mating and gestation time).

After all pregnant dams had littered, 25 litters and their associated dams and sires were randomly selected for continuation on the study. Excess plug-positive dams that did not produce litters and mated dams that did not produce litters and were not designated as sentinel animals were transferred to the pathology lab for euthanasia and processing of the uteri for determination of resorption sites. 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, but fostered pups were not used as breeders for the next generation and thus were not included among animals necropsied for histopathology. 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. Pups to be used for breeding to produce the next generation were selected by the Multigeneration Support System at this time. These pups were selected randomly, with the stipulations that the maximum number of available litters be represented and no more than two pups of each sex from any one litter be selected. Breeding pairs could not be siblings. One female from each litter was identified for monitoring of vaginal cytology for 14 consecutive days starting 3 days after vaginal opening was observed. The animals designated for vaginal cytology monitoring beginning 3 days after vaginal opening were identified by tail tattoo and pair housed with another animal from the same exposure group. Animals designated as breeders were marked with a unique number by tail tattoo and housed individually. All animals not selected for breed-

ing or for monitoring of vaginal smears were assigned to approved addenda to the protocol or euthanized. On PND 56, or no later than PND 60, the 35 male pups selected by the Multigeneration Support System for breeding were placed in wire breeding cages for acclimation. Males and females from the same exposure group were paired when they were between 70 and 84 days old. Similar procedures for mating and litter selection were followed for the F_1 through F_4 generations. The procedures for the F_3 generation differed somewhat, in that all litters produced were held to ensure that there were 50 pups per sex per exposure group for the 2-year study (NTP, 2009) conducted with this generation.

Animals were maintained on soy- and alfalfa-free Purina 5K96 feed throughout the study. Animals in the exposed groups were fed dosed feed continuously from PND 42 of the parental generation (F_0) through weaning of the F_3 generation. At weaning, all animals in the F_3 generation were placed on 5K96 control feed. Purina 5K96 feed and Millipore®-filtered tap water were available *ad libitum* until the day before sacrifice when feed was withheld overnight. The 5K96 diet underwent routine analyses as well as periodic analyses for isoflavone concentrations as described above. Feeders were gently agitated daily with a vibrating tool (Dremel, Racine, WI) to prevent caking and were changed once per week. Feed consumption was measured weekly (F_0 animals: from PND 42 to termination; F_1 through F_4 animals: from PND 21 to termination) except during the 21-day nursing period in each generation when dam feed and water consumption were measured daily. Cages were changed weekly, and racks were changed every 28 days. Further details of animal maintenance are given in Table 2. Information on feed composition and contaminants is provided in Appendix N.

In-life Examinations and Pathology

The data collected during the in-life phase of the study and at necropsy are detailed in Table 2. Twice daily morbidity and mortality checks were performed, and any animals that were found moribund or dead were transported to Pathology and subjected to a complete necropsy. Body weights of F_0 animals at allocation to exposure groups on PND 42 were recorded. Thereafter, body weights and clinical findings were recorded weekly until the animals were terminated. For the F_1 through F_4 generations, body weights and clinical findings were recorded weekly from PND 21 through termination; in addition, pup body weights were measured on PNDs 2, 4, 7, and 14.

For the F_1 through F_5 generations, the date on which pups were born was designated as PND 1. The last daily check for litters was made between 1400 and 1430 hours, and littering had to have been completed by that time in order for it to be recorded as the delivery day. On PND 2, the number of pups alive and dead, sex ratio (ratio of males to females), and total live litter weight by sex were recorded, and any gross malformations were noted. The litters were randomly standardized to four male and four female pups per litter (pups with gross malformations were excluded), and the pups were marked with paw tattoos. For litter standardization, males and females were lined up on opposite sides of a cage. The first male was designated "number one," and the remaining males were numbered sequentially, followed by the females, starting with the uppermost. A computer-generated random number list was then used to select the pups. After standardization, individual body weights of the retained pups were recorded. In addition, anogenital distances (AGDs) were measured on the retained pups from 10 randomly selected litters. Individual pup body weights were recorded on PNDs 4, 7, 14, and 21.

For the F_1 through F_4 generations, all male pups were examined for nipple retention, and beginning on PND 14, males were monitored for testicular descent. On PND 21, pups were weaned and those selected for breeding, monitoring of vaginal smears, or assignment to other approved studies were given unique tail tattoo identification numbers. Females were monitored for vaginal opening from PND 21. After vaginal opening occurred, the estrous cycle of one female in each litter was monitored by vaginal cytology for 14 consecutive days, starting 3 days after vaginal opening was observed. These females were not used for breeding and were assigned to the chronic phase of the study, to other approved experiments, or euthanized after the vaginal smear monitoring phase was completed. Males were monitored for preputial separation beginning on PND 35.

For the F_0 through F_4 generations, mating and pregnancy parameters were measured for each litter. Sperm analyses were performed on single male animals from each litter at necropsy on PND 140. Vaginal cytology assessments on one female animal from each litter were performed for 9 or 10 consecutive days prior to scheduled sacrifice on PND 140. Ovarian follicle counts were recorded from eight females in each exposure group at scheduled sacrifice. Litters produced from the

breeding of the F_4 generation (F_5 generation) were euthanized at weaning following collection of basic litter information.

At study termination, all surviving animals from the F_0 through F_4 generations were euthanized by exposure to carbon dioxide and complete necropsies and microscopic examinations were performed. Complete necropsies were also performed on five animals that were removed prior to study termination as either dead or moribund. The adrenal gland, brain, epididymis, kidney, liver, ovary, spleen, testis, thymus, and uterus were weighed as soon as possible after dissection. The pituitary gland, prostate gland, seminal vesicle/coagulating gland, and thyroid gland were weighed after fixation. The left epididymis and testis from each male were frozen after dissection and weighing and used for assessment of testicular spermatid head counts, caudal epididymal sperm counts, and caudal epididymal sperm morphology. Sperm from the left vas deferens were collected in a prewarmed (38° C) solution of 1% bovine serum albumin dissolved in phosphate buffered saline for assessment of sperm motility. All protocol-specified tissues were examined grossly for visible lesions, removed, and fixed and preserved in 10% neutral buffered formalin with the exception of the testis which was placed in Bouin's fixative. The protocol-designated tissues were trimmed, processed, and embedded in Tissue Prep II, sectioned to a thickness of 4 to 6 microns, and stained, with the exception of the testis, with hematoxylin and eosin for microscopic examination. In addition, 5 step sections of both ovaries from eight females per exposure group were used to obtain counts of small, growing, and antral follicles. Periodic acid-Schiff stain was used for testis and rete testis evaluations to better aid in the characterization of sperm maturation. Tissues examined microscopically are listed in Table 2.

Histopathology samples collected during the course of the study were stored in the NCTR archives. Microscopic evaluations of tissues designated in the protocol were performed by two Study Pathologists, one for males and one for females, for generations F_0 through F_4. An in-house review of the histopathology findings from the current study was conducted. Except for F_1 animals, all neoplasms from all exposure groups and all generations along with target organs (mammary gland, kidney, and all reproductive organs) from 5% of the animals in all exposure groups and all generations were reviewed. In the F_1 animals, target organs were reviewed from 15 animals from the female control and 50 ppb groups and all tissues were reviewed in 5% of the animals in all exposure groups. The Quality Control (QC) pathologist evaluated the Gross Individual Animal Necropsy Report, the Gross to Microscopic Correlations, and Histopathology for each case, and the concurrence or nonconcurrence was documented. In the case of nonconcurrence, the QC Pathologist consulted with the Study Pathologist to attempt resolution of differences. The pathology staff decided any unresolved differences.

Statistical Methods

Reproductive Dose Range-Finding Study

For F_0 dams, total body weight gains and feed consumption during pregnancy and lactation were analyzed by one way analysis of variance (ANOVA). Daily body weight and feed consumption during pregnancy and weekly body weights and feed consumption after parturition were analyzed by repeated measures ANOVA using a mixed model approach. Dunnett's (1955) test was used to make comparisons between control and ethinyl estradiol-exposed groups, and contrasts were used to test for linear exposure concentration trends at each time interval.

For the pups, body weight, feed consumption, pup organ weights, and measures of sexual maturation (vaginal opening and preputial separation) were analyzed separately by sex using a nested mixed model ANOVA. If a likelihood ratio test indicated that there was a litter effect, birth dam nested within exposure concentration was included in the model as a random factor to account for the litter effect. For body weights, feed consumption, and organ weights, tests for linear and quadratic dose trends were conducted using contrasts and, for all endpoints, two-sided Dunnett's tests were used to compare ethinyl estradiol-exposed group means to the control group means.

Table 2
Experimental Design and Materials and Methods in the Feed Studies of Ethinyl Estradiol[a]

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Study Laboratory National Center for Toxicological Research (NCTR) (Jefferson, AR)	National Center for Toxicological Research (Jefferson, AR)
Strain and Species Sprague-Dawley/CD23/NCTR BR rats	Sprague-Dawley/CD23/NCTR BR rats
Animal Source NCTR breeding colony (Jefferson, AR)	NCTR breeding colony
Acclimation Time 2 weeks for F_0 animals prior to mating	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 Study Began Gestational day 7 (GD 7)	F_0: 6 weeks F_1 through F_5: 0 weeks (on study from conception)
Date of First Exposure[b] October 19-27, 1998	F_0 September 19, 2000 F_1 October 22, 2000 F_2 January 28, 2001 F_3 May 13, 2001 F_4 August 26, 2001 F_5 December 9, 2001
Duration of Exposure 64 days (GD 7 through PND 49)	F_0 From PND 42 to PND 140 (98 days) F_1 From conception to PND 140 (161 days) F_2 From conception to PND 140 (161 days) F_3 From conception to PND 21, fed control feed from PND 21 to PND 140 (161 days total, 42 days on dosed feed) F_4 No exposure; control feed from conception to PND 140 (161 days total, no dosed feed) F_5 No exposure; control feed from conception to PND 21 (42 days total, no dosed feed)
Date of Last Exposure[c] January 4-11, 1999	F_0 January 2, 2001 F_1 April 6, 2001 F_2 July 16, 2001 F_3 October 29, 2001 F_4 February 11, 2002 F_5 February 3, 2002
Necropsy Dates January 5-12, 1999	

Table 2
Experimental Design and Materials and Methods in the Feed Studies of Ethinyl Estradiol

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Average Age at Necropsy	
50 days	20 weeks
Size of Study Groups	
Five litters each consisting of four male and four female pups	35 mated pairs in the F_0, F_1, F_3, and F_4 generations; 40 mated pairs in the F_2 generation to provide extra pups for the chronic study reported elsewhere (NTP, 2009); 25 rats per sex from each generation (F_0 through F_4) were selected for in-life studies and necropsy on PND 140
Method of Distribution	
Vaginal plug-positive dams were randomly assigned to exposure groups on GD 6; litters were randomly culled to eight (four males and four females) on PND 2.	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.
Animals per Cage	
Pregnant dams were housed individually. Pups were kept with their mothers and then were housed in same sex pairs after weaning on PND 21.	F_0 animals were held two per cage from weaning until allocation to the exposure groups on PND 42, then housed individually. In subsequent generations, all animals were housed individually after weaning except the females in the F_1 through F_4 generations designated for study of vaginal cytology shortly after vaginal opening.
Method of Animal Identification	
Paw tattoo, tail tattoo	Tail tattoo; newborns were identified by paw tattoo until tail tattoo identification at weaning
Diet	
Purina 5K96 rodent chow, irradiated (Test Diets, Purina Mills, Inc., Richmond, IN), available *ad libitum* until the day before sacrifice	Same as Reproductive Dose Range-Finding Study
Water	
Millipore-filtered tap water (Jefferson, AR, municipal supply) via water bottle, available *ad libitum*	Same as Reproductive Dose Range-Finding Study
Cages	
Solid-bottom polycarbonate (Allentown Caging Equipment Co., Allentown, NJ), changed weekly	Same as Reproductive Dose Range-Finding Study
Bedding	
Heat-treated hardwood chips (P.J. Murphy Forest Products, Corp., Montville, NJ), changed weekly	Same as Reproductive Dose Range-Finding Study
Cage Bonnets	
Microisolator tops (Lab Products, Inc., Maywood, NJ)	Same as Reproductive Dose Range-Finding Study
Racks	
Metal animal cage racks (Allentown Caging Equipment Co., Allentown, NJ), changed every 28 days	Same as Reproductive Dose Range-Finding Study

Table 2
Experimental Design and Materials and Methods in the Feed Studies of Ethinyl Estradiol

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Animal Room Environment	
Temperature: 23° ± 3° C Relative humidity: 50% ± 20% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour	Temperature: 23° ± 3° C Relative humidity: 50% ± 20% Room fluorescent light: 12 hours/day Room air changes: at least 10/hour
Exposure Concentrations	
0, 0.1, 1, 5, 25, 100, or 200 ppb in feed, available *ad libitum*	0, 2, 10, or 50 ppb in feed, available *ad libitum*
Type and Frequency of Observation[d]	
From GD 7 until parturition, the dams were observed twice daily, and body weights and feed consumption of nonsentinel dams were recorded daily. Reproductive performance of the dams was recorded at parturition. Feed consumption and body weights of the dams were measured weekly during the nursing period. All nonsentinel dams were sacrificed without further analysis when the pups were weaned on PND 21. Pups were observed twice daily and weighed on PNDs 2, 4, 7, 14, and 21, weekly until PND 49, and at sacrifice on PND 50. Clinical findings were recorded once weekly, and feed consumption was measured weekly from PND 21 to 49. Reproductive and developmental endpoints were recorded at various time points from PNDs 1 to 49.	Observed twice daily; F_0 animals were weighed weekly from week 6 through termination, and F_1 through F_4 animals were weighed on PNDs 2, 4, and 7, and then weekly through termination. Clinical findings were recorded weekly. Feed consumption was recorded weekly except during the nursing period when dam feed and water consumption were measured daily. During the mating period, females were checked twice daily for vaginal plugs (*in situ* or in pan below cage). After mating, the time from pairing to detection of a vaginal plug, proportion of vaginal plug-positive dams giving birth, time from plug detection to birth, and proportion of mated females delivering litters were recorded. For the F_1 through F_5 litters, litter size, litter weight, number of live and dead pups of each sex, and sex ratio were determined. Anogenital distance was measured on 10 litters per exposure group in the F_1 through F_5 generations after standardization of litters to four male and four female pups each on PND 2. Time of testicular descent and body weight at preputial separation and vaginal opening were recorded for litters in generations F_1 through F_4.
Method of Sacrifice	
For two pups/sex per litter: anesthetized with carbon dioxide/oxygen, bled by cardiac puncture, and asphyxiated with carbon dioxide, following overnight fasting with water only. For one pup/sex per litter: decapitation, following overnight fasting with water only. (The brain tissue from these animals was transferred to the NCTR Division of Neurotoxicology for studies not reported here). The fourth pup of each sex in each litter was overdosed with sodium pentobarbital and then perfused transcardially with 0.9% saline followed by 10% buffered formalin. The brain was then prepared for three-dimensional reconstruction and volume measurement (Reports in support of these studies are on file in the NCTR archives).	Carbon dioxide asphyxiation following overnight fasting with water only

Table 2
Experimental Design and Materials and Methods in the Feed Studies of Ethinyl Estradiol

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Necropsy	
Necropsies were performed on three pups/sex per litter. Organs weighed were brain, epididymis, liver, left and right ovary/oviduct, pituitary gland, preputial gland, dorsolateral and ventral prostate gland, seminal vesicle/coagulating gland, spleen, left and right testis, thymus, thyroid gland, uterus, and vagina.	Necropsies were performed on all animals of the F_0 through F_4 generations plus five animals removed prior to study termination as either dead or moribund. The uterus of any dam detected as vaginal plug-positive but not littering was examined for resorption sites. Organs weighed prior to fixation were adrenal gland, brain, epididymis, kidney, liver, left and right ovary, spleen, left and right testis, thymus, and uterus. Organs weighed after fixation were: pituitary gland; dorsal, lateral and ventral prostate gland (lobes were separated after fixation); seminal vesicle with coagulating gland; and thyroid gland. The right femur was removed and fixed in neutral buffered formalin.
Clinical Pathology	
Blood was collected by cardiac puncture from two males and two females per litter surviving to the end of the studies for hematology and clinical chemistry. ***Hematology:*** hematocrit; hemoglobin concentration; erythrocyte, reticulocyte, and platelet counts; mean cell volume; mean cell hemoglobin; mean cell hemoglobin concentration; and leukocyte count and differentials ***Clinical chemistry:*** albumin, calcium, chloride, cholesterol, glucose, phosphorous, potassium, sodium, total protein, and triglyceride	None
Histopathology	
Complete histopathology was performed on pups in the 0 and 200 ppb groups. In addition to gross lesions and tissue masses, the following tissues were examined: adrenal gland, bone with marrow, clitoral gland, coagulating gland, heart, kidney, liver, lung, mammary gland, ovary, oviduct, penis, pituitary gland, preputial gland, dorsolateral and ventral prostate gland, spleen, left testis with epididymis and seminal vesicle, thymus, thyroid gland, ureter, urethra, urinary bladder, uterus, and vagina. Except for the penis, the reproductive organs, accessory sex organs, and mammary gland were examined in the remaining exposed groups; other organs were examined in the remaining exposed groups if increased incidences or severities of lesions were noted in the 200 ppb group.	For the surviving animals in each of the F_0 through F_4 generations and the five additional animals removed from study as either dead or moribund, complete histopathology was performed on all gross lesions, reproductive organs, mammary glands, and kidneys (females only). In addition, the following tissues were examined in the control and 50 ppb groups of these generations: adrenal gland, bone (femur), bone marrow, kidney (males), liver, pituitary gland, skin, spleen, thymus, and thyroid gland. In the case of the male kidney, the 10 ppb group was evaluated in the F_1 and F_2 generations.

Table 2
Experimental Design and Materials and Methods in the Feed Studies of Ethinyl Estradiol

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Sperm Analysis and Vaginal Cytology	
None	On PND 140, sperm samples were collected from surviving male animals in generations F_0 through F_4 for sperm evaluations. The following parameters were evaluated: sperm motility, epididymal sperm count, testicular spermatid head count, and sperm morphology. Vaginal samples were collected from designated females for 14 consecutive days starting 3 days after vaginal opening (F_1 through F_4 generations) and for 9 or 10 consecutive days prior to PND 140 (F_0 through F_4 generations) for vaginal cytology evaluations. Separate sets of pair-housed females, littermates of the animals maintained as breeders and designated for necropsy, were used for the 14-day analysis. The 10-day analysis was performed on animals selected for necropsy. The evaluations included the percentage of time spent in the various estrous cycle stages; number and percentages of abnormal cycles of estrus, diestrus, and the sum of the abnormal cycles of estrus and diestrus; and estrous cycle length.
Ovarian Follicle Counts	
None	For the F_0 through F_4 generations at necropsy on PND 140, two investigators counted small, growing, and antral follicles on five step sections of the left and right ovaries from eight animals per exposure group per generation.

[a] All animal use and procedures were conducted under a protocol reviewed and approved by the NCTR Institutional Animal Care and Use Committee.
[b] For the Multigenerational Reproductive Toxicology Study, the first date of exposure was the date of conception for the F_1 through F_5 generations.
[c] For the Multigenerational Reproductive Toxicology Study, the dates shown are dates of last exposure (F_0 through F_2) and/or necropsy (F_0 through F_5).
[d] For the Multigenerational Reproductive Toxicology Study, the statements describe the F_0 through F_4 generations unless otherwise indicated.

Histopathology data were analyzed for ethinyl estradiol effects on lesion incidences and severities by the Jonckheere-Terpstra (JT) test (Jonckheere, 1954; Hollander and Wolfe, 1973). Williams' modification of Shirley's test (Shirley, 1977; Williams, 1986) was used to compare exposed groups to the control group. All statistical tests were conducted at the $\alpha = 0.05$ level. Summary statistics only were determined for clinical chemistry, bone parameters, mean live pup weight, and anogenital distance. When inspection of the summary statistics indicated a possible effect that could affect exposure concentration selection, further analyses as specified in the table legends were conducted by the Principal Investigator and/or Study Director.

Multigenerational Reproductive Toxicology Study

Nonhistopathologic Data

The majority of data collected were analyzed by mixed models ANOVA. The experiment was evaluated as a two-way fixed effect treatment structure with exposure concentration ("dose") and generation ("generation") as the treatments. This evaluation was selected in order to test exposure effects as well as generation and exposure by generation interaction (D×G) effects. A "carry over" of an exposure effect from the exposed generations [F_0 through F_3 (until weaning)] into the nonexposed generations [F_3 (after weaning), F_4, and F_5] could be meas-

ured and tested within this two-way layout. It should be noted that a confounding effect on the exposure concentration effect running through the generations was the litter or family line influence in the study. The F_1 control dose group animals were direct descendants of the F_0 control group. The F_2 control group animals were direct descendants of the F_1 control group; this pattern continued for the control groups of successive generations. Similarly, each exposed group in each successive generation was the direct progeny of animals exposed to the same concentration of ethinyl estradiol in the preceding generation.

There were 37 original sires and 37 original dams that gave rise to the F_0 generation; from these mating pairs, all animals in the F_0 generation arose. There were 280 animals in the F_0 generation arising from these 37 pairs (35 animals $\times$ 4 exposure groups $\times$ 2 sexes). Consequently, an F_0 mother random effect, an F_0 father random effect, and an interaction of F_0 mother and F_0 father random effects 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 the 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.

The reason that F_0 mother and F_0 father random effects were included in the model was to dispense with nuisance variation. 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.

For data collected from the 25 animals of each sex that were carried to terminal sacrifice, no other ancestors were considered as possible random effects in this study. The reason was that for virtually all generations, only one animal per sex per litter was kept in the study. Consequently, intralitter variation was zero (calculated from a random sample of one), rather than positive (calculated from a random sample of greater than one).

In cases where analyses included data from all litters born into the study, another set of three random variables was tested via a log-likelihood ratio test for inclusion in the model. In short, there was a random variable for each unique female lineage beginning with F_0's mother through each applicable generation and, similarly, for each unique male lineage. Also, there was an interaction of the unique female and unique male lineages that was considered. Because of the very minor effect inclusion of any of these effects had on the results of the analyses, and because the simpler model selecting random effects from F_0's mother, F_0's father, and their interaction explained the dose and generation effects equally as well, these other three random effects were not employed. The sole exception was the analysis of the females' anogenital distance with body weight as the covariate. For this endpoint, the females' unique lineage random variable was included in the model used in the analyses.

Body weights, organ weights, feed consumption, and water consumption are historically considered to be normally distributed, and the raw data were analyzed after removal of outliers. Three models were used in the analysis of organ weight data: absolute organ weight, ratio of organ weight to body weight (relative weight), and analysis of covariance with body weight as the covariate applied to the absolute organ weight.

For some endpoints, transformations of the data were used to stabilize variance and bring the data closer to normality. Square root transformations were applied for ovarian follicle count and litter size analyses, and a natural log transformation was applied for the sex ratio analysis. The untransformed data for these endpoints are reported in the summary tables in the current report regardless of whether the statistical analysis was conducted on actual or transformed data.

Anogenital distance was analyzed both by analysis of covariance with body weight as the covariate and as the ratio of anogenital distance to the cube root of body weight (Gallavan *et al.,* 1999). Also, the model for newborn pup weights had a covariate of litter size included in the model.

Three *post hoc* tests were performed. First, Dunnett's tests on exposure concentration were done by generation or, in the case of repeated measures, generation and time

interval. These tests compare the control group with each exposed group and make an adjustment for the fact that several comparisons are being carried out concurrently. Secondly, Holm's-adjusted independent *t*-tests (Holm, 1979) on generation were done by exposure concentration or, in the case of repeated measures, by exposure concentration and time interval. All possible pairwise comparisons of the different generations were made, and the Holm's adjustment corrected for the fact that several comparisons were being carried out concurrently. Finally, in cases (fertility, mating, and pregnancy indices and gestational length) where the data were analyzed by logistic regression (Myers *et al.,* 2001), pairwise Chi-square test comparisons of controls to each exposed group were adjusted for simultaneous inference with Holm's adjustment.

Testing for linear and quadratic exposure concentration trends was accomplished using contrasts, and the results are reported in the data summary tables throughout the current report. Because the unequal spacing of the exposure concentrations (0, 2, 10, and 50 ppb) could lead to undue influence of the highest exposure concentration on trend analyses, trend analyses for those endpoints analyzed by ANOVA (except for repeated measures analyses of body weight, feed consumption, and water consumption) were also conducted using the natural log of the actual exposure concentration plus one, which resulted in a more evenly spaced scale of 0, 1.1, 2.4, and 3.9.

Nonparametric ANOVA was used in cases where data were not normally distributed (age at testicular descent, age at vaginal opening, age at preputial separation, vaginal cytology endpoints, and sperm parameter data). Two-way nonparametric ANOVAs were performed on all data except the sperm data, followed by one-way nonparametric ANOVAs (Kruskal-Wallis' tests; Kruskal and Wallis, 1952) by generation and exposure concentration. Nonparametric pairwise comparisons (Wilcoxon's tests; Wilcoxon, 1945) of exposure concentrations within generation or of generations within exposure concentration, with Holm's correction for multiple comparisons, were used for *post hoc* tests. For sperm parameters (testicular spermatid head counts, caudal epididymal sperm counts, caudal epididymal sperm motility, and sperm morphology) non-parametric ANOVAs (Kruskal-Wallis' tests) were conducted within generations.

Vaginal cytology endpoints examined were percentage of days in each stage of the estrous cycle, number and percentage of abnormal cycles, and length of cycle. An abnormal cycle was defined as 3 or more consecutive days of estrus or 4 or more consecutive days of diestrus in a cycle (Cooper and Goldman, 1999). The JT nonparametric test for monotonic increasing or monotonic decreasing trend was used to analyze exposure concentration effects on length of estrous cycle.

The probability of survival from the time of litter culling to weaning was estimated by the Kaplan-Meier procedure (Kaplan and Meier, 1958). Log-rank tests were used to test for an exposure concentration effect in each generation separately, as well as for an exposure concentration effect across all generations.

Where data on a particular endpoint were collected from both sexes, analyses were conducted separately by sex. All statistical tests (except for the random effects described previously) were conducted at the $\alpha = 0.05$ level. In cases where a significant dose main effect or a significant dose $\times$ generation interaction was observed, plots of adjusted (least squares) means were generated to examine the data further for potential nonmonotonic effects.

Histopathologic Data

The incidences of neoplasms or nonneoplastic lesions are presented in Tables A1a to A1e, A2a to A2e, B1a to B1e, and B2a to B2e as the number of animals bearing such lesions at a specific anatomic site and the numbers of animals with that site examined microscopically. There were no treatment-related neoplastic lesions observed during the microscopic evaluation of tissues from the current multigenerational reproductive toxicology study that was terminated at PND 140. Observed nonneoplastic lesions were recorded with their severity scores and analyzed by a JT test for exposure concentration trends along with Shirley's test for pairwise comparisons of exposed groups to the controls. These tests allow both incidence and severity information to be used. If the JT test indicated a positive exposure concentration trend, Shirley's test was used to test for a monotonic increase in response. If the JT test indicated a negative exposure concentration trend, Shirley's test was used to test for a monotonic decrease in response.

To examine the data more thoroughly for possible nonmonotonic responses, a Kruskal-Wallis' ANOVA was used to detect if differences exist, and Wilcoxon's test (Wilcoxon, 1945) was used to compare, in a pairwise

fashion, each exposed group to the control group. Exact P values were obtained using Monte Carlo simulations. The JT/Shirley's and Kruskal-Wallis'/Wilcoxon's tests were run for each generation separately; no cross-generation comparisons were made. This approach was necessary for these data since the lesions were sparse and in many cases existed in only some of the generations tested.

During the micropathology examinations, the Pathology Group also determined the estrous cycle stage (proestrus, estrus, metestrus, and diestrus) for the three major female sex organs: ovary, uterus, and vagina. The effect of ethinyl estradiol on synchrony of the stages in these three organs and the prevalence of each stage were examined. For analysis of synchrony, scores were assigned based on the level of desynchrony observed (number of organs out of synchrony, desynchrony due to adjacent or nonadjacent cycle stages), resulting in nine categories. For analysis of estrous cycle prevalence, a weighted least-squares analysis was used to model the estrous stage prevalence as a function of exposure concentration. Contrasts were also used to separate out the effect of exposure concentration for each stage (proestrus, estrus, metestrus, and diestrus), to compare exposed populations to controls, and to test for linear exposure concentration trends.

Quality Assurance Methods

The reproductive dose range-finding study and the multigenerational reproductive toxicology study were 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 studies. 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

Reproductive Dose Range-Finding Study

Body Weight and Feed Consumption of Dams During Pregnancy and After Delivery

Body weights and feed consumption of the dams during pregnancy are shown in Tables 3 through 6. Body weights during pregnancy were affected by exposure to ethinyl estradiol in the 100 and 200 ppb groups. Although there was not a strictly linear decrease in body weights with increasing exposure concentration, the linear exposure concentration trend test was significant starting on GD 8 and continuing through GD 21 (Table 3). Over this time period, body weights of 100 and 200 ppb dams were approximately 5% to 10% and

Table 3
Body Weights of Dams During Pregnancy in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	10	6	7	9	7	8	9
Gestation Day							
7[b]	278.37 ± 4.51	264.60 ± 7.90	285.90 ± 7.95	276.68 ± 4.76	288.73 ± 7.39	278.45 ± 4.26	279.93 ± 6.91
8	286.74 ± 5.34	270.28 ± 8.08	295.73 ± 7.29	283.19 ± 4.00	286.06 ± 7.47	271.90 ± 4.80	272.25 ± 7.32[c]
9	291.74 ± 5.25	275.12 ± 8.16	298.81 ± 7.51	287.96 ± 4.00	289.29 ± 7.04	274.19 ± 4.64	271.74 ± 6.85
10	296.70 ± 5.72	282.70 ± 7.60	305.49 ± 7.91	293.26 ± 4.10	294.23 ± 6.89	272.83 ± 5.58	272.02 ± 6.10*
11	300.83 ± 5.65	286.07 ± 7.75	310.34 ± 8.05	298.48 ± 4.14	297.21 ± 6.63	277.13 ± 4.65	274.10 ± 6.12*
12	304.82 ± 6.03	289.57 ± 7.49	312.67 ± 9.16	302.67 ± 4.30	299.11 ± 6.27	278.91 ± 5.20*	269.81 ± 5.24*[c]
13	309.81 ± 6.23	293.52 ± 7.56	317.90 ± 8.98	307.96 ± 4.32	303.59 ± 7.39	278.61 ± 6.07*	274.01 ± 5.87*
14	313.21 ± 6.27	297.98 ± 7.70	320.64 ± 9.18	312.41 ± 4.04	303.06 ± 7.03	279.43 ± 5.82*	272.52 ± 5.88*
15	321.99 ± 6.60	307.23 ± 8.58	328.73 ± 9.18	321.17 ± 3.89	312.50 ± 7.83	286.16 ± 6.47*	278.52 ± 7.39*
16	331.88 ± 6.73	316.40 ± 7.83	338.93 ± 9.83	332.12 ± 4.38	324.14 ± 8.81	297.35 ± 6.76*	287.19 ± 7.01*
17	343.75 ± 7.17	329.35 ± 7.80	350.37 ± 9.49	342.96 ± 5.18	337.79 ± 7.87	308.81 ± 6.34*	298.20 ± 7.58*
18	356.62 ± 7.52	341.70 ± 7.92	361.03 ± 10.05	358.41 ± 4.51	351.70 ± 8.85	322.16 ± 6.70*	311.27 ± 8.06*
19	367.26 ± 7.85	353.47 ± 8.48	374.70 ± 11.88[d]	369.96 ± 4.01	368.34 ± 10.94	335.41 ± 7.30*	319.17 ± 6.78*
20	377.84 ± 8.06	362.58 ± 7.45	384.94 ± 10.40	384.70 ± 4.05	376.93 ± 9.61	343.26 ± 7.54*	330.22 ± 8.34*
21	382.11 ± 9.11	367.22 ± 10.04	392.26 ± 10.82	388.10 ± 4.45[c]	380.17 ± 8.64	345.33 ± 9.61*[e]	329.56 ± 9.09*[c]

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Data given as the mean ± standard error in g/dam. GD 0 is the first day a dam was observed to be vaginal plug positive
[b] All days except day 7 showed a significant ($P \leq 0.05$) linear exposure concentration trend
[c] n = 8
[d] n = 6
[e] n = 7

5% to 14% lower, respectively, than those in the control group. Pairwise comparisons with the control group indicated significantly lower body weights in the 100 ppb group beginning on GD 12 and in the 200 ppb group beginning on GD 10. Daily feed consumption was also affected by exposure to ethinyl estradiol in the 100 and 200 ppb groups. In the early days of exposure (GD 8 to GD 14), the 100 and 200 ppb dams exhibited significant exposure concentration-related decreases in mean feed consumption, ranging from 27% to 60%, compared to controls (Table 4). Compared to controls, total body weight gain and total feed consumption were signifi-

Table 4
Feed Consumption by Dams During Pregnancy in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	10	6	7	9	7	8	9
Gestation Day							
8[b,c]	27.56 ± 1.37[d]	19.85 ± 2.44*	27.99 ± 0.88	26.50 ± 0.90[e]	20.56 ± 1.18	15.12 ± 1.53*	11.17 ± 1.54*[f]
9[b]	25.45 ± 1.18	21.36 ± 1.08	24.41 ± 1.47	22.99 ± 0.95	18.28 ± 1.62*	16.78 ± 1.13*	14.89 ± 1.47*
10[b]	24.79 ± 1.20	28.65 ± 5.51	27.24 ± 3.32	23.75 ± 0.78	21.39 ± 2.18	16.18 ± 1.19*	14.51 ± 1.05*
11[b]	23.02 ± 1.10	21.99 ± 1.28[g]	23.87 ± 2.40	22.97 ± 0.63	19.82 ± 2.05	19.73 ± 3.11	16.45 ± 1.89
12[b]	24.60 ± 1.63	25.10 ± 2.21	24.90 ± 3.62	25.05 ± 1.79	22.30 ± 1.52	22.48 ± 2.35	16.21 ± 1.59*[e]
13[b]	29.32 ± 3.08	25.77 ± 1.85	31.13 ± 2.74	28.12 ± 1.20	25.61 ± 1.58	20.09 ± 1.38*	17.02 ± 1.17*
14	26.01 ± 3.10[d]	25.02 ± 2.25	19.88 ± 3.30	24.00 ± 0.76	19.29 ± 2.57	20.48 ± 1.68	19.01 ± 1.94*
15	27.22 ± 1.78	24.90 ± 1.36	26.99 ± 1.43	26.60 ± 0.90	26.71 ± 1.20	26.59 ± 3.81	23.53 ± 2.40
16[c]	24.60 ± 0.99	23.33 ± 1.74	25.24 ± 1.44	27.74 ± 1.01	27.04 ± 0.91	31.65 ± 2.94*	25.03 ± 2.50
17	27.82 ± 1.52	26.98 ± 1.77	27.68 ± 1.73	28.36 ± 1.60	27.07 ± 1.43	28.63 ± 2.40	27.32 ± 2.59
18	29.39 ± 1.09	27.73 ± 1.13	26.76 ± 1.50	27.31 ± 2.51	26.73 ± 2.43	27.63 ± 3.10	25.67 ± 2.15
19	23.68 ± 1.11	24.28 ± 1.42	26.79 ± 2.61[f]	25.43 ± 1.42	27.83 ± 2.07	28.71 ± 1.68	23.31 ± 1.83
20[b,c]	23.26 ± 1.81	21.82 ± 1.37	27.29 ± 3.17	25.73 ± 2.13	20.70 ± 0.90	17.79 ± 2.73	19.04 ± 2.85
21[c]	15.67 ± 2.41	16.09 ± 1.51	21.00 ± 2.27	16.28 ± 1.26[e]	14.84 ± 2.29	9.76 ± 2.04[h]	18.25 ± 1.36[e]

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Data given as the mean ± standard error in g/dam. GD 0 is the first day a dam was observed to be vaginal plug positive
[b] Significant ($P \leq 0.05$) linear exposure concentration trend
[c] Significant ($P \leq 0.05$) quadratic exposure concentration trend
[d] n = 9
[e] n = 8
[f] n = 6
[g] n = 5
[h] n = 7

cantly decreased during the gestational period in 100 and 200 ppb dams (Tables 5 and 6). Compared to the controls, the 100 and 200 ppb groups exhibited significant exposure concentration-related decreases in total body weight gains of approximately 33% and 50%, respectively, and in feed consumption of 13% and 24%, respectively.

Body weights and feed consumption of the dams were measured on a weekly basis after delivery until the pups were weaned and the dams terminated. Other than a linear negative trend in body weights in the first week, no significant alterations of body weight or feed consumption were detected in the dams after delivery (Tables 7 and 8).

Table 5
Total Body Weight Gains of Dams During Pregnancy in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	10	6	7	9	7	7	8
	103.74 ± 5.32	102.62 ± 3.43	106.36 ± 6.27	110.71 ± 1.83	91.44 ± 6.12	69.39 ± 8.42*	52.24 ± 7.49*

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Data given as the mean ± standard error in g/dam. Significant (P≤0.01) linear exposure concentration trend

Table 6
Total Feed Consumption by Dams During Pregnancy in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	10	6	7	9	7	8	9
	347.04 ± 13.51	329.20 ± 7.33	357.35 ± 9.92	346.07 ± 8.54	318.17 ± 5.09	300.39 ± 15.50*	263.85 ± 14.45*

* Significantly different (P≤0.05) from the control group by Dunnett's test
[a] Data given as the mean ± standard error in g/dam. Significant (P≤0.05) linear exposure concentration trend

Table 7
Body Weights of Dams After Parturition until Weaning in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

Week	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	5	5	5	5	5	5	4
1[b]	298.56 ± 14.05	278.28 ± 7.21	307.48 ± 7.92	286.44 ± 9.90	291.16 ± 3.88	270.56 ± 10.23	272.34 ± 8.37[c]
2	297.32 ± 11.99	265.98 ± 13.68	290.16 ± 10.24	276.48 ± 6.56	283.82 ± 7.57	266.06 ± 7.53	270.05 ± 12.27
3	304.86 ± 9.59	282.42 ± 5.39	293.38 ± 10.06	279.80 ± 9.10	292.78 ± 3.56	275.18 ± 12.85	290.85 ± 9.64

[a] Data given as the mean ± standard error in g/dam.
[b] Significant ($P \leq 0.01$) linear exposure concentration trend
[c] n = 5

Table 8
Feed Consumption by Dams After Parturition until Weaning in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

Week	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Dams	5	5	5	5	5	5	5
1	53.84 ± 4.71	58.81 ± 5.31	57.83 ± 2.79	54.37 ± 1.75	53.23 ± 1.30	60.68 ± 2.91	51.72 ± 2.52
2	85.17 ± 2.59	77.39 ± 9.53	82.61 ± 4.34	82.98 ± 5.48	84.53 ± 5.89	89.67 ± 2.13	89.19 ± 3.96[b]
3	87.63 ± 9.93	94.32 ± 11.21	85.35 ± 7.31	93.60 ± 13.65	88.84 ± 13.33	90.52 ± 14.61	75.25 ± 12.30

[a] Data given as the mean ± standard error in g/dam per day.
[b] n = 4

Litter Production, Gestation Duration, and Litter Parameters

Data on the proportion of vaginal plug-positive dams assigned to the study that produced litters, gestation duration, and other litter parameters are summarized in Table 9. No significant treatment effects were observed on the proportion of dams producing litters, gestation duration, litter size, proportion of stillborn pups, or sex ratio. There was a significant effect of treatment on pup birth weight, with approximately 15% lower weights in the 100 and 200 ppb groups compared to that in the control group. This is also consistent with results obtained in the immunotoxicity and behavior studies of ethinyl estradiol (data not shown). Those studies did not include

Table 9
Litter Data for Rats in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Litters/Plug-Positive Females	10/12	6/10	7/10	9/10	7/10	8/10	9/12
Gestation Duration (days)[a]	22.7 ± 0.2	22.5 ± 0.2	22.7 ± 0.2	22.4 ± 0.2	22.6 ± 0.2	22.3 ± 0.2	22.3 ± 0.2
Total Pups/Litter[a]	13.30 ± 1.05	12.83 ± 1.92	12.57 ± 2.03	15.56 ± 0.53	15.57 ± 1.23	14.75 ± 0.45	13.00 ± 1.42
Stillborn Pups/ Total Pups	4/133	1/77	2/88	3/140	5/109	2/118	4/117
Mean Live Pup Weight (g)[a,b]	6.05 ± 0.34	5.70 ± 0.52	5.72 ± 0.22	5.65 ± 0.12	5.76 ± 0.30	5.12 ± 0.21*	5.15 ± 0.16*
Male Pups (%)[a]	0.54 ± 0.04	0.49 ± 0.06	0.46 ± 0.09	0.53 ± 0.03	0.50 ± 0.05	0.47 ± 0.04	0.48 ± 0.07
Anogenital Distance (mm)[c]							
n	5	6	5	6	6	7	7
Male	3.54 ± 0.04	3.50 ± 0.03	3.52 ± 0.04	3.40 ± 0.04	3.50 ± 0.03	3.54 ± 0.07	3.50 ± 0.04
Female	2.24 ± 0.05	2.20 ± 0.03	2.22 ± 0.06	2.13 ± 0.04	2.13 ± 0.03	2.16 ± 0.03	2.13 ± 0.04
n	4	5	4	4	4	6	7
Male	3.52 ± 0.05	3.48 ± 0.02	3.50 ± 0.04	3.40 ± 0.04	3.48 ± 0.03	3.53 ± 0.08	3.50 ± 0.04
Female	2.25 ± 0.06	2.20 ± 0.03	2.20 ± 0.07	2.13 ± 0.06	2.10 ± 0.04	2.15 ± 0.03	2.13 ± 0.04

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Mean ± standard error, data from all litters born are included.
[b] Significant ($P \leq 0.05$) main effect of Dose by ANCOVA with litter size as the covariate
[c] Litter means ± standard error are presented for all litters in which anogenital distance (AGD) was measured. Two values are presented for each sex. The first is for all litters on which AGD was measured, and the second is for litters on which AGD was measured and for which litter body weights were available. The former data were analyzed by ANOVA, while the latter data were analyzed by ANCOVA with body weight as the covariate. Plugged dams were delivered to the study over a 2-week period from the NCTR breeding colony. The dams were randomly allocated to exposure groups on arrival, and approximately 80% of the dams were expected to litter. Since it was not known which of the allocated dams would litter and become a part of the five litters per exposure group kept on study, the AGD of all litters that could potentially be assigned to the study were measured. The five litter positions per exposure group were filled with the first available litters that did not contain fostered pups or have an inattentive dam. If a litter was born to an exposure group that already had five litters assigned, AGD was not measured.

a 100 ppb group, but mean birth weights of the 200 ppb groups were found to be 14% and 20% less than control weights in the immunotoxicity and behavior studies, respectively. In the immunotoxicity study, there was an apparent effect of treatment on the proportion of stillborn pups as indicated by a significant Chi-square test. However, it appears that this was most likely due to an abnormally low proportion of stillborn pups in the 25 ppb group and a slightly elevated proportion in the 200 ppb group (0 ppb, 2.7%; 5 ppb, 2.7%; 25 ppb, 0.9%; 200 ppb, 4.1%). Together with the lack of effect observed in the current reproductive and unshown behavior studies, it is concluded that ethinyl estradiol did not affect the proportion of stillborn pups under the conditions of these studies.

Anogenital Distance, Pup Developmental Landmarks, Body Weight, and Feed Consumption

Ethinyl estradiol had no apparent effect on anogenital distance measured on PND 2 in either sex (Table 9). Developmental landmarks for the pups are presented in Table 10. Preputial separation, a measure of male puberty, was significantly accelerated by approximately 1.9 and 2.6 days in the 5 and 25 ppb groups, respectively.

Table 10
Developmental Landmarks in Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Male							
Number of Litters	5	5	5	5	5	5	5
Righting Reflex	1.98 ± 0.84	1.50 ± 0.41	1.66 ± 0.39	1.78 ± 0.30	1.02 ± 0.38	2.12 ± 0.12	1.62 ± 0.45
Fur Development	9.26 ± 0.85	10.00 ± 0.84	9.50 ± 0.55	9.46 ± 0.84	9.90 ± 0.78	10.76 ± 0.77	10.60 ± 0.98
Eye Opening	14.80 ± 0.46	15.56 ± 0.42	15.70 ± 0.54*	14.80 ± 0.58	14.76 ± 0.35	15.40 ± 0.51	15.50 ± 0.39
Ear Unfolding	16.82 ± 0.53	17.46 ± 0.43	17.83 ± 0.28[b]	17.02 ± 0.68	16.76 ± 0.45	18.26 ± 0.37*	17.70 ± 0.44
Incisor Eruption	10.42 ± 0.38	10.28 ± 0.35	10.16 ± 0.46	10.62 ± 0.52	10.12 ± 0.26	11.66 ± 0.38*	11.02 ± 0.42
Testicular Descent	21.46 ± 0.47	22.42 ± 0.33	21.76 ± 0.58	21.50 ± 0.22	21.36 ± 0.49	22.86 ± 0.78	24.76 ± 1.23
Preputial Separation	43.06 ± 0.70	42.39 ± 0.84	41.79 ± 0.38[b]	41.20 ± 0.55*	40.47 ± 0.28*	43.38 ± 0.52	44.00 ± 2.00[c]
Animals showing preputial separation at PND 50/number examined	17/20	18/20	19/20	20/20	19/20	16/18	4/20[d]
Female							
Number of Litters	5	5	5	5	5	5	5
Righting Reflex	1.94 ± 1.10	1.88 ± 0.45	1.08 ± 0.40	2.22 ± 0.45	1.32 ± 0.48	2.20 ± 0.19	2.46 ± 0.57
Fur Development	9.86 ± 0.92	10.00 ± 0.84	9.40 ± 0.68	10.02 ± 0.91	10.20 ± 0.74	10.80 ± 0.80	10.60 ± 0.98
Eye Opening	14.76 ± 0.41	15.46 ± 0.47	15.46 ± 0.50	14.80 ± 0.58	14.82 ± 0.34	15.40 ± 0.51	15.46 ± 0.39
Ear Unfolding	17.08 ± 0.39	17.86 ± 0.16	17.70 ± 0.41[b]	17.06 ± 0.64	17.06 ± 0.58	18.16 ± 0.33*	17.70 ± 0.44
Incisor Eruption	11.12 ± 0.49	10.54 ± 0.35	10.12 ± 0.37	10.42 ± 0.65	10.18 ± 0.26	11.98 ± 0.40	11.20 ± 0.37
Vaginal Opening	32.53 ± 0.78	34.45 ± 1.21	32.37 ± 0.84	32.75 ± 1.16	28.84 ± 2.15	28.10 ± 1.43	27.15 ± 1.34*

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] All landmarks except preputial separation ratio at PND 50 given as litter mean ± standard error in days.
[b] n = 4
[c] n = 2
[d] Significantly different from the control group by Chi-square test

There was no significant effect on the time of preputial separation in the 200 ppb group when animals showing preputial separation were examined; however, only four (20%) of the animals in the 200 ppb group showed preputial separation at scheduled sacrifice on PND 50 compared to 85% to 100% in all of the other exposure groups. Chi-square analysis of the proportion of animals showing preputial separation indicated a significant effect of treatment. When the analysis was run with the 200 ppb group excluded, there was no effect of treatment. Thus, it appears that 200 ppb ethinyl estradiol did affect (i.e., delay) preputial separation. The time of testicular descent was not significantly affected by treatment, although the mean time of observation of this event in 200 ppb males was 3.3 days later than the time of observation in controls. In females, the time of vaginal opening was accelerated in an exposure concentration-related fashion in the 25, 100, and 200 ppb groups, but was significantly decreased only in the 200 ppb group. While a few sporadic statistically significant increases or decreases in the mean time of occurrence of other developmental landmarks were observed, no patterns suggestive of biological significance were evident.

Body weights for the male and female pups remaining after culling of the litters that were designated for continuation on the study are shown in Table 11. For both males and females, the only significant body weight

Table 11
Body Weights of Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

Postnatal Day	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Male							
Number of Pups	20	20	20	20	20	18	20
2	6.30 ± 0.28	6.68 ± 0.59	6.90 ± 0.52	6.76 ± 0.77	6.50 ± 0.30	6.46 ± 0.35	6.68 ± 0.63
4	8.24 ± 0.47	9.30 ± 1.33	9.40 ± 1.33	9.30 ± 1.30	8.86 ± 0.93	8.82 ± 0.79	9.16 ± 1.39
7	9.59 ± 0.51	11.83 ± 1.55	10.49 ± 0.69	10.26 ± 0.68	9.92 ± 0.38	9.62 ± 0.41	9.66 ± 0.90
14	24.62 ± 1.98	24.43 ± 2.16	26.04 ± 0.99	23.99 ± 0.76	25.09 ± 0.68	24.29 ± 0.88	22.86 ± 1.07[b]
21	39.34 ± 2.45	38.93 ± 2.48	40.65 ± 1.39	38.69 ± 0.72	39.92 ± 0.88	37.77 ± 2.73	38.32 ± 2.81
28	72.91 ± 5.30	76.58 ± 5.34	78.34 ± 2.27	73.31 ± 2.74	76.45 ± 1.55	71.46 ± 3.76	69.74 ± 3.43
35	116.02 ± 8.02	122.65 ± 7.66	122.71 ± 3.63	115.44 ± 3.86	122.20 ± 1.80	112.51 ± 6.17	107.28 ± 5.20
42	161.59 ± 8.88	171.41 ± 7.86	167.98 ± 3.15	150.97 ± 4.82	169.02 ± 2.55	160.16 ± 8.98	147.81 ± 6.01*
49	201.93 ± 9.44	210.91 ± 10.29	210.56 ± 2.06	201.98 ± 4.61	208.42 ± 3.97	198.52 ± 7.40	180.78 ± 7.74*
Female							
Number of Pups	19	20	20	20	20	20	20
2	5.86 ± 0.29	5.84 ± 0.41	6.26 ± 0.21	5.74 ± 0.35	5.76 ± 0.33	5.50 ± 0.31	5.50 ± 0.38
4	7.40 ± 0.46	7.38 ± 0.59	7.84 ± 0.25	7.80 ± 0.56	7.30 ± 0.37	7.26 ± 0.41	6.72 ± 0.70
7	8.50 ± 0.50	9.59 ± 0.94	9.95 ± 0.39	9.06 ± 0.55	9.03 ±0.34	8.99 ± 0.41	8.69 ± 0.77
14	22.25 ± 1.18	22.62 ± 2.10	24.24 ± 1.24	22.78 ± 0.47	23.00 ± 0.53	23.34 ± 0.94	21.07 ± 1.48[b]
21	36.18 ± 1.60	36.52 ± 2.63	38.57 ± 1.56	35.97 ± 0.64	36.62 ± 0.70	36.62 ± 2.23	35.55 ± 2.49
28	64.57 ± 2.89	67.71 ± 5.23[c]	70.70 ± 2.14	64.09 ± 1.21	67.12 ± 0.85[c]	65.74 ± 2.51	63.49 ± 3.12
35	99.43 ± 5.50	103.74 ± 6.94	106.04 ± 2.69	97.51 ± 3.68	101.39 ± 2.49[d]	99.28 ± 4.00	91.54 ± 3.65
42	134.35 ± 7.65	136.62 ± 6.55	139.16 ± 3.21	130.20 ± 4.10	131.79 ± 2.55[c]	129.79 ± 5.40	121.92 ± 4.89*
49	152.36 ± 8.30	155.46 ± 7.96	159.29 ± 3.00	148.75 ± 5.34	149.47 ± 3.84[c]	146.27 ± 3.90	139.49 ± 5.24*

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Data given as the mean ± standard error in grams.
[b] n = 16
[c] n = 19
[d] n = 17

effects were 8% to 10% decreases relative to the controls in the 200 ppb groups at the last two time points measured, PNDs 42 and 49. Other than a significant negative linear exposure concentration trend from PND 29 to 35 for females, no significant effects on feed consumption were observed (Table 12). There were no significant effects on total body weight gain of male pups or total feed consumption of pups of either sex between weaning and termination of the experiment; total body weight gains of female pups showed significant negative linear and quadratic exposure concentration trends (Tables 13 and 14).

Table 12
Feed Consumption by Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

Postnatal Days	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Cages	10	10	10	10	10	10	10
Male							
21-28	9.03 ± 0.52	9.20 ± 0.39	9.72 ± 0.32	9.52 ± 0.38	9.69 ± 0.22	10.82 ± 1.48	8.92 ± 0.54
29-35	13.36 ± 0.79	13.37 ± 0.63	13.79 ± 0.49	13.26 ± 0.34	14.46 ± 0.84	13.76 ± 0.54	12.41 ± 0.51
36-42	17.03 ± 0.91	17.36 ± 0.71	17.74 ± 0.46	16.98 ± 0.28	16.82 ± 0.50	17.23 ± 0.86	15.84 ± 0.57
43-49	19.90 ± 0.78	19.84 ± 0.87	20.52 ± 0.62	20.36 ± 0.59	19.63 ± 0.56	19.68 ± 1.42	18.38 ± 1.04
Female							
21-28	8.63 ± 0.37	7.94 ± 0.64	8.87 ± 0.30	8.97 ± 0.33	9.89 ± 0.56	8.62 ± 0.18	7.93 ± 0.54
29-35[b]	11.79 ± 0.46	11.60 ± 0.60	12.23 ± 0.20	11.83 ± 0.36	12.27 ± 0.47[c]	11.64 ± 0.26	10.98 ± 0.29
36-42	14.78 ± 0.63	14.16 ± 0.39	14.75 ± 0.27	14.14 ± 0.28	13.27 ± 0.78	14.02 ± 0.33	13.54 ± 0.45
43-49	15.23 ± 0.77	14.24 ± 0.72	15.40 ± 0.45	15.41 ± 0.42	14.76 ± 0.32	14.93 ± 0.54	14.35 ± 0.61

[a] Data given as the animal mean ± standard error in g/day.
[b] Significant ($P \leq 0.05$) linear exposure concentration trend
[c] n = 9

Table 13
Total Body Weight Gains of Rat Pups After Weaning in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Pups	20	20	20	20	20	18	20
Male	192.34 ± 8.86	199.08 ± 9.27	200.06 ± 1.62	191.72 ± 4.00	198.49 ± 3.68	188.90 ± 7.25	171.12 ± 6.77
Number of Pups	19	20	20	20	19	20	20
Female[b,c]	143.85 ± 7.61	145.87 ± 6.95	149.34 ± 2.97	139.68 ± 4.85	140.56 ± 3.51	137.28 ± 3.65	130.80 ± 4.47

[a] Data given as the mean ± standard error in grams for the period from PND 21 through PND 50.
[b] Significant ($P \leq 0.05$) linear exposure concentration trend
[c] Significant ($P \leq 0.01$) quadratic exposure concentration trend

Table 14
Total Feed Consumption by Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Number of Cages	10	10	10	10	10	10	10
Male	391.16 ± 13.62	389.87 ± 11.01	405.40 ± 6.51	398.45 ± 6.36	403.03 ± 5.44	409.45 ± 21.34	367.25 ± 12.12
Female	335.51 ± 10.21	316.27 ± 10.61	339.61 ± 4.83	335.92 ± 5.73	331.38 ± 12.85	328.21 ± 6.04	310.75 ± 8.00

[a] Data given as the animal mean ± standard error in grams for the period from PND 21 through PND 50.

Terminal Body Weights and Absolute and Relative Organ Weights

Males

The mean terminal body weight of 200 ppb males was less than that of the controls (Table 15). The majority of significant organ weight effects were observed in the 200 ppb group. The exceptions were the mean weights of the dorsolateral prostate gland, which showed 13% to 33% increases in the intermediate exposure concentration groups, and were significantly increased in the 5 ppb group; these increases were significant regardless of the statistical model used for the analysis (absolute weight, ratio of organ weight to body weight, or body weight as covariate). Pituitary gland weights showed positive exposure concentration trends by both the ratio and covariance models, and relative pituitary gland weight was significantly increased in the 200 ppb group. Testis and ventral prostate gland weights were significantly decreased in 200 ppb males. Relative brain weight was significantly increased in 200 ppb males, and this may reflect the fact that brain weight is generally not affected by alterations in body weight.

Table 15
Organ Weights and Organ-Weight to-Body-Weight Ratios for Male Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
n	15	15	15	15	15	13	15
Necropsy Body Wt	191.8 ± 6.2	194.6 ± 5.5	198.0 ± 2.9	191.5 ± 3.6	193.9 ± 2.9	183.3 ± 4.0	169.6 ± 4.8*
Brain							
Absolute	1.827 ± 0.050[d]	1.808 ± 0.028[e]	1.825 ± 0.009[d]	1.888 ± 0.046[d]	1.870 ± 0.017[d]	1.791 ± 0.055[e]	1.876 ± 0.033[d]
Relative[b,c]	9.340 ± 0.293[d]	9.486 ± 0.469[e]	9.204 ± 0.300[d]	9.774 ± 0.242[d]	9.660 ± 0.233[d]	9.796 ± 0.146[e]	11.234 ± 0.535*[d]
ANCOVA[b,c]		–	–	–	–	–	–
Epididymis							
Absolute[b,c]	0.298 ± 0.005	0.314 ± 0.023	0.311 ± 0.016	0.308 ± 0.012	0.325 ± 0.005	0.300 ± 0.020	0.263 ± 0.014
Relative	1.586 ± 0.083	1.615 ± 0.095	1.569 ± 0.060	1.610 ± 0.034	1.675 ± 0.032	1.643 ± 0.074	1.549 ± 0.033
ANCOVA		–	–	–	–	–	–
Liver							
Absolute[b]	7.029 ± 0.308	7.310 ± 0.348	7.634 ± 0.350	7.186 ± 0.263	7.215 ± 0.259	7.050 ± 0.571[g]	6.487 ± 0.380
Relative	36.619 ± 0.990	37.614 ± 0.341	38.480 ± 1.328	37.447 ± 0.594	37.218 ± 0.808	38.264 ± 1.486[g]	38.214 ± 0.837
ANCOVA		–	–	–	–	–	–
Pituitary Gland							
Absolute	8.5 ± 0.3	8.8 ± 0.6	8.8 ± 0.4	8.4 ± 0.6	8.7 ± 0.5	9.1 ± 0.8	9.5 ± 0.3
Relative[b,c]	0.044 ± 0.002	0.045 ± 0.002	0.044 ± 0.001	0.044 ± 0.003	0.045 ± 0.003	0.050 ± 0.004	0.056 ± 0.003*
ANCOVA[b]		–	–	–	–	–	*
Preputial Gland							
Absolute[c,h]	104.7 ± 4.6	105.8 ± 13.5	92.3 ± 8.8	101.4 ± 7.1	120.3 ± 9.9	124.5 ± 8.0	84.9 ± 6.3
Relative[c,h]	0.549 ± 0.026	0.538 ± 0.050	0.469 ± 0.052	0.529 ± 0.032	0.627 ± 0.066	0.678 ± 0.047	0.503 ± 0.030
ANCOVA[c,h]		–	–	–	–	–	–
Dorsolateral Prostate Gland							
Absolute[b,c]	0.126 ± 0.021	0.143 ± 0.008	0.160 ± 0.010	0.168 ± 0.006*	0.146 ± 0.007	0.151 ± 0.013	0.120 ± 0.011
Relative[c]	0.658 ± 0.100	0.736 ± 0.033	0.809 ± 0.037	0.879 ± 0.038*	0.753 ± 0.033	0.824 ± 0.054	0.700 ± 0.031
ANCOVA[c]		–	–	*	–	–	–
Ventral Prostate Gland							
Absolute[b,c]	0.172 ± 0.027	0.169 ± 0.018	0.179 ± 0.020[f]	0.190 ± 0.132	0.190 ± 0.020	0.155 ± 0.019	0.101 ± 0.016*
Relative[b]	0.886 ± 0.106	0.852 ± 0.055	0.892 ± 0.090[f]	0.989 ± 0.058	0.973 ± 0.094	0.845 ± 0.772	0.583 ± 0.068*
ANCOVA[b,c]		–	–	–	–	–	–

Table 15
Organ Weights and Organ-Weight to-Body-Weight Ratios for Male Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Seminal Vesicle/Coagulating Gland							
Absolute[b]	0.139 ± 0.016	0.160 ± 0.029	0.185 ± 0.031	0.154 ± 0.013	0.180 ± 0.015	0.145 ± 0.018	0.086 ± 0.016
Relative[b]	0.714 ± 0.065	0.804 ± 0.132	0.925 ± 0.148	0.802 ± 0.055	0.918 ± 0.064	0.790 ± 0.077	0.495 ± 0.074
ANCOVA		–	–	–	–	–	–
Spleen							
Absolute	0.515 ± 0.008	0.541 ± 0.054	0.550 ± 0.032	0.536 ± 0.009	0.526 ± 0.013	0.525 ± 0.018	0.465 ± 0.026
Relative	2.705 ± 0.139	2.773 ± 0.205	2.777 ± 0.174	2.809 ± 0.105	2.719 ± 0.063	2.869 ± 0.104	2.747 ± 0.053
ANCOVA		–	–	–	–	–	–
L. and R. Testis							
Absolute[b,c]	2.107 ± 0.068	2.206 ± 0.152	2.236 ± 0.024	2.222 ± 0.036[f]	2.175 ± 0.040	1.972 ± 0.114	1.545 ± 0.057*
Relative[b,c]	11.063 ± 0.262	11.289 ± 0.283	11.288 ± 0.102	11.634 ± 0.232[f]	11.230 ± 0.209	10.782 ± 0.207	9.143 ± 0.278*
ANCOVA[b,c]		–	–	–	–	–	*
Thymus							
Absolute	0.720 ± 0.013	0.716 ± 0.043	0.681 ± 0.036	0.671 ± 0.025	0.677 ± 0.042	0.663 ± 0.015	0.678 ± 0.024
Relative	3.805 ± 0.198	3.741 ± 0.360	3.447 ± 0.127	3.520 ± 0.157	3.494 ± 0.190	3.628 ± 0.113	4.013 ± 0.076
ANCOVA		–	–	–	–	–	–
Thyroid Gland							
Absolute	18.7 ± 0.2	20.8 ± 1.5[f]	19.6 ± 0.9	20.6 ± 1.9[f]	20.2 ± 2.4	17.8 ± 1.3	18.9 ± 1.3
Relative	0.100 ± 0.006	0.108 ± 0.008[f]	0.099 ± 0.005	0.107 ± 0.011[f]	0.105 ± 0.016	0.097 ± 0.005	0.111 ± 0.006
ANCOVA		–	–	–	–	–	–

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test;
a dash on the ANCOVA line indicates not significant by Dunnett's test

[a] Mean ± standard error. The values given are based on individual pups. Absolute organ weights are given in grams except for pituitary gland, preputial gland, and thyroid gland which are given in milligrams.
Relative organ weights are given as (grams organ weight/grams body weight) × 1,000.
For ANCOVA analyses, body weight was the covariate.

[b] Significant ($P \leq 0.05$) linear exposure concentration trend

[c] Significant ($P \leq 0.05$) main effect of Dose concentration

[d] n = 10

[e] n = 9

[f] n = 14

[g] n = 12

[h] Significant ($P \leq 0.05$) quadratic exposure concentration trend

Females

As in males, the mean terminal body weight of 200 ppb females was significantly decreased (Table 16). Relative liver weight was increased in the 200 ppb group. The only other significant organ weight effects in females were decreases in absolute and relative weights of the ovary (30% and 25%, respectively) in the 200 ppb group.

Table 16
Organ Weights and Organ-Weight-to-Body-Weight Ratios for Female Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
n	14	15	15	15	14	15	15
Necropsy Body Wt	142.9 ± 4.9	144.1 ± 4.4	149.2 ± 2.9	140.3 ± 2.7	139.3 ± 2.6	136.3 ± 3.0	129.8 ± 4.24*
Brain							
Absolute	1.732 ± 0.014[d]	1.717 ± 0.024[d]	1.707 ± 0.065[d]	1.747 ± 0.032[d]	1.794 ± 0.032[d]	1.733 ± 0.045[d]	1.780 ± 0.045[d]
Relative[b,c]	12.271 ± 0.744[d]	12.001 ± 0.402[d]	11.394 ± 0.189[d]	12.466 ± 0.337[d]	13.095 ± 0.466[d]	12.716 ± 0.271[d]	13.667 ± 0.433[d]
ANCOVA[b,c]		–	–	–	–	–	–
Liver							
Absolute	5.041 ± 0.394	5.196 ± 0.273	5.440 ± 0.169	4.902 ± 0.136	5.073 ± 0.185	4.950 ± 0.238	4.955 ± 0.216
Relative[b,c]	35.119 ± 0.954	36.063 ± 0.780	36.387 ± 0.509	34.936 ± 0.222	36.409 ± 0.993	36.263 ± 0.567	38.25 ± 0.41*
ANCOVA[b,c]		–	–	–	–	–	*
L. and R. Ovary							
Absolute[b,c]	0.10 ± 0.008	0.09 ± 0.005	0.10 ± 0.005	0.10 ± 0.003	0.09 ± 0.006	0.08 ± 0.005	0.07 ± 0.005*[e]
Relative[b,c]	0.67 ± 0.03	0.64 ± 0.02	0.68 ± 0.03	0.72 ± 0.02	0.63 ± 0.04	0.58 ± 0.04	0.50 ± 0.02*[e]
ANCOVA[b,c]		–	–	–	–	–	–
Pituitary Gland							
Absolute	8.6 ± 0.6	8.0 ± 0.7[e]	9.0 ± 0.9	9.3 ± 0.9	9.6 ± 0.6[f]	8.7 ± 0.7	7.9 ± 0.6
Relative	60.1 ± 2.5	55.3 ± 3.0[e]	60.3 ± 6.0	65.9 ± 5.1	69.5 ± 5.8[f]	64.2 ± 4.3	61.3 ± 2.4
ANCOVA		–	–	–	–	–	–

Table 16
Organ Weights and Organ-Weight to-Body-Weight Ratios for Female Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
n	14	15	15	15	14	15	15
Necropsy Body Wt	142.9 ± 4.9	144.1 ± 4.4	149.2 ± 2.9	140.3 ± 2.7	139.3 ± 2.6	136.3 ± 3.0	129.8 ± 4.24*
Spleen							
Absolute[c]	0.408 ± 0.010	0.430 ± 0.033	0.425 ± 0.020	0.412 ± 0.016	0.376 ± 0.017	0.397 ± 0.017	0.356 ± 0.020
Relative	2.88 ± 0.12	2.97 ± 0.13	2.84 ± 0.08	2.94 ± 0.13	2.70 ± 0.10	2.91 ± 0.06	2.75 ± 0.05
ANCOVA		–	–	–	–	–	–
Thymus							
Absolute	0.606 ± 0.024	0.573 ± 0.020	0.577 ± 0.023	0.562 ± 0.026	0.563 ± 0.036	0.561 ± 0.029	0.625 ± 0.052
Relative[c]	4.28 ± 0.23	4.01 ± 0.22	3.87 ± 0.17	4.01 ± 0.24	4.05 ± 0.25	4.12 ± 0.14	4.80 ± 0.27
ANCOVA[c]		–	–	–	–	–	–
Thyroid Gland							
Absolute	16.9 ± 0.9	20.9 ± 1.5	17.9 ± 0.5	16.9 ± 1.2	17.8 ± 2.0[f]	17.7 ± 0.9	18.3 ± 1.5
Relative	0.119 ± 0.008	0.147 ± 0.010	0.120 ± 0.004	0.121 ± 0.008	0.128 ± 0.014[f]	0.130 ± 0.008	0.144 ± 0.017
ANCOVA		–	–	–	–	–	–
Uterus							
Absolute	0.262 ± 0.023	0.242 ± 0.026	0.308 ± 0.015	0.319 ± 0.015	0.274 ± 0.014	0.257 ± 0.028	0.253 ± 0.028
Relative	1.853 ± 0.135	1.681 ± 0.164	2.071 ± 0.086	2.287 ± 0.145	1.968 ± 0.079	1.885 ± 0.208	1.956 ± 0.154
ANCOVA		–	–	–	–	–	–
Vagina							
Absolute	0.144 ± 0.010	0.129 ± 0.010	0.140 ± 0.008	0.145 ± 0.005	0.153 ± 0.006	0.138 ± 0.005	0.130 ± 0.07
Relative	1.008 ± 0.0185	0.895 ± 0.043	0.946 ± 0.054	1.034 ± 0.041	1.107 ± 0.067	1.021 ± 0.053	1.010 ± 0.060
ANCOVA		–	–	–	–	–	–

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test; a dash on the ANCOVA line indicates not significant by Dunnett's test.
[a] Mean ± standard error. The values given are based on individual pups. Absolute organ weights are given in grams except for pituitary gland and thyroid gland which are given in milligrams. Relative organ weights are given as (grams organ weight/grams body weight) × 1,000. For ANCOVA analyses, body weight was the covariate.
[b] Significant ($P \leq 0.05$) main effect of Dose
[c] Significant ($P \leq 0.05$) linear exposure concentration trend
[d] n = 10
[e] n = 14
[f] n = 13

Clinical Chemistry and Hematology Parameters and Sperm Counts

Examination of the summary statistics for hematology and clinical chemistry parameters (not shown) suggested that none of these parameters were affected by exposure to a degree sufficient to impact exposure concentration selection for the multigenerational reproductive toxicology or chronic studies, and statistical analysis confirmed the general lack of significant treatment differences.

Examination of the summary statistics for testicular spermatid head counts and epididymal sperm counts suggested that ethinyl estradiol may have affected spermatid head counts in the testis and spermatocyte counts in the head and body (combined) of the epididymis, and these data were further evaluated by ANOVA (Table 17). A significant decrease in testicular spermatid head counts in the 200 ppb group was observed. Epididymal sperm counts were low, as would be expected in males of this age, and nonparametric analysis of the data indicated no significant differences from controls.

Pathology and Statistical Analysis

Incidences and severities of lesions in those organs showing treatment-related effects are discussed here and listed in Tables 18 and 19 for males and females, respectively.

Table 17
Testicular Spermatid Head Counts and Epididymal Sperm Counts in Male Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol[a]

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
n	15	15	15	15	15	13	15
Testicular spermatid head counts per g tissue[b]	56.9 ± 5.1	60.1 ± 5.8	66.5 ± 4.4	66.4 ± 4.7	58.5 ± 3.7	48.2 ± 4.2	33.5 ± 5.0*
Epididymal spermatocyte counts per g tissue[b]	3.3 ± 0.3	3.5 ± 0.6	4.3 ± 0.6	4.3 ± 0.4	3.9 ± 0.4	3.0 ± 0.6	2.0 ± 0.4

* Significantly different ($P \leq 0.05$) from the control group by Dunnett's test
[a] Data are given as mean $\times 10^6$ ± standard error.
[b] Significant ($P \leq 0.05$) main effect of Dose

Males

The incidences of minimal to moderate mineralization of the renal tubules were significantly increased in 100 and 200 ppb males (Table 18). Significantly increased incidences of hyperplasia of the ducts and terminal end buds of the mammary gland occurred in males exposed to 25 ppb or greater and 100 ppb or greater, respectively. Mammary gland effects occurred at a lower exposure concentration than any of the other treatment-related histological changes.

In the reproductive tract, ethinyl estradiol affected the testis, epididymis, and seminal vesicle in groups exposed to 100 and/or 200 ppb. Most of the lesions observed in the testis were subtle degenerative changes in and depletion of different generations of germ cells. Incidences of degeneration of pachytene spermatocytes in the 100 and 200 ppb groups and degeneration of round spermatids in the 200 ppb group were significantly increased relative to those in the control group. Incidences of depletion of elongated spermatids were significantly increased in the

Table 18
Incidences of Selected Nonneoplastic Lesions in Male Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol

	0 ppb		0.1 ppb		1 ppb		5 ppb		25 ppb		100 ppb		200 ppb	
Kidney[a]	15		15		15		15		15		13		15	
Mineralization, Renal Tubule[b,c]	0		0		0		0		1	(1.0)[d]	6	(1.0)**	14	(1.4)**
Mammary Gland	15		14		15		15		15		13		15	
Hyperplasia, Duct[c]	3	(1.0)	2	(1.0)	2	(1.5)	6	(1.0)	8	(1.5)*	12	(1.8)**	14	(1.8)**
Hyperplasia, Terminal End Buds[c]	2	(1.0)	0		0		1	(1.0)	3	(1.3)	11	(1.3)**	13	(1.5)**
Testis	14		15		15		15		15		13		15	
Degeneration Pachytene Spermatocyte[c]	5	(1.2)	6	(1.0)	7	(1.3)	6	(1.2)	6	(1.5)	11	(1.3)**	15	(1.6)**
Degeneration, Round Spermatid[e]	1	(1.0)	3	(1.0)	5	(1.2)	6	(1.0)	4	(1.0)	4	(1.0)	10	(1.0)**
Depletion, Elongated Spermatid[c]	3	(2.7)	5	(2.0)	2	(2.5)	3	(2.0)	4	(2.0)	11	(2.5)**	15	(3.8)**
Epididymis	15		15		15		15		15		13		15	
Hypospermia/Aspermia, Head[f,g]	14	(2.7)	7	(2.3)	5	(1.8)	7	(3.1)	8	(2.1)	7	(3.9)	14	(4.6)*
Exfoliated Germ Cells[c]	15	(1.1)	10	(1.1)	15	(1.0)	14	(1.0)	15	(1.0)	13	(1.0)	15	(1.7)**
Seminal Vesicle	15		15		15		15		15		13		15	
Atrophy[c]	0		0		0		0		0		0		7	(1.0)**
Depletion, Secretory[c]	3	(2.3)	2	(3.0)	2	(2.0)	1	(2.0)	4	(2.3)	8	(2.9)**	13	(3.5)**

* Significantly different ($P \leq 0.05$) from the control group by Shirley-Williams' test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with lesion
[c] Significant exposure concentration trend ($P \leq 0.001$) by the Jonckheere-Terpstra test
[d] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked
[e] Significant exposure concentration trend ($P \leq 0.01$) by the Jonckheere-Terpstra test
[f] Significant exposure concentration trend ($P \leq 0.05$) by the Jonckheere-Terpstra test
[g] Aspermia, the absolute end stage of hypospermia was given a severity grade of 5 for the purpose of calculating an overall severity grade for these combined endpoints.

100 and 200 ppb groups, and the severity of this lesion was relatively increased in the 200 ppb group. Depletion was most obvious and marked in Stage VII tubules, but decreased numbers were apparent in some tubules in Stages I-VI and Stages XII-XIV. Depletion of elongated spermatids also occurred in the control group.

Sperm production was not at full capacity even in control rats at the time of sacrifice (PND 50), so sperm numbers in the epididymis were generally low (Table 17). Still, the severity of hypospermia/aspermia in the head region and exfoliated germ cells was significantly increased in the 200 ppb group relative to the controls (Table 18). Cell size and chromatin pattern in the nucleus suggested that most of the germ cells were pachytene spermatocytes with fewer round spermatids. This observation is consistent with the increase in the testis of degeneration of pachytene spermatocytes and round spermatids. Both lesions occurred in the 200 ppb group, and degeneration of pachytene spermatocytes also occurred in the 100 ppb group.

In the accessory glands (prostate gland, seminal vesicle, preputial gland), only the seminal vesicle exhibited detectable lesions. Incidences of secretory depletion were significantly increased in the 100 and 200 ppb groups relative to the controls. Atrophy of the seminal vesicle only occurred in 200 ppb males, and the incidence in this group was significantly increased. Atrophy was characterized by a decrease in cell size due to an apparent reduction of cytoplasm.

Females

Ethinyl estradiol disrupted normal estrous cycles and caused morphologic changes in the ovary, uterus, and vagina, primarily in the 200 ppb group, although some lesions also occurred in the 100 ppb group (Table 19). Except for those of one animal that appeared to be in normal proestrus, the ovaries of the 200 ppb animals were similar in indicating abnormal cycling and were diagnosed as exhibiting anestrus. Incidences of anestrus were significantly increased in the 100 and 200 ppb groups. Affected ovaries were characterized by only one generation of corpora lutea, which appeared most like those in diestrus, though the individual luteal cells may have been somewhat smaller; reduced numbers of corpora lutea; a large proportion of degenerating antral follicles; and inactive and poorly developed interstitial glands. The uterus and vagina of several 200 ppb animals also appeared to be histologically abnormal and the incidences of uterine atrophy and vaginal mucocyte metaplasia and dystrophy were significantly increased in this group.

Table 19
Incidences of Selected Nonneoplastic Lesions in Female Rat Pups in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol

	0 ppb	0.1 ppb	1 ppb	5 ppb	25 ppb	100 ppb	200 ppb
Ovary[a]	14	15	15	15	15	15	15
Anestrus[b,c]	0	0	0	0	0	2*	14**
Uterus	14	15	15	15	15	15	15
Atrophy[c]	0	0	0	0	0	0	6 (1.7)**[d]
Vagina	14	15	15	15	15	15	15
Metaplasia, Mucocyte[c]	0	1 (1.0)	0	0	0	3 (3.0)	9 (2.1)**
Dystrophy[c]	0	0	0	0	0	0	5 (1.8)**

* Significantly different ($P \leq 0.05$) from the control group by the Shirley-Williams' test
** $P \leq 0.01$
[a] Number of animals with tissue examined microscopically
[b] Number of animals with condition/lesion
[c] Significant exposure concentration trend ($P \leq 0.001$) by the Jonckheere-Terpstra test
[d] Average severity grade of lesions in affected animals: 1 = minimal, 2 = mild, 3 = moderate, 4 = marked

Multigenerational Reproductive Toxicology Study

Body Weights, Feed Consumption, and Water Intake during Lactation

Female and male growth curves from the start of dosing of the F_0 generation through the termination of the F_4 generation are shown in Figures 2 through 10, and body weight data and detailed statistical results are tabulated in Tables D1a through D11.

Effects of ethinyl estradiol on postweaning body weights, when animals were directly ingesting ethinyl estradiol, were seen in females in the 50 ppb groups in the F_0, F_1, and F_2 generations. In the F_0 generation, females in the 50 ppb group showed body weights that were significantly less than those of the controls (mean difference of 14%) in 7 of 8 weeks prior to litter delivery and for all weeks for which data were collected after delivery (Figure 2 and Table D1a). Significantly decreased body weights relative to controls in the F_1 and F_2 generations, the generations in which ethinyl estradiol exposure was continuous from conception to termination, were also observed in the 50 ppb female groups for all 15 weeks measured (Figures 3 and 5; Tables D1b and D1c). The 10 ppb F_0 and F_1 female groups also had body weights that were significantly less than those of the controls for 8 of 12 weeks and 8 of 15 weeks, respectively (mean differences of 6% and 4%, respectively, for the 8 weeks affected; Figures 3 and 5; Tables D1a and D1b). In the F_3 generation, which was exposed only until weaning, and in the unexposed F_4 generation, no biologically meaningful significant body weight effect between any exposed group of females and controls was observed (Figures 7 and 9; Tables D1d and D1e). That the exposure concentration effect in females was predominant in the F_0, F_1, and F_2 generations and strongest in the F_0 generation is also evident from the total body weight gains in the predelivery period where significant body weight gain decreases in the 50 ppb groups were found for the F_0 through F_2 generations and also in the 10 ppb group for the F_0 generation (Table D5).

Effects of ethinyl estradiol exposure in females in the preweaning period were also evident (Figures 3, 5, 7, and 9; Table D2), particularly in the F_1 and F_2 generations where body weights in the 50 ppb groups were significantly less than those of the control groups by 9% to 14% at PNDs 14 and 21. In the F_1 generation, body weights of the 2 and 10 ppb female groups were also significantly less than those in the controls by 8% and 5%, respectively, on PND 21. In the F_3 generation, the body weight of the 50 ppb female group was significantly less than that of the controls (5%) only on PND 21. Total body weight gains prior to weaning in females in the 50 ppb groups were significantly less than those in the controls in the F_1 and F_2 generations (Table D7).

The effects of ethinyl estradiol on body weights in males during the postweaning period showed a similar pattern to that in females, with significant body weight decreases relative to the control groups of 7% to 11% in the majority of weeks monitored for the 50 ppb groups in the F_0, F_1, and F_2 generations (Figures 2, 4, and 6; Tables D3a, D3b, and D3c). Significantly decreased body weights relative to controls were also observed in the 2 and 10 ppb male groups (6% and 4% mean differences, respectively) in the F_2 generation from weeks 9 through 19 (Table D3c). Total body weight gains were significantly less than those in the controls for the 50 ppb groups of F_0, F_1, and F_2 males, as well as in the 10 ppb groups of F_0 and F_2 males and the 2 ppb group of F_2 males (Table D8). Male preweaning body weights in the 50 ppb groups were significantly less than those of the controls (8% to 10%) on PND 14 and PND 21 in the F_1, F_2, and F_3 generations (Figures 6, 8, and 10; Table D4). Total body weight gains of males during the preweaning period showed significant negative linear exposure concentration trends as well as significant decreases in the 50 ppb groups relative to the control groups in the F_1 (10%) and F_2 (11%) generations; although total preweaning body weight gains were less than those of the controls in the 50 ppb groups of the F_3 (10%) and F_4 (6%) generations, these differences were not statistically significant (Table D7).

Terminal body weights of males and females in the 50 ppb groups were significantly less than control body weights by 8% to 15% in the F_0, F_1, and F_2 generations (Table D9). F_0 females in the 10 ppb group and F_2 males in the 2 and 10 ppb groups also had terminal body

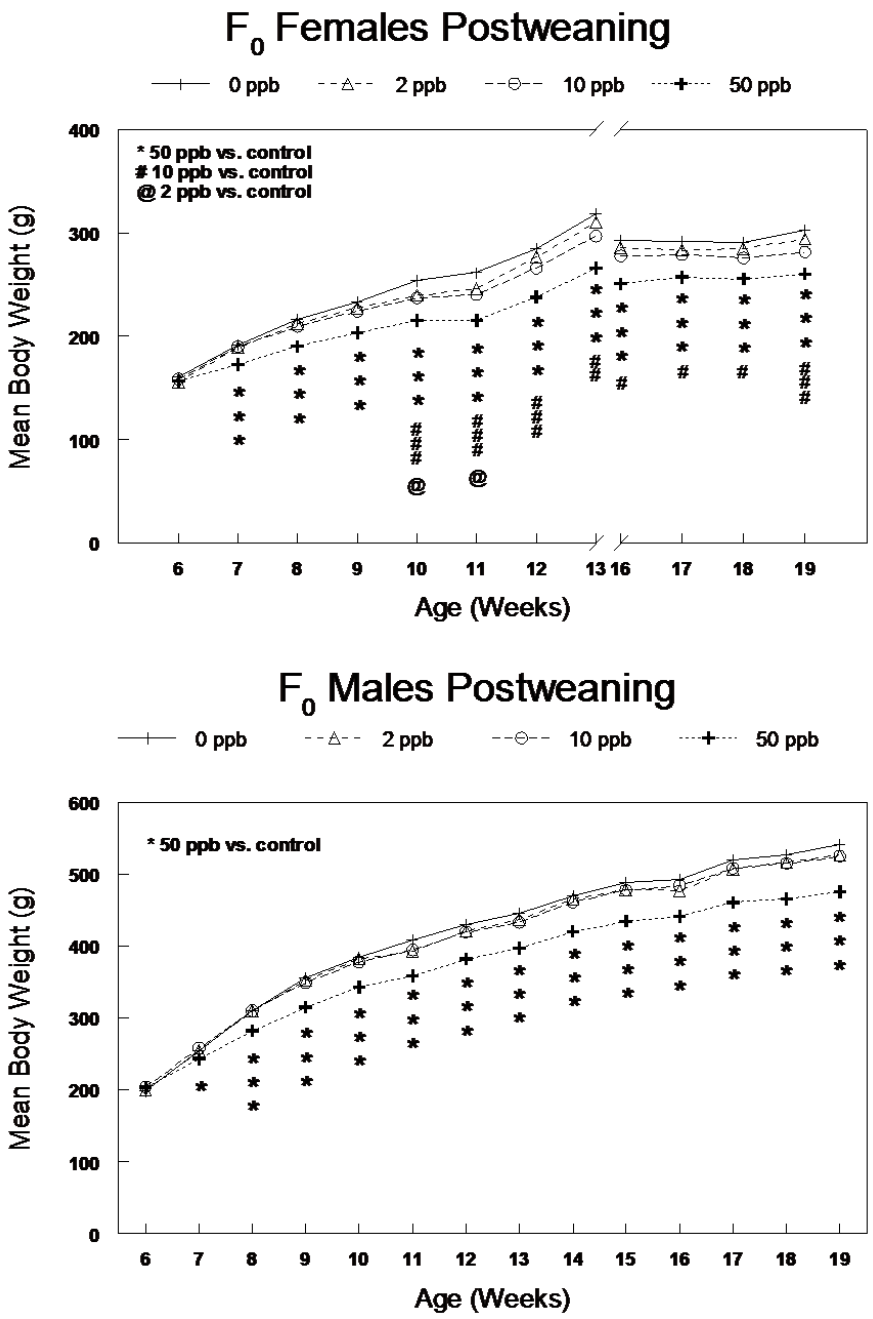


FIGURE 2
Postweaning Growth Curves for F_0 Rats Exposed to Dietary Ethinyl Estradiol

Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 50 ppb, 10 ppb, and 2 ppb groups, respectively. *, #, @, $P \leq 0.05$; ##, $P \leq 0.01$; ***, ###, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D1a (females) and D3a (males).

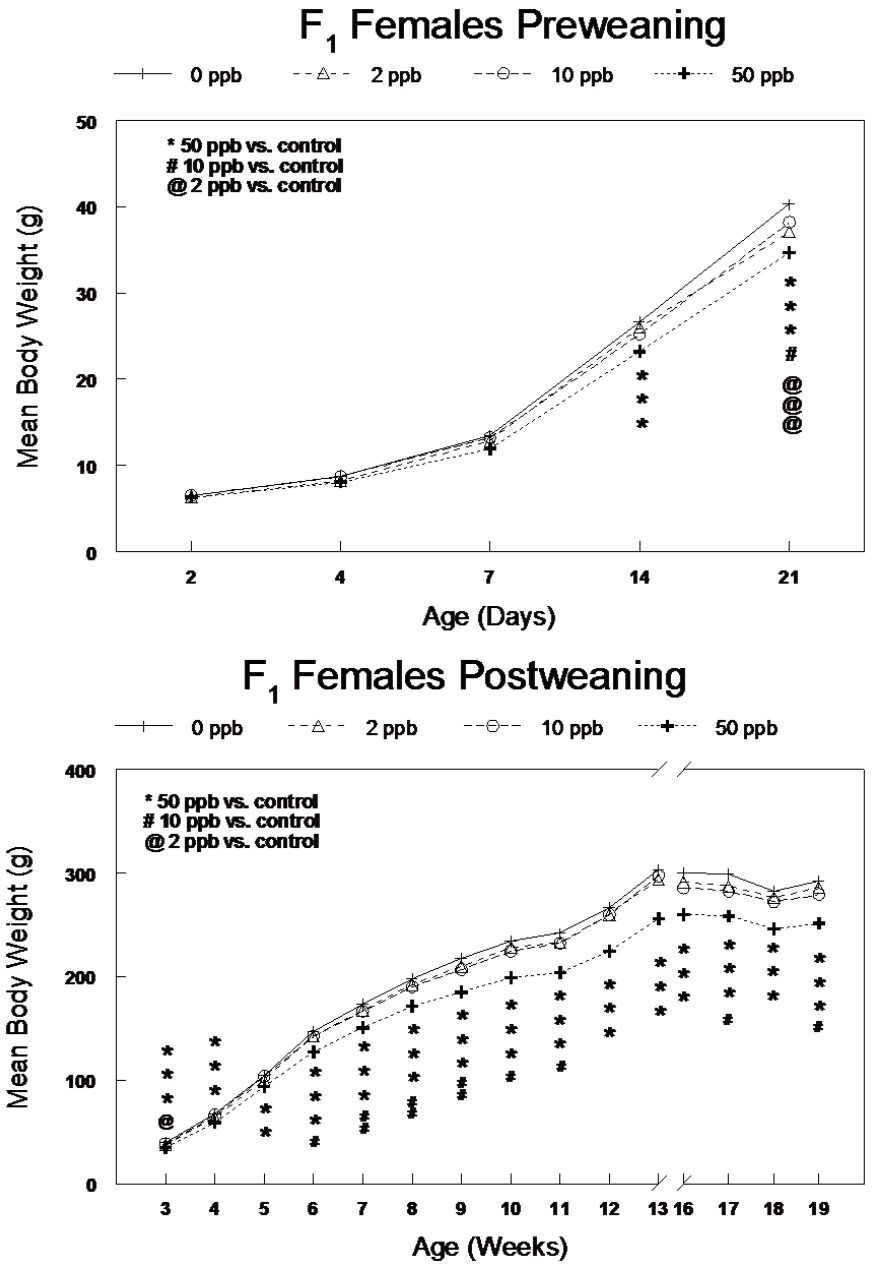


FIGURE 3

Preweaning and Postweaning Growth Curves for F_1 Female Rats Exposed to Dietary Ethinyl Estradiol

Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 50 ppb, 10 ppb, and 2 ppb groups, respectively. #, @, $P \leq 0.05$; **, ##, $P \leq 0.01$; ***, ###, @@@, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1b (postweaning).

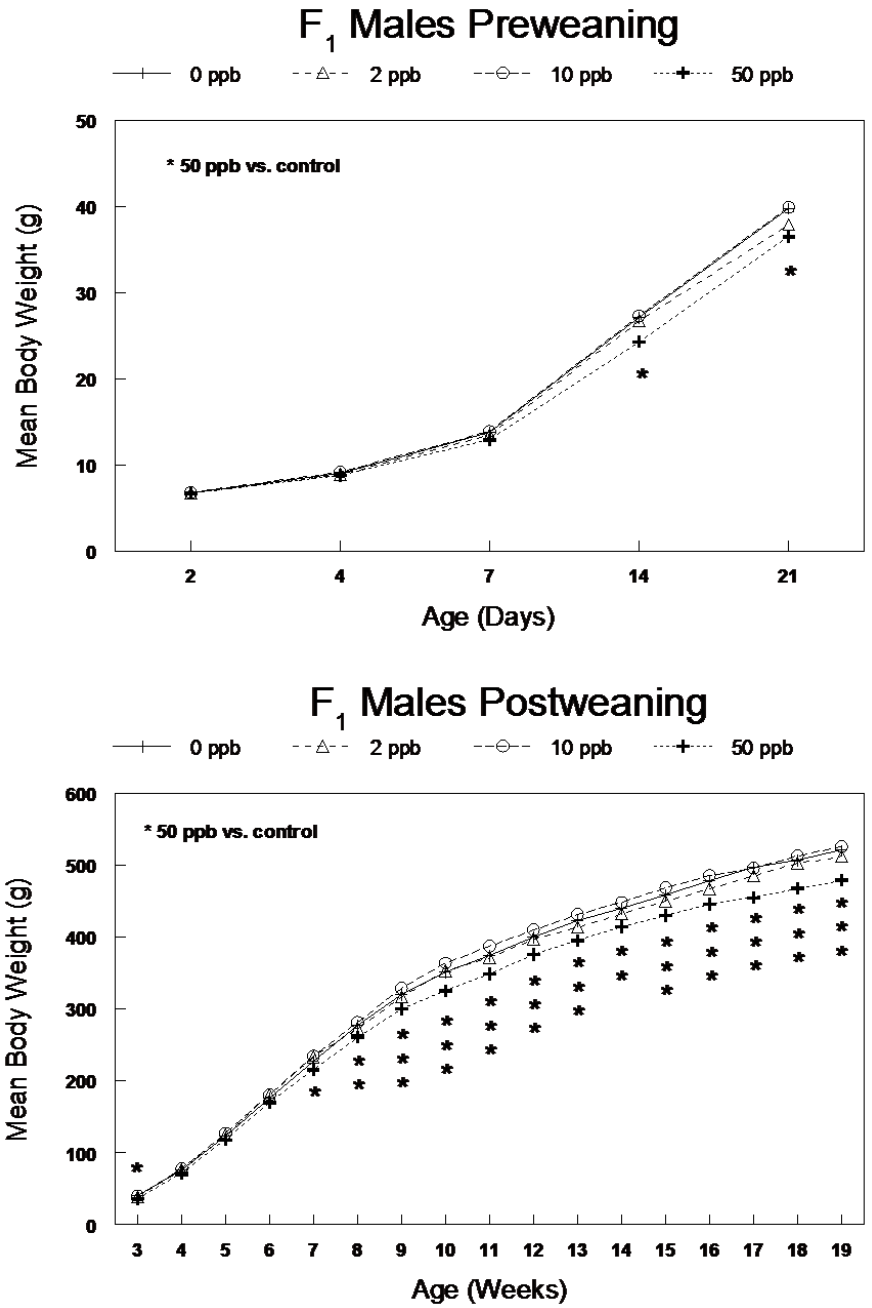


FIGURE 4
Preweaning and Postweaning Growth Curves for F_1 Male Rats Exposed to Dietary Ethinyl Estradiol
Asterisks (*) indicate significant differences between controls the 50 ppb group. *, P≤0.05; **, P≤0.01; ***, P≤0.001. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3b (postweaning).

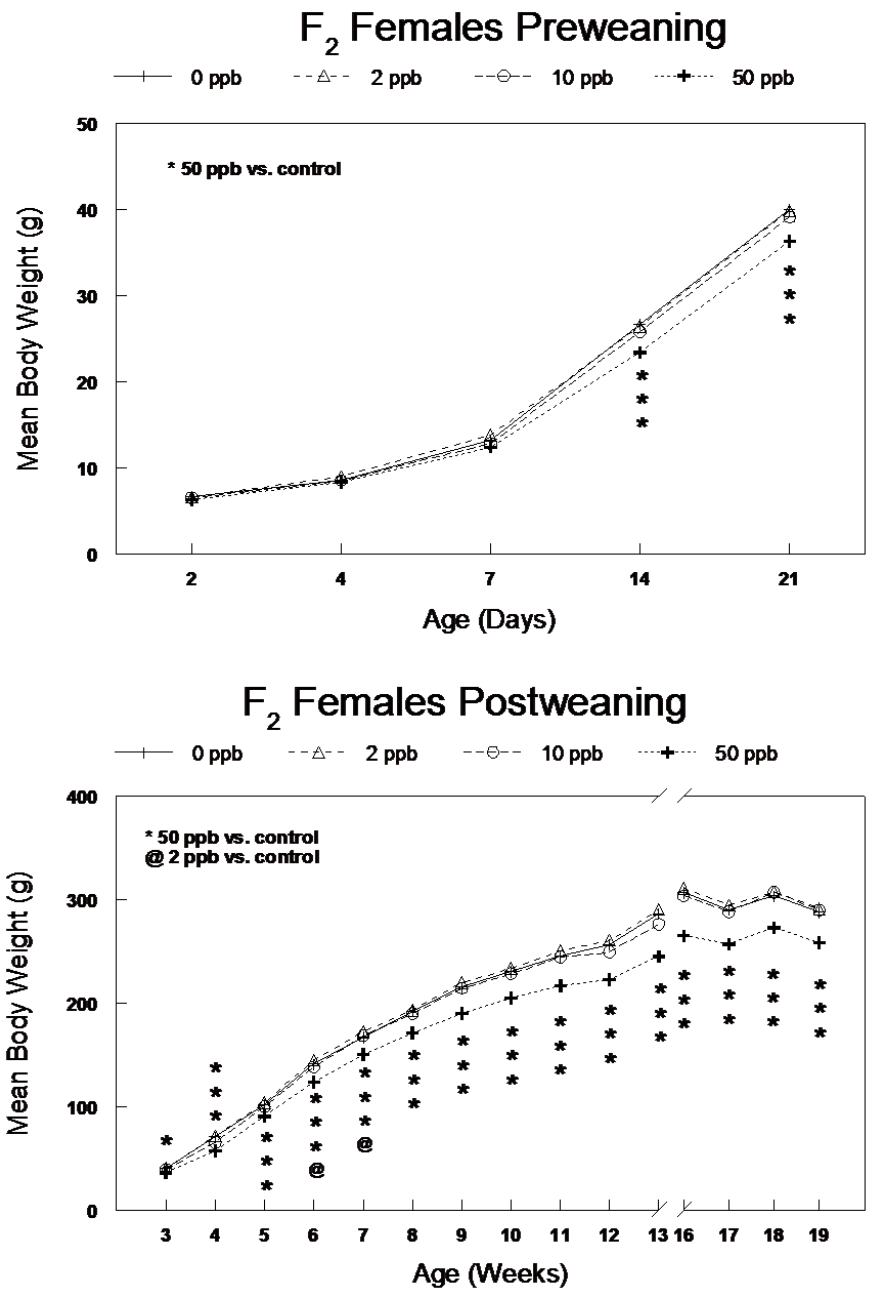


Figure 5

Preweaning and Postweaning Growth Curves for F_2 Female Rats Exposed to Dietary Ethinyl Estradiol

Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*) and "at" signs (@) indicate significant differences between controls and the 50 ppb and 2 ppb groups, respectively.
*, @, $P \leq 0.05$; ***, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1c (postweaning).

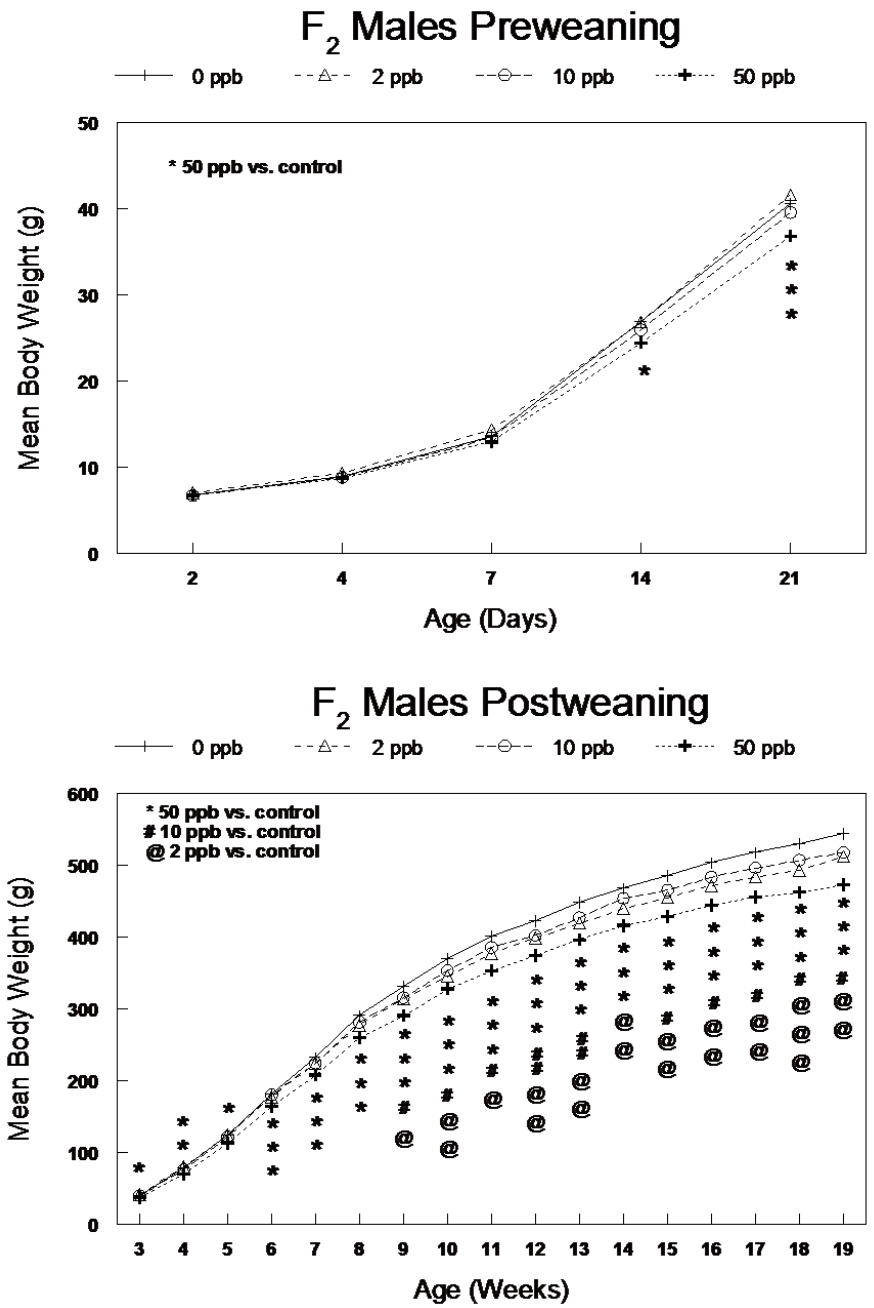


Figure 6

Preweaning and Postweaning Growth Curves for F_2 Male Rats Exposed to Dietary Ethinyl Estradiol

Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 50 ppb, 10 ppb, and 2 ppb groups, respectively. *, #, @, $P \leq 0.05$; **, ##, @@, $P \leq 0.01$; ***,@@@, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3c (postweaning).

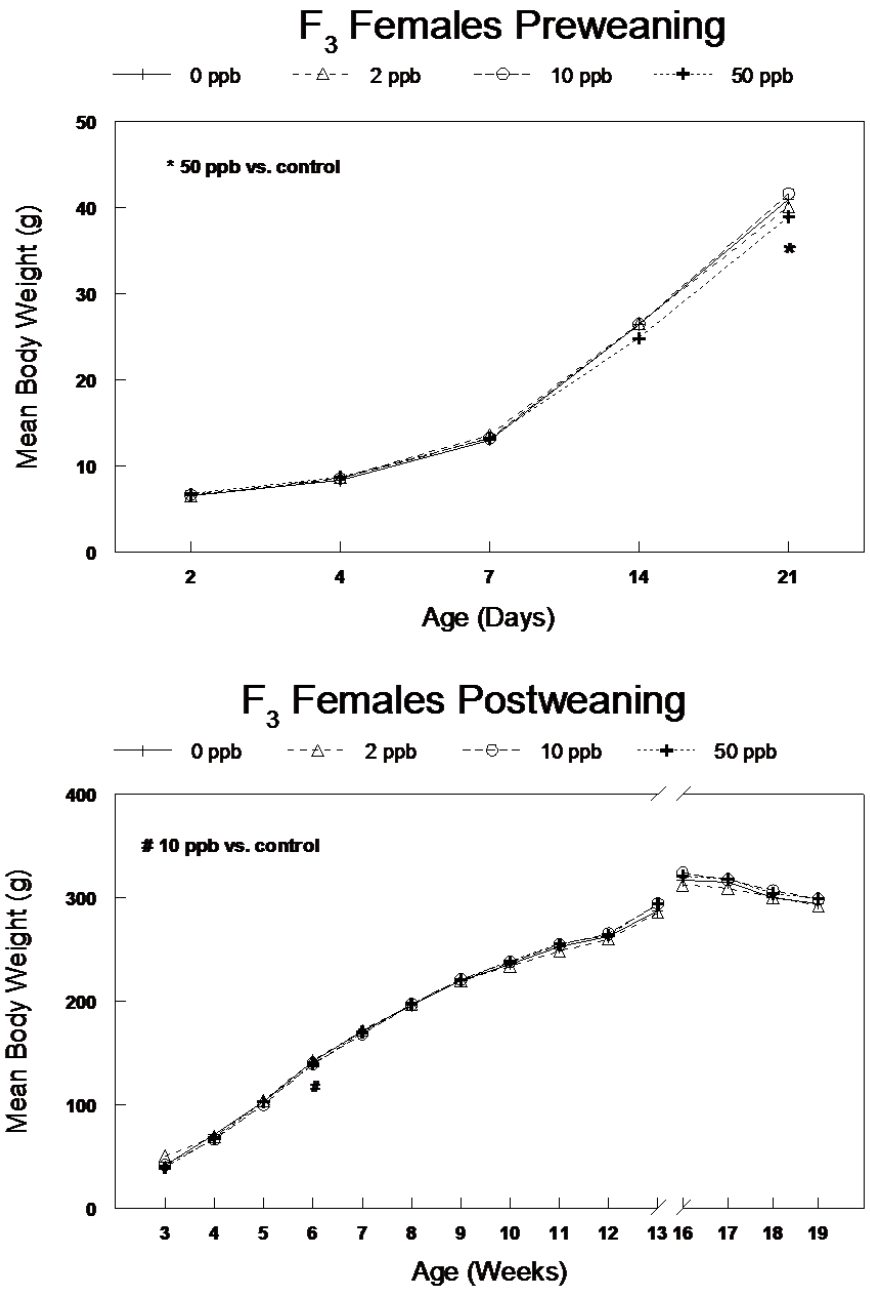


FIGURE 7
Preweaning and Postweaning Growth Curves for F_3 Female Rats Exposed to Dietary Ethinyl Estradiol
Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*) and pound signs (#) indicate significant differences between controls and the 50 ppb and 10 ppb groups, respectively. *, #, $P \leq 0.05$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1d (postweaning).

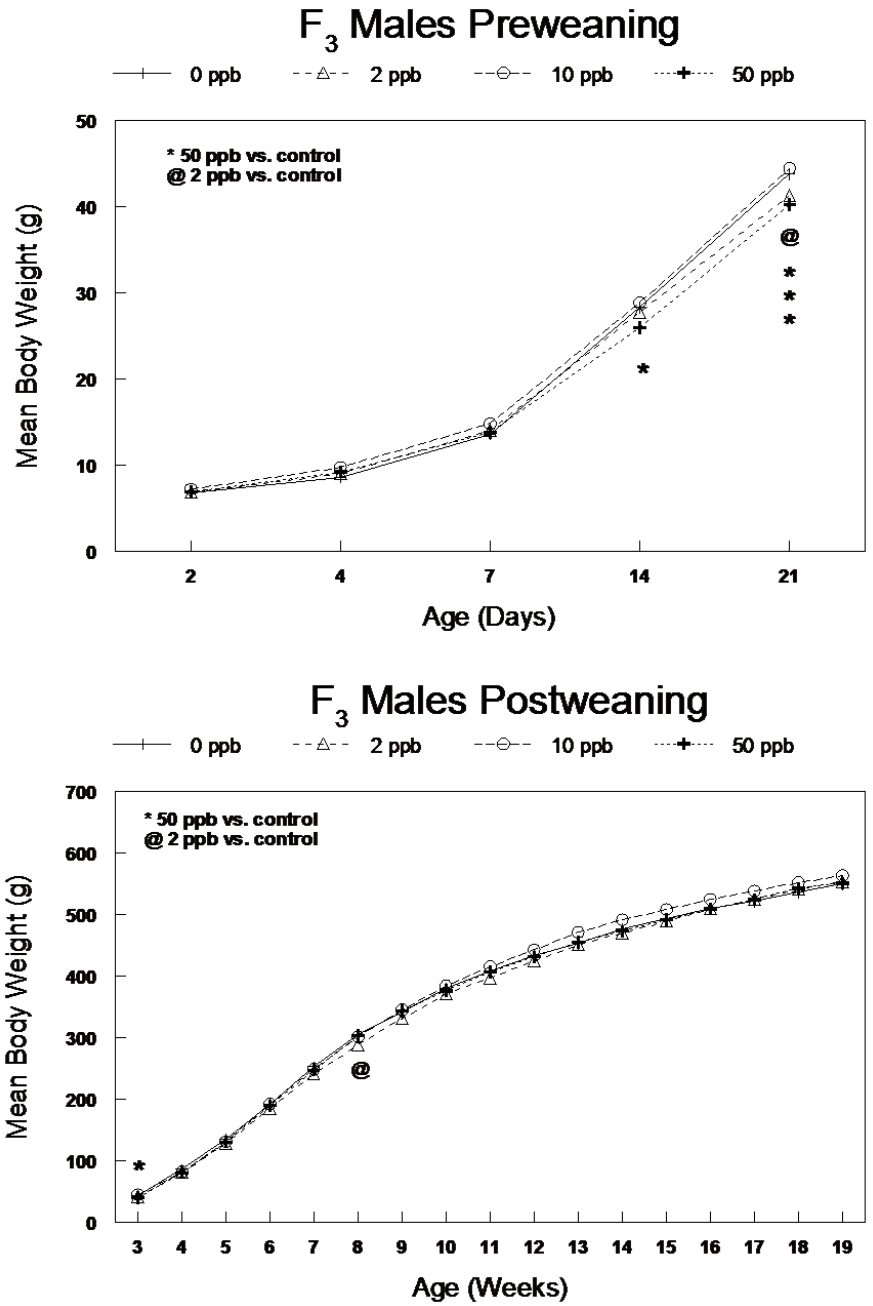


FIGURE 8
Preweaning and Postweaning Growth Curves for F_3 Male Rats Exposed to Dietary Ethinyl Estradiol
Asterisks (*) and "at" signs (@) indicate significant differences between controls and the 50 ppb and 2 ppb groups, respectively. *, @, $P \leq 0.05$; ***, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3d (postweaning).

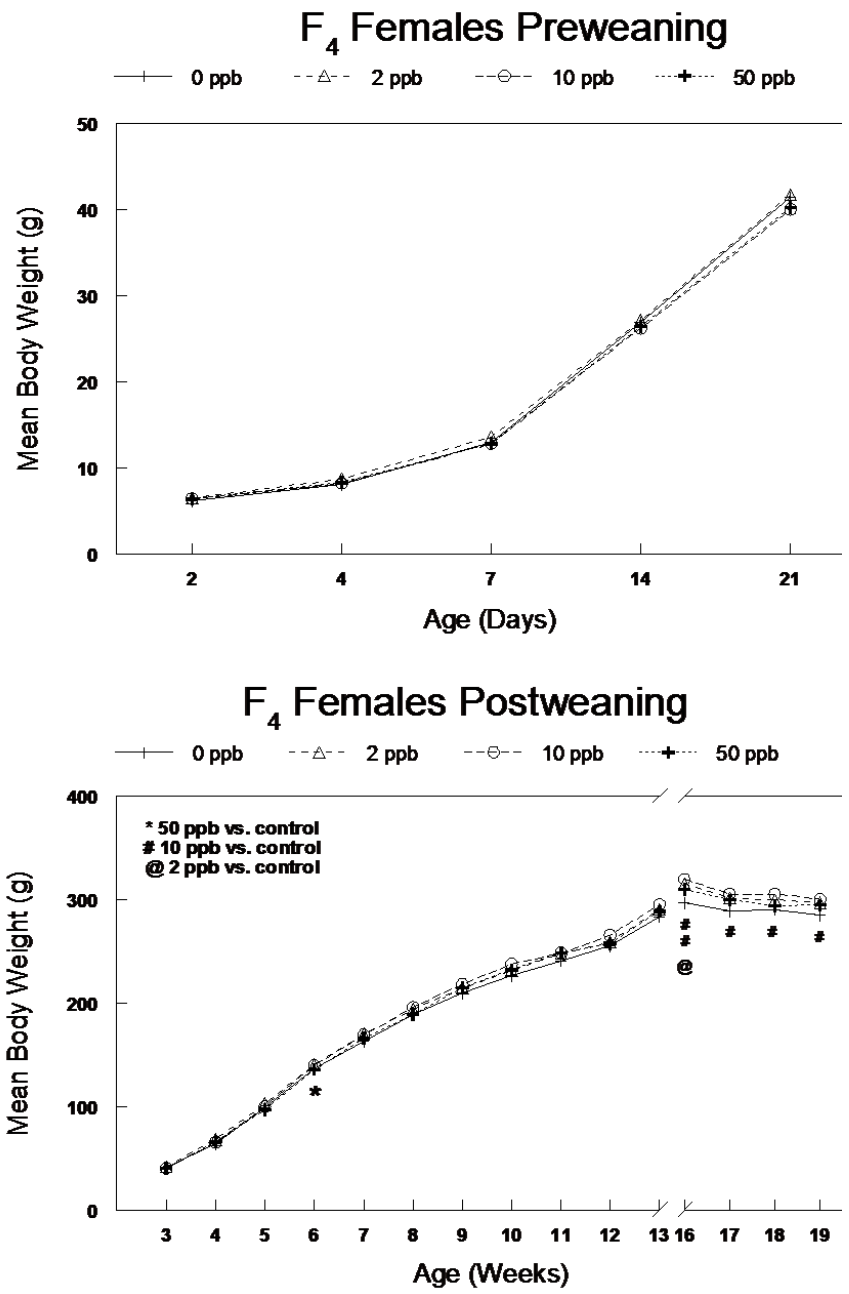


FIGURE 9
Preweaning and Postweaning Growth Curves for F_4 Female Rats Exposed to Dietary Ethinyl Estradiol

Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 50 ppb, 10 ppb, and 2 ppb groups, respectively. *, #, @, $P \leq 0.05$; ##, $P \leq 0.01$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1e (postweaning).

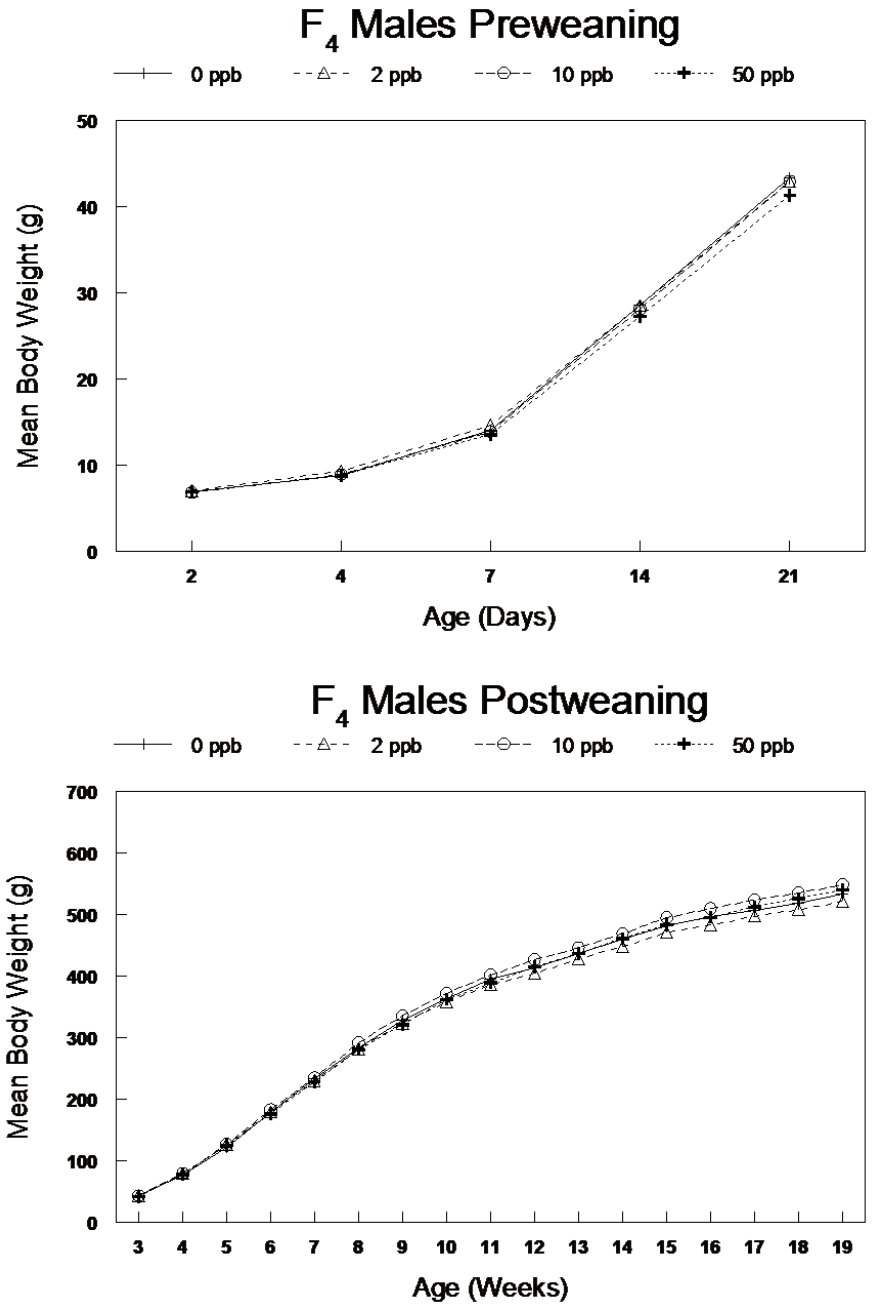


Figure 10
Preweaning and Postweaning Growth Curves for F_4 Male Rats Exposed to Dietary Ethinyl Estradiol
There were no significant differences between exposed groups and the control group. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3e (postweaning).

weights significantly less (5% to 7%) than controls. A significantly greater (6%) terminal body weight in 10 ppb F_4 females compared to the control group was the only significant difference in terminal body weight for either sex in the F_3 and F_4 generations (Table D9).

Significant differences between generations in body weights at particular ages within exposure groups for both females and males are tabulated in Tables D2, D4, D10, and D11 and Figures D1 to D8. In the control groups for both females and males, the F_0 animals were generally heavier than animals in subsequent generations at early time points. The difference between F_0 animals and subsequent generations was that the dams of the F_0 animals were fed standard chow diet (NIH 31) until the F_0 animals were weaned, whereas the dams of subsequent generations were fed 5K96 diet throughout the experiment. This early diet difference may have contributed to the observed body weight differences between F_0 animals and subsequent generations. Other generational body weight differences, particularly those greater than 10%, generally reflected the observed treatment effects on body weights in the F_0 through F_2 generations, but not the F_3 or F_4 generations, that were discussed above.

Feed consumption data and statistical analyses of those data for females and males of the F_0 through F_4 generations are summarized in Tables E1 through E7. The feed consumption data were not corrected for spillage and are thus approximate. While significant effects of treatment on feed consumption were observed, these effects were not well correlated with the treatment-related body weight decreases that were described earlier, with significant decreases in feed consumption occurring in the absence of body weight decreases and significant body weight decreases occurring without a significant decrease in feed consumption. Thus, although estrogens are known to have anorectic activity (Wade and Schneider, 1992), there was no evidence that the body weight depression observed under the conditions of the present study was due to appetite suppression. Estrogens are also known to reduce metabolic efficiency, that is, the change of body weight per unit feed consumed over a given time period (Wade and Schneider, 1992). Analysis of the metabolic efficiency data in the present study indicated a clear significant reduction in metabolic efficiency only for females of the F_1 generation. Generation differences in feed consumption within exposure groups are presented in Tables E6 and E7 and Figures E1 through E4. There was no clear pattern across generations, although feed consumption in F_0 males and females appeared to be generally higher than that in subsequent generations.

Water intake of dams during gestation in each generation of this experiment is reported in Table F1. Water intake is known to increase significantly during lactation, and estrogen has been reported to affect this increased intake (Fujisawa *et al.,* 2001; Speth *et al.,* 2002). Ethinyl estradiol had no significant effect on this endpoint under the conditions of the present study.

Mating and Pregnancy

Results for the mating, fertility, and pregnancy indices, time to mating and gestation time are reported in Table G1. No significant exposure concentration-related effects were observed for these endpoints. Results of examination of the uteri of mated females that did not litter within 24 days after removal from the breeding cages and did not show weight gain consistent with pregnancy were examined for resorption (data not shown). Of 68 animals examined in the F_1 through F_4 generations, only two were found to have resorption sites or nonviable or viable fetuses, and both of these were F_4 control animals. Thus ethinyl estradiol under the conditions of this study had no effect on mating or pregnancy parameters.

Litter and Perinatal Pup Parameters

Measurements recorded for the F_1 through F_5 litters and for newborn pups along with a summary of the statistical analyses are reported in Table H1. Analyses for main effect of Dose or Dose × Generation interaction (indicative of a treatment effect that varied across generations) were performed on the data for total pups born, live pups born (total or by sex), stillbirths, male and female pup birth weights, and sex ratio; the only statistically significant effect was an overall Dose effect on total pups born. There were no significant differences between any exposed group and the controls for any generation for these endpoints.

There were no significant overall Dose or Dose × Generation interactions for anogenital distance in males, although there was a significant negative exposure concentration trend in the F_3 generation, and the mean value for the 50 ppb group was significantly less than that in the F_3 controls in the ANCOVA analysis. However, this difference was approximately 4%, and no similar exposure concentration-related decreases in male anogenital distances were observed in the other generations. In females, significant exposure concentration trends were observed for anogenital distance in the F_2 and F_3 generations with the 50 ppb groups significantly different from controls in both the ANCOVA and ratio statistical models. However, these differences were in opposite directions in the two generations (increasing in F_2, decreasing in F_3), and the magnitude of the differences was less than 10%. Thus, ethinyl estradiol did not produce a biologically significant change in anogenital distance in either sex under the conditions of this study.

Survival of the pups between the time of litter standardization and the time of weaning was not significantly affected by ethinyl estradiol exposure (data not shown).

Markers of Sexual Development

The time and body weight at vaginal opening are shown in Figure 11 and in Table I1. For age at vaginal opening, significant overall effects of Dose were observed in the F_1, F_2, and F_3 generations, with vaginal opening occurring approximately 4, 6, and 3 days earlier in the 50 ppb groups than in the respective controls. When the body weight at vaginal opening was examined, significant negative linear exposure concentration trends were observed in the F_1, F_2, and F_3 generations, with vaginal opening occurring when the animals were 68%, 60%, and 84% of the weight of controls in the 50 ppb groups of the F_1, F_2, and F_3 generations, respectively. The body weight at vaginal opening in the 10 ppb group in the F_1 generation was significantly lower (10%) than controls in that generation. Within the control group, neither the day of vaginal opening nor the body weight at vaginal opening differed significantly across generations.

Ethinyl estradiol did not show consistent biologically significant effects on the markers of male maturation that were monitored, preputial separation (Table I2) and testicular descent (Table I3). Statistically significant effects were confined to a 1.5-day delay in preputial separation in the 50 ppb group of the F_2 generation, a significant negative linear exposure concentration trend in body weight at preputial separation in the F_1 generation, a 1-day delay in testicular descent in the 2 ppb group of the F_1 generation, and a 0.2-day decrease (beyond the resolving power of the study, where testicular descent was monitored daily) in testicular descent in the 2 ppb group of the F_4 generation. Thus, while ethinyl estradiol exposure under the conditions of this study showed a highly significant effect on the onset of puberty in females, there were no convincing effects on this endpoint in males.

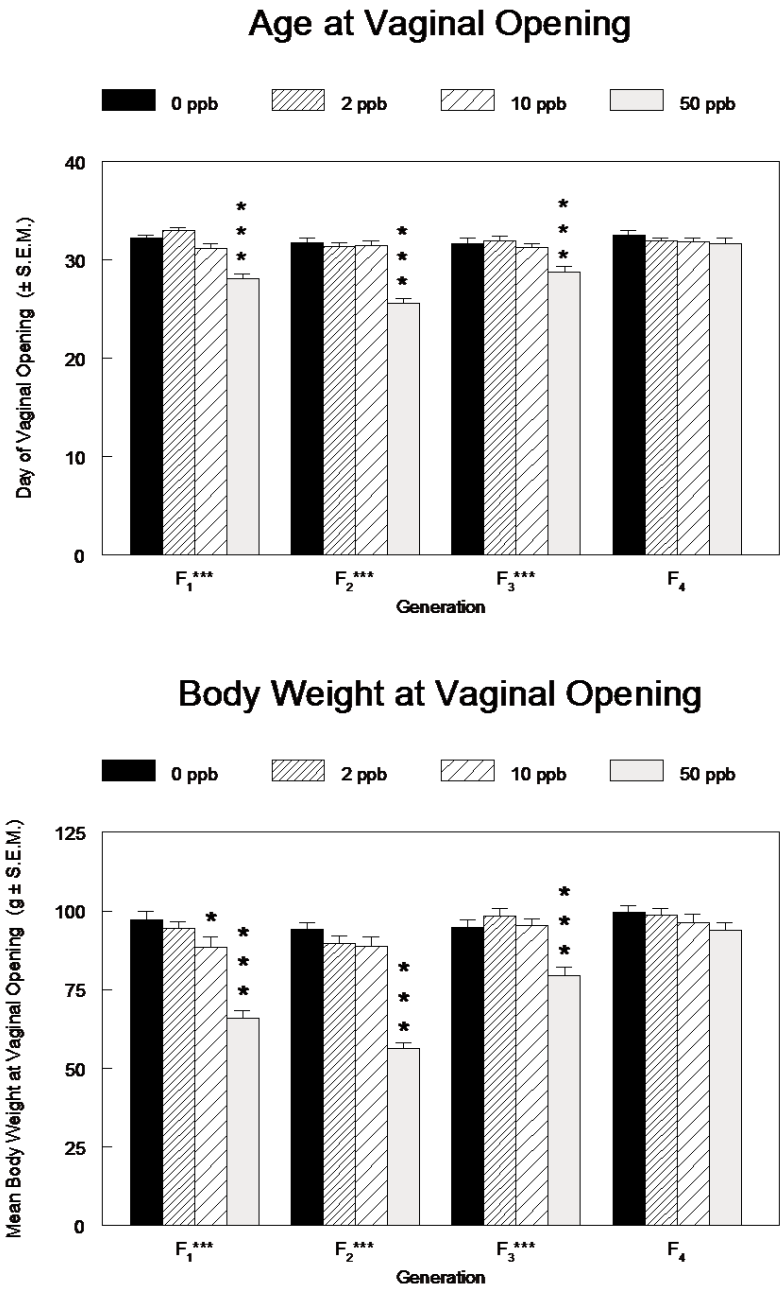


Figure 11
Effects of Dietary Ethinyl Estradiol on Age (top panel) and Body Weights (bottom panel) at Vaginal Opening

Results of nonparametric analyses within generations are presented for the age at vaginal opening (mean ± standard error). Asterisks on the x-axis indicate a significant overall Kruskal-Wallis' test for the marked generation while asterisks above the data bars indicate a significant difference between the means of the marked group and the control group in that generation (Holm's-adjusted Wilcoxon's test). Body weight at vaginal opening (mean ± standard error) was analyzed by ANOVA. Asterisks on the x-axis indicate a significant linear exposure concentration trend within the marked generation. Asterisks above the data bars indicate a significant difference between the means of the marked group and the control group in that generation (Dunnett's test). These data are tabulated in Table I1. *, $P \leq 0.05$; ***, $P \leq 0.001$.

The Estrous Cycle

Data were analyzed as percent of time in each of the stages of diestrus, estrus, and proestrus, number and percentage of abnormal cycles (defined as three or more consecutive days in estrus or four or more consecutive days in diestrus), and length of cycles (Table J1). In the F_1 and F_2 generations, significant increases in the percentage of time in estrus relative to controls were observed in the 50 ppb groups (Figure 12 and Table J1). The percent time in estrus was significantly decreased relative to controls in the 10 ppb group in the F_1 generation only. The mean percentages of time in proestrus and diestrus were decreased relative to the control group in the 50 ppb groups of the F_1 and F_2 generations. The number and percentage of abnormal cycles were significantly increased relative to the controls in the 50 ppb groups of the F_1 and F_2 generations, and these effects were largely due to an increased duration of estrus (Figure 13 and Table J1). All abnormal cycles in control animals were due to prolonged diestrus. The significant increase in the percentage of abnormal cycles due to prolonged diestrus in the 50 ppb group of the F_1 generation was due in part to a low incidence of abnormal cycles in the controls of that generation. Length of cycle was analyzed by two nonparametric methods, a Kruskal-Wallis ANOVA on ranks followed by pairwise comparisons of exposed groups to control by Wilcoxon's test and a more powerful Jonckheere-Terpstra trend test (Figure 14 and Table J1). Length of cycle was significantly increased in the 50 ppb groups relative to controls in the continuously exposed F_1 (5.6 days or a 122% increase) and F_2 (5.4 days or a 100% increase) generations and in the 2 and 10 ppb groups of the F_1 generation. There were no significant exposure-related effects in the F_3 or F_4 generations.

Vaginal smears were also obtained from breeder females from each generation (F_0 through F_4) for 10 consecutive days prior to necropsy, and the estrous cycle data were compiled and analyzed in the manner described above (Table J2). There were no statistically significant exposure concentration-related differences in the percentage of time in various estrous stages, the number or percentage of abnormal cycles, or the length of the cycle.

The ovaries, uteri, and vaginas taken from animals at necropsy were evaluated for stage of cycle and analyzed to determine if the organs were in synchrony (Tables B2a through B2e). No significant effects of ethinyl estradiol on estrous cycle synchrony in these organs were found. In all three tissues in the F_3 generation, there was an increase in the prevalence of proestrus relative to diestrus, and in the F_4 generation, there appeared to be a trend toward decreased prevalence of estrus with increasing exposure concentration.

Organ Weights

Organ weight data are summarized in Appendix K. While there were multiple statistically significant effects [significant Dose or Dose × Generation effects, differences in pairwise comparisons to controls, or significant exposure concentration trends for absolute organ weight and/or organ-weight-to-body-weight ratio (relative weight)] in the organs weighed in both sexes, there was little evidence for treatment-related toxicity given that the majority of these significant differences were confined to a single generation, did not follow a consistent pattern across ethinyl estradiol-exposed generations, or reflected the 8% to 15% treatment-related body weight decrease in the 50 ppb groups of the F_0 through F_2 generations that was presented earlier. In addition, mean values obtained in exposed groups were generally within the ranges of means measured in control animals across generations, and exposure concentration effects within a generation were often small (differences less than 10%).

In males, the most consistent organ weight effects were observed in the brain (Table K2), pituitary gland (Table K6), and spleen (Table K11) where positive linear exposure concentration and natural log exposure concentration trends in relative organ weights in the F_0, F_1, and F_2 generations occurred, and the 50 ppb group means were significantly greater than those of the control groups in each of these generations. Relative brain weights were also increased in the 2 and 10 ppb groups of the F_2 generation of males; significant body weight decreases also occurred in these exposed groups. Of these increases, the relative pituitary gland weight increases were greatest in magnitude, with increases of 24%, 22%, and 15% in the F_0, F_1, and F_2 generations, respectively. Few consistent effects were observed in male reproductive organs. Relative dorsal prostate gland (Table K7) and lateral prostate gland (Table K8) weights exhibited positive linear concentration and/or natural log exposure concentration trends in the F_0 and F_2 generations and the F_1 and F_2 generations, respectively, but the only significant difference from controls in these generations was a 22% greater lateral prostate gland weight in the 50 ppb group of the F_2 generation. A positive linear exposure concentration/natural log exposure concentration trend occurred for the relative epididymis weight only in the F_2 generation, with a significant relative

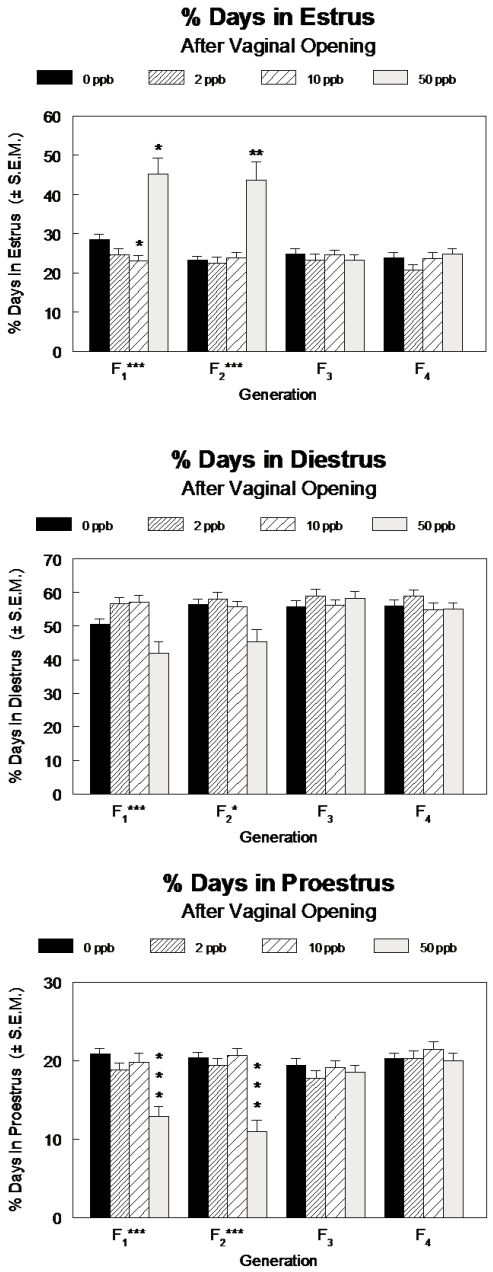


FIGURE 12

Effect of Dietary Ethinyl Estradiol on the Percentage of Days in Estrus, Diestrus, and Proestrus in Females Monitored Shortly after Vaginal Opening

Data were analyzed within generations by Kruskal-Wallis' nonparametric ANOVA and Holm's-adjusted Wilcoxon's tests for pairwise comparisons with the controls. Asterisks on the x-axis indicate significant overall Kruskal-Wallis' tests for the marked generation, while asterisks above the data bars indicate a significant difference between the marked group and the control group. These data are tabulated in Table J1. *, P≤0.05; **, P≤0.01; ***, P≤0.001.

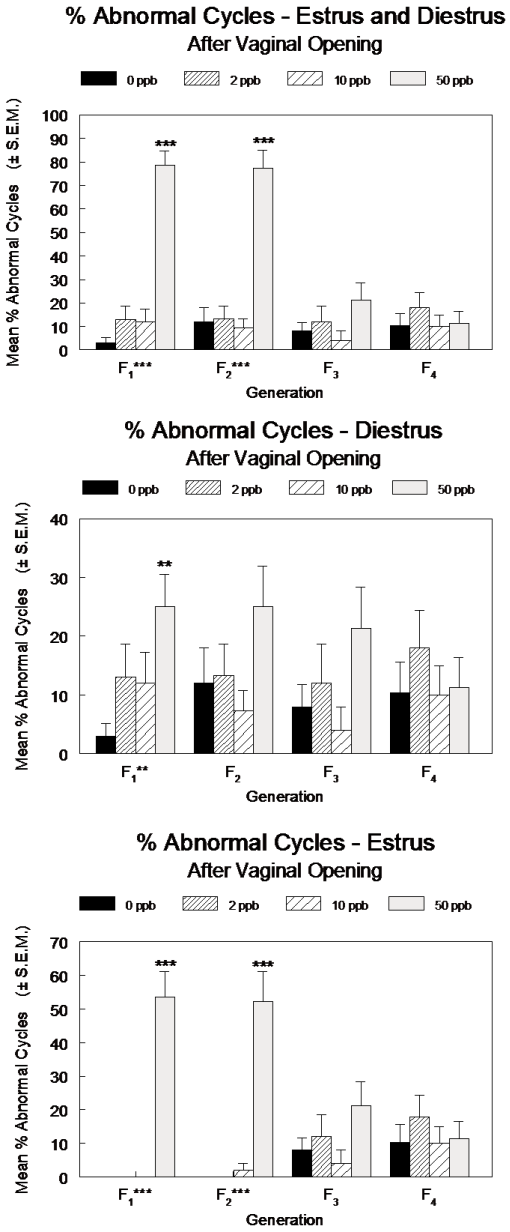


FIGURE 13
Effect of Dietary Ethinyl Estradiol on the Percentage of Abnormal Cycles in Females Monitored Shortly After Vaginal Opening

Abnormal cycles were defined as 3 or more consecutive days of estrus or 4 or more consecutive days of diestrus. The top panel gives the percentage of abnormal cycles due to either prolonged diestrus or estrus, the middle panel gives the percentage of abnormal cycles due to prolonged diestrus, and the bottom panel the percentage of abnormal cycles due to prolonged estrus. Data were analyzed within generations by the Kruskal-Wallis' nonparametric ANOVA and Holm's-adjusted Wilcoxon's tests for pairwise comparisons with the controls. Asterisks on the x-axis indicate significant overall Kruskal-Wallis' tests for the designated generation, while asterisks above the data bars indicate a significant difference between the marked group and the control group. **, $P \leq 0.01$; ***, $P \leq 0.001$.

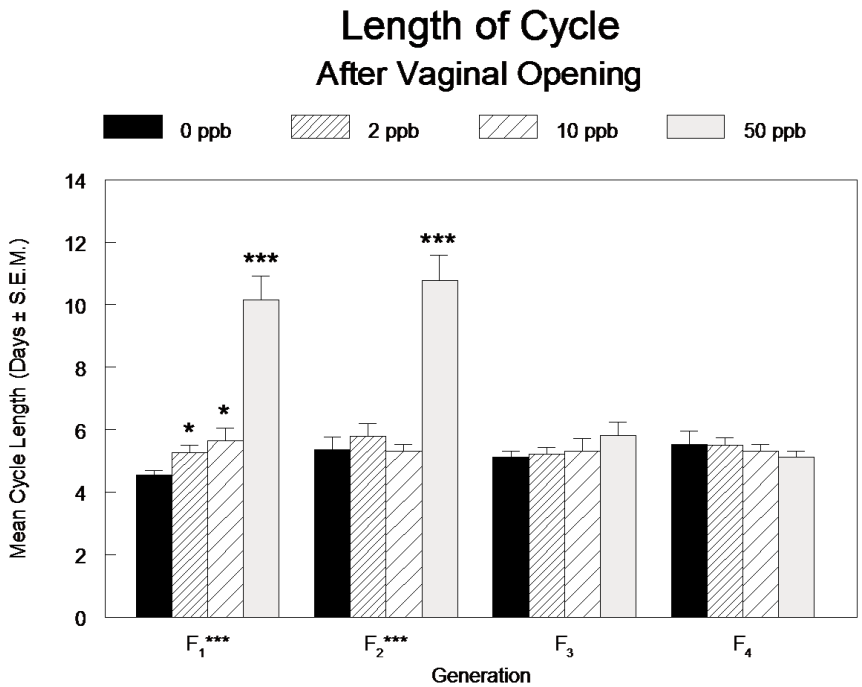


FIGURE 14
Effects of Dietary Ethinyl Estradiol on Mean Cycle Length of Females Examined for 14 Days Beginning 3 Days after Vaginal Opening

Asterisks on the x-axis indicate significant overall Kruskal-Wallis' tests within generations. Asterisks above the data bars indicate significant differences between the marked group and the control group for that generation. *, $P \leq 0.05$; ***, $P \leq 0.001$. Significant positive trends ($P \leq 0.001$) for the F_1 and F_2 generations were also found using the Jonckheere-Terpstra trend test.

weight increase in the 50 ppb group (Table K3). The low control value for the relative epididymal weight appeared to contribute to the significant effects observed in this generation. Relative weights of seminal vesicle/coagulating gland (Table K10) and testis (Table K12) showed significant exposure-concentration effects (positive linear exposure concentration/natural log exposure concentration trends and 50 ppb groups significantly increased relative to the control group by 13% to 21%) in the F_0 and F_2 generations, but not in F_1. Relative seminal vesicle/coagulating gland weights were also significantly increased in the 2 and 10 ppb groups of the F_2 generation, and a relatively low control value for this endpoint in this generation appeared to contribute to this effect.

In females, the most consistent observations across the exposed generations were the significant negative linear exposure concentration/natural log exposure concentration trends in absolute kidney (Table K17) and liver (Table K18) weights and positive linear exposure concentration/natural log exposure concentration trends in relative brain (Table K16) weights in the F_0 through F_2 generations. In all of these generations, the 50 ppb groups were significantly different from the controls (brain, greater than controls; liver and kidney, less than controls). Absolute kidney weights in the 2 and 10 ppb groups were also significantly decreased in the F_0 generation females, and the relatively high kidney weight of the F_0 control females appeared to contribute to these observations. Absolute kidney weight in the 10 ppb group of F_4 generation females was significantly greater than that in the control group, but this was likely a chance observation of no biological significance. There were few significant exposure concentration-related effects in female reproductive organ weights. There were negative linear exposure concentration and/or natural log exposure concentration trends in the absolute ovary weight in the F_0 through F_2 generations and a positive linear natural log exposure concentration trend in the relative ovary weight in the F_0 generation; in pairwise comparisons, only the absolute ovary weight in the

50 ppb group of the F_0 generation was significantly different (12% less) than that in the controls (Table K19). Absolute spleen weights were decreased in the 50 ppb groups of females in the F_0 and F_1 generations and increased in the 2 ppb group of the F_2 generation, with significant linear and/or quadratic exposure concentration and/or natural log exposure concentration trends in these generations (Table K21). Relative spleen weights were increased in the 2 ppb group of females in the F_1 generation and all exposed groups of the F_2 generation. Relative thymus weights were significantly increased (by 16% to 21%) in the 2 and 50 ppb groups of females in the F_1 generation and the 50 ppb groups of the F_2 and F_4 generations (Table K22). There was some evidence for increased absolute and relative thyroid gland weights in females (significant differences of 21% to 30% from control and/or significant quadratic trends) in the 2 and 10 ppb groups of the F_0 and F_1, but not the F_2 or F_3 generations (Table K23). Absolute thyroid gland weight was also significantly increased (23%) in the 10 ppb group of the unexposed F_4 generation, where significant positive linear natural log exposure concentration and quadratic exposure concentration trends occurred.

Sperm Parameters

Sperm parameter data are presented in Tables L1 through L4. There were no significant effects of ethinyl estradiol treatment in any generation on sperm motility (Table L1) or sperm morphology (Table L4). In the F_2 generation, epididymal sperm counts were significantly increased in the 10 and 50 ppb groups (48% and 49%) (Figure 15 and Table L2). Increased epididymal sperm counts were also observed in the 10 and 50 ppb groups in the F_1 generation (16% and 20% increases, respectively), in the F_3 generation (36% and 14% increases, respectively), and the 10 ppb group of the F_4 generation (40% increase); however, these increases were not statistically significant. The only significant treatment effect on testicular spermatid head counts was a significant decrease (16%) in the 50 ppb F_1 group (Figure 16 and Table L3).

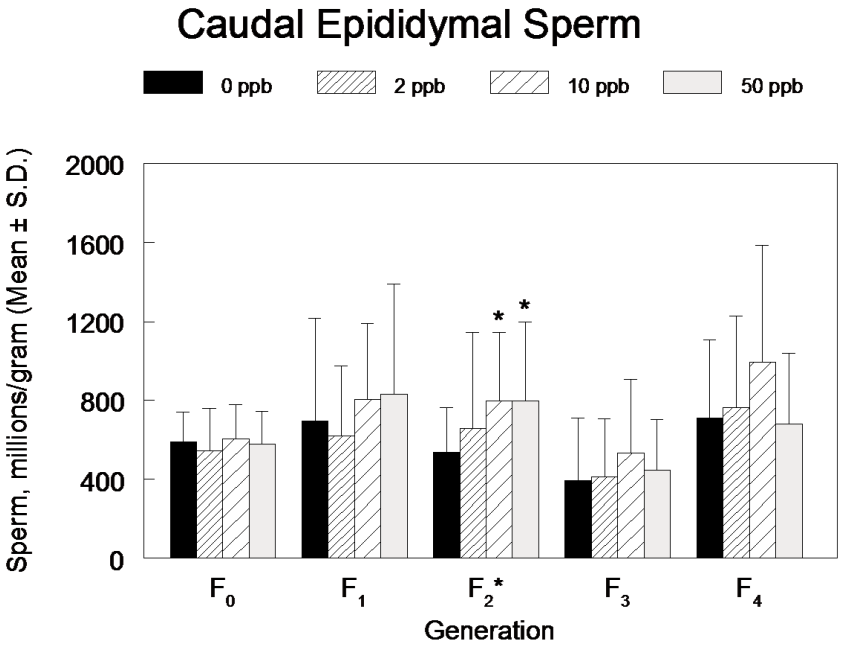


FIGURE 15
Effect of Dietary Ethinyl Estradiol on Caudal Epididymal Sperm Counts

Data were analyzed within each generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the control. An overall significant exposure concentration effect within a generation is indicated by an asterisk on the x-axis label. Significant differences between exposed groups and the control are indicated by asterisks above the bars. *, $P \leq 0.05$.

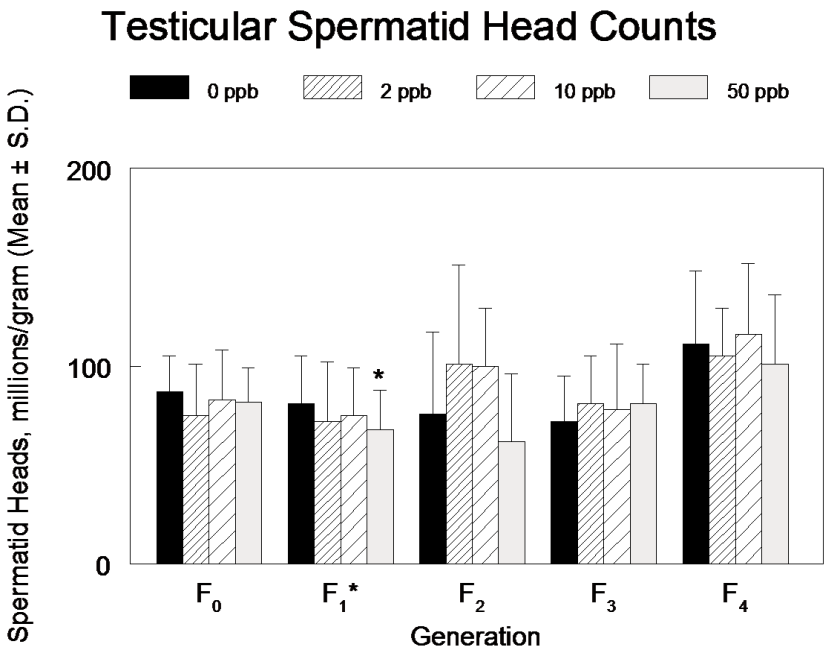


FIGURE 16
Effect of Dietary Ethinyl Estradiol on Testicular Spermatid Head Counts

Data were analyzed within each generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the control. An overall significant exposure concentration effect within a generation is indicated by an asterisk on the x-axis label. Significant differences between exposed groups and the control are indicated by asterisks above the bars. *, $P \leq 0.05$.

Ovarian Follicle Counts

Ovarian follicle counts are presented in Figure 17 and Table M1. A significant overall Dose effect was observed for antral follicle counts, and significant Dose × Generation interactions (indicating differences in the effect of treatment across generations) were observed for the small, antral, and combined (both small and growing follicles combined and all follicles) categories of follicle counts. In the F_0 generation, a significant positive linear exposure concentration (and natural log exposure concentration) trend occurred for counts of small follicles, as well as small and growing follicles combined and all follicles, and the count in the 50 ppb group was significantly greater than that in the controls. A significant negative natural log exposure concentration trend occurred in these categories in the F_4 generation. In the F_4 generation, significant negative linear exposure concentration and natural log exposure concentration trends occurred for counts of growing follicles, and the count in the 50 ppb group was significantly less than that in the controls. For antral follicle counts, significant positive linear exposure concentration and natural log exposure concentration trends occurred in the F_0 and F_1 generations, respectively, and significant positive quadratic trends occurred in the F_1 (exposure concentration and natural log exposure concentration) and F_2 (exposure concentration) generations; antral follicle counts showed significant increases relative to controls in the 10 ppb groups of the F_1 and F_2 generations. Only the F_1 10 ppb mean antral follicle count was outside the range of mean control antral follicle counts.

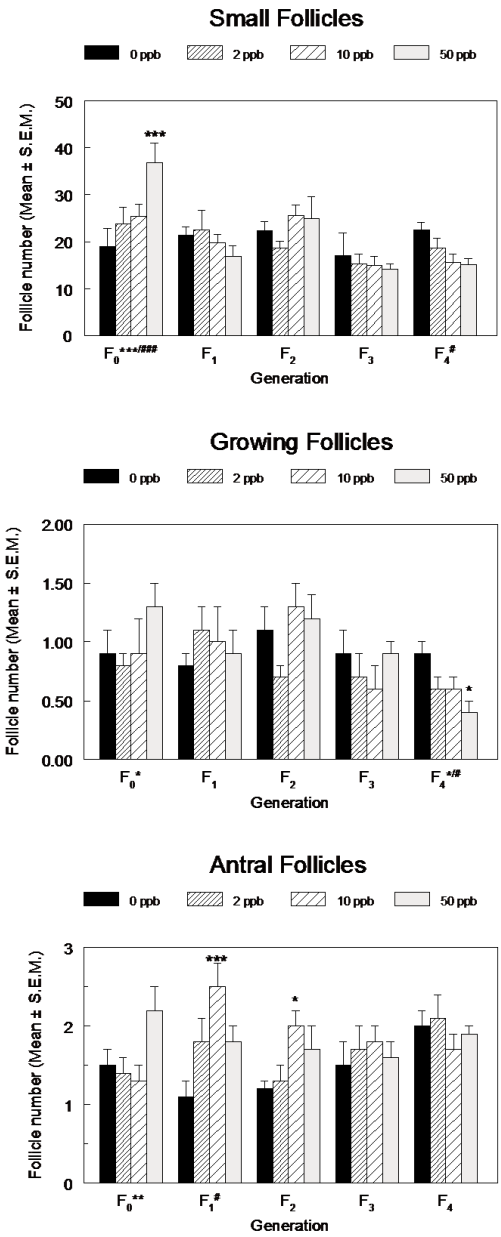


FIGURE 17
Effect of Dietary Ethinyl Estradiol on Ovarian Follicle Counts

Asterisks over bars indicate a significant difference between that exposed group and the controls in the same generation by Dunnett's test. Asterisks and pound signs on x-axis labels indicate significant linear exposure concentration or natural log exposure concentration plus one trends, respectively. *, #, $P \leq 0.05$; **, $P \leq 0.01$; ***, ###, $P \leq 0.001$. Significant quadratic exposure concentration trends were also seen for antral follicles in the F_1 and F_2 generations but are not labeled here.

Pathology and Statistical Analyses

This section describes the statistically significant or biologically noteworthy changes in the incidences of nonneoplastic lesions of the mammary gland and kidney. Summaries of the incidences of neoplasms and nonneoplastic lesions are presented in Appendix A for male rats and Appendix B for female rats.

Mammary Gland: Incidences of alveolar/ductal hyperplasia occurred with positive linear exposure concentration trends in F_0 through F_3 generation males, and the incidences of this lesion were significantly increased (compared to same-generation controls) in the 50 ppb groups of the F_0 through F_3 generations, the 2 and 10 ppb groups of the F_1 generation, and the 10 ppb group of the F_2 generation (Tables 20 and A2a through A2e). The slight increase in the incidence of mammary gland alveolar hyperplasia that occurred in the 50 ppb F_4 males was not statistically significant. Thus, males in the F_1 and F_2 generations that were continuously exposed to ethinyl estradiol from conception through termination at PND 140 showed greater exposure concentration effects

Table 20
Incidences and Severities of Nonneoplastic Lesions of the Mammary Gland in Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Lesion	Generation	0 ppb	2 ppb	10 ppb	50 ppb
Male					
Alveolar Hyperplasia					
	F_0***,##	1/24 (1.0)	2/24 (2.0)	5/25 (2.2)*	10/25 (1.8)***,##
	F_1***,#	1/25 (3.0)	7/24 (1.6)*,#	11/25 (1.8)**,##	10/26 (2.6)**,##
	F_2***,###	3/25 (1.7)	3/25 (1.7)	7/25 (1.9)	13/25 (3.0)***,###
	F_3*	2/25 (1.5)	5/25 (1.8)	4/24 (2.0)	7/24 (2.0)*
	F_4	3/25 (1.0)	5/25 (2.2)	5/25 (2.0)	7/25 (1.9)
Ductal Hyperplasia					
	F_0	2/24 (1.0)	1/24 (1.0)	2/25 (1.0)	2/25 (1.0)
	F_1***,###	0/25 (0.0)	1/24 (2.0)	5/25 (1.6)**	10/26 (2.0)***,###
	F_2***,###	0/25 (0.0)	5/25 (1.2)**	8/25 (1.8)**,##	12/25 (1.8)***,###
	F_3	1/25 (2.0)	1/25 (2.0)	3/24 (1.0)	1/24 (2.0)
	F_4	1/25 (1.0)	0/25 (0.0)	2/25 (1.0)	0/25 (0.0)
Combined Alveolar/Ductal Hyperplasia					
	F_0***,###	3/24 (1.0)	3/24 (1.7)	7/25 (1.9)	12/25 (1.7)**,##
	F_1***,###	1/25 (3.0)	8/24 (1.6)**,#	16/25 (1.8)***,###	20/26 (2.3)***,###
	F_2***,###	3/25 (1.7)	8/25 (1.4)	15/25 (1.8)***,###	25/25 (2.4)***,###
	F_3*	3/25 (1.7)	6/25 (1.8)	7/24 (1.6)	8/24 (2.0)*
	F_4	4/25 (1.0)	5/25 (2.2)	7/25 (1.7)	7/25 (1.9)

Table 20
Incidences and Severities of Nonneoplastic Lesions of the Mammary Gland in Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Lesion	Generation	0 ppb	2 ppb	10 ppb	50 ppb
Female					
Lobular Hyperplasia					
	F_0	0/25	0/25	0/25	0/25
	F_1	0/25	0/25	0/25	0/25
	F_2*	7/25 (1.1)	10/25 (1.8)	13/25 (1.5)*	13/25 (1.5)*
	F_3	7/25 (1.7)	4/25 (1.8)	6/25 (1.3)	8/25 (1.5)
	F_4#	4/25 (1.0)	10/25 (1.3)	2/26 (1.5)	4/25 (1.3)
Alveolar Hyperplasia					
	F_0	1/25 (1.0)	4/25 (1.3)	3/25 (1.0)	4/25 (1.0)
	F_1	5/25 (1.2)	6/25 (1.2)	5/25 (1.2)	5/25 (1.4)
	F_2	14/25 (1.8)	15/25 (1.5)	12/25 (1.5)	11/25 (1.5)
	F_3	9/25 (1.7)	4/25 (1.8)	11/25 (1.6)	9/25 (1.3)
	F_4	11/25 (1.7)	14/25 (1.4)	7/26 (1.1)	9/25 (1.4)

[a] All mammary glands for males and females received in pathology were examined microscopically except in cases where this was precluded by autolysis or insufficient glandular tissue in the section. Lesion severity was graded on an ordinal scale as follows: no lesion, 0; minimal, 1; mild, 2; moderate, 3; marked, 4. The number of animals with a lesion is listed to the left of the slash, the total number of animals examined is listed to the right of the slash, and the average severity grade of the lesion in affected animals in the exposure group is given in parentheses. Data were analyzed by two statistical methods: 1) Results of a one-sided Jonckheere-Terpstra linear exposure concentration trend test and pairwise comparisons to the controls using Shirley's test are indicated by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Significant Jonckheere-Terpstra trend test results are indicated in the Generation column. Shirley's test results are indicated in the exposed group columns; this test indicates that the incidence and/or severity of the lesion in the marked group differs significantly from that in the control group. The Jonckheere-Terpstra trend test determines whether a monotonic exposure relationship is present. Shirley's test assumes a monotonic exposure concentration response. 2) In order to test for possible nonmonotonic exposure concentration responses, two-sided Kruskal-Wallis' tests with Wilcoxon's tests for pairwise comparisons of exposed groups to controls were also run. The results of these tests are indicated by pound signs (#). The Kruskal-Wallis' test results are indicated in the Generation column, while the Wilcoxon's test results are indicated in the exposed group columns: #, $P \leq 0.05$; ##, $P \leq 0.01$; ###, $P \leq 0.001$.

on the mammary gland than the generations that were exposed only as adults (F_0) or only through weaning (F_3).

The most important criteria for distinguishing ductal and alveolar hyperplasia of the mammary gland in male rats were the size of the gland in sections and the density of the mammary gland structures (ducts, alveoli, or both) in exposed animals compared to controls. Ductal hyperplasia was observed as a relative increase in the number of branching ducts. Small budding alveoli were also present within the hypodermis. The alveoli were either attached or adjacent to ducts. The ducts along with the alveoli formed a tubuloalveolar pattern of development. These tubuloalveolar units often had distinct lumens that were usually lined by one layer of cuboidal epithelium or two layers of stratified or pseudostratified cuboidal epithelium, occasionally with minimal to mild hypertrophy. The cytoplasm of the ductal epithelium was often vacuolated. The lumens were occasionally dilated and often contained secretory material. Varying amounts of fibrous connective tissue surrounded the ducts and alveoli, diminishing gradually as branching progressed from the primary to tertiary ducts of the gland.

Alveolar hyperplasia in the males was seen histologically as an increase in alveoli, most often with a predominance of lobuloalveolar development, but with some tubuloalveolar patterns of growth as well. The lobuloalveolar growth pattern was characterized by more contiguous lobules of glands with ducts and alveolar lumens being less prominent or completely indistinct. In contrast, the tubuloalveolar pattern of hyperplasia was characterized by prominent clusters of large alveoli often with wide, distinct lumens. Both lobuloalveolar and tubuloalveolar growth patterns demonstrated vacuolization of the alveolar and ductal epithelium with either patent or collapsed indistinct lumens. Lumens sometimes contained secretory material and/or numerous blebs of apoptotic epithelial cells. In some cases, either lobuloalveolar or tubuloalveolar growth patterns were present nearly exclusively, but both patterns were often present in the same specimen. Both of these morphologic patterns were present in the control male rats as well; however, with hyperplasia, the number of prominent alveoli appeared to be increased per unit area of section. This increased alveolar density appeared to correlate positively with the severity of hyperplasia. The hyperplasia described did not appear to be "feminization" of the glands in that the male glands remained morphologically distinct from the mammary glands of females of the same age. First, the females did not have the distinct lobuloalveolar growth that was a feature in the males (see description above). Secondly, the alveoli forming lobules generally had smaller diameters than those present in males, were lined by a single layer of basophilic cuboidal epithelial cells with a decreased cytoplasmic-to-nuclear ratio, and the alveolar lumens were small compared to males. A more complete discussion which includes photomicrographs of the morphology of male mammary gland hyperplasia and its relationship to female mammary gland morphology, is presented in Appendix R.

Incidences of lobular hyperplasia occurred with a positive linear exposure concentration trend in F_2 females, and the incidences of this lesion in the 10 and 50 ppb groups were significantly greater than those in the F_2 controls (Tables 20 and B2a through B2e). Incidences of lobular and alveolar hyperplasia of the mammary gland in females were highly variable across all exposure concentrations and generations, and this rendered a treatment effect difficult to determine. Females had recently nursed litters, and the variability in the time since the termination of lactation may have contributed to the variations in the incidences of hyperplasia.

Kidney: Incidences of renal tubule mineralization (nephrocalcinosis) occurred with positive exposure concentration trends in the continuously exposed F_1 and F_2 generations of males, and the incidences of this lesion in the 50 ppb F_1 and F_2 males were significantly greater than those in the same-generation controls (Tables 21 and A2a through A2e). Renal tubule mineralization consisted of intratubular calcified deposits mainly at the corticomedullary junction but also in the medulla.

Renal tubule mineralization is a high-incidence background lesion in females of this strain of rat under the dietary conditions of this study, and across the genera-

Table 21
Incidences and Severities of Renal Tubule Mineralization in Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	Generation	0 ppb	2 ppb	10 ppb	50 ppb
Male					
	F_0	1/24 (1.0)	0/4	—	1/25 (1.0)
	F_1***, ###	0/25	0/2	0/25	9/26 (1.3)***, ##
	F_2***, ###	1/25 (1.0)	—	0/25	10/25 (1.2)***, ##
	F_3	0/25	—	0/2	0/25
	F_4	0/25	—	0/1	1/25 (1.0)
Female					
	F_0	20/25 (1.8)	22/25 (1.6)	24/25 (1.2)	17/25 (1.5)
	F_1*,#	21/25 (1.9)	18/25 (2.0)	21/25 (1.7)	17/25 (1.2)**,##
	F_2	18/25 (1.6)	23/25 (1.4)	20/25 (1.8)	15/25 (1.5)
	F_3	19/25 (1.2)	19/25 (1.3)	18/25 (1.4)	14/25 (1.1)
	F_4	24/25 (1.5)	19/25 (1.3)*	19/26 (1.7)	19/25 (1.3)*,#

[a] Kidneys from animals in the 50 ppb and control groups were examined microscopically in all generations. Intermediate exposure groups were examined only if an effect was observed in the 50 ppb group or if a gross lesion was noted; a dash indicates no kidneys were examined in the exposed group. Lesion severity was graded on an ordinal scale as follows: no lesion, 0; minimal, 1; mild, 2; moderate, 3; marked, 4. The number of animals with a lesion is listed to the left of the slash, the total number of animals examined is listed to the right of the slash, and the average severity grade of the lesion in affected animals in the exposure group is given in parentheses. Data were analyzed by two statistical methods: 1) Results of a one-sided Jonckheere-Terpstra linear exposure concentration trend test and pairwise comparisons to the controls using Shirley's test are indicated by asterisks: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Significant Jonckheere-Terpstra trend test results are indicated in the Generation column. Shirley's test results are indicated in the exposed group columns; this test indicates that the incidence and/or severity of the lesion in the marked group differs significantly from that in the control group. The Jonckheere-Terpstra trend test determines whether a monotonic exposure relationship is present. Shirley's test assumes a monotonic exposure concentration response. 2) In order to test for possible nonmonotonic exposure concentration responses, two-sided Kruskal-Wallis' tests with Wilcoxon's tests for pairwise comparisons of exposed groups to controls were also run. The results of these tests are indicated by pound signs (#). The Kruskal-Wallis' test results are indicated in the Generation column, while the Wilcoxon's test results are indicated in the exposed group columns: #, $P \leq 0.05$; ##, $P \leq 0.01$; ###, $P \leq 0.001$.

tions, there were generally lower incidences and severities of this lesion in the 50 ppb female groups (Tables 21 and B2a through B2e). Incidences of renal tubule mineralization in 50 ppb F_1 and F_4 females were significantly less than those in the respective controls.

Miscellaneous Lesions: An unusual observation in the male rats in this study was a high background incidence of developmental malformations of the coagulating gland (Table 22). Whereas the coagulating gland is normally attached to the concave side of the seminal vesicle and is approximately 4 to 6 mm in length with five to six tubules noted grossly, only a small portion of the gland was evident at the base of the seminal vesicle in animals diagnosed with developmental malformations of this gland. While there was a significant treatment effect observed in the F_3 generation evidenced by a significant positive exposure concentration trend and significantly increased incidence in the 50 ppb group, the incidences of this lesion in control groups from the various generations varied from a low of 8% to a high of 42% and were also highly variable in the other exposed groups. Thus, the occurrence of this lesion was not related to treatment and its origin is unknown. There were no other abnormalities noted in the male reproductive tract that would suggest a broader aberration in development.

Table 22
Incidences of Coagulating Gland Malformation in Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generation	0 ppb	2 ppb	10 ppb	50 ppb
F_0	10/24	8/25	8/25	8/24
F_1	6/25	3/24	2/25	5/26
F_2	8/25	5/23	8/25	8/25
F_3*	2/25	8/25	6/25	9/25*,#
F_4	10/25	9/25	6/26	11/24

a The number of animals with a lesion is listed to the left of the slash, the total number of animals examined is listed to the right of the slash. Data were analyzed by two statistical methods: 1) Results of a one-sided Jonckheere-Terpstra linear exposure concentration trend test and pairwise comparisons to the controls using Shirley's test are indicated by asterisks: *, $P \leq 0.05$. Significant Jonckheere-Terpstra trend test results are indicated in the Generation column. Shirley's test results are indicated in the exposed group columns; this test indicates that the incidence of the lesion in the marked group differs significantly from that in the control group. The Jonckheere-Terpstra trend test determines whether a monotonic exposure relationship is present. Shirley's test assumes a monotonic exposure concentration response. 2) In order to test for possible nonmonotonic exposure concentration responses, two-sided Kruskal-Wallis' tests with Wilcoxon's tests for pairwise comparisons of exposed groups to controls were also run. The results of these tests are indicated by pound signs (#). The Wilcoxon's test results are indicated in the exposed group columns: #, $P \leq 0.05$.

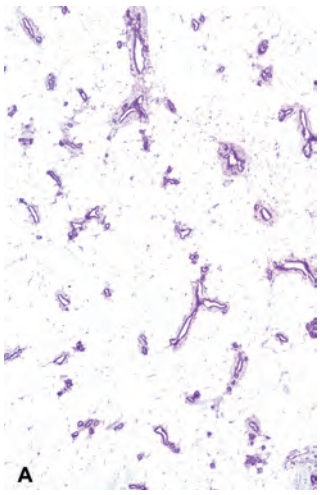


Plate 1
Normal mammary gland tissue from an F_1 control group male at PND 50. H&E

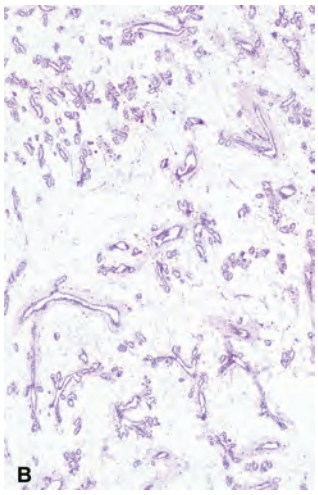


Plate 2
Ductal hyperplasia at PND 50 in the mammary gland of an F_1 male exposed to 50 ppb ethinyl estradiol in feed. H&E

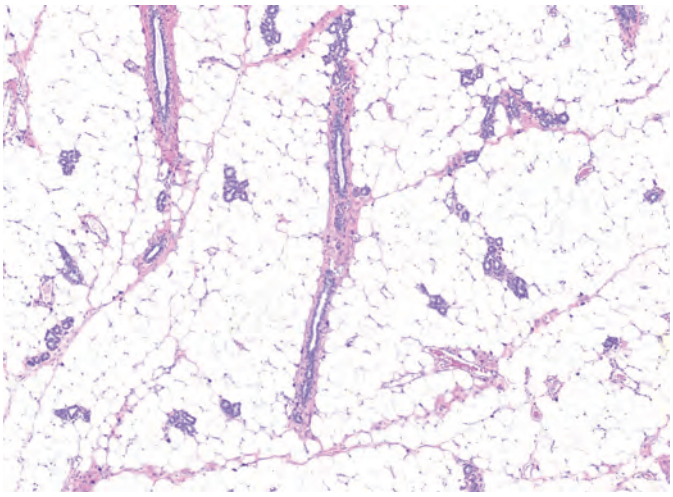


Plate 3
Normal mammary gland tissue from control group female at PND 65, from another unrelated study. H&E

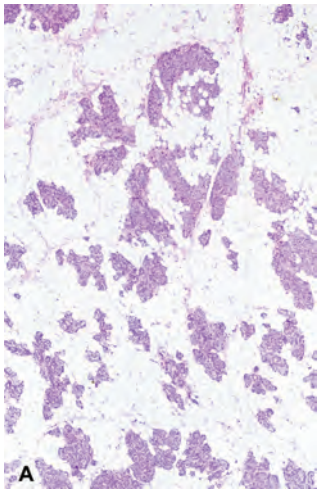


Plate 4
Normal mammary gland tissue from an F_1 control group male at PND 90. H&E

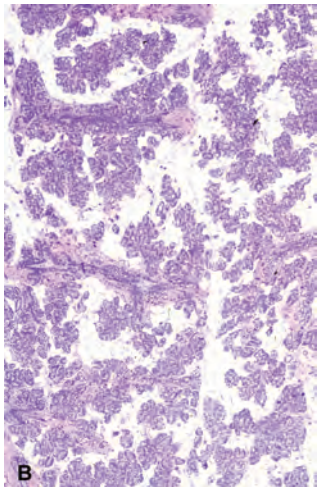


Plate 5
Lobuloalveolar and tubuloalveolar alveolar hyperplasia at PND 90 in an F_1 male exposed to 50 ppb ethinyl estradiol in feed. H&E

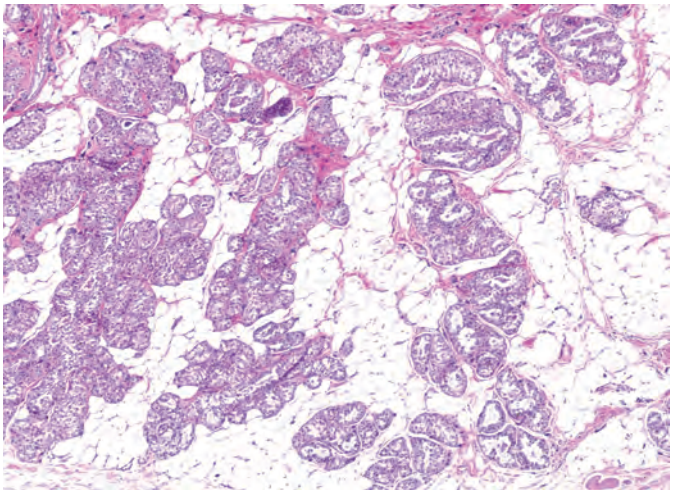


Plate 6
Lobuloalveolar and tubuloalveolar alveolar hyperplasia at PND 140 in an F_1 male exposed to 50 ppb ethinyl estradiol in feed. H&E

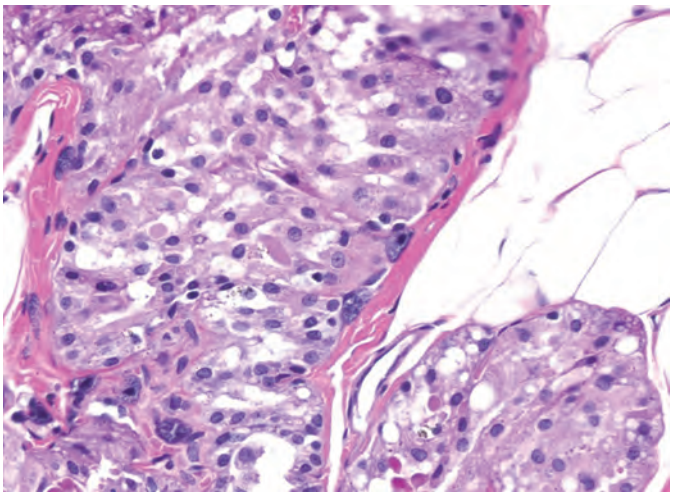


Plate 7
Higher magnification of lobuloalveolar alveolar hyperplasia at PND 140 in the same F_1 male shown in Plate 6. H&E

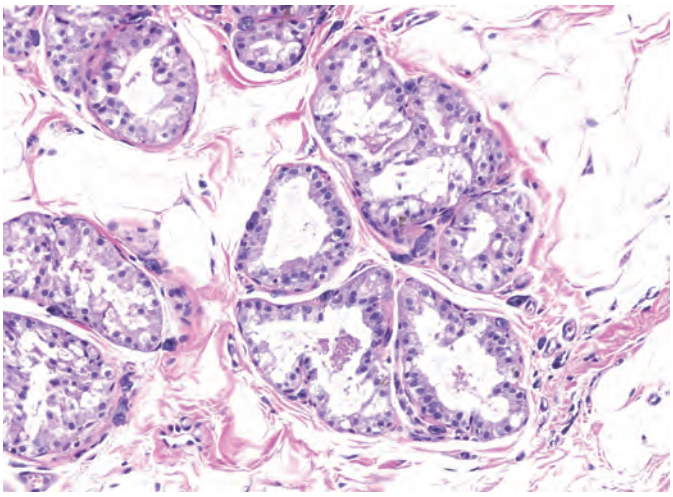


Plate 8
Higher magnification of tubuloalveolar alveolar hyperplasia at PND 140 in the same F_1 male shown in Plates 6 and 7. H&E.

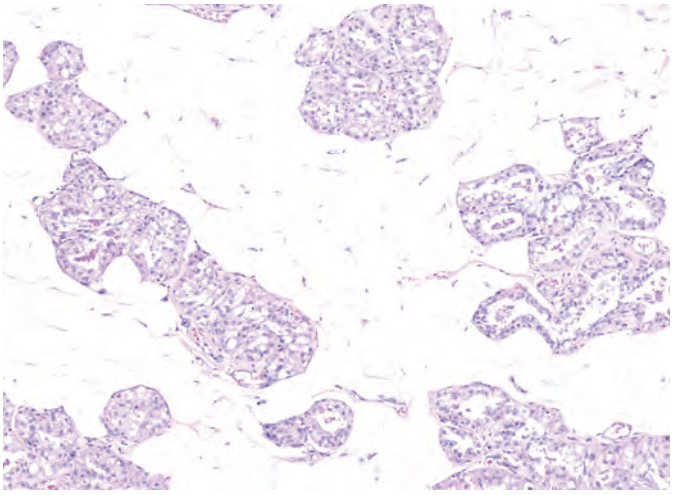


Plate 9
Lobuloalveolar and tubuloalveolar alveolar hyperplasia at PND 140 in a male control group rat. H&E

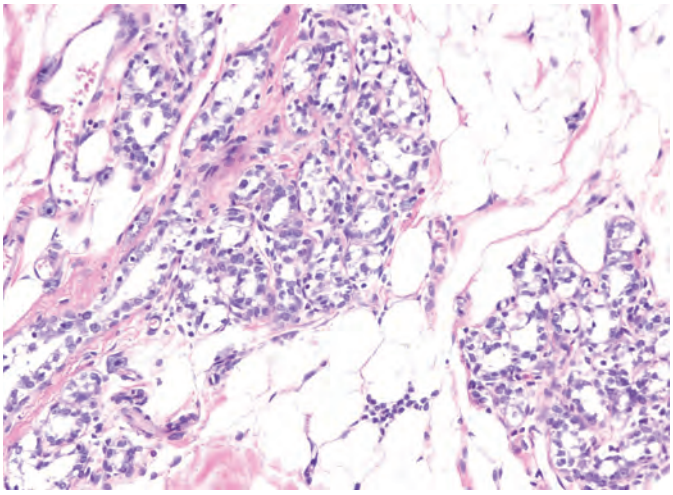


Plate 10
Tubuloalveolar alveolar hyperplasia at PND 140 in a female control group rat. H&E

DISCUSSION

Ethinyl estradiol is a well known potent synthetic estrogen commonly used in pharmaceuticals because of its improved oral bioavailability over 17β-estradiol. The current study defines the activity of ethinyl estradiol on administration in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats over several generations. This experimental system was also used in parallel studies, reported elsewhere, with genistein (NTP, 2008) and nonylphenol (TPA, 2005) to allow direct comparisons of the toxicities of compounds of varying estrogenic potencies. Data from a reproductive dose range-finding study of ethinyl estradiol (presented in this Technical Report) were used to select dietary exposure concentrations of 0, 2, 10, and 50 ppb for the current multigenerational reproductive toxicology study. In keeping with the goals of the study, outlined in the Introduction, to examine a dose range that demonstrated only subtle effects in the first generation, higher doses that were tested in the reproductive dose range-finding study were ruled out for use in the multigenerational reproductive toxicology study due to effects on body weight and the reproductive tract of males and females. The dietary exposure concentrations of 0, 2, 10, or 50 ppb resulted in ingested ethinyl estradiol doses of approximately 0, 0.1, 0.7, or 4 µg ethinyl estradiol/kg body weight per day for males and 0, 0.2, 1, or 6 µg/kg for females during the time that the rats were directly consuming dosed feed. Serum concentrations of ethinyl estradiol in these animals, even in the 50 ppb group, were below 10 pg/mL, the limit of detection of the liquid chromatography-mass spectrophotometric method used for the analysis (Twaddle *et al.,* 2003). This result is consistent with the low oral bioavailability of ethinyl estradiol in rats relative to humans (Dusterberg *et al.*, 1986). For example, in contrast to the low serum concentrations of ethinyl estradiol in 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 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 in the multigenerational reproductive toxicology study, there was 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 (Yasuda *et al.,* 1977a,b, 1981, 1985,a,b, 1986,a,b, 1987, 1988; Thayer *et al.,* 2001). 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 the dams reported less than 0.1% of the total dose in the bodies of the pups at each of the three time intervals (0 to 4, 0 to 24, and 24 to 48 hours) examined (Cargill *et al.,* 1969).

Despite the low serum concentrations of ethinyl estradiol resulting from the dietary consumption of ethinyl estradiol, there were clear effects of exposure in the animals of the multigenerational reproductive toxicology study, including body weight reductions, acceleration of vaginal opening, prolonged and aberrant estrous cycles in young females, increased incidences of hyperplasia of the mammary gland, and mineralization of the renal tubules in males. In general, the exposure concentration range over which these effects were observed is consistent with that reported for uterotrophic or gene expression changes in immature rodents orally dosed with ethinyl estradiol (Kanno *et al.,* 2001; Naciff *et al.,* 2002, 2003, 2005). Given that Masutomi *et al.* (2004a) reported that the effects of a 500 ppb dose of ethinyl estradiol administered from gestational day (GD) 15 through postnatal day (PND) 10 on female Sprague-Dawley rat pups were exacerbated by a soy-containing diet, it should be stressed that the current study was conducted with a soy- and alfalfa-free diet.

In the multigenerational reproductive toxicology study, significant treatment-related effects on body weights of 10% or greater were confined to the 50 ppb group in the continuously exposed generations (F_0, F_1, and F_2), although significant effects of lesser magnitude were observed in some cases in the 2 and 10 ppb groups of both sexes. While the feed consumption values were generally less in the exposed groups that had decreased body weights, the decreases were not always statistically significant, and in some cases, feed consumption values were unchanged or increased relative to control values during periods when body weights of exposed groups were less than those of the controls. Consumption measurements of the meal feed were not corrected for spillage, and thus the approximate nature of these values may contribute to the lack of strict correlation between feed consumption and body weight in the exposed groups. On the other hand, while estrogens have been demonstrated to be anorectic in several species (Wade and Schneider, 1992), they have also been shown to modulate metabolism without direct effects on feed consumption (Toth *et al.,* 2001; Wallen *et al.,* 2001). The observations in the current study indicate that the 50 ppb dietary dose of ethinyl estradiol clearly reduces growth in both sexes without a similarly clear reduction in feed consumption.

Acceleration of vaginal opening and the induction of persistent estrus and aberrant estrous cycles are expected estrogenic effects that were produced in the 50 ppb groups. While a significant effect on the time of vaginal opening was observed in the F_3 generation where exposure was terminated at weaning (PND 21) as well as in the continuously exposed F_1 and F_2 generations, there were no significant effects of ethinyl estradiol exposure on the estrous cycle of animals in the F_3 generation. Despite the high incidences of aberrant and prolonged cycles detected in the females immediately after vaginal opening, these effects were not sufficiently severe to impair fertility as determined in the breeding that occurred several weeks after these data were collected. In addition, cycles evaluated prior to termination, after the dams had completed nursing of their litters, were not adversely affected by the exposure concentrations used. No convincing treatment effects on the timing of puberty in males were observed, although there was evidence in an ancillary study of a transient depression of serum testosterone concentrations at PND 50 in males treated continuously with 10 or 50 ppb ethinyl estradiol and in males treated until PND 21 with 50 ppb ethinyl estradiol (Appendix Q).

No clearly exposure concentration-related lesions were found in microscopic evaluations of female tissues, and exposure concentration-related lesions in males were confined to the mammary gland and kidney. Consistent with the observations in the reproductive dose range-finding study, ethinyl estradiol induction of hyperplasia in the male mammary gland was among the most sensitive affected endpoints in the multigenerational reproductive toxicology study. In the reproductive dose range-finding study, a treatment-related increase in the incidences of male mammary gland hyperplasia was observed in groups exposed to 25 ppb or greater at PND 50 in animals exposed from GD 7. In the multigenerational reproductive toxicology study, there was clear evidence of hyperplasia in the male mammary glands in the continuously exposed F_1 and F_2 generations in the 10 and 50 ppb groups and some evidence of effects in the 2 ppb groups. In the F_0 generation, with exposure from postnatal week 6, and the F_3 generation, with exposure discontinued at PND 21, there were significantly increased incidences of hyperplasia only in the 50 ppb groups. This pattern of induction of hyperplasia across generations, with the strongest effects seen in the 10 and 50 ppb groups of the continuously exposed F_1 and F_2 generations, indicates that both developmental and post-weaning exposures contribute to this effect. Late pubertal and adult exposure, as in the F_0 generation, or developmental only exposure, as in the F_3 generation, produced lesser effects. Studies in a subset of animals from the F_1 and F_2 generations evaluated at PNDs 50 and 90 (Appendix Q) confirmed both the observation of effects at 2 ppb and a gradual lessening of the hyperplastic effect with time after cessation of dosing at PND 21. In females, significantly increased incidences of mammary gland hyperplasia were noted in the 10 and 50 ppb groups of the F_2 generation only, but the variable time since lactation was terminated in the female rats made true treatment effects difficult to distinguish. In previous studies of dietary 17β-estradiol (10 and 50 ppm; Biegel *et al.,* 1998) and 17α-ethinyl estradiol (0.08 ppm; Schardein, 1980), examination of the mammary glands in adult males indicated feminization. Similar observations in 28-day gavage studies of ethinyl estradiol in Wistar rats were reported by Andrews *et al.* (2002) at doses as low as 10 µg/kg per day, although Yamasaki et al. (2002a) reported only atrophy of the male mam-

mary gland in Sprague-Dawley rats at 200 µg/kg per day in a study of similar design. This author also reported that treatment of male rats with a dopamine antagonist resulted in male mammary glands with a tubuloalveolar structure typical of females and speculated that an increase in prolactin resulting from the drug treatment may have been responsible for the feminizing effect. This author also suggested that the male mammary gland may be a valuable marker tissue for endocrine-active compounds. Although the present study does not indicate ethinyl estradiol-induced feminization, the present results along with the results of Andrews *et al.* (2002) with ethinyl estradiol, the previously reported studies with genistein (Delclos *et al.,* 2001; NTP, 2008), and the studies of genistein and methoxychlor (You *et al.,* 2002; Wang *et al.,* 2006) have all found the male mammary gland to be a sensitive tissue for the detection of the activity of these compounds. The apparent disparity between the results of the current studies regarding feminization compared with some other reports of endocrine active chemical studies may be due to differences in sampling technique, sample size, dose, age, method or duration of exposure, and potentially different modes of action and effects of specific chemicals.

Also, consistent with the results of the reproductive dose range-finding study, a mild degree of mineralization of renal tubules, or nephrocalcinosis, was observed in males, with the increase confined to the continuously exposed F_1 and F_2 generations at an exposure concentration of 50 ppb. Nephrocalcinosis is a gender-related lesion common in untreated female rats, and its occurrence is influenced by diet composition (Ritskes-Hoitinga and Beynen, 1992). This lesion has been reported to be induced by estrogen treatment in males (Ritskes-Hoitinga and Beynen, 1992). On the other hand, treatment-related increased incidences of nephrocalcinosis in males were noted after dietary administration of 17β-estradiol or 17α-ethinyl estradiol to rats (Schardein, 1980; Biegel *et al.,* 1998), and this response could be modulated by the base diet used in the various studies.

Summary

Ethinyl estradiol administered at exposure concentrations of 2, 10, or 50 ppb in a low phytoestrogen diet to NCTR CD (Sprague-Dawley) rats showed clear biological activity including potentially adverse effects. Both preweaning and postweaning body weights of males and females were decreased during periods of direct exposure to dosed feed. Ethinyl estradiol accelerated the attainment of puberty of females under continuous exposure conditions (F_1 and F_2) and of animals where dosing was terminated at weaning (F_3). Perturbation of the estrous cycle (prolonged cycles, aberrant cycles, time in estrus) in young females after vaginal opening and prior to mating was observed in the F_1 and F_2 generations. In males, statistically significant inductions of male mammary gland hyperplasia (F_0 through F_3 generations) and mild mineralization of renal tubules (F_1 and F_2 generations) were observed. The majority of these effects were observed at 50 ppb, but significant effects on body weight reduction and male mammary gland hyperplasia were observed at the lowest exposure concentration (2 ppb). With the possible exception of a 1.5-day delay of preputial separation in the F_2 males, effects of ethinyl estradiol did not appear to be magnified across exposed generations.

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APPENDIX A
SUMMARY OF LESIONS IN MALE RATS IN THE MULTIGENERATIONAL REPRODUCTIVE TOXICOLOGY FEED STUDY OF ETHINYL ESTRADIOL

TABLE A1a Summary of the Incidence of Neoplasms in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 104

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TABLE A2a Summary of the Incidence of Nonneoplastic Lesions in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 109

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Table A1a
Summary of the Incidence of Neoplasms in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary[a]				
Animals initially in study	25	25	25	26
Early deaths				
Natural deaths	1			1
Survivors				
Terminal sacrifice	24	25	25	25
Animals examined microscopically	25	25	25	26

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

[a] Animals initially in study refers to either the original breeders (F_0 animals) assigned to the study from the NCTR breeding colony or, for subsequent generations, animals that were born into the study. Pups were randomly selected for continuation on the study and were necropsied in pathology if they survived to terminal sacrifice or died or became moribund prior to scheduled necropsy.

Table A1b
Summary of the Incidence of Neoplasms in F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	26
Early deaths				
Moribund				1
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	26

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

Table A1c
Summary of the Incidence of Neoplasms in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

Table A1d
Summary of the Incidence of Neoplasms in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppm	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

Table A1e
Summary of the Incidence of Neoplasms in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	26	25
Early deaths				
Moribund			1	
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	26	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

Table A2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		26	
Early deaths								
Natural deaths	1						1	
Survivors								
Terminal sacrifice	24		25		25		25	
Animals examined microscopically	25		25		25		26	
Alimentary System								
Liver	(24)		(1)		(2)		(25)	
Basophilic focus							1	(4%)
Developmental malformation			1	(100%)				
Hepatodiaphragmatic nodule	1	(4%)			2	(100%)		
Infiltration cellular, lymphocyte	6	(25%)					8	(32%)
Inflammation, chronic active	3	(13%)					4	(16%)
Pancreas	(1)		(0)		(0)		(0)	
Acinus, degeneration	1	(100%)						
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(24)		(0)		(0)		(26)	
Vacuolization cytoplasmic	1	(4%)						
Pituitary gland	(24)		(0)		(0)		(25)	
Pars distalis, cyst							2	(8%)
Thyroid gland	(24)		(0)		(0)		(25)	
Cyst, squamous, multiple							1	(4%)
Cyst, squamous	4	(17%)					3	(12%)
General Body System								
None								
Genital System								
Coagulating gland	(24)		(25)		(25)		(24)	
Developmental malformation	7	(29%)	7	(28%)	8	(32%)	8	(33%)
Bilateral, developmental malformation	3	(13%)	1	(4%)				
Epididymis	(25)		(25)		(25)		(26)	
Atrophy	1	(4%)						
Hypospermia	1	(4%)						
Infiltration cellular, lymphocyte	2	(8%)	3	(12%)			1	(4%)
Preputial gland	(0)		(2)		(3)		(2)	
Infiltration cellular, lymphocyte					1	(33%)		
Inflammation, suppurative			2	(100%)	2	(67%)	1	(50%)
Duct, dilatation			1	(50%)	2	(67%)	1	(50%)
Prostate, dorsal/lateral lobe	(24)		(25)		(25)		(26)	
Infiltration cellular, lymphocyte	3	(13%)	1	(4%)	4	(16%)	1	(4%)
Inflammation, suppurative	2	(8%)	4	(16%)	4	(16%)	8	(31%)

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

Table A2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Prostate, ventral lobe	(24)		(25)		(25)		(26)	
Infiltration cellular, lymphocyte	20	(83%)	18	(72%)	19	(76%)	21	(81%)
Rete testes	(25)		(23)		(24)		(25)	
Dilatation	1	(4%)	1	(4%)	1	(4%)		
Seminal vesicle	(24)		(25)		(25)		(25)	
Depletion secretory	3	(13%)	2	(8%)	1	(4%)	2	(8%)
Testes	(25)		(25)		(25)		(26)	
Seminiferous tubule, degeneration	6	(24%)	2	(8%)	6	(24%)	1	(4%)
Hematopoietic System								
Spleen	(24)		(0)		(0)		(25)	
Hyperplasia, lymphoid	1	(4%)					1	(4%)
Pigmentation	2	(8%)					7	(28%)
Integumentary System								
Mammary gland	(24)		(24)		(25)		(25)	
Alveolus, dilatation					1	(4%)		
Alveolus, hyperplasia	1	(4%)	2	(8%)	5	(20%)	10	(40%)
Duct, hyperplasia	2	(8%)	1	(4%)	2	(8%)	2	(8%)
Skin	(1)		(1)		(0)		(1)	
Hyperkeratosis			1	(100%)				
Epidermis, hyperplasia			1	(100%)				
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
Eye	(1)		(0)		(0)		(0)	
Autolysis	1	(100%)						
Urinary System								
Kidney	(24)		(4)		(0)		(25)	
Hyaline droplet	2	(8%)					6	(24%)
Infiltration cellular, lymphocyte	17	(71%)	2	(50%)			19	(76%)
Cortex, cyst	4	(17%)	1	(25%)			4	(16%)
Interstitium, fibrosis	2	(8%)	1	(25%)			1	(4%)
Pelvis, dilatation							1	(4%)
Renal tubule, degeneration	2	(8%)	2	(50%)			3	(12%)
Renal tubule, dilatation	6	(25%)	1	(25%)			9	(36%)
Renal tubule, mineralization	1	(4%)					1	(4%)
Renal tubule, regeneration	10	(42%)	2	(50%)			12	(48%)

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		26	
Early death								
Moribund							1	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		26	
Alimentary System								
Liver	(25)		(1)		(1)		(26)	
Hepatodiaphragmatic nodule			1	(100%)	1	(100%)	3	(12%)
Infiltration cellular, lymphocyte	5	(20%)						
Inflammation, chronic active	6	(24%)					10	(38%)
Vacuolization cytoplasmic							1	(4%)
Mesentery	(1)		(0)		(1)		(0)	
Fat, necrosis	1	(100%)			1	(100%)		
Oral mucosa	(0)		(0)		(0)		(1)	
Abscess							1	(100%)
Cardiovascular System								
Heart	(0)		(0)		(0)		(1)	
Cardiomyopathy							1	(100%)
Endocrine System								
Adrenal cortex	(25)		(0)		(0)		(26)	
Vacuolization cytoplasmic	3	(12%)					1	(4%)
Pituitary gland	(24)		(0)		(0)		(26)	
Pars distalis, cyst							1	(4%)
Pars distalis, cyst, multiple							2	(8%)
Thyroid gland	(25)		(0)		(0)		(26)	
Cyst, squamous, multiple	1	(4%)					1	(4%)
Cyst, squamous	2	(8%)						
General Body System								
None								
Genital System								
Coagulating gland	(25)		(24)		(25)		(26)	
Developmental malformation	4	(16%)	3	(13%)	2	(8%)	4	(15%)
Bilateral, developmental malformation	2	(8%)					1	(4%)
Epididymis	(25)		(25)		(25)		(26)	
Atrophy			3	(12%)	1	(4%)		
Hypospermia			3	(12%)	1	(4%)	1	(4%)
Infiltration cellular, lymphocyte	2	(8%)	2	(8%)	5	(20%)		
Epithelium, degeneration					1	(4%)		

Table A2b
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Preputial gland	(3)		(2)		(3)		(1)	
Atrophy	2	(67%)	1	(50%)	1	(33%)		
Infiltration cellular, lymphocyte			1	(50%)				
Inflammation, suppurative	1	(33%)	1	(50%)				
Bilateral, atrophy					1	(33%)		
Prostate, dorsal/lateral lobe	(25)		(25)		(25)		(26)	
Infiltration cellular, lymphocyte	3	(12%)	1	(4%)	5	(20%)		
Inflammation, suppurative	6	(24%)	2	(8%)	5	(20%)	4	(15%)
Prostate, ventral lobe	(25)		(25)		(25)		(26)	
Infiltration cellular, lymphocyte	17	(68%)	16	(64%)	19	(76%)	17	(65%)
Inflammation, suppurative	2	(8%)	1	(4%)				
Rete testes	(25)		(23)		(24)		(25)	
Dilatation			4	(17%)	2	(8%)		
Seminal vesicle	(25)		(25)		(25)		(26)	
Depletion secretory	2	(8%)			1	(4%)		
Testes	(25)		(25)		(25)		(26)	
Seminiferous tubule, degeneration	2	(8%)	5	(20%)	3	(12%)	2	(8%)
Hematopoietic System								
Spleen	(25)		(0)		(0)		(26)	
Hematopoietic cell proliferation	1	(4%)						
Hyperplasia, lymphoid							1	(4%)
Pigmentation	1	(4%)					4	(15%)
Integumentary System								
Mammary gland	(25)		(24)		(25)		(26)	
Alveolus, hyperplasia	1	(4%)	7	(29%)	11	(44%)	10	(38%)
Duct, hyperplasia			1	(4%)	5	(20%)	10	(38%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								

TABLE A2b
Summary of the Incidence of Nonneoplastic Lesions in F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Urinary System								
Kidney	(25)		(2)		(25)		(26)	
Congestion	2	(8%)	1	(50%)			2	(8%)
Developmental malformation			1	(50%)				
Hyaline droplet	4	(16%)			1	(4%)	5	(19%)
Infiltration cellular, lymphocyte	18	(72%)	1	(50%)	12	(48%)	17	(65%)
Inflammation, chronic	1	(4%)			1	(4%)	1	(4%)
Cortex, cyst	4	(16%)	1	(50%)	3	(12%)	1	(4%)
Cortex, cyst, multiple							1	(4%)
Interstitium, fibrosis	4	(16%)					1	(4%)
Renal tubule, degeneration							1	(4%)
Renal tubule, dilatation	9	(36%)			6	(24%)	11	(42%)
Renal tubule, hyperplasia					1	(4%)	1	(4%)
Renal tubule, mineralization							9	(35%)
Renal tubule, regeneration	9	(36%)			4	(16%)	13	(50%)

Table A2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Intestine small, ileum	(0)		(1)		(0)		(0)	
Hyperplasia, lymphoid			1	(100%)				
Liver	(25)		(1)		(1)		(25)	
Erythrophagocytosis							1	(4%)
Hematopoietic cell proliferation							1	(4%)
Hepatodiaphragmatic nodule			1	(100%)	1	(100%)	1	(4%)
Infiltration cellular, lymphocyte	3	(12%)					1	(4%)
Inflammation, chronic active	10	(40%)					12	(48%)
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(25)		(0)		(0)		(25)	
Vacuolization cytoplasmic	1	(4%)						
Pituitary gland	(23)		(0)		(0)		(25)	
Pars distalis, cyst, multiple	1	(4%)						
Pars distalis, hypertrophy							1	(4%)
Thyroid gland	(24)		(0)		(0)		(25)	
Cyst, squamous, multiple							2	(8%)
Cyst, squamous	4	(17%)					3	(12%)
Ectopic thymus	1	(4%)						
General Body System								
None								
Genital System								
Coagulating gland	(25)		(23)		(25)		(25)	
Developmental malformation	8	(32%)	5	(22%)	8	(32%)	8	(32%)
Epididymis	(25)		(25)		(25)		(25)	
Atrophy	2	(8%)	2	(8%)	1	(4%)	1	(4%)
Hypospermia	3	(12%)	2	(8%)	1	(4%)	1	(4%)
Infiltration cellular, lymphocyte	1	(4%)	1	(4%)			1	(4%)
Preputial gland	(0)		(2)		(0)		(0)	
Inflammation, suppurative			2	(100%)				
Duct, dilatation			1	(50%)				
Prostate, dorsal/lateral lobe	(25)		(25)		(25)		(25)	
Infiltration cellular, lymphocyte	8	(32%)	10	(40%)	15	(60%)	10	(40%)
Inflammation, suppurative	4	(16%)	1	(4%)	2	(8%)	3	(12%)
Prostate, ventral lobe	(25)		(25)		(25)		(25)	
Infiltration cellular, lymphocyte	9	(36%)	8	(32%)	8	(32%)	11	(44%)
Inflammation, suppurative			4	(16%)	1	(4%)	4	(16%)

Table A2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Rete testes	(24)		(24)		(23)		(25)	
Dilatation	2	(8%)			1	(4%)	1	(4%)
Seminal vesicle	(25)		(25)		(25)		(25)	
Depletion secretory	1	(4%)	1	(4%)			1	(4%)
Testes	(25)		(25)		(25)		(25)	
Seminiferous tubule, degeneration	4	(16%)	3	(12%)	2	(8%)	1	(4%)
Hematopoietic System								
Bone marrow	(25)		(0)		(0)		(25)	
Erythroid cell, hyperplasia							1	(4%)
Myeloid cell, hyperplasia	2	(8%)						
Spleen	(25)		(0)		(0)		(25)	
Hematopoietic cell proliferation	1	(4%)					1	(4%)
Pigmentation	1	(4%)					4	(16%)
Thymus	(25)		(1)		(0)		(25)	
Hemorrhage	1	(4%)	1	(100%)				
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Alveolus, hyperplasia	3	(12%)	3	(12%)	7	(28%)	13	(52%)
Duct, hyperplasia			5	(20%)	8	(32%)	12	(48%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								
Urinary System								
Kidney	(25)		(0)		(25)		(25)	
Casts protein	1	(4%)						
Hyaline droplet	7	(28%)			1	(4%)	8	(32%)
Infiltration cellular, lymphocyte	21	(84%)			7	(28%)	21	(84%)
Inflammation, chronic	1	(4%)			1	(4%)		
Cortex, cyst	4	(16%)			2	(8%)	4	(16%)
Interstitium, fibrosis							1	(4%)
Pelvis, dilatation					2	(8%)		
Renal tubule, degeneration					1	(4%)	1	(4%)
Renal tubule, dilatation	5	(20%)			2	(8%)	8	(32%)
Renal tubule, mineralization	1	(4%)					10	(40%)
Renal tubule, regeneration	12	(48%)			6	(24%)	10	(40%)

Table A2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Liver	(25)		(0)		(1)		(25)	
Hepatodiaphragmatic nodule					1	(100%)		
Infiltration cellular, lymphocyte	4	(16%)					3	(12%)
Inflammation, chronic active	9	(36%)					5	(20%)
Hepatocyte, vacuolization cytoplasmic							1	(4%)
Mesentery	(0)		(0)		(0)		(1)	
Inflammation, granulomatous							1	(100%)
Fat, necrosis							1	(100%)
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(25)		(0)		(0)		(25)	
Vacuolization cytoplasmic	2	(8%)					2	(8%)
Pituitary gland	(25)		(0)		(0)		(25)	
Pars distalis, cyst	1	(4%)					1	(4%)
Thyroid gland	(25)		(0)		(0)		(25)	
Cyst, squamous, multiple							1	(4%)
Cyst, squamous	4	(16%)					4	(16%)
Infiltration cellular, lymphocyte	1	(4%)					1	(4%)
General Body System								
None								
Genital System								
Coagulating gland	(25)		(25)		(25)		(25)	
Developmental malformation	2	(8%)	7	(28%)	5	(20%)	8	(32%)
Bilateral, developmental malformation			1	(4%)	1	(4%)	1	(4%)
Epididymis	(25)		(25)		(25)		(25)	
Atrophy			3	(12%)	1	(4%)	1	(4%)
Hypospermia			3	(12%)	1	(4%)	1	(4%)
Infiltration cellular, lymphocyte	1	(4%)	2	(8%)	1	(4%)		
Preputial gland	(0)		(0)		(2)		(1)	
Inflammation, suppurative					1	(50%)	1	(100%)
Duct, dilatation					1	(50%)	1	(100%)
Prostate, dorsal/lateral lobe	(25)		(25)		(25)		(25)	
Infiltration cellular, lymphocyte	2	(8%)	3	(12%)			1	(4%)
Inflammation, suppurative	7	(28%)	9	(36%)	8	(32%)	8	(32%)

Table A2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Prostate, ventral lobe	(25)		(25)		(25)		(25)	
Infiltration cellular, lymphocyte	17	(68%)	17	(68%)	14	(56%)	16	(64%)
Inflammation, suppurative	1	(4%)					1	(4%)
Rete testes	(25)		(24)		(25)		(25)	
Dilatation			3	(13%)			2	(8%)
Seminal vesicle	(25)		(25)		(25)		(25)	
Depletion secretory					1	(4%)	1	(4%)
Testes	(25)		(25)		(25)		(25)	
Seminiferous tubule, degeneration	2	(8%)	5	(20%)	2	(8%)	1	(4%)
Hematopoietic System								
Bone marrow	(25)		(0)		(0)		(25)	
Myeloid cell, hyperplasia	1	(4%)					2	(8%)
Spleen	(25)		(0)		(0)		(25)	
Hyperplasia, lymphoid	1	(4%)					2	(8%)
Pigmentation	1	(4%)					3	(12%)
Thymus	(24)		(0)		(0)		(25)	
Congestion	1	(4%)						
Integumentary System								
Mammary gland	(25)		(25)		(24)		(24)	
Alveolus, hyperplasia	2	(8%)	5	(20%)	4	(17%)	7	(29%)
Duct, hyperplasia	1	(4%)	1	(4%)	3	(13%)	1	(4%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								
Urinary System								
Kidney	(25)		(0)		(2)		(25)	
Casts protein							1	(4%)
Congestion	2	(8%)			2	(100%)		
Hyaline droplet	2	(8%)					2	(8%)
Infiltration cellular, lymphocyte	21	(84%)					19	(76%)
Bilateral, pelvis, dilatation	1	(4%)						
Interstitium, fibrosis							2	(8%)
Renal tubule, dilatation	3	(12%)					6	(24%)
Renal tubule, regeneration	10	(40%)					9	(36%)

Table A2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		26		25	
Early death								
Moribund					1			
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		26		25	
Alimentary System								
Liver	(25)		(1)		(2)		(25)	
Cyst	1	(4%)						
Hepatodiaphragmatic nodule					1	(50%)		
Infiltration cellular, lymphocyte	1	(4%)			1	(50%)	1	(4%)
Inflammation, chronic active	13	(52%)					10	(40%)
Vacuolization cytoplasmic							1	(4%)
Bile duct, hyperplasia					1	(50%)	1	(4%)
Hepatocyte, degeneration					1	(50%)		
Hepatocyte, necrosis					1	(50%)		
Pancreas	(0)		(0)		(1)		(0)	
Infiltration cellular, lymphocyte					1	(100%)		
Acinar cell, degeneration					1	(100%)		
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(25)		(0)		(1)		(25)	
Accessory adrenal cortical nodule	1	(4%)						
Vacuolization cytoplasmic	4	(16%)					2	(8%)
Pituitary gland	(25)		(0)		(2)		(25)	
Pars distalis, cyst					1	(50%)	2	(8%)
Thyroid gland	(25)		(0)		(0)		(25)	
Cyst, squamous	6	(24%)					4	(16%)
General Body System								
None								
Genital System								
Coagulating gland	(25)		(25)		(26)		(24)	
Developmental malformation	9	(36%)	9	(36%)	6	(23%)	10	(42%)
Hypoplasia					1	(4%)		
Bilateral, developmental malformation	1	(4%)					1	(4%)
Epididymis	(25)		(25)		(26)		(25)	
Atrophy							1	(4%)
Hypoplasia					1	(4%)		
Hypospermia							1	(4%)
Preputial gland	(0)		(2)		(1)		(0)	
Inflammation, suppurative			2	(100%)	1	(100%)		
Duct, dilatation			2	(100%)				

Table A2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Prostate, dorsal/lateral lobe	(25)		(25)		(26)		(25)	
Hypoplasia					1	(4%)		
Inflammation, suppurative	6	(24%)	7	(28%)	9	(35%)	6	(24%)
Prostate, ventral lobe	(25)		(25)		(26)		(25)	
Hypoplasia					1	(4%)		
Infiltration cellular, lymphocyte	14	(56%)	9	(36%)	13	(50%)	16	(64%)
Inflammation, suppurative	1	(4%)	1	(4%)				
Rete testes	(25)		(24)		(26)		(25)	
Dilatation							1	(4%)
Seminal vesicle	(25)		(25)		(26)		(25)	
Hypoplasia					1	(4%)		
Testes	(25)		(25)		(26)		(25)	
Hypoplasia					1	(4%)		
Seminiferous tubule, degeneration	1	(4%)	3	(12%)			1	(4%)
Hematopoietic System								
Spleen	(25)		(0)		(1)		(25)	
Congestion					1	(100%)		
Hyperplasia, lymphoid					1	(100%)	1	(4%)
Thymus	(25)		(0)		(1)		(25)	
Atrophy					1	(100%)		
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Alveolus, hyperplasia	3	(12%)	5	(20%)	5	(20%)	7	(28%)
Duct, hyperplasia	1	(4%)			2	(8%)		
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
Lung	(0)		(0)		(1)		(0)	
Infiltration cellular, histiocyte					1	(100%)		
Peribronchiolar, infiltration cellular, lymphocyte					1	(100%)		
Special Senses System								
None								

Table A2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb	10 ppb		50 ppb	
Urinary System							
Kidney	(25)		(0)	(1)		(25)	
Hyaline droplet						4	(16%)
Infiltration cellular, lymphocyte	14	(56%)		1	(100%)	13	(52%)
Inflammation, chronic	1	(4%)					
Cortex, cyst	1	(4%)				4	(16%)
Interstitium, fibrosis				1	(100%)	2	(8%)
Pelvis, epithelium, hyperplasia						1	(4%)
Renal tubule, dilatation	2	(8%)				5	(20%)
Renal tubule, mineralization						1	(4%)
Renal tubule, regeneration	8	(32%)				11	(44%)

APPENDIX B
SUMMARY OF LESIONS IN FEMALE RATS IN THE MULTIGENERATIONAL REPRODUCTIVE TOXICOLOGY FEED STUDY OF ETHINYL ESTRADIOL

TABLE B1a Summary of the Incidence of Neoplasms in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 122
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TABLE B1a
Summary of the Incidence of Neoplasms in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary[a]				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

[a] Animals initially in study refers to either the original breeders (F_0 animals) assigned to the study from the NCTR breeding colony or, for subsequent generations, animals that were born into the study. Pups were randomly selected for continuation on the study and were necropsied in pathology if they survived to terminal sacrifice or died or became moribund prior to scheduled necropsy.

Table B1b
Summary of the Incidence of Neoplasms in F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE B1c
Summary of the Incidence of Neoplasms in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Systems Examined with No Neoplasms Observed				
Alimentary System				
Cardiovascular System				
Endocrine System				
General Body System				
Genital System				
Hematopoietic System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				
Urinary System				
Integumentary System				
Mammary gland	(25)	(25)	(25)	(25)
Adenoma	1 (4%)			
Neoplasm Summary				
Total animals with primary neoplasms[b]	1			
Total primary neoplasms	1			
Total animals with benign neoplasms	1			
Total benign neoplasms	1			

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Primary neoplasms: all neoplasms except metastatic neoplasms

Table B1d
Summary of the Incidence of Neoplasms in F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	25	25
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25

Systems Examined with No Neoplasms Observed

Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

TABLE B1e
Summary of the Incidence of Neoplasms in F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	0 ppb	2 ppb	10 ppb	50 ppb
Disposition Summary				
Animals initially in study	25	25	26	25
Early deaths				
Natural death			1	
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	26	25

Systems Examined with No Neoplasms Observed
Alimentary System
Cardiovascular System
Endocrine System
General Body System
Genital System
Hematopoietic System
Integumentary System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System
Urinary System

Systemic Lesions				
Multiple organs[b]	(25)	(25)	(26)	(25)
Lymphoma malignant			1 (4%)	
Neoplasm Summary				
Total animals with primary neoplasmsc			1	
Total primary neoplasms			1	
Total animals with malignant neoplasms			1	
Total malignant neoplasms			1	

[a] Number of animals examined microscopically at the site and the number of animals with neoplasm
[b] Number of animals with any tissue examined microscopically
[c] Primary neoplasms: all neoplasms except metastatic neoplasms

Table B2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Liver	(25)		(2)		(2)		(25)	
Developmental malformation	3	(12%)	1	(50%)	2	(100%)		
Fatty change, focal	1	(4%)						
Hepatodiaphragmatic nodule			1	(50%)				
Inflammation, chronic active	4	(16%)					3	(12%)
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(25)		(0)		(0)		(25)	
Accessory adrenal cortical nodule	1	(4%)						
Unilateral, accessory adrenal cortical nodule							1	(4%)
Pituitary gland	(25)		(0)		(0)		(25)	
Cyst	1	(4%)						
General Body System								
None								
Genital System								
Clitoral gland	(2)		(1)		(2)		(0)	
Distended	2	(100%)	1	(100%)	2	(100%)		
Inflammation, chronic active	2	(100%)	1	(100%)				
Ovary	(25)		(25)		(25)		(25)	
Cyst			1	(4%)	1	(4%)		
Diestrus	7	(28%)	6	(24%)	8	(32%)	6	(24%)
Estrus	8	(32%)	8	(32%)	6	(24%)	11	(44%)
Metestrus	4	(16%)	4	(16%)	3	(12%)	1	(4%)
Proestrus	6	(24%)	7	(28%)	8	(32%)	6	(24%)
Corpus luteum, cyst	1	(4%)	1	(4%)				
Corpus luteum, depletion							1	(4%)
Follicle, cyst					1	(4%)		
Follicle, cyst, multiple							1	(4%)
Oviduct	(24)		(25)		(25)		(25)	
Mucosa, hyperplasia			1	(4%)				

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

Table B2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Uterus	(25)		(25)		(25)		(25)	
Diestrus	7	(28%)	7	(28%)	9	(36%)	5	(20%)
Estrus	7	(28%)	7	(28%)	6	(24%)	12	(48%)
Metestrus	4	(16%)	4	(16%)	3	(12%)	1	(4%)
Proestrus	7	(28%)	7	(28%)	7	(28%)	7	(28%)
Endometrial glands, dilatation			1	(4%)				
Endometrial glands, hyperplasia					1	(4%)		
Vagina	(25)		(25)		(25)		(25)	
Diestrus	6	(24%)	5	(20%)	6	(24%)	6	(24%)
Estrus	6	(24%)	8	(32%)	6	(24%)	9	(36%)
Metestrus	7	(28%)	5	(20%)	3	(12%)	4	(16%)
Proestrus	6	(24%)	7	(28%)	10	(40%)	5	(20%)
Epithelium, hyperplasia							1	(4%)
Hematopoietic System								
Spleen	(25)		(0)		(0)		(25)	
Fibrosis, focal	1	(4%)						
Pigmentation	3	(12%)					6	(24%)
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Alveolus, hyperplasia	1	(4%)	4	(16%)	3	(12%)	4	(16%)
Skin	(2)		(3)		(1)		(0)	
Hyperkeratosis, focal			1	(33%)				
Inflammation, focal, chronic	1	(50%)						
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb	2 ppb	10 ppb	50 ppb
Urinary System				
Kidney	(25)	(25)	(25)	(25)
Infarct	1 (4%)	1 (4%)	1 (4%)	
Inflammation, chronic active		1 (4%)		
Nephropathy	2 (8%)			3 (12%)
Arteriole, nuclear alteration				1 (4%)
Cortex, cyst	3 (12%)	3 (12%)		
Renal tubule, degeneration, focal		1 (4%)		
Renal tubule, dilatation, focal			2 (8%)	
Renal tubule, mineralization	20 (80%)	22 (88%)	24 (96%)	17 (68%)

TABLE B2b
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Liver	(25)		(0)		(0)		(25)	
Basophilic focus, multiple							1	(4%)
Developmental malformation	1	(4%)					2	(8%)
Inflammation, chronic active	5	(20%)					1	(4%)
Cardiovascular System								
None								
Endocrine System								
Adrenal cortex	(25)		(0)		(0)		(25)	
Hypertrophy, focal							1	(4%)
Thyroid gland	(25)		(1)		(0)		(25)	
Ectopic thymus			1	(100%)				
Ultimobranchial cyst	2	(8%)					1	(4%)
General Body System								
None								
Genital System								
Clitoral gland	(1)		(2)		(0)		(1)	
Cyst							1	(100%)
Distended	1	(100%)	2	(100%)				
Inflammation, chronic active	1	(100%)	2	(100%)				
Ovary	(25)		(25)		(25)		(25)	
Asynchrony					1	(4%)		
Cyst					1	(4%)	1	(4%)
Diestrus	9	(36%)	6	(24%)	6	(24%)	11	(44%)
Estrus	4	(16%)	2	(8%)	6	(24%)	3	(12%)
Hyperplasia, sertoliform							3	(12%)
Metestrus	5	(20%)	7	(28%)	6	(24%)	3	(12%)
Proestrus	7	(28%)	10	(40%)	6	(24%)	8	(32%)
Corpus luteum, cyst	1	(4%)					1	(4%)
Follicle, cyst	1	(4%)						
Rete ovarii, dilatation							1	(4%)
Uterus	(25)		(25)		(25)		(25)	
Cyst					1	(4%)		
Diestrus	9	(36%)	6	(24%)	7	(28%)	11	(44%)
Estrus	4	(16%)	3	(12%)	6	(24%)	3	(12%)
Metestrus	5	(20%)	6	(24%)	6	(24%)	3	(12%)
Proestrus	7	(28%)	10	(40%)	6	(24%)	8	(32%)

Table B2b
Summary of the Incidence of Nonneoplastic Lesions in F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Vagina	(24)		(25)		(25)		(25)	
Diestrus	10	(42%)	6	(24%)	5	(20%)	11	(44%)
Estrus	4	(17%)	4	(16%)	6	(24%)	6	(24%)
Metestrus	4	(17%)	7	(28%)	8	(32%)	3	(12%)
Proestrus	6	(25%)	8	(32%)	6	(24%)	5	(20%)
Hematopoietic System								
Spleen	(25)		(0)		(0)		(25)	
Pigmentation	2	(8%)					2	(8%)
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Alveolus, hyperplasia	5	(20%)	6	(24%)	5	(20%)	5	(20%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
Eye	(0)		(0)		(1)		(1)	
Bilateral, cataract							1	(100%)
Cornea, edema					1	(100%)		
Urinary System								
Kidney	(25)		(25)		(25)		(25)	
Infarct			1	(4%)				
Nephropathy					1	(4%)		
Cortex, cyst			3	(12%)			6	(24%)
Pelvis, dilatation	1	(4%)	1	(4%)				
Renal tubule, mineralization	21	(84%)	18	(72%)	21	(84%)	17	(68%)

Table B2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Intestine small, ileum	(1)		(0)		(0)		(0)	
Peyer's patch, hyperplasia, lymphoid	1	(100%)						
Liver	(25)		(0)		(3)		(25)	
Clear cell focus							1	(4%)
Developmental malformation	2	(8%)			1	(33%)		
Infiltration cellular, mast cell, focal	1	(4%)						
Inflammation, chronic					1	(33%)		
Proliferation connective tissue, focal	1	(4%)			1	(33%)		
Cardiovascular System								
None								
Endocrine System								
Pituitary gland	(25)		(0)		(0)		(25)	
Cyst							1	(4%)
Thyroid gland	(25)		(0)		(0)		(25)	
Ultimobranchial cyst							1	(4%)
General Body System								
None								
Genital System								
Clitoral gland	(1)		(2)		(1)		(0)	
Abscess			1	(50%)				
Distended	1	(100%)	1	(50%)	1	(100%)		
Inflammation, chronic			1	(50%)				
Inflammation, chronic active	1	(100%)	1	(50%)	1	(100%)		
Ovary	(25)		(25)		(25)		(25)	
Cyst	1	(4%)			1	(4%)		
Diestrus	12	(48%)	14	(56%)	13	(52%)	8	(32%)
Estrus	5	(20%)	4	(16%)	5	(20%)	5	(20%)
Metestrus	2	(8%)	4	(16%)	2	(8%)	5	(20%)
Proestrus	6	(24%)	3	(12%)	5	(20%)	7	(28%)
Corpus luteum, cyst			1	(4%)				
Uterus	(25)		(25)		(25)		(25)	
Diestrus	10	(40%)	12	(48%)	11	(44%)	8	(32%)
Estrus	4	(16%)	4	(16%)	5	(20%)	5	(20%)
Metestrus	6	(24%)	6	(24%)	4	(16%)	5	(20%)
Proestrus	5	(20%)	3	(12%)	5	(20%)	7	(28%)

Table B2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Genital System (continued)								
Vagina	(25)		(25)		(25)		(23)	
Diestrus	11	(44%)	12	(48%)	10	(40%)	7	(30%)
Estrus	6	(24%)	4	(16%)	5	(20%)	7	(30%)
Metestrus	3	(12%)	6	(24%)	5	(20%)	4	(17%)
Proestrus	5	(20%)	3	(12%)	5	(20%)	5	(22%)
Mucocyte, hyperplasia			2	(8%)				
Hematopoietic System								
Spleen	(25)		(0)		(0)		(25)	
Pigmentation	1	(4%)					1	(4%)
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Alveolus, hyperplasia	14	(56%)	15	(60%)	12	(48%)	11	(44%)
Lobules, hyperplasia	7	(28%)	10	(40%)	13	(52%)	13	(52%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								
Urinary System								
Kidney	(25)		(25)		(25)		(25)	
Infarct					2	(8%)		
Inflammation, chronic active			1	(4%)			1	(4%)
Nephropathy					1	(4%)		
Cortex, cyst	3	(12%)	1	(4%)	3	(12%)	2	(8%)
Renal tubule, mineralization	18	(72%)	23	(92%)	20	(80%)	15	(60%)

TABLE B2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		25		25	
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		25		25	
Alimentary System								
Intestine small, jejunum	(0)		(1)		(0)		(0)	
Peyer's patch, hyperplasia			1	(100%)				
Liver	(25)		(0)		(0)		(25)	
Developmental malformation	1	(4%)						
Inflammation, chronic active							2	(8%)
Cardiovascular System								
None								
Endocrine System								
Pituitary gland	(25)		(0)		(0)		(25)	
Cyst	2	(8%)						
Thyroid gland	(25)		(0)		(0)		(25)	
Keratin cyst							2	(8%)
General Body System								
None								
Genital System								
Clitoral gland	(0)		(3)		(0)		(0)	
Distended			1	(33%)				
Inflammation, chronic active			3	(100%)				
Ovary	(25)		(25)		(24)		(25)	
Cyst	2	(8%)						
Diestrus	10	(40%)	9	(36%)	3	(13%)	4	(16%)
Estrus	5	(20%)	4	(16%)	5	(21%)	6	(24%)
Hyperplasia, sertoliform					1	(4%)		
Metestrus	6	(24%)	5	(20%)	7	(29%)	4	(16%)
Proestrus	4	(16%)	7	(28%)	9	(38%)	11	(44%)
Corpus luteum, cyst	1	(4%)						
Uterus	(25)		(25)		(25)		(25)	
Diestrus	8	(32%)	9	(36%)	4	(16%)	3	(12%)
Estrus	5	(20%)	4	(16%)	5	(20%)	6	(24%)
Metestrus	8	(32%)	5	(20%)	6	(24%)	5	(20%)
Proestrus	4	(16%)	7	(28%)	10	(40%)	11	(44%)
Vagina	(25)		(25)		(25)		(25)	
Diestrus	10	(40%)	8	(32%)	3	(12%)	4	(16%)
Estrus	4	(16%)	4	(16%)	7	(28%)	5	(20%)
Metestrus	8	(32%)	6	(24%)	7	(28%)	5	(20%)
Proestrus	3	(12%)	7	(28%)	8	(32%)	11	(44%)
Mucocyte, hyperplasia			1	(4%)				

TABLE B2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System								
Bone marrow	(25)		(0)		(0)		(25)	
Pigmentation	1	(4%)						
Spleen	(25)		(0)		(0)		(25)	
Congestion	1	(4%)						
Pigmentation	4	(16%)					1	(4%)
Integumentary System								
Mammary gland	(25)		(25)		(25)		(25)	
Lactation	2	(8%)	1	(4%)	1	(4%)		
Alveolus, hyperplasia	9	(36%)	4	(16%)	11	(44%)	9	(36%)
Lobules, hyperplasia	7	(28%)	4	(16%)	6	(24%)	8	(32%)
Skin	(0)		(0)		(0)		(1)	
Inflammation, focal, chronic							1	(100%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
None								
Special Senses System								
None								
Urinary System								
Kidney	(25)		(25)		(25)		(25)	
Hydronephrosis					1	(4%)		
Infarct					2	(8%)		
Nephropathy, focal							1	(4%)
Cortex, cyst	3	(12%)	1	(4%)	3	(12%)	3	(12%)
Renal tubule, mineralization	19	(76%)	19	(76%)	18	(72%)	14	(56%)

TABLE B2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Disposition Summary								
Animals initially in study	25		25		26		25	
Early death								
Natural death					1			
Survivors								
Terminal sacrifice	25		25		25		25	
Animals examined microscopically	25		25		26		25	
Alimentary System								
Liver	(25)		(0)		(1)		(25)	
Developmental malformation	1	(4%)						
Pancreas	(0)		(0)		(1)		(0)	
Cardiovascular System								
None								
Endocrine System								
Thyroid gland	(25)		(0)		(1)		(25)	
Keratin cyst	1	(4%)					1	(4%)
Bilateral, keratin cyst	1	(4%)						
General Body System								
None								
Genital System								
Clitoral gland	(3)		(0)		(0)		(0)	
Distended	3	(100%)						
Inflammation, chronic active	3	(100%)						
Ovary	(25)		(25)		(26)		(25)	
Cyst	2	(8%)	1	(4%)				
Diestrus	6	(24%)	7	(28%)	9	(35%)	8	(32%)
Estrus	7	(28%)	11	(44%)	2	(8%)	2	(8%)
Metestrus	4	(16%)	3	(12%)	3	(12%)	6	(24%)
Proestrus	8	(32%)	4	(16%)	12	(46%)	9	(36%)
Corpus luteum, cyst							1	(4%)
Follicle, cyst	1	(4%)					1	(4%)
Uterus	(25)		(25)		(26)		(25)	
Diestrus	5	(20%)	6	(24%)	7	(27%)	7	(28%)
Estrus	7	(28%)	11	(44%)	2	(8%)	2	(8%)
Metestrus	5	(20%)	4	(16%)	5	(19%)	7	(28%)
Proestrus	8	(32%)	4	(16%)	12	(46%)	9	(36%)
Vagina	(25)		(25)		(26)		(25)	
Diestrus	5	(20%)	6	(24%)	7	(27%)	7	(28%)
Estrus	8	(32%)	7	(28%)	4	(15%)	5	(20%)
Metestrus	5	(20%)	8	(32%)	5	(19%)	5	(20%)
Proestrus	7	(28%)	4	(16%)	10	(38%)	8	(32%)

TABLE B2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

	0 ppb		2 ppb		10 ppb		50 ppb	
Hematopoietic System								
Bone marrow	(25)		(0)		(1)		(25)	
Lymph node	(0)		(0)		(1)		(0)	
Lymph node, mandibular	(0)		(0)		(1)		(0)	
Spleen	(25)		(0)		(2)		(25)	
Thymus	(25)		(0)		(1)		(25)	
Integumentary System								
Mammary gland	(25)		(25)		(26)		(25)	
Alveolus, hyperplasia	11	(44%)	14	(56%)	7	(27%)	9	(36%)
Lobules, hyperplasia	4	(16%)	10	(40%)	2	(8%)	4	(16%)
Musculoskeletal System								
None								
Nervous System								
None								
Respiratory System								
Lung	(0)		(0)		(1)		(0)	
Special Senses System								
None								
Urinary System								
Kidney	(25)		(25)		(26)		(25)	
Cyst					1	(4%)		
Nephropathy			1	(4%)	1	(4%)	2	(8%)
Cortex, cyst	2	(8%)					2	(8%)
Renal tubule, mineralization	24	(96%)	19	(76%)	19	(73%)	19	(76%)

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) which was used in the reproductive dose range-finding study and the multigenerational reproductive toxicology study. 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 studies 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 (EI) 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 17 α-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-NMR, ^{13}C-NMR, and MS spectra are presented in Figures C1, C2, and C3, respectively.

Before, during, and after the studies, the purity of lot 57H1178 was determined using ^{1}H-NMR (based on –CH groups), GC-EI MS, and/or 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 multigenerational reproductive toxicology 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 studies 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 studies 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 NIH-31 feed and contains casein in place of soy and alfalfa. 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 studies, native isoflavone content was determined for several lots of 5K96 feed using high-performance liquid chromatography (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 studies, an additional 27 consecutive lots of 5K96 feed were analyzed by two HPLC MS systems. 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, Torrance, CA). The column parameters were 250 mm $\times$ 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 to 80%A:20%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. 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 of this system 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 every 9 weeks or as needed by mixing ethinyl estradiol with feed (Table C2). For the 0, 1, 5, 25, 100, and 200 ppb dose formulations in the reproductive dose range-finding study and the 0, 10, and 50 ppb dose formulations in the multigenerational reproductive toxicology study, 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 0.1 ppb (reproductive dose range-finding study) and 2 ppb (multigenerational reproductive toxicology study) dose formulations were prepared by 1:10 and 1:5 dry dilutions, respectively, of the 1 and 10 ppb dose formulations previously prepared for the two studies. 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: the 1 and 5 ppb dose formulations were analyzed using GC-EI MS by system A (Table C1), the 10 and 50 ppb dose formulations were analyzed using GC with electron capture (EC) detection by system D, and the 200 ppb dose formulation was analyzed by HPLC-fluorescence. HPLC-fluorescence was performed on a Waters instrument (Waters Corporation, Milford, MA) 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 nm; emission 304 nm). Stability studies of the 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 using GC-EI MS by system A (reproductive dose range-finding study) or GC-EC by system D (multigenerational reproductive toxicology study). Because of the very low exposure concentrations utilized in these studies, 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 studies. For the reproductive dose range-finding study, specifications for the dose formulations were set as being within 50% of the target concentration with a coefficient of variation of ± 20%. For the multigenerational reproductive toxicology study, these specifications were set as being within 30% ± 20% of the target concentrations. Prior to and during the reproductive dose range-finding study, the dose formulations were analyzed approximately monthly (Table C3); all five of the dose formulations analyzed met the study specifications. During the multigenerational reproductive toxicology study, the dose formulations were generally analyzed every 6 weeks (Table C4). All 51 of the dose formulations analyzed and used in the study were within the study specifications. Periodic analysis of samples from the animal cage feeders confirmed that the animals were receiving the appropriate doses.

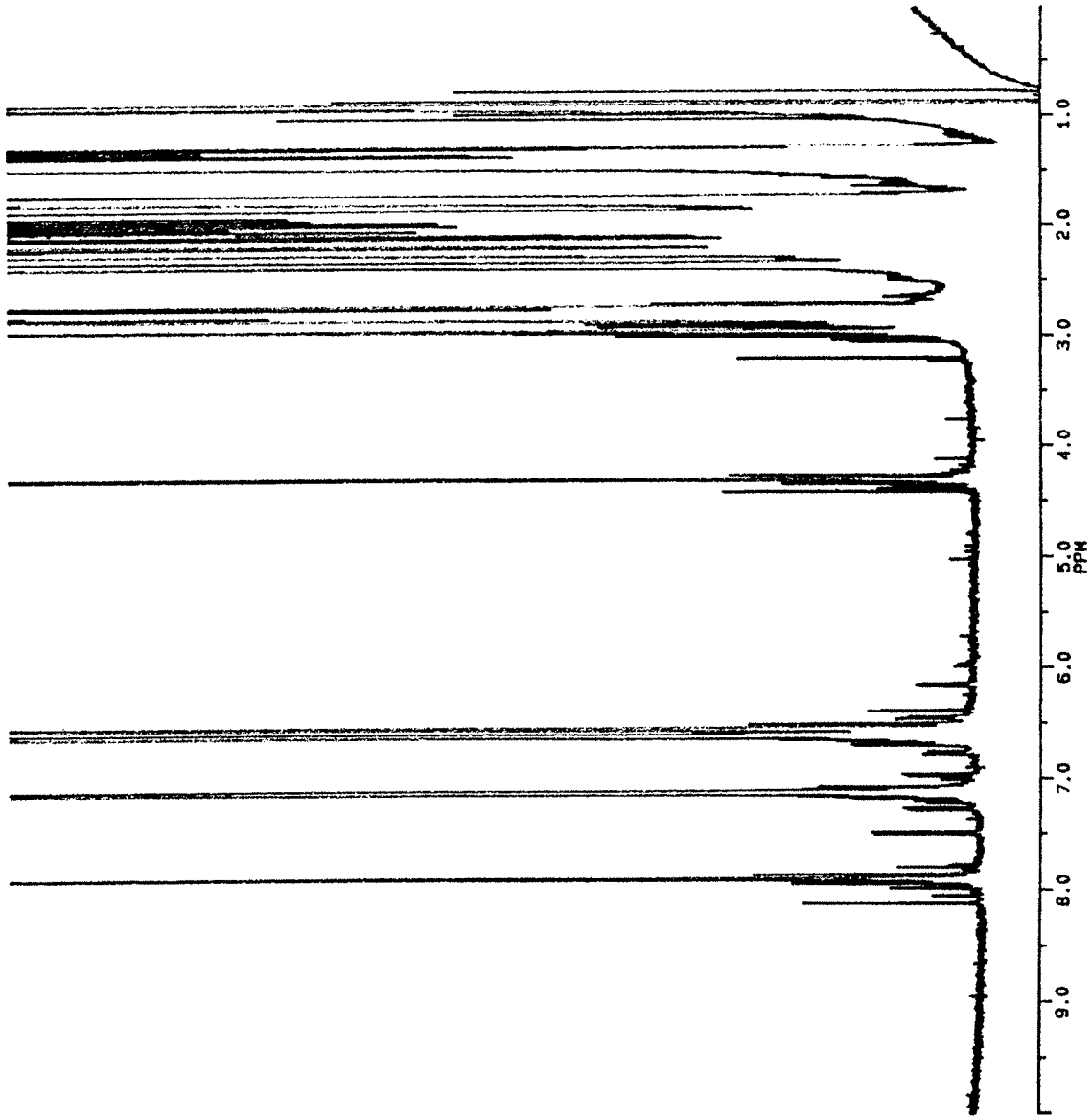

FIGURE C1
^{1}H-Nuclear Magnetic Resonance Spectrum of Ethinyl Estradiol

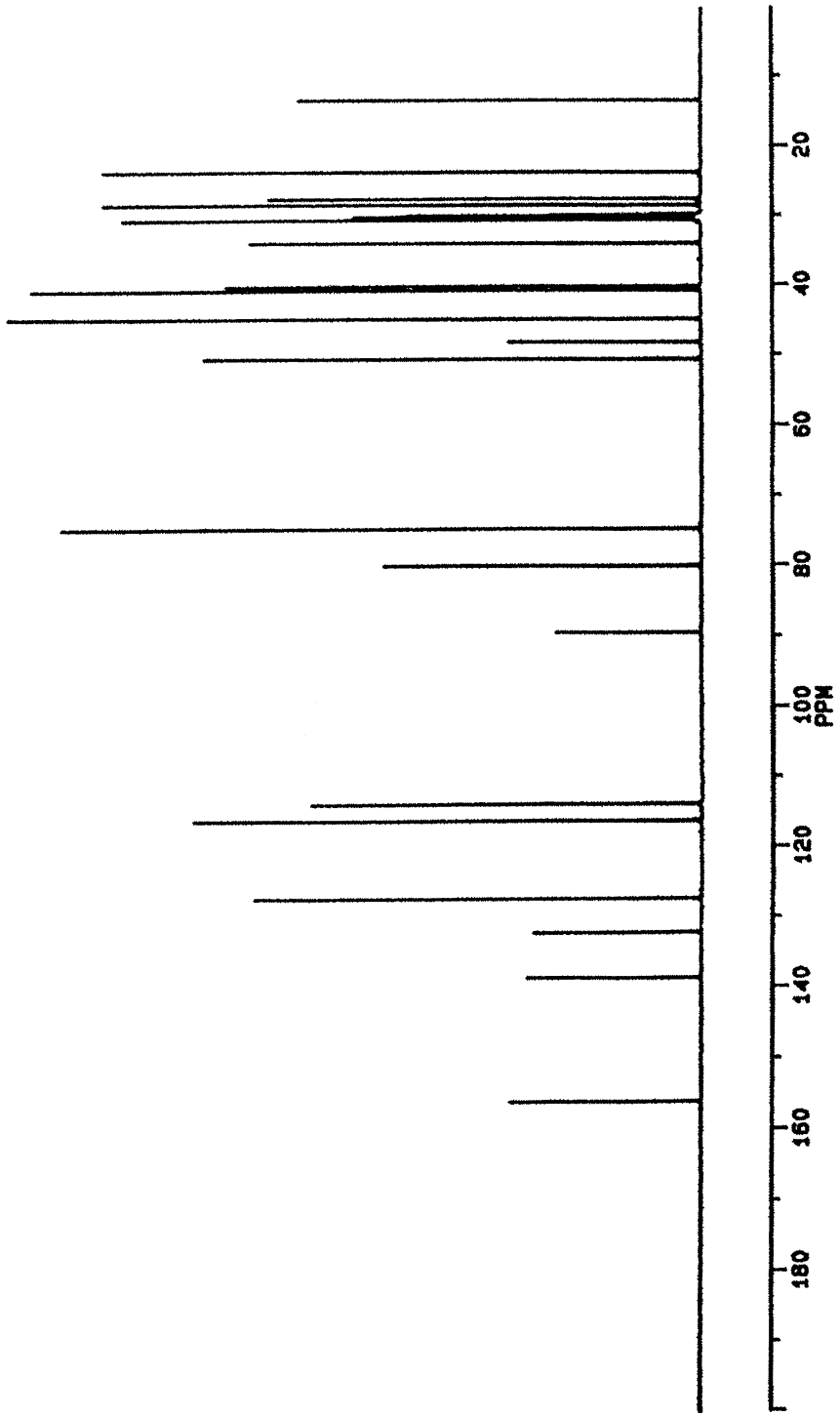

FIGURE C2
^{13}C- Nuclear Magnetic Resonance Spectrum of Ethinyl Estradiol

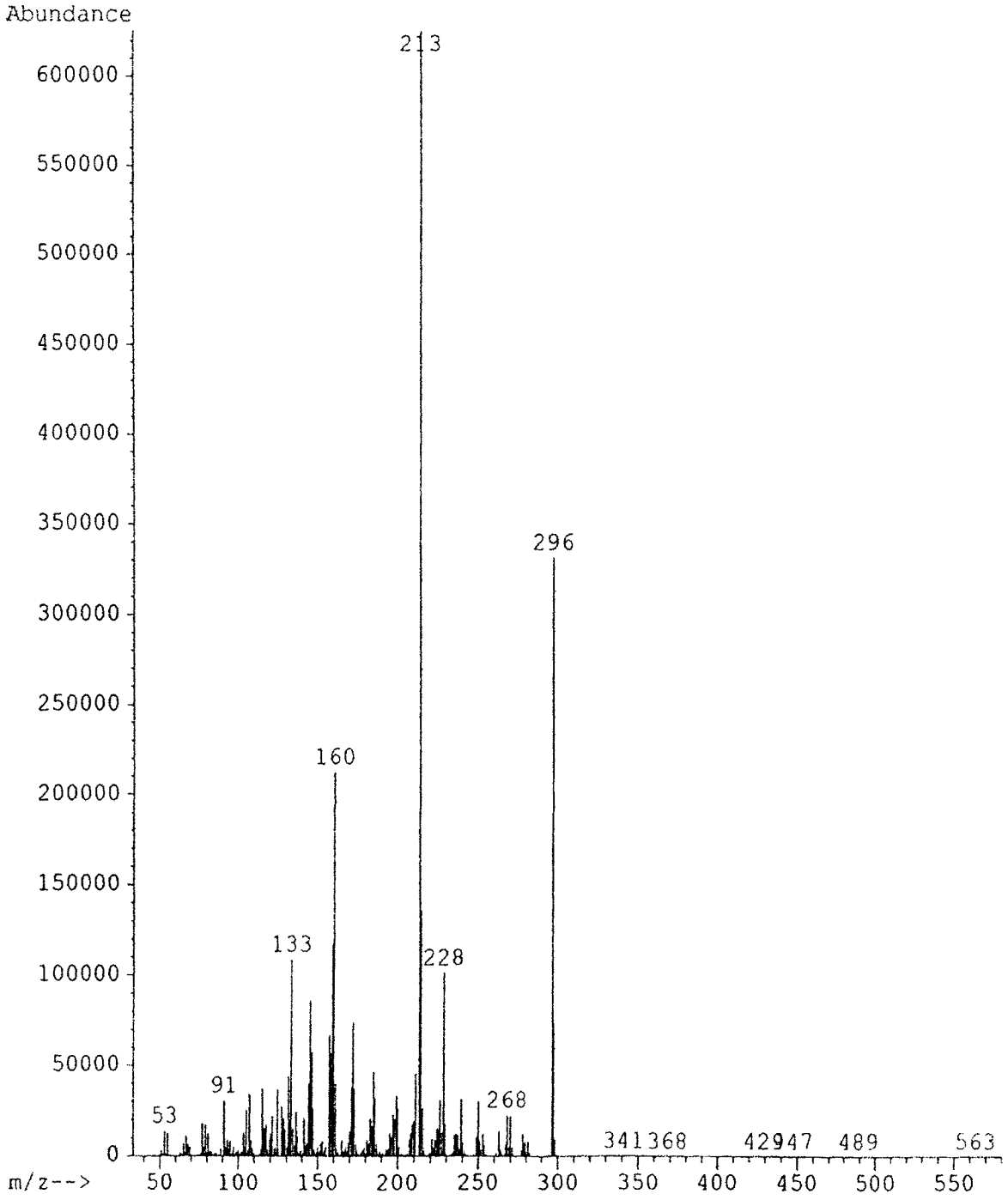

FIGURE C3
Mass Spectrum of Ethinyl Estradiol

TABLE C1
Gas Chromatography Systems Used in the Feed Studies 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° C 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° C 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 (Palo Alto, CA); 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 Feed Studies of Ethinyl Estradiol

Reproductive Dose Range-Finding Study	Multigenerational Reproductive Toxicology Study
Preparation	
Intermediate solutions were prepared by weighing the appropriate amounts of ethinyl estradiol and blending with 95% ethanol for the 0, 1, 5, 25, 100, and 200 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 0.1 ppb dose formulation, a 1:10 dry dilution was made by adding the appropriate amounts of 1 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 every 9 weeks or as needed.	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 every 9 weeks or as needed.
Chemical Lot Number	
57H1178	57H1178
Maximum Storage Time	
9 weeks	9 weeks
Storage Conditions	
Stainless steel cans with lids secured with tie downs at 4° C ± 2° C.	Same as Reproductive Dose Range-Finding Study
Study Laboratory	**Study Laboratory**
National Center for Toxicological Research (Jefferson, AR)	National Center for Toxicological Research (Jefferson, AR)

TABLE C3
Results of Analyses of Dose Formulations Administered to Rats in the Reproductive Dose Range-Finding Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
August 31, 1998	1	0.568 ± 0.08	–43
September 21, 1998	1	0.840 ± 0.13	–16
September 22, 1998	1	0.590 ± 0.08	–41
September 23, 1998	100	81.4 ± 15.2	–19
September 29, 1998	5	4.72 ± 0.22	–6

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

TABLE C4
Results of Analyses of Dose Formulations Administered to Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration[a] (ppb)	Difference from Target (%)
August 14, 2000	10	9.84 ± 1.0	–2
	50	48.3 ± 4.8	–3
August 29, 2000	2	1.7 ± 0.18	–15
	10	7.71 ± 0.34	–23
	10	10.99 ± 1.95	+10
September 7, 2000	10	10.23 ± 1.47	+2
October 25, 2000	10	9.7 ± 0.5	–3
	10	9.5 ± 0.013	–5
November 27, 2000	10	10.6 ± 0.8	+6
	10	11.6 ± 0.4	+16
November 28, 2000	10	12.6 ± 1.8	+26
	50	58.1 ± 4.6	+16
December 19, 2000	10	9.7 ± 0.3	–3
	10	11.6 ± 1.5	+16
December 20, 2000	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, 2001	10	9.5 ± 1.2	–5
	10	9.5 ± 1.1	–5
	10	9.4 ± 0.7	–6
January 23, 2001	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, 2001	10	9.3 ± 0.8	–7
March 6, 2001	10	8.7 ± 1.3	–13
	50	41.0 ± 7.8	–18

TABLE C4
Results of Analyses of Dose Formulations Administered to Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Date Prepared	Target Concentration (ppb)	Determined Concentration (ppb)	Difference from Target (%)
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, 2001	10	11.0 ± 1.7	+10
	10	10.8 ± 1.0	+8
April 4, 2001	50	45.7 ± 1.6	–9
April 24, 2001	10	10.1 ± 1.0	+1
April 25, 2001	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
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
Animal Room Samples [b]			
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

[a] Results of triplicate analyses (mean ± standard deviation)
[b] Results of quadruplicate analyses (mean ± standard deviation); dates shown are sampling dates

APPENDIX D
BODY WEIGHTS

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TABLE D1a
Postweaning Body Weights of F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
6*	160.4 ± 3.3	155.9 ± 2.9	158.6 ± 3.0	156.7 ± 3.6
7***	192.1 ± 3.8	188.8 ± 3.2	190.0 ± 3.9	172.5 ± 3.1***
8***	216.6 ± 4.8	212.2 ± 3.4	209.4 ± 3.5	190.3 ± 3.7***
9***	233.1 ± 5.1	227.5 ± 3.6	224.0 ± 3.8	203.5 ± 4.0***
10***	253.8 ± 5.7	239.6 ± 4.0*	236.9 ± 3.6***	215.2 ± 4.1***
11***,#	261.6 ± 5.8	246.1 ± 4.1*	240.3 ± 3.7***	215.4 ± 4.1***
12***	285.3 ± 5.9	276.7 ± 4.7	266.1 ± 3.9***	238.0 ± 3.9***
13***,#	318.5 ± 6.4	310.1 ± 4.6	296.9 ± 4.2**	266.3 ± 4.2***
16***	293.2 ± 6.3	285.7 ± 5.4	277.6 ± 5.5*	250.7 ± 3.4***
17***	292.0 ± 4.7	283.0 ± 3.8	279.1 ± 3.4*	257.2 ± 3.6***
18***	290.8 ± 5.3	284.8 ± 3.5	275.6 ± 3.4*	255.7 ± 3.5***
19***,##	302.4 ± 6.3	294.3 ± 4.0	281.5 ± 3.6***	260.2 ± 4.0*** (24)

TABLE D1a
Postweaning Body Weights of F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001.

[b] Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for that generation. Therefore, in order to conduct tests of generation effects within dose groups (results shown in Table D10), two sets of statistical analyses were conducted for females for the interval prior to delivery of their litters: the first included data from week 6 to the start of littering for all generations (F_0 to F_4), and the second included all data from birth to the start of littering for generations F_1 to F_4. The statistical results reported in this table for weeks 3, 4, and 5 are from the latter analysis, while results from weeks 6 to 13 are from the former analysis. All postweaning data (weaning on PND 21) are included in this table. Data from the weeks during which the dams were littering (weeks 14 and 15) were excluded from the analysis. Data from dams in the F_0 to F_4 generations after delivery of their litters (weeks 16 to 19) were analyzed separately, and those results are also reported in this table. Preweaning data (birth to PND 21) for females are tabulated separately (Table D2).

[c] Body weights were analyzed using a repeated measures approach to a mixed model ANOVA. The ANOVA results for each analysis were as follows:
1) Dam predelivery (weeks 6 to 13) body weights, F_0 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P<0.001; weeks × Generation, P<0.001; weeks × dose × Generation, P<0.001. Random effects of the F_0 breed father, the F_0 breed mother, and the interaction between the F_0 breed mother and F_0 breed father were significant at P<0.50 and were included in the model.
2) Dam predelivery (birth to week 13) body weights, F_1 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P<0.001; weeks × Generation, P<0.001; weeks × dose × Generation, P<0.001. No random effects for the F_0 birth parents were included in the statistical model.
3) Dam postdelivery (weeks 16-19) body weights, F_0 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P=0.356; weeks × Generation, P<0.001; weeks × dose × Generation, P=0.944. Random effects of the F_0 breed father, the F_0 breed mother, and the interaction between the F_0 breed mother and F_0 breed father were significant at P<0.50 and were included in the statistical model.

[d] Asterisks in the shaded cells in the age column indicate significant linear exposure concentration trends within a given week as determined by contrasts: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Pound signs indicate significant quadratic exposure concentration trend. #, P≤0.05; ##, P≤0.01.

TABLE D1b
Postweaning Body Weights of F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3***	40.3 ± 1.0	37.1 ± 1.0*	38.2 ± 0.9	34.7 ± 0.7***
4***	67.4 ± 1.7 (24)	64.2 ± 1.9	66.2 ± 1.4 (24)	58.8 ± 1.5***
5***	104.3 ± 2.4	100.0 ± 2.4	103.7 ± 2.3	93.9 ± 2.1**
6***	146.5 ± 2.9	142.5 ± 2.6	142.4 ± 3.1*	127.2 ± 2.7***
7***	173.3 ± 3.3	167.6 ± 2.8	166.3 ± 3.4**	150.4 ± 2.8***
8***	198.3 ± 3.8	192.4 ± 3.0	190.1 ± 3.6**	171.3 ± 3.1***
9***	217.7 ± 4.1	210.0 ± 3.5	206.7 ± 3.7**	184.9 ± 2.8***
10***	234.7 ± 4.5	228.1 ± 3.8	224.5 ± 3.5*	199.2 ± 3.1***
11***	241.8 ± 4.6	233.3 ± 3.8	232.5 ± 3.8*	204.1 ± 3.0***
12***	266.8 ± 5.3	260.0 ± 3.8	259.8 ± 4.5	224.5 ± 3.3***
13***	303.4 ± 6.7	294.2 ± 4.3	297.3 ± 5.4	256.0 ± 3.2***
16***	300.1 ± 5.2	291.3 ± 3.7	285.7 ± 3.8	260.0 ± 3.2***
17***	299.1 ± 5.7	287.7 ± 3.7	282.4 ± 4.6*	258.4 ± 3.8***
18***	282.6 ± 5.2	276.3 ± 4.6	272.4 ± 3.4	246.2 ± 3.1***
19***	292.5 ± 5.2	286.5 ± 3.3	279.4 ± 3.7*	251.7 ± 2.9***

The footnotes for this table are defined in Table D1a.

TABLE D1c
Postweaning Body Weights of F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3**	40.0 ± 0.9	39.9 ± 0.9	39.1 ± 0.8	36.3 ± 1.1*
4***	70.6 ± 1.9	71.1 ± 2.2	65.1 ± 1.8	57.6 ± 1.5***
5***	101.6 ± 1.8	103.7 ± 2.4	99.8 ± 2.3	90.3 ± 2.0***
6***	140.7 ± 2.6	144.8 ± 3.2*	138.2 ± 3.0	123.7 ± 2.5***
7***	167.5 ± 2.7	172.4 ± 3.3*	168.3 ± 3.5	150.1 ± 2.9***
8***	192.3 ± 2.8	193.1 ± 3.5	189.8 ± 3.6	171.0 ± 3.2***
9***	215.5 ± 3.1	219.9 ± 4.0	214.1 ± 3.9	189.8 ± 3.5***
10***	230.8 ± 3.3	233.5 ± 3.8	228.5 ± 3.9	204.5 ± 3.5***
11***	245.3 ± 3.4 (24)	250.8 ± 4.2	244.3 ± 4.4 (24)	217.0 ± 3.6***
12***	256.7 ± 4.3	260.6 ± 5.3	249.2 ± 4.2	222.6 ± 3.8***
13***	286.4 ± 5.3	290.2 ± 6.6	276.1 ± 4.4	244.9 ± 3.9***
16***	307.0 ± 3.5	310.9 ± 4.4	303.4 ± 5.0	265.1 ± 4.5***
17***	290.1 ± 4.4	294.4 ± 4.3	288.1 ± 3.8	256.9 ± 4.4***
18***	303.5 ± 5.1	307.3 ± 5.1	307.0 ± 4.1	272.9 ± 4.0***
19***	288.0 ± 3.0	291.3 ± 3.3	289.6 ± 4.6	258.3 ± 3.9***

The footnotes for this table are defined in Table D1a.

TABLE D1d
Postweaning Body Weights of F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3	41.0 ± 1.0	40.1 ± 0.8	41.6 ± 0.8	38.9 ± 0.9
4	70.6 ± 2.0	69.0 ± 1.8	66.3 ± 1.9	67.4 ± 1.6
5	102.7 ± 2.1	103.5 ± 1.9	99.7 ± 2.0	102.5 ± 2.0
6[#]	141.6 ± 2.2	142.1 ± 2.9	139.2 ± 2.4*	138.7 ± 2.5
7[#]	170.9 ± 2.3	171.3 ± 3.2	168.0 ± 2.8	169.7 ± 2.8
8	195.6 ± 2.5	196.6 ± 3.4	196.5 ± 3.0	197.3 ± 3.6
9	219.3 ± 2.8	219.8 ± 3.5	220.9 ± 3.5	220.4 ± 3.6
10	236.0 ± 3.2	233.7 ± 3.7	237.9 ± 3.9	238.2 ± 3.9
11	252.3 ± 3.6	248.4 ± 3.6	254.3 ± 4.0	255.1 ± 4.7
12	262.1 ± 3.4	259.5 ± 3.8	265.2 ± 4.1	263.7 ± 4.5
13	287.2 ± 3.4	285.5 ± 4.1	293.7 ± 4.1	294.3 ± 4.6
16	316.9 ± 5.5	312.0 ± 5.1	323.8 ± 5.3	321.0 ± 6.1
17	314.3 ± 4.3	308.9 ± 5.5	317.2 ± 4.8	317.8 ± 4.6
18	300.1 ± 5.2	300.0 ± 5.7	306.9 ± 4.5	303.7 ± 4.3
19	294.3 ± 3.8	292.3 ± 4.5	298.1 ± 3.8	298.7 ± 3.8

The footnotes for this table are defined in Table D1a.

TABLE D1e
Postweaning Body Weights of F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3	41.4 ± 0.9	41.7 ± 1.1	40.0 ± 0.9	40.2 ± 0.9
4	64.1 ± 2.2	68.3 ± 1.9	65.4 ± 2.0	66.4 ± 1.6
5	98.9 ± 2.2	102.7 ± 2.3	100.0 ± 2.4	96.6 ± 1.9
6**	136.2 ± 2.9	140.5 ± 2.3	139.6 ± 2.6	135.6 ± 2.4* (23)
7	163.5 ± 2.7	169.6 ± 2.5	169.2 ± 2.9	166.0 ± 3.8
8*	189.2 ± 2.9	193.6 ± 2.8	195.8 ± 3.3	188.8 ± 3.0
9	209.3 ± 3.0 (24)	214.6 ± 3.1	218.2 ± 3.4	214.3 ± 3.6
10#	226.4 ± 3.1	231.7 ± 3.4	237.6 ± 3.7	232.0 ± 3.8
11	240.8 ± 3.4	247.8 ± 3.8	248.3 ± 4.0	248.3 ± 4.0
12	255.2 ± 3.6	258.7 ± 3.8	265.7 ± 4.2	257.5 ± 4.3
13	283.4 ± 5.1	290.6 ± 4.4	295.2 ± 4.3	288.5 ± 4.7
16##	296.8 ± 3.9	315.1 ± 5.8*	319.5 ± 3.3**	310.0 ± 4.8
17#	288.7 ± 4.5	301.4 ± 4.3	305.4 ± 3.6*	299.8 ± 5.4
18#	290.5 ± 4.5	299.8 ± 5.0	305.3 ± 4.3*	293.9 ± 5.5
19#	285.0 ± 3.6	296.7 ± 3.7	300.2 ± 3.5*	294.9 ± 3.8

The footnotes for this table are defined in Table D1a.

TABLE D2
Preweaning Body Weights of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Generation	Age	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_1	PND 2	6.5 ± 0.1	6.3 ± 0.2	6.5 ± 0.1	6.3 ± 0.2
	PND 4	8.7 ± 0.2	8.2 ± 0.3	8.7 ± 0.2	8.1 ± 0.2
	PND 7	13.5 ± 0.3 (24)	12.9 ± 0.4	13.2 ± 0.2	11.9 ± 0.3
	PND 14***	26.7 ± 0.7	26.0 ± 0.8	25.2 ± 0.6 (23)	23.2 ± 0.5*** (23) [4]
	PND 21***	40.3 ± 1.0	37.1 ± 1.0*** [2,3,4]	38.2 ± 0.9* [3]	34.7 ± 0.7*** [3,4]
F_2	PND 2	6.6 ± 0.1	6.6 ± 0.1	6.5 ± 0.1	6.3 ± 0.1
	PND 4	8.6 ± 0.2 (22)	9.0 ± 0.2	8.5 ± 0.2	8.3 ± 0.2
	PND 7	13.2 ± 0.3	13.8 ± 0.3	12.8 ± 0.3	12.4 ± 0.3
	PND 14***	26.7 ± 0.5	26.4 ± 0.5	25.8 ± 0.6	23.4 ± 0.6*** [4]
	PND 21***	40.0 ± 0.9	39.9 ± 0.9 [1]	39.1 ± 0.8 [3]	36.3 ± 1.1*** [3,4]

TABLE D2
Preweaning Body Weights of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Generation	Age	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_3	PND 2	6.5 ± 0.1	6.5 ± 0.1	6.6 ± 0.1	6.7 ± 0.1
	PND 4	8.3 ± 0.2	8.6 ± 0.2	8.5 ± 0.2	8.7 ± 0.2
	PND 7	13.0 ± 0.4	13.6 ± 0.3	13.2 ± 0.4	13.2 ± 0.3
	PND 14*	26.4 ± 0.6	26.5 ± 0.4	26.4 ± 0.5	24.8 ± 0.4
	PND 21*	41.0 ± 1.0	40.1 ± 0.8 [1]	41.6 ± 0.8 [1,2]	38.9 ± 0.9* [1,2]
F_4	PND 2	6.2 ± 0.1	6.5 ± 0.1	6.4 ± 0.1	6.4 ± 0.1
	PND 4	8.1 ± 0.2	8.7 ± 0.2	8.2 ± 0.2	8.3 ± 0.2
	PND 7	13.0 ± 0.3	13.6 ± 0.3	12.8 ± 0.4	12.8 ± 0.4
	PND 14	26.9 ± 0.6	27.1 ± 0.6	26.2 ± 0.6	26.4 ± 0.7 [1,2]
	PND 21#	41.4 ± 0.9	41.7 ± 1.1 [1]	40.0 ± 0.9	40.2 ± 0.9 [1,2]

ANOVA results (P values for main effects and their interactions): dose, P=0.012; Generation, P=0.086; dose × Generation, P=0.700; Days, P<0.001; Days × Generation, P<0.001; Days × dose, P<0.001; Days × Generation × dose, P=0.018.

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; ***, P≤0.001. Asterisks adjacent to age designations indicate significant linear exposure concentration trends within a generation as determined by contrasts: *, P≤0.05; ***, P≤0.001; a single pound sign indicates a significant (P≤0.05) quadratic exposure concentration trend. Significant differences between generations within an exposure concentration group are indicated by generation numbers in brackets.

[b] There were significant random F_0 breed mother and interaction between F_0 breed mother and F_0 breed father effects that were included in the statistical model.

TABLE D3a
Postweaning Body Weights of F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
6	198.9 ± 5.7 (24)	199.3 ± 4.5	204.0 ± 5.0	203.3 ± 5.1
7**	254.7 ± 6.1 (24)	254.1 ± 4.4	258.1 ± 5.8	242.6 ± 4.5*
8***	310.3 ± 6.4 (24)	309.9 ± 4.7	310.0 ± 5.7	282.2 ± 4.3***
9***	355.9 ± 7.4 (24)	352.2 ± 4.8	349.5 ± 5.6	315.2 ± 4.1***
10***	383.5 ± 6.6 (24)	382.4 ± 4.7	378.0 ± 5.6	343.0 ± 4.7***
11***	408.6 ± 6.4 (24)	393.1 ± 6.1	394.9 ± 6.1	358.4 ± 4.8***
12***	429.6 ± 7.0 (24)	421.3 ± 6.4	419.4 ± 5.9	382.1 ± 4.0***
13***	445.4 ± 6.6 (24)	437.0 ± 5.5	433.1 ± 5.9	397.0 ± 4.3***
14***	470.3 ± 6.7 (24)	465.6 ± 6.5	461.3 ± 6.4	420.3 ± 4.8***
15***	488.3 ± 7.3 (24)	478.7 ± 6.1	478.0 ± 6.6	434.9 ± 4.8***
16***	493.0 ± 7.0 (24)	477.3 ± 6.4	483.8 ± 6.4	441.4 ± 5.3***
17***	520.2 ± 7.9 (24)	506.7 ± 7.1	508.1 ± 7.5	460.6 ± 5.4***
18***	527.1 ± 8.3 (24)	517.2 ± 7.2	515.1 ± 6.8	465.5 ± 5.7***
19***	541.7 ± 8.3 (24)	526.5 ± 7.2	525.1 ± 7.2	475.8 ± 5.9***

TABLE D3a
Postweaning Body Weights of F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001.

[b] Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for that generation. Therefore, in order to conduct tests of generation effects within dose groups (results shown in Table D11), two sets of statistical analyses were conducted for males: the first included data from week 6 to the end of the experiment for all generations (F_0 to F_4), and the second included all data from birth to the end of the experiment for generations F_1 to F_4. The statistical results reported in this table for weeks 3, 4, and 5 are from the latter analysis, while results from weeks 6 to 19 are from the former analysis. All postweaning data (weaning on PND 21) are included in this table. Preweaning data (birth to PND 21) for males are tabulated separately (Table D4).

[c] Body weights were analyzed using a repeated measures approach to a mixed model ANOVA. ANOVA results for each analysis were as follows:
1) Male body weights, weeks 6 to 19, F_0 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P<0.001; weeks × Generation, P<0.001; weeks × dose × Generation, P<0.001. Random effects of the F_0 breed father, the F_0 breed mother, and the interaction between the F_0 breed mother and F_0 breed father were significant at P<0.50 and were included in the statistical model.
2) Male body weights, birth to week 19, F_1 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P<0.001; weeks × Generation, P<0.001; weeks × dose × Generation, P=0.005. No random effects for the F_0 breed parents were included in the statistical model.

[d] Asterisks in the shaded cells in the age column indicate significant linear exposure concentration trends within a given week as determined by contrasts: *, P≤0.05; **, P≤0.01; ***, P≤0.001. A single pound sign indicates a significant (P≤0.05) quadratic exposure concentration trend.

TABLE D3b
Postweaning Body Weights of F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3*	39.8 ± 1.0	37.9 ± 0.9	39.9 ± 0.7	36.5 ± 1.0*
4	75.1 ± 2.3	76.0 ± 2.0	77.8 ± 1.8	71.6 ± 2.1
5	122.7 ± 3.4	124.3 ± 3.0	127.0 ± 2.8	118.2 ± 2.9
6**	175.5 ± 4.0	180.2 ± 3.3	180.3 ± 3.4	169.6 ± 3.3
7***	226.5 ± 5.0	232.8 ± 4.2	234.3 ± 4.4	215.5 ± 4.7*
8***	277.8 ± 5.2	272.6 ± 4.0	281.2 ± 4.4	260.6 ± 4.9**
9***	319.9 ± 5.5	316.3 ± 3.9	328.7 ± 5.0	300.0 ± 5.3***
10***,#	351.3 ± 6.6	352.6 ± 4.4	363.9 ± 5.8	325.0 ± 5.7***
11***,#	374.1 ± 5.9	371.4 ± 4.6	387.2 ± 6.0	348.6 ± 6.0***
12***	400.3 ± 5.8	397.4 ± 4.6	409.9 ± 5.9	376.0 ± 6.5***
13***	423.2 ± 6.1	413.5 ± 4.6	431.6 ± 5.4	395.2 ± 6.9***
14***	439.5 ± 6.5	432.1 ± 4.9	448.4 ± 6.0	414.3 ± 7.0**
15***	458.3 ± 7.4	449.8 ± 4.8	468.4 ± 6.4	430.4 ± 7.9***
16***	479.0 ± 7.3	467.5 ± 4.6	485.3 ± 6.6	445.6 ± 7.8***
17***	497.2 ± 7.2	485.0 ± 4.7	495.9 ± 6.9	454.8 ± 8.6***
18***	507.5 ± 7.8	502.7 ± 5.1	513.2 ± 7.0	467.6 ± 8.9***
19***	520.8 ± 8.0	512.2 ± 5.4	526.0 ± 7.6	479.4 ± 9.4***

The footnotes for this table are defined in Table D3a.

TABLE D3c
Postweaning Body Weights of F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3***	40.6 ± 0.8	41.6 ± 1.2	39.5 ± 0.8	36.8 ± 1.0*
4***	79.0 ± 1.9	79.7 ± 2.4	75.7 ± 2.1	69.8 ± 2.1**
5**	124.5 ± 2.4	124.4 ± 3.6	121.4 ± 2.9	112.8 ± 3.0*
6***	181.1 ± 3.3	177.3 ± 3.6	180.0 ± 3.4	164.4 ± 3.9***
7***	231.8 ± 3.7	224.2 ± 5.3	224.1 ± 4.2	207.6 ± 5.0***
8***	290.7 ± 3.5	277.2 ± 4.6	281.1 ± 3.9	259.8 ± 5.1***
9***	330.7 ± 3.7	313.6 ± 5.1*	315.0 ± 3.8*	290.4 ± 4.8***
10***	370.0 ± 4.1 (24)	345.3 ± 5.0** (23)	352.7 ± 4.0*	327.3 ± 5.3***
11***	401.0 ± 4.7 (24)	376.8 ± 5.0* (23)	385.1 ± 4.1*	353.0 ± 5.6***
12***	422.2 ± 5.0 (23)	398.6 ± 7.1** (20)	401.7 ± 5.6** (20)	374.1 ± 7.2*** (19)
13***	448.1 ± 5.3	418.5 ± 6.1** (23)	426.8 ± 4.2**	396.0 ± 5.8***
14***	467.9 ± 5.6	439.9 ± 5.7**	453.2 ± 4.8	415.7 ± 6.4***
15***	485.4 ± 5.7	454.6 ± 5.9**	464.7 ± 4.8*	428.1 ± 6.4***
16***	503.0 ± 5.7	471.4 ± 5.7**	483.0 ± 4.9*	444.0 ± 7.0***
17***	517.6 ± 5.5	482.9 ± 5.9**	495.7 ± 5.3*	455.9 ± 7.1***
18***	530.0 ± 6.0	493.0 ± 6.0***	506.3 ± 5.7*	462.0 ± 7.6***
19***	543.4 ± 6.1	511.6 ± 6.2**	517.3 ± 6.1*	472.7 ± 7.1***

The footnotes for this table are defined in Table D3a.

TABLE D3d
Postweaning Body Weights of F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3*	43.8 ± 0.8	41.3 ± 0.8	44.4 ± 1.0 (24)	40.2 ± 0.7*
4	85.6 ± 2.1	80.5 ± 1.9	81.7 ± 2.3 (24)	80.3 ± 1.5
5	135.2 ± 3.3	128.3 ± 2.6	131.0 ± 3.1	130.8 ± 2.4
6	191.7 ± 3.4	184.8 ± 3.7	192.1 ± 2.7	189.5 ± 2.7
7	252.6 ± 4.7	240.8 ± 4.8	248.9 ± 4.2	247.0 ± 3.4
8	304.5 ± 4.7	288.4 ± 5.0*	300.9 ± 4.7	303.1 ± 4.0
9	341.0 ± 5.4	330.3 ± 5.7	345.3 ± 4.9	343.1 ± 4.5
10	379.9 ± 6.0	371.2 ± 5.9	384.0 ± 5.5	375.9 ± 4.9
11	408.7 ± 6.5	396.9 ± 5.6	415.6 ± 5.4	406.7 ± 5.0
12	433.4 ± 6.7	424.7 ± 5.9	442.7 ± 5.4	430.8 ± 5.2
13#	453.8 ± 7.3	451.0 ± 6.6	471.2 ± 5.7	455.2 ± 5.9
14#	476.1 ± 7.2	470.1 ± 6.8	491.2 ± 5.7	472.8 ± 6.8
15	493.4 ± 7.2	489.2 ± 7.3	507.8 ± 5.8	491.8 ± 6.6
16	510.2 ± 7.4	508.8 ± 7.9	524.3 ± 6.1	509.1 ± 6.7
17	521.0 ± 7.8	524.5 ± 8.3	538.2 ± 6.0	524.2 ± 7.2
18	536.8 ± 8.1	541.0 ± 8.3	552.0 ± 7.7	542.6 ± 7.1
19	549.3 ± 8.1	553.4 ± 8.7	563.2 ± 7.2	551.0 ± 7.0

The footnotes for this table are defined in Table D3a.

TABLE D3e
Postweaning Body Weights of F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)[d]	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
3	43.3 ± 1.2	42.9 ± 1.8	42.9 ± 0.8	41.3 ± 1.0
4	77.2 ± 2.4	79.5 ± 2.6	79.0 ± 1.7	77.8 ± 2.2
5	122.7 ± 3.9	126.2 ± 3.8	126.5 ± 2.3	124.8 ± 3.2
6	178.1 ± 3.3	179.4 ± 4.6	183.2 ± 2.6	176.1 ± 4.0
7	232.6 ± 4.8	229.9 ± 6.5	234.7 ± 3.6	227.9 ± 5.5
8	283.9 ± 4.4	281.4 ± 6.7	291.6 ± 3.5	280.2 ± 5.7
9	328.6 ± 4.9	323.6 ± 7.2	335.6 ± 4.2	320.9 ± 6.0
10	364.1 ± 4.6	357.9 ± 7.6	371.9 ± 4.4	362.0 ± 6.6
11	395.5 ± 4.3	385.5 ± 7.6	401.8 ± 5.1	388.9 ± 7.7
12[#]	413.4 ± 4.9	404.2 ± 8.0	426.9 ± 5.2	414.5 ± 7.3
13	435.8 ± 5.4	428.2 ± 8.2	446.0 ± 5.3	436.8 ± 7.3
14	460.6 ± 5.3	447.9 ± 8.1	468.9 ± 5.4	460.8 ± 7.3
15[#]	481.5 ± 5.5	470.6 ± 8.4	494.8 ± 5.6	483.1 ± 8.3
16[#]	497.4 ± 5.8	483.1 ± 8.1	509.3 ± 5.6	495.3 ± 8.2
17[#]	506.1 ± 6.6	496.9 ± 8.6	523.8 ± 6.0	512.5 ± 8.6
18[#]	518.2 ± 6.5	508.2 ± 9.3	535.2 ± 5.7	525.8 ± 8.4
19	533.0 ± 6.5	520.8 ± 9.3	548.3 ± 6.1	539.6 ± 8.7

The footnotes for this table are defined in Table D3a.

TABLE D4
Preweaning Body Weights of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Generation	Age	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_1	PND 2	6.7 ± 0.2	6.7 ± 0.1	6.8 ± 0.1	6.7 ± 0.2
	PND 4	9.1 ± 0.3	8.9 ± 0.2	9.2 ± 0.2	8.8 ± 0.2
	PND 7	13.8 ± 0.4 (24)	13.5 ± 0.4	13.9 ± 0.2	12.9 ± 0.3
	PND 14***	27.1 ± 0.7	26.7 ± 0.6	27.3 ± 0.5 (24)	24.3 ± 0.5** [4]
	PND 21***	39.8 ± 1.0 [3,4]	37.9 ± 0.9 [2,3,4]	39.9 ± 0.7 [3,4]	36.5 ± 1.0** [3,4]
F_2	PND 2	6.8 ± 0.1	7.0 ± 0.1	6.7 ± 0.1	6.7 ± 0.1
	PND 4	8.9 ± 0.2	9.3 ± 0.2	8.8 ± 0.2	8.7 ± 0.2
	PND 7	13.6 ± 0.3	14.3 ± 0.3	13.4 ± 0.4	12.9 ± 0.4
	PND 14**	26.9 ± 0.6	26.8 ± 0.5	25.9 ± 0.6 [3]	24.4 ± 0.6* [4]
	PND 21***	40.6 ± 0.8 [3,4]	41.6 ± 1.2 [1]	39.5 ± 0.8 [3,4]	36.8 ± 1.0*** [3,4]

TABLE D4
Preweaning Body Weights of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

F_3	PND 2	6.8 ± 0.1	6.8 ± 0.1	7.2 ± 0.1	6.9 ± 0.1
	PND 4	8.6 ± 0.2	9.0 ± 0.2	9.7 ± 0.3	9.2 ± 0.2
	PND 7	13.6 ± 0.3	14.0 ± 0.3	14.8 ± 0.4	13.8 ± 0.2
	PND 14**	28.3 ± 0.5	27.7 ± 0.4	28.8 ± 0.6 [2]	25.9 ± 0.4*
	PND 21***,#	43.8 ± 0.8 [1,2]	41.3 ± 0.8* [1]	44.4 ± 1.0 [1,2]	40.2 ± 0.7*** [1,2]
F_4	PND 2	6.9 ± 0.2	7.0 ± 0.1	6.8 ± 0.1	6.9 ± 0.1
	PND 4	8.8 ± 0.2	9.3 ± 0.3	8.9 ± 0.2	8.8 ± 0.3
	PND 7	14.0 ± 0.4	14.6 ± 0.4	13.9 ± 0.3	13.5 ± 0.5
	PND 14	28.5 ± 0.6	28.5 ± 0.8	28.1 ± 0.5	27.2 ± 0.6 [1,2]
	PND 21*	43.3 ± 1.2 [1,2]	42.9 ± 1.8 [1]	42.9 ± 0.8 [1,2]	41.3 ± 1.0 [1,2]

ANOVA results (P values for main effects and their interactions): dose, P=0.039; Generation, P<0.001; dose × Generation, P=0.780; Days, P<0.001; Days × dose, P<0.001; Days × Generation, P<0.001; Days × dose × Generation, P=0.129.

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Asterisks adjacent to age designations indicate significant linear exposure concentration trends within a generation as determined by contrasts: *, P≤0.05; **, P≤0.01; ***, P≤0.001; a single pound sign indicates a significant (P≤0.05) quadratic exposure concentration trend. Significant differences between generations within an exposure group on a given day are indicated by generation numbers in brackets.

[b] A random F_0 breed mother × F_0 breed father interaction effect was significant and was included in the statistical model.

TABLE D5
Predelivery Total Body Weight Gains of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generations covered[b]	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_0 – F_4 Dose P<0.001 Gen P<0.001 D x G P<0.001	F_0***,#	158.1 ± 4.7 [1,2,3,4]	154.2 ± 3.4 [1,2,3,4]	138.3 ± 4.1*** [2,3,4]	109.6 ± 3.3*** [1,2,3,4]
	F_1***	156.8 ± 6.0 [0,2,3]	151.7 ± 3.7 [0,2,3,4]	154.9 ± 4.6 [0,3,4]	128.8 ± 1.7*** [0,3]
	F_2***	145.7 ± 5.0 [0,1]	145.3 ± 5.2 [0,1]	137.9 ± 2.9 [0,3]	121.2 ± 2.4*** [0,3]
	F_3	145.6 ± 2.8 [0,1]	143.5 ± 2.7 [0,1]	154.5 ± 3.0 [0,2]	155.6 ± 3.6 [0,1,2]
	F_4	147.2 ± 3.5 [0]	150.1 ± 3.0 [0]	155.6 ± 2.8 [0]	151.7 ± 3.6 [0]
F_1 – F_4 Dose P<0.001 Gen P<0.001 D x G P<0.001	F_1***	263.0 ± 6.3 [2,3,4]	257.1 ± 4.4	259.1 ± 5.0 [2]	221.3 ± 3.0*** [3,4]
	F_2***	246.4 ± 5.1 [1]	250.3 ± 6.1	237.1 ± 4.0 [1,4]	208.6 ± 3.5*** [3,4]
	F_3	246.2 ± 3.4 [1]	245.4 ± 3.9	252.1 ± 3.7	255.4 ± 4.4 [1,2]
	F_4	242.0 ± 4.5 [1]	249.0 ± 4.0	255.2 ± 3.9 [2]	248.3 ± 4.1 [1,2]

[a] Mean body weight gain prior to delivery of litters (g) ± standard error. Time period covered: weeks 6 to 13 (F_0 to F_4) and weeks 3 to 13 (F_1 to F_4). Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: ***, P≤0.001. Asterisks adjacent to generation designations indicate significant linear exposure concentration trends within a generation as determined by contrasts: ***, P≤0.001. Significant differences (P≤0.05) between generations within an exposure group are indicated by generation numbers in brackets.

[b] Results of two-way ANOVA with main effects generation (Gen), dose, and dose × generation interaction (D × G) are indicated. Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for that generation. Therefore, in order to conduct tests of generation effects within exposure groups, two sets of statistical analyses were conducted for females prior to the start of delivery of litters: the first included data from week 6 to the start of litter delivery for all generations (F_0 to F_4), and the second included all data from birth to the start of litter delivery for generations F_1 to F_4. The results from these two separate analyses are reported here. For the F_0 to F_4 analysis, the significant (P<0.50) random effects of F_0 breed mother, F_0 breed father, and the interaction between F_0 breed mother and F_0 breed father were included in the statistical model. For the F_1 to F_4 analysis, no random effects were included in the statistical model.

TABLE D6
Postdelivery Total Body Weight Gains of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

ANOVA Results[b]	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_0 - F_4 Dose P=0.323 Gen P<0.001 D x G P=0.272	F_0	9.2 ± 4.1 [1,2,3,4]	8.6 ± 4.2 [1,2,3,4]	3.9 ± 3.4 [2,3,4]	9.9 ± 2.8 (24) [1,2,3,4]
	F_1	-7.5 ± 2.3 [0,2,3]	-4.7 ± 2.6 [0,2,3,4]	-6.3 ± 2.0 [3,4]	-8.3 ± 1.7 [0,3]
	F_2**	-19.0 ± 3.0 [0,1]	-19.6 ± 2.0 [0,1]	-13.8 ± 2.2 [0,3]	-6.8 ± 2.1* [0,3]
	F_3	-22.6 ± 3.3 [0,1]	-19.4 ± 2.7 (23) [0,1]	-25.7 ± 2.6 [0,1,2]	-22.3 ± 6.0 [0,1,2]
	F_4	-11.7 ± 3.6 [0]	-18.4 ± 4.3 [0,1]	-19.3 ± 2.2 [0,1]	-15.1 ± 2.6 [0]

[a] Mean body weight gain after delivery of litters (g) ± standard error. Time period covered: weeks 16 to 19. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05. Asterisks adjacent to generation designations indicate significant linear exposure concentration trends within a generation as determined by contrasts: **, P≤0.01. Significant differences (P≤0.05) between generations within an exposure group are indicated by generation numbers in brackets.

[b] Results of two-way ANOVA with main effects generation (Gen), dose, and dose × generation interaction (D × G) are indicated. The significant (P<0.50) random effects of F_0 breed mother, F_0 breed father, and the interaction between F_0 breed mother and F_0 breed father were included in the statistical model.

TABLE D7
Preweaning Total Body Weight Gains of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Sex[b]	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Female Dose P<0.001 Gen P<0.001 D x G P=0.567	F_1***	33.8 ± 0.9	30.8 ± 0.9 [3,4]	31.8 ± 0.8	28.4 ± 0.7*** [3,4]
	F_2***	33.4 ± 0.9	33.2 ± 0.9	32.6 ± 0.8	30.1 ± 1.0** [4]
	F_3*	34.4 ± 0.9	33.6 ± 0.8 [1]	35.0 ± 0.7	32.2 ± 0.8 [1]
	F_4	35.2 ± 0.9	35.1 ± 1.1 [1]	33.6 ± 0.9	33.8 ± 0.8 [1,2]
Male Dose P<0.001 Gen P<0.001 D x G P=0.196	F_1*	33.0 ± 0.9 [3]	31.3 ± 0.8 [2,3,4]	33.1 ± 0.7 [3]	29.7 ± 0.9* [3,4]
	F_2***	33.9 ± 0.8	34.7 ± 1.2 [1]	32.8 ± 0.7 [3]	30.1 ± 1.0* [3,4]
	F_3	36.9 ± 0.7 [1]	34.5 ± 0.8 [1]	37.3 ± 0.9 (24) [1,2]	33.3 ± 0.7 [1,2]
	F_4	36.4 ± 1.1	35.9 ± 1.8 [1]	36.2 ± 0.7	34.4 ± 0.9 [1.2]

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group column indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Asterisks adjacent to generation designations indicate significant linear trends within a generation as determined by contrasts: *, P≤0.05; ***, P≤0.001. Significant differences between generations within an exposure group are indicated by generation numbers in brackets.

[b] Results of two-way ANOVA with main effects Generation (Gen), dose, and dose × Generation interaction (D × G) are indicated. For both females and males, significant random effects for F_0 breed mother and the interaction between F_0 breed father and F_0 breed mother were included in the model.

TABLE D8
Total Body Weight Gains of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Generations covered[b]	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
$F_0 – F_4$ Dose P<0.001 Gen P<0.001 D x G P<0.001	F_0***	342.8 ± 7.1 (24)	327.2 ± 7.0 [3]	321.1 ± 6.2* [1,3,4]	272.5 ± 4.7*** [1,2,3,4]
	F_1***	345.3 ± 6.2	332.1 ± 4.4 [3]	345.7 ± 7.2 [0,3]	309.8 ± 6.5*** [0,3,4]
	F_2***	362.3 ± 6.7	334.2 ± 5.6* [3]	337.3 ± 4.2* [3,4]	308.4 ± 5.2*** [0,3,4]
	F_3	357.5 ± 6.4	368.6 ± 7.3 [0,1,2]	371.1 ± 7.0 [0,1,2]	361.5 ± 5.7 [0,1,2]
	F_4	354.9 ± 6.3	341.4 ± 6.8	365.1 ± 4.8 [0,2]	363.5 ± 7.4 [0,1,2]
$F_1 – F_4$ Dose P<0.001 Gen P<0.001 D x G P<0.001	F_1***	481.0 ± 7.8	474.3 ± 5.2 [3]	486.1 ± 7.7 [3]	442.9 ± 8.8*** [3,4]
	F_2***	502.8 ± 6.0	469.9 ± 6.1** [3]	477.8 ± 4.7* [3,4]	435.9 ± 6.5*** [3,4]
	F_3	505.5 ± 7.6	512.0 ± 8.5 [1,2,4]	520.0 ± 7.2 (24) [1,2]	510.9 ± 7.0 [1,2]
	F_4	489.7 ± 6.3	477.9 ± 8.7 [3]	505.4 ± 5.9 [2]	498.3 ± 8.6 [1,2]

[a] Mean body weight gain (g) ± standard error. Time period covered: weeks 6 to 19 (F_0 to F_4) and weeks 3 to 19 (F_1 to F_4). Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Asterisks adjacent to generation designations indicate significant linear exposure concentration trends within a generation as determined by contrasts: ***, P≤0.001. Significant differences between generations within an exposure group are indicated by generation numbers in brackets.

[b] Results of two-way ANOVA with main effects generation (Gen), dose, and dose × generation interaction (D × G) are indicated. Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for that generation. Therefore, in order to conduct tests of generation effects within exposure groups, two sets of statistical analyses were conducted for males: the first included data from week 6 to the end of the experiment for all generations (F_0 to F_4), and the second included all data from birth to the end of the experiment for generations F_1 to F_4. The results from these two separate analyses are reported here. For the F_0 to F_4 analysis, significant random effects of F_0 breed mother, F_0 breed father, and the interaction between F_0 breed mother and F_0 breed father were included in the statistical model. For the F_1 to F_4 analysis, the significant random effect of F_0 breed father was included in the statistical model.

TABLE D9
Terminal Body Weights of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Sex	Generation	Dietary Ethinyl Estradiol (ppb)				Trends	
		0	2	10	50	Linear	Quad
Female[b] Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0	296.6 ± 7.1 [4][c]	284.6 ± 4.6	274.7 ± 3.9*** [3,4]	251.8 ± 4.0*** [3,4]	***/ ###	*
	F_1	281.3 ± 4.7	276.4 ± 3.2	269.2 ± 3.9 [3,4]	243.2 ± 2.8*** [3,4]	***/ ###	#
	F_2	281.0 ± 3.0	285.2 ± 3.3	280.4 ± 4.2	250.5 ± 3.7*** [3,4]	***/ ###	###
	F_3	282.6 ± 3.3	283.7 ± 3.9	291.4 ± 3.8 [0,1]	291.3 ± 3.4 [0,1,2]	-	-
	F_4	275.3 ± 3.2 [0]	287.7 ± 4.0	291.9 ± 3.3** [0,1]	286.9 ± 3.8 [0,1,2]	-	*/#
Male[b] Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0	536.4 ± 8.8 [1]	528.8 ± 7.4 [1]	530.3 ± 8.3	474.9 ± 5.8*** [3,4]	***/ ###	##
	F_1	505.6 ± 8.1 [0,3]	498.6 ± 5.2 [0,3]	511.1 ± 7.6 [3,4]	466.1 ± 8.9*** [3,4]	***/ ###	##
	F_2	534.7 ± 6.2	501.0 ± 6.2** [3]	508.4 ± 5.0* [3,4]	466.7 ± 7.1*** [3,4]	***/ ###	-
	F_3	537.7 ± 8.5 [1]	543.0 ± 8.9 [1,2,4]	552.4 ± 7.2 [1,2]	537.9 ± 7.3 [0,1,2]	-	-
	F_4	525.6 ± 7.0	509.4 ± 10.2 [3]	540.5 ± 6.5 [1,2]	529.8 ± 8.8 [0,1,2]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by numbers in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test; asterisks in the trends column indicate significant linear or quadratic (quad) exposure concentration trends as determined by contrasts: *, P≤0.05; **, P≤0.01; ***, P≤0.001. A dash in the trend column indicates no statistical significance as determined by contrasts. Pound signs indicate significant exposure concentration trends determined for a scale using the natural logarithm of the dose plus 1: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

[b] Results of two-way ANOVA with main effects Generation (Gen), dose, and dose × Generation interaction (D × G) are indicated.

[c] Significant differences between generations within a dose group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.

TABLE D10
Generational Effects in Postweaning Body Weights of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
3	NA	NSD	NA	1v4** ↑12%	NA	NSD	NA	1v3** ↑12% 1v4*** ↑16% 2v4* ↑11%
4	NA	NSD	NA	1v2* ↑11%	NA	NSD	NA	1v3** ↑15% 1v4** ↑13% 2v3*** ↑17% 2v4** ↑15%
5	NA	NSD	NA	NSD	NA	NSD	NA	1v3* ↑9% 2v3*** ↑14%
6	**0v1*** ↓9%** **0v2*** ↓12%** **0v3*** ↓12%** **0v4*** ↓15%**	1v4* ↓7%	**0v1*** ↓9%** **0v2* ↓7%** **0v3*** ↓9%** **0v4*** ↓10%** 2v4** ↓3%	NSD	**0v1*** ↓10%** **0v2*** ↓13%** **0v3*** ↓12%** **0v4*** ↓12%**	NSD	**0v1*** ↓19%** **0v2*** ↓21%** **0v3*** ↓11%** **0v4*** ↓13%** 1v3*** ↑9% 1v4* ↑7% 2v3*** ↑12% 2v4** ↑10%	1v3* ↑9% 2v3*** ↑12% 2v4** ↑10%
7	**0v1*** ↓10%** **0v2*** ↓13%** **0v3*** ↓11%** **0v4*** ↓15%**	NSD	**0v1*** ↓11%** **0v2** ↓9%** **0v3*** ↓9%** **0v4*** ↓10%** 2v4* ↓2%	NSD	**0v1*** ↓12%** **0v2*** ↓11%** **0v3*** ↓12%** **0v4*** ↓11%**	NSD	**0v1*** ↓13%** **0v2*** ↓13%** **0v4* ↓4%** 1v3*** ↑13% 1v4* ↑10% 2v3*** ↑13% 2v4** ↑11%	1v3*** ↑13% 1v4*** ↑10% 2v3*** ↑13% 2v4*** ↑11%
8	**0v1*** ↓8%** **0v2*** ↓11%** **0v3*** ↓10%** **0v4*** ↓13%**	NSD	**0v1*** ↓9%** **0v2*** ↓9%** **0v3*** ↓7%** **0v4*** ↓9%**	NSD	**0v1*** ↓9%** **0v2*** ↓9%** **0v3*** ↓6%** **0v4** ↓6%**	NSD	**0v1*** ↓10%** **0v2*** ↓10%** 1v3*** ↑15% 1v4*** ↑10% 2v3*** ↑15% 2v4*** ↑10% 3v4* ↓4%	1v3*** ↑15% 1v4*** ↑10% 2v3*** ↑15% 2v4*** ↑10%

TABLE D10
Generational Effects in Postweaning Body Weights of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Age (weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
9	**0v1** ↓7% **0v2** ↓8% **0v3*** ↓6% **0v4*** ↓10%	NSD	**0v1*** ↓8% **0v4*** ↓6% 1v2* ↑5% 2v4* ↓2%	NSD	**0v1*** ↓8%** 1v3* ↑7 % 1v4** ↑6 %	NSD	**0v1*** ↓9%** **0v2** ↓7%** **0v3** ↑8 %** 1v3*** ↑19% 1v4*** ↑16% 2v3*** ↑16% 2v4*** ↑13%	1v3*** ↑19% 1v4*** ↑16% 2v3*** ↑16% 2v4*** ↑13%
10	**0v1*** ↓8%** **0v2*** ↓9%** **0v3** ↓7%** **0v4*** ↓11%**	NSD	**0v1* ↓5%** **0v4* ↓3%**	NSD	**0v1*** ↓5%** 1v3* ↑6 % 1v4** ↑6 %	NSD	**0v1*** ↓7%** **0v2* ↓5%** **0v3*** ↑11 %** **0v4* ↑8 %** 1v3*** ↑20% 1v4*** ↑16% 2v3*** ↑16% 2v4*** ↑13%	1v3*** ↑20% 1v4*** ↑16% 2v3*** ↑16% 2v4*** ↑13%
11	**0v1** ↓8%** **0v2* ↓6%** **0v4** ↓8%**	NSD	**0v1* ↓5%** 1v2*** ↑8% 1v3* ↑6%	1v2** ↑8% 1v3* ↑6% 1v4* ↑6%	1v3*** ↑9% 1v4* ↑7%	1v3*** ↑9% 1v4* ↑7%	**0v1*** ↓5%** **0v3*** ↑18 %** **0v4* ↑15 %** 1v2* ↑6 % 1v3*** ↑25% 1v4*** ↑22% 2v3*** ↑18% 2v4*** ↑14%	1v2* ↑6 % 1v3*** ↑25% 1v4*** ↑22% 2v3*** ↑18% 2v4*** ↑14%
12	**0v1** ↓6%** **0v2*** ↓10%** **0v3** ↓8%** **0v4*** ↓11%**	NSD	**0v1** ↓6%** **0v3** ↓6%** **0v4*** ↓7%**	NSD	**0v2** ↓6%** 2v4* ↑7 %	2v3* ↑6 % 2v4* ↑7 %	**0v1** ↓6%** **0v2** ↓6%** **0v3*** ↑11 %** **0v4* ↑8 %** 1v3*** ↑17% 1v4*** ↑15% 2v3*** ↑18% 2v4*** ↑16%	1v3*** ↑17% 1v4*** ↑15% 2v3*** ↑18% 2v4*** ↑16%

TABLE D10
Generational Effects in Postweaning Body Weights of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Age (weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
13	**0v2*** ↓10%** **0v3** ↓10%** **0v4*** ↓11%** 1v4* ↓7%	1v4* ↓ 7%	**0v1* ↓5%** **0v2* ↓6%** **0v3*** ↓8%** **0v4*** ↓6%**	NSD	**0v2** ↓7%** 1v2* ↓7% 2v4* ↑7 %	1v2* ↓7% 2v3* ↑6 % 2v4* ↑7 %	**0v2** ↓8%** **0v3*** ↑11 %** **0v4* ↑8 %** 1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑20% 2v4*** ↑18%	1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑20% 2v4*** ↑18%
16	**0v3*** ↑8%** 3v4* ↓6%	NA	**0v2*** ↑9%** **0v3*** ↑9%** **0v4*** ↑10%** 1v3* ↑7 % 1v4** ↑8 %	NA	**0v2*** ↑9%** **0v3*** ↑17%** **0v4*** ↑15%** 1v2* ↑6 % 1v3*** ↑13% 1v4*** ↑12% 2v3* ↑7% 2v4* ↑5%	NA	**0v3*** ↑28%** **0v4*** ↑24%** 1v3*** ↑23% 1v4*** ↑19% 2v3* ↑21% 2v4* ↑17%	NA
17	**0v3*** ↑8%** 2v3** ↑8 % 3v4* ↓8%	NA	**0v3*** ↑9%** **0v4** ↑7%** 1v3** ↑7 % 2v3* ↑5%	NA	**0v3*** ↑14%** **0v4*** ↑9%** 1v3*** ↑12% 1v4*** ↑8% 2v3*** ↑10% 2v4** ↑8%	NA	**0v3*** ↑24%** **0v4*** ↑17%** 1v3*** ↑23% 1v4*** ↑16% 2v3*** ↑24% 2v4*** ↑17% 3v4** ↓6%	NA
18	1v2*** ↑7 % 1v3* ↑6%	NA	**0v2** ↑8%** **0v3* ↑5%** **0v4* ↑5%** 1v2*** ↑11% 1v3** ↑9 % 1v4** ↑9%	NA	**0v2*** ↑11%** **0v3*** ↑11%** **0v4*** ↑11%** 1v2*** ↑13% 1v3*** ↑13% 1v4*** ↑12%	NA	**0v2* ↑7%** **0v3*** ↑19 %** **0v4*** ↑15 %** 1v2*** ↑11% 1v3*** ↑23% 1v4*** ↑19% 2v3*** ↑11% 2v4* ↑8%	NA
19	NSD	NA	NSD	NA	**0v4*** ↑7%** 1v3* ↑7% 1v4*** ↑7%	NA	**0v3*** ↑15 %** **0v4*** ↑13%** 1v3*** ↑19% 1v4*** ↑17% 2v3*** ↑16% 2v4*** ↑14%	NA

[a] Results of Holm's-adjusted t-tests of body weight differences are indicated. Only comparisons showing significant differences between generations within an exposure group are shown. Generations are indicated by their subscripts, so that "0v1" means F_0 versus F_1. Asterisks indicate the level of significance: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Arrows indicate the direction of the difference of the second listed generation relative to the first, and the percentage difference is given. Comparisons involving the F_0 generation are bolded. NA, not applicable; NSD, no significant differences.

[b] Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for this generation. Therefore, in order to conduct tests of generation effects within exposure groups, two sets of statistical analyses were conducted for females for the interval prior to delivery of their litters: the first included data from week 6 to the start of littering for all generations (F_0 to F_4), and the second included all data from birth to the start of littering for generations F_1 to F_4. The statistical results reported in these tables for weeks 3, 4, and 5 are from the latter analysis, while results from weeks 6 to 13 are from the former analysis. All postweaning data (weaning on PND 21) are included in this table. Data from the weeks during which the dams were littering (weeks 14 and 15) were excluded from the analysis. Data from dams in the F_0 to F_4 generations after delivery of their litters (weeks 16 to 19) were analyzed separately, and those results are also reported in this table. Preweaning data (birth to PND 21) are tabulated separately (Table D2).

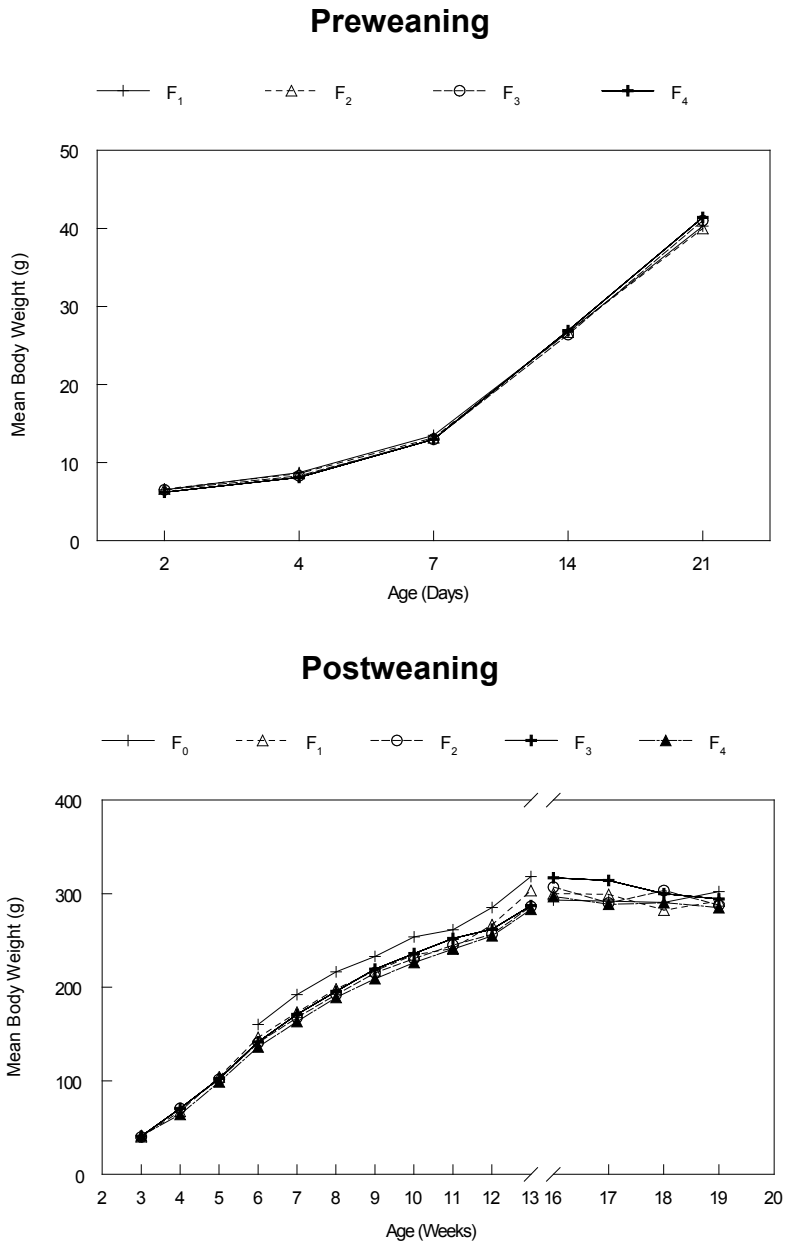

FIGURE D1
Body Weights of 0 ppb Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

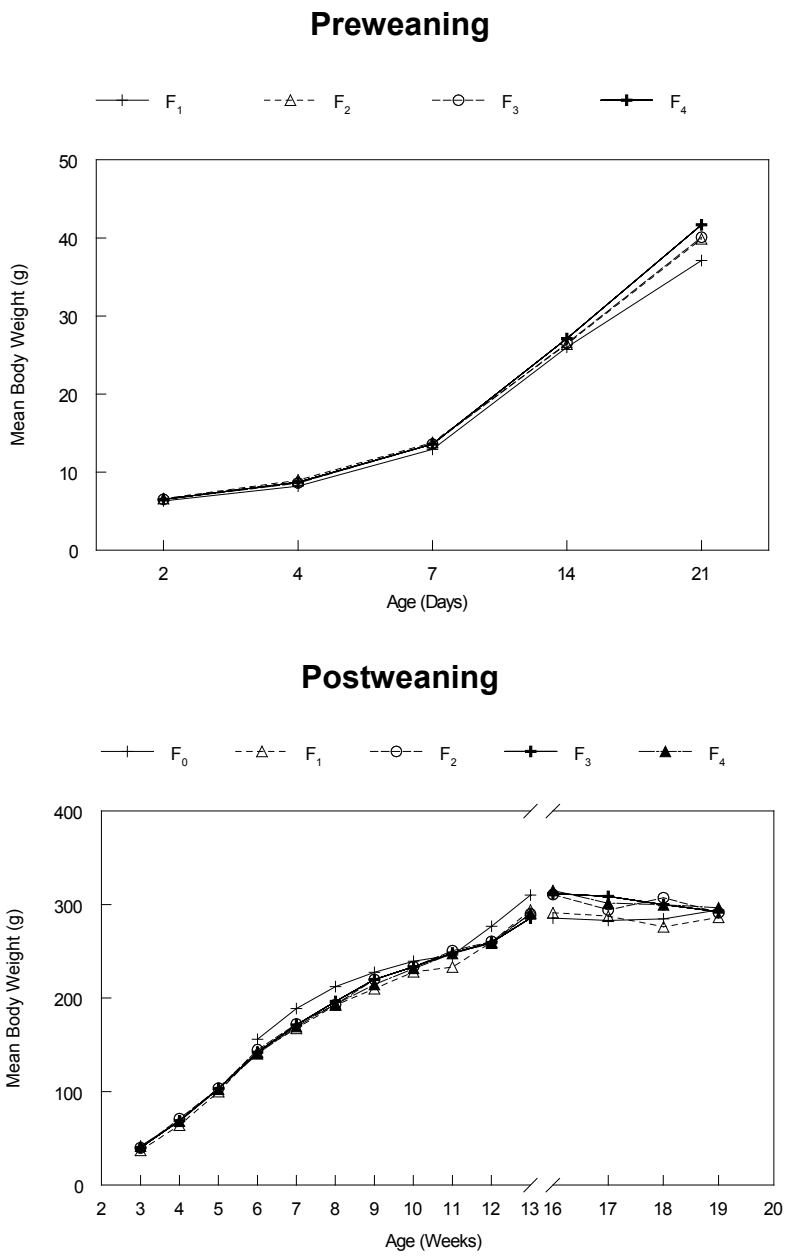

FIGURE D2
Body Weights of 2 ppb Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

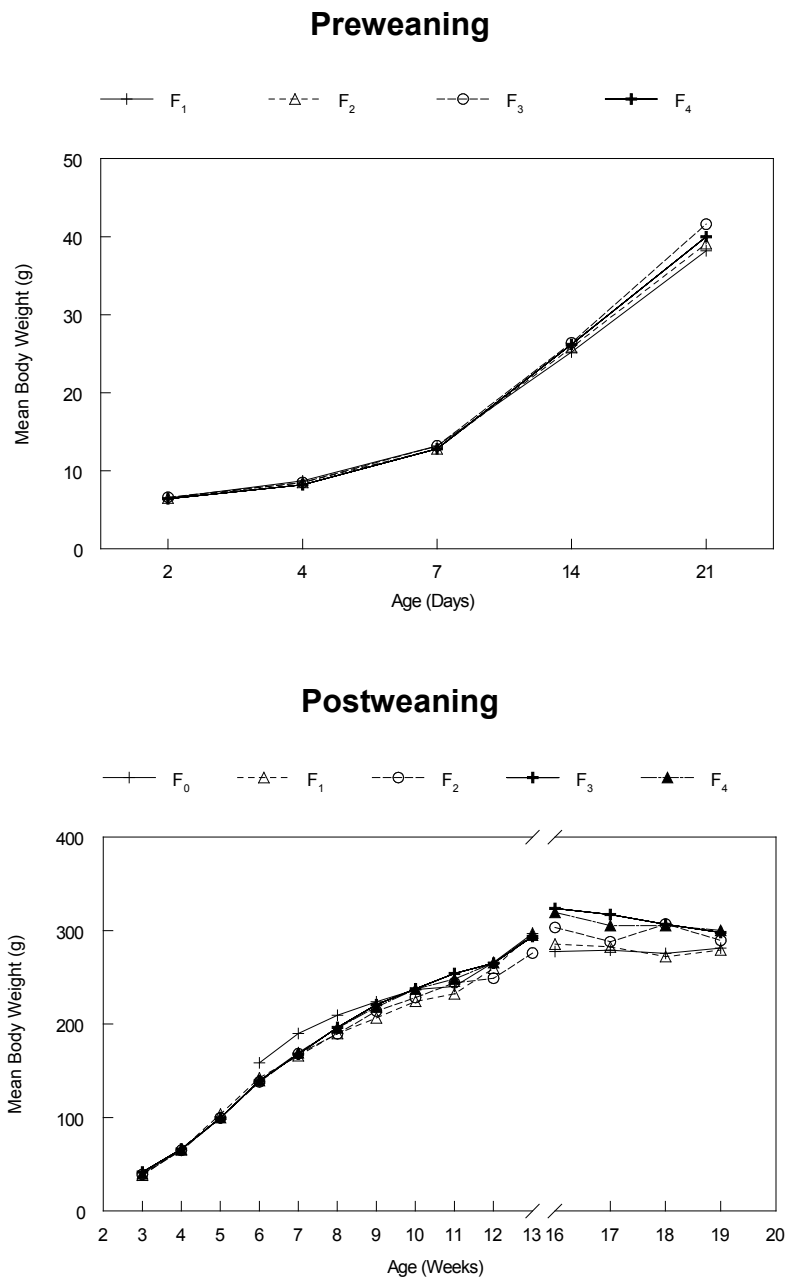

FIGURE D3
Body Weights of 10 ppb Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

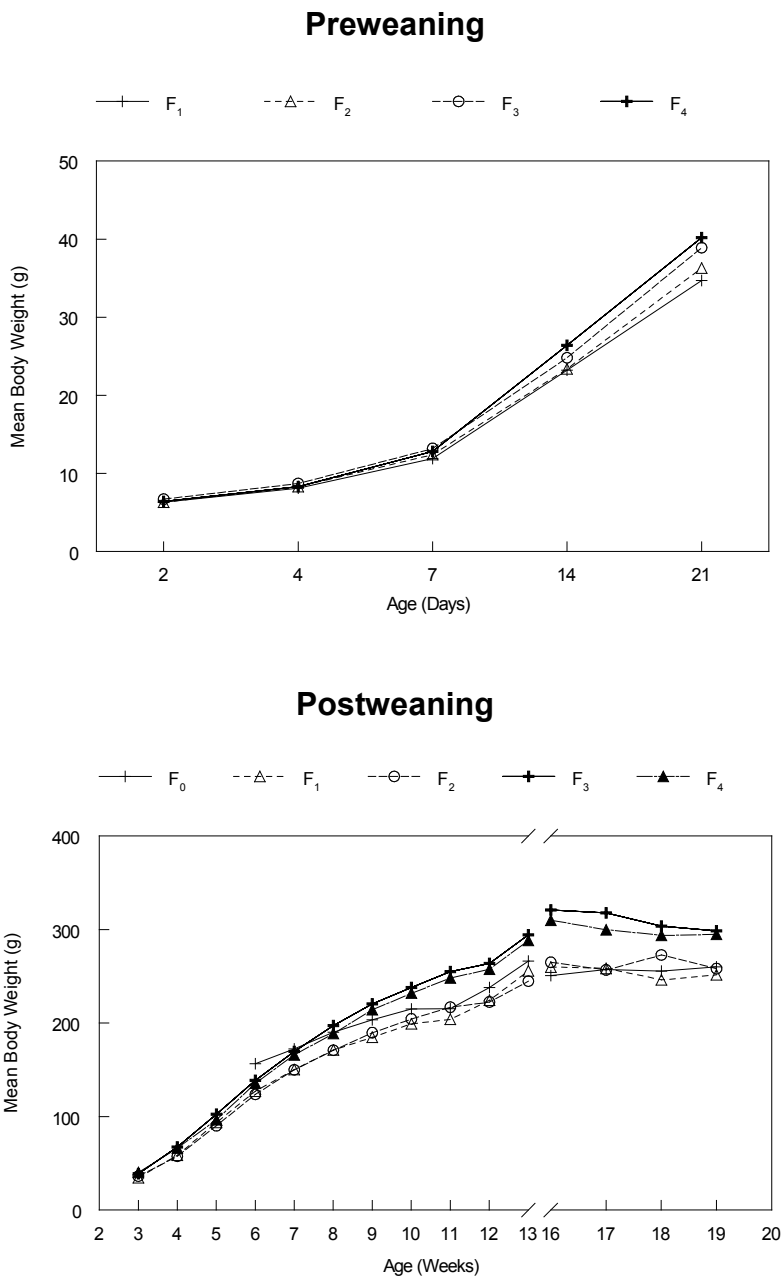

FIGURE D4
Body Weights of 50 ppb Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

TABLE D11
Generational Effects in Postweaning Body Weights of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
3	NA	1v3* ↑10%	NA	1v2* ↑10% 1v4** ↑13%	NA	1v3** ↑11% 2v3** ↑12%	NA	1v3* ↑10% 1v4** ↑13% 2v4** ↑12%
4	NA	1v3** ↑14% 3v4* ↓10%	NA	NSD	NA	NSD	NA	1v3* ↑12% 2v3** ↑15% 2v4* ↑11%
5	NA	1v3* ↑10% 3v4* ↓9%	NA	NSD	NA	NSD	NA	1v3* ↑11% 2v3***↑16% 2v4* ↑11%
6	**0v1*** ↓12%** **0v2*** ↓9%** **0v4*** ↓10%** 1v3** ↑9% 2v3* ↑6% 3v4** ↓7%	1v3** ↑9% 3v4* ↓7%	**0v1*** ↓10%** **0v2*** ↓11%** **0v3*** ↓7%** **0v4*** ↓10%**	NSD	**0v1*** ↓12%** **0v2*** ↓12%** **0v3*** ↓6%** **0v4*** ↓10%** 1v3** ↑7% 2v3** ↑7%	NSD	**0v1*** ↓17%** **0v2*** ↓19%** **0v3*** ↓7%** **0v4*** ↓13%** 1v3*** ↑12% 2v3*** ↑15% 2v4* ↑7% 3v4*** ↓7%	1v3*** ↑12% 2v3***↑15% 3v4* ↓7%
7	**0v1*** ↓11%** **0v2*** ↓9%** **0v4*** ↓9%** 1v3*** ↑12% 2v3*** ↑9% 3v4** ↓8%	1v3*** ↑12% 2v3** ↑9% 3v4* ↓8%	**0v1*** ↓8%** **0v2*** ↓12%** **0v3*** ↓5%** **0v4*** ↓10%** 2v3*** ↑7%	NSD	**0v1*** ↓9%** **0v2*** ↓13%** **0v4*** ↓9%** 1v3* ↑6% 2v3*** ↑11%	2v3** ↑11%	**0v1*** ↓11%** **0v2*** ↓14%** **0v4** ↓6%** 1v3*** ↑15% 2v3*** ↑19% 2v4** ↑10% 3v4** ↓8%	1v3***↑15% 2v3***↑19% 2v4** ↑10% 3v4* ↓8%
8	**0v1*** ↓10%** **0v2*** ↓6%** **0v4*** ↓9%** 1v3*** ↑10% 2v3* ↑5% 3v4** ↓7%	1v3*** ↑10% 3v4** ↓7%	**0v1*** ↓12%** **0v2*** ↓11%** **0v3*** ↓7%** **0v4*** ↓9%** 1v3* ↑6%	NSD	**0v1*** ↓9%** **0v2*** ↓9%** **0v4** ↓6%** 1v3** ↑7% 2v3*** ↑7%	1v3* ↑7% 2v3* ↑7%	**0v1*** ↓8%** **0v2*** ↓8%** **0v3** ↑7%** 1v3*** ↑16% 1v4** ↑8% 2v3*** ↑17% 2v4** ↑8% 3v4*** ↓8%	1v3***↑16% 1v4** ↑8% 2v3***↑17% 2v4** ↑8% 3v4** ↓8%

TABLE D11
Generational Effects in Postweaning Body Weights of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
9	**0v1*** ↓10%** **0v2*** ↓7%** **0v3* ↓4%** **0v4*** ↓8%** 1v3* ↑7%	1v3* ↑7%	**0v1*** ↓10%** **0v2*** ↓11%** **0v3*** ↓6%** **0v4*** ↓8%** 2v3* ↑5%	NSD	**0v1*** ↓6%** **0v2*** ↓10%** **0v4* ↓4%** 1v3* ↑5% 2v3*** ↑10% 2v4* ↑7%	2v3*** ↑10% 2v4* ↑7%	**0v1* ↓5%** **0v2*** ↓8%** **0v3*** ↑9%** 1v3*** ↑14% 1v4** ↑7% 2v3*** ↑18% 2v4*** ↑11% 3v4** ↓6%	1v3***↑14% 1v4** ↑7% 2v3***↑18% 2v4*** ↑11% 3v4** ↓6%
10	**0v1*** ↓8%** **0v4* ↓5%** 1v3*** ↑8%	1v3** ↑8%	**0v1*** ↓8%** **0v2*** ↓10%** **0v4*** ↓6%** 1v3* ↑5% 2v3** ↑8%	2v3** ↑8%	**0v1* ↓4%** **0v2*** ↓7%** 1v3* ↑6% 2v3*** ↑9% 2v4* ↑5%	1v3** ↑6% 2v3*** ↑9%	**0v1* ↓5%** **0v2* ↓5%** **0v3*** ↑10%** 1v3*** ↑16% 1v4** ↑11% 2v3*** ↑15% 2v4*** ↑11%	1v3*** ↑16% 1v4*** ↑11% 2v3*** ↑15% 2v4*** ↑11%
11	**0v1*** ↓8%** 1v2** ↑7% 1v3*** ↑9% 1v4* ↑6%	1v2** ↑7% 1v3*** ↑9% 1v4* ↑6%	**0v1** ↓6%** 1v3* ↑7%	1v3* ↑7%	1v3*** ↑7% 2v3*** ↑8%	1v3** ↑7% 2v3*** ↑8%	**0v3*** ↑13%** **0v4*** ↑9%** 1v3*** ↑17% 1v4*** ↑12% 2v3*** ↑15% 2v4*** ↑10% 3v4* ↓4%	1v3*** ↑17% 1v4*** ↑12% 2v3*** ↑15% 2v4*** ↑10%
12	**0v1*** ↓7%** 1v2* ↑5% 1v3*** ↑8%	1v2* ↑5% 1v3*** ↑8%	**0v1** ↓6%** **0v2** ↓5%** 1v3** ↑7% 2v3** ↑7%	1v3** ↑7% 2v3** ↑7%	**0v2* ↓4%** 1v3*** ↑8% 2v3*** ↑10% 2v4** ↑6%	1v3*** ↑8% 2v3*** ↑10% 2v4* ↑6%	**0v3*** ↑13%** **0v4*** ↑8%** 1v3*** ↑15% 1v4*** ↑10% 2v3*** ↑15% 2v4*** ↑11%	1v3*** ↑15% 1v4*** ↑10% 2v3*** ↑15% 2v4*** ↑11%
13	**0v1* ↓5%** 1v2* ↑6% 1v3** ↑7%	1v2* ↑6% 1v3** ↑7%	**0v1** ↓5%** **0v2* ↓4%** 1v3*** ↑9% 2v3*** ↑8% 3v4* ↓5%	1v3*** ↑9% 2v3*** ↑8% 3v4* ↓5%	**0v3*** ↑9%** 1v3*** ↑9% 2v3*** ↑10% 2v4* ↑4% 3v4* ↓5%	1v3*** ↑9% 2v3*** ↑10% 3v4* ↓5%	**0v3*** ↑15%** **0v4*** ↑10%** 1v3*** ↑15% 1v4*** ↑11% 2v3*** ↑15% 2v4*** ↑10% 3v4* ↓4%	1v3*** ↑15% 1v4*** ↑11% 2v3*** ↑15% 2v4*** ↑10%

TABLE D11
Generational Effects in Postweaning Body Weights of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
14	**0v1*** ↓7%** 1v2** ↑6% 1v3*** ↑8%	1v2** ↑6% 1v3*** ↑8%	**0v1*** ↓7%** **0v2** ↓6%** 1v3*** ↑9% 2v3** ↑7%	1v3*** ↑9% 2v3** ↑7% 3v4* ↓5%	**0v3* ↑6%** 1v3*** ↑10% 1v4* ↑5% 2v3*** ↑8%	1v3*** ↑10% 2v3*** ↑8% 3v4* ↓5%	**0v3*** ↑12%** **0v4*** ↑10%** 1v3*** ↑14% 1v4*** ↑11% 2v3*** ↑14% 2v4*** ↑11%	1v3*** ↑14% 1v4*** ↑11% 2v3*** ↑14% 2v4*** ↑11%
15	**0v1** ↓6%** 1v2* ↑6% 1v3*** ↑8%	1v2* ↑6% 1v3** ↑8%	**0v1*** ↓6%** **0v2** ↓5%** 1v3*** ↑9% 2v3** ↑8%	1v3*** ↑9% 2v3** ↑8%	**0v3* ↑6%** 1v3*** ↑8% 1v4** ↑6% 2v3*** ↑9% 2v4** ↑6%	1v3*** ↑8% 1v4* ↑6% 2v3*** ↑9% 2v4* ↑6%	**0v3*** ↑13%** **0v4*** ↑11%** 1v3*** ↑14% 1v4*** ↑12% 2v3*** ↑15% 2v4*** ↑13%	1v3*** ↑14% 1v4*** ↑12% 2v3*** ↑15% 2v4*** ↑13%
16	1v3** ↑7%	1v3** ↑7%	**0v3* ↑7%** 1v3*** ↑9% 2v3*** ↑8% 3v4* ↓5%	1v3*** ↑9% 2v3*** ↑8% 3v4* ↓5%	**0v3*** ↑8%** 1v3*** ↑8% 1v4* ↑5% 2v3*** ↑9% 2v4** ↑5%	1v3*** ↑8% 1v4* ↑5% 2v3*** ↑9% 2v4* ↑5%	**0v3*** ↑15%** **0v4*** ↑12%** 1v3*** ↑14% 1v4*** ↑11% 2v3*** ↑15% 2v4*** ↑12%	1v3*** ↑14% 1v4*** ↑11% 2v3*** ↑15% 2v4*** ↑12%
17	NSD	NSD	**0v1* ↓4%** **0v2* ↓5%** 1v3*** ↑8% 2v3*** ↑9% 3v4* ↓5%	1v3*** ↑8% 2v3*** ↑9% 3v4** ↓5%	**0v3* ↑6%** 1v3*** ↑9% 1v4** ↑6% 2v3*** ↑9% 2v4** ↑6%	1v3*** ↑9% 1v4* ↑6% 2v3*** ↑9% 2v4* ↑6%	**0v3*** ↑14%** **0v4*** ↑11%** 1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑15% 2v4*** ↑12%	1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑15% 2v4*** ↑12%
18	1v3* ↑6%	1v3* ↑6%	**0v2* ↓5%** 1v3*** ↑8% 2v3*** ↑10% 3v4** ↓6%	1v3** ↑8% 2v3*** ↑10% 3v4** ↓6%	**0v3** ↑7%** 1v3*** ↑8% 2v3*** ↑9% 2v4** ↑6%	1v3*** ↑8% 2v3*** ↑9% 2v4* ↑6%	**0v3*** ↑17%** **0v4*** ↑13%** 1v3*** ↑16% 1v4*** ↑12% 2v3*** ↑17% 2v4*** ↑14%	1v3*** ↑16% 1v4*** ↑12% 2v3*** ↑17% 2v4*** ↑14%
19	NSD	1v3* ↑5%	1v3*** ↑8% 2v3*** ↑8% 3v4* ↓6%	1v3*** ↑8% 2v3*** ↑8% 3v4** ↓6%	**0v3** ↑7%** 1v3*** ↑7% 2v3*** ↑9% 2v4** ↑6%	1v3** ↑7% 2v3*** ↑9% 2v4* ↑6%	**0v3*** ↑16%** **0v4*** ↑13%** 1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑17% 2v4*** ↑14%	1v3*** ↑15% 1v4*** ↑13% 2v3*** ↑17% 2v4*** ↑14%

[a] Results of Holm's-adjusted t-tests of body weight differences are indicated. Only comparisons showing significant differences between generations within an exposure group are shown. Generations are indicated by their subscripts, so that "0v1" means F_0 versus F_1. Asterisks indicate the level of significance: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Arrows indicate the direction of the difference of the second listed generation relative to the first, and the percentage difference is given. Comparisons involving the F_0 generation are bolded. NA, not applicable; NSD, no significant differences.

[b] Because the F_0 generation was started on dosed feed at 6 weeks of age, data from earlier times were not available for this generation. Therefore, in order to conduct tests of generation effects within dose groups, two sets of statistical analyses were conducted for males: the first included data from week 6 to the end of the experiment for all generations (F_0 to F_4), and the second included all data from birth to the end of the experiment for generations F_1 to F_4. The statistical results reported in this table for weeks 3, 4, and 5 are from the latter analysis, while results from weeks 6 to 19 are from the former analysis. All postweaning data (weaning on PND 21) are included in this table. Preweaning data (birth to PND 21) are tabulated separately (Table D4).

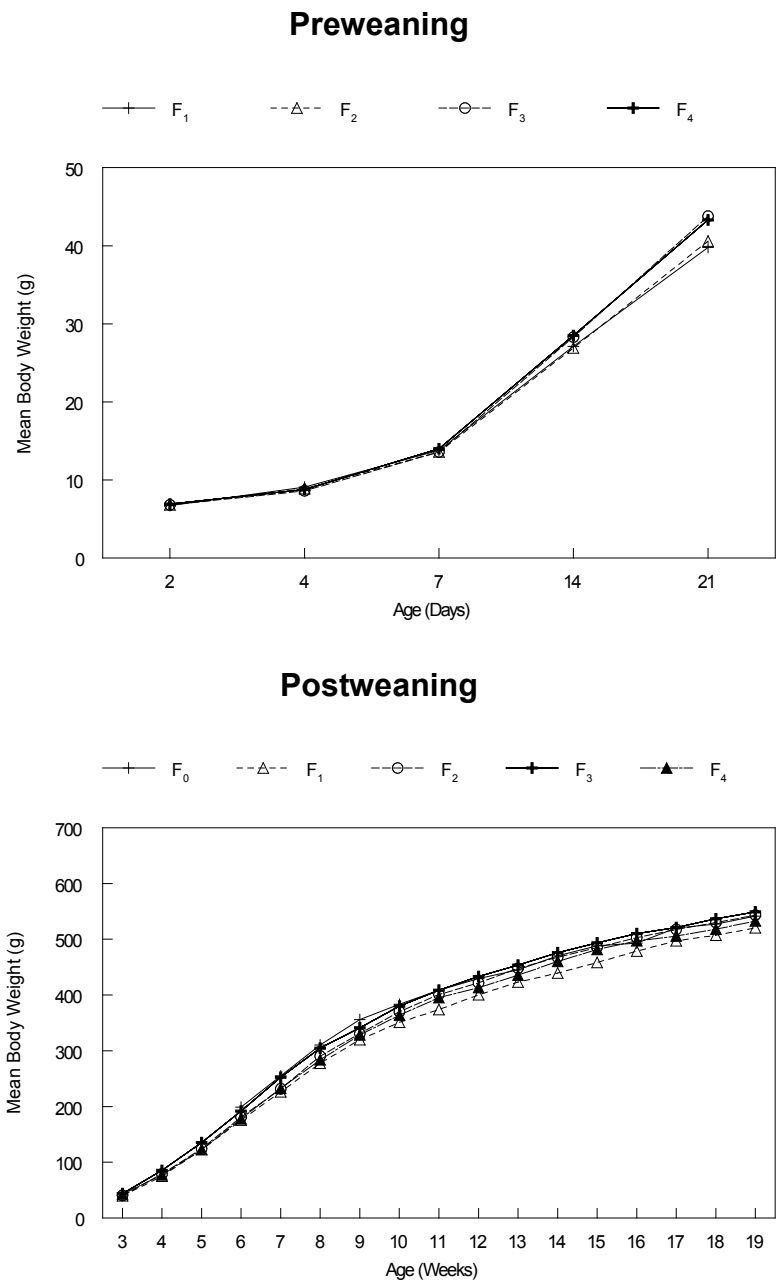

FIGURE D5
Body Weights of 0 ppb Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

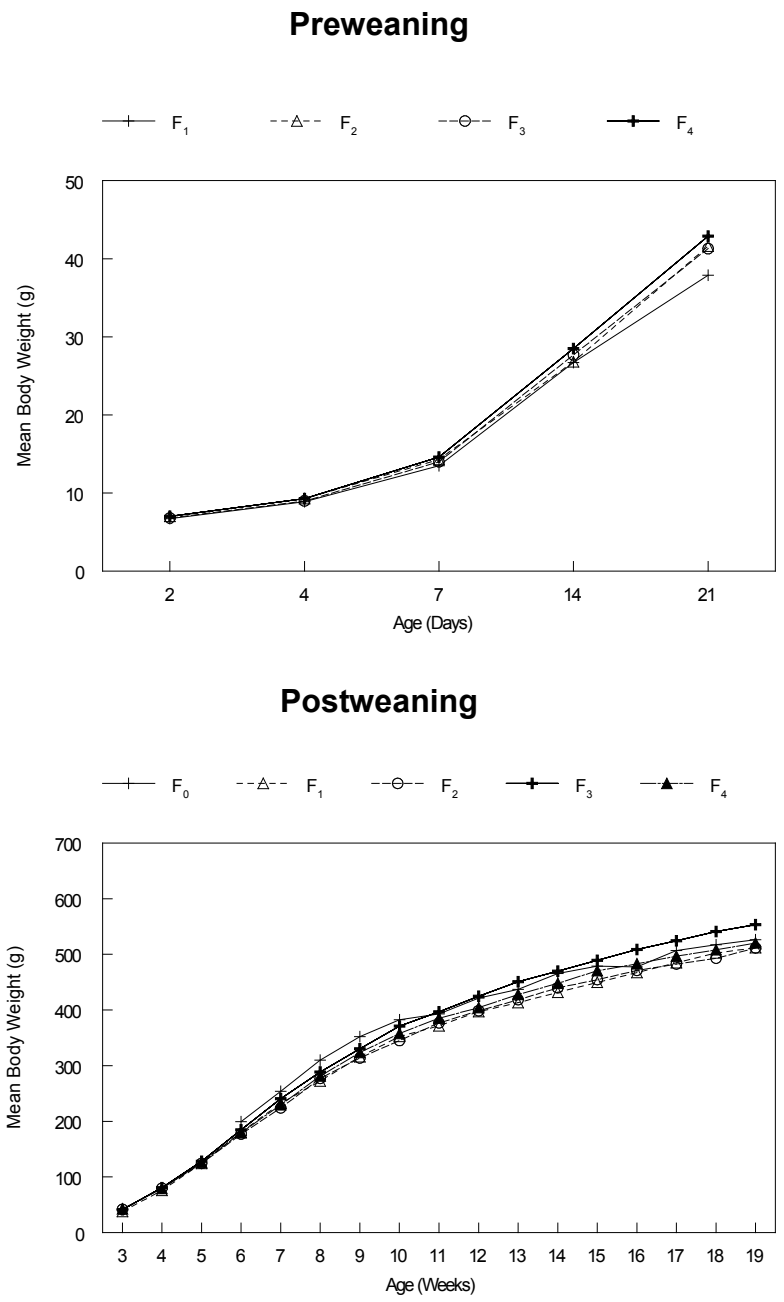

FIGURE D6
Body Weights of 2 ppb Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

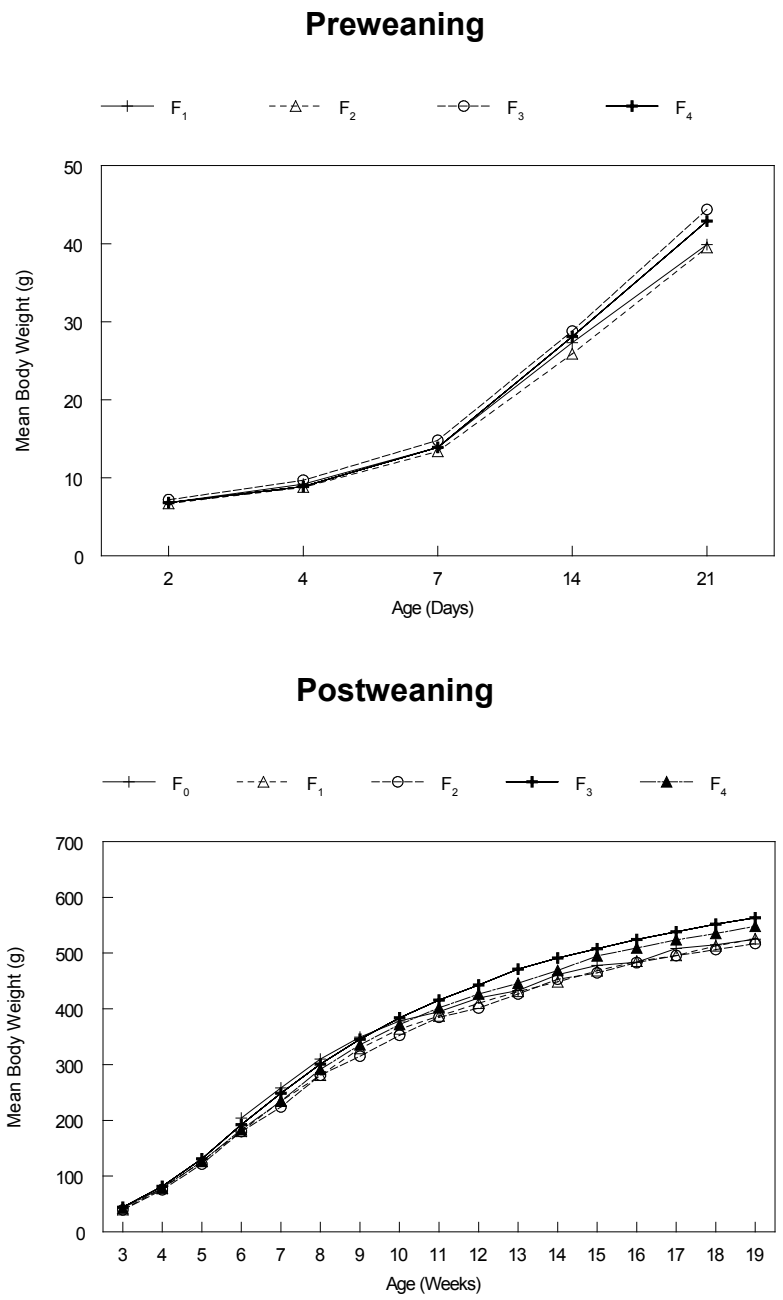

FIGURE D7
Body Weights of 10 ppb Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

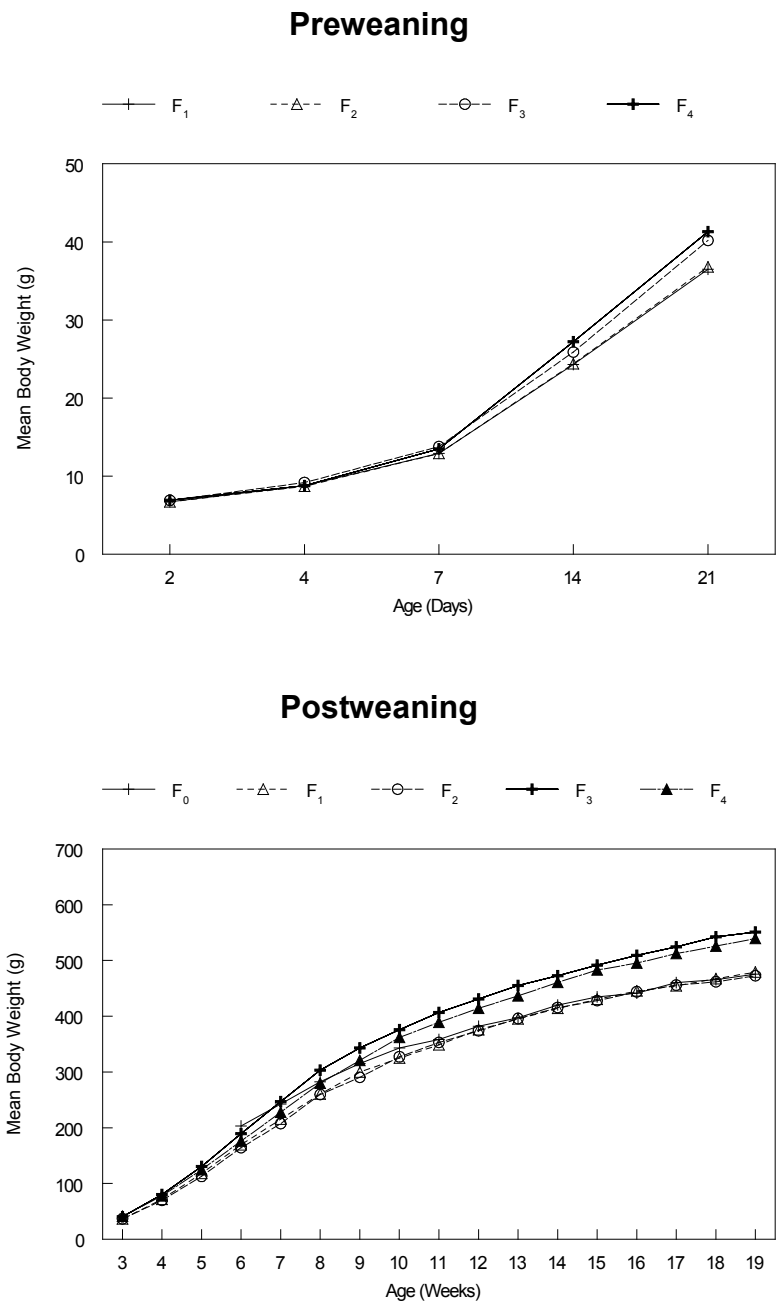

FIGURE D8
Body Weights of 50 ppb Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

APPENDIX E
FEED CONSUMPTION

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TABLE E1a
Predelivery Feed Consumption by F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
8***	21.4 ± 0.7	20.9 ± 0.8	23.0 ± 0.6	23.9 ± 1.2*
9***,#	24.6 ± 1.0	20.9 ± 0.6***	23.0 ± 0.7	27.4 ± 2.0**
10***,##	27.1 ± 1.1	21.2 ± 0.6***	24.5 ± 0.7**	29.5 ± 1.5*
11	24.1 ± 0.8	22.1 ± 0.8*	23.3 ± 0.9	22.2 ± 0.8
13***,#	22.4 ± 1.8	18.6 ± 1.8* (24)	19.6 ± 1.9	24.9 ± 1.9
14	27.0 ± 0.7	23.5 ± 0.6**	24.9 ± 0.4	24.9 ± 0.6
15***	30.3 ± 0.9	29.6 ± 0.8	30.2 ± 0.8	26.2 ± 0.8**
16*	43.9 ± 1.3	43.5 ± 1.2	45.8 ± 1.5	41.4 ± 0.8

[a] Mean daily feed consumption (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test. Asterisks and pound signs in shaded cells in the age column indicate significant linear or quadratic exposure concentration trends, respectively. *, P≤0.05; **, P≤0.01; ***, P≤0.001; #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

[b] Because the F_0 generation entered the experiment at a later age than subsequent generations, data from that generation do not completely overlap data from the F_1 to F_4 generations Therefore, in order to conduct tests of generation effects within dose groups (results shown in Table E6), two sets of statistical analyses were conducted for food consumption for females: the first included data from postnatal week 8 to the start of litter delivery for all generations (F_0 to F_4) and the second included all data from postnatal week 4 to the start of litter delivery for generations F_1 to F_4. The statistical results reported in this table for weeks 4, 5, 6, and 7 are from the latter analysis, while results from postnatal weeks 8 to 16 are from the former analysis. In both analyses, data from postnatal week 12, during which the majority of males and females were paired for mating, were not included. Data from postnatal weeks 19 and 20 (after delivery and nursing of litters) are presented separately (Table E2).

[c] Food consumption data were analyzed using a repeated measures approach to a mixed model ANOVA. Random effects for F_0 breed mother, F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. The ANOVA results for each analysis were as follows:

1) Female feed consumption, postnatal weeks 8 to 16, F_0 to F_4: dose, P=0.002 ; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P = 0.004; weeks × Generation, P<0.001; weeks × dose × Generation, P<0.001. Random effects of the F_0 breed mother and the interaction between the F_0 breed mother and the F_0 breed father were significant at P<0.50 and were incorporated into the model.
2) Female feed consumption, postnatal weeks 4 to 16, F_1 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P=0.097; weeks × Generation, P<0.001; weeks × dose × Generation, P<0.001. Random effects of the F_0 breed father, the F_0 breed mother, and the interaction between the F_0 breed mother and the F_0 breed father were significant at P<0.50 and were incorporated into the model.

TABLE E1b
Predelivery Feed Consumption by F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4*, ##	9.7 ± 0.5 (21)	10.5 ± 0.9 (17)	12.1 ± 0.8* (19)	11.2 ± 0.4* (24)
5	14.1 ± 0.5 (20)	12.7 ± 1.7 (14)	16.1 ± 1.2 (16)	14.6 ± 1.2 (17)
6	17.8 ± 0.5	15.1 ± 0.7**	18.2 ± 1.0	15.4 ± 0.6*
7**	19.3 ± 0.7	17.0 ± 0.3**	18.5 ± 0.5	16.2 ± 0.6***
8	19.1 ± 0.4	17.1 ± 0.3	19.4 ± 0.9	19.4 ± 1.0
9	20.1 ± 0.4	18.9 ± 0.5	19.9 ± 0.5	18.9 ± 0.6
10	21.5 ± 0.6	19.5 ± 0.5*	21.3 ± 0.7	20.8 ± 0.6
11	21.7 ± 0.8 (11)	21.4 ± 1.6 (20)	21.1 ± 0.7 (16)	21.5 ± 1.5 (14)
13	22.9 ± 0.9 (21)	21.9 ± 0.6 (22)	24.3 ± 0.6 (24)	23.0 ± 0.7 (24)
14	25.7 ± 0.6	24.2 ± 0.7	25.5 ± 0.6	25.3 ± 0.9
15	32.6 ± 1.3	28.9 ± 0.9**	33.5 ± 1.1	33.0 ± 1.0
16	43.7 ± 1.1	40.0 ± 1.4*	44.6 ± 0.9	43.5 ± 0.8

The footnotes for this table are defined in Table E1a.

TABLE E1c
Predelivery Feed Consumption by F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4	10.7 ± 0.7 (17)	9.8 ± 0.5 (17)	10.7 ± 0.5 (22)	10.1 ± 0.6 (19)
5	13.9 ± 1.2	13.1 ± 0.5	13.7 ± 0.4	13.0 ± 0.6
6**	16.9 ± 0.4 (24)	17.6 ± 1.2	16.9 ± 0.4	15.7 ± 0.6
7**, #	17.5 ± 0.3	17.7 ± 0.4 (23)	19.0 ± 1.0	16.4 ± 0.6
8*	19.3 ± 1.4	17.9 ± 0.7	17.7 ± 0.3 (24)	16.6 ± 0.4**
9*	19.1 ± 0.3 (24)	18.7 ± 0.5	20.2 ± 0.6	17.2 ± 0.4
10*	19.2 ± 0.5	18.0 ± 0.4	18.2 ± 0.3	16.8 ± 0.4*
11*	18.7 ± 0.3 (24)	19.5 ± 0.4	19.2 ± 0.4	17.5 ± 0.4
13*	18.8 ± 1.2 (19)	18.7 ± 1.4 (13)	16.6 ± 1.0 (15)	15.1 ± 1.2 (16)
14#	21.4 ± 1.4 (23)	21.6 ± 1.5 (19)	23.4 ± 1.2 (22)	21.0 ± 1.0
15*	28.2 ± 0.8	26.4 ± 1.1	26.6 ± 0.8	24.8 ± 0.8*
16*	33.9 ± 1.5	33.6 ± 1.4	33.3 ± 1.0	31.1 ± 0.9

The footnotes for this table are defined in Table E1a.

TABLE E1d
Predelivery Feed Consumption by F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4	9.5 ± 0.3 (19)	10.0 ± 0.4 (19)	9.7 ± 0.4 (22)	9.6 ± 0.3 (21)
5[#]	12.3 ± 0.6	13.6 ± 0.5	15.0 ± 0.6*	14.1 ± 0.7
6	16.3 ± 0.4	17.7 ± 0.4	18.0 ± 0.5	17.5 ± 0.5
7	18.0 ± 0.4	18.5 ± 0.4	18.8 ± 0.5	19.6 ± 0.5
8	19.7 ± 0.4	21.1 ± 0.4	20.4 ± 0.4	21.7 ± 0.6
9	21.2 ± 0.4	22.1 ± 0.5	22.4 ± 0.4	23.3 ± 0.7
10	20.1 ± 0.4	20.7 ± 0.5	20.9 ± 0.4	21.5 ± 0.6
11	21.2 ± 0.5 (24)	21.0 ± 0.5	21.2 ± 0.4	22.2 ± 0.6
13	22.9 ± 0.8 (16)	22.1 ± 0.9 (13)	23.5 ± 0.5 (22)	23.8 ± 0.8 (19)
14	24.6 ± 1.4 (18)	25.1 ± 0.7 (18)	26.6 ± 0.5 (23)	25.6 ± 0.7 (21)
15	23.8 ± 0.8 (24)	24.9 ± 0.9	25.7 ± 0.9	25.7 ± 0.7
16[#]	28.6 ± 0.8	31.9 ± 1.0	33.2 ± 0.8*	30.8 ± 0.9

The footnotes for this table are defined in Table E1a.

TABLE E1e
Predelivery Feed Consumption by F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4###	11.6 ± 0.6 (23)	14.1 ± 0.6* (18)	17.4 ± 0.6*** (23)	13.1 ± 0.5 (22)
5#	14.5 ± 0.5	15.4 ± 0.8	12.7 ± 0.6	14.5 ± 0.5
6	16.8 ± 0.5	17.5 ± 0.5	17.8 ± 0.5	16.6 ± 0.5
7##	17.3 ± 0.5	19.1 ± 0.5**	19.7 ± 0.3*	19.2 ± 0.4
8	18.5 ± 0.3	19.8 ± 0.4	19.7 ± 0.3	19.8 ± 0.6
9	18.8 ± 0.3	19.9 ± 0.4	19.6 ± 0.3	19.3 ± 0.4
10	20.6 ± 0.3	21.3 ± 0.6	20.8 ± 0.4	20.3 ± 0.3 (24)
11	20.2 ± 0.3 (22)	21.0 ± 0.5 (21)	21.8 ± 0.4 (24)	20.8 ± 0.3 (19)
13	21.4 ± 0.7 (17)	21.7 ± 0.6 (16)	21.6 ± 0.6 (19)	20.9 ± 0.6 (13)
14	23.4 ± 0.6 (24)	24.7 ± 0.8 (23)	24.2 ± 0.6 (24)	23.4 ± 0.6 (23)
15**	25.8 ±1.1	26.9 ± 0.6	27.4 ± 0.9	23.5 ± 0.7
16	34.9 ± 1.3	30.7 ± 0.9*	33.3 ± 1.2	33.9 ± 1.2

The footnotes for this table are defined in Table E1a.

TABLE E2
Feed Consumption by Female Rats during Postnatal Weeks 19 and 20 in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Gen[b]	Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
F_0	19	25.3 ± 0.8 [2,3]	20.5 ± 0.5 [2,3,4]	21.7 ± 0.6 [2,3,4]	23.0 ± 0.8 (24) [2,3,4]
	20	21.5 ± 0.8 (5)	21.1 ± 2.6 (5)	22.0 ± 1.5 (5)	24.3 ± 1.9 (5)
F_1	19	23.8 ± 1.4 [2,3,4]	22.7 ± 1.9 [2,3,4]	21.4 ± 0.5 [2,3,4]	18.4 ± 0.7 [2,3,4]
	20	20.7 ± 0.5 [3]	20.0 ± 0.5 [3]	20.4 ± 0.4	20.4 ± 0.4
F_2	19	36.7 ± 2.9 [0,1]	39.2 ± 3.4 [0,1]	39.1 ± 3.1 [0,1,3,4]	33.4 ± 3.6 [0,1]
	20	21.5 ± 0.8 [3]	22.5 ± 0.5	20.3 ± 0.7	20.2 ± 0.9
F_3	19	35.2 ± 2.6 [0]	36.8 ± 2.7 [0,1]	31.1 ± 1.9 [0,1,2]	35.6 ± 2.1 [0,1]
	20#	25.9 ± 2.3 [1,2,4]	25.6 ± 2.3 [1]	22.7 ± 0.7*	23.4 ± 0.8
F_4	19	32.6 ± 2.3	40.1 ± 2.8 [0,1]	31.1 ± 2.0 [0,1,2]	36.6 ± 2.4 [0,1]
	20	21.8 ± 0.7 [3]	22.8 ± 0.6	23.4 ± 0.8	22.5 ± 0.8

[a] Mean daily food consumption ± standard error for the weeks indicated. Twenty-five animals in each group except where indicated by number in parentheses. Data from weeks 17 and 18 during the lactation period were not included since the data were hightly variable due to the pups disturbing the feed. Data from postnatal weeks 19 and 20 were analyzed by ANOVA with dose, generation, and Time (weeks) as factors. The random effect for the F_0 breed father and the interaction of F_0 breed father with F_0 breed mother were significant in a log-likelihood test at P≤0.50 and were incorporated into the statistical model. The overall ANOVA results were as follows: dose, P=0.157; generation, P<0.001; and dose × generation, P=0.114; weeks, P<0.001; weeks × dose, P=0.320; weeks × generation, P<0.001; weeks × dose × generation, P=0.162. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicated the generations whose means are significantly different from the given mean value at P≤0.05. The pound sign indicates a significant quadratic exposure concentration trend #, P<0.05; and the asterisk indicates a significant difference (Dunnett's test, *, P<0.05) between an exposure group and the controls for that generation and week.

[b] Gen = generation

TABLE E3
Predelivery Total Feed Consumption by Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generations covered[b]	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
$F_0 – F_4$[c] Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0	1544.9 ± 27.8 [1,2,3,4]	1396.3 ± 26.9*** [2,3,4]	1499.7 ± 26.3 [2,3,4]	1543.2 ± 41.2 [1,2,3,4]
	F_1#	1340.9 ± 26.6 [0,2,3,4]	1295.0 ± 23.9 [2]	1408.0 ± 21.5 [2,4]	1365.2 ± 24.6 [0,2,4]
	F_2*	1195.3 ± 30.6 [0,1]	1122.3 ± 39.6 [0,1]	1156.0 ± 30.6 [0,1,3,4]	1082.4 ± 28.5* [0,1,3]
	F_3*, ###	1155.7 ± 33.6 [0,1]	1198.7 ± 28.8 [0]	1322.7 ± 27.8*** [0,2]	1293.5 ± 31.3** [0,2,4]
	F_4*	1213.5 ± 29.7 [0,1]	1210.8 ± 33.2 [0]	1269.5 ± 28.7 [0,1,2]	1149.3 ± 35.9 [0,1,3]
$F_1 – F_4$[d] Dose P<0.001 Gen P<0.001 DxG P=0.002	F_1#	1737.0 ± 32.1 [2,3]	1619.4 ± 31.6	1801.8 ± 32.7 [2]	1731.9 ± 28.5 [2,4]
	F_2*	1580.3 ± 35.5 [1]	1497.6 ± 45.6 [4]	1569.5 ± 37.4 [1,3,4]	1451.8 ± 38.9* [1,3,4]
	F_3*, ###	1532.5 ± 39.7 [1]	1600.4 ± 35.5	1744.6 ± 36.7*** [2]	1708.2 ± 41.0** [2,4]
	F_4#	1628.6 ± 30.7	1645.7 ± 44.4 [2]	1732.0 ± 37.0 [2]	1582.1 ± 41.0 [1,2,3]

[a] Total feed consumed per animal (g) ± standard error in the period before litters were delivered. Time period covered: weeks 8 to 16 (F_0 to F_4) and weeks 4 to 16 (F_1 to F_4). Week 12 was excluded for both analyses since males and females were paired at that time. Twenty-five animals in each group. Asterisks in shaded cells in an exposure group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. An asterisk in a shaded cell in the generation column indicates a significant (P≤0.05) linear exposure concentration trend. Pound signs indicate significant quadratic exposure concentration trends #, P≤0.05; ###, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05. Because the F_0 animals were started on the experiment at a later age than were the subsequent generations, some data are missing for the F_0 generation, and two separate analyses covering the overlapping periods of generations F_0 to F_4 and the overlapping periods of F_1 to F_4 were conducted.

[b] Results of two-way ANOVA with main effects generation (Gen), dose, and dose × generation interaction (D × G) are indicated.

[c] ANOVA results for the F_0 to F_4 analysis are indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father are significant at P≤0.50 and were incorporated into the model.

[d] ANOVA results for the F_1 to F_4 analysis are indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father are significant at P≤0.50 and were incorporated into the model.

TABLE E4a
Feed Consumption by F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
7	25.6 ± 0.5 (24)	24.0 ± 0.3	24.9 ± 0.5	23.9 ± 0.8
8***,#	31.6 ± 1.0 (24)	27.3 ± 0.5***	29.0 ± 0.6**	32.5 ± 1.2
9***	30.6 ± 0.9 (24)	29.0 ± 1.0*	30.1 ± 0.7	32.9 ± 1.0*
10***	28.7 ± 0.7 (24)	27.2 ± 0.5	27.9 ± 0.4	30.7 ± 0.9
13**	27.7 ± 0.9 (24)	25.8 ± 0.9	26.9 ± 0.7	29.6 ± 0.9
14#	32.8 ± 0.7 (24)	31.1 ± 0.7	33.7 ± 1.0	30.8 ± 0.7
15	27.4 ± 1.0 (24)	25.8 ± 0.4	27.6 ± 1.6	25.9 ± 0.7
16	25.4 ± 0.7 (24)	23.3 ± 0.9*	24.9 ± 0.7	24.6 ± 0.6
17	30.7 ± 0.8 (24)	27.4 ± 0.6***	29.4 ± 0.5	29.8 ± 1.1
18***,#	28.8 ± 0.7 (24)	26.2 ± 0.5***	26.0 ± 0.5***	24.6 ± 0.4***
19***,###	32.1 ± 0.8 (24)	28.3 ± 0.8***	25.9 ± 0.5***	26.4 ± 0.5***
20	28.9 ± 1.1 (5)	24.7 ± 0.4* (5)	27.3 ± 1.2 (5)	28.7 ± 1.6 (5)

[a] Mean daily feed consumption (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in exposed group columns indicate significant difference from controls at the same age in the same generation as determined by Dunnett's test. Asterisks and pound signs in shaded cells in age column indicate significant linear or quadratic exposure concentration trends, respectively *, P≤0.05; **, P≤0.01; ***, P≤0.001; #, P≤0.05; ##, P≤0.01; ###, P≤0.001

[b] Because the F_0 generation entered the experiment at a later age than subsequent generations, data from that generation do not completely overlap data from the F_1 to F_4 generations Therefore, in order to conduct tests of generation effects within dose groups (results shown in Table E7), two sets of statistical analyses were conducted for feed consumption for males: the first included data from postnatal week 7 to the end of the experiment for all generations (F_0 to F_4), and the second included all data from postnatal week 4 to the end of the experiment for generations F_1 to F_4. The statistical results reported in these tables for weeks 4, 5, and 6 are from the latter analysis, while results from week 7 to 20 are from the former analysis. In both analyses, data from postnatal week 12, during which males and females were paired for mating, were not included.

[c] Food consumption data were analyzed using a repeated measures approach to a mixed model ANOVA. Random effects for F_0 breed mother, F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. In the case of feed consumption of the males, both analyses incorporated significant random effects for F_0 breed mother, F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father. The ANOVA results for each analysis were as follows:

1) Male food consumption, postnatal weeks 7 to 20, F_0 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P<0.001; weeks, P<0.001; weeks × dose, P=0.001; weeks × Generation, P<0.001 ; weeks × dose × Generation, P<0.001.
2) Male food consumption, postnatal week 4 to postnatal week 20, F_1 to F_4: dose, P<0.001; Generation, P<0.001; dose × Generation, P=0.006; weeks, P<0.001; weeks × dose, P<0.001; weeks × Generation, P<0.001; weeks × dose × generation, P<0.001.

TABLE E4b
Feed Consumption by F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4[#]	15.9 ± 2.1 (10)	16.4 ± 2.4 (5)	12.4 ±1.1 (10)	13.6 ± 1.0 (8)
5	15.5 ± 1.0	12.3 ± 0.7**	14.9 ± 0.5	15.7 ± 0.8
6*, [#]	20.2 ± 0.5	19.3 ± 0.8	21.7 ± 0.8	21.9 ± 1.1
7*	22.9 ± 0.6	21.6 ± 0.6	23.2 ± 0.9	24.3 ± 1.3
8	25.1 ± 0.6	23.9 ± 0.5	24.3 ± 0.4	23.6 ± 0.8
9	27.3 ± 0.4	23.7 ± 0.4***	25.2 ± 0.4	25.5 ± 0.9
10	29.1 ± 0.5	26.7 ± 0.3*	28.7 ± 0.5	27.5 ± 1.0
11***, [# #]	30.4 ± 0.8 (16)	28.5 ± 0.4 (20)	30.8 ± 0.6	26.3 ± 0.6*** (22)
13	27.0 ± 0.6 (20)	25.6 ± 0.5 (19)	25.6 ± 0.5 (22)	25.8 ± 0.8 (22)
14	26.1 ± 0.6 (24)	24.1 ± 0.6	24.9 ± 0.9	24.1 ± 0.5 (24)
15	25.9 ± 0.5	24.3 ± 0.5	25.1 ± 0.5	24.7 ± 0.5
16	26.2 ± 0.4	24.7 ± 0.5	26.5 ± 0.4	25.3 ± 0.6
17	26.0 ± 0.4	25.1 ± 0.4	26.0 ± 0.6	25.2 ± 0.6
18*	27.0 ± 0.6	26.0 ± 0.4	25.4 ± 0.7	25.1 ± 0.6
19[#]	26.5 ± 0.7	26.9 ± 0.5	27.8 ± 0.4	25.7 ± 0.5
20	26.1 ± 0.5	25.3 ± 0.6	26.3 ± 0.5	24.8 ± 0.6

The footnotes for this table are defined in Table E4a.

TABLE E4c
Feed Consumption by F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4	9.2 ± 1.8 (5)	8.9 ± 0.8 (14)	10.7 ± 0.4 (11)	9.9 ± 0.7 (11)
5	12.2 ± 0.5	12.3 ± 0.6	13.4 ± 0.6	12.1 ± 0.7
6	18.4 ± 0.4	17.3 ± 0.4	18.6 ± 0.5	17.4 ± 0.6
7	21.1 ± 0.3	20.1 ± 0.5	21.6 ± 0.5	19.8 ± 0.4
8	23.4 ± 0.3	22.5 ± 0.8	23.0 ± 0.4	21.7 ± 0.6
9***	25.3 ± 0.5	23.3 ± 0.4	24.6 ± 0.4	22.0 ± 0.8**
10	25.9 ± 0.4	22.6 ± 0.6**	23.9 ± 0.3	24.7 ± 1.0
11	25.9 ± 0.5	24.9 ± 0.8	25.4 ± 0.5	25.4 ± 0.6
13*	26.3 ± 1.2 (18)	25.1 ± 1.1 (15)	24.4 ± 1.9 (10)	22.8 ± 1.1 (19)
14*	28.6 ± 0.6 (23)	26.0 ± 0.7* (22)	27.6 ± 1.2 (20)	25.6 ± 0.9** (21)
15*	30.0 ± 0.9	27.0 ± 0.7*	28.1 ± 0.7	26.4 ± 0.7**
16	25.5 ± 0.9	24.7 ± 0.5	25.1 ± 0.5	25.0 ± 0.6
17*	27.1 ± 0.5	26.2 ± 1.4	26.2 ± 0.5	25.0 ± 0.5
18*	26.7 ± 0.5	23.5 ± 0.6** (24)	24.7 ± 0.7	23.6 ± 0.7***
19	24.2 ± 0.6	23.1 ± 0.7	23.8 ± 0.6	22.6 ± 0.6
20	26.3 ± 0.4 (24)	25.4 ± 0.5 (21)	25.3 ± 0.5 (22)	25.5 ± 1.1 (20)

The footnotes for this table are defined in Table E4a.

TABLE E4d
Feed Consumption by F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4#	11.0 ± 0.5 (7)	11.3 ± 0.6 (10)	13.2 ± 1.5 (17)	11.3 ± 0.4 (8)
5	13.1 ± 0.6	13.7 ± 0.6	15.0 ± 0.6	13.8 ± 0.6
6##	19.4 ± 0.5	18.6 ± 0.7	21.5 ± 0.6	19.0 ± 0.5
7	23.3 ± 0.4	23.0 ± 0.4	23.6 ± 0.4	23.0 ± 0.4
8	24.5 ± 0.4	24.1 ± 0.4	24.7 ± 0.4	24.4 ± 0.4
9	25.2 ± 0.5	25.0 ± 0.5	26.4 ± 0.4	25.7 ± 0.4
10	26.1 ± 0.4	26.2 ± 0.8	27.4 ± 0.5	26.4 ± 0.4
11	27.3 ± 0.4	27.5 ± 0.5	26.6 ± 0.5	26.6 ± 0.5
13*	21.8 ± 1.4 (12)	27.1 ± 3.6* (12)	25.2 ± 0.6 (19)	21.8 ± 1.1 (15)
14#	26.7 ± 0.6 (20)	27.3 ± 0.4 (19)	28.6 ± 0.6 (23)	27.5 ± 0.7 (21)
15	26.0 ± 0.6	27.3 ± 0.7	27.7 ± 0.6	26.6 ± 0.7
16	27.2 ± 0.5	27.4 ± 0.6	27.0 ± 0.5	27.1 ± 0.4
17	26.6 ± 0.4	28.0 ± 0.5	27.0 ± 0.5	26.4 ± 0.5
18	26.0 ± 0.4	27.6 ± 0.5	26.8 ± 0.5	26.1 ± 0.4
19	25.2 ± 0.5	27.9 ± 0.4**	25.7 ± 0.9	26.0 ± 0.5
20	27.4 ± 0.5	29.5 ± 1.1*	28.2 ± 0.4	27.2 ± 0.5

The footnotes for this table are defined in Table E4a.

TABLE E4e
Feed Consumption by F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b,c]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
4	11.0 ± 0.4 (18)	10.6 ± 0.7 (9)	12.7 ± 0.6 (13)	11.4 ± 0.9 (10)
5	15.5 ± 1.3	13.2 ± 0.5*	15.1 ± 0.5	14.0 ± 0.4
6[##]	18.8 ± 0.5	17.2 ± 0.5	20.0 ± 0.5	17.0 ± 0.7
7	23.2 ± 0.5	21.1 ± 0.7*	21.6 ± 0.9	21.4 ± 1.0
8*,[##]	25.0 ± 0.3	24.1 ± 0.6	26.4 ± 0.8	23.4 ± 0.5
9*	27.1 ± 0.6	26.6 ± 0.7	27.2 ± 0.5	25.5 ± 0.5
10	27.2 ± 0.5	27.4 ± 0.6	27.6 ± 0.4	27.3 ± 0.7
11	27.0 ± 0.3	25.5 ± 0.6	27.5 ± 0.5	26.4 ± 0.5
13	27.1 ± 0.5 (14)	27.5 ± 0.9 (13)	26.3 ± 0.6 (14)	26.0 ± 0.7 (11)
14*	26.7 ± 0.5 (21)	27.2 ± 0.7 (23)	25.7 ± 0.5 (22)	24.9 ± 0.6 (21)
15	26.9 ± 0.5	26.5 ± 0.8	27.5 ± 0.7	26.8 ± 0.7
16[#]	27.3 ± 0.5	27.5 ± 0.6	29.0 ± 0.8	28.1 ± 1.2
17***	26.3 ± 0.5	26.3 ± 0.7	26.0 ± 0.5	23.1 ± 0.6***
18[#]	25.8 ± 0.6	27.8 ± 0.5*	27.9 ± 0.6*	27.0 ± 0.6
19	26.1 ± 0.6	26.5 ± 0.8	27.0 ± 0.5	26.8 ± 0.5
20	27.0 ± 0.4	27.3 ± 0.8	27.8 ± 0.4	27.2 ± 0.6

The footnotes for this table are defined in Table E4a.

TABLE E5
Total Feed Consumption by Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generations covered	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
$F_0 – F_4$[c] Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0	2291.4 ± 46.3 (24) [2,3,4]	2102.9 ± 31.0*** [2]	2175.9 ± 32.4* [2]	2222.0 ± 44.0 [1,2,3,4]
	F_1	2161.3 ± 31.9	2041.7 ± 20.1 [2]	2142.0 ± 24.8 [2]	2083.6 ± 42.0 [0,2]
	F_2**,#	2098.1 ± 31.2 [0]	1898.8 ± 34.5*** [0,1,3,4]	1926.1 ± 32.8** [0,1,3,4]	1891.3 ± 42.9*** [0,1,3,4]
	F_3##	2026.3 ± 32.9 [0]	2099.5 ± 46.1 [2]	2169.9 ± 32.3 [2]	2065.5 ± 41.7 [0,2]
	F_4*	2096.9 ± 39.7 [0]	2103.4 ± 54.8 [2]	2137.4 ± 31.6 [2]	2023.6 ± 30.8 [0,2]
$F_1 – F_4$[d] Dose P<0.001 Gen P<0.001 DxG P=0.015	F_1	2592.1 ± 39.8	2445.4 ± 25.5*	2631.7 ± 32.0 [2]	2539.3 ± 54.0 [2]
	F_2*	2506.8 ± 36.0	2314.5 ± 38.8* [3,4]	2360.8 ± 42.0 [1,3,4]	2306.0 ± 50.0** [1,3]
	F_3###	2466.3 ± 41.4	2550.1 ± 53.2 [2]	2674.2 ± 45.1** [2]	2506.2 ± 51.5 [2]
	F_4	2574.1 ± 42.4	2521.0 ± 65.9 [2]	2622.0 ± 39.7 [2]	2457.2 ± 40.8

[a] Total feed consumed per animal (g) ± standard error. Time period covered: weeks 7 to 20 (F_0 to F_4) and weeks 4 to 20 (F_1 to F_4). Week 12 was excluded from both analyses since males and females were paired at that time. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks in shaded cells in the exposed group columns indicate significant differences from controls at the same age in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Asterisks and pound signs in shaded cells in the generation column indicate significant linear or quadratic exposure concentration trends. *, P≤0.05; **, P≤0.01; ***, P≤0.001; #, P≤0.05; ##, P≤0.01; ###, P≤0.001 Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05. Because the F_0 animals were started on the experiment at a later age than were the subsequent generations, some data were missing for the F_0 generation and two separate analyses covering the overlapping periods of generations F_0 to F_4 and the overlapping periods of F_1 to F_4 were conducted.

[b] Results of two-way ANOVA with main effects generation (Gen), dose, and dose × generation interaction (D × G) are indicated.

[c] ANOVA results for the F_0 to F_4 analysis are indicated. The random effect for the interaction between the F_0 breed mother and F_0 breed father is significant at P≤0.50 and was incorporated into the model.

[d] ANOVA results for the F_1 to F_4 analysis are indicated. Random effects for the F_0 breed mother, and the interaction between the F_0 breed mother and F_0 breed father are significant at P≤0.50 and were incorporated into the model.

TABLE E6
Generational Effects in Predelivery Feed Consumption by Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
4	NA	NSD	NA	1v4** ↑34% 2v4*** ↑44% 3v4** ↑41%	NA	1v4*** ↑44% 2v4*** ↑63% 3v4*** ↑79%	NA	2v4** ↑30% 3v4*** ↑36%
5	NA	NSD	NA	NSD	NA	NSD	NA	NSD
6	NA	NSD	NA	NSD	NA	NSD	NA	NSD
7	NA	NSD	NA	NSD	NA	NSD	NA	1v3* ↑21% 2v3** ↑20% 2v4* ↑17%
8	**0v4* ↓14%**	NSD	**0v1*** ↓18%** **0v2*** ↓14%** 1v3*** ↑23% 1v4* ↑16% 2v3** ↑18%	1v3*** ↑23%	**0v1** ↓16%** **0v2*** ↓23%** **0v3* ↓11%** **0v4** ↓14%**	NSD	**0v1*** ↓19%** **0v2*** ↓31%** **0v3* ↓9%** **0v4*** ↓17%** 1v2* ↓14% 2v3*** ↑31% 2v4** ↑19%	2v3*** ↑31% 2v4* ↑19%
9	**0v1*** ↓18%** **0v2*** ↓22%** **0v3** ↓14%** **0v4*** ↓24%**	3v4* ↓11%	1v3** ↑17% 2v3** ↑18%	1v3*** ↑17% 2v3*** ↑18%	**0v1* ↓13%** **0v2* ↓12%** **0v4* ↓15%** 3v4* ↓13%	1v3* ↑13% 3v4* ↓13%	**0v1*** ↓31%** **0v2*** ↓37%** **0v3*** ↓15%** **0v4*** ↓30%** 1v3*** ↑23% 2v3*** ↑35% 3v4*** ↓17%	1v3*** ↑23% 2v3*** ↑35% 3v4*** ↓17%
10	**0v1*** ↓21%** **0v2*** ↓29%** **0v3** ↓26%** **0v4*** ↓24%**	NSD	**0v2** ↓15%** 2v3** ↑15% 2v4** ↑18%	2v3* ↑15% 2v4** ↑18%	**0v1** ↓13%** **0v2*** ↓26%** **0v3** ↓15%** **0v4*** ↓15%** 1v2** ↓15% 2v3* ↑15% 2v4* ↑14%	1v2** ↓15% 2v4* ↑14%	**0v1*** ↓29%** **0v2*** ↓43%** **0v3*** ↓27%** **0v4*** ↓31%** 1v2*** ↓19% 2v3*** ↑28% 2v4*** ↑21%	1v2*** ↓19% 2v3*** ↑28% 2v4*** ↑21%
11	**0v2*** ↓22%** **0v3** ↓12%** **0v4*** ↓16%**	NSD	**0v2* ↓12%**	NSD	**0v2*** ↓18%** 2v4* ↑14%	NSD	**0v2*** ↓21%** 1v2*** ↓19% 2v3*** ↑27% 2v4** ↑19%	1v2*** ↓19% 2v3*** ↑27% 2v4* ↑19%
13	NSD	1v2* ↓18%	NSD	NSD	**0v1* ↑24%** 1v2*** ↓32% 2v3*** ↑42% 2v4* ↑30%	1v2*** ↓32% 2v3*** ↑42% 2v4** ↑30%	**0v2*** ↓39%** 1v2*** ↓34% 2v3*** ↑58% 2v4* ↑38%	1v2*** ↓34% 2v3*** ↑58% 2v4*** ↑38%
14	**0v2*** ↓21%** **0v4* ↓13%** 1v2*** ↓17% 2v3* ↑15%	NSD	NSD	NSD	NSD	NSD	**0v2** ↓16%** 1v2*** ↓17% 2v3*** ↑22%	1v2* ↓17% 2v3* ↑22%
15	**0v3*** ↓21%** **0v4** ↓15%** 1v2** ↓13% 1v3*** ↓27% 1v4*** ↓21% 2v3** ↓16%	1v3*** ↓27% 1v4*** ↓21%	**0v3** ↓16%**	NSD	**0v1* ↑11%** **0v2* ↓12%** **0v3** ↓15%** 1v2*** ↓21% 1v3*** ↓23% 1v4*** ↓18%	1v2*** ↓21% 1v3*** ↓23% 1v4*** ↓18%	**0v1*** ↑26%** 1v2*** ↓25% 1v3*** ↓22% 1v4*** ↓29%	1v2*** ↓25% 1v3*** ↓22% 1v4*** ↓29%

TABLE E6
Generational Effects in Predelivery Feed Consumption by Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
16	**0v2*** ↓23%** **0v3** ↓35%** **0v4*** ↓21%** 1v2*** ↓22% 1v3*** ↓35% 1v4*** ↓20% 2v3** ↓16% 3v4*** ↑22%	1v2*** ↓22% 1v3*** ↓35% 1v4*** ↓20% 3v4*** ↑22%	**0v2*** ↓23%** **0v3*** ↓27%** **0v4*** ↓29%** 1v2*** ↓16% 1v3*** ↓20% 1v4*** ↓23%	1v2* ↓16% 1v3*** ↓20% 1v4*** ↓23%	**0v2*** ↓27%** **0v3*** ↓28%** **0v4*** ↓27%** 1v2*** ↓25% 1v3*** ↓26% 1v4*** ↓25%	1v2*** ↓25% 1v3*** ↓26% 1v4*** ↓25%	**0v2*** ↓25%** **0v3*** ↓26%** **0v4*** ↓18%** 1v2*** ↓29% 1v3*** ↓29% 1v4*** ↓22%	1v2*** ↓29% 1v3*** ↓29% 1v4*** ↓22%

[a] Results of Holm's adjusted t-tests of feed concumption differences are indicated. Only comparisons showing significant differences between generations within an exposure group are shown. generations are indicated by their subscripts, so that "0v1" means F_0 versus F_1. Asterisks indicate the level of significance: *, P≤0.05; **, P≤0.01; ***, P≤0.001). Arrows indicate the direction of the difference of the second listed generation relative to the first, and the percentage difference is given. Comparisons involving the F_0 generation are bolded. NA, not applicable; NSD, no significant differences.

[b] Because the F_0 generation entered the experiment at a later age than subsequent generations, data from that generation do not completely overlap data from the F_1 to F_4 generations. Therefore, in order to conduct tests of generation effects within exposure groups, two sets of statistical analyses were conducted for feed consumption for females: the first included data from postnatal week 8 to the start of litter delivery for all generations (F_0 to F_4), and the second included all data from postnatal week 4 to the start of litter delivery for generations F_1 to F_4. The statistical results reported in this table for postnatal weeks 4, 5, 6, and 7 are from the latter analysis, while results from weeks 8 to 16 are from the former analysis. In both analyses, data from postnatal week 12, during which the majority of males and females were paired for mating, were not included.

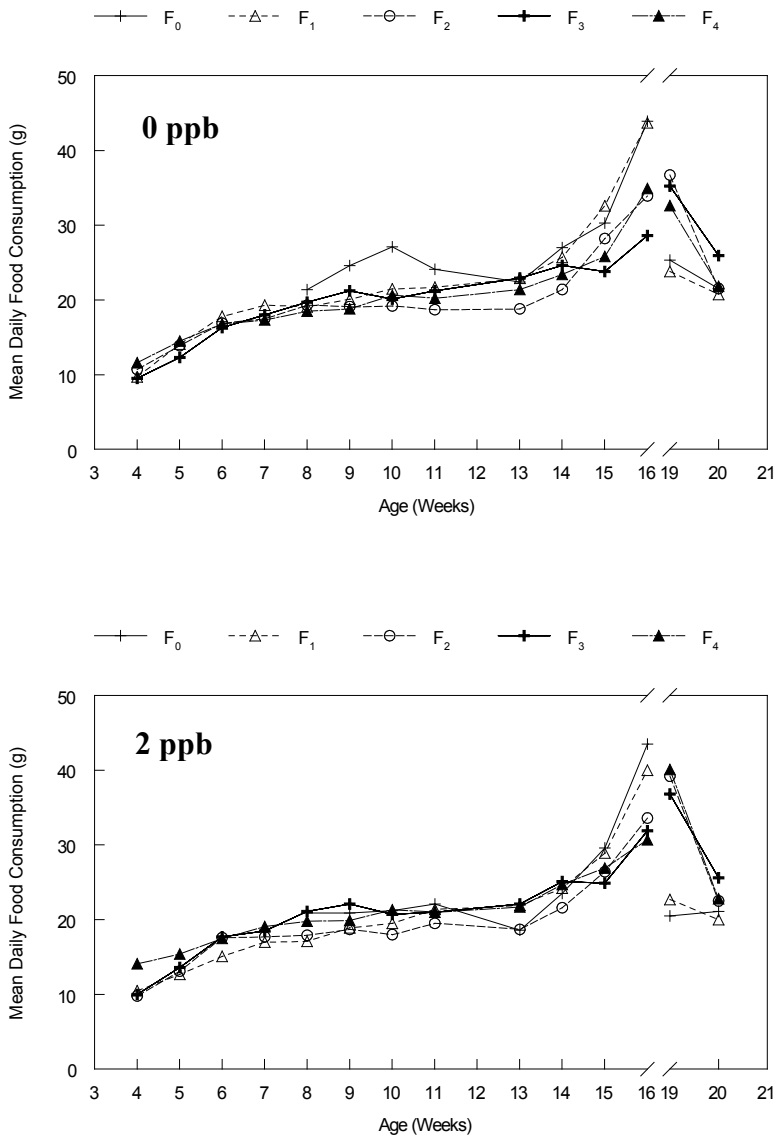

FIGURE E1
Feed Consumption by 0 and 2 ppb Female Rats
in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

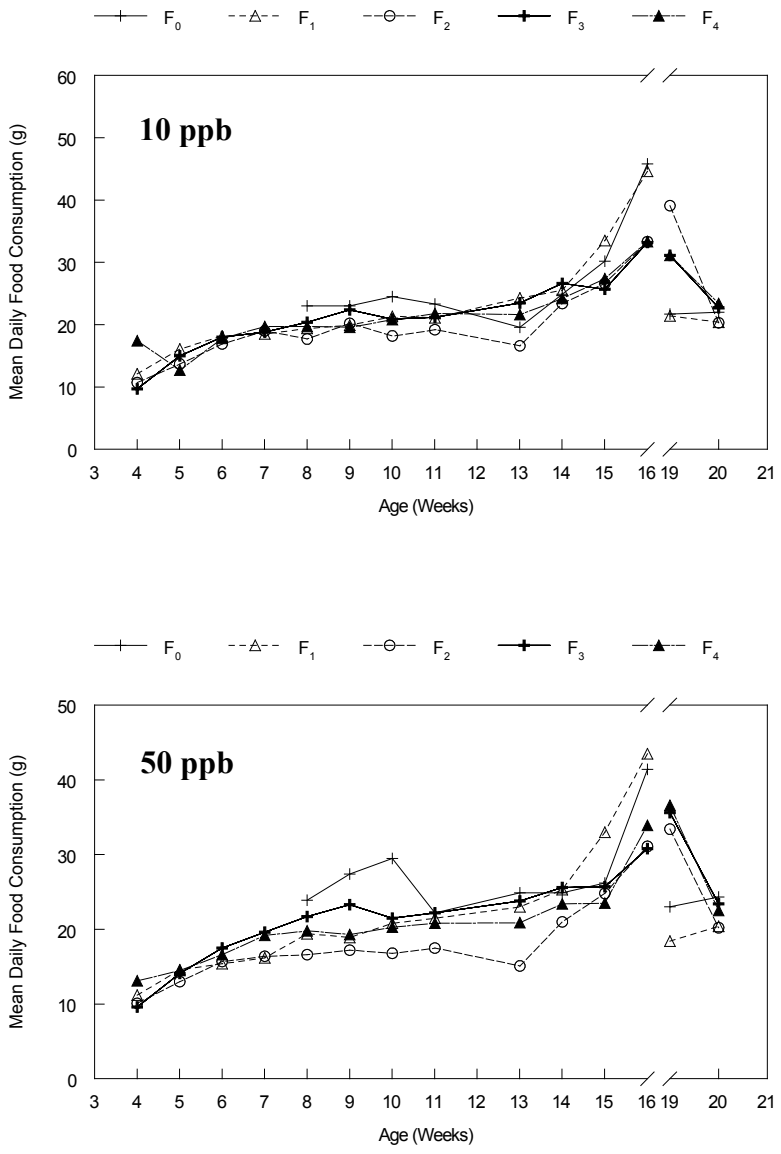

FIGURE E2
Feed Consumption by 10 and 50 ppb Female Rats
in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

TABLE E7
Generational Effects in Predelivery Feed Consumption by Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
4	NA	1v2** ↓42% 1v3** ↓31% 1v4** ↓31%	NA	1v2*** ↓46% 1v3** ↓31% 1v4** ↓35%	NA	NSD	NA	1v2* ↓27%
5	NA	1v2** ↓21% 2v4** ↑27% 3v4* ↑18%	NA	NSD	NA	NSD	NA	1v2** ↓23%
6	NA	NSD	NA	NSD	NA	1v2** ↓14% 2v3* ↑16%	NA	1v2*** ↓21% 1v3** ↓13%
7	**0v1** ↓11%** **0v2*** ↓18%** **0v3* ↓9%** **0v4* ↓9%** 2v4* ↑10%	NSD	**0v2*** ↓16%** **0v4* ↓12%** 2v3* ↑14%	2v3* ↑14%	**0v2** ↓13%** **0v4** ↓13%**	NSD	**0v2*** ↓17%** **0v4* ↓10%** 1v2*** ↓19% 1v4** ↓12% 2v3** ↑16%	1v2*** ↓19% 1v4** ↓12% 2v3** ↑16%
8	**0v1*** ↓21%** **0v2*** ↓26%** **0v3*** ↓22%** **0v4*** ↓21%**	NSD	**0v1** ↓12%** **0v2*** ↓18%** **0v3*** ↓12%** **0v4** ↓12%**	NSD	**0v1*** ↓16%** **0v2*** ↓21%** **0v3*** ↓15%** **0v4* ↓9%** 1v4* ↑9% 2v4*** ↑15% 3v4* ↑7%	1v4* ↑9% 2v4*** ↑15% 3v4* ↑7%	**0v1*** ↓27%** **0v2*** ↓33%** **0v3*** ↓25%** **0v4*** ↓28%** 2v3* ↑12%	2v3** ↑12%
9	**0v1*** ↓11%** **0v2*** ↓17%** **0v3*** ↓18%** **0v4*** ↓11%**	1v3* ↓8% 2v4* ↑7% 3v4* ↑8%	**0v1*** ↓18%** **0v2*** ↓20%** **0v3*** ↓14%** 1v4** ↑12% 2v4** ↑14%	1v4*** ↑12% 2v4*** ↑14% 3v4* ↑6%	**0v1*** ↓16%** **0v2*** ↓18%** **0v3*** ↓12%** **0v4** ↓10%** 2v4** ↑11%	1v4* ↑8% 2v4** ↑11%	**0v1*** ↓22%** **0v2*** ↓33%** **0v3*** ↓22%** **0v4*** ↓22%** 1v2*** ↓14% 2v3*** ↑17% 2v4*** ↑16%	1v2*** ↓14% 2v3*** ↑17% 2v4*** ↑16%
10	**0v2** ↓10%** **0v3** ↓9%** 1v2** ↓11% 1v3** ↓10%	1v2** ↓11% 1v3** ↓10%	**0v2*** ↓17%** 1v2** ↓15% 2v3* ↑16% 2v4** ↑21%	1v2*** ↓15% 2v3*** ↑16% 2v4*** ↑21%	**0v2*** ↓14%** 1v2*** ↓17% 2v3*** ↑15% 2v4*** ↑15%	1v2*** ↓17% 2v3*** ↑15% 2v4*** ↑15%	**0v1*** ↓10%** **0v2*** ↓20%** **0v3*** ↓14%** **0v4*** ↓11%** 1v2** ↓10% 2v4* ↑11%	1v2** ↓10% 2v4** ↑11%
11	NSD	1v2*** ↓15% 1v3*** ↓10% 1v4*** ↓11%	NSD	1v2*** ↓13% 1v4*** ↓11% 2v3** ↑10%	NSD	1v2*** ↓18% 1v3*** ↓14% 1v4*** ↓11% 2v4* ↑8%	NSD	NSD
13	**0v3*** ↓21%** 1v3* ↓19% 3v4* ↑24%	1v3** ↓19% 2v3* ↓17% 3v4** ↑24%	NSD	NSD	NSD	NSD	**0v1* ↓13%** **0v2*** ↓23%** **0v3*** ↓26%** 1v3* ↓16% 3v4* ↑19%	1v3* ↓16% 3v4* ↑19%

TABLE E7
Generational Effects in Predelivery Feed Consumption by Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Age (Weeks)	Dietary Ethinyl Estradiol (ppb)							
	0		2		10		50	
	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4	F_0-F_4	F_1-F_4
14	**0v1*** ↓20%** **0v2*** ↓13%** **0v3*** ↓19%** **0v4*** ↓19%** 1v2* ↑10%	1v2* ↑10%	**0v1*** ↓23%** **0v2*** ↓16%** **0v3*** ↓12%** **0v4*** ↓13%** 1v3* ↑13% 1v4* ↑13%	1v3* ↑13% 1v4** ↑13%	**0v1*** ↓26%** **0v2*** ↓18%** **0v3*** ↓15%** **0v4*** ↓24%** 1v2* ↑11% 1v3** ↑15% 3v4* ↓10%	1v2* ↑11% 1v3** ↑15% 3v4* ↓10%	**0v1*** ↓22%** **0v2*** ↓17%** **0v3** ↓11%** **0v4*** ↓19%** 1v3** ↑14%	1v3** ↑14%
15	1v2*** ↑16% 2v3*** ↓13% 2v4* ↓10%	1v2*** ↑16% 2v3*** ↓13% 2v4** ↓10%	NSD	1v2* ↑11% 1v3* ↑12%	1v2* ↑12%	1v2** ↑12% 1v4* ↑10%	NSD	NSD
16	NSD	NSD	**0v3*** ↑18%** **0v4*** ↑18%** 1v3* ↑11% 1v4* ↑11% 2v4* ↑11%	1v3* ↑11% 1v4* ↑11% 2v3* ↑11% 2v4* ↑11%	**0v4*** ↑16%** 1v4* ↑9% 2v4*** ↑16% 3v4* ↑7%	1v4* ↑9% 2v4*** ↑16% 3v4* ↑7%	**0v4** ↑14%** 1v4* ↑11% 2v4** ↑12%	1v4** ↑11% 2v4** ↑12%
17	**0v1*** ↓15%** **0v2*** ↓12%** **0v3*** ↓13%** **0v4*** ↓14%**	NSD	NSD	1v3* ↑12%	**0v1*** ↓12%** **0v2** ↓11%** **0v3* ↓8%** **0v4** ↓12%**	NSD	**0v1*** ↓15%** **0v2*** ↓16%** **0v3*** ↓11%** **0v4*** ↓22%** 3v4** ↓13%	3v4*** ↓13%
18	**0v1* ↓6%** **0v2* ↓7%** **0v3*** ↓10%** **0v4*** ↓10%**	NSD	**0v2* ↓10%** 1v2* ↓10% 2v3*** ↑17% 2v4*** ↑18%	1v2* ↓10% 2v3*** ↑17% 2v4*** ↑18%	1v4** ↑10% 2v4*** ↑13%	1v4** ↑10% 2v4*** ↑13%	**0v4* ↑10%** 2v3* ↑11% 2v4*** ↑14%	2v3* ↑11% 2v4*** ↑14%
19	**0v1*** ↓17%** **0v2* ↓25%** **0v3*** ↓21%** **0v4*** ↓19%**	NSD	**0v2*** ↓18%** 1v2*** ↓14% 2v3*** ↑21% 2v4*** ↑15%	1v2*** ↓14% 2v3*** ↑21% 2v4*** ↑15%	1v2*** ↓14% 2v4*** ↑13%	1v2*** ↓14% 1v3* ↓8% 2v4*** ↑13%	**0v2*** ↓14%** 1v2** ↓12% 2v3** ↑15% 2v4*** ↑19%	1v2** ↓12% 2v3*** ↑15% 2v4*** ↑19%
20	NSD	NSD	**0v3** ↑19%** 1v3*** ↑17% 2v3*** ↑16%	1v3*** ↑17% 2v3*** ↑16%	2v3* ↑11% 2v4* ↑10%	2v3* ↑11% 2v4* ↑10%	NSD	1v3* ↑10% 1v4* ↑10%

[a] Results of Holm's-adjusted t-tests of feed consumption differences are indicated. Only comparisons showing significant differences between generations within an exposure group are shown. Generations are indicated by their subscripts, so that "0v1" means F_0 versus F_1. Asterisks indicate the level of significance: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Arrows indicate the direction of the difference of the second listed generation relative to the first, and the percentage difference is given. Comparisons involving the F_0 generation are bolded. NA, not applicable; NSD, no significant differences.

[b] Because the F_0 generation entered the experiment at a later age than subsequent generations, data from that generation do not completely overlap data from the F_1 to F_4 generations. Therefore, in order to conduct tests of generation effects within exposure groups, two sets of statistical analyses were conducted for feed consumption for males: the first included data from week 7 to the end of the experiment for all generations (F_0 to F_4), and the second included all data from week 4 to the end of the experiment for generations F_1 to F_4. The statistical results reported in this table for weeks 4, 5, and 6 are from the latter analysis, while results from weeks 7 to 20 are from the former analysis. In both analyses, data from week 12, during which the majority of males and females were paired for mating, were not included.

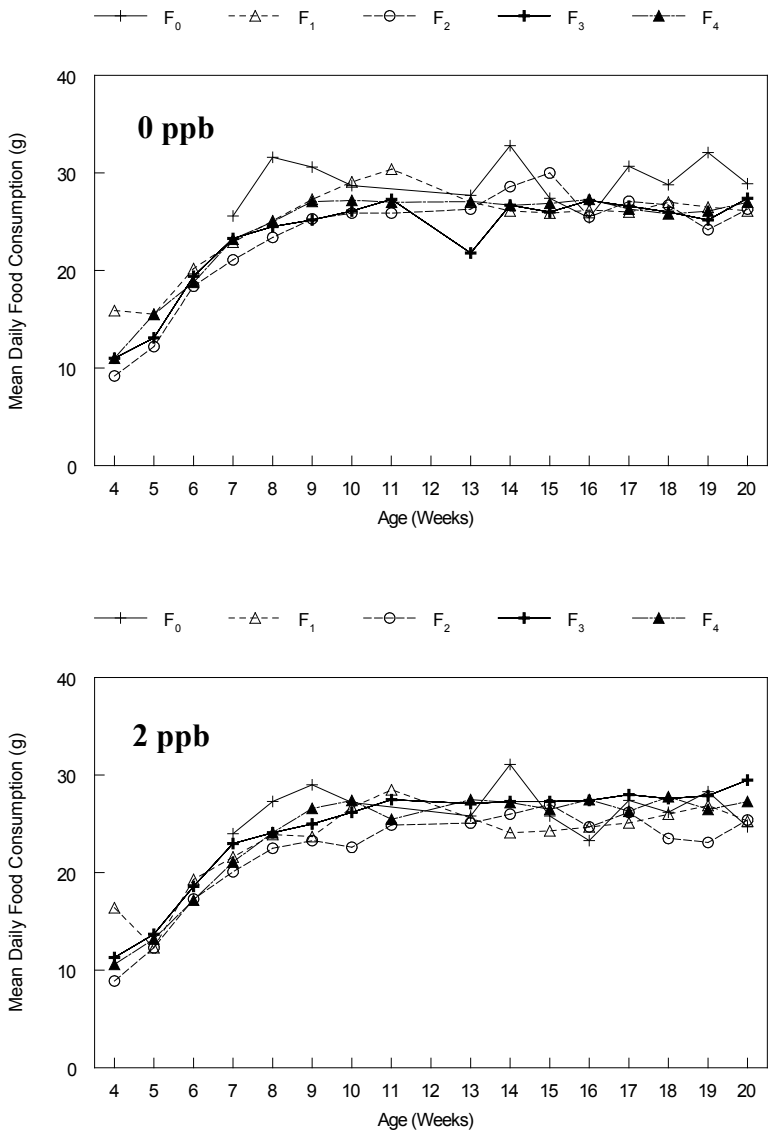

FIGURE E3
Feed Consumption by 0 and 2 ppb Male Rats
in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

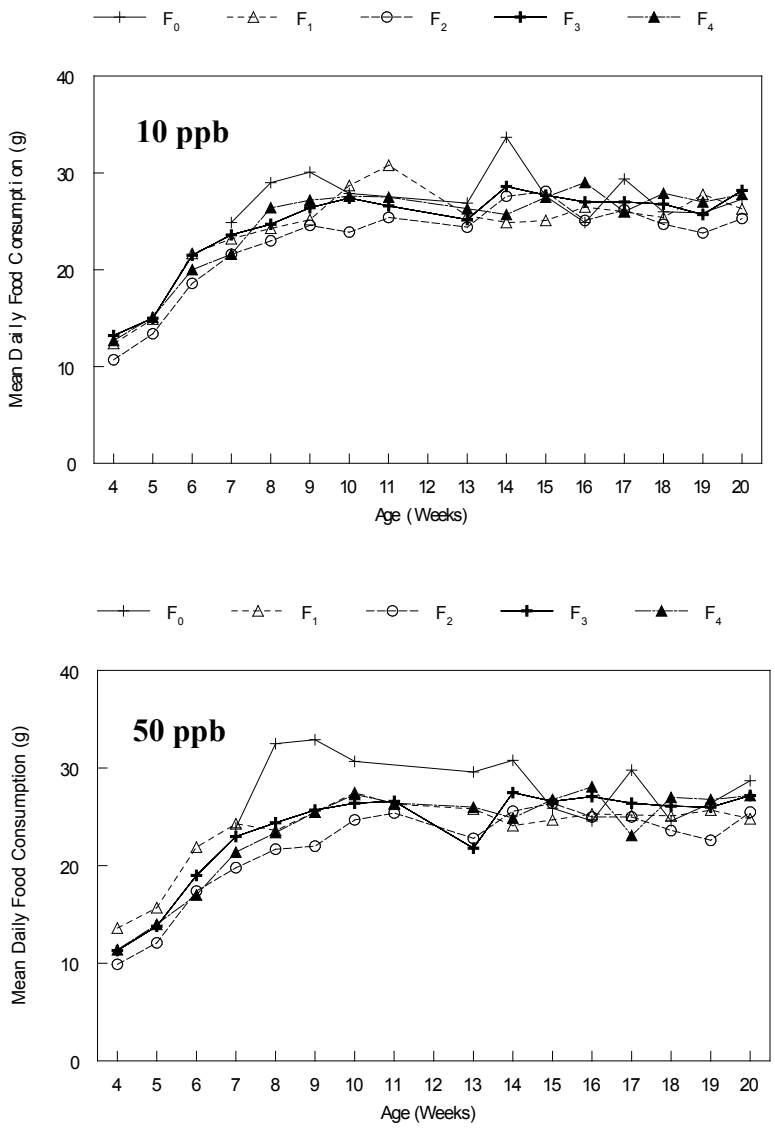

FIGURE E4
Feed Consumption by 10 and 50 ppb Male Rats
in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

APPENDIX F
WATER CONSUMPTION

TABLE F1a Water Consumption by F_0 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 210
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TABLE F1a
Water Consumption by F_0 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Postnatal Day[b]	Dietary Ethinyl Estradiol (ppb)[c,d]			
	0	2	10	50
3#	46.5 ± 1.7 (24) [3,4]	46.9 ± 2.5 [1,3]	51.5 ± 2.4 (22) [3]	48.5 ± 2.0 (24) [3]
4	50.9 ± 1.9	49.2 ± 2.2 [1,2,3,4]	50.1 ± 1.7 [3,4]	48.4 ± 2.0 [3,4]
5#	51.6 ± 1.3	51.2 ± 2.3 [1,2,4]	56.8 ± 1.8	50.4 ± 1.4 [3]
6*	61.5 ± 5.2	61.2 ± 7.1 (24)	55.4 ± 2.3 [2]	51.1 ± 1.5 (24) [2]
7	58.8 ± 2.2 (24) [4]	61.2 ± 4.9	59.4 ± 1.7	56.4 ± 1.5 (24)
8#	56.6 ± 1.9 [2,4]	63.7 ± 7.3	66.1 ± 2.6*	58.3 ± 1.4 [2]
9*	60.8 ± 3.0 [4]	63.6 ± 3.6	59.0 ± 1.7	54.8 ± 1.4
10	58.4 ± 2.2 [2,4]	60.0 ± 3.9	58.2 ± 1.9	58.1 ± 1.1
11	62.4 ± 2.5	66.3 ± 6.2	62.2 ± 1.9	58.3 ± 1.5 [4]
12*	65.8 ± 2.9	68.6 ± 6.7	60.0 ± 1.8	58.0 ± 1.8
13	63.1 ± 2.4	64.5 ± 3.1	60.8 ± 3.1	63.8 ± 2.2
14	64.4 ± 2.9	66.1 ± 3.6	66.8 ± 2.7	61.1 ± 2.3 [3]
15	60.6 ± 2.0 [1,2,4]	66.9 ± 4.8	70.0 ± 2.4	67.4 ± 2.2 [2,4]
16	61.4 ± 1.7	59.4 ± 3.3 [1,2,4]	66.9 ± 1.6	64.0 ± 1.9
17	66.0 ± 2.0	72.8 ± 4.1	68.1 ± 1.9	65.8 ± 1.6
18	68.4 ± 2.0	69.8 ± 3.7 [4]	66.0 ± 1.7 [2]	66.1 ± 1.8 [4]
19#	69.4 ± 2.4	65.0 ± 3.3 [1,2,3,4]	75.4 ± 2.4 [3]	70.2 ± 2.2
20	71.2 ± 2.2 [1,2,3,4]	82.0 ± 4.6*	76.4 ± 2.7	73.5 ± 2.9 [3,4]

TABLE F1a
Water Consumption by F_0 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

[a] Mean mL of water consumed per day ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Dams' water consumption during days 3 to 20 of the lactation period was analyzed using a repeated measures approach to analysis of variance. Significant (P<0.50) random effects of the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the statistical model. The results of the ANOVA were as follows: dose, P=0.068; generation, P<0.001; dose × generation, P=0.019; Days, P<0.001; Days × dose, P=0.024; Days × generation, P<0.001; Days × dose × generation, P=0.091.
[c] Asterisks and pound signs in shaded cells in the "Postnatal Day" column indicate significant linear or quadratic exposure concentration trends, respectively, on that day in that generation as determined by contrasts; asterisks in the exposed group columns indicate significant differences from controls on that day in that generation as determined by Dunnett's test. *, P≤0.05; **, P≤0.01; ***, P≤0.001; #, P≤0.05; ##, P≤0.01; ###, P≤0.001.
[d] Numbers in brackets indicate significant differences (P≤0.05) between generations within that exposure group on that day. The numbers (0, 1, 2, etc.) are abbreviations for the generations (F_0, F_1, F_2, etc.) with which there are significant differences.

TABLE F1b
Water Consumption by F_1 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Postnatal Day[b]	Dietary Ethinyl Estradiol (ppb)[c,d]			
	0	2	10	50
3###	52.4 ± 1.8	52.8 ± 2.6 (24) [0]	45.1 ± 1.5* [2,3]	53.3 ± 2.4
4	54.1 ± 1.7	55.8 ± 2.4 [0]	55.0 ± 2.4	53.9 ± 2.4
5##	57.5 ± 1.5	62.5 ± 2.9 [0]	50.8 ± 1.1 [2,3]	56.0 ± 1.6
6	57.5 ± 2.1 (24)	59.6 ± 2.1	53.6 ± 1.1	58.0 ± 1.4
7	55.8 ± 2.6 [2,4]	63.2 ± 2.1*	57.3 ± 1.8 [2]	56.5 ± 1.9
8	56.6 ± 1.7 [2,4]	61.0 ± 1.6	57.3 ± 1.9 [2]	61.8 ± 1.7
9#	59.9 ± 1.5 [4]	63.7 ± 2.3	53.9 ± 1.8 [2,4]	58.7 ± 1.6
10	58.2 ± 1.7 [2,4]	60.8 ± 1.5	55.3 ± 1.2 [2]	57.8 ± 1.5
11	60.6 ± 1.9 (24)	64.3 ± 2.3	58.5 ± 1.9	58.1 ± 1.7 [4]
12	64.6 ± 1.9	66.8 ± 1.8	61.3 ± 1.6	63.5 ± 1.6
13	64.8 ± 2.5	65.7 ± 2.4	63.1 ± 1.8	66.2 ± 2.0
14	67.4 ± 3.2	67.5 ± 3.9	63.8 ± 3.2	64.5 ± 2.5
15	77.7 ± 2.7 [0,3]	78.1 ± 3.1	75.3 ± 3.2	72.2 ± 2.4
16	68.6 ± 2.7	70.6 ± 2.5 [0]	68.6 ± 2.4	69.6 ± 2.6
17	69.7 ± 2.3	73.0 ± 2.4	74.0 ± 3.1	67.6 ± 1.9
18	72.8 ± 3.0	73.4 ± 1.6 [4]	73.9 ± 2.3	69.1 ± 1.8
19	75.4 ± 2.4	80.1 ± 1.7 [0]	76.1 ± 2.5 [3]	72.8 ± 2.3
20*	82.3 ± 2.4 [0]	88.2 ± 1.9	84.0 ± 2.3	78.5 ± 1.9

The footnotes for this table are defined in Table F1a.

TABLE F1c
Water Consumption by F_2 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Postnatal Day[b]	Dietary Ethinyl Estradiol (ppb)[c,d]			
	0	2	10	50
3	50.0 ± 3.2 (24)	53.3 ± 2.8	56.0 ± 2.0 (24) [1]	54.3 ± 1.8
4	54.0 ± 1.7	58.7 ± 3.2 [0]	54.6 ± 1.4 (24)	53.2 ± 1.8 (24)
5	59.2 ± 5.6	60.1 ± 2.2 [0]	58.5 ± 1.7 (23) [1]	57.0 ± 1.7 (19)
6	59.7 ± 2.8	67.2 ± 6.8	58.7 ± 2.0	67.6 ± 8.1 [0]
7*	65.6 ± 2.8 [1]	65.3 ± 2.7	68.0 ± 3.7 (24) [0,1]	58.7 ± 1.7 (24)
8	68.6 ± 4.5 [0,1]	66.5 ± 2.9 (24)	69.7 ± 2.5 (24) [1]	71.4 ± 4.1 [0]
9	67.0 ± 3.2 [4]	70.7 ± 6.4 (24)	64.2 ± 2.2 [1]	63.9 ± 2.1
10	68.8 ± 3.0 [0,1]	65.8 ± 2.5 (24)	63.9 ± 2.3 [1]	62.1 ± 2.2
11	55.3 ± 3.4	64.2 ± 2.9	59.8 ± 3.9	63.1 ± 1.9
12	62.3 ± 3.2	57.9 ± 3.1	60.5 ± 3.9	63.9 ± 8.1
13	59.5 ± 2.2	71.0 ± 3.7	69.9 ± 4.4	59.2 ± 3.1 (24)
14[#]	62.8 ± 3.8	63.4 ± 2.5	70.6 ± 1.9	66.9 ± 2.6 (24)
15	71.9 ± 2.9 [0]	68.4 ± 2.9	71.1 ± 2.0	71.0 ± 2.5 [0,3]
16	70.1 ± 2.5	76.4 ± 2.9 [0]	74.0 ± 1.8	78.7 ± 5.4
17	68.4 ± 2.8	69.7 ± 2.0	73.0 ± 1.6	70.9 ± 3.8 (24)
18	70.0 ± 2.5	71.8 ± 2.0 [4]	75.3 ± 2.0 [0]	73.9 ± 2.2
19	73.5 ± 3.3	78.0 ± 2.8 [0]	80.6 ± 2.1	73.3 ± 2.3
20	83.5 ± 3.8 [0]	81.5 ± 3.1	83.1 ± 2.5	82.3 ± 3.1

The footnotes for this table are defined in Table F1a.

TABLE F1d
Water Consumption by F_3 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Postnatal Day[b]	Dietary Ethinyl Estradiol (ppb)[c,d]			
	0	2	10	50
3	53.9 ± 2.8 [0]	56.7 ± 2.0 [0]	61.2 ± 2.9 (22) [0,1,4]	60.1 ± 2.2 (23) [0,4]
4	54.2 ± 1.7	60.9 ± 2.1 [0]	58.2 ± 2.7 [0]	59.9 ± 2.3 [0]
5[#]	53.9 ± 1.4	57.7 ± 1.7	62.2 ± 4.5* [1]	59.2 ± 1.5 (24) [0]
6	57.1 ± 1.7	62.5 ± 2.5	60.0 ± 2.5	60.1 ± 1.9
7	58.8 ± 1.6	62.3 ± 1.9	62.3 ± 1.8	62.7 ± 2.1
8	60.5 ± 1.9	61.2 ± 2.4	65.4 ± 2.2	62.9 ± 1.9
9	61.8 ± 2.2	63.1 ± 1.9	63.9 ± 1.9	61.0 ± 1.9
10	64.4 ± 2.6 [4]	63.2 ± 1.7	62.1 ± 1.8	64.0 ± 1.6
11	65.8 ± 2.8	64.3 ± 2.6	66.4 ± 2.1	65.3 ± 2.5
12	70.5 ± 3.5	68.4 ± 2.8	64.9 ± 2.9	63.5 ± 2.9
13	72.1 ± 6.1	68.3 ± 4.3	74.2 ± 4.4	69.1 ± 3.8
14	73.4 ± 3.8	74.0 ± 3.6	68.7 ± 4.1	74.1 ± 3.3 [0]
15*	64.1 ± 2.3 [1,4]	69.8 ± 3.0	68.4 ± 2.4	75.2 ± 5.1* [2]
16	65.4 ± 2.1	65.7 ± 2.1	69.4 ± 2.5	65.7 ± 1.5
17	70.5 ± 2.8	71.9 ± 2.2	67.6 ± 2.5	69.9 ± 1.9 (24)
18	72.8 ± 3.2 (24)	74.3 ± 3.7 [4]	71.5 ± 3.1	73.5 ± 3.2
19[###]	76.2 ± 3.5	78.3 ± 2.9 [0]	91.3 ± 7.9** [0,1]	76.6 ± 2.7
20	84.5 ± 3.3 (24) [0]	83.1 ± 3.2 (24)	84.3 ± 3.2 (24)	86.8 ± 2.8 [0]

The footnotes for this table are defined in Table F1a.

TABLE F1e
Water Consumption by F_4 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Postnatal Day[b]	Dietary Ethinyl Estradiol (ppb)[c,d]			
	0	2	10	50
3	53.3 ± 2.1 (24) [0]	51.8 ± 1.6	49.2 ± 1.7 (23) [3]	49.7 ± 2.6 [3]
4	55.7 ± 1.3 (24)	56.0 ± 1.3 [0]	56.0 ± 1.9 [0]	56.7 ± 1.7 [0]
5	57.5 ± 1.6	59.1 ± 1.8 [0]	57.3 ± 1.9	58.3 ± 1.7
6	60.3 ± 1.4	60.4 ± 1.4	58.5 ± 2.1 (24)	61.9 ± 2.0
7	66.6 ± 2.0 [0,1]	63.5 ± 2.0	64.4 ± 3.0	63.5 ± 2.8
8	67.2 ± 1.8 [0,1]	64.7 ± 1.9	63.2 ± 2.2	65.0 ± 1.9
9**, #	79.1 ± 7.4 [0,1,2,3]	63.8 ± 2.2***	64.3 ± 2.2** (24) [1]	62.4 ± 1.8***
10	70.4 ± 6.8 [0,1]	59.9 ± 1.3**	62.1 ± 1.7	65.1 ± 1.9
11	65.9 ± 2.3 (24)	66.5 ± 7.2 (24)	67.5 ± 3.5	73.1 ± 5.5 [0,1]
12	62.2 ± 2.6	61.7 ± 3.2	59.3 ± 2.6	65.5 ± 3.5
13	73.0 ± 5.2	78.1 ± 6.8	74.0 ± 7.0	66.6 ± 3.7 (23)
14	64.2 ± 2.6	72.9 ± 4.3	64.3 ± 2.8	63.3 ± 3.2
15	78.8 ± 7.0 [0,3]	71.1 ± 3.2	75.6 ± 2.8	70.5 ± 2.9 [0]
16	69.6 ± 2.8	71.4 ± 2.8 [0]	66.2 ± 2.4	76.1 ± 7.1
17*	74.8 ± 4.6	79.9 ± 6.7	71.9 ± 2.2	69.2 ± 2.7
18	75.1 ± 2.9	85.4 ± 4.5* (24) [0,1,2,3]	73.8 ± 2.7	77.4 ± 2.8 (24) [0]
19	76.4 ± 2.8	79.6 ± 2.8 (24) [0]	77.9 ± 2.9	80.9 ± 2.6
20	84.9 ± 3.0 [0]	85.4 ± 3.1	83.7 ± 3.0	89.0 ± 4.4 [0]

The footnotes for this table are defined in Table F1a.

APPENDIX G
MATING AND PREGNANCY PARAMETERS

TABLE G1 Mating and Pregnancy Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 218

TABLE G1
Mating and Pregnancy Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Mating Index[a]	F_0	0.74 (34)	0.83 (35)	0.77 (35)	0.85 (33)
	F_1	0.69 (35)	0.74 (34)	0.83 (35)	0.83 (35)
Dose P=0.402	F_2	0.85 (40)	0.75 (40)	0.75 (40)	0.80 (40)
Gen P=0.808	F_3	0.77 (35)	0.74 (35)	0.86 (35)	0.83 (35)
DxG P=0.694	F_4	0.66 (35)	0.83 (35)	0.68 (34)	0.80 (35)
Mating Time[b]	F_0	3.6 ± 0.5 (24)	2.8 ± 0.4 (25)	2.6 ± 0.6 (21)	3.2 ± 0.5 (23)
	F_1	3.7 ± 0.7 (21)	3.9 ± 0.9 (20)	2.5 ± 0.2 (26)	3.2 ± 0.5 (24)
Dose P=0.496	F_2	3.4 ± 0.5 (32)	3.7 ± 0.6 (28)	3.6 ± 0.6 (21)	2.6 ± 0.3 (24)
Gen P=0.874	F_3	4.0 ± 0.7 (21)	3.0 ± 0.6 (21)	3.1 ± 0.4 (26)	3.8 ± 0.7 (24)
DxG P=0.755	F_4	3.2 ± 0.4 (18)	3.5 ± 0.3 (24)	3.0 ± 0.3 (20)	3.4 ± 0.4 (21)
Fertility Index[c]	F_0	0.96 (25)	0.86 (29)	0.78 (27)	0.82 (28)
	F_1	0.96 (24)	0.80 (25)	0.97 (29)	0.83 (29)
Dose P=0.100	F_2[#]	0.94 (34)	0.93 (30)	0.73 (30)	0.81 (32)
Gen P=0.376	F_3	0.78 (27)	0.81 (26)	0.87 (30)	0.83 (29)
DxG P=0.127	F_4	0.83 (23)	0.83 (29)	0.91 (23)	0.75 (28)
Pregnancy Index[d]	F_0	0.74 (34)	0.83 (35)	0.77 (35)	0.85 (33)
	F_1	0.69 (35)	0.74 (34)	0.83 (35)	0.83 (35)
Dose P=0.997	F_2	0.85 (40)	0.75 (40)	0.75 (40)	0.80 (40)
Gen P=0.673	F_3	0.77 (35)	0.74 (35)	0.86 (35)	0.83 (35)
DxG P=0.238	F_4	0.66 (35)	0.83 (35)	0.68 (34)	0.80 (35)

TABLE G1
Mating and Pregnancy Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)[g]			
		0	2	10	50
Gestation Time[e] Dose P=0.013 Gen P=0.001 DxG P= 0.503	F_0**	22.3 ± 0.09 (24)	22.1 ± 0.06 (25) [3]	22.0 ± 0.10 (21) [3]	22.0 ± 0.04 (23)
	F_1	22.4 ± 0.11 (21)	22.2 ± 0.11 (20) [3]	22.1 ± 0.06 (26)	22.2 ± 0.08 (24) [3]
	F_2	22.5 ± 0.10 (32)	22.3 ± 0.10 (28)	22.4 ± 0.13 (21)	22.3 ± 0.09 (24)
	F_3	22.5 ± 0.13 (21)	22.7 ± 0.11 (21) [0,1]	22.5 ± 0.13 (26) [0]	22.7 ± 0.10 (24) [1]
	F_4	22.4 ± 0.15 (18)	22.3 ± 0.09 (24)	22.2 ± 0.14 (20)	22.5 ± 0.11 (21)

[a] The mating index is the ratio of vaginal plug-positive and/or littering dams to the number of potentially mating pairs. The number of potentially mating pairs is given in parentheses. The results of a logistic regression analysis are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There are no significant effects of exposure concentration in pairwise Chi-square comparisons of exposed groups to the controls or in exposure concentration trend tests. There are also no significant generation effects within exposure groups.

[b] Mating time is the time from cohabitation of the male and female breeders to the detection of a vaginal plug. Only those pairs for which a vaginal plug was detected (number given in parentheses) were included in the analysis. Values given are means ± standard error. Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G) are given. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. The F_0 breed mother and the interaction between the F_0 breed mother and F_0 breed father were significant random effects that were incorporated into the analysis model. There are no significant exposure concentration effects within generations as determined by Dunnett's tests, no significant trends, and no significant generation effects within exposure groups indicated by Holm's-adjusted t-tests.

[c] The fertility index is the ratio of the number of dams littering to the number of vaginal plug-positive dams. The number of vaginal plug-positive dams (given in parentheses) includes all dams with either a vaginal plug detected or those producing a litter, regardless of whether or not the vaginal plug was detected. The results of a logistic regression analysis are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There are no significant exposure concentration effects within generations and no significant generation effects within exposure groups. A significant quadratic exposure concentration trend within the F_2 generation is indicated by a pound sign: #, $P \leq 0.05$.

[d] The pregnancy index is the ratio of dams producing litters to the number of potentially mating pairs. The number of potentially mating pairs is given in parentheses. The results of a logistic regression analysis are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There are no significant exposure concentration effects within generations as determined by Holm's-adjusted Chi-square test and no significant generation effects within exposure groups.

[e] The gestation time is the number of days from the detection of a vaginal plug to the birth of a litter. Only those dams for which a vaginal plug was detected and that produced litters were included in the analysis (number given in parentheses). Values given are means ± standard error. Results of a logistic regression analysis are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There are no significant exposure concentration effects within generations as determined by Holm's-adjusted pairwise Chi-square tests. Numbers in brackets indicate generations that differ significantly within an exposure group as determined by Holm's-adjusted pairwise Chi-square tests. The Jonckheere-Terpstra test was used to test the monotonic exposure concentration trends within each generation; a significant linear exposure concentration trend withing the F_0 generation is indicated by asterisks: **, $P \leq 0.01$.

APPENDIX H
LITTER AND PERINATAL PUP PARAMETERS

TABLE H1 Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 222

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Total Pups Born[b,c,d] Dose P=0.018 Gen P<0.001 DxG P=0.224	F_1	12.94 ± 0.54 (32)	13.32 ± 0.56 (31) [2]	13.07 ± 0.45 (28)	11.86 ± 0.37 (28)
	F_2[#]	11.39 ± 0.67 (33)	11.15 ± 0.56 (27) [1]	12.18 ± 0.44 (34)	10.39 ± 0.49 (28) [4,5]
	F_3	11.36 ± 0.50 (36)	12.24 ± 0.41 (37)	12.06 ± 0.37 (32)	10.79 ± 0.44 (34) [4]
	F_4	12.50 ± 0.53 (26)	11.57 ± 0.44 (28)	13.37 ± 0.30 (30)	13.30 ± 0.40 (30) [2,3]
	F_5	12.64 ± 0.40 (28)	12.20 ± 0.45 (30)	13.21 ± 0.45 (29)	12.73 ± 0.41 (26) [2]
Live Pups Born[b,c,d] Dose P<0.205 Gen P<0.001 DxG P<0.281	F_1	12.94 ± 0.54 (32)	13.32 ± 0.56 (31)	13.07 ±0.45 (28)	11.86 ± 0.37 (28)
	F_2	11.39 ± 0.67 (33)	11.15 ± 0.56 (27)	12.18 ± 0.44 (34)	10.39 ± 0.49 (28) [4]
	F_3	11.36 ± 0.50 (36)	12.24 ± 0.41 (37)	11.66 ± 0.53 (32)	10.79 ± 0.44 (34) [4]
	F_4	12.50 ± 0.53 (26)	11.57 ± 0.44 (28)	13.23 ± 0.35 (30)	13.30 ± 0.40 (30) [2,3]
	F_5	12.36 ± 0.54 (28)	12.20 ± 0.45 (30)	12.79 ± 0.57 (29)	12.46 ± 0.48 (26)

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Female Live Births[b,c,e]	F_1	6.78 ± 0.52 (32)	7.10 ± 0.41 (31)	6.39 ± 0.42 (28)	5.82 ± 0.3 (28)
	F_2	6.03 ± 0.41 (33)	5.70 ± 0.49 (27)	5.88 ± 0.33 (34)	5.21 ± 0.40 (28)
	F_3	5.92 ± 0.36 (36)	5.65 ± 0.32 (37)	5.97 ± 0.39 (32)	5.65 ± 0.41 (34)
Dose P<0.380 Gen P=0.014 DxG P=0.730	F_4	6.73 ± 0.42 (26)	5.50 ± 0.38 (28)	6.37 ± 0.32 (30)	6.40 ± 0.39 (30)
	F_5	6.21 ± 0.35 (28)	5.83 ± 0.38 (30)	6.10 ± 0.34 (29)	6.42 ± 0.47 (26)
Male Live Births[b,c,e]	F_1	6.16 ± 0.43 (32)	6.23 ± 0.43 (31)	6.68 ± 0.43 (28)	6.04 ± 0.38 (28)
	F_2	5.36 ± 0.40 (33)	5.44 ± 0.33 (27)	6.29 ± 0.35 (34)	5.18 ± 0.48 (28) [4]
	F_3	5.44 ± 0.30 (36)	6.59 ± 0.35 (37)	5.69 ± 0.33 (32)	5.15 ± 0.35 (34) [4]
Dose P=0.065 Gen P=0.011 DxG P=0.369	F_4	5.77 ± 0.41 (26)	6.07 ± 0.42 (28)	6.87 ± 0.42 (30)	6.90 ± 0.42 (30) [2,3]
	F_5	6.14 ± 0.42 (28)	6.37 ± 0.39 (30)	6.69 ± 0.47 (29)	6.04 ± 0.37 (26)

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Pups Born Dead	F_1	0	0	0	0
	F_2	0	0	0	0
	F_3	0	0	1	0
	F_4	0	0	0	0
	F_5	0	0	0	0
Male Pup Weight[c,f] Litter Size P<0.001 Dose P=0.498 Gen P<0.001 DxG P=0.927	F_1	6.3 ± 0.1 (31)	6.2 ± 0.1 (30) [3,4]	6.3 ± 0.1 (28)	6.1 ± 0.1 (28) [4]
	F_2	6.6 ± 0.1 (33)	6.6 ± 0.1 (27)	6.4 ± 0.1 (33)	6.6 ± 0.1 (28)
	F_3	6.6 ± 0.1 (36)	6.7 ± 0.1 (37) [1]	6.6 ± 0.1 (32)	6.6 ± 0.1 (34)
	F_4	6.5 ± 0.1 (26)	6.7 ± 0.1 (28) [1]	6.5 ± 0.1 (30)	6.5 ± 0.1 (30) [1]
	F_5	6.4 ± 0.1 (28)	6.5 ± 0.1 (30)	6.4 ± 0.1 (29)	6.5 ± 0.1 (26)

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Female Pup Weight[c,f] Litter Size P<0.001 Dose P=0.641 Gen P<0.001 DxG P=0.448	F_1	5.9 ± 0.1 (31)	5.8 ± 0.1 (31) [4]	5.8 ± 0.1 (28) [3]	5.8 ± 0.1 (27) [3,5]
	F_2	6.2 ± 0.1 (32)	6.2 ± 0.1 (26)	6.1 ± 0.1 (33)	6.0 ± 0.1 (27)
	F_3	6.3 ± 0.1 (34)	6.2 ± 0.1 (36)	6.2 ± 0.1 (31) [1]	6.2 ± 0.1 (34) [1]
	F_4	6.0 ± 0.1 (26)	6.3 ± 0.1 (28) [1]	6.1 ± 0.1 (30)	6.1 ± 0.1 (30)
	F_5	6.0 ± 0.1 (28)	6.1 ± 0.1 (30)	5.9 ± 0.1 (29)	6.2 ± 0.1 (26) [1]
Sex Ratio[c,e,g] Dose P=0.545 Gen P=0.958 DxG P=0.467	F_1	1.2 ± 0.2 (31)	1.1 ± 0.2 (31)	1.2 ± 0.1 (28)	1.5 ± 0.3 (28)
	F_2	1.4 ± 0.4 (33)	1.3 ± 0.2 (27)	1.3 ± 0.1 (33)	1.4 ± 0.3 (28)
	F_3	1.5 ± 0.3 (36)	1.4 ± 0.2 (37)	1.3 ± 0.3 (32)	1.1 ± 0.1 (34)
	F_4	1.0 ± 0.3 (28)	1.6 ± 0.3 (28)	1.2 ± 0.1 (30)	1.4 ± 0.2 (30)
	F_5	1.1 ± 0.1 (28)	1.3 ± 0.2 (30)	1.2 ± 0.1 (29)	1.3 ± 0.2 (26)

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Male Anogenital Distance[c,e,h] Body Weight P< 0.001 Dose P=0.899 Gen P=0.010 DxG P=0.278	F_1	3.14 ± 0.06	3.23 ± 0.05	3.25 ± 0.04	3.27 ± 0.04 [3]
	F_2	3.18 ± 0.02	3.21 ± 0.04	3.12 ± 0.06	3.22 ± 0.07
	F_3*	3.23 ± 0.06	3.16 ± 0.04	3.15 ± 0.05	3.09 ± 0.02* [1]
	F_4	3.15 ± 0.04	3.19 ± 0.08	3.14 ± 0.03	3.13 ± 0.04
	F_5	3.13 ± 0.02	3.08 ± 0.02	3.17 ± 0.05	3.09 ± 0.02
Male Anogenital Distance Ratio[c,e,h] Dose P=0.700 Gen P=0.010 DxG P=0.510	F_1	1.72 ± 0.03	1.78 ± 0.03	1.74 ± 0.02	1.75 ± 0.01
	F_2	1.73 ± 0.02	1.72 ± 0.03	1.70 ± 0.04	1.76 ± 0.04
	F_3	1.74 ± 0.03	1.68 ± 0.03	1.68 ± 0.02	1.66 ± 0.02
	F_4	1.75 ± 0.02	1.72 ± 0.03	1.72 ± 0.02	1.72 ± 0.04
	F_5	1.70 ± 0.02	1.68 ± 0.02	1.72 ± 0.01	1.67 ± 0.02

TABLE H1
Litter and Perinatal Pup Parameters of Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Female Anogenital Distance[c,d,h]	F_1	1.98 ± 0.04	2.01 ± 0.02	2.00 ± 0.02	1.99 ± 0.02
	F_2*	1.91 ± 0.04	2.01 ± 0.03	1.94 ± 0.04	2.03 ± 0.04* [3]
Body Weight P=0.876	F_3*,#	2.03 ± 0.04	1.98 ± 0.04	1.90 ± 0.04*	1.90 ± 0.03* [2]
	F_4	2.01 ± 0.03	1.97 ± 0.04	1.96 ± 0.02	1.98 ± 0.02
Dose P=0.296 Gen P=0.553 DxG P=0.053	F_5	2.01 ± 0.03	1.96 ± 0.02	1.98 ± 0.03	1.95 ± 0.02
Female Anogenital Distance Ratio[c,f,h]	F_1	1.09 ± 0.03	1.10 ± 0.02	1.07 ± 0.01	1.07 ± 0.01
	F_2*	1.04 ± 0.02	1.08 ± 0.02	1.06 ± 0.02	1.11 ± 0.03* [3]
	F_3*	1.09 ± 0.02	1.05 ± 0.03	1.02 ± 0.03	1.02 ± 0.02* [2]
Dose P=0.320 Gen P=0.080 DxG P=0.070	F_4	1.12 ± 0.02	1.06 ± 0.02	1.07 ± 0.02	1.09 ± 0.03
	F_5	1.10 ± 0.02	1.07 ± 0.02	1.07 ± 0.01	1.06 ± 0.02

[a] Asterisks (*) and pound signs (#) in shaded cells in the generation column indicate significant linear or quadratic exposure concentration trends, respectively, within a generation as determined by contrasts: asterisks in shaded cells in the exposed group columns indicate significant differences from controls in the same generation as determined by Dunnett's test: * or #, P≤0.05.
[b] Statistical analyses were run on square root transformations of the raw data to stabilize variance.
[c] Mean ± standard error reported. Numbers in parentheses are the numbers of litters. Numbers in brackets indicate significant differences (P≤0.05) between the indicated generations within an exposure group as determined by Holm's-adjusted t-tests. Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G) are given; for ANCOVA, the covariates were litter size (for pup weights) or pup body weight (for anogenital distance). Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes for the specific endpoints.
[d] Significant random effect of the F_0 breed mother was incorporated into the statistical model.
[e] No significant random effects were incorporated into the statistical model.
[f] Significant random effects of the F_0 breed mother, the F_0 breed father, and the F_0 breed mother × F_0 breed father interaction were incorporated into the statistical model.
[g] The sex ratio is the ratio of males to females per litter; statistical analyses were run on natural log transformations of the ratio to stabilize variance.
[h] All anogenital distance measurements were made on the pups in 10 litters after culling to four pups per sex; the data presented are for these 10 litters. The data were analyzed by ANCOVA with pup body weight as the covariate or as the ratio of measured anogenital distance to the cube root of body weight.

APPENDIX I
MARKERS OF SEXUAL DEVELOPMENT

TABLE I1 **Age and Body Weight at Vaginal Opening of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol** **230**
TABLE I2 **Age and Body Weight at Preputial Separation of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol** **231**
TABLE I3 **Age at Testicular Descent of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol** **232**

TABLE I1
Age and Body Weight at Vaginal Opening of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Age (Postnatal Day) at vaginal opening[b]						
F_1***	32.2 ± 0.4 (32)	33.0 ± 0.3 [2]	31.2 ± 0.5	28.1 ± 0.5*** [2,4]	NA	NA
F_2***	31.8 ± 0.4 (37)	31.4 ± 0.4 (40) [1]	31.5 ± 0.5 (40)	25.6 ± 0.5*** (40) [1,3,4]	NA	NA
F_3***	31.7 ± 0.5 (32)	32.0 ± 0.4	31.3 ± 0.4	28.8 ± 0.6*** [1,2,4]	NA	NA
F_4	32.6 ± 0.4 (32)	32.0 ± 0.3	31.9 ± 0.4	31.7 ± 0.5 [1,2,3]	NA	NA
Body Weight (g) at vaginal opening[c]						
F_1	97.3 ± 2.6 (32)	94.4 ± 2.2	88.5 ± 3.2*	66.0 ± 2.3*** [2,3,4]	***	-
F_2	94.3 ± 2.1 (37)	89.7 ± 2.3 (40)	88.7 ± 3.0 (40)	56.4 ± 1.6*** (40) [1,3,4]	***	-
F_3	94.8 ± 2.3 (32)	98.5 ± 2.3	95.4 ± 2.2	79.5 ± 2.7*** [1,2,4]	***	-
F_4	99.6 ± 2.1 (32)	98.7 ± 2.2	96.4 ± 2.5	94.0 ± 2.1 [1,2,3]	-	-

[a] Mean ± standard error. Thirty-five animals in each group except where indicated by numbers in parentheses. Numbers in brackets indicate significant differences (P≤0.05) between generations within an exposure group.

[b] For age at vaginal opening, a two-way nonparametric ANOVA was conducted. The overall dose effect, overall generation, and overall dose × generation interaction were all significant at P≤0.001. *Post hoc* one-way nonparametric ANOVAs (Kruskal-Wallis' tests) were performed on dose, holding generation constant, and on generation, holding dose constant. Holm's-adjusted Wilcoxon's tests were used for *post hoc* pairwise comparisons. The Holm's adjustment was used in order to control the Type I error rate for simultaneous inference with the Wilcoxon's tests. Exposure concentration trend tests were not conducted as indicated by NA (not applicable). Asterisks adjacent to generation designations indicate a significant overall Kruskal-Wallis' test; asterisks in shaded cells in the exposed group columns indicate a significant difference from the controls in the same generation: ***, P≤0.001.

[c] For body weight at vaginal opening, a two-way ANOVA was conducted. Significant (P<0.50) random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were included in the statistical model. The overall dose effect, the overall generation effect, and the overall dose × generation interaction were all significant at P<0.001. Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends, and Dunnett's tests were used to compare exposed group means to control means within a generation. Holm's-adjusted t-tests were used to compare means within an exposure group across generations. Asterisks in shaded cells in the exposed group columns indicate a significant difference from the control value in the same generation, and asterisks in the trend columns indicate significant exposure concentration trends within a generation: *, P≤0.05; ***, P≤0.001. A dash in the trend column indicates that the exposure concentration trend test was not significant (P>0.05).

TABLE I2
Age and Body Weight at Preputial Separation of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	Dietary Ethinyl Estradiol (ppb)				Trends	
	0	2	10	50	Linear	Quad
Age[b]						
F_1	42.3 ± 0.4 (32) [3]	43.3 ± 0.4 [3,4]	42.3 ± 0.4 [2,3]	42.7 ± 0.3 [2]	NA	NA
F_2*	43.2 ± 0.5 (37) [3]	43.9 ± 0.3 (40) [3,4]	44.2 ± 0.4 (39) [3,4]	44.7 ± 0.3* (40) [1,3,4]	NA	NA
F_3	40.7 ± 0.2 (32) [1,2]	41.6 ± 0.3 [1,2]	41.4 ± 0.4 [1,2]	41.6 ± 0.3 [2]	NA	NA
F_4	41.6 ± 0.3 (31)	41.8 ± 0.4 [1,2]	42.0 ± 0.4 (34) [2]	42.4 ± 0.4 [2]	NA	NA
Body Weight[c]						
F_1	181.3 ± 2.9 (32)	182.1 ± 3.0	180.2 ± 2.7 [2]	171.7 ± 3.1 [2,3,4]	**	-
F_2	191.3 ± 2.8 (37)	191.2 ± 2.3 (40)	195.6 ± 3.2 (39) [1]	186.9 ± 2.6 (40) [1]	-	-
F_3	186.1 ± 2.9 (32)	188.1 ± 3.0	190.0 ± 2.2	188.4 ± 2.6 [1]	-	-
F_4	183.0 ± 3.2 (31)	184.2 ± 3.0	187.6 ± 2.6 (34)	187.7 ± 2.4 [1]	-	-

[a] Mean ± standard error. Thirty-five animals in each group except where indicated by numbers in parentheses. Numbers in brackets indicate significant differences (P≤0.05) between generations within an exposure group.

[b] For age at preputial separation, a two-way nonparametric ANOVA was conducted. The overall dose effect and overall generation effect were significant at P=0.001; the overall dose × generation interaction was not significant at P=0.537. *Post hoc* one-way nonparametric ANOVAs (Kruskal-Wallis' tests) were performed on dose, holding generation constant, and on generation, holding dose constant. Holm's-adjusted Wilcoxon's tests were used for *post hoc* pairwise comparisons of exposed groups to controls within generations and of all generations within an exposure group. The Holm's adjustment was used in order to control the Type I error rate for simultaneous inference with the Wilcoxon's tests. Exposure concentration trend tests were not conducted, as indicated by NA (not applicable). Asterisks adjacent to generation designations indicate a significant overall Kruskal-Wallis' test; asterisks in shaded cells in the exposed group columns indicate a significant difference from the controls in the same generation: *, P≤0.05

[c] For body weight at preputial separation, a two-way ANOVA was conducted. Significant (P<0.50) random effects of the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were included in the statistical model. The overall dose effect was not significant at P<0.168; the overall generation effect was significant at P<0.001, and the overall dose × generation interaction was not significant at P=0.317. Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends, and Dunnett's tests were used to compare exposed group means to control means within a generation. Holm's-adjusted t-tests were used to compare means within an exposure group across generations. The Holm's adjustment was used in order to control the Type I error rate for simultaneous inference with the Wilcoxon's tests. Asterisks in trend columns indicate significant exposure concentration trends within a generation: **, P≤0.01. A dash in the trend column indicates that the exposure concentration trend test was not significant (P>0.05).

TABLE I3
Age at Testicular Descent of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
F_1*	22.3 ± 0.2 (32)	23.3 ± 0.3* [2,3,4]	22.6 ± 0.2 [2,4]	23.3 ± 0.4 [4]
F_2	21.8 ± 0.2 (37)	21.9 ± 0.2 (39) [1]	21.9 ± 0.2 (40) [1]	22.7 ± 0.4 (40) [4]
F_3	22.2 ± 0.2 (32)	22.0 ± 0.2 [1]	21.8 ± 0.3 (33)	22.0 ± 0.3
F_4	21.5 ± 0.2 (32)	21.3 ± 0.3 [1]	22.0 ± 0.5 [1]	21.3 ± 0.3 [1,2]

[a] Mean day of testicular descent ± standard error. Thirty-five animals in each group except where indicated by numbers in parentheses. Numbers in brackets indicate significant differences (P≤0.05) between generations within an exposure group. A two-way nonparametric ANOVA was conducted. The overall dose effect was not significant at P=0.581; the overall generation effect was significant at P=0.001; and the overall dose × generation interaction was not significant at P=0.225. *Post hoc* one-way nonparametric ANOVAs (Kruskal-Wallis' tests) were performed on dose, holding generation constant, and on generation, holding dose constant. Wilcoxon's tests were used for *post hoc* pairwise comparisons. The Holm's adjustment was used in order to control the Type I error rate for simultaneous inference with the Wilcoxon's tests. Asterisks adjacent to generation designations indicate a significant overall Kruskal-Wallis' test; asterisks in shaded cells in the exposed group columns indicate a significant difference from the control value in the same generation: *, P≤0.05.

APPENDIX J
ESTROUS CYCLE CHARACTERIZATION

TABLE J1 Estrous Cycle Characterization after Vaginal Opening for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 234
TABLE J2 Estrous Cycle Characterization prior to Scheduled Sacrifice for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 238

TABLE J1
Estrous Cycle Characterization after Vaginal Opening for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Number of animals (n)	F_1	25	23	25	25
	F_2	25	25	25	25
	F_3	25	25	25	25
	F_4	24	25	25	25
% Time in cycle stages[b]					
% Time in Diestrus	F_1***	50.57 ± 1.57	56.52 ± 1.85	57.19 ± 1.89	41.93 ± 3.38 [3,4]
	F_2*	56.44 ± 1.54	58.00 ± 1.95	55.58 ± 1.70	45.30 ± 3.74 [3]
	F_3	55.71 ± 1.80	59.03 ± 1.86	56.29 ± 1.45	58.31 ± 1.91 [1,2]
	F_4	55.95 ± 1.76	58.86 ± 1.86	54.86 ± 1.92	55.14 ± 1.78 [1]
% Time in Estrus	F_1***	28.57 ± 1.36	24.69 ± 1.47	23.00 ± 1.37*	45.19 ± 4.00* [3,4]
	F_2***	23.21 ± 1.11	22.57 ± 1.41	23.78 ± 1.41	43.78 ± 4.55** [3,4]
	F_3	24.86 ± 1.31	23.19 ± 1.60	24.57 ± 1.17	23.18 ± 1.46 [1,2]
	F_4	23.81 ± 1.34	20.86 ± 1.16	23.71 ± 1.47	24.86 ± 1.31 [1,2]
% Time in Proestrus	F_1***	20.85 ± 0.66	18.79 ± 0.90	19.81 ± 1.10	12.88 ± 1.30*** [3,4]
	F_2***	20.35 ± 0.68	19.43 ± 0.88	20.64 ± 0.96	10.92 ± 1.46*** [3,4]
	F_3	19.43 ± 0.88	17.78 ± 0.95	19.14 ± 0.80	18.52 ± 0.85 [1,2]
	F_4	20.24 ± 0.70	20.29 ± 0.98	21.43 ± 0.92	20.00 ± 0.92 [1,2]

TABLE J1
Estrous Cycle Characterization after Vaginal Opening for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Number of Abnormal Cycles[b]					
# of Abnormal Cycles - Diestrus	F_1**	0.08 ± 0.06	0.26 ± 0.11	0.28 ± 0.12	0.56 ± 0.12**
	F_2	0.24 ± 0.12	0.28 ± 0.11	0.16 ± 0.08	0.56 ± 0.14
	F_3	0.16 ± 0.08	0.24 ± 0.13	0.08 ± 0.08	0.44 ± 0.14
	F_4	0.21 ± 0.10	0.36 ± 0.13	0.20 ± 0.10	0.24 ± 0.10
# of Abnormal Cycles - Estrus	F_1***	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	1.36 ± 0.22*** [3,4]
	F_2***	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	1.32 ± 0.24*** [3,4]
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1,2]
	F_4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1,2]
# of Abnormal Cycles – Diestrus and Estrus	F_1***	0.08 ± 0.06	0.26 ± 0.11	0.28 ± 0.12	1.92 ± 0.20*** [3,4]
	F_2***	0.24 ± 0.12	0.28 ± 0.11	0.20 ± 0.08	1.88 ± 0.22*** [3,4]
	F_3	0.16 ± 0.08	0.24 ± 0.13	0.08 ± 0.08	0.44 ± 0.14 [1,2]
	F_4	0.21 ± 0.10	0.36 ± 0.13	0.20 ± 0.10	0.24 ± 0.10 [1,2]

TABLE J1
Estrous Cycle Characterization after Vaginal Opening for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Percentage of Abnormal Cycles[b]					
% of Abnormal Cycles - Diestrus	F_1**	3.00 ± 2.20	13.04 ± 5.64	12.00 ± 5.32	25.00 ± 5.53**
	F_2	12.00 ± 5.97	13.33 ± 5.27	7.33 ± 3.48	25.00 ± 6.87
	F_3	8.00 ± 3.74	12.00 ± 6.63	4.00 ± 4.00	21.33 ± 7.04
	F_4	10.42 ± 5.20	18.00 ± 6.38	10.00 ± 5.00	11.33 ± 5.07
% of Abnormal Cycles - Estrus	F_1***	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	53.67 ± 7.49*** [3,4]
	F_2***	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	52.33 ± 8.76*** [3,4]
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1,2]
	F_4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1,2]
% of Abnormal Cycles – Diestrus and Estrus	F_1***	3.00 ± 2.20	13.04 ± 5.64	12.00 ± 5.32	78.67 ± 6.12*** [3,4]
	F_2***	12.00 ± 5.97	13.33 ± 5.27	9.33 ± 3.86	77.33 ± 7.81*** [3,4]
	F_3	8.00 ± 3.74	12.00 ± 6.63	4.00 ± 4.00	21.33 ± 7.04 [1,2]
	F_4	10.42 ± 5.20	18.00 ± 6.38	10.00 ± 5.00	11.33 ± 5.07 [1,2]

TABLE J1
Estrous Cycle Characterization after Vaginal Opening for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Length of Cycle[b,c]					
Length of Cycle (Days)	F_1***,###	4.57 ± 0.13	5.28 ± 0.22*	5.65 ± 0.41*	10.17 ± 0.76*** [3,4]
	F_2***,###	5.37 ± 0.40	5.79 ± 0.41	5.32 ± 0.21	10.78 ± 0.82*** [3,4]
	F_3	5.13 ± 0.19	5.23 ± 0.20	5.32 ± 0.39	5.83 ± 0.42 [1,2]
	F_4	5.54 ± 0.42	5.51 ± 0.23	5.32 ± 0.21	5.13 ± 0.19 [1,2]

[a] Starting 3 days after vaginal opening was observed, vaginal smears were taken for 14 consecutive days for determination of stage of the estrous cycle. The number of animals for which data were available for analysis in each exposure group of each generation is indicated under "Number of animals." The following endpoints were analyzed: percentage of days in diestrus, estrus, or proestrus; number and percentage of abnormal cycles; and length of cycle. An abnormal cycle was defined as 4 or more consecutive days of diestrus or 3 or more consecutive days of estrus. Abnormal cycles due to prolonged diestrus or prolonged estrus were evaluated both separately and combined.

[b] Separate nonparametric one-way ANOVAs (Kruskal-Wallis' tests) were run on exposure concentration within each generation and on generation within each exposure group. Holm's-adjusted pairwise Wilcoxon's tests were run to compare exposed groups to the controls within generations or to compare all generations within an exposure group. For the analysis of exposure concentration effects within generations, overall significant Kruskal-Wallis' tests are indicated by asterisks in shaded cells in the generation column; exposed groups that differ significantly from the controls in the same generation by Holm's-adjusted Wilcoxon's tests are indicated by asterisks in shaded cells in the exposed group columns: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences (P≤0.05) between generations within an exposure group are indicated by numbers in brackets.

[c] For the length of cycle endpoint, a Jonckheere-Terpstra linear exposure concentration trend test was run to evaluate trends within each generation. Significant exposure concentration trend tests are indicated by pound signs in shaded cells in the generation column: ###, P≤0.001.

TABLE J2
Estrous Cycle Characterization prior to Scheduled Sacrifice for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Number of Animals (n)	F_0	25	25	25	25
	F_1	25	25	25	25
	F_2	25	25	25	25
	F_3	25	25	25	25
	F_4	25	25	25	25
% Time in cycle stages[b]					
% Time in Diestrus	F_0	48.98 ± 1.42	42.80 ± 2.27 [3]	44.40 ± 2.09	50.40 ± 2.34
	F_1	48.80 ± 1.45	48.98 ± 1.64	48.80 ± 1.66	47.60 ± 2.02
	F_2	48.62 ± 1.19	47.82 ± 1.62	48.98 ± 1.65	52.04 ± 1.42
	F_3	50.40 ± 2.41	53.60 ± 2.07 [0]	48.40 ± 1.49	53.71 ± 1.87
	F_4	50.80 ± 1.44	47.60 ± 1.45	47.20 ± 2.20	52.00 ± 1.92
% Time in Estrus	F_0	28.18 ± 1.70	33.60 ± 2.76	33.20 ± 2.93	28.00 ± 1.92
	F_1	28.80 ± 1.33	29.78 ± 1.64	28.40 ± 1.60	30.40 ± 2.04
	F_2	27.87 ± 1.13	27.11 ± 1.37	27.96 ± 1.48	26.22 ±1.63
	F_3	27.60 ± 1.66	24.40 ± 1.54	26.80 ± 1.38	23.94 ± 1.52
	F_4	26.18 ± 1.61	26.40 ± 1.40	30.40 ± 2.20	26.80 ± 1.38
% Time in Proestrus	F_0	22.84 ± 1.21	23.60 ± 1.14	22.40 ± 1.56	21.60 ± 1.11
	F_1	22.40 ± 1.05	21.24 ± 1.03	22.80 ± 1.23	22.00 ± 1.00
	F_2	23.51 ± 0.98	25.07 ± 1.14	23.07 ± 1.06	21.73 ± 1.04
	F_3	22.00 ± 1.29	22.00 ± 1.29	24.80 ± 1.02	22.34 ± 1.03
	F_4	23.02 ± 1.12	26.00 ± 1.16	22.40 ± 1.05	21.20 ± 1.20

TABLE J2
Estrous Cycle Characterization prior to Scheduled Sacrifice for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Number of Abnormal Cycles[b]					
# of Abnormal Cycles - Diestrus	F_0	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.06
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_3	0.08 ± 0.06	0.16 ± 0.10	0.04 ± 0.04	0.08 ± 0.06
	F_4	0.04 ± 0.04	0.00 ± 0.00	0.04 ± 0.04	0.12 ± 0.07
# of Abnormal Cycles - Estrus	F_0	0.00 ± 0.00	0.16 ± 0.10	0.16 ± 0.11	0.00 ± 0.00
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.08
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.04 ± 0.04
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_4	0.04 ± 0.04	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00
# of Abnormal Cycles – Diestrus and Estrus	F_0	0.00 ± 0.00	0.16 ± 0.10	0.20 ± 0.12	0.08 ± 0.06
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.08
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.04 ± 0.04
	F_3	0.08 ± 0.06	0.16 ± 0.10	0.04 ± 0.04	0.08 ± 0.06
	F_4	0.08 ± 0.06	0.00 ± 0.00	0.08 ± 0.06	0.12 ± 0.07

TABLE J2
Estrous Cycle Characterization prior to Scheduled Sacrifice for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Percentage of Abnormal Cycles[b]					
% of Abnormal Cycles - Diestrus	F_0	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 4.00	8.00 ± 5.54
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_3	8.00 ± 5.54	12.00 ± 6.63	2.00 ± 2.00	6.00 ± 4.40
	F_4	2.00 ± 2.00	0.00 ± 0.00	2.00 ± 2.00	10.00 ± 5.77
% of Abnormal Cycles - Estrus	F_0	0.00 ± 0.00	8.00 ± 4.73	8.00 ± 5.54	0.00 ± 0.00
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 4.00
	F_2	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	2.00 ± 2.00
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_4	2.00 ± 2.00	0.00 ± 0.00	2.00 ± 2.00	0.00 ± 0.00
% of Abnormal Cycles – Diestrus and Estrus	F_0	0.00 ± 0.00	8.00 ± 4.73	12.00 ± 6.63	8.00 ± 5.54
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	4.00 ± 4.00
	F_2	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	2.00 ± 2.00
	F_3	8.00 ± 5.54	12.00 ± 6.63	2.00 ± 2.00	6.00 ± 4.40
	F_4	4.00 ± 2.77	0.00 ± 0.00	4.00 ± 2.77	10.00 ± 5.77

TABLE J2
Estrous Cycle Characterization prior to Scheduled Sacrifice for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Endpoint	Generation	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Length of Cycle [b,c]					
Length of Cycle (Days)	F_0	4.87 ± 0.09	4.80 ± 0.26	5.27 ± 0.38	5.20 ± 0.31
	F_1	4.80 ± 0.11	4.80 ± 0.11	4.87 ± 0.09	5.00 ± 0.24
	F_2	4.47 ± 0.16	4.20 ± 0.17 [3]	4.27 ± 0.17	4.33 ± 0.17 [3]
	F_3	5.07 ± 0.33	5.40 ± 0.36 [2]	4.73 ± 0.12	5.33 ± 0.29 [2]
	F_4	5.00 ± 0.24	4.60 ± 0.14	4.73 ± 0.12	5.13 ± 0.32

[a] Starting 10 days prior to the scheduled sacrifice date, daily vaginal smears were taken for determination of stage of the estrous cycle. The number of animals for which data were available for analysis in each exposure group of each generation is indicated under "Number of Animals." The following endpoints were analyzed: percentage of days in diestrus, estrus, or proestrus; number and percentage of abnormal cycles; and length of cycle. An abnormal cycle was defined as 4 or more consecutive days of diestrus or 3 or more consecutive days of estrus. Abnormal cycles due to prolonged diestrus or prolonged estrus were evaluated both separately and combined.

[b] Separate nonparametric one-way ANOVAs (Kruskal-Wallis' tests) were run on exposure concentration within each generation and on generation within each exposure group. Holm's-adjusted pairwise Wilcoxon's tests were run to compare exposed groups to the controls within generations or to compare all generations within an exposure group. No statistically significant exposure concentration effects were observed. Significant differences ($P \leq 0.05$) between generations within an exposure group are indicated by numbers in brackets.

[c] For the length of cycle endpoint, a Jonckheere-Terpstra linear exposure concentration trend test was run to evaluate trends within each generation; no significant trend tests were observed.

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

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TABLE K1
Adrenal Gland Weights and Adrenal Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	54.3 ± 1.2 (24)	54.4 ± 1.2	54.2 ± 1.9	55.8 ± 1.7	-	-
	F_1	55.2 ± 1.7	53.4 ± 1.7	58.0 ± 1.7	54.5 ± 1.5 (24)	-	-
Dose P=0.051	F_2	57.1 ± 2.6	52.5 ± 1.5	55.6 ± 1.7	55.2 ± 1.6	-	-
Gen P=0.160	F_3	57.2 ± 1.8	57.2 ± 1.9	58.0 ± 1.8	55.0 ± 1.9	-	-
DxG P=0.452	F_4	52.8 ± 1.7	50.5 ± 1.1	58.2 ± 1.9	53.9 ± 1.5	-	# #
Relative[e]	F_0	101.4 ± 2.2 (24)	103.3 ± 2.8	102.1 ± 3.2	118.0 ± 4.0** [3,4]	***/ # # #	#
	F_1	109.8 ± 3.9	107.0 ± 3.2	113.5 ± 3.0	118.2 ± 4.1 (24) [3,4]	-	-
Dose P=0.013	F_2	106.6 ± 4.3	104.9 ± 2.9	109.6 ± 3.4	118.8 ± 3.5 [3,4]	**/ # #	-
Gen P=0.001	F_3	106.9 ± 3.8	105.6 ± 3.6	105.4 ± 3.6	102.5 ± 3.5 [0,1,2]	-	-
DxG P=0.047	F_4	100.7 ± 3.0	100.0 ± 2.9	108.3 ± 3.8	102.4 ± 3.4 [0,1,2]	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	-	-	-	-	-
Dose P=0.107	F_2	-	-	-	-	-	-
Gen P=0.189	F_3	-	-	-	-	-	-
DxG P=0.401	F_4	-	-	-	-	-	*
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the ANCOVA analysis with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.436), F_0 breed father (P=0.017), and F_0 breed mother × F_0 breed father interaction (P=0.026) random effects incorporated into the analysis model.
[e] F_0 breed father (P=0.003) and F_0 breed mother × F_0 breed father interaction (P=0.003) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.011) and F_0 breed mother × F_0 breed father interaction (P=0.017) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: **, P≤0.01. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K2
Brain Weights and Brain Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	2128.8 ± 27.1 (24)	2189.0 ± 15.4 (23)	2171.6 ± 17.9	2160.6 ± 22.1	-	#
	F_1	2156.7 ± 18.4	2170.0 ± 21.9	2176.1 ± 22.9	2137.6 ± 25.5	-	-
Dose P=0.002	F_2	2109.6 ± 20.5	2154.9 ± 18.9	2167.7 ± 16.8	2126.4 ± 17.5	-	-
Gen P=0.001	F_3	2122.2 ± 25.0	2198.8 ± 20.8 (24)	2199.5 ± 23.9	2174.8 ± 23.0	-	-
DxG P=0.954	F_4	2083.7 ± 22.6	2155.6 ± 27.5 (24)	2109.0 ± 18.5	2108.6 ± 12.7	-	-
Relative[e]	F_0	3988.6 ± 72.0 (24) [1]	4132.9 ± 69.2 (23)	4112.9 ± 56.2	4559.6 ± 54.9*** [3,4]	***/ # # #	#
	F_1	4287.3 ± 67.0 [0,2,3,4]	4362.7 ± 62.1 [3]	4273.0 ± 60.2 [3,4]	4612.4 ± 73.2*** [3,4]	***/ # # #	-
Dose P<0.001	F_2	3957.4 ± 57.5 [1]	4316.2 ± 63.5**	4271.3 ± 43.3** [3,4]	4572.7 ± 52.4*** [3,4]	***/ # # #	-
Gen P<0.001	F_3	3963.3 ± 59.7 [1]	4076.8 ± 72.4 (24) [1]	4001.6 ± 76.5 [1,2]	4060.8 ± 69.1 [0,1,2]	-	-
DxG P<0.001	F_4	3980.2 ± 65.8 [1]	4260.3 ± 84.9 (24)	3916.3 ± 60.9 [1,2]	4002.1 ± 59.0 [0,1,2]	-	-
ANCOVA[f]	F_0	-	*	-	* [4]	#	-
	F_1	-	-	-	-	-	-
Dose P<0.001	F_2	-	*	-	-	-	* / #
Gen P<0.001	F_3	-	-	-	-	-	-
DxG P=0.823	F_4	-	*	-	- [0]	-	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.027), F_0 breed father (P=0.011), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.213), F_0 breed father (P=0.012), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.291), F_0 breed father (P=0.014), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ###, P≤0.001.

TABLE K3
Epididymis Weights and Epididymis Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	1370.8 ± 92.2 (23) [2]	1241.1 ± 54.9 (24)	1270.6 ± 31.6	1237.5 ± 40.0 (24)	-	-
	F_1	1245.3 ± 25.6	1160.9 ± 40.0 (24)	1353.4 ± 128.1	1166.0 ± 22.1 (23)	-	**
Dose P= 0.143	F_2	1179.4 ± 33.5 [0]	1206.0 ± 31.2	1215.8 ± 24.2	1188.3 ± 20.3	-	-
Gen P=0.055	F_3	1295.0 ± 29.0	1234.3 ± 38.5	1266.8 ± 30.4	1280.9 ± 33.7 (24)	-	-
DxG P=0.296	F_4	1218.3 ± 16.2	1205.6 ± 23.1	1232.6 ± 25.5	1214.3 ± 22.2 (24)	-	-
Relative[e]	F_0	2562.7 ± 175.3 (23)	2349.8 ± 105.2 (24)	2405.3 ± 60.5	2608.5 ± 86.9 (24)	-	#
	F_1	2478.4 ± 66.8	2334.2 ± 80.8 (24)	2668.5 ± 266.7	2539.2 ± 57.0 (23)	-	*
Dose P=0.143	F_2	2210.5 ± 65.3	2411.4 ± 64.6	2394.2 ± 48.1	2557.8 ± 52.9*	*/ # #	-
Gen P=0.055	F_3	2417.2 ± 55.3	2283.3 ± 72.6	2303.5 ± 64.8	2387.0 ± 69.9 (24)	-	-
DxG P=0.296	F_4	2325.1 ± 36.5	2379.5 ± 48.2	2284.4 ± 47.1	2311.2 ± 54.6 (24)	-	-
ANCOVA[f]	F_0	- [2]	-	-	-	-	-
	F_1	-	-	-	-	-	**
Dose P= 0.289	F_2	- [0]	-	-	-	-	-
Gen P=0.170	F_3	-	-	-	-	-	-
DxG P=0.339	F_4	-	-	-	-	-	-
BW P=0.010							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.205) and F_0 breed father (P=0.002) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.354) and F_0 breed father (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.259) and F_0 breed father (P<0.001) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01.

TABLE K4
Kidney Weights and Kidney Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	3514.0 ± 65.7 (24)	3475.3 ± 47.8 [2]	3489.8 ± 67.6 [2]	3284.3 ± 65.4**	***/ # #	-
	F_1	3315.6 ± 49.8	3523.6 ± 60.6	3295.0 ± 58.1	3063.5 ± 57.1** [3,4]	***/ # #	-
Dose P<0.001 Gen P<0.001 DxG P=0.079	F_2	3418.0 ± 61.4	3183.3 ± 56.6* [0]	3240.5 ± 41.5* [0]	3088.9 ± 56.5*** [3,4]	**/ # # #	-
	F_3	3352.7 ± 69.5	3393.0 ± 68.1	3477.6 ± 71.5	3388.6 ± 43.7 [1,2]	-	-
	F_4	3380.1 ± 41.6	3321.2 ± 73.5	3446.6 ± 60.6	3331.2 ± 81.9 [1,2]	-	-
Relative[e]	F_0	6555.7 ± 75.3 (24)	6580.2 ± 67.2	6582.1 ± 77.7	6910.2 ± 94.8* [3,4]	**/ # #	-
	F_1	6576.1 ± 93.9	6532.0 ± 120.3	6454.9 ± 89.7	6589.3 ± 94.4	-	-
Dose P<0.001 Gen P<0.001 DxG P=0.064	F_2	6394.2 ± 88.5	6356.4 ± 90.0	6384.4 ± 90.1	6621.2 ± 76.1	*	-
	F_3	6244.8 ± 105.2	6254.0 ± 89.4	6297.7 ± 107.2	6317.4 ± 91.6 [0]	-	-
	F_4	6443.2 ± 72.8	6528.2 ± 88.8	6382.4 ± 102.0	6286.0 ± 111.6 [0]	-	-
ANCOVA[f]	F_0	-	-	-	- [3,4]	-	-
	F_1	-	-	-	-	-	-
Dose P=0.917 Gen P<0.001 DxG P=0.889 BW P<0.001	F_2	-	-	-	-	-	-
	F_3	-	-	-	- [0]	-	-
	F_4	-	-	-	- [0]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P<0.001), F_0 breed father (P=0.208), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.073), F_0 breed father (P=0.013), and F_0 breed mother × F_0 breed father interaction (P=0.237) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.009), F_0 breed father (P=0.041), and F_0 breed mother × F_0 breed father interaction (P=0.244) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: ##, P≤0.01; ###, P≤0.001.

TABLE K5
Liver Weights and Liver Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P<0.001 Gen P=0.052 DxG P=0.021	F_0	17821 ± 412.2 (24) [3]	17381 ± 421.1 [4]	17678 ± 325.3 (24)	15826 ± 313.1***	***/ # # #	#
	F_1	16932 ± 335.9	16592 ± 291.7	17584 ± 433.9	16061 ± 441.3	*	*/ #
	F_2	17573 ± 340.0 (24)	16066 ± 374.0*	16917 ± 286.6	15863 ± 301.8**	*/ # #	-
	F_3	16257 ± 402.2 [0]	16702 ± 415.0	16742 ± 351.9	16303 ± 360.8	-	-
	F_4	16345 ± 355.7	15559 ± 450.7 [0]	17037 ± 344.9	16406 ± 378.2	-	-
Relative[e] Dose P=0.053 Gen P<0.001 DxG P=0.801	F_0	33169 ± 333.6 (24) [3,4]	32836 ± 571.8 [3,4]	33515 ± 373.2 (24) [3,4]	33299 ± 444.1 [3,4]	-	-
	F_1	33555 ± 597.8 [3,4]	33295 ± 536.6 [3,4]	34405 ± 688.3 [3,4]	34377 ± 512.2 [3,4]	-	-
	F_2	32968 ± 449.0 (24) [3,4]	32026 ± 531.3	33278 ± 462.9 [3,4]	33972 ± 307.7 [3,4]	-	-
	F_3	30227 ± 531.7 [0,1,2]	30711 ± 442.7 [0,1]	30257 ± 366.3 [0,1,2]	30294 ± 479.6 [0,1,2]	-	-
	F_4	31040 ± 364.6 [0,1,2]	30483 ± 441.2 [0,1]	31496 ± 447.5 [0,1,2]	30936 ± 413.1 [0,1,2]	-	-
ANCOVA[f] Dose P=0.048 Gen P<0.001 DxG P=0.654 BW P<0.001	F_0	- [3,4]	- [3,4]	- [3,4]	- [3,4]	-	-
	F_1	- [3,4]	- [3,4]	- [3,4]	- [3,4]	#	-
	F_2	- [3,4]	- [3]	- [3,4]	- [3,4]	**/ #	
	F_3	- [0,1,2]	- [0,1,2]	- [0,1,2]	- [0,1,2]	-	-
	F_4	- [0,1,2]	- [0,1]	- [0,1,2]	- [0,1,2]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.005) and F_0 breed mother × F_0 breed father interaction (P=0.042) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.019) and F_0 breed mother × F_0 breed father interaction (P=0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.007) and F_0 breed mother × F_0 breed father interaction (P=0.018) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K6
Pituitary Gland Weights and Pituitary Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.034 Gen P<0.001 DxG P=0.344	F_0	13.6 ± 0.7 (24)	14.0 ± 0.7	14.6 ± 0.4	14.9 ± 0.6 (23)	-	-
	F_1	13.4 ± 0.7 (24)	14.4 ± 0.4	15.1 ± 0.4*	15.0 ± 0.5 (24)	#	-
	F_2	14.8 ± 0.3	15.0 ± 0.4	15.2 ± 0.5 (24)	14.9 ± 0.5	-	-
	F_3	15.8 ± 0.6	16.0 ± 0.6	16.7 ± 0.5	15.2 ± 0.3	-	-
	F_4	16.0 ± 0.7	14.8 ± 0.7	16.9 ± 0.6	15.3 ± 0.5	-	-
Relative[e] Dose P<0.001 Gen P=0.019 DxG P=0.002	F_0	25.4 ± 1.4 (24)	26.5 ± 1.2	27.7 ± 0.7	31.5 ± 1.3*** (23)	***/ # # #	-
	F_1	26.5 ± 1.3 (24)	28.9 ± 0.9	29.7 ± 0.8	32.4 ± 0.8*** (24)	***/ # # #	-
	F_2	27.8 ± 0.7	30.1 ± 0.9	30.0 ± 1.1 (24)	31.9 ± 1.1* (25)	*/ # #	-
	F_3	29.3 ± 0.9	29.6 ± 1.1	30.3 ± 0.9	28.5 ± 0.8	-	-
	F_4	30.5 ± 1.2	29.1 ± 1.2	31.4 ± 1.2	29.0 ± 0.9	-	-
ANCOVA[f] Dose P=0.012 Gen P=0.005 DxG P=0.041 BW P<0.001	F_0	-	-	-	**	**/ # # #	-
	F_1	-	-	-	**	**/ # # #	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.149), F_0 breed father (P=0.068), and F_0 breed mother × F_0 breed father interaction (P=0.005) random effects incorporated into the analysis model.
[e] F_0 breed father (P=0.094) and F_0 breed mother × F_0 breed father interaction (P=0.019) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.087) and F_0 breed mother × F_0 breed father interaction (P=0.012) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K7
Dorsal Prostate Gland Weights and Dorsal Prostate Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.913 Gen P<0.001 DxG P=0.823	F_0	283.0 ± 13.5 (24) [2]	264.5 ± 10.1	261.0 ± 14.8	285.2 ± 10.2 [1,2]	-	-
	F_1	245.7 ± 10.6 [3]	236.3 ± 12.1 [3,4]	232.0 ± 11.3 [3]	232.8 ± 10.8 (24) [0,3,4]	-	-
	F_2	226.0 ± 11.3 [0,3,4]	222.2 ± 11.9 [3,4]	252.9 ± 12.5 (24)	234.3 ± 12.1 [0,3,4]	-	-
	F_3	314.9 ± 20.0 [1,2]	309.7 ± 15.7 [1,2]	300.4 ± 14.6 [1]	300.2 ± 15.1 [1,2]	-	-
	F_4	280.2 ± 16.9 [2]	291.5 ± 11.5 [1,2]	275.1 ± 13.8	289.2 ± 17.7 [1,2]	-	-
Relative[e] Dose P=0.115 Gen P<0.001 DxG P=0.103	F_0	527.3 ± 23.7 (24) [2]	502.7 ± 20.3	490.4 ± 25.4	603.1 ± 23.3 [1,2]	**/#	# #
	F_1	489.6 ± 24.2	472.6 ± 21.6 [3,4]	457.1 ± 24.3	499.6 ± 22.1 (24) [0]	-	-
	F_2	423.6 ± 21.7 [0,3,4]	445.2 ± 23.7 [3,4]	499.6 ± 25.0 (24)	504.1 ± 26.9 [0]	#	-
	F_3	585.6 ± 35.4 [2]	575.5 ± 31.6 [1,2]	543.5 ± 25.2	560.8 ± 28.7	-	-
	F_4	531.7 ± 29.5 [2]	576.5 ± 24.5 [1,2]	509.9 ± 25.0	546.1 ± 31.8	-	-
ANCOVA[f] Dose P=0.644 Gen P<0.001 DxG P=0.514 BW P=0.001	F_0	- [2]	-	-	-	-	#
	F_1	- [3]	- [3,4]	- [3]	-	-	-
	F_2	- [0,3,4]	- [3,4]	-	-	-	-
	F_3	- [1,2]	- [1,2]	- [1]	-	-	-
	F_4	- [2]	- [1,2]	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed father (P=0.400) random effect incorporated into the analysis model.
[e] F_0 breed mother (P=0.399), F_0 breed father (P=0.187), and F_0 breed mother × F_0 breed father interaction (P=0.271) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.285) and F_0 breed mother × F_0 breed father interaction (P=0.495) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01.

TABLE K8
Lateral Prostate Gland Weights and Lateral Prostate Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	318.5 ± 15.5 (24) [1,2,3]	291.2 ± 12.4	322.4 ± 14.4 [2,3]	299.2 ± 15.4 [3]	-	-
	F_1	257.8 ± 11.8 [0]	280.4 ± 18.6	304.6 ± 16.0 [3]	275.8 ± 13.5 (24)	-	*
Dose P=0.003	F_2	238.7 ± 14.1 [0]	236.1 ± 15.1 [4]	261.6 ± 11.0 [0,4]	252.4 ± 11.4 (24)	-	-
Gen P<0.001	F_3	252.5 ± 14.4 [0]	248.4 ± 18.1	238.3 ± 12.1 [0,1,4]	221.1 ± 9.9 [0]	-	-
DxG P=0.098	F_4	280.6 ± 18.7	304.2 ± 14.2 [2]	354.2 ± 16.0*** [2,3]	273.4 ± 12.5	-	***/ ###
Relative[e]	F_0	596.1 ± 29.4 (24) [2,3]	551.0 ± 22.8	609.2 ± 26.9 [3]	633.8 ± 35.1 [3,4]	-	-
	F_1	510.4 ± 22.4	564.6 ± 38.1	599.9 ± 34.2 [3]	591.9 ± 29.2 (24) [3]	#	-
Dose P=0.038	F_2	447.0 ± 26.6 [0]	473.2 ± 30.5 [4]	517.5 ± 23.5 [4]	544.7 ± 25.5* [3]	*/ ##	-
Gen P<0.001	F_3	472.4 ± 27.9 [0]	463.5 ± 35.8 [4]	433.7 ± 23.3 [0,1,4]	411.6 ± 18.1 [0,1,2,4]	-	-
DxG P=0.007	F_4	531.8 ± 34.0	596.9 ± 25.1 [2,3]	655.7 ± 28.6** [2,3]	519.1 ± 25.9 [0,3]	-	***/ ###
ANCOVA[f]	F_0	- [2,3]	-	- [2,3]	- [3]	-	-
	F_1	-	-	- [3]	- [3]	-	*
Dose P=0.012	F_2	- [0]	- [4]	- [0,4]	-	-	-
Gen P<0.001	F_3	- [0]	- [4]	- [0,1,4]	- [0,1]	-	-
DxG P=0.060	F_4	-	- [2,3]	** [2,3]	-	-	***/ ###
BW P=0.057							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.382) and F_0 breed mother × F_0 breed father interaction (P=0.080) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.368) and F_0 breed mother × F_0 breed father interaction (P=0.058) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.438) and F_0 breed mother × F_0 breed father interaction (P=0.080) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K9
Ventral Prostate Gland Weights and Ventral Prostate Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.019 Gen P<0.001 DxG P=0.998	F_0	669.7 ± 35.0 (24) [4]	632.9 ± 24.6 [4]	651.6 ± 31.9 [4]	638.0 ± 30.2	-	-
	F_1	608.6 ± 34.8 (24) [4]	582.8 ± 26.5 [4]	588.3 ± 30.8 [4]	556.8 ± 29.8 [4]	-	-
	F_2	703.8 ± 40.4	621.5 ± 33.6 [4]	644.7 ± 26.7 [4]	616.4 ± 29.6	-	-
	F_3	722.9 ± 38.3	651.6 ± 22.7	680.0 ± 38.1	658.2 ± 30.2	-	-
	F_4	829.9 ± 48.8 [0,1]	769.0 ± 41.2 [0,1,2]	806.7 ± 50.4 [0,1,2]	720.5 ± 42.0 (24) [1]	-	-
Relative[e] Dose P=0.390 Gen P<0.001 DxG P=0.683	F_0	1249.8 ± 63.6 (24) [4]	1198.5 ± 45.6 [4]	1234.5 ± 61.6	1346.1 ± 65.9	-	-
	F_1	1215.3 ± 72.7 (24) [4]	1169.5 ± 52.5 [4]	1153.6 ± 60.4 [4]	1207.8 ± 72.6	-	-
	F_2	1323.6 ± 79.9	1246.6 ± 69.4 [4]	1273.1 ± 55.6	1321.5 ± 61.2	-	-
	F_3	1353.0 ± 74.7	1206.1 ± 44.2 [4]	1235.9 ± 71.8	1229.2 ± 58.0	-	-
	F_4	1572.6 ± 83.7 [0,1]	1530.7 ± 91.7 [0,1,2,3]	1486.6 ± 85.5 [1]	1357.3 ± 76.8 (24)	*/ #	-
ANCOVA[f] Dose P=0.101 Gen P<0.001 DxG P=0.988 BW P=0.033	F_0	- [4]	- [4]	- [4]	-	-	-
	F_1	- [4]	- [4]	- [4]	-	-	-
	F_2	-	- [4]	- [4]	-	-	-
	F_3	-	-	-	-	-	-
	F_4	- [0,1]	- [0,1,2]	- [0,1,2]	-	*	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother × F_0 breed father interaction (P=0.129) random effect incorporated into the analysis model.
[e] F_0 breed mother × F_0 breed father interaction (P=0.032) random effect incorporated into the analysis model.
[f] F_0 breed mother × F_0 breed father interaction (P=0.092) random effect incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05.

TABLE K10
Seminal Vesicle/Coagulating Gland Weights and Seminal Vesicle/Coagulating Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.008 Gen P<0.001 DxG P=0.648	F_0	1387.0 ± 53.1 (24)	1401.8 ± 44.1 [4]	1486.8 ± 52.6	1395.9 ± 35.6 (24) [1,4]	-	*
	F_1	1249.9 ± 54.1 (24) [3,4]	1337.3 ± 47.6 (20) [4]	1360.6 ± 41.0 (24) [4]	1217.5 ± 53.7 (23) [0,3,4]	-	*/# #
	F_2	1215.6 ± 45.7 [3,4]	1339.9 ± 44.6 [4]	1372.2 ± 44.4* (24) [4]	1283.4 ± 60.5 [4]	-	#
	F_3	1442.9 ± 33.6 [1,2]	1450.0 ± 37.4	1464.0 ± 47.4	1424.4 ± 52.4 [1,4]	-	-
	F_4	1534.8 ± 37.4 [1,2]	1566.6 ± 37.2 [0,1,2]	1569.5 ± 53.6 [1,2]	1598.6 ± 47.9 [0,1,2,3]	-	-
Relative[e] Dose P<0.001 Gen P<0.001 DxG P=0.109	F_0	2595.7 ± 101.2 (24)	2658.5 ± 84.8 [4]	2813.7 ± 102.9	2945.1 ± 89.2** (24)	***/ # # #	-
	F_1	2504.5 ± 120.0 (24) [4]	2690.2 ± 96.7 (20)	2667.8 ± 80.8 (24)	2641.2 ± 131.9 (23)	-	-
	F_2	2281.0 ± 90.6 [3,4]	2683.5 ± 92.9**	2698.4 ± 100.0** (24)	2770.4 ± 143.2***	*/ # # #	*/ #
	F_3	2702.2 ± 81.7 [2]	2705.0 ± 104.0	2661.0 ± 94.7	2663.6 ± 107.5	-	-
	F_4	2919.3 ± 59.6 [1,2]	3098.4 ± 87.0 [0]	2907.0 ± 98.5	3039.1 ± 106.3	-	-
ANCOVA[f] Dose P=0.009 Gen P<0.001 DxG P=0.644 BW P=0.747	F_0	-	- [4]	-	- [1,4]	-	*
	F_1	- [3,4]	- [4]	-	- [0,3,4]	-	*/ #
	F_2	- [3,4]	- [4]	*	- [4]	-	#
	F_3	- [1,2]	-	-	- [1,4]	-	-
	F_4	- [1,2]	- [0,1,2]	-	- [0,1,2,3]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.192), F_0 breed father (P=0.164), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.026), F_0 breed father (P=0.018), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.188), F_0 breed father (P=0.160), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K11
Spleen Weights and Spleen Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	812.2 ± 20.6 (24)	827.4 ± 14.7	845.7 ± 18.5	789.3 ± 16.4	*	#
	F_1	769.5 ± 16.9 [2]	817.7 ± 18.1	807.4 ± 18.6	808.5 ± 18.8	-	-
Dose P=0.215	F_2	852.3 ± 22.7 [1]	836.3 ± 19.2	824.3 ± 15.0	816.8 ± 17.9	-	-
Gen P=0.230	F_3	805.7 ± 22.0	831.0 ± 16.9	839.0 ± 19.1	818.4 ± 17.8	-	-
DxG P=0.337	F_4	792.6 ± 18.1	835.6 ± 16.0	825.9 ± 19.0	831.8 ± 17.9	-	-
Relative[e]	F_0	1518.7 ± 38.4 (24)	1569.9 ± 31.2	1595.4 ± 26.5	1664.9 ± 34.3** [3]	***/ # # #	-
	F_1	1531.8 ± 43.2	1639.9 ± 31.2	1580.1 ± 29.1	1743.3 ± 43.7*** [3,4]	***/ # # #	-
Dose P<0.001	F_2	1597.1 ± 42.8	1670.5 ± 34.4	1623.5 ± 29.8	1750.9 ± 30.7** [3,4]	**/ #	-
Gen P<0.001	F_3	1497.4 ± 31.0	1534.5 ± 28.3	1518.7 ± 29.1	1523.3 ± 29.6 [0,1,2]	-	-
DxG P=0.091	F_4	1508.9 ± 30.1	1650.2 ± 35.1*	1528.0 ± 29.2	1576.3 ± 36.4 [1,2]	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	*	-	**	**/ # #	-
Dose P<0.001	F_2	-	-	-	- [3]	-	-
Gen P=0.002	F_3	-	-	-	- [2]	-	-
DxG P=0.567	F_4	-	*	-	-	-	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P<0.001), F_0 breed father (P=0.144), and F_0 breed mother × F_0 breed father interaction (P=0.001) random effects incorporated into the analysis model.
[e] F_0 breed mother (P<0.001), F_0 breed father (P=0.002), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P<0.001), F_0 breed father (P=0.025), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects could not be incorporated into the analysis model due to computational unfeasibility.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K12
Right and Left Testis Weights and Testis Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P<0.001 Gen P=0.006 DxG P=0.765	F_0	3454.9 ± 65.1 (24)	3302.8 ± 112.0	3577.8 ± 86.6 (24)	3483.5 ± 104.9 [1]	-	*
	F_1	3403.7 ± 65.1	3271.9 ± 124.2	3340.4 ± 59.8	3162.0 ± 91.4 (24) [0]	-	-
	F_2	3305.4 ± 143.1	3179.2 ± 74.8	3334.5 ± 60.7	3251.9 ± 57.1	-	-
	F_3	3454.1 ± 52.6	3215.2 ± 97.7	3415.5 ± 43.3 (24)	3296.0 ± 48.5	-	-
	F_4	3389.0 ± 47.5	3359.2 ± 65.3	3485.2 ± 29.3	3249.0 ± 42.4	-	-
Relative[e] Dose P=0.014 Gen P<0.001 DxG P<0.001	F_0	6466.4 ± 138.3 (24)	6258.6 ± 212.0	6606.8 ± 176.1 (24)	7345.3 ± 222.1*** [3,4]	***/ # # #	#
	F_1	6777.1 ± 177.1	6586.4 ± 269.1	6567.4 ± 147.7	6863.0 ± 224.3 (24) [3,4]	-	-
	F_2	6182.6 ± 255.4	6362.6 ± 159.3	6564.0 ± 113.8	6991.6 ± 130.0** [3,4]	***/ # # #	-
	F_3	6448.6 ± 111.8	5957.4 ± 203.8	6200.7 ± 124.0 (24)	6149.2 ± 111.1 [0,1,2]	-	-
	F_4	6462.0 ± 91.8	6624.4 ± 124.7	6468.6 ± 89.9	6164.6 ± 110.5 [0,1,2]	-	-
ANCOVA[f] Dose P=0.010 Gen P=0.025 DxG P=0.398 BW P<0.001	F_0	-	-	-	- [4]	#	-
	F_1	-	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	- [0]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] No significant F_0 parental generation random effects incorporated in the model.
[e] F_0 breed father (P=0.386) and F_0 breed mother × F_0 breed father interaction (P=0.314) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.462) random effect incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ###, P≤0.001.

TABLE K13
Thymus Weights and Thymus Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	370.2 ± 18.8 (24)	380.0 ± 18.3 [2,3,4]	365.8 ± 16.9	333.7 ± 20.0 [3,4]	*	-
	F_1	428.9 ± 20.7	436.3 ± 15.3	436.0 ± 19.1	371.8 ± 13.6 [4]	**/ #	-
Dose P=0.002	F_2	415.8 ± 17.5	448.1 ± 20.6 [0]	417.1 ± 18.2	391.7 ± 20.1	-	-
Gen P<0.001	F_3	395.4 ± 14.8	483.4 ± 20.7** [0]	426.8 ± 23.8	415.0 ± 16.6 [0]	-	#
DxG P=0.112	F_4	409.7 ± 15.9	449.7 ± 22.4 [0]	423.6 ± 18.2	460.0 ± 24.3 [0,1]	-	-
Relative[e]	F_0	687.3 ± 30.2 (24) [1]	716.3 ± 30.4 [1,2,3,4]	690.8 ± 30.3 [1]	703.0 ± 40.5 [2,4]	-	-
	F_1	851.9 ± 41.5 [0]	876.1 ± 31.0 [0]	854.2 ± 35.3 [0]	806.8 ± 37.0	-	-
Dose P=0.002	F_2	778.0 ± 31.8	893.2 ± 37.8 [0]	820.1 ± 34.6	837.4 ± 40.2 [0]	-	-
Gen P<0.001	F_3	738.3 ± 28.0	892.5 ± 39.0** [0]	775.5 ± 44.6	769.7 ± 26.0	-	-
DxG P=0.535	F_4	780.3 ± 29.1	887.0 ± 44.1 [0]	784.1 ± 32.8	874.9 ± 49.3 [0]	-	-
ANCOVA[f]	F_0	-	- [1,2,3,4]	-	- [4]	-	-
	F_1	-	- [0]	-	-	-	-
Dose P=0.003	F_2	-	- [0]	-	-	-	-
Gen P<0.001	F_3	-	** [0]	-	-	-	-
DxG P=0.437	F_4	-	- [0]	-	- [0]	-	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.040), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.005), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.010), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: **, P≤0.01. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means were significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05.

TABLE K14
Thyroid Gland Weights and Thyroid Gland Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	36.2 ± 2.2 (24)	36.6 ± 2.0	37.7 ± 1.6	35.5 ± 2.1 (24)	-	-
	F_1	40.0 ± 3.3	35.0 ± 1.6	33.0 ± 1.7*	28.9 ± 1.4*** (24) [4]	***/ # # #	-
Dose P=0.047	F_2	38.1 ± 2.4	34.6 ± 2.0	39.0 ± 2.7	33.7 ± 1.4	-	-
Gen P=0.083	F_3	35.6 ± 1.4	35.1 ± 1.4	39.4 ± 1.5	34.4 ± 1.5	-	-
DxG P=0.035	F_4	36.7 ± 2.0	37.2 ± 1.6	39.2 ± 1.7	39.9 ± 1.7 [1]	-	-
Relative[e]	F_0	68.0 ± 4.4 (24)	69.4 ± 3.8	71.8 ± 3.3	74.6 ± 4.3 (24)	-	-
	F_1	80.1 ± 7.1	70.3 ± 3.4	64.4 ± 3.1**	62.1 ± 2.7*** (24)	**/ # # #	*
Dose P=0.798	F_2	71.8 ± 4.8	69.4 ± 4.3	76.5 ± 5.1	72.5 ± 3.0	-	-
Gen P=0.139	F_3	66.2 ± 2.6	64.7 ± 2.5	71.4 ± 2.6	64.4 ± 3.0	-	-
DxG P=0.057	F_4	69.7 ± 3.7	74.1 ± 4.0	72.9 ± 3.3	75.9 ± 3.6	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	-	*	*** [4]	***/ # # #	-
Dose P=0.142	F_2	-	-	-	-	-	-
Gen P=0.155	F_3	-	-	-	-	-	-
DxG P=0.050	F_4	-	-	-	- [1]	-	-
BW P=0.230							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed father (P=0.015) and F_0 breed mother × F_0 breed father interaction (P=0.020) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.406), F_0 breed father (P=0.033), and F_0 breed mother × F_0 breed father interaction (P=0.013) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.016) and F_0 breed mother × F_0 breed father interaction (P=0.018) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means were significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: ###, P≤0.001.

TABLE K15
Adrenal Gland Weights and Adrenal Gland Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	68.5 ± 2.3	64.6 ± 1.4	64.8± 1.4	62.7 ± 1.8 (24)	#	-
	F_1	63.3 ± 1.9	65.4 ± 1.7	63.4 ± 2.2	60.7 ± 1.4 [3,4]	-	-
Dose P=0.048	F_2	65.3 ± 2.1	71.0 ± 1.7	67.2 ± 1.8	60.8 ± 1.8 [3]	**/ #	# #
Gen P<0.001	F_3	68.3 ± 2.2	67.3 ± 2.1	69.3 ± 1.8	67.9 ± 1.7 [1,2]	-	-
DxG P=0.031	F_4	63.4 ± 1.4	68.7 ± 1.7	70.0 ± 2.1	67.2 ± 1.5 [1]	-	#
Relative[e]	F_0	231.7 ± 6.8	227.4 ± 4.9	236.9 ± 5.7	249.2 ± 7.6 (24)	*/ #	-
	F_1	225.9 ± 7.0	237.9 ± 7.3	236.2 ± 8.5	250.0 ± 5.8*	*/ #	-
Dose P=0.076	F_2	232.7 ± 7.5	249.9 ± 6.8	239.7 ± 5.4	242.6 ± 6.2	-	-
Gen P=0.773	F_3	241.7 ± 7.2	237.3 ± 7.0	238.3 ± 6.6	233.0 ± 5.1	-	-
DxG P=0.466	F_4	231.2 ± 6.0	239.1 ± 5.6	240.2 ± 6.9	235.2 ± 5.9	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	-	-	-	-	-
Dose P=0.477	F_2	-	-	-	-	-	-
Gen P=0.182	F_3	-	-	-	-	-	-
DxG P=0.613	F_4	-	-	-	-	-	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P<0.001), F_0 breed father (P=0.044), and F_0 breed mother × F_0 breed father interaction (P=0.055) random effects incorporated into the analysis model.
[e] F_0 breed mother (P<0.001), F_0 breed father (P=0.127), and F_0 breed mother × F_0 breed father interaction (P=0.009) random effects incorporated into the analysis model.
[f] F_0 breed mother (P<0.001), F_0 breed father (P=0.057), and F_0 breed mother × F_0 breed father interaction (P=0.026) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05. There were no significant generation effects in pairwise comparisons for the adrenal gland of female rats.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ## P≤0.01.

TABLE K16
Brain Weights and Brain Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	1979.3 ± 22.7 (24)	1939.8 ± 21.5 [3]	2004.6 ± 17.1	1939.2 ± 18.5	-	-
	F_1	1972.8 ± 18.8	1995.4 ± 15.4 (24)	1974.4 ± 17.9	1928.7 ± 24.9	*/ #	-
Dose P=0.001	F_2	1910.1 ± 20.0	1982.3 ± 19.9	1966.6 ± 19.9	1888.8 ± 17.6 [3]	*	# #
Gen P<0.001	F_3	1969.4 ± 20.7	2028.5 ± 37.8 [0]	1981.2 ± 18.7	1979.6 ± 19.0 [2]	-	-
DxG P=0.154	F_4	1891.6 ± 16.3	1985.2 ± 17.5*	1958.6 ± 26.5	1912.8 ± 14.1	-	#
Relative[e]	F_0	6722.8± 155.4 (24)	6848.5 ± 112.7	7340.7 ± 140.3*** [3,4]	7746.1 ± 137.0*** [3,4]	***/ # # #	**
	F_1	7048.3 ± 103.6	7197.6 ± 88.8 (24)	7372.6 ± 133.5 [3,4]	7940.7 ± 101.6*** [3,4]	***/ # # #	#
Dose P<0.001	F_2	6817.4 ± 107.9	6962.9 ± 76.7	7035.2 ± 82.4	7562.6 ± 83.6*** [3,4]	***/ # # #	-
Gen P<0.001	F_3	6993.3 ± 110.8	7174.3 ± 153.0	6827.7 ± 117.0 [0,1]	6814.3 ± 90.8 [0,1,2]	-	-
DxG P<0.001	F_4	6887.0 ± 82.6	6926.7 ± 98.5	6722.5 ± 96.3 [0,1]	6688.2 ± 83.8 [0,1,2]	-	-
ANCOVA[f]	F_0	-	- [3]	-	-	-	*
	F_1	-	-	-	-	-	-
D P=0.041	F_2	-	-	-	-	-	#
Gen P<0.001	F_3	-	- [0]	-	-	-	-
DxG P=0.283	F_4	-	-	-	-	-	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.049), F_0 breed father (P=0.010), and F_0 breed mother × F_0 breed father interaction (P=0.003) random effects incorporated into the analysis model.
[e] F_0 breed mother (P<0.001), F_0 breed father (P=0.001), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.210), F_0 breed father (P=0.002), and F_0 breed mother × F_0 breed father interaction (P=0.004) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ## P≤0.01; ### P≤0.001.

TABLE K17
Kidney Weights and Kidney Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	2056.8 ± 55.1 [4]	1902.8 ± 38.0**	1885.1 ± 33.4***	1718.0 ± 28.8*** [3,4]	***/ # # #	-
	F_1	1925.5 ± 37.3	1876.0 ± 31.7	1858.2 ± 60.9 [4]	1599.7 ± 27.1*** [3,4]	***/ # # #	#
Dose P<0.001 Gen P<0.001 DxG P<0.001	F_2	1910.0 ± 31.5	1946.3 ± 34.7	1942.6 ± 39.4	1673.7 ± 35.8*** [3,4]	***/ # # #	# #
	F_3	1912.6 ± 29.7	1982.8 ± 42.1	1992.3 ± 51.5	1985.8 ± 40.0 [0,1,2]	-	-
	F_4	1861.8 ± 28.1 [0]	1975.8 ± 36.0	2024.4 ± 40.8* [1]	1960.6 ± 39.2 [0,1,2]	-	*/ #
Relative[e]	F_0	6942.5 ± 113.3	6686.9 ± 88.2	6867.2 ± 88.3	6838.1 ± 97.7	-	-
	F_1	6850.4 ± 86.3	6797.7 ± 118.8	6896.2 ± 185.4	6579.8 ± 92.1	-	-
Dose P=0.190 Gen P=0.739 DxG P=0.668	F_2	6802.3 ± 99.9	6817.5 ± 75.5	6926.9 ± 96.3	6674.8 ± 83.8	-	-
	F_3	6778.3 ± 100.7	6990.4 ± 120.9	6826.1 ± 127.7	6814.4 ± 104.0	-	-
	F_4	6771.3 ± 98.0	6867.7 ± 80.6	6928.9 ± 96.3	6830.9 ± 96.4	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	-	-	-	-	-
Dose P=0.175 Gen P=0.702 DxG P=0.669 BW P<0.001	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P<0.001), F_0 breed father (P=0.209), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.020), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.005) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.020), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.005) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ## P≤0.01; ### P≤0.001.

TABLE K18
Liver Weights and Liver Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	9556.7 ± 295.8 [4]	9170.5 ± 231.3	8957.1 ± 166.5	8429.6 ± 257.3*** [3]	***/ # # #	-
	F_1	9524.3 ± 232.7 [4]	9526.3 ± 170.9	9070.4 ± 190.9	8402.4 ± 176.8*** [3]	***/ # # #	-
Dose P<0.001 Gen P=0.138 DxG P<0.001	F_2	9331.3 ± 192.2 [4]	9986.1 ± 209.8 [3]	9755.9 ± 233.5	8513.4 ± 252.4* [3]	***/ # #	# # #
	F_3	8880.4 ± 257.1	8923.1 ± 154.0 [2]	9029.6 ± 184.0	9551.1 ± 265.8 [0,1,2]	*	-
	F_4	8468.6 ± 166.3 [0,1,2]	9339.7 ± 211.9*	9570.3 ± 225.8**	9181.2 ± 239.6	#	**/ # #
Relative[e]	F_0	32202 ± 546.2	32204 ± 595.9 [2]	32660 ± 532.7	33454 ± 813.8	-	-
	F_1	33877 ± 651.0 [4]	34537 ± 672.7 [3]	33731 ± 600.1 [3]	34526 ± 569.5 [4]	-	-
Dose P=0.122 Gen P<0.001 DxG P=0.395	F_2	33207 ± 596.5	34996 ± 580.8 [0,3,4]	34723 ± 492.3 [3]	33896 ± 737.8	-	#
	F_3	31419 ± 820.1	31501 ± 495.4 [1,2]	30975 ± 452.2 [1,2]	32689 ± 654.2	-	-
	F_4	30787 ± 569.4 [1]	32435 ± 500.1 [2]	32744 ± 577.8	31921 ± 613.3 [1]	-	*
ANCOVA[f]	F_0	-	- [1,2]	-	-	*/ #	-
	F_1	- [4]	- [0,3]	- [3]	- [4]	-	-
Dose P=0.058 Gen P<0.001 DxG P=0.321 BW P<0.001	F_2	-	- [0,3,4]	- [3]	-	-	-
	F_3	-	- [1,2]	- [1,2]	-	-	-
	F_4	- [1]	- [2]	-	- [1]	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.070), F_0 breed father (P=0.490), and F_0 breed mother × F_0 breed father interaction (P=0.151) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.278), F_0 breed father (P=0.245), and F_0 breed mother × F_0 breed father interaction (P=0.194) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.173), F_0 breed father (P=0.191), and F_0 breed mother × F_0 breed father interaction (P=0.142) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ## P≤0.01; ### P≤0.001.

TABLE K19
Left and Right Ovary Weights and Ovary Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.009 Gen P<0.001 DxG P=0.138	F_0	169.5 ± 6.2	159.3 ± 4.6	156.6 ± 3.1	155.2 ± 4.5 [1]	#	-
	F_1	153.6 ± 5.3 [3]	151.8 ± 4.2 [2,4]	152.5 ± 5.0 (24) [3]	135.0 ± 4.8** [0,2,3,4]	***/ # #	-
	F_2	166.1 ± 6.2	171.7 ± 6.7 [1]	168.1 ± 5.5	153.0 ± 4.8 [1]	**/ #	-
	F_3	173.7 ± 6.5 (23) [1]	164.4 ± 5.8	173.7 ± 5.6 [1]	172.7 ± 5.0 [1]	-	-
	F_4	158.3 ± 3.2	168.8 ± 4.5 [1]	169.3 ± 5.4	162.3 ± 2.7 [1]	-	-
Relative[e] Dose P=0.869 Gen P<0.001 DxG P=0.656	F_0	574.9 ± 20.3	560.6 ± 15.3	572.6 ± 13.6	616.6 ± 15.0 [1]	*	-
	F_1	546.4 ± 16.5	550.0 ± 15.2	568.7 ± 18.1 (24)	554.9 ± 18.6 [0]	-	-
	F_2	591.3 ± 21.3	603.6 ± 24.4	601.3 ± 20.0	612.2 ± 19.0	-	-
	F_3	611.9 ± 20.0 (23)	579.3 ± 19.0	595.5 ± 16.3	592.1 ± 14.9	-	-
	F_4	576.8 ± 13.7	588.9 ± 17.0	580.0 ± 17.4	567.9 ± 11.7	-	-
ANCOVA[f] Dose P=0.832 Gen P<0.001 DxG P=0.834 BW P<0.001	F_0	-	-	-	- [1]	-	-
	F_1	- [3]	-	-	- [0,3]	-	-
	F_2	-	-	-	-	-	-
	F_3	- [1]	-	-	- [1]	-	-
	F_4	-	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.162), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.033) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.132), F_0 breed father (P=0.005), and F_0 breed mother × F_0 breed father interaction (P=0.111) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.273), F_0 breed father (P=0.001), and F_0 breed mother × F_0 breed father interaction (P=0.110) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: **, P≤0.01. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05, ##, P≤0.01.

TABLE K20
Pituitary Gland Weights and Pituitary Gland Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.007 Gen P<0.001 DxG P=0.291	F_0	18.4 ± 0.9	19.0 ± 0.7 [1]	18.6 ± 0.6 (23) [1]	16.6 ± 0.8	**/ #	-
	F_1	16.1 ± 0.7 (24)	15.8 ± 0.4 (22) [0]	15.3 ± 0.5 (24) [0,3,4]	14.8 ± 0.6 (24) [3]	-	-
	F_2	16.7 ± 0.9 (24)	17.0 ± 0.6	17.0 ± 0.7 (24)	15.0 ± 0.6 [3]	*/ #	-
	F_3	16.6 ± 0.5	16.7 ± 0.8	18.7 ± 0.7 [1]	18.2 ± 0.8 [1,2]	-	-
	F_4	17.4 ± 0.5	17.8 ± 0.6	19.0 ± 0.7 [1]	16.9 ± 0.6	-	-
Relative[e] Dose P=0.436 Gen P<0.001 DxG P=0.445	F_0	61.9 ± 2.9	67.0 ± 2.4 [1,3]	67.5 ± 1.9 (23) [1]	66.4 ± 3.4	-	-
	F_1	57.2 ± 2.4 (24)	57.6 ± 1.7 (22) [0]	57.3 ± 2.0 (24) [0]	61.1 ± 2.4 (24)	-	-
	F_2	59.0 ± 2.9 (24)	59.4 ± 2.0	60.6 ± 2.1 (24)	59.8 ± 2.4	-	-
	F_3	59.0 ± 2.2	58.6 ± 2.4 [0]	63.8 ± 2.0	62.2 ± 2.6	-	-
	F_4	63.3 ± 2.0	61.8 ± 1.9	65.1 ± 2.1	58.9 ± 1.8	-	-
ANCOVA[f] Dose P=0.363 Gen P<0.001 DxG P=0.505 BW P<0.001	F_0	-	- [1]	- [1]	-	-	-
	F_1	-	- [0]	- [0]	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.001), F_0 breed father (P=0.220), and F_0 breed mother × F_0 breed father interaction (P=0.003) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.002), F_0 breed father (P=0.008), and F_0 breed mother × F_0 breed father interaction (P=0.004) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.003), F_0 breed father (P=0.022), and F_0 breed mother × F_0 breed father interaction (P=0.004) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05.

TABLE K21
Spleen Weights and Spleen Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0	570.5 ± 15.1	545.7 ± 13.1 [1,2,3,4]	526.3 ± 11.2 [2,3,4]	508.7 ± 14.7** [3,4]	**/ # # #	-
	F_1	572.0 ± 17.8	603.2 ± 11.1 [0]	577.2 ± 15.9	513.8 ± 10.9** [3,4]	***/ # # #	# # #
	F_2	565.7 ± 12.6	631.2 ± 13.8*** [0]	600.4 ± 11.3 [0]	546.8 ± 14.2	***	# # #
	F_3	573.5 ± 14.0	600.7 ± 15.3 [0]	594.0 ± 8.9 [0]	584.7 ± 11.0 [0,1]	-	-
	F_4	552.3 ± 10.9	596.5 ± 12.1 [0]	593.9 ± 11.9 (24) [0]	576.9 ± 10.5 [0,1]	-	#
Relative[e] Dose P=0.003 Gen P<0.001 DxG P=0.099	F_0	1932.2 ± 45.0	1918.0 ± 36.2 [1,2,3]	1915.5 ± 28.6 [1,2]	2019.4 ± 46.6	*/ #	-
	F_1	2032.4 ± 49.2	2187.1 ± 43.4* [0]	2142.0 ± 45.9 [0]	2112.2 ± 37.8	-	#
	F_2	2015.8 ± 42.8	2221.3 ± 56.7*** [0,4]	2145.8 ± 38.2* [0]	2186.4 ± 54.8** [4]	#	#
	F_3	2035.2 ± 54.1	2116.6 ± 43.3 [0]	2043.9 ± 35.0	2011.0 ± 38.6	-	-
	F_4	2009.0 ± 38.6	2080.5 ± 46.5 [2]	2034.7 ± 37.0 (24)	2015.2 ± 37.9 [2]	-	-
ANCOVA[f] Dose P=0.003 Gen P<0.001 DxG P=0.188 BW P<0.001	F_0	-	- [1,2,3]	- [1,2]	-	-	-
	F_1	-	* [0]	- [0]	-	-	# #
	F_2	-	*** [0]	* [0]	-		# # #
	F_3	-	- [0]	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.

[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.

[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.

[d] F_0 breed mother (P=0.028), F_0 breed father (P=0.003), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.

[e] F_0 breed mother (P<0.001), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.

[f] F_0 breed mother (P=0.012), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.

[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05. There were no significant generation effects in pairwise comparisons for the spleen of female rats.

[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01; ***, P≤0.001. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K22
Thymus Weights and Thymus Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	329.4 ± 13.5	317.5 ± 10.7 [1]	314.4 ± 12.5 [1,2]	307.8 ± 12.4 [3,4]	-	-
	F_1	350.8 ± 16.0	398.1 ± 17.0 [0]	378.0 ± 15.9 [0]	356.1 ± 16.0	-	#
Dose P=0.002	F_2	328.2 ± 13.4	367.7 ± 15.8	374.6 ± 14.6 [0]	353.1 ± 13.6	-	#
Gen P<0.001	F_3	333.0 ± 16.3	369.0 ± 14.1 (24)	368.3 ± 13.5	368.4 ± 12.5 [0]	-	-
DxG P=0.176	F_4	314.2 ± 13.3	377.2 ± 20.4**	359.2 ± 11.4	378.6 ± 14.9** [0]	*/ # #	-
Relative[e]	F_0	1115.7 ± 42.7	1121.6 ± 40.9 [1]	1145.3 ± 42.5 [1]	1221.0 ± 42.8 [1]	-	-
	F_1	1247.1 ± 51.7	1443.9 ± 61.5* [0]	1403.8 ± 56.5 [0]	1459.1 ± 57.4* [0]	#	-
Dose P<0.001	F_2	1169.5 ± 48.2	1293.6 ± 56.6	1339.3 ± 52.4	1419.6 ± 61.4**	**/ # # #	-
Gen P<0.001	F_3	1178.3 ± 55.2	1301.9 ± 50.1 (24)	1271.6 ± 54.6	1266.5 ± 42.4	-	-
DxG P=0.833	F_4	1142.2 ± 46.3	1306.9 ± 64.9	1231.7 ± 37.2	1326.5 ± 54.9*	*/ #	-
ANCOVA[f]	F_0	-	- [1]	- [1]	-	-	-
	F_1	-	- [0]	- [0]	-	-	-
Dose P<0.001	F_2	-	-	-	*	#	-
Gen P<0.001	F_3	-	-	-	-	-	-
DxG P=0.849	F_4	-	*	-	*	#	-
BW P<0.001							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.019), F_0 breed father (P=0.100), and F_0 breed mother × F_0 breed father interaction (P=0.008) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.002), F_0 breed father (P=0.057), and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.008), F_0 breed father (P=0.069), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05. There were no significant generation effects in pairwise comparisons for the thymus of female rats.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K23
Thyroid Gland Weights and Thyroid Gland Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d] Dose P=0.013 Gen P=0.003 DxG P=0.007	F_0	27.8 ± 1.3 (24) [3]	32.6 ± 1.1	33.7 ± 1.5* (24)	26.8 ± 1.6	*	*/ # # #
	F_1	29.3 ± 1.5 (24) [3]	33.8 ± 1.9 (22) [2]	33.0 ± 1.5 (23)	26.7 ± 1.9 (24)	**	# #
	F_2	29.1 ± 1.7 (24) [3]	27.4 ± 1.5 [1]	29.6 ± 1.2	30.4 ± 1.3 (24)	-	-
	F_3	35.6 ± 1.9 [0,1,2,4]	32.6 ± 2.0	32.8 ± 1.1	31.6 ± 1.5	-	-
	F_4	26.5 ± 1.5 [3]	30.6 ± 1.6	32.6 ± 1.4*	31.9 ± 1.4	#	*
Relative[e] Dose P=0.073 Gen P=0.017 DxG P=0.003	F_0	94.6 ± 3.7 (24) [3]	114.8 ± 3.9*	123.0 ± 4.9** (24)	106.6 ± 6.2	-	**/ # #
	F_1	104.9 ± 5.9 (24)	122.4 ± 6.5 (22) [2]	123.8 ± 6.1 (23)	110.0 ± 7.7 (24)	-	# #
	F_2	103.4 ± 5.7 (24) [3]	96.4 ± 5.3 [1]	106.2 ± 4.4	121.5 ± 5.4 (24)	**/ #	-
	F_3	126.2 ± 6.7 [0,2,4]	115.1 ± 7.3	112.5 ± 3.7	108.5 ± 5.0	#	-
	F_4	96.5 ± 5.5 [3]	106.2 ± 5.1	111.9 ± 5.0	110.9 ± 4.4	-	-
ANCOVA[f] Dose P=0.068 Gen P=0.013 DxG P=0.006 BW P<0.001	F_0	- [3]	-	**	-	-	**/ # # #
	F_1	-	- [2]	-	-	-	# #
	F_2	- [3]	- [1]	-	-	*	-
	F_3	- [0,2,4]	-	-	-	-	-
	F_4	- [3]	-	-	-	-	-

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother × F_0 breed father interaction (P=0.018) random effect incorporated into the analysis model.
[e] F_0 breed mother × F_0 breed father interaction (P=0.012) random effect incorporated into the analysis model.
[f] F_0 breed mother × F_0 breed father interaction (P=0.017) random effect incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ###, P≤0.001.

TABLE K24
Uterus Weights and Uterus Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Ethinyl Estradiol (ppb)[g]				Trends[h]	
		0	2	10	50	Linear	Quad
Absolute[d]	F_0	520.0 ± 28.6 (24)	498.3 ± 31.2	551.9 ± 44.1	484.3 ± 15.6	-	-
	F_1	560.1 ± 39.3	597.8 ± 44.0	553.3 ± 35.1 (24)	561.2 ± 40.1 (23)	-	-
Dose P=0.373	F_2	538.1 ± 33.7	476.7 ± 20.2	545.9 ± 33.2	535.1 ± 33.0	-	-
Gen P=0.050	F_3	535.4 ± 42.3	520.3 ± 27.1	600.2 ± 36.1	601.2 ± 31.4	#	-
DxG P=0.611	F_4	548.2 ± 24.5	501.9 ± 17.8	521.1 ± 27.5	546.7 ± 22.0	-	-
Relative[e]	F_0	1778.7 ± 120.2 (24)	1738.4 ± 97.6	2025.8 ± 167.7	1926.0 ± 55.8	-	-
	F_1	1988.5 ± 132.1	2167.5 ± 160.4	2062.8 ± 129.7 (24)	2301.3 ± 159.2 (23)	-	-
Dose P=0.021	F_2	1911.2 ± 114.5	1682.2 ± 80.9	1955.3 ± 121.2	2138.2 ± 131.2	*	-
Gen P=0.014	F_3	1905.7 ± 156.5	1841.6 ± 98.8	2066.6 ± 124.1	2073.1 ± 114.2	-	-
DxG P=0.632	F_4	2007.5 ± 103.1	1755.9 ± 69.4	1800.5 ± 112.1	1908.5 ± 75.0	-	-
ANCOVA[f]	F_0	-	-	-	-	-	-
	F_1	-	-	-	-	-	-
Dose P=0.296	F_2	-	-	-	-	-	-
Gen P=0.045	F_3	-	-	-	-	-	-
DxG P=0.699	F_4	-	-	-	-	-	-
BW P=0.304							

[a] Mean (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses.
[b] Organ weights in mg; relative organ weights in mg/kg body weight. For the analysis of covariance with body weight as the covariate, only statistical significance or lack of significance (-) are indicated.
[c] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G); for ANCOVA, terminal body weight (BW) is indicated. Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. Any random effects incorporated are indicated in footnotes d, e, and f for the absolute, relative, and ANCOVA models, respectively.
[d] F_0 breed mother (P=0.456) and F_0 breed mother × F_0 breed father interaction (P=0.129) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.294), F_0 breed father (P=0.298), and F_0 breed mother × F_0 breed father interaction (P=0.040) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.440) and F_0 breed mother × F_0 breed father interaction (P=0.113) random effects incorporated into the analysis model.
[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05. Because of the unequal spacing of concentrations, trends were also determined for a scale using the natural logarithm of the dose +1. These "log dose" trends are indicated with pound signs as follows: #, P≤0.05

APPENDIX L
SPERM PARAMETERS

TABLE L1 **Sperm Motility of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 270**
TABLE L2 **Epididymal Sperm Count of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 271**
TABLE L3 **Testicular Spermatid Head Count of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 272**
TABLE L4 **Sperm Morphology of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 273**

TABLE L1
Sperm Motility of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generation	Dietary Ethinyl Estradiol (ppb) [b]			
	0	2	10	50
F_0	96 ± 4 (24)	95 ± 7 (25)	97 ± 2 (25)	97 ± 2 (25)
F_1	91 ± 11 (25)	90 ± 17 (25)	90 ± 16 (25)	95 ± 6 (25)
F_2	92 ± 7 (25)	88 ± 19 (24)	91 ± 10 (25)	91 ± 7 (25)
F_3	92 ± 7 (25)	92 ± 4 (25)	91 ± 5 (25)	92 ± 4 (25)
F_4	88 ± 6 (25)	90 ± 6 (25)	88 ± 7 (25)	86 ± 8 (25)

[a] Mean percent motile ± standard deviation. Number of animals given in parentheses.
[b] Data were analyzed within generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the controls. No significant effects were observed.

TABLE L2
Epididymal Sperm Count of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generation	Dietary Ethinyl Estradiol (ppb) [b]			
	0	2	10	50
F_0	590 ± 151 (24)	546 ± 213 (25)	605 ± 172 (24)	580 ± 166 (25)
F_1	694 ± 522 (23)	621 ± 353 (23)	805 ± 385 (25)	831 ± 557 (24)
F_2	536 ± 227 (25)	656 ± 487 (25)	796 ± 348* (25)	799 ± 400* (25)
F_3	393 ± 318 (25)	414 ± 294 (25)	534 ± 373 (25)	447 ± 257 (25)
F_4	711 ± 395 (25)	765 ± 461 (25)	994 ± 589 (25)	679 ± 360 (25)

[a] Mean count (10^6/g) ± standard deviation. Number of animals given in parentheses.
[b] Data were analyzed within generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the controls. Significant differences between exposed groups and the controls are indicated in shaded cells as follows: *, $P \leq 0.05$.

TABLE L3
Testicular Spermatid Head Count of Male Rats
in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generation	Dietary Ethinyl Estradiol (ppb) [b]			
	0	2	10	50
F_0	87 ± 18 (24)	75 ± 26 (25)	83 ± 25 (24)	82 ± 17 (25)
F_1	81 ± 24 (25)	72 ± 30 (25)	75 ± 24 (25)	68 ± 20* (25)
F_2	76 ± 41 (25)	101 ± 50 (25)	100 ± 40 (25)	62 ± 34 (24)
F_3	72 ± 23 (25)	81 ± 24 (25)	78 ± 33 (24)	81 ± 20 (25)
F_4	111 ± 37 (25)	105 ± 24 (25)	116 ± 36 (24)	101 ± 35 (24)

[a] Mean count (10^6/g) ± standard deviation. Number of animals given in parentheses.
[b] Data were analyzed within generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the controls. Significant differences between exposed groups and the controls are indicated in shaded cells as follows: *, $P \leq 0.05$.

TABLE L4
Sperm Morphology of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Generation	Dietary Ethinyl Estradiol (ppb) [b]			
	0	2	10	50
F_0	0.4 ± 0.5 (24)	0.3 ± 0.3 (25)	0.4 ± 0.4 (24)	0.3 ± 0.4 (25)
F_1	0.3 ± 0.4 (23)	0.3 ± 0.4 (23)	0.2 ± 0.3 (25)	0.2 ± 0.3 (24)
F_2	0.2 ± 0.4 (25)	0.1 ± 0.3 (25)	0.3 ± 0.3 (25)	0.2 ± 0.3 (25)
F_3	0.2 ± 0.4 (25)	0.2 ± 0.3 (25)	0.2 ± 0.2 (25)	0.2 ± 0.3 (25)
F_4	0.2 ± 0.3 (25)	0.2 ± 0.3 (25)	0.2 ± 0.3 (25)	0.2 ± 0.3 (25)

[a] Mean percent abnormal ± standard deviation. Number of animals given in parentheses.
[b] Data were analyzed within generation by Kruskal-Wallis' nonparametric ANOVA. If this test was significant at $P \leq 0.05$, Wilcoxon's tests were run to compare exposed groups to the controls. No significant effects were observed.

APPENDIX M
OVARIAN FOLLICLE COUNTS

TABLE M1 Ovarian Follicle Counts of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol 276

TABLE M1
Ovarian Follicle Counts of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol[a]

Follicle Class	Generation	Dietary Ethinyl Estradiol (ppb)				Trends[d]	
		0	2	10	50	Linear	Quad
Small[b,c] Dose P=0.957 Gen P<0.001 DxG P=0.002	F_0	18.9 ± 3.9 (7)	23.7 ± 3.6	25.4 ± 2.6	36.8 ± 4.1*** [1,3,4]	***/ # # #	-
	F_1	21.4 ± 1.7	22.5 ± 4.2	19.7 ± 1.8	16.9 ± 2.2 [0]	-	-
	F_2	22.4 ± 1.9	18.7 ± 1.5	25.6 ± 2.2	25.0 ± 4.5	-	-
	F_3	17.1 ± 4.8	15.2 ± 2.1	14.9 ± 1.9	14.2 ± 1.1 [0]	-	-
	F_4	22.6 ± 1.5	18.6 ± 2.1	15.5 ± 1.8	15.1 ± 1.3 [0]	#	-
Growing[b,c] Dose P=0.580 Gen P<0.001 DxG P=0.073	F_0	0.9 ± 0.2 (7)	0.8 ± 0.1	0.9 ± 0.3	1.3 ± 0.2 [4]	*	-
	F_1	0.8 ± 0.1	1.1 ± 0.2	1.0 ± 0.3	0.9 ± 0.2	-	-
	F_2	1.1 ± 0.2	0.7 ± 0.1	1.3 ± 0.2	1.2 ± 0.2 [4]	-	-
	F_3	0.9 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.9 ± 0.1	-	-
	F_4	0.9 ± 0.1	0.6 ± 0.1	0.6 ± 0.1	0.4 ± 0.1* [0,2]	*/ #	-

TABLE M1
Ovarian Follicle Counts of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Follicle Class	Generation	Dietary Ethinyl Estradiol (ppb)				Trends	
		0	2	10	50	Linear	Quad
Small & Growing Combined [b,c]	F_0	19.8 ± 4.0 (7)	24.5 ± 3.7	26.3 ± 2.7	38.1 ± 4.2*** [1,3,4]	***/ # # #	-
	F_1	22.2 ± 1.7	23.6 ± 4.3	20.8 ± 2.0	17.7 ± 2.4 [0]	-	-
Dose P=0.939	F_2	23.5 ± 2.0	19.4 ± 1.6	26.9 ± 2.2 [3]	26.2 ± 4.6	-	-
Gen P<0.001	F_3	18.0 ± 4.9	15.8 ± 2.1	15.5 ± 2.0 [2]	15.0 ± 1.2 [0]	-	-
DxG P=0.002	F_4	23.5 ± 1.5	19.2 ± 2.1	16.1 ± 1.8	15.6 ± 1.2 [0]	#	-
Antral[b,c]	F_0	1.5 ± 0.2 (7)	1.4 ± 0.2	1.3 ± 0.2 [1]	2.2 ± 0.3	**	-
	F_1	1.1 ± 0.2	1.8 ± 0.3	2.5 ± 0.3*** [0]	1.8 ± 0.2	#	***/ # #
Dose P=0.016	F_2	1.2 ± 0.1	1.3 ± 0.2	2.0 ± 0.2*	1.7 ± 0.3	-	**
Gen P=0.068	F_3	1.5 ± 0.3	1.7 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	-	-
DxG P=0.012	F_4	2.0 ± 0.2	2.1 ± 0.3	1.7 ± 0.2	1.9 ± 0.1	-	-

TABLE M1
Ovarian Follicle Counts of Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Ethinyl Estradiol

Follicle Class	Generation	Dietary Ethinyl Estradiol (ppb)				Trends	
		0	2	10	50	Linear	Quad
All [b] Dose P=0.897 Gen P<0.001 DxG P=0.001	F_0	21.3 ± 4.2 (7)	26.0 ± 3.8	27.6 ± 2.9	40.3 ± 4.3*** [1,3,4]	***/ # # #	-
	F_1	23.3 ± 1.8	25.3 ± 4.4	23.3 ± 2.3	19.5 ± 2.3 [0]	-	-
	F_2	24.7 ± 2.1	20.7 ± 1.7	28.9 ± 2.3 [3]	27.9 ± 4.8	-	-
	F_3	19.4 ± 5.1	17.6 ± 2.2	17.3 ± 2.1 [2]	16.7 ± 1.3 [0]	-	-
	F_4	25.5 ± 1.6	21.3 ± 2.3	17.8 ± 1.8	17.5 ± 1.2 [0]	#	-

[a] Mean ± standard error. Eight animals were in each group, except where indicated by number in parentheses. Five step sections of both ovaries were evaluated by two independent reviewers (counters).

[b] Results of two-way ANOVA: dose, generation (Gen), and dose × generation interaction (D×G). Random effects for the F_0 breed mother, the F_0 breed father, and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model where computationally feasible when any of these effects were significant via a log-likelihood ratio test at an α of 0.50. The high α value of 0.50 was selected to guard against Type II error. The following random effects were significant and were incorporated in the analysis model: F_0 breed mother for Small, Growing, Small and Growing Combined, and All Follicles; the F_0 breed mother and the interaction between F_0 breed mother and F_0 breed father for Antral Follicles.

[c] Significant differences between exposed groups and the controls within a generation given by Dunnett's tests are indicated in shaded exposed group cells as follows: *, P≤0.05; ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's-adjusted t-tests; numbers in brackets indicate the generations whose means are significantly different from the given mean value at P≤0.05.

[d] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells as follows: *, P≤0.05; **, P≤0.01;.***, P≤0.001. Because of the unequal spacing of doses, trends were also determined for a scale using the natural logarithm of the exposure concentration + 1. These "ln dose" trends are indicated with pound signs as follows: #, P≤0.05; ##, P≤0.01; ##, P≤0.001. A dash indicates no statistical significance (P>0.05).

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

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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 N1
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	1,800 ppm	choline chloride
Folic acid	2.7 ppm	folic acid
Pyridoxine	10 ppm	pyridoxine hydrocloride
Biotin	0.3 ppm	
Vitamin B_{12}	44 mcg/kg	cyanocobalamin
Vitamin A	25 IU/gm	vitamin A acetate
Vitamin E	93 IU/kg	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 N2
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 N3
Contaminant Levels in Purina 5K96 Rat Ration

Contaminant	Mean ± Standard Deviation	# Lots / # Lots positive
Arsenic, ppm	0.18 ± 0.13	32/18
Cadmium, ppm[a]	0.14 ± 0.04	32 / 2
Lead, ppm	0.54 ± 0.16	32 / 22
Total Fumonisin, ppb[b]	205 ± 209	32 / 32
Aflatoxin B_1, ppb	< MDL	32 / 0
Aflatoxin B_2, ppb	< MDL	32 / 0
Aflatoxin G_1, ppb	< MDL	32 / 0
Aflatoxin G_2, ppb	< MDL	32 / 0

[a] Results of <MDL or <0.10 ppm are not included.
[b] One diet contained 4,400 ppb total fumonisin and was destroyed. That result is not included here.

APPENDIX O
SENTINEL ANIMAL PROGRAM

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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 multigenerational reproductive toxicology study. Blood from each animal was collected and allowed to clot, and the serum was separated. Samples were processed appropriately at the National Center for Toxicological Research Division of Microbiology (Jefferson, AR) 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]
RATS	
ELISA	
H-1 (Toolan's H-1 virus)	22, 25, 35, 40, 50, 64, 70, and 80 weeks
KRV (Kilham Rat Virus)	22, 25, 35, 40, 50, 64, 70, and 80 weeks
Mycoplasma arthritides	22, 25, 35, 40, 50, 64, 70, and 80 weeks
Mycoplasma pulmonis	22, 25, 35, 40, 50, 64, 70, and 80 weeks
PVM (pneumonia virus of mice)	22, 25, 35, 40, 50, 64, 70, and 80 weeks
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	22, 25, 35, 40, 50, 64, 70, and 80 weeks
Sendai	22, 25, 35, 40, 50, 64, 70, and 80 weeks

RESULTS

For the multigenerational reproductive toxicology study in rats, all serology tests were negative.

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

APPENDIX P
ASSOCIATED PUBLICATIONS

The following publications relate to the current study in that the studies reported in these publications either used extra animals from the study described in this Technical Report or were conducted with similarly treated animals to provide data relevant to the interpretation of the multigenerational reproductive toxicology feed study. The results from these studies are discussed in the Discussion section of this Technical Report as appropriate.

Ferguson, S.A., Delclos, K.B., Newbold, R.R., and Flynn, K.M. (2003). Dietary ethinyl estradiol exposure during development causes increased voluntary sodium intake and mild maternal and offspring toxicity in rats. *Neurotoxicol. Teratol.* **25**, 491-501.

Guo, T.L., Germolec, D.R., Musgrove, D.L., Delclos, K.B., Newbold, R.R., Weis, C., and White, K.L., Jr. (2005). Myelotoxicity in genistein-, nonylphenol-, methoxychlor-, vinclozolin- or ethinyl estradiol-exposed F1 generations of Sprague-Dawley rats following developmental and adult exposures. *Toxicology* **211**, 207-219.

Laurenzana, E.M., Weis, C.C., Bryant, C.W., Newbold, R., and Delclos, K.B. (2002). Effect of dietary administration of genistein, nonylphenol or ethinyl estradiol on hepatic testosterone metabolism, cytochrome P-450 enzymes, and estrogen receptor alpha expression. *Food Chem. Toxicol.* **40**, 53-63.

Twaddle, N.C., Churchwell, M.I., Newbold, R.R., Delclos, K.B., and Doerge, D.R. (2003). Determination using liquid-chromatography-electrospray tandem mass spectroscopy of ethinylestradiol serum pharmacokinetics in adult Sprague-Dawley rats. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **793**, 309-315.

APPENDIX Q
SUPPLEMENTAL
REPRODUCTIVE TOXICITY STUDIES
IN MALE RATS

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ABSTRACT

The reproductive dose range-finding study described in this Technical Report indicated that ethinyl estradiol administered in a soy-and alfalfa-free diet from gestation day (GD) 7 through termination of the experiment at postnatal day (PND) 50 resulted in hyperplasia of the male mammary gland at exposure concentrations of 25 ppb or greater. In addition, the dorsolateral prostate gland weight was significantly increased relative to controls at the intermediate exposure concentration of 5 ppb (approximately 1 µg/kg body weight per day). The current study was conducted to determine if these effects were reproducible, persisted into adulthood, and persisted after termination of exposure. The parental generation received a soy- and alfalfa-free diet containing 0, 2, 10, or 50 ppb ethinyl estradiol starting 28 days prior to mating. Exposure continued throughout pregnancy and lactation. F_1 pups were fed the same diet as their parents until sacrifice; one male from each of 18 litters was sacrificed at PND 50 and another at PND 90. F_2 pups were removed from exposure at weaning (PND 21) and sacrificed according to the same schedule as the F_1 pups. Inguinal mammary glands from all pups were removed, fixed in 10% neutral buffered formalin (NBF), and processed for microscopic evaluation. Prostate glands were also removed from the animals, and the ventral and dorsolateral lobes were separated and weighed. Six randomly selected glands from each exposure group were fixed in 10% NBF for histopathological evaluation. Blood was taken at necropsy and the serum was evaluated for testosterone concentrations.

Hyperplasia of the mammary gland in the F_1 rats was evident at PND 50 and PND 90. Mostly mammary gland ducts were affected at PND 50 (0 ppb, 2/18; 2 ppb, 5/18; 10 ppb, 6/18; 50 ppb, 14/18), while both ducts and alveoli were hyperplastic in an exposure concentration dependent-manner at PND 90. In the F_2 generation, which was removed from exposure at weaning, there was still a significant trend of ductal hyperplasia at PND 50 (3/18, 4/18, 6/18, 7/17), but by PND 90, exposed groups were similar to the controls. Terminal body weights were decreased in 50 ppb F_1 animals at PND 50 and in 10 and 50 ppb animals at PND 90. In F_2 animals, body weights were significantly decreased in the 2 and 50 ppb groups at PND 50. There were no significant treatment-related effects on dorsolateral prostate gland weights. Absolute ventral prostate gland weights were decreased in the 50 ppb groups of the F_1 and F_2 generations at PND 50, and ventral prostate gland weight relative to body weight was decreased in the 50 ppb group of the F_2 generation. There were no treatment-related microscopic lesions in the prostate glands of any ethinyl estradiol exposed group. Serum testosterone concentrations were significantly decreased in PND 50 animals of the F_1 (10 and 50 ppb) and F_2 (50 ppb) generations, but there were no significant treatment effects at PND 90 in either generation.

From the results of the current study it is concluded that hyperplasia of the mammary gland in male rats is a sensitive indicator of the estrogenic activity of ethinyl estradiol, particularly in continuously exposed animals. The apparent nonmonotonic effect on dorsolateral prostate gland weight, with significantly higher weight in the low exposure concentration range that was observed in the reproductive dose range-finding study was not reproduced here, and effects on serum testosterone concentrations and ventral prostate gland weights (decreases) were transient.

INTRODUCTION

The reproductive dose range-finding study, described in this Technical Report indicated that ethinyl estradiol induced hyperplasia in the mammary glands of males that had been exposed to 25, 100, or 200 ppb ethinyl estradiol from gestation day 7 through termination of the experiment at PND 50. A significant increase in dorsolateral prostate gland weight at the intermediate exposure concentration of 5 ppb was observed, with adjacent exposure concentrations showing nonsignificant increases. The current study was conducted to determine if these effects were reproducible, persisted into adulthood, and persisted after termination of exposure. In addition, prostate gland histopathology and serum testosterone concentrations were evaluated.

Estrogen treatment of neonatal rodents has been shown to induce permanent effects on the prostate gland (reviewed in Huang *et al.,* 2004), and the doses at which such permanent effects can be elicited have been controversial (NTP,

2001). Of particular relevance for the current study is the report of Thayer *et al.* (2001) that indicated that subclinical doses of orally administered ethinyl estradiol (20 ng/kg body weight per day to pregnant mice) produced a statistically significant increase in prostate gland weights in male pups at 50 days and 5 months of age and a decrease in daily sperm production at the earlier, but not the later, time point. Similarly administered oral doses of 100 ng/kg per day to CD1 mice on GDs 14 to 18 were reported to produce a significant increase in the number of ducts in the dorsolateral prostate gland, an increase in dorsolateral prostate gland duct volume, and increased proliferation in the basal epithelial cells of these ducts in near term male fetuses (Timms *et al.,* 2005).

The male mammary gland also appears to be a sensitive target for compounds with estrogenic activity. In addition to the results obtained in the reproductive dose range-finding study of ethinyl estradiol, the growth-stimulating and feminizing effects of ethinyl estradiol and 17α-estradiol on the male mammary gland have been noted in other studies (Schardein, 1980; Biegel *et al.,* 1998; Andrews *et al.,* 2002). Weaker xenoestrogens, such as genistein and methoxychlor, have also been reported to stimulate male mammary gland growth (Delclos *et al.,* 2001; You *et al.,* 2002; NTP, 2008a). Cardy (1991) has demonstrated the feminizing effect of dopamine antagonists on the male mammary gland and suggested the utility of this tissue as an indicator of endocrine active substances.

The exposure concentrations utilized for the multigenerational reproductive toxicology study of ethinyl estradiol described in this Technical Report were 2, 10, and 50 ppb. These exposure concentrations covered the range over which the effects on the male mammary gland and prostate gland had been observed in the reproductive dose range-finding study. In the multigenerational reproductive toxicology study, all animals were scheduled to be terminated at PND 140, so that transient effects at earlier ages, including PND 50 evaluated in the reproductive dose range-finding toxicity study, would not be detected. For the current study, male pups that would otherwise have been discarded from the F_1 and F_2 generations of the multigenerational reproductive toxicology study were utilized to evaluate potential effects on the mammary gland and prostate gland at younger ages (PNDs 50 and 90).

Experimental Design and Methods

The experimental design and endpoints evaluated are summarized in Table Q1. Sprague-Dawley rats (NCTR breeding colony strain CD23) were placed on a soy- and alfalfa-free diet (5K96, Purina Mills, Inc., Richmond, IN) at weaning. At 6 weeks of age, approximately 28 days prior to breeding, the parental (F_0) generation was placed on dosed feed containing 0, 2, 10, or 50 ppb ethinyl estradiol. Exposure continued throughout pregnancy and lactation. F_1 pups were fed the same diet as their parents until termination and are thus designated hereafter as F_1C (for F_1, continuously dosed). One male from each of 18 litters was sacrificed at PND 50, and a littermate was sacrificed at PND 90. F_2 pups were removed from exposure at weaning (PND 21) and sacrificed according to the same schedule as the F_1C pups. The F_2 generation is designated hereafter as F_2T21 (for F_2, dosing truncated at PND 21). The animals were housed with a 12 hour light/12 hour dark schedule with lights on at 0700. Necropsies were started at 0800 and completed by 1200. Under carbon dioxide anesthesia, the animals were exsanguinated by cardiac puncture. Serum was prepared from the blood for measurement of serum testosterone concentrations. The prostate gland was removed, and the ventral and dorsolateral lobes were dissected and weighed separately. In each exposure group, prostate gland lobes from six animals were fixed in 10% NBF, embedded in paraffin (Tissue Prep II) and processed for histopathology. The remaining prostate glands were snap frozen in liquid nitrogen and stored at –80°C for possible future biochemical assays. The inguinal mammary gland from all animals was removed, fixed in 10% NBF, oriented in a frontal plane, and processed for routine microscopic evaluation. Severity scores for hyperplasia were assigned as follows: grade 1, minimally more than expected normally; grade 2, mildly increased above normal or slightly more than grade 1; grade 3, moderately increased above normal or slightly more than grade 2; grade 4, markedly increased above normal or slightly more than grade 3.

Total serum testosterone concentration (bound + free) was measured in duplicate using a Coat-a-Count Total Testosterone, I125 RIA kit (Diagnostic Products Corporation, Los Angeles, CA) according to the manufacturer's directions. Radioactivity was measured with a Cobra II gamma counter (Packard Instrument Co., Meriden, CT). In addition to the PND 50 and PND 90 animals, serum from available PND 2 culled pups from the multigenerational reproductive toxicology study was also analyzed.

Continuous data (body and organ weights, serum testosterone concentration) were analyzed within each generation and age group using one-way analysis of variance. Pairwise comparisons of exposed groups to controls were accomplished using Dunnett's test (Dunnett, 1955). Data were assessed for homogeneity of variance using Levene's test (Levene, 1960). Data that failed this test were transformed using a natural log transformation to stabilize variance prior to analysis. Histopathology data were analyzed using an exact Jonckheere-Terpstra trend test (Jonckheere, 1954). The test was run as a one-sided test for positive trend. If the trend was significant with all exposure groups included in the analysis, the high exposure group was dropped and the trend test was rerun. If this test was also significant, this procedure was repeated with the middle exposure group dropped.

RESULTS

The most important criteria for distinguishing ductal and alveolar hyperplasia of the mammary gland were the size of the sections and the density of the mammary gland structures – ducts, alveoli, or both. Ductal hyperplasia was observed as a relative increase in the number of branching ducts (Plate Q1). Alveolar hyperplasia was seen as a histologic increase of predominantly tubuloalveolar and lobuloalveolar patterns of growth in the mammary gland (Plate Q2).

The incidences and severities of alveolar and ductal hyperplasia and the combined incidences of these lesions in the F_1C and F_2T21 generations at PNDs 50 and 90 are given in Table Q2. In the F_1C generation at PND 50, the incidences of ductal hyperplasia increased in an exposure concentration responsive manner with minimal alveolar growth. By PND 90, the incidences and severities of ductal and alveolar hyperplasia in exposed groups were increased compared to those of the PND 50 rats. In the F_2T21 rats, in which exposure was terminated at PND 21, ductal and alveolar responses at PND 50 were similar to those observed in the continuously exposed F_1C generation, except that both incidence and severity were decreased in the high exposure concentration group of the F_2T21 generation compared to the F_1C rats. In contrast to the F_1C rats at PND 90, the F_2T21 generation at PND 90 showed significantly less growth of both ducts and alveoli, suggesting regression of mammary gland growth to essentially normal in male rats following cessation of exposure to ethinyl estradiol.

Terminal body weights and absolute and relative ventral and dorsolateral prostate gland weights are shown in Tables Q3 and Q4 for F_1C and F_2T21 rats, respectively. For F_1C animals, terminal body weights in the 50 ppb groups were 11% and 8% less than those of controls at PNDs 50 and 90, respectively, and were also 6% less than controls in the 10 ppb group at PND 90. The absolute ventral prostate gland weight was 20% less than controls in the 50 ppb group at PND 50 but was not significantly less at PND 90. The ventral prostate gland weight relative to body weight did not differ from controls at any exposure concentration at either age. For F_2T21 animals at PND 50, terminal body weights were 8% and 10% less than those of controls in the 2 and 50 ppb groups, respectively. Both absolute and relative ventral prostate gland weights were also significantly less than controls, by 22% and 13%, respectively, in the 50 ppb groups at PND 50. There were no statistically significant treatment effects on body or ventral prostate gland weights at PND 90 in F_2T21 animals or on absolute or relative dorsolateral prostate gland weights in F_1C or F_2T21 animals at either age tested. In addition, while microscopic evaluation indicated some inflammation in both the dorsolateral and, more prominently, the ventral prostate glands of F_1C and F_2T21 animals, this was not related to treatment with ethinyl estradiol (Table Q5).

Serum testosterone concentrations measured in PND 2 culls from the multigenerational reproductive toxicology study and in PND 50 and PND 90 males from both the F_1C and F_2T21 generations are tabulated in Table Q6. Statistically significant treatment effects were confined to PND 50 animals, with 61% and 76% decreases relative to controls, respectively, in the 10 and 50 ppb groups of the F_1C generation and a 66% decrease relative to controls in the 50 ppb group of the F_2T21 generation.

DISCUSSION

The results of the current study confirm the sensitivity of the male mammary gland to ethinyl estradiol and indicate that a continuous exposure regimen is most effective in inducing and maintaining hyperplasia of the male mammary gland ducts and alveoli. Under continuous exposure conditions, a significant effect was detected at PND 90 at an exposure concentration of 2 ppb, which resulted in an ingested dose of approximately 0.1 µg/kg body weight per day (Table 1). A similar induction of male mammary gland hyperplasia was observed in a feed study conducted under identical conditions with the soy isoflavone genistein (NTP, 2008a), and while some hyperplasia persisted for 2 years in animals exposed continuously or for up to 20 weeks of age, no neoplastic lesions were detected (NTP, 2008b). Results of the 2-year feed study with ethinyl estradiol will be reported separately (NTP, 2009).

The results of the reproductive dose range-finding study conducted with ethinyl estradiol as a prelude to the multigenerational reproductive toxicology study reported in this Technical Report suggested a possible acceleration of preputial separation in male pups in the intermediate dose range as well as a significant increase in dorsolateral prostate gland weight at 5 ppb in animals evaluated at PND 50. Studies by Putz *et al.* (2001a,b), reported while the present study was underway, indicated an acceleration of puberty and transient increases (that is, an elevation observed at PND 35, but not at PND 90) in prostate gland weights at the low end of the exposure concentrations of subcutaneously administered estradiol benzoate in neonatal Sprague-Dawley rats. In the multigenerational reproductive toxicology study of dietary ethinyl estradiol, no effect of ethinyl estradiol on preputial separation was observed at 2, 10, or 50 ppb (Table I2). The current study also indicates that there was no significant effect of ethinyl estradiol over the exposure concentration range tested on dorsolateral prostate gland weight.

Significant reductions of ventral prostate gland weights occurred in F_1C and F_2T21 50 ppb groups at PND 50, and a decreased relative ventral prostate gland weight occurred only in the F_2T21 animals. These effects did not persist through PND 90 in either generation. Estrogens have been reported to decrease testosterone concentrations in both developing and adult male rats (Cook *et al.,* 1998; Atanassova *et al.,* 1999; Kaneto *et al.,* 1999; Goyal *et al.,* 2003; Della Seta *et al.,* 2006). At the exposure concentrations used here, decreased serum testosterone concentrations were observed in PND 50 animals of both generations (at 10 and 50 ppb for F_1C and 50 ppb for F_2T21), but no significant effect was observed in PND 2 or PND 90 animals in either generation. While the concentrations of testosterone measured at PND 90 are somewhat lower than some literature reports in control adult male Sprague-Dawley rats (Atanassova *et al.,* 1999; Goyal *et al.,* 2003; Horvath *et al.,* 2004; Della Seta *et al.,* 2006), they are consistent with those reported in other studies (Cook *et al.,* 1998), including a study conducted under identical conditions (Laurenzana *et al.,* 2002). In any case, the depression of testosterone concentrations appeared transient and did not result in persistent adverse effects detectable in this study or in the main multigenerational reproductive toxicology study.

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Table Q1
Experimental Design Summary

F_0 generation placed on soy- and alfalfa-free (control) feed (5K96) at weaning
- Placed on 5K96 feed containing 0, 2, 10, or 50 ppb ethinyl estradiol 28 days prior to mating

F_1 generation, F_1C (18 litters, two male pups selected per litter)
- Continuously exposed to dosed feed from conception to termination
- One pup per litter sacrificed on PND 50
- One pup per litter sacrificed on PND 90

F_2 generation, F_2T21 (18 litters, two male pups selected per litter)
- Exposed to dosed feed until weaning at PND 21, then fed control feed until termination
- One pup per litter sacrificed on PND 50
- One pup per litter sacrificed on PND 90

Endpoints (F_1C and F_2T21 males)
- Terminal body weights
- Ventral and dorsolateral prostate gland weights
- Serum testosterone
- Histopathology; prostate gland and mammary gland

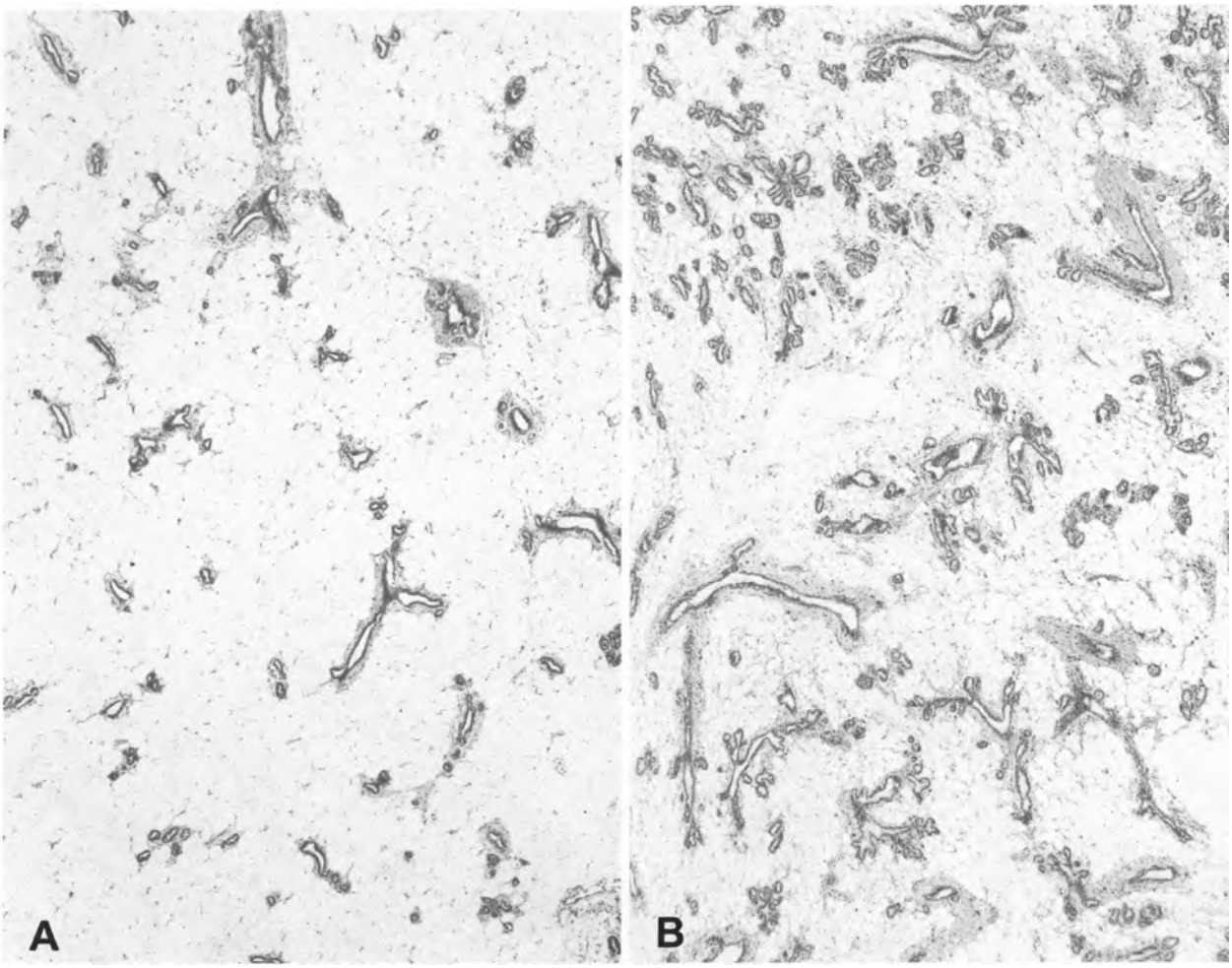

PLATE Q1
F_1C Male Rat Mammary Gland at PND 50
A) Normal mammary gland ducts (control)
B) Grade 3 mammary gland ductal hyperplasia (50 ppb)
H&E

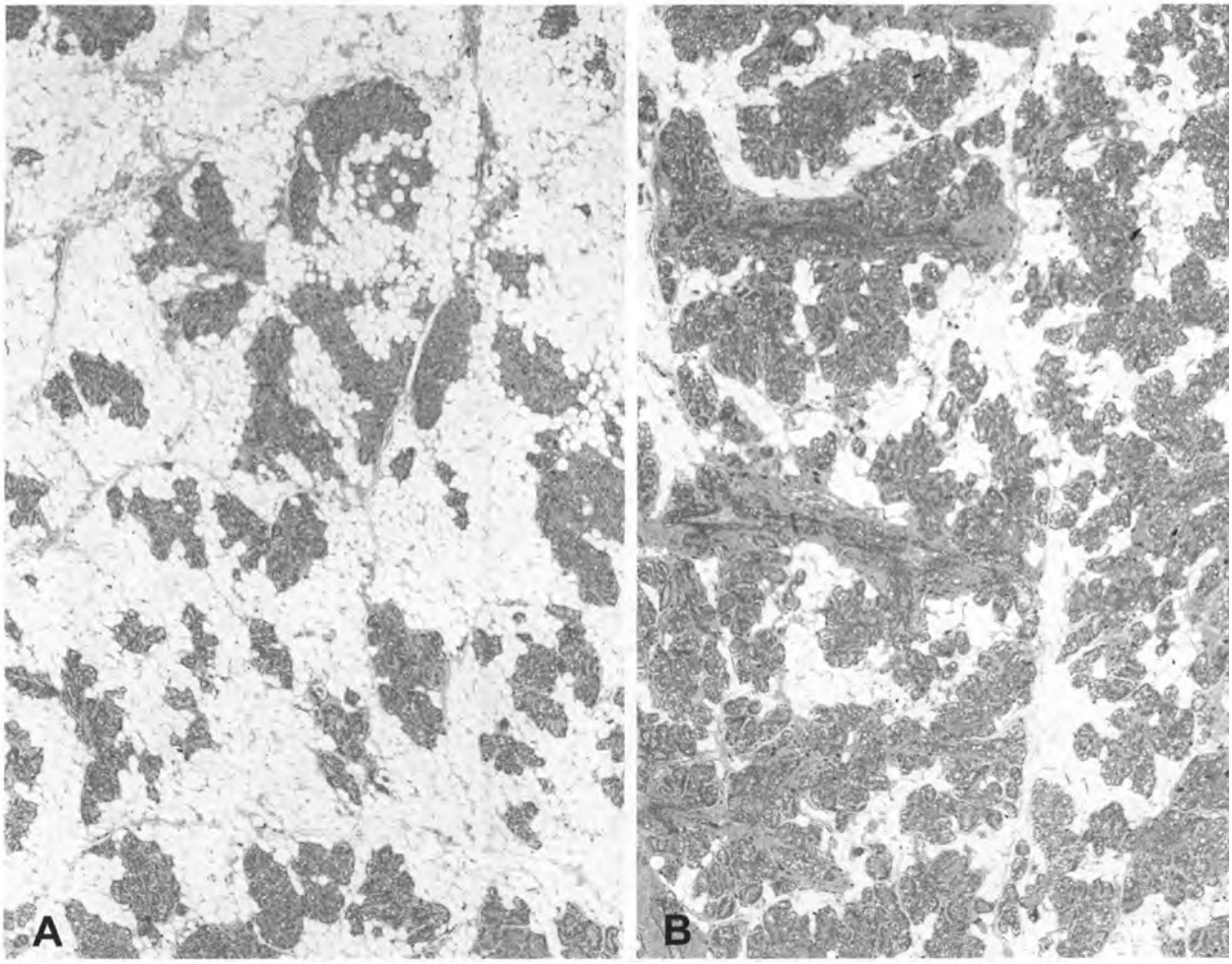

Plate Q2
F_1C Male Rat Mammary Gland at PND 90
A) Normal mammary gland alveoli (control)
B) Grade 3 mammary gland alveolar hyperplasia (50 ppb)
H&E

TABLE Q2
Microscopic Mammary Gland Lesions in Male Rats Exposed to Ethinyl Estradiol in Feed[a]

Lesion	Generation/Age	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Hyperplasia, alveolus	F_1C, PND 50	0/18	0/18	0/18	1/18 (1.0)
	F_1C, PND 90	0/18	6/18** (1.8)	5/18* (2.2)	11/18*** (2.5)
	F_2T21, PND 50	0/18	1/18 (1.0)	3/18 (1.0)	0/17
	F_2T21, PND 90	1/18 (2.0)	3/18 (1.3)	1/18 (2.0)	4/17 (1.8)
Hyperplasia, duct	F_1C, PND 50	2/18 (1.0)	5/18 (1.4)	6/18 (1.5)	14/18*** (2.1)
	F_1C, PND 90	0/18	2/18 (2.0)	5/18* (1.6)	13/18*** (2.9)
	F_2T21, PND 50	3/18 (1.3)	4/18 (1.2)	6/18 (1.7)	7/17* (1.3)
	F_2T21, PND 90	0/18	0/18	2/18 (1.5)	0/17
Hyperplasia, alveolus or duct	F_1C, PND 50	2/18 (1.0)	5/18 (1.4)	6/18 (1.5)	14/18*** (2.1)
	F_1C, PND 90	0/18	8/18** (1.9)	8/18** (1.9)	15/18*** (3.0)
	F_2T21, PND 50	3/18 (1.3)	4/18 (1.2)	7/18 (1.6)	7/17* (1.3)
	F_2T21, PND 90	1/18 (2.0)	3/18 (2.0)	2/18 (1.5)	4/17 (1.8)

[a] The number before the slash mark represents the number of animals with a diagnosis of hyperplasia while the number following the slash mark is the total number of animals evaluated in that exposure group. Six animals per exposure group were evaluated. The numbers in parentheses are the mean severity grades for affected animals: minimal, 1; mild, 2; moderate, 3; marked, 4. Data were analyzed with a Jonckheere-Terpstra test for positive linear trend. The trend test was run in a sequential fashion, with the top exposure concentration eliminated in each sequential run. Shaded cells indicate significant positive trends when the indicated exposure concentration was the highest exposure concentration in the analysis: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

TABLE Q3
Terminal Body and Prostate Gland (Ventral and Dorsolateral) Weights of F_1C Male Rats Exposed to Ethinyl Estradiol in Feed[a]

	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
F_1C, PND 50				
Body weight (g)	232.6 ± 7.3	225.7 ± 5.6	222.1 ± 5.1	207.0 ± 4.4**
Ventral Prostate Gland				
Absolute (mg)	213.3 ± 7.7	193.6 ± 11.9	187.0 ± 7.5	169.7 ± 12.1**
Relative (mg/g)	0.92 ± 0.03	0.85 ± 0.04	0.84 ± 0.03	0.82 ± 0.05
Dorsolateral Prostate Gland				
Absolute (mg)	139.6 ± 7.3	146.1 ± 6.7	128.3 ± 5.9	118.2 ± 6.2
Relative (mg/g)	0.60 ± 0.02	0.65 ± 0.03	0.58 ± 0.02	0.57 ± 0.03
F_1C, PND 90				
Body weight (g)	373.4 ± 5.4	359.1 ± 7.0	349.3 ± 4.3**	344.1 ± 4.0***
Ventral Prostate Gland				
Absolute (mg)	444.9 ± 22.7	405.5 ± 19.2	403.7 ± 18.6	412.9 ± 13.6
Relative (mg/g)	1.19 ± 0.06	1.13 ± 0.04	1.15 ± 0.05	1.20 ± 0.04
Dorsolateral Prostate Gland				
Absolute (mg)	289.9 ± 8.4	281.1 ± 10.4	288.7 ± 16.2	274.8 ± 10.8
Relative (mg/g)	0.78 ± 0.02	0.78 ± 0.03	0.82 ± 0.04	0.80 ± 0.03

[a] Values are mean ± standard error; n=18. Asterisks in shaded cells indicate significant differences from controls by Dunnett's test: **, P≤0.01; ***, P≤0.001.

TABLE Q4
Terminal Body and Prostate Gland (Ventral and Dorsolateral) Weights of F_2T21 Male Rats Exposed to Ethinyl Estradiol in Feed[a]

	Dietary Ethinyl Estradiol (ppb)			
	0	2	10	50
F_2T21, PND 50				
Body weight (g)	200.2 ± 4.3	183.9 ± 5.4*	194.9 ± 4.7	180.3 ± 4.8*
Ventral Prostate Gland				
Absolute (mg)	163.8 ± 6.1	143.7 ± 7.6	153.6 ± 6.8	128.2 ± 5.8**
Relative (mg/g)	0.82 ± 0.03	0.78 ± 0.03	0.79 ± 0.02	0.71 ± 0.02*
Dorsolateral Prostate Gland				
Absolute (mg)	102.4 ± 3.7	90.2 ± 4.2	94.8 ± 2.5	89.0 ± 5.6
Relative (mg/g)	0.51 ± 0.01	0.49 ± 0.02	0.49 ± 0.01	0.49 ± 0.02
F_2T21, PND 90				
Body weight (g)	404.4 ± 6.8	396.2 ± 7.4	389.5 ± 6.0	392.4 ± 9.4
Ventral Prostate Gland				
Absolute (mg)	411.4 ± 13.1	416.8 ± 19.5	406.0 ± 18.0	430.1 ± 28.3
Relative (mg/g)	1.02 ± 0.03	1.05 ± 0.05	1.05 ± 0.05	1.09 ± 0.06
Dorsolateral Prostate Gland				
Absolute (mg)	295.7 ± 11.0	302.4 ± 18.0	276.4 ± 8.6	277.2 ± 9.8
Relative (mg/g)	0.73 ± 0.02	0.77 ± 0.05	0.71 ± 0.02	0.71 ± 0.02

[a] Values are mean ± standard error; n=18, except in the 50 ppb group, where n=17. Asterisks in shaded cells indicate significant differences from controls by Dunnett's test: *, P≤0.05; **, P≤0.01.

TABLE Q5
Microscopic Prostate Gland Lesions in Male Rats Exposed to Ethinyl Estradiol in Feed[a]

Lesion	Generation/Age	Dietary Ethinyl Estradiol (ppb)			
		0	2	10	50
Dorsolateral Prostate Gland, Inflammation	F_1C, PND 50	0/6	1/6 (1.0)	0/6	0/6
	F_1C, PND 90	1/6 (1.0)	1/6 (1.0)	2/6 (1.5)	1/6 (3.0)
	F_2T21, PND 50	2/6 (1.0)	0/6	1/6 (1.0)	0/6
	F_2T21, PND 90	2/6 (1.0)	1/6 (2.0)	1/6 (1.0)	1/6 (2.0)
Ventral Prostate Gland, Inflammation	F_1C, PND 50	2/6 (1.5)	0/6	0/6	0/6
	F_1C, PND 90	4/6 (1.2)	5/6 (1.2)	6/6 (1.3)	2/6 (1.5)
	F_2T21, PND 50	2/6 (1.0)	0/6	0/6	1/6 (1.0)
	F_2T21, PND 90	6/6 (1.0)	5/6 (1.4)	5/6 (1.0)	5/6 (1.2)

[a] The number before the slash mark represents the number of animals with a diagnosis of inflammation while the number following the slash mark is the total number of animals evaluated in that exposure group. Six animals per exposure group were evaluated. The numbers in parentheses are the mean severity grades for affected animals: minimal, 1; mild, 2; moderate, 3; marked, 4. There were no significant treatment effects indicated by the Jonckheere-Terpstra test.

TABLE Q6
Serum Testosterone Concentrations in Male Rats Exposed to Ethinyl Estradiol in Feed[a]

	Dietary Ethinyl Estradiol (ppb)			
Generation/Age	**0**	**2**	**10**	**50**
F_1C, PND 2	0.15 ± 0.03 (13)	0.13 ± 0.05 (12)	0.13 ± 0.04 (9)	0.27 ± 0.08 (10)
F_1C, PND 50	0.98 ± 0.21	0.81 ± 0.21	0.38 ± 0.11*	0.24 ± 0.03**
F_1C, PND 90	0.38 ± 0.10	0.34 ± 0.06	0.65 ± 0.22 (16)	0.45 ± 0.12
F_2T21, PND 2	0.08 ± 0.02 (8)	0.12 ± 0.04 (12)	0.11 ± 0.03 (17)	0.15 ± 0.07 (6)
F_2T21, PND 50	1.00 ± 0.21	0.62 ± 0.12	0.79 ± 0.21 (17)	0.34 ± 0.14* (17)
F_2T21, PND 90	0.71 ± 0.11	0.73 ± 0.15	0.85 ± 0.16	0.61 ± 0.22 (17)

[a] Mean concentration (ng/mL) ± standard error; n=18 except where indicated by numbers in parentheses. PND 2 animals were culls from the main multigenerational reproductive toxicology study. Shaded cells are significantly different from the corresponding control group by Dunnett's test: *, P<0.05; **, P<0.01.

APPENDIX R
SUPPLEMENTAL ANALYSES
OF RAT MAMMARY GLAND LESIONS

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BACKGROUND **304**
RESULTS **304**

Background

Additional histopathologic examinations of mammary gland tissue from ethinyl estradiol exposed and control male and female Sprague-Dawley rats were conducted by Dr. J.R. Latendresse of Pathology Associates International.

Results

As a result of these investigations, it was determined that the most important factors for distinguishing ductal and alveolar hyperplasia of the mammary gland in male rats were the size of the gland in sections and the density of the mammary gland structures – ducts, alveoli, or both in treated animals compared to normal controls.

Ductal hyperplasia was observed as a relative increase in the number of branching ducts. This was the predominant ethinyl estradiol effect in male rats treated from conception to sacrifice at postnatal day 50 (PND 50) (Plate R1-B, 50 ppb; compare with PND 50 control, Plate R1-A). Relatively few small budding alveoli were also present within the hypodermis. The alveoli were either attached or adjacent to ducts. The ducts together with alveoli formed a tubuloalveolar pattern of growth. These tubuloalveolar units often had distinct lumens that were usually lined by one layer of cuboidal epithelium or two layers of stratified or pseudostratified cuboidal to columnar epithelium. The cytoplasm of the ductal and alveolar epithelium was often vacuolated. The lumens were occasionally dilated and contained secretory material and/or blebs of apoptotic epithelial cells. Varying amounts of fibrous connective tissue surrounded the ducts and alveoli, diminishing gradually as branching progressed from the primary to tertiary ducts of the gland. This tubuloalveolar pattern of predominantly elongated and branched ducts was similar to mammary gland from a PND 65 control virgin female Sprague-Dawley rat from an unrelated study (Plate R2).

However, by PND 90, ethinyl estradiol had induced significant alveolar hyperplasia in male Sprague-Dawley rats (Plate R3-B, 50 ppb; compare with PND 90 control, Plate R3-A). Alveolar hyperplasia in the males was seen histologically as an increase in alveoli most often with a predominance of lobuloalveolar development. Some tubuloalveolar growth was also present (Plate R4, PND 140). The lobuloalveolar pattern was characterized by more contiguous lobules of glands with ducts and alveolar lumens being less prominent or completely indistinct (Plate R5-A). In contrast, the tubuloalveolar pattern of hyperplasia was characterized by clusters of large or medium alveoli often with prominent lumens (Plate R5-B). Both growth patterns demonstrated moderate to marked vacuolization of the alveolar and ductal epithelium with either patent or collapsed lumens. Patent lumens sometimes contained secretory material and/or blebs of apoptotic epithelial cells. One or the other pattern usually predominated, but a mixed pattern was common in the same specimen (Plate R4). Both of these growth patterns were present in the normal control male rats as well (Plate R6); however, with hyperplasia, the number of prominent alveoli appeared to be increased per unit area of section, and this increased alveolar density correlated positively with the severity of hyperplasia.

Not until PND 140 were there age-matched female rats in the F_1 through F_4 generations for comparison of mammary gland growth in males. The severity of hyperplasia of the mammary gland in the females was much more variable, presumably due to physiologic hormonal modulation resulting from different intervals from lactation or weaning to the time of sacrifice. The stage of estrous cycle at the time of sacrifice could also have contributed to the degree of hyperplasia in some cases.

By PND 140, the females manifested a tubuloalveolar pattern of mammary gland growth that could be readily differentiated from the males. First, the females did not have the lobuloalveolar growth that was a feature in the males (see description above). Secondly, the alveoli forming lobules were generally smaller in diameter than those present in males and also had smaller lumens (Plate R7; compare with Plate R5-B). With these features, the gender could be derived from the glandular architecture in virtually 100% of the specimens examined. Using the criteria above, mammary glands from 10 male and 10 female F_0 rats on test for 140 days were examined without knowledge of gender. Males were distinguished from females without error using the above criteria.

Groups of three control and three 50 ppb males from the F_4 generation (parents recieved 50 ppb ethinyl estradiol) diagnosed with hyperplasia of the mammary gland were randomly chosen for microscopic evaluation, and there was no morphological difference in the observed hyperplasia.

Hyperplastic mammary glands in groups of three or more 50 ppb males from the F_1, F_2, F_3, and F_4 (parental exposure) generations were microscopically examined, and there was no difference in morphologic patterns among the various generations – all retained sexual dimorphism.

Because the supplemental study (described in Appendix Q) used only male rats, it was not possible to include mammary gland specimens from age-matched control females from that study for comparison. To provide this comparison, mammary gland tissue harvested and processed from a virgin control female Sprague-Dawley rat 65 days of age from an unrelated study is illustrated for comparison (Plate R2). To further investigate gender differences, random sets of both male and female rats (n=3) from control and 50 ppb groups for generations F_1 through F_3 and control and 50 ppb (parental exposure) groups from the F_4 generation were examined microscopically for sexual dimorphism at PND 140, the earliest time point in the ethinyl estradiol multigenerational reproductive toxicology study that had age-matched male and female rats sacrificed. Both normal and hyperplastic mammary glands from female subjects were morphologically distinct from the male mammary glands examined.

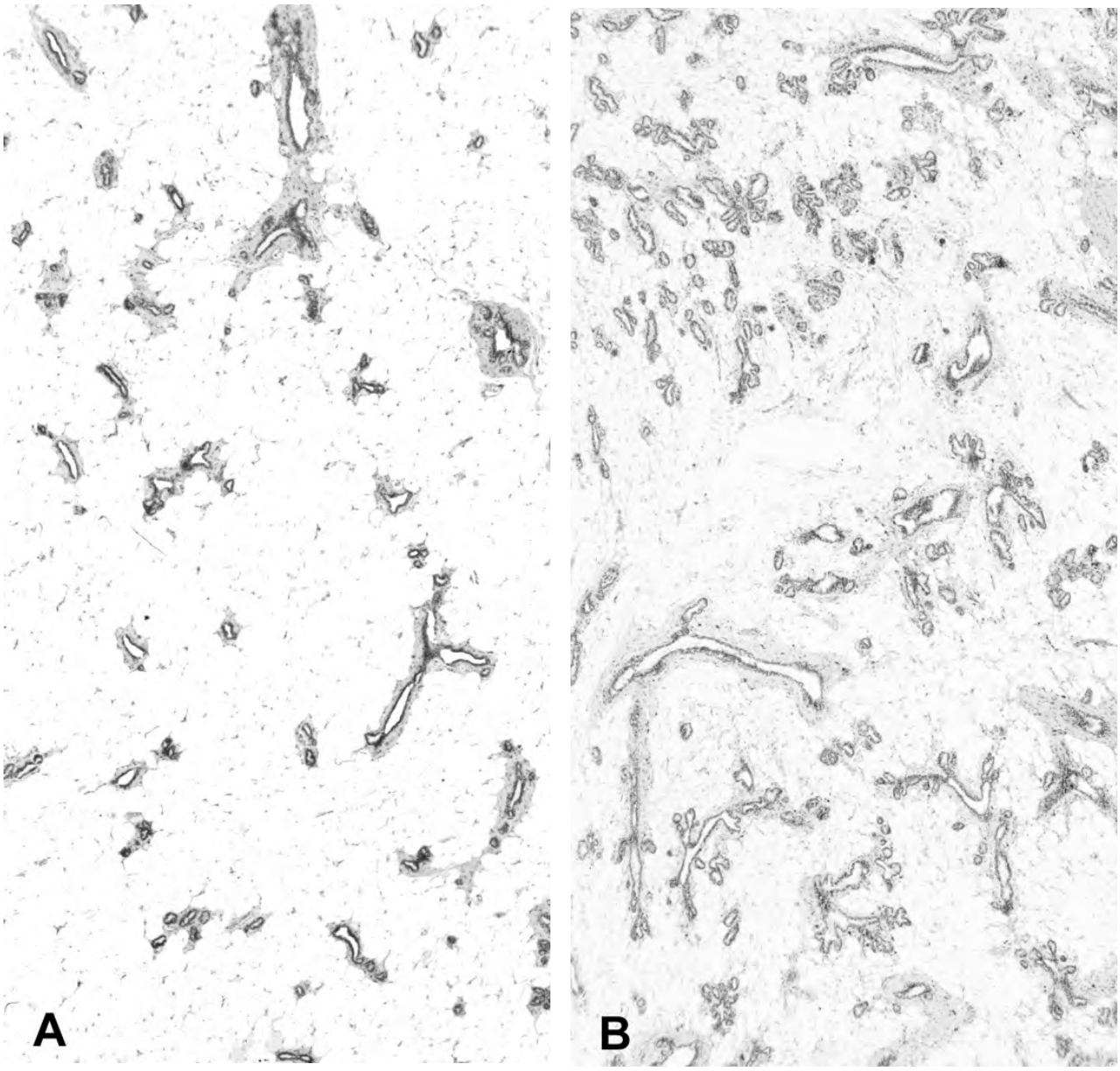


Plate R1
F_1C Male Rat Mammary Gland at PND 50; higher magnification of Plate Q1
A) Normal mammary gland ducts (control)
B) Mammary gland ductal hyperplasia (50 ppb)
H&E

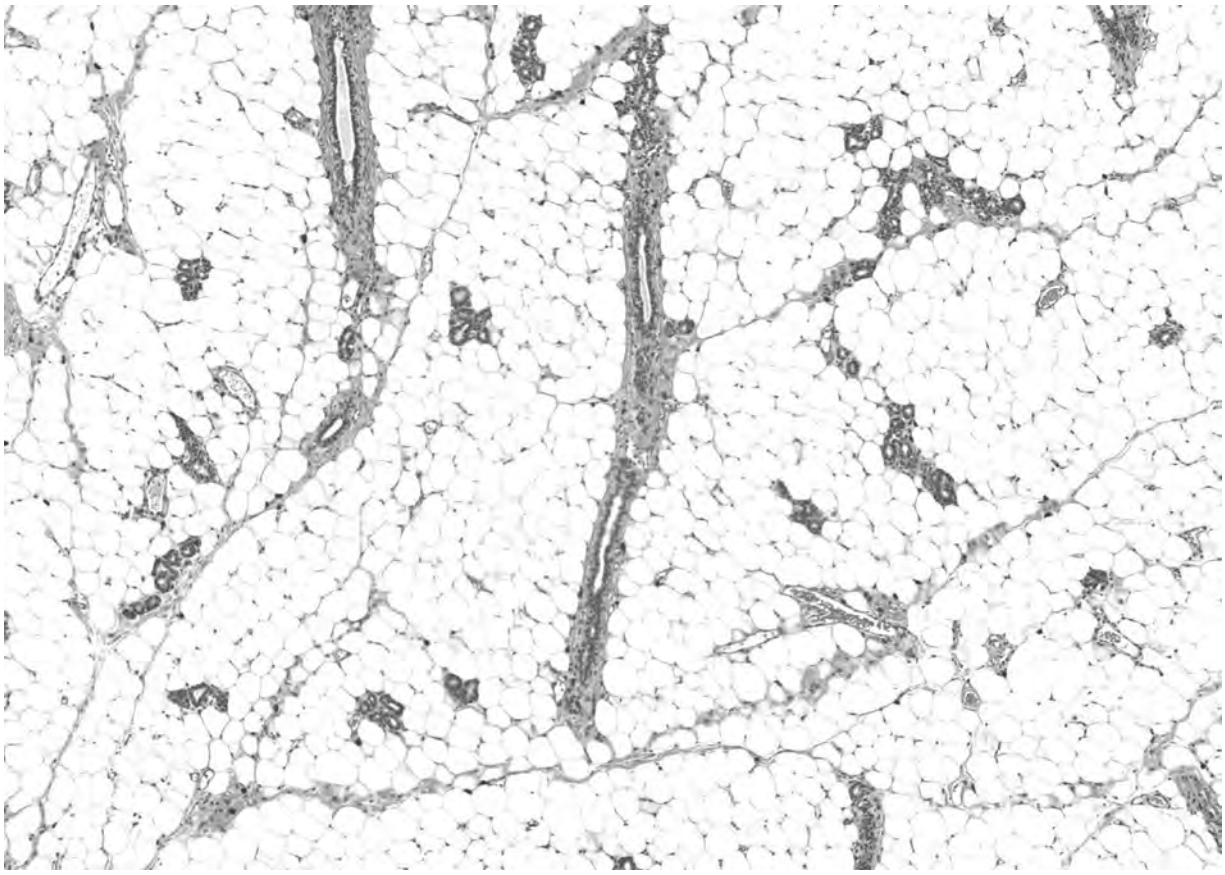


Plate R2
Female Rat Mammary Gland at PND 65 (control)
H&E

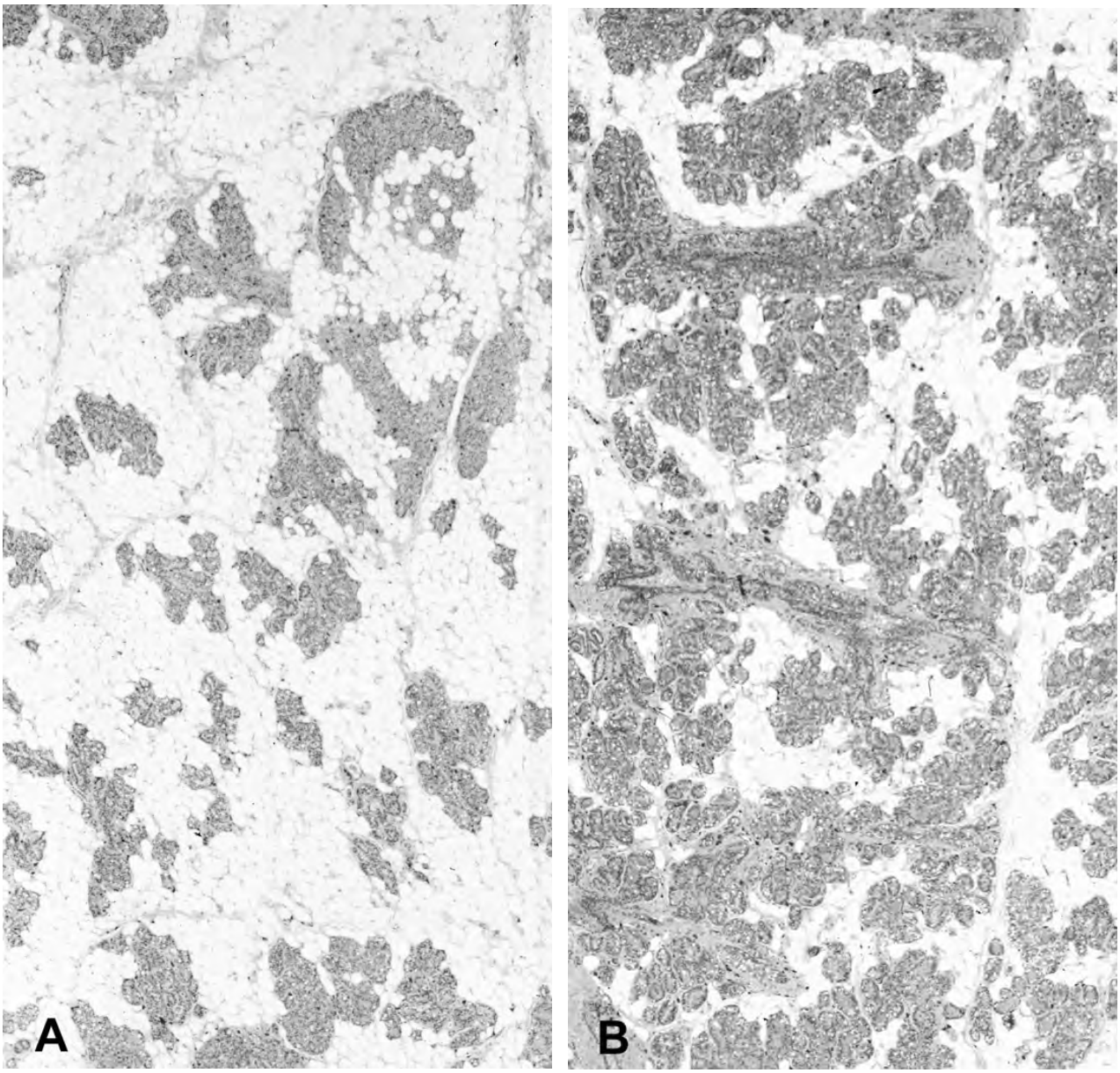


Plate R3
F_1C Male Rat Mammary Gland at PND 90; higher magnification of Plate Q2
A) Normal mammary gland alveoli (control)
B) Mammary gland alveolar hyperplasia (50 ppb) showing lobuloalveolar and tubuloaveolar growth patterns
H&E

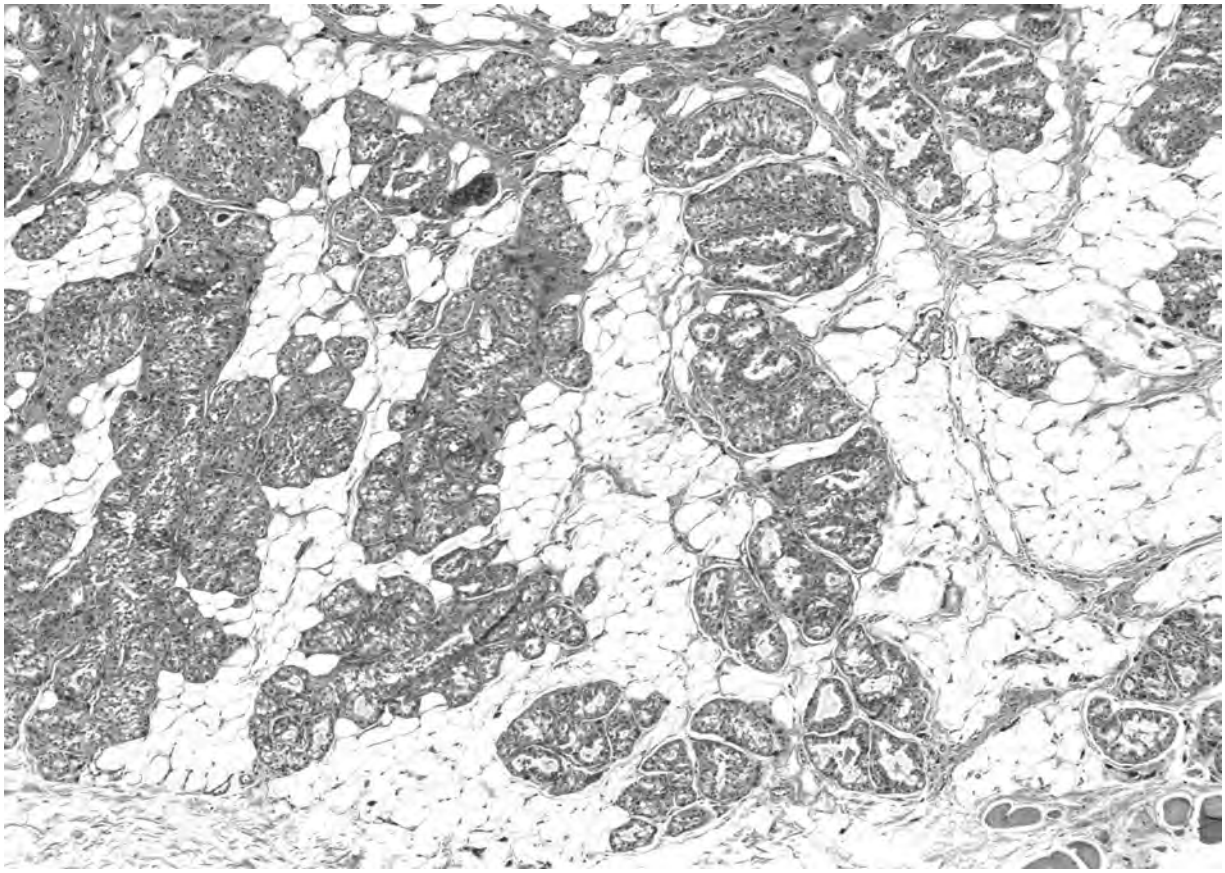
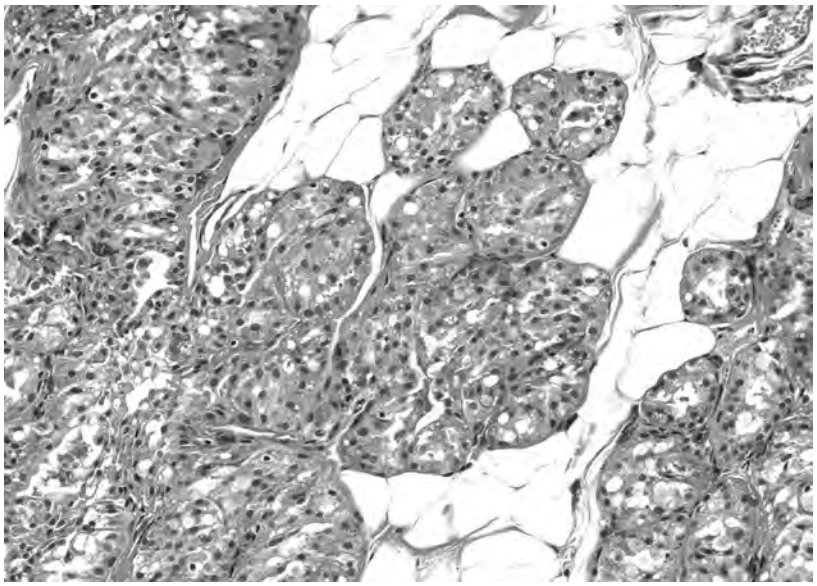


Plate R4
F_1C Male Rat Mammary Gland at PND 140
Mammary gland alveolar hyperplasia (50 ppb) with lobuloalveolar and tubuloalveolar growth patterns
H&E



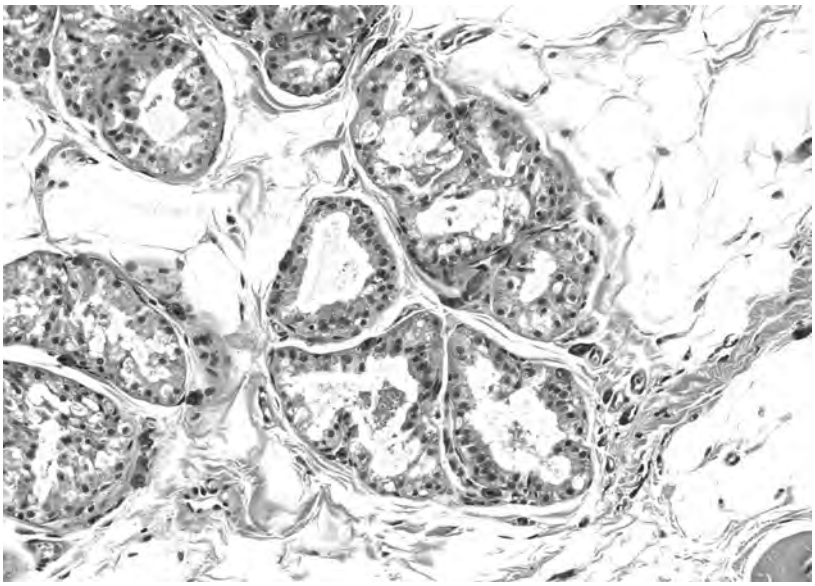


Plate R5
F_1C Male Rat Mammary Gland at PND 140
A) Mammary gland alveolar hyperplasia with lobuloalveolar growth (50 ppb)
B) Mammary gland alveolar hyperplasia with tubuloaveolar growth (same rat as in A)
H&E

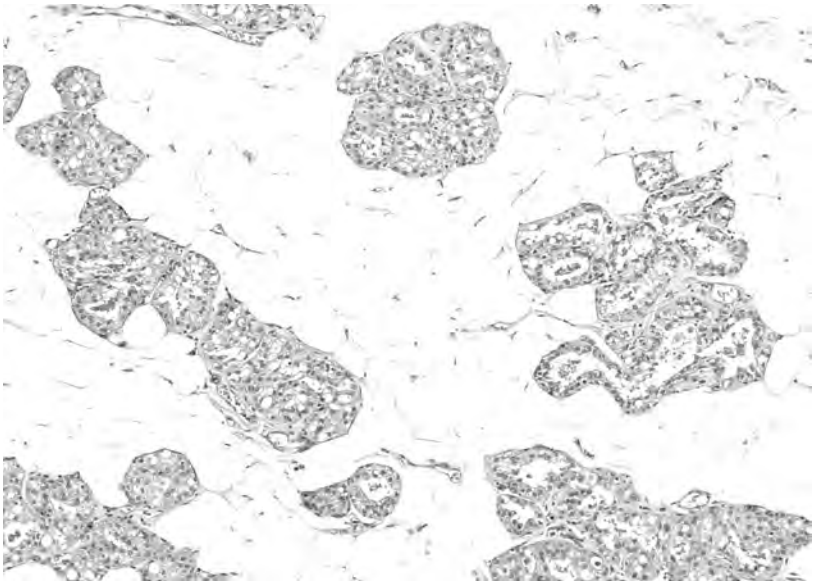


PLATE R6
F_1C Male Rat Mammary Gland at PND 140
Note the lobuloalveolar and tubuloalveolar growth patterns (control)
H&E

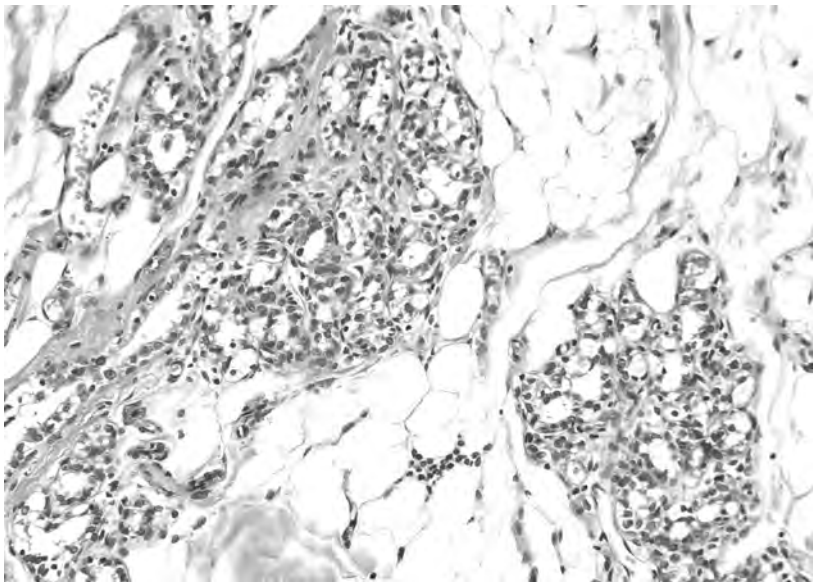


PLATE R7
F_1C Female Rat Mammary Gland at PND 140
Mammary gland alveolar hyperplasia with tubuloalveolar growth (50 ppb)
H&E