NTP TECHNICAL REPORT

ON THE

MULTIGENERATIONAL REPRODUCTIVE TOXICOLOGY

STUDY OF GENISTEIN

(CAS NO. 446-72-0)

IN SPRAGUE-DAWLEY RATS

(FEED STUDY)

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

March 2008

NTP TR 539

NIH Publication No. 08-4477

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

FOREWORD

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

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

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

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CONTRIBUTORS

The study on genistein 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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CONTENTS

SUMMARY

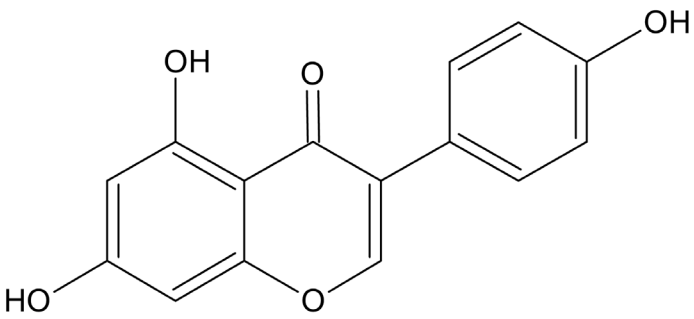
Background
Genistein is an isoflavone that occurs in soy products including soy-based infant formulas. Genistein is one of a class of chemicals known as "environmental estrogens," which can affect the hormone activities and possibly reproductive function of wildlife and humans through exposure. The NTP conducted a series of studies on three such chemicals to detect if exposure to such chemicals over the course of multiple generations could have any cumulative effect on animals' reproductive systems. This report describes the results of a set of studies in which several generations of rats were exposed to genistein through their feed and/or through exposure from their mothers through gestation and weaning.

Methods
The study extended over five generations of rats following a parental group of rats that were exposed to genistein in their feed starting at the age of 6 weeks. The first and second generations of offspring were exposed to genistein during conception through their mothers, during weaning through their mothers' milk, and during their lifetimes through feed containing genistein. The third generation was exposed just during gestation and weaning, and the fourth and fifth generations were not exposed directly, to see if any carryover effects resided from exposure of earlier generations. The dosed feed contained 5, 100, or 500 parts per million (ppm) of genistein. The primary measures examined during each generation were body weights, development of reproductive organs, and number of offspring per litter after each cycle of mating.

Results
Female rats given 500 ppm in their feed had lower body weights, accelerated sexual maturation, and altered estrous cyclicity compared to unexposed animals. Male rats given 500 ppm in the feed had lower body weights in the first generation but not in the second generation, with similar chemical exposure. For animals continuously exposed to genistein, there was some reduction in litter size in the first two generations. Male rats exposed to 100 or 500 ppm had increased rates of mammary gland hyperplasia and calcification of renal tubules. In the later generations, the only observed effects on offspring of exposed animals were smaller body weight gains in pups before weaning.

Conclusions
We conclude that exposure to 500 ppm of genistein caused lower body weights and some alterations in the reproductive system of female rats. Exposure to genistein caused lower body weights in one generation of male rats and increases in mammary gland hyperplasia and renal tubule calcification. Except for lower body weights in pups, there was no evidence for a carryover of genistein effects into unexposed generations.

ABSTRACT



GENISTEIN

CAS No. 446-72-0

Chemical Formula: $C_{15}H_{10}O_5$ Molecular Weight: 270.23

Synonym: 4′,5,7-Trihydroxyisoflavone

Genistein is a naturally occurring isoflavone that interacts with estrogen receptors and multiple other molecular targets. Human exposure to genistein is predominantly through consumption of soy products, including soy-based infant formula and dietary supplements. Consumption of soy and genistein has been associated with a variety of beneficial effects in animals and humans, but concerns have also been raised concerning potential adverse effects of genistein, particularly with regard to reproductive toxicity and the induction or potentiation of carcinogenesis, due primarily to its weak estrogenic activity. Because of these concerns, genistein was selected as one of the compounds to be examined in a protocol utilizing Sprague-Dawley rats to evaluate the effects of multigenerational and long-term exposures to doses of estrogenic agents that produce subtle reproductive tract lesions in developmentally exposed Sprague-Dawley rat pups. Results from the multigenerational reproductive toxicology feed study are reported in this report, and results of the 2-year feed study are reported separately (NTP, 2008a). Data from a preliminary reproductive dose range-finding feed study (NTP, 2007) that utilized exposure concentrations of up to 1,250 ppm genistein were used to select dietary exposure concentrations of 0, 5, 100, and 500 ppm for the current study. These dietary doses resulted in ingested genistein doses of approximately 0, 0.3, 7, or 35 mg genistein/kg body weight per day for males and 0, 0.5, 10, or 51 mg/kg per day for females during the time that the rats were directly consuming dosed feed. The current study was a multigenerational study (F_0 through F_4, with F_5 litters terminated at weaning) that focused on reproductive endpoints. Animals were continuously exposed to genistein 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 sacrificed and necropsied on postnatal day 140 (PND 140). 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.

For this study, 140 animals of each sex were obtained from the NCTR CD (Sprague-Dawley) rat colony at weaning and placed on a soy- and alfalfa-free diet that was used throughout the study in an attempt to maintain consistently low background exposure to phytoestrogens. 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 and then necropsied at termination at 20 weeks of age (PND 140). Similar procedures were used to produce each generation.

Results of the current study are summarized below. In the postweaning period, exposure to 500 ppm genistein reduced body weights predominantly in females of generations in which rats were ingesting the compound throughout adulthood (F_0 through F_2). In the unexposed F_4 generation, female body weight was also depressed, although to a lesser extent than in the earlier generations. In the F_1 generation, postweaning body weights were reduced in all 100 and 500 ppm groups, with a more pronounced effect in the females. While pup birth weights were not significantly affected by genistein in the F_1 through F_4 generations (with the exception of 100 ppm males in the F_1 generation), both sexes showed depressed body weight gains during the preweaning period in the 500 ppm groups in all of these generations. Male pup preweaning body weight gains were also depressed in the 5 and 100 ppm groups in the F_1 generation. In the unexposed F_5 generation, pup birth weights in all exposed groups of both sexes were significantly lower than those in the controls, although it seems likely that this is a chance observation rather than a carryover effect from exposures in earlier generations.

Measures of fertility were not adversely affected by genistein except for litter size. Litter size of the 500 ppm group in the F_2 generation was significantly smaller than that in the corresponding control group. The litter sizes in the F_1, F_2, and F_3 generations showed negative exposure concentration trends. Male and female 500 ppm pups in the F_1 generation had slightly reduced anogenital distances (AGDs) relative to controls when covaried by body weight. Female pups also had reduced AGDs in the F_2 (500 ppm) and F_3 (100 ppm) generations, although the statistical significance was dependent on the analysis method applied. Females exposed to 500 ppm showed an accelerated time of vaginal opening (approximately 3 days) in the F_1 and F_2 generations, while the 5 ppm group showed an earlier time of vaginal opening (1.3 days) in the F_3 generation. Body weight at vaginal opening was lower in 500 ppm females of the F_1 through F_3 generations and in 5 ppm females of the F_1 generation. When examined shortly after vaginal opening, estrous cycles of 500 ppm females in the F_1 and F_2 generations were significantly longer (approximately 3 days and 1 day, respectively) than those of their respective control groups. Other estrous cycle disturbances (with the exception of decreased time in diestrus for 100 ppm females in the F_4 generation) were confined to the 500 ppm group of the F_1 generation and included reduced time in proestrus and an increase in the number and percentage of aberrant cycles. When the estrous cycles of older animals were examined prior to termination, the sole significant effects were a decreased time in estrus and increased time in diestrus in 5 ppm females of the F_2 generation and an increased number of abnormal cycles in 500 ppm females of the F_3 generation. No effects of genistein on male sexual development were noted with the exception of an increased time to testicular descent in 500 ppm males of the F_3 generation. Significant organ weight effects in both sexes were largely confined to single exposed groups in single generations; no clear patterns indicating toxicity to reproductive or nonreproductive organs were observed.

Exposure-related microscopic lesions were confined to males, with the mammary gland and kidney affected. Incidences of mammary gland alveolar/ductal hyperplasia were significantly increased in 500 ppm males in the F_0 through F_2 generations and in 100 ppm males in the F_1 and F_2 generations. In the F_3 generation, a significant positive linear exposure concentration trend in the incidences of mammary gland hyperplasia occurred, but no exposed group differed significantly from the controls in pairwise comparisons. The more pronounced effect of genistein on the incidences of male mammary gland hyperplasia in the continuously exposed F_1 and F_2 generations as compared to the late adolescent and adult exposures of the F_0 generation and the preweaning-only exposure of the F_3 generation indicates that both developmental and adult exposures contribute to the maintenance of this effect into adulthood. Statistically significant effects of genistein on the incidences of generally minimal to mild kidney lesions in males were confined to the continuously exposed F_1 and F_2 generations.

Incidences of renal tubule mineralization were significantly increased in 100 and 500 ppm males in the F_1 and F_2 generations, and incidences of inflammation and renal tubule regeneration were significantly increased in 500 ppm males in the F_1 generation.

In addition to the results reported above for animals from the main study, ancillary studies were conducted with pups derived from the current study or from animals treated under similar conditions. These results have been reported elsewhere (Appendix P) and are not presented in detail in this report. Of particular importance are the data on blood and tissue genistein concentrations obtained from adult animals in the F_1 generation (Chang *et al.*, 2000), from dams and fetuses (Doerge *et al.*, 2001), and from dams and nursing pups (Doerge *et al.*, 2006). These data provide measures of the internal dose resulting from the dietary exposure concentrations used in the current study and indicate that while fetal and adult exposures to genistein were at concentrations relevant to the full range of human exposures, only very low exposures were achieved during the early neonatal period when the pups were receiving exposures exclusively from the milk. The minimal exposure to genistein during this critical developmental period must be considered in the interpretation of the data derived from the current study.

In summary, although genistein did show adverse effects with dietary exposures of 100 or 500 ppm, there were no clear adverse effects on the reproductive or developmental parameters measured at genistein concentrations ranging from less than 1 ppm (control diet) to 100 ppm, a range of doses producing serum concentrations achievable from the phytoestrogen content of human diets. There were few clear, overtly toxic effects that carried over across directly exposed generations or appeared to be imprinted to carry over into unexposed descendants under the conditions of exposure in this study.

Summary

Under the conditions of this study, dietary exposure to 500 ppm genistein (approximately 35 mg genistein/kg body weight per day in males and 51 mg/kg per day in females) decreased body weights, accelerated vaginal opening, decreased anogenital distance, and altered estrous cyclicity in females continuously ingesting genistein. Significant decreases in postweaning body weight and decreases in anogenital distance in males were confined to the F_1 generation and were not seen in the similarly exposed F_2 generation. In animals exposed to 500 ppm, there was some evidence for reduced litter size in the F_1 and F_2 generations that were continuously exposed to the test chemical. No other impacts on fertility and no histopathologic lesions were observed in females. The male reproductive tract did not show significant alterations, but increased incidences of hyperplasia of the mammary gland and calcification of renal tubules were observed in continuously exposed 100 and 500 ppm males examined at 20 weeks of age. Weaker effects on the incidences of male mammary gland hyperplasia were observed in 500 ppm males exposed only as adults or exposed only *in utero* and through lactation. Other than decreased body weight gains in preweaning pups, there was no evidence for a carryover of genistein effects into unexposed generations.

A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

Summary of the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

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	↓ (500)	↓ (500)	↓ (500)	↓ (500)	NA
Postweaning	↓ (500)	↓ (100, 500)	↓ (500)	-	↓ (500)	NA
Male						
Preweaning	NA	↓ (5, 100, 500)	↓ (500)	↓ (500)	↓ (500)	NA
Postweaning	-	↓ (100, 500)	-	-	-	NA
Feed Consumption						
Female	↓ (500)	↓ (500)	↓ (500)	-	↓ (500)	NA
Litter Size	NA	-	↓ (500)	-	-	-
Pup Birth Weight						
Male	NA	↓ (100)	-	-	-	↓ (5, 100, 500)
Female	NA	-	-	-	-	↓ (5, 100, 500)
Anogenital Distance						
Male PND 2						
ANCOVA	NA	↓ (500)	-	-	-	-
Female PND 2						
ANCOVA	NA	↓ (500)	↓ (500)	↓ (100)		
Ratio	NA	↓ (500)	-	-		
Vaginal Opening						
Age	NA	↓ (500)	↓ (500)	↓ (5)	-	NA
Body Weight	NA	↓ (5, 500)	↓ (500)	↓ (500)	-	NA
Testicular Descent						
Age	NA	-	-	↑ (500)	-	NA
Vaginal Cytology After Vaginal Opening						
% Time Diestrus	NA	-	-	-	↓ (100)	NA
% Time Proestrus	NA	↓ (500)	-	-	-	NA
% Abnormal Cycles	NA	↑ (500)	-	-	-	NA
Number Abnormal Cycles	NA	↑ (500)	-	-	-	NA
Length of Cycle	NA	↑ (500)	↑ (500)	-	-	NA
Vaginal Cytology Before Termination						
% Time Estrus	-	-	↓ (5)	-	-	NA
% Time Diestrus	-	-	↑ (5)	-	-	NA
Number Abnormal Cycles	-	-	-	↑ (500)	-	NA
Terminal Body Weight						
Male	-	↓ (500)	-	-	-	NA
Female	↓ (500)	↓ (500)	↓ (500)	-	-	NA

Summary of the Multigenerational Reproductive Toxicology Feed Study of Genistein

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)
Male Organ Weights						
Adrenal Gland						
Absolute	↑ (5)	-	-	-	-	NA
Relative	↑ (5)	-	-	-	-	NA
ANCOVA	↑ (5)	-	-	-	-	NA
Brain						
Absolute	-	-	↓ (500)	-	↓ (500)	NA
Relative	-	-	↓ (5)	-	-	NA
ANCOVA	-	-	↓ (500)	-	-	NA
Kidney						
Relative	-	↑ (500)	-	-	-	NA
ANCOVA	-	↑ (500)	-	-	-	NA
Liver						
Absolute	-	-	-	-	↓ (500)	NA
Relative	-	↑ (500)	-	-	↓ (500)	NA
ANCOVA	-	↑ (500)	-	-	↓ (500)	NA
Pituitary Gland						
Absolute	-	-	↑ (500)	-	-	NA
Relative	-	-	↑ (500)	-	-	NA
ANCOVA	-	-	↑ (500)	-	-	NA
Spleen						
Absolute	↑ (5)	-	↑ (5)	-	-	NA
Relative	↑ (5)	-	-	-	-	NA
ANCOVA	↑ (5)	-	-	-	-	NA
Testis						
Absolute	↑ (500)	-	-	-	-	NA
Relative	↑ (500)	-	-	-	-	NA
ANCOVA	↑ (500)	-	-	-	-	NA
Thymus						
Absolute	-	-	↓ (100)	-	↓ (100)	NA
Relative	-	-	↓ (100)	-	-	NA
ANCOVA	-	-	↓ (100)	-	-	NA

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	-	-	↓ (5)	-	-	NA
Brain						
Relative	↑ (500)	↑ (500)	-	-	-	NA
Kidney						
Absolute	-	↓ (500)	-	-	-	NA
Liver						
Relative	-	↑ (5, 500)	↑ (100)	-	-	NA
ANCOVA	-	↑ (5, 100, 500)	-	-	-	NA
Pituitary Gland						
Absolute	↑ (100)	-	-	-	-	NA
Relative	↑ (100)	↑ (500)	-	-	-	NA
ANCOVA	↑ (100)	-	-	-	-	NA
Spleen						
Absolute	-	↑ (5)	-	-	-	NA
Relative	-	↑ (5)	-	-	-	NA
ANCOVA	-	↑ (5)	-	-	-	NA
Thymus						
Absolute	-	-	-	↓ (100)	-	NA
Relative	-	-	-	↓ (100)	-	NA
ANCOVA	-	-	-	↓ (100)	-	NA
Thyroid Gland						
Absolute	-	-	↓ (500)	-	-	NA
Relative	-	↑ (500)	-	-	-	NA
ANCOVA	-	-	↓ (500)	-	-	NA
Histopathology						
Male						
Mammary Gland, Alveolar/ Ductal Hyperplasia	↑ (500)	↑ (100, 500)	↑ (100, 500)	↑ (Trend)	-	NA
Renal Tubule, Mineralization	-	↑ (100, 500)	↑ (100, 500)	-	-	NA
Kidney, Inflammation	-	↑ (500)	-	-	-	NA
Renal Tubule, Regeneration	-	↑ (500)	-	-	-	NA

[a] 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, or, where indicated, significant overall exposure concentration trend; "-", no exposed group significantly different from the control group in that generation in pairwise comparisons

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 genistein on June 12, 2006, 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 June 12, 2006, the draft Technical Report on the multigenerational toxicology study of genistein 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, Research Triangle Park, NC.

Dr. K.B. Delclos, National Center for Toxicology Research (NCTR), introduced the multigenerational reproductive toxicology studies of genistein by providing some background on the joint NIEHS-NCTR series of endocrine disruptor studies. He described the forms of genistein present in soy products and resulting from metabolism, human exposures, and the short-term reproductive range-finding study that served as the basis of exposure concentration selection for the multigenerational reproductive toxicology and chronic rodent studies. He then described the exposure regimen for the multigenerational reproductive toxicology study and the effects of genistein on survival, body weights, and reproductive endpoints in exposed animals.

The proposed summary for the multigenerational reproductive toxicology study was: "Under the conditions of this study, dietary exposure to 500 ppm genistein (approximately 35 mg genistein/kg body weight per day in males and 51 mg/kg per day in females) decreased body weights, accelerated vaginal opening, decreased anogenital distance, and altered estrous cyclicity in females continuously ingesting genistein. Significant decreases in postweaning body weight and decreases in anogenital distance in males were confined to the F_1 generation and were not seen in the similarly exposed F_2 generation. In animals exposed to 500 ppm, there was some evidence for reduced litter size in the F_1 and F_2 generations that were continuously exposed to the test chemical. No other impacts on fertility and no histopathologic lesions were observed in females. The male reproductive tract did not show significant alterations, but increased incidences of hyperplasia of the mammary gland and calcification of renal tubules were observed in continuously exposed 100 and 500 ppm males examined at 20 weeks of age. Weaker effects on the incidences of male mammary gland hyperplasia were observed in 500 ppm males exposed only as adults or exposed only *in utero* and through lactation. Other than decreased body weight gains in preweaning pups, there was no evidence for a carryover of genistein effects into unexposed generations."

Dr. Walker, the first principal reviewer, did not have substantial scientific criticisms. He noted that the complex multigenerational design was very different from other NTP studies.

Dr. Daston, the second principal reviewer, noted that the use of different windows of exposure permitted determination of whether responses were primary pharmacologic effects or adaptive responses. He also noted that the control diet and exposure concentration selection spanned the varied range of human exposures and suggested the diet and phytoestrogen concentrations be specified in more detail. He asked whether the time in estrus or proestrus was varied. He suggested that mention of the known anorectic effect of estrogen be added to the results text. He also noted the limited precision of measures of estrous cycle phases. For the Discussion, he asked for comparison of doses in literature studies with those in the current studies and comparison of human consumption levels. He also thought it worth noting that in the exposure range of 1 to 100 ppm phytoestrogen, the reproductive and developmental parameters remained relatively normal.

Dr. Crump, the third principal reviewer, thought the complicated statistical analyses were presented clearly, and he agreed with the summary presented at the end of the discussion section.

Ad hoc reviewer Dr. Cooke thought the studies were very thorough and well organized and presented. He thought it should be emphasized that the study design did not parallel the human situation for soy-fed infants.

Dr. Delclos agreed that while fetal exposure was significant, postnatal exposure was less, and he would note that in the abstract.

Dr. Birt asked for some comparison of the responses in other strains as part of the rationale for selection of the Sprague-Dawley rat and for more detail on the composition and control of the diet formulation. Ms. R.R. Newbold, NIEHS, noted that the diets used were specially prepared to have low concentrations of phytoestrogens.

Dr. Kerkvliet inquired about the corresponding times of rodent and human gestational stages. Dr. Delclos responded that postnatal days 1 to 5 for rodents corresponded roughly to the second trimester for humans. Ms. Newbold added that developmental events in various tissues or systems are more continuous and not restricted to particular prenatal windows.

Dr. Daston moved, and Dr. Walker seconded, that the summary be accepted as written. The motion was accepted unanimously with 10 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 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. 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 Sprague-Dawley 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). The sensitivity of the NCTR CD rat to the potent estrogen ethinyl estradiol was evaluated as part of this series of studies and is being reported separately (NTP 2008b,c).

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 Reproductive Toxicology and Chronic Studies Conducted Subsequent to the Reproductive Dose Range-finding Studies

As in the short-term studies, the multigenerational reproductive toxicology and chronic studies were conducted with the NCTR CD (Sprague-Dawley) rat and test compounds were administered in the soy- and alfalfa-free 5K96 diet. The design of the multigenerational reproductive toxicology and chronic studies is outlined in Figure 1. For the multigenerational reproductive toxicology studies, males and females of the original parental generation (F_0) were placed on 5K96 diet at weaning, and dosed feed was administered starting on postnatal day (PND) 42, 4 to 6 weeks before breeding. The F_0 generation was maintained on dosed feed until termination at PND 140. For breeding, one male was cohabited with one female for 14 days or until a vaginal plug (*in situ* or in pan below cage) was detected. Subsequent generations (F_1 through F_4) were bred similarly. The F_1 and F_2 generations were exposed to the test compound administered in the diet continuously from conception through termination at PND 140; the F_3 generation was removed from exposure at weaning (PND 21) and continued on control feed until PND 140, while the F_4 generation received no dietary exposure to the test compound. The F_4 generation was bred to produce an unexposed F_5 generation. The F_5 litters were terminated at weaning following collection of basic litter information. Thus, this design incorporated an evaluation of the magnification (or reduction) of effects 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 was also examined for two test compounds (ethinyl estradiol and genistein) and reported separately (NTP, 2008a,c). Three exposure windows were examined in the chronic studies (Figure 1): 1) continuous exposure from conception through 2 years

(designated F_1 continuous, or F_1C) to evaluate the effects of lifelong exposure, 2) exposure from conception through PND 140 followed by control diet to 2 years (designated F_1 truncated at PND 140, or F_1T140) to determine if effects observed in the multigeneration study led to long term adverse effects, and 3) exposure from conception through weaning followed by control diet to 2 years (designated F_3 truncated at PND 21, or F_3T21) to evaluate the long term effects of developmental exposure. The F_3 designation for the F_3T21 exposure 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.

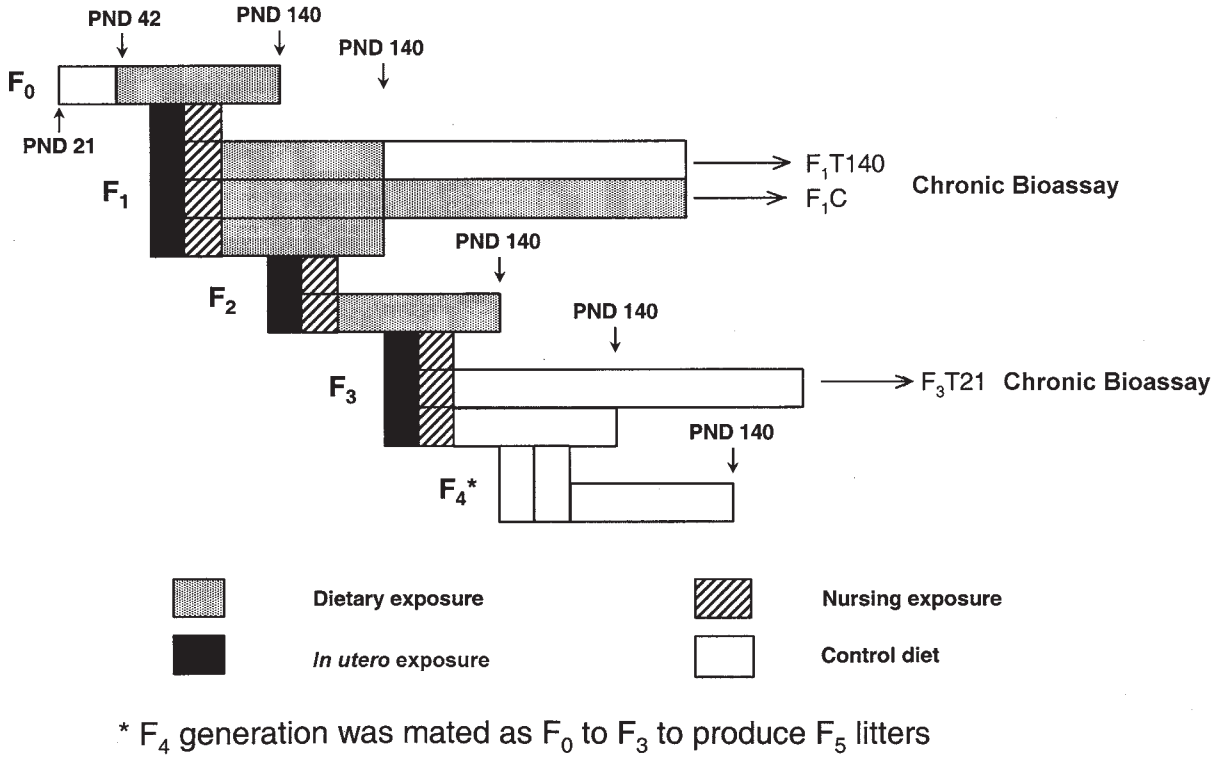
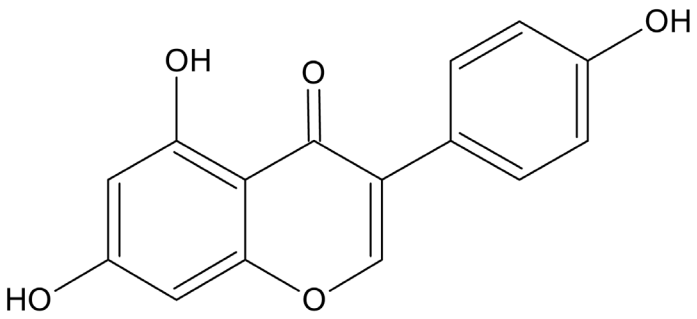


FIGURE 1
Dosing Schedule for the Multigenerational Reproductive Toxicology and Chronic Studies

INTRODUCTION



GENISTEIN

CAS No. 446-72-0

Chemical Formula: $C_{15}H_{10}O_5$ Molecular Weight: 270.23

Synonym: 4′,5,7-Trihydroxyisoflavone

PHYSICAL PROPERTIES, PRODUCTION, USE, AND EXPOSURE

Genistein belongs to the class of chemicals designated isoflavones. It has a molecular weight of 270.23 and in pure form is a pale-yellow crystalline solid that is practically insoluble in water but freely soluble in methanol and ethanol (*Merck*, 1996). In nature, genistein is primarily found in legumes where it is produced by a branch of the phenylpropanoid pathway of secondary metabolism through the action of the enzyme isoflavone synthase on the flavanone intermediate naringenin (Dixon and Ferreira, 2002; Jackson and Rupasinghe, 2002). Products derived from soybeans are the primary source of human exposure to genistein. Genistein content of soybeans varies according to the cultivar and season, and processing of the soybean and soy foods further affects both the genistein content and the form of genistein present (Gugger, 2002; Jackson and Rupasinghe, 2002). The aglycone genistein (shown above) is present primarily in fermented products such as miso and tempeh, while genistein exists predominantly as the glucoside conjugate (genistin) or acetyl or malonyl derivatives of genistin in whole soybean and nonfermented products such as tofu or soy drinks. Glucosides and glucoside derivatives are hydrolyzed to the aglycone genistein in the gut by gut bacteria or gut wall enzymes. This metabolism of the glucoside has been shown to be a critical factor in the absorption of orally ingested isoflavones (Setchell *et al.*, 2002). In rats, the oral administration of the aglycone has been shown to result in faster uptake than the glucoside, although the exposures measured by the area under the curve (AUC) are similar (King *et al.,* 1996). Similar results have been reported in humans, although administration of the glucoside appears to result in a higher AUC than does administration of the aglycone (Setchell *et al.,* 2001). Regardless of whether the glucoside or the aglycone is administered, the predominant circulating forms of genistein in rats, and humans are glucuronide conjugates (Chang *et al.,* 2000; Setchell *et al.*, 2001) and similar effects of the aglycone and glucoside at doses resulting in equivalent serum concentrations would be expected (Allred *et al.,* 2001; Satoh *et al.,* 2006).

Intake patterns and isoflavone content of ingested products vary widely, but the Committee on Toxicity of Chemicals in Food, Consumer Products and the Environment (COT, 2003) of the United Kingdom has

recently estimated an approximate rank order of daily isoflavone exposure as follows: infant on soy formula (40 mg genistein/day), average Japanese consumer (25 to 100 mg/day), vegetarian consumer (3 mg/day), and the average British consumer (1 mg/day). The typical ingestion by the average consumer in the United States is likely to be similar to that by a consumer in the United Kingdom. Data on isoflavone intake from dietary supplements are sparse, but the COT estimated that manufacturers' recommended daily dosages would result in exposures of 29 to 88 mg isoflavones per day, or about 0.4 to 1.3 mg/kg per day for a 70 kg person. On a body weight basis, infants consuming soy formula are exposed to the highest doses, with mean doses estimated at 6 to 9 mg/kg per day (Setchell *et al.*, 1997).

The consumption of diets with high levels of soy has been proposed to have multiple beneficial effects, including chemopreventive activities against various cancers and alleviation of some of the adverse consequences of menopause, although the epidemiological evidence for many of these beneficial effects is controversial (Adlercreutz, 2002; Messina *et al.,* 2006; Sacks *et al.,* 2006; Trock *et al.,* 2006; Williamson-Hughes *et al.,* 2006). Diets high in soy contain multiple agents that may contribute to these effects, and consumption of these diets is also associated with lower calorie and fat intake. Nonetheless, much research attention has focused on the isoflavones, and particularly genistein, as the active components contributing to (or responsible for) the beneficial effects of soy. This is due to the demonstrated interaction of soy isoflavones, particularly genistein, with estrogen receptors, effects on hormone synthesis and metabolism and sex hormone binding proteins, and genistein's ability to inhibit multiple enzymes involved in growth regulation, including tyrosine kinases and topoisomerases. These activities have been extensively reviewed (see above references). Genistein has been demonstrated in numerous studies to act as an estrogen by stimulating uterine growth in immature or ovariectomized rodents and has been shown to induce a similar, though not identical, pattern of gene expression as ethinyl estradiol in the developing rat uterus (Naciff *et al.*, 2002) and in developing rat testes and epididymides (Naciff *et al.*, 2005). Recent studies, published after the present work was completed, have also indicated that genistein, at concentrations above 1 μM, can modulate the expression of androgen-regulated genes and peroxisome proliferator activated receptor α- and γ-regulated genes (Dang *et al.*, 2003; Mezei *et al.*, 2003; Takahashi *et al.*, 2004; Kim *et al.*, 2005), thus adding to the potential complexity of genistein-mediated effects. The association of diets containing soy with lower rates of many common Western health problems has led to the development of concentrated isoflavone-containing plant extracts for use as dietary supplements (Hodgson *et al.*, 1998; Nestel *et al.*, 1999; Kurzer, 2003). In addition, soy-based infant formulas have been available for decades, and infants consuming soy formula have been shown to have concentrations of circulating isoflavones as high as 5 to 10 μM (Setchell *et al.*, 1997).

Research assessing the potential adverse effects associated with isoflavone consumption is directed toward defining any potential risk from exposure to a range of doses of isoflavones during different life stages. Developmental stages are of particular concern because of the demonstrated adverse consequences of exposure to hormonally active agents such as diethylstilbestrol during development (Bern, 1992; Newbold, 1995; NIH, 1999), although potential adverse stimulatory effects of genistein on reproductive and breast tissues of postmenopausal women also require particular attention (Petrakis *et al.*, 1996; Hargreaves *et al.,* 1999).

Adverse effects of soy-containing foods and soy components on reproductive processes of animals had been reported prior to the initiation of this study (East, 1955; Stob, 1983; Price and Fenwick, 1985), and some human studies had suggested that the consumption of soy products could have hormonal effects in women (Wilcox *et al.*, 1990; Cassidy *et al.*, 1994; Baird *et al.*, 1995; Cassidy and Bingham, 1995; Nagata *et al.*, 1997, 1998; Xu *et al.*, 1998; Duncan *et al.*, 1999). It has further been suggested, based on studies in ovariectomized rodents and nonhuman primates, that beneficial effects of soy and its component isoflavones on the cardiovascular system and bone occur at doses that do not adversely affect the reproductive tract (Anthony *et al.*, 1996; Ishimi *et al.*, 1999). In addition, inhibition of chemically induced mammary gland cancer in rats has been reported at doses that did not produce adverse effects on reproductive tissues (Murrill *et al.*, 1996; Fritz *et al.*, 1998; Lamartiniere *et al.*, 1998a). Given the potential range of effects of soy and its components and the magnitude of human exposure, it was important to conduct comprehensive toxicologic evaluations of these agents to better understand potential adverse effects that could result from their use in products such as dietary supplements and soy infant formula.

Dose Selection for the Multigenerational Reproductive Toxicology Feed Study of Genistein

Results from the short-term reproductive dose range-finding feed study of genistein and the rationale for exposure concentration selection for the multigenerational reproductive toxicology and 2-year studies are presented in NTP Toxicity Study Report 79 (NTP, 2007). Dietary exposures of 5, 25, 100, 250, 625, and 1,250 ppm were evaluated in the reproductive dose range-finding study. Pups in the 1,250 ppm groups had significantly decreased body weights relative to controls at the time of sacrifice (males, 9% decrease; females, 12% decrease). The most pronounced organ weight effects in the pups were decreased ventral prostate gland weight in 1,250 ppm males (absolute weight, 28% decrease; relative weight, 20% decrease) and a trend toward higher relative pituitary gland weights in both sexes. Histopathologic examination of female pups revealed increased incidences of mammary gland ductal/alveolar hyperplasia at 250 ppm or greater. Increased incidences of mammary gland ductal/alveolar hyperplasia and hypertrophy occurred in exposed males, with significant increases seen at exposure concentrations of 25 ppm or greater for hypertrophy and 250 ppm or greater for hyperplasia. In 625 and 1,250 ppm females, the incidences of abnormal cellular maturation (mucocyte metaplasia) in the vagina were significantly increased; in addition, the incidence of abnormal ovarian antral follicles was significantly increased in 1,250 ppm females. In 1,250 ppm males, the incidence of aberrant or delayed spermatogenesis in the seminiferous tubules was significantly increased. Histologic evaluation indicated a deficit of sperm in the epididymis of 625 and 1,250 ppm males relative to controls, although testicular spermatid head counts and epididymal spermatozoa counts did not show significant differences from controls at these exposure concentrations. Control females showed a high incidence of renal tubule mineralization, and the severities of this lesion were significantly increased in groups exposed to 250 ppm or greater. Males showed no renal tubule mineralization below 250 ppm, but incidences and severities increased with exposures of 250 ppm or greater. Based on these results, a 1,250 ppm exposure concentration was clearly ruled out for further testing based on the effects on body weights, histopathologic observations in males and females, and a reduction in the proportion of mated dams producing litters. While the effects observed at 625 ppm would not be predicted to impair reproduction significantly, the observation of significant effects at 250 ppm (hyperplasia in the mammary gland of both sexes), together with the suggestion of subtle effects at this exposure concentration and lower in the parallel immunotoxicity and neuroanatomical studies indicated that a high exposure concentration between 250 ppm and 625 ppm would be appropriate for the purposes of the current study. Accordingly, the highest exposure concentration for the multigenerational reproductive toxicology study was set at 500 ppm. A low exposure concentration of 5 ppm, where no significant effects were observed in the reproductive dose range-finding study, and an intermediate exposure concentration of 100 ppm were also selected. The calculated ingested doses of genistein by animals consuming these dietary concentrations are given in Table 1.

TABLE 1
Ingested Doses of Genistein in Rats Exposed to 5, 100, or 500 ppm Genistein in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

Sex/Dosing Period	Generation	Mean Dose (mg/kg per day) ± Standard Error		
		5 ppm	100 ppm	500 ppm
Male, Entire Feeding Period				
	F_0	0.3 ± 0.03 (12)	5.9 ± 0.5 (12)	28.9 ± 2.5 (12)
	F_1	0.4 ± 0.03 (17)	7.1 ± 0.5 (17)	37.6 ± 2.6 (17)
	F_2	0.3 ± 0.03 (17)	7.4 ± 0.7 (17)	35.7 ± 2.6 (17)
	F_0 - F_2 inclusive	0.3 ± 0.02 (46)	6.9 ± 0.3 (46)	34.6 ± 1.6 (17)
Female, Entire Feeding Period				
	F_0	0.5 ± 0.06 (12)	10.0 ± 1.2 (12)	50.4 ± 6.0 (12)
	F_1	0.5 ± 0.03 (16)	9.8 ± 0.7 (16)	50.6 ± 3.8 (17)
	F_2	0.5 ± 0.04 (17)	10.2 ± 0.7 (17)	50.7 ± 3.7 (17)
	F_0 - F_2 inclusive	0.5 ± 0.02 (45)	10.0 ± 0.5 (45)	50.6 ± 2.4 (46)
Female, Nonlactating				
	F_0	0.4 ± 0.01 (9)	8.0 ± 0.4 (9)	39.8 ± 1.8 (9)
	F_1	0.4 ± 0.02 (13)	9.0 ± 0.7 (13)	45.4 ± 2.7 (14)
	F_2	0.5 ± 0.03 (14)	9.3 ± 0.6 (14)	45.0 ± 2.4 (14)
	F_0 - F_2 inclusive	0.4 ± 0.02 (36)	8.9 ± 0.4 (36)	43.9 ± 1.4 (37)
Female, Lactating				
	F_0	0.8 ± 0.08 (3)	16.1 ± 1.8 (3)	82.2 ± 9.2 (3)
	F_1	0.6 ± 0.08 (3)	13.3 ± 1.7 (3)	74.6 ± 8.5 (3)
	F_2	0.7 ± 0.04 (3)	14.4 ± 1.3 (3)	77.2 ± 3.6 (3)
	F_0 - F_2 inclusive	0.7 ± 0.04 (9)	14.6 ± 0.9 (9)	78.0 ± 3.9 (9)

[a] The mean ingested dose was calculated for each week by multiplying the dietary concentration of genistein by the mean measured amount of feed ingested weekly and dividing the result by the mean body weight for the week. These values were divided by 7 to give the mean daily dose listed 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 nonlactating period, and the period during which the dams were lactating. 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 GENISTEIN

Genistein was obtained from Toronto Research Chemicals, Inc. (North York, Ontario, Canada), in one lot (2-BP-136-6). 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 genistein study are on file at the NCTR.

The chemical, a pale-yellow crystalline solid, was identified as genistein by proton nuclear magnetic resonance spectroscopy. The purity of lot 2-BP-136-6 was determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) and mass spectrophotometric (MS) detection, by gas chromatography (GC) with MS detection, and by probe/MS methods. HPLC/UV and HPLC/MS spectra indicated a purity of essentially 100%. GC/MS spectra indicated one major peak and minor impurities with a purity greater than 99%. Probe/MS testing indicated one major component with two minor components, suggesting little to no impurities. The overall purity of lot 2-BP-136-6 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at –70° C, protected from light in the original shipping containers. Purity was periodically measured during the study using the methods listed above; no degradation of the bulk chemical was detected.

BACKGROUND ISOFLAVONE CONTENT OF THE BASE DIET

The base diet used for the current study was an irradiated soy- and alfalfa-free rodent feed, designated 5K96, obtained from Purina Mills, Inc. (Richmond, IN), in an attempt to maintain consistently low background exposure to phytoestrogens. In some associated publications resulting from this study (Appendix P), this feed is referred to as NIH-31C because it maintains the nutritional specifications of the NIH-31 feed and contains casein. The composition of this diet and the results of the routine monitoring of the diet conducted throughout the study are presented in Appendix N. The control feed was routinely assayed for total isoflavone content after acid hydrolysis by the study laboratory using HPLC/MS methods. Analysis of 10 consecutive lots of 5K96 feed by these methods indicated 0.417 ± 0.213 ppm genistein and 0.271 ± 0.161 ppm daidzein. These results were consistent with an earlier study of four lots of 5K96 feed assayed at the study laboratory using liquid chromatography/tandem mass spectrometry that yielded concentrations of 0.54 ± 0.31 ppm genistein and 0.48 ± 0.21 ppm daidzein (Doerge *et al.*, 2000). It should be noted that animals consuming control feed were ingesting a concentration of genistein approximately 10-fold lower than that of the groups exposed to the lowest experimental exposure concentration, a concentration consistent with the isoflavone intake of individuals consuming typical Western diets.

PREPARATION AND ANALYSIS OF DOSE FORMULATIONS

The dose formulations were prepared every 5 weeks or as needed by mixing genistein with feed (Table C1). Homogeneity (analysis of three samples each from the bottom, middle, and top of blends) and stability studies of a 5 ppm dose formulation using lot 1-BP-118-3 were conducted by the study laboratory as part of the reproductive dose range-finding study (NTP, 2007) using HPLC/UV. Homogeneity was confirmed, and stability in stainless steel cans was confirmed for up to 17 days at ambient temperature and for up to 32 weeks at 2° ± 8° C.

Periodic analyses of the dose formulations of genistein (analysis of one sample each from the top, middle, and bottom of blends) were conducted by the study laboratory using HPLC/UV. The dose formulations were analyzed at intervals of 1 to 4 weeks (Table C2). All dose formulations analyzed and used in the study were within 10% of the target concentrations.

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, 5, 100, or 500 ppm genistein 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 postnatal day 140 (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 NIH-31 diet has been reported to contain approximately 30 ppm of each of the soy-derived isoflavones genistein and daidzein, which are present predominantly in the form of the glucosides genistin and daidzin (Thigpen *et al.*, 1999).

The NCTR CD rat strain was founded in 1972 from Sprague-Dawley rats from Charles River Laboratories and has been maintained in the NCTR breeding facility since that time. Rats of the F_0 generation were acclimated to the Purina 5K96 diet for 3 weeks from PND 21 to PND 42 and were 6 weeks old at the beginning of the study. Animals in the F_1 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, 5, 100, or 500 ppm genistein. 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 ra ndomly 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. Each of the selected animals was marked with a unique number by tail tattoo and housed individually. 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 housed individually. All animals not selected for breeding 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, 2008a) 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. 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 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.

For the F_1 through F_4 generations, daily monitoring of males for testicular descent was begun on PND 14. 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 scheduled necropsy on PND 140. Vaginal cytology assessments on one female animal from each litter were performed for 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 four animals that were removed prior to study termination as either dead or moribund. The adrenal gland, brain, epididymis, kidney, liver, left and right ovary, seminal vesicle/coagulating gland, spleen, left and right testis, thymus, and uterus were weighed as soon as possible after dissection. The pituitary gland, prostate gland (separated dorsolateral and ventral lobes), and thyroid gland were weighed after fixation. The left epididymis and testis from each male were not fixed but were instead 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 exceptions of the male and female reproductive organs and accessory glands, which were 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 µm, and stained, with the exception of the testis, with hematoxylin and eosin for microscopic examination. In addition, five 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 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. All neoplasms from all exposure groups and all generations along with target organs (mammary gland, kidney, and reproductive organs) from 5% of all animals in all exposure groups and all generations were reviewed. The Quality Control (QC) Pathologist evaluated the Gross Individual Animal Necropsy Report, the Gross to Microscopic Corre-lations, 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.

TABLE 2
Experimental Design and Materials and Methods in the Multigenerational Reproductive Toxicology Feed Study of Genistein

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

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

Animal Source
NCTR breeding colony (Jefferson, AR)

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

Average Age When Study Began
F_0: 6 weeks
F_1 through F_5: 0 weeks (on study from conception)

Date of First Exposure
F_0 August 25, 1998
F_1 September 22, 1998
F_2 December 10, 1998
F_3 April 18, 1999
F_4 August 1, 1999
F_5 November 14, 1999

Duration of Exposure
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
F_0 December 10, 1998
F_1 March 8, 1999
F_2 June 23, 1999
F_3 October 4, 1999
F_4 January 24, 2000

Average Age at Necropsy
20 weeks

Size of Study Groups
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, 2008a); 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
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
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.

TABLE 2
Experimental Design and Materials and Methods in the Multigenerational Reproductive Toxicology Feed Study of Genistein

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

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

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

Cages
Solid-bottom polycarbonate cages (Allentown Caging Equipment Co., Allentown, NJ), changed weekly

Bedding
Heat-treated hardwood chips (P.J. Murphy Forest Products Corp., Montville, NJ), changed weekly

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

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

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

Exposure Concentrations
0, 5, 100, or 500 ppm in feed, available *ad libitum*

Type and Frequency of Observation (F_0 through F_4 generations unless otherwise indicated)
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. Times 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
Carbon dioxide asphyxiation

Necropsy
Necropsies were performed on all animals of the F_0 through F_4 generations plus four 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, seminal vesicle with coagulating gland, spleen, left and right testis, thymus, and uterus. Organs weighed after fixation were: pituitary gland, dorsolateral and ventral prostate gland (lobes were separated after fixation), and thyroid gland. The right femur was removed and fixed in neutral buffered formalin.

Histopathology
For the surviving animals in each of the F_0 through F_4 generations and the four additional animals removed from study either dead or moribund, complete histopathology was performed on all gross lesions, reproductive organs, mammary glands, and kidneys (except for the kidneys of F_0, F_3, and F_4 generation males). In addition, the following tissues were examined in the control and 500 ppm groups of these generations: adrenal gland, bone (femur), bone marrow, liver, pituitary gland, skin, spleen, thymus, and thyroid gland.

TABLE 2
Experimental Design and Materials and Methods in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Sperm Analysis and Vaginal Cytology
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 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
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.

STATISTICAL METHODS

The majority of data collected was analyzed by mixed models ANOVA. The experiment was evaluated as a two-way fixed effect treatment structure with exposure concentration ("dose") and 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 measured and tested within this two-way layout. 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 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 genistein in the preceding generation.

There were 38 original sires and 38 original dams that gave rise to the F_0 generation; from these mating pairs, all animals in the F_0 generation arose. There were 35 animals × 4 exposure groups × 2 genders = 280 animals in the F_0 generation arising from these original 38 pairs of rats. 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 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 body weights of females at the time of vaginal opening for the 35 or 40 litters. 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 a normality assumption. 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 or not 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.

Two *post hoc* tests were performed. First, Dunnett's tests (Dunnett, 1955) 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.

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, 5, 100, and 500 ppm) could lead to undue influence of the highest exposure concentration on trend analyses, trend analyses for endpoints analyzed by ANOVA, except for ovarian follicle counts and the repeated measures analyses of body weight, feed consumption, and water consumption, were also conducted using the natural log of the actual exposure concentration plus 1, which resulted in a more evenly spaced scale of 0, 1.8, 4.6, and 6.2.

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 (caudal epididymal sperm motility, caudal epididymal sperm counts, testicular spermatid head counts, and sperm morphology) nonparametric 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 Jonckheere-Terpstra (JT) (Jonckheere, 1954; Hollander and Wolfe, 1973) nonparametric test for monotonic

increasing or monotonic decreasing trend was used to analyze exposure concentration effects on length of estrous cycle.

In order to confirm the mixed-models ANOVA results, Kruskal-Wallis' tests and logistic regression (Myers *et al.*, 2001) were conducted and yielded equivalent statistical significances in the main effects and their interaction for the mating time and gestational length endpoints. Index data (mating index, pregnancy index, and fertility index) were analyzed by logistic regression. Here, the response variable was the proportion of animals observed positive for the endpoint. Poisson regressions (Myers *et al.*, 2001) were also conducted for these three results using the raw count of the number of animals observed positive as the response variable. Both approaches yielded equivalent results for the main effects and their interaction for the fertility, mating, and pregnancy indexes.

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 made at an α equal to the 0.05 level. In cases where a significant dose main effect or a significant dose × generation interaction was observed, plots of adjusted (least squares) means were generated to examine the data further for potential nonmonotonic effects.

Statistical Analysis of Histopathology 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 numbers 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 trend along with Shirley's test (Shirley, 1977; Williams, 1986) 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 a Wilcoxon's test 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 all three major genital system organs within the females: ovary, uterus, and vagina. The effect of genistein 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

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

RESULTS

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 D4. In females, the effects of genistein on postweaning body weight were seen in the 500 ppm F_0 through F_2 groups when the animals were directly ingesting genistein (Figures 2, 3, and 5; Tables D1a to D1c). In the F_0 generation, mean postweaning body weights of 500 ppm females were significantly less than those of the controls (mean difference of 8%) in each of the 7 weeks prior to litter delivery and for 3 of the 4 weeks for which data were collected after delivery. Decreased postweaning body weights in the F_1 and F_2 generations, the generations in which genistein exposure was continuous from conception to termination, were also evident, with the most pronounced effect in the F_1 generation. The mean difference in mean postweaning body weights of 500 ppm F_1 females and their controls for the 14 weeks in which significant differences were observed was approximately 15%. In the F_2 females, a lesser difference (mean of 8%) was observed in 9 of the 15 postweaning weeks. Mean postweaning body weights of 100 ppm F_1 females were significantly less than those of the controls for 5 of the 11 weeks measured prior to litter delivery (mean difference of 6%). In the F_3 generation, exposed to genistein only until weaning, no significant differences in mean postweaning body weights were observed between any exposed group and the controls. For mean postweaning body weights of the unexposed F_4 generation, significant negative linear exposure concentration trends were found in 9 of the 11 predelivery weeks and all 4 of the postdelivery weeks measured, with a mean difference of 6% between the mean postweaning body weights of the 500 ppm group and the controls in the 8 weeks for which significant differences between these groups were found. That the exposure effect on the growth of females was predominant in the F_0 through F_2 generations and strongest in the F_1 generation is also evident in total body weight gains in the predelivery period; significant decreases in body weight gain were found for 500 ppm females in the F_0 through F_2 generations (Table D5).

Exposure effects in females in the preweaning period were also evident (Figures 3, 5, 7, and 9; Table D2). In the F_1 through F_4 generations, mean preweaning body weights of 500 ppm females were significantly less than those of the control groups by 6% to 14% on PND 21. In the F_1 generation, the mean preweaning body weight of 500 ppm females was 12% less than that of the controls on PND 14. Total body weight gains of 500 ppm females prior to weaning were significantly less than those of controls in all generations except the F_2 generation (Table D7). Females lost weight after delivery of their litters, and significant positive linear exposure concentration trends (toward less body weight loss) were seen in the F_1 and F_4 generations, with the 500 ppm F_1 females losing significantly less weight than controls in that generation (Table D6).

The effects of genistein on body weight in males during the postweaning period were largely confined to the 100 and 500 ppm groups of the F_1 generation (Figures 2, 4, 6, 8, and 10; Tables D3a through D3e); only sporadic differences with no evidence of a consistent pattern were observed between exposed and control groups in the other generations. Mean postweaning body weights of 100 ppm F_1 males were significantly less than those of the controls in 10 of the 17 weeks for which measurements were collected after weaning, with a mean difference of 6% between the exposed and control groups. Mean postweaning body weights of 500 ppm F_1 males were significantly less than those of the controls in 11 of these 17 weeks, with a mean difference of 7% between the groups. Total body weight gains of 100 and 500 ppm F_1 males were significantly less (5% to 6%) than those of the controls (Table D8, analysis of the F_1 through F_4 generations). In addition, total body weight gain of 500 ppm F_3 males was significantly less than that of the controls. Preweaning body weights of males, like preweaning body weights of females, showed exposure effects at PNDs 14 (F_1, F_2, and F_4 generations) and 21

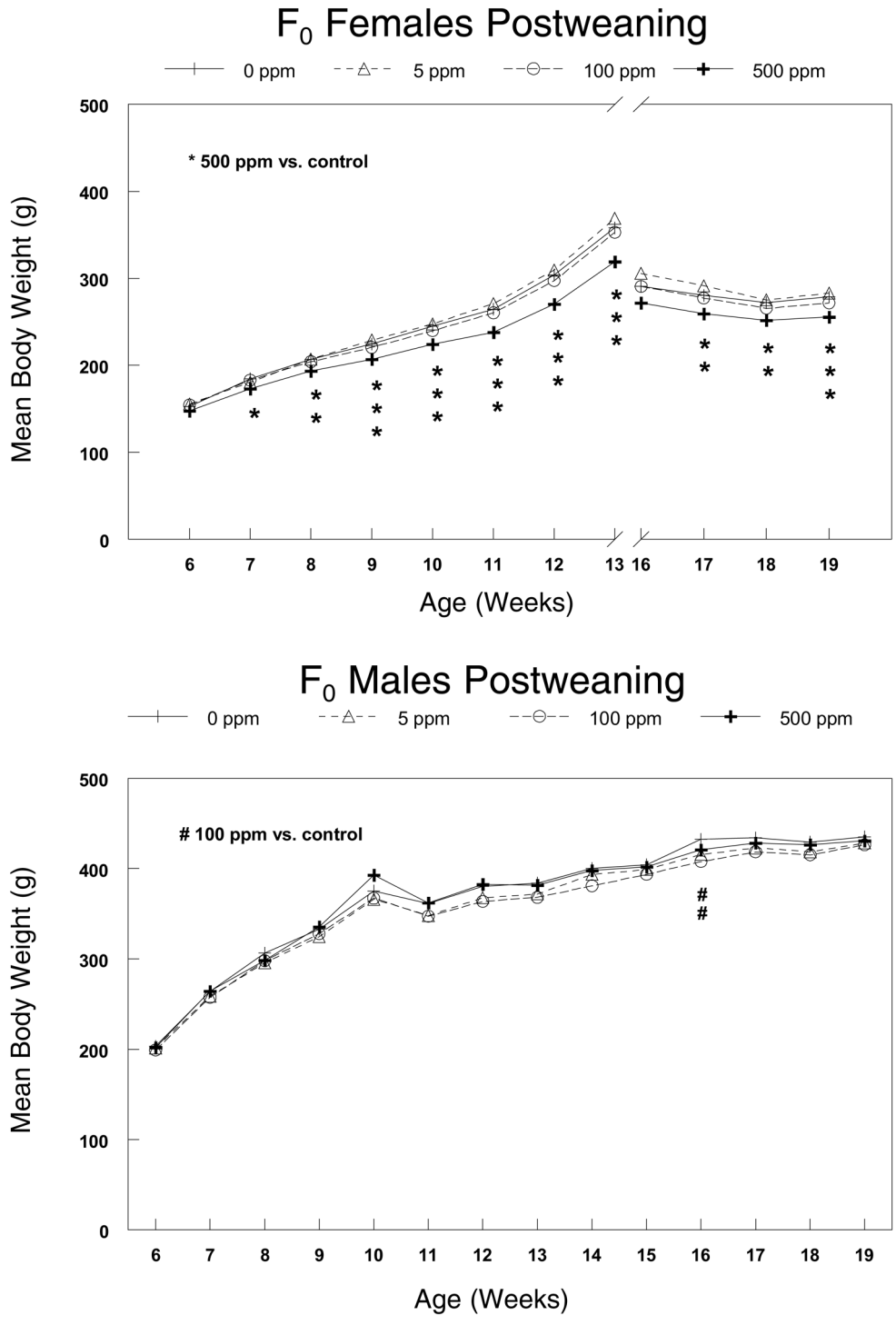


FIGURE 2
Postweaning Growth Curves for F_0 Rats Exposed to Dietary Genistein
Data are not included for weeks 14 and 15 for females as they were delivering litters during that period. Asterisks (*) and pound signs (#) indicate significant differences between controls and the 500 and 100 ppm groups, respectively. *, $P \leq 0.05$; ** or # #, $P \leq 0.01$; ***, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D1a (females) and D3a (males).

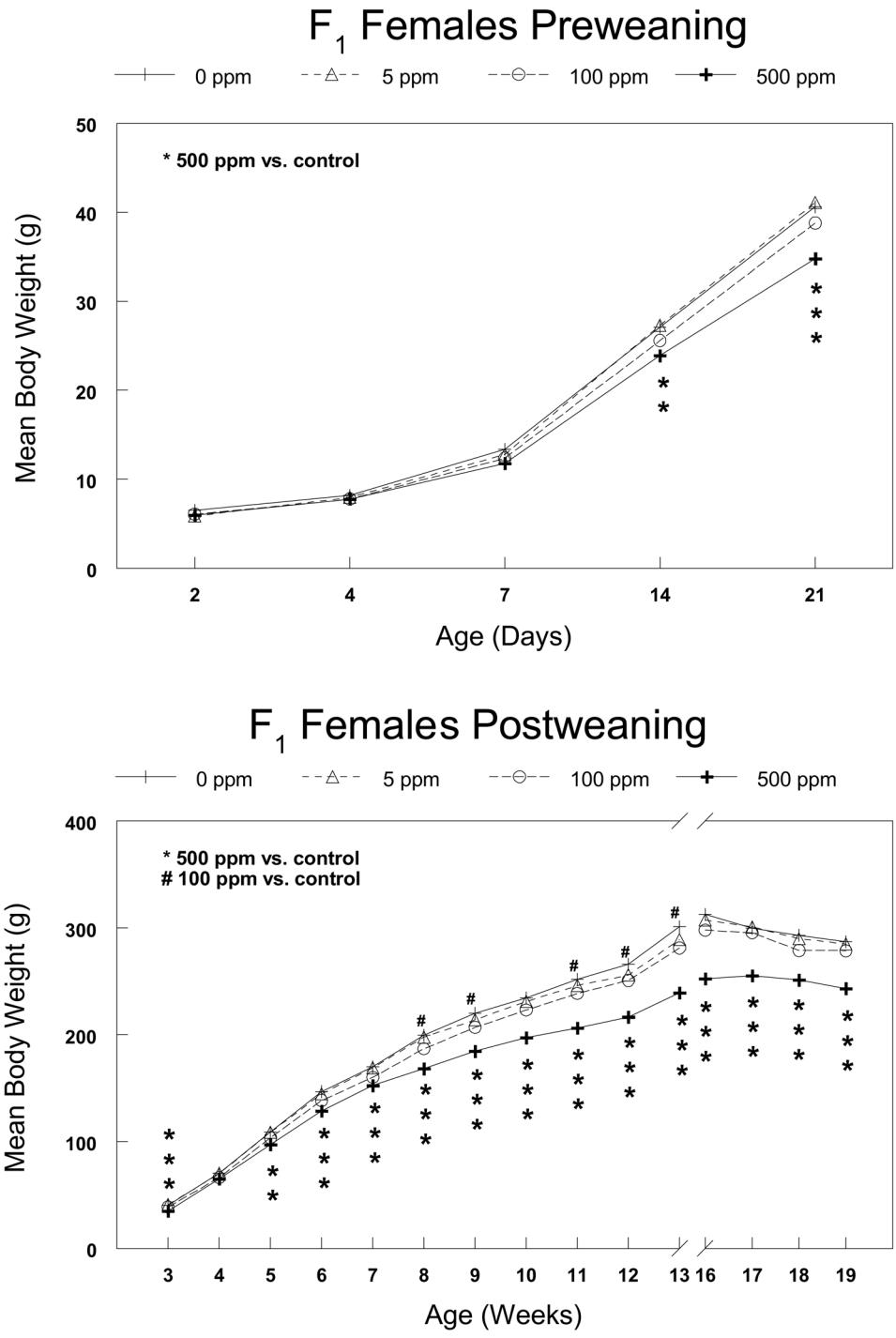


FIGURE 3
Preweaning and Postweaning Growth Curves for F_1 Female Rats Exposed to Dietary Genistein
Data are not included for weeks 14 and 15 as the rats were delivering litters during that period. Asterisks (*) and pound signs (#) indicate significant differences between controls and the 500 and 100 ppm 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).

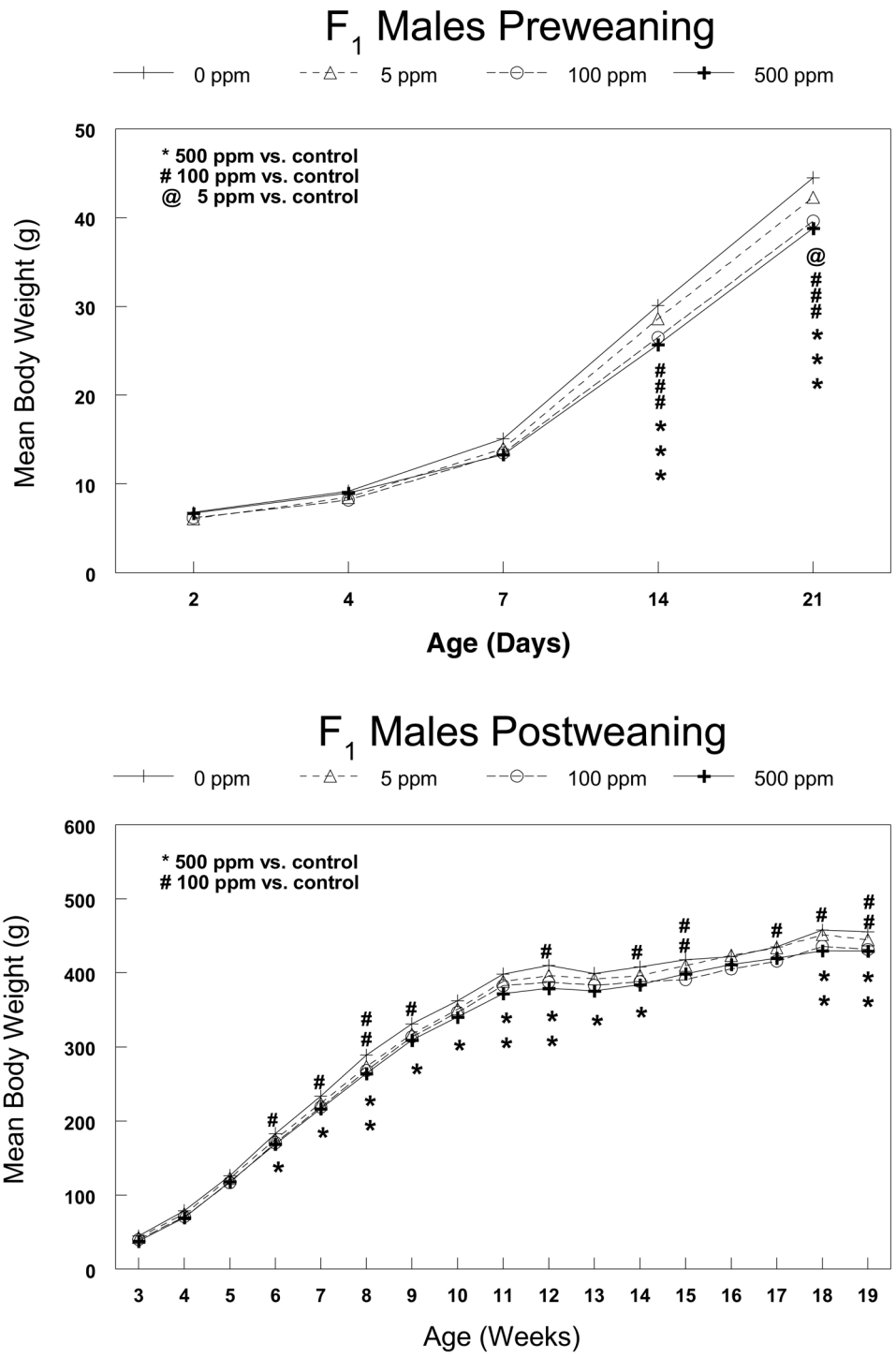


Figure 4
Preweaning and Postweaning Growth Curves for F_1 Male Rats Exposed to Dietary Genistein
Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 500, 100, and 5 ppm groups, respectively. *, #, or @, $P \leq 0.05$; ** or # #, $P \leq 0.01$; *** or # # #, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3b (postweaning).

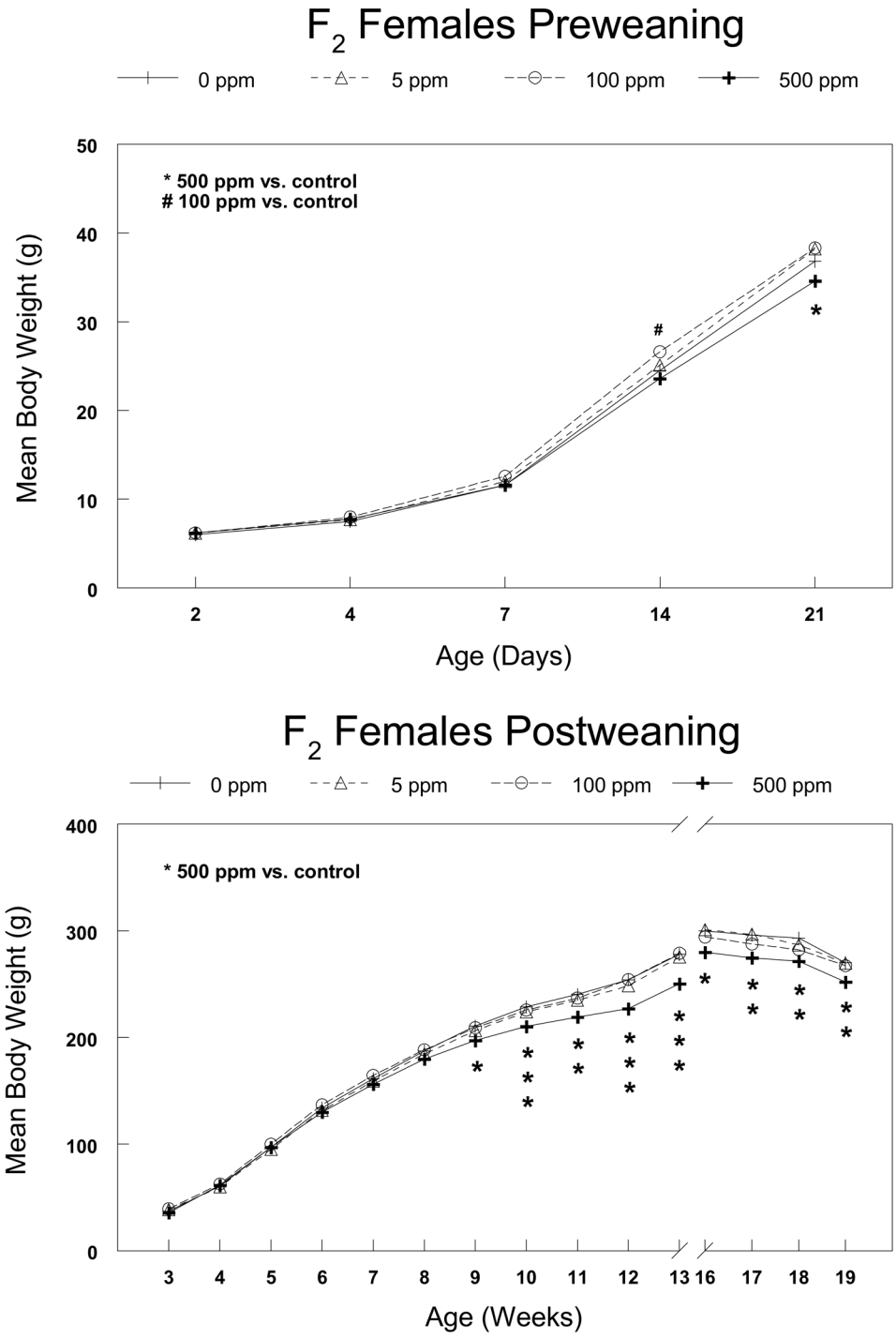


FIGURE 5
Preweaning and Postweaning Growth Curves for F_2 Female Rats Exposed to Dietary Genistein
Data are not included for weeks 14 and 15 as the rats were delivering litters during that period. Asterisks (*) and pound signs (#) indicate significant differences between controls and the 500 and 100 ppm groups, respectively. * or #, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1c (postweaning).

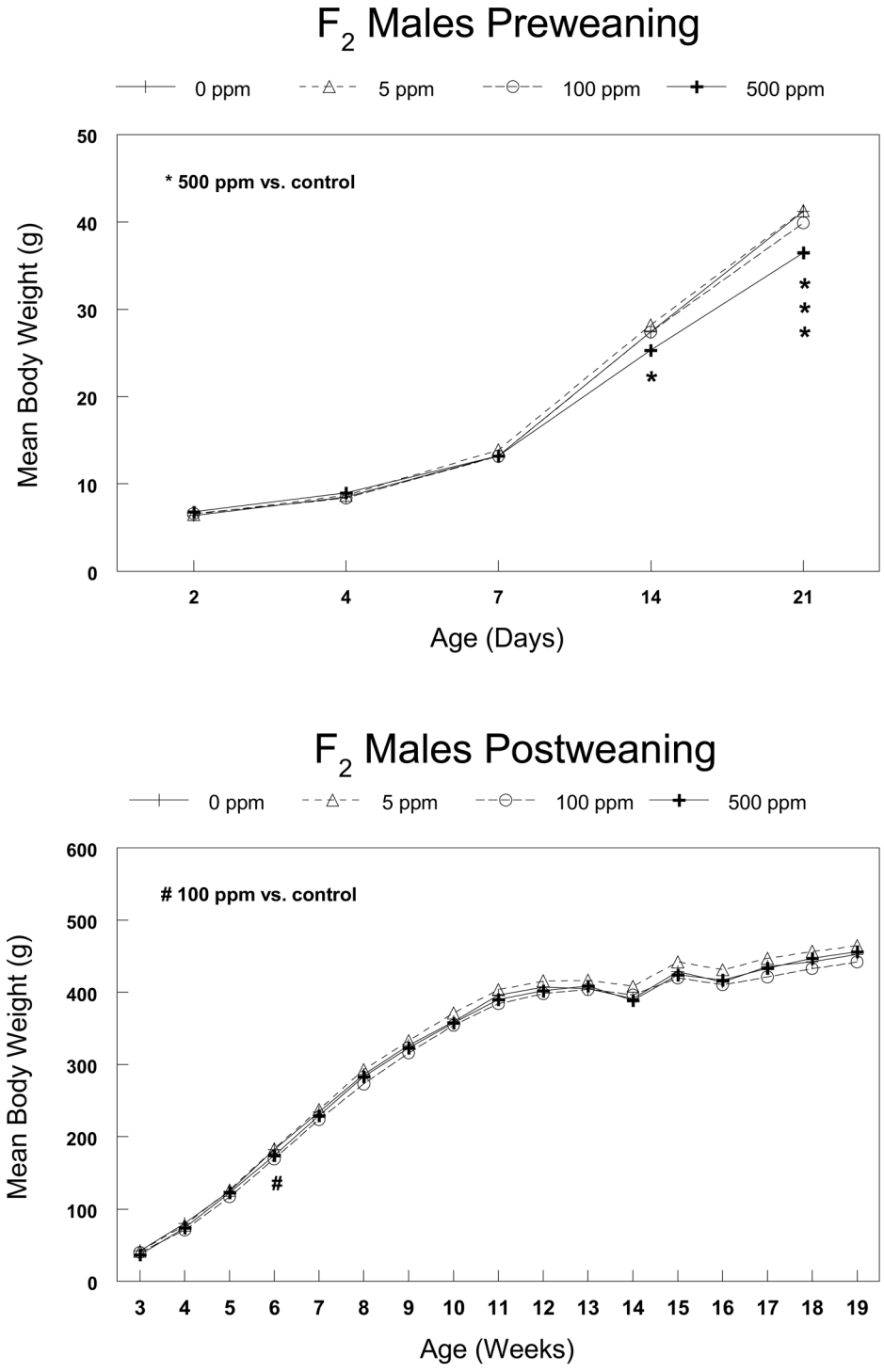


FIGURE 6
Preweaning and Postweaning Growth Curves for F_2 Male Rats Exposed to Dietary Genistein
Asterisks (*) and pound signs (#) indicate significant differences between controls and the 500 and 100 ppm groups, respectively. * or #, $P \leq 0.05$; ***, $P \leq 0.001$. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3c (postweaning).

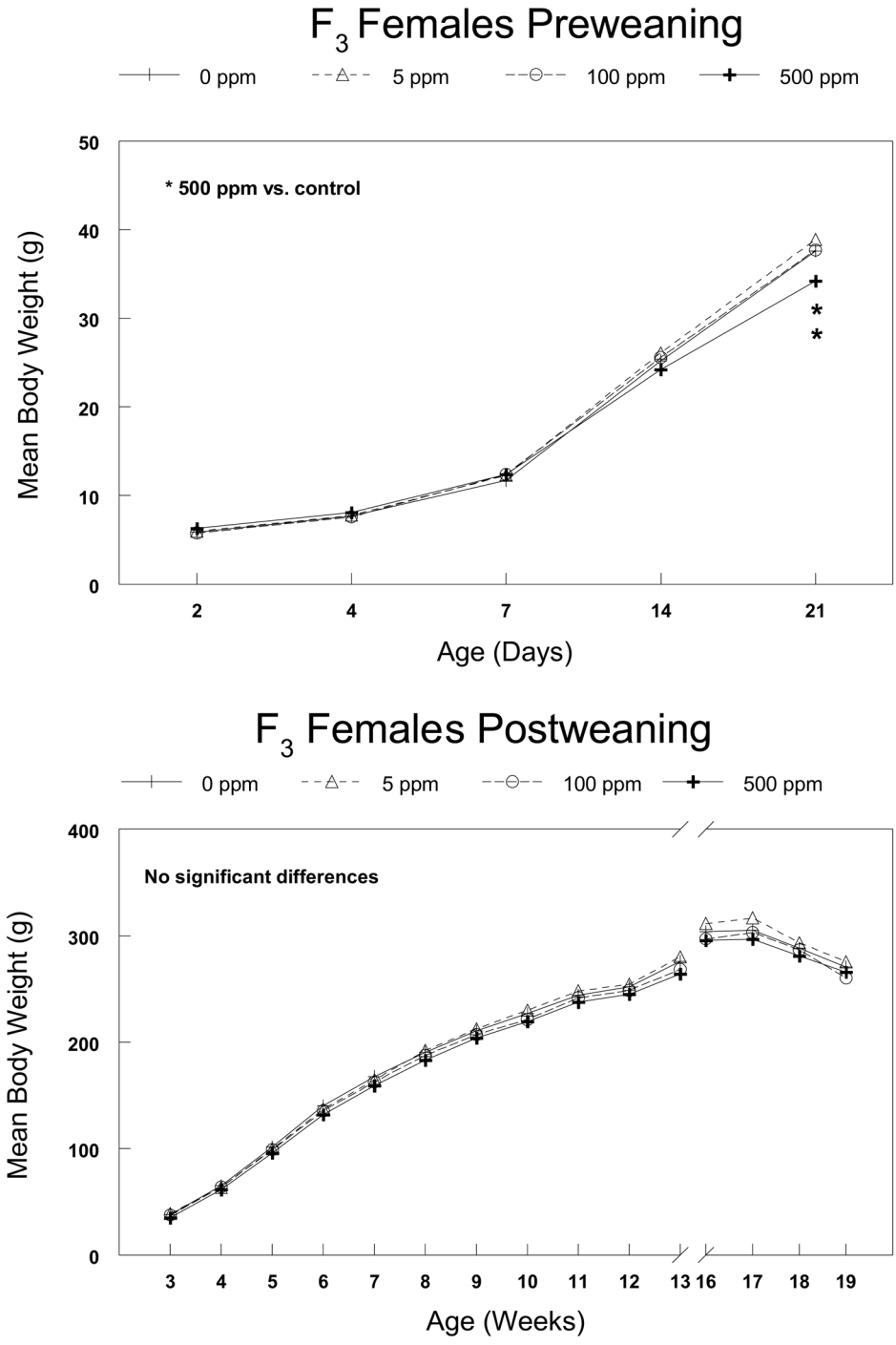


FIGURE 7
Preweaning and Postweaning Growth Curves for F_3 Female Rats Exposed to Dietary Genistein
Data are not included for weeks 14 and 15 as the rats were delivering litters during that period. Asterisks (*) indicate a significant difference between the control and 500 ppm groups. **, P≤0.01. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1d (postweaning).

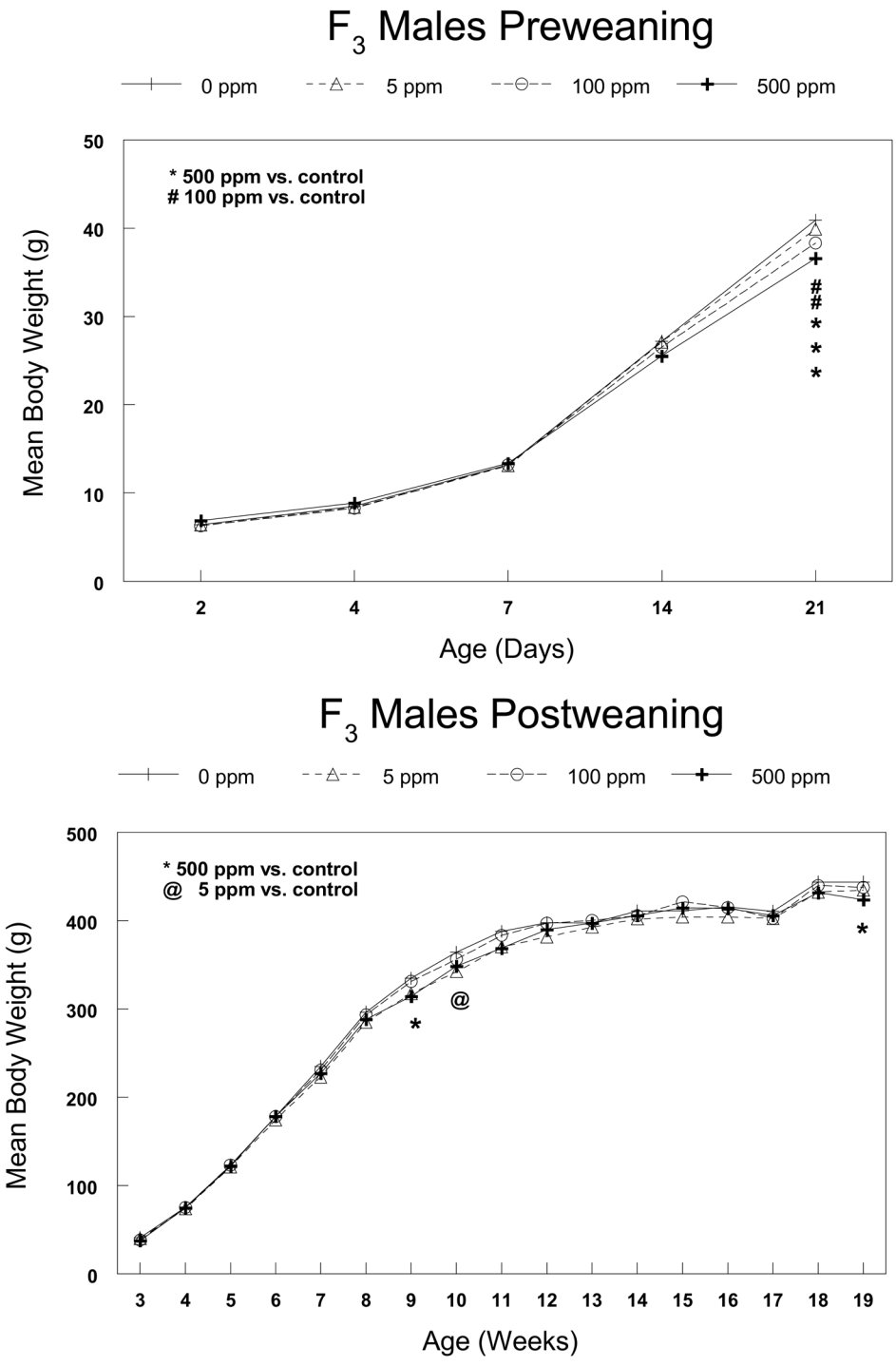


FIGURE 8
Preweaning and Postweaning Growth Curves for F_3 Male Rats Exposed to Dietary Genistein
Asterisks (*), pound signs (#), and "at" signs (@) indicate significant differences between controls and the 500, 100, and 5 ppm groups, respectively. * or @, P≤0.05; # #, P≤0.01; ***, P≤0.001. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3d (postweaning).

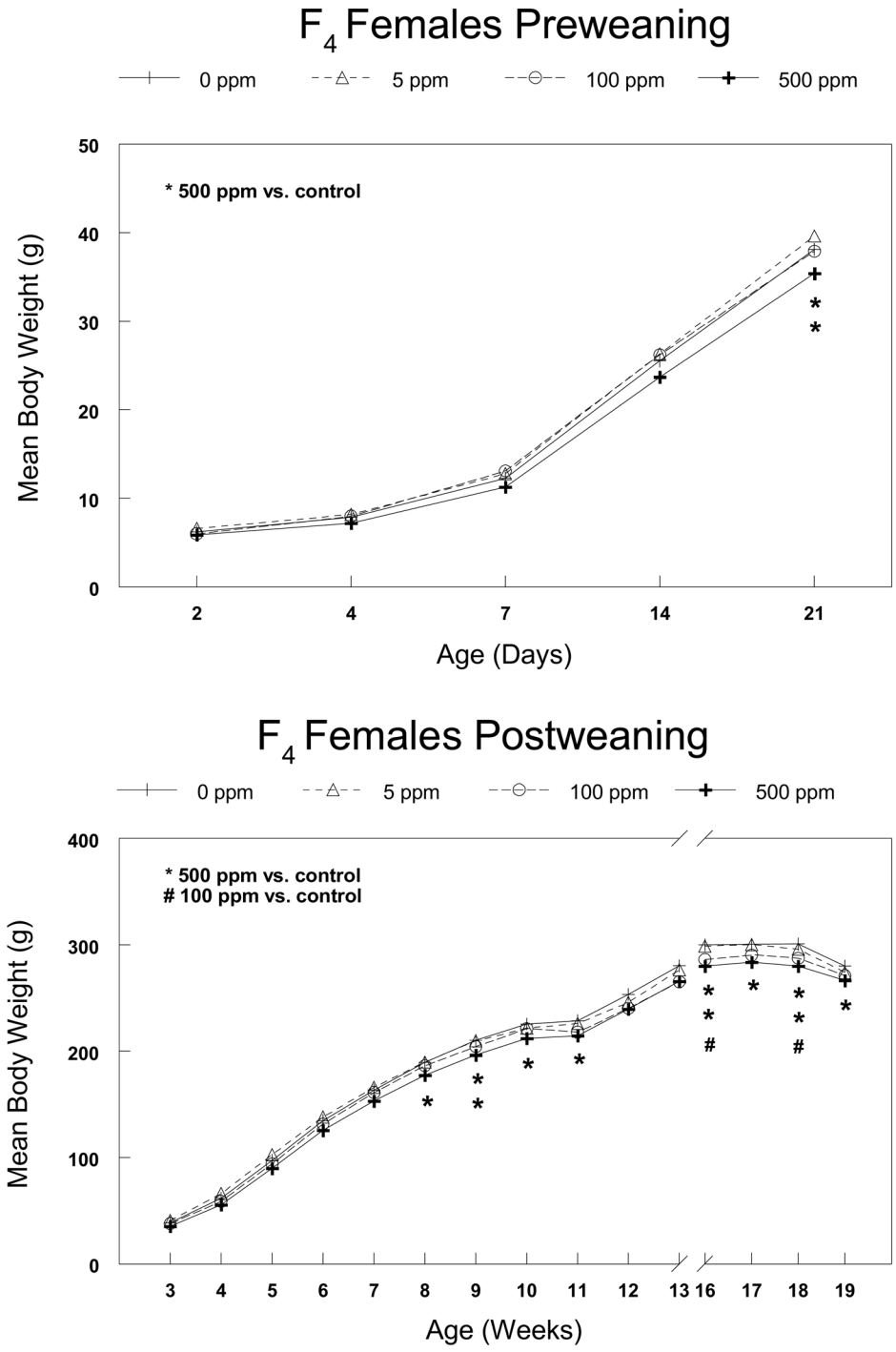


FIGURE 9
Preweaning and Postweaning Growth Curves for F_4 Female Rats Exposed to Dietary Genistein
Data are not included for weeks 14 and 15 as the rats were delivering litters during that period. Asterisks (*) and pound signs (#) indicate significant differences between controls and the 500 and 100 ppm groups, respectively. * or #, $P \leq 0.05$; **, $P \leq 0.01$. Means, number of animals, and standard error are given in Tables D2 (preweaning) and D1e (postweaning).

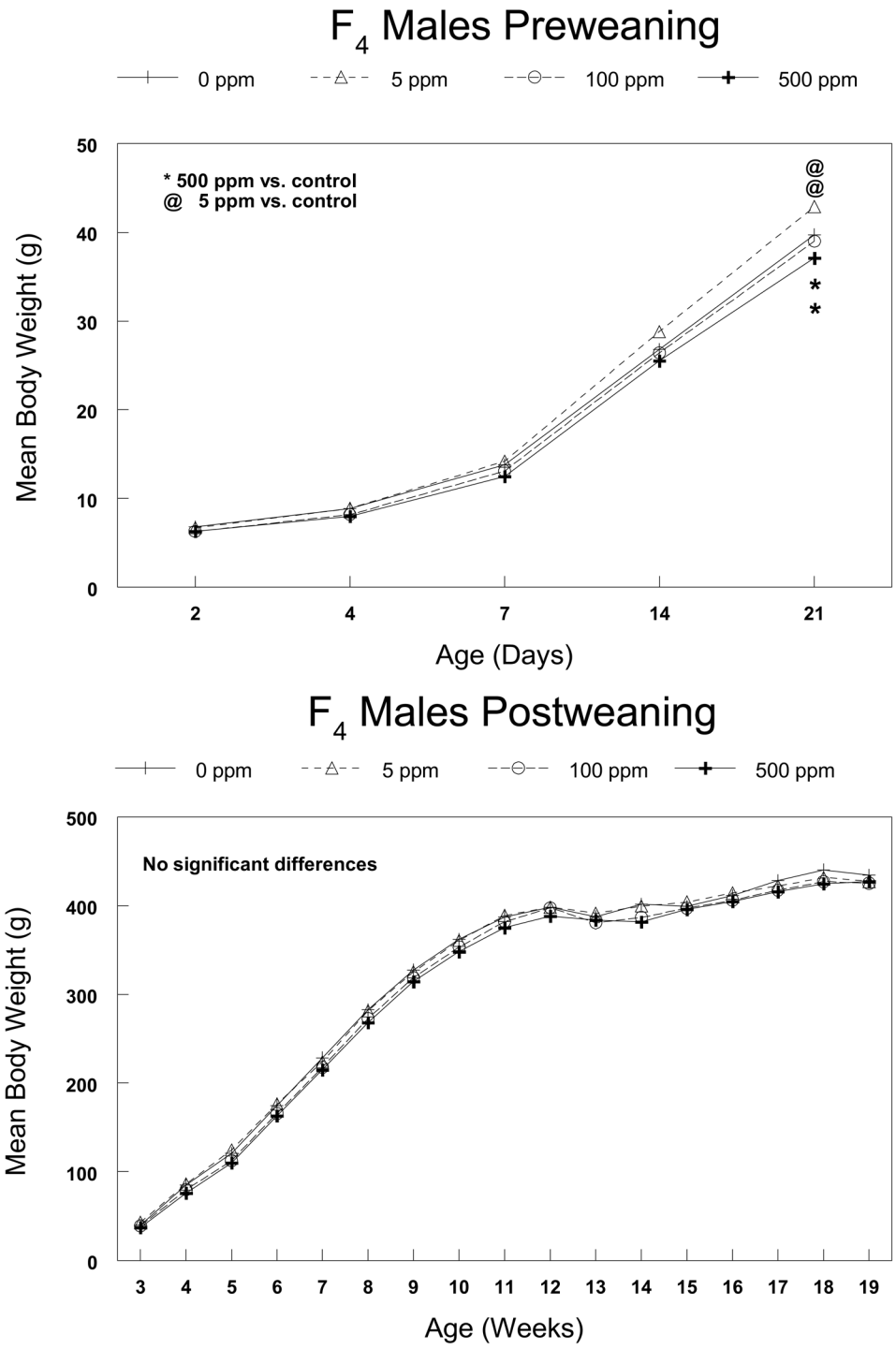


Figure 10
Preweaning and Postweaning Growth Curves for F_4 Male Rats Exposed to Dietary Genistein
Asterisks (*) and "at" signs (@) indicate significant differences between controls and the 500 and 5 ppm groups, respectively. ** or @ @, $P \leq 0.01$. Means, number of animals, and standard error are given in Tables D4 (preweaning) and D3e (postweaning).

(F_1 through F_4 generations) (Table D4). Total body weight gains of males during the preweaning period (Table D7) showed significant negative linear exposure concentration trends as well as significant decreases in the 500 ppm group relative to controls in all generations (14% to 15% decreases in the F_1 through F_3 generations and 7% decrease in the F_4 generation). Total preweaning body weight gain of 100 ppm F_1 males was significantly less than that of the controls (11% difference). The mean terminal (PND 140) body weights of 500 ppm F_1 males and 500 ppm F_0, F_1, and F_2 females were significantly less than those of the controls (9%, 14%, 6%, and 6% differences, respectively) (Table D9).

Significant differences between generations in body weights at particular ages within exposure groups for both males and females were largely ≤10% (Tables D2, D4, D10, and D11; Figures D1 through D8). There were evident exceptions; the most prominent occurred in female body weights in all exposure groups at 12 and 13 weeks of age. This difference was an experimental artifact that resulted from the fact that animals in the F_0 generation were bred at earlier ages than the animals in subsequent generations (mean age at mating for the F_0 generation was 73 days; mean age at mating for the F_1 through F_4 generations was 83 days). Thus the body weights at these ages in the F_0 generation, but not in subsequent generations, reflected weight gains due to pregnancy. The discrepancy in breeding age arose from a change in the procedures after the F_0 generation to limit the breeding window for subsequent generations to a 2-week period in order to avoid logistical problems that would have resulted from having the spacing of litter births increase over the generations. The greater body weight depression in 500 ppm F_1 females than in the 500 ppm females of the other generations is also evident from these generation comparisons. In males, generation differences greater than 10% in body weights were also largely confined to differences between the F_0 generation and later generations. The direction of these differences was not consistent throughout the study, with higher body weights in F_0 males relative to other generations at early postweaning times and lower body weights near the breeding period (10 to 12 weeks of age). These observed generational differences in body weights do not interfere with the interpretation of the effects of genistein exposure.

Feed consumption data and statistical analyses of those data for F_0 through F_4 generation male and female rats are summarized in Tables E1a through E5. There were significant effects and patterns of genistein exposure that generally corresponded to the body weight gain effects and are generally consistent with the known anorectic effect of estrogens (Wade and Schneider, 1992). That is, females showed greater effects than males with the most pronounced effect in F_1 generation females and in the 500 ppm exposure groups. This is evident both from the weekly feed consumption tables (Tables E1a through E2 for females; Tables E4a through E4e for males) and from the total feed consumption tables (Table E3 for females; Table E5 for males). Generation differences within exposure groups were observed (Tables E6 and E7; Figures E1 through E4), with the most pronounced difference occurring between F_0 females and later generations during weeks 14 to 16. As noted earlier for the body weight differences between F_0 females and later generations of females, this can be explained by the fact that the F_0 generation was bred at a younger age, so that F_0 females started lactating earlier and showed the spike in feed consumption due to lactation earlier than other generations.

Water consumption of dams during lactation in each generation of this study is reported in Tables F1a through F1e. 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). Genistein had no consistent significant effect on this endpoint in the current study.

Mating and Pregnancy

Data for mating, fertility, and pregnancy indexes and the mating and gestation times are reported in Table G1. The only statistically significant effect found was for gestation time, where there was a significant difference among generations and significant linear exposure concentration trends in the F_0 and F_3 generations. However, the trends in these two generations were in opposite directions, and the maximum mean difference between exposed and control groups in both generations was 0.3 days, or approximately 1.5% of the total mean gestation time, which is below the resolving power of the study given that litters not completely born by 1430 hours were not recorded as "delivered" until the following day. The uteri of 108 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 resorptions (data not shown). Ten of these animals were found to have resorption sites or nonviable or viable fetuses. Three of these were control animals (one in the F_2 generation and two in the F_4 generation)

with one or two resorption sites or fetuses. All animals with greater than two resorption sites were in continuously exposed groups: one 100 ppm F_0 animal had five resorption sites, one 500 ppm F_1 animal had 12 resorption sites, and one 5 ppm F_2 animal had three resorption sites. No relationship between genistein exposure and resorptions was evident.

Litter and Perinatal Pup Parameters

Statistical analyses of litter parameters and perinatal pup measurements for the F_1 through F_5 generations are reported in Table H1. The effect of genistein exposure varied across generations for all of the measures associated with litter size (total pups born, live pups, and male and female live births). Statistically significant negative linear exposure concentration trends were detected in the F_1 through F_3 generations (the generations whose parents were fed genistein throughout adulthood) for total pups born (Figure 11) and live births, and these trends appeared to be largely determined by the 12% to 31% reduction in litter size in the 500 ppm groups of those generations. The mean litter size of the 500 ppm F_2 group was significantly less than that of the F_2 control group and also significantly less than the litter size of the 500 ppm group of any other generation. There was no effect of genistein exposure on the incidences of stillbirth.

There was a significant difference among exposure concentrations for male pup weights. Statistically significant decreases (ranging from 6% to 9% compared to controls) were confined to all exposed groups in the F_5 generation (whose parents were not exposed to dosed feed) and to the 100 ppm F_1 group. For female pup weights, significant decreases relative to controls occurred in all exposed groups in the F_5 generation. A significant difference among exposure concentrations and significant positive exposure concentration trends in the F_1 and F_4 generations were detected for the sex ratio (ratio of males to females), but values for this parameter varied considerably [the range of the means was 0.88 to 2.57, with these extremes found in the same (F_4) generation], and there were no significant differences between exposed groups and their controls within any generation.

The mean anogenital distance (AGD) of 500 ppm F_1 males on PND 2 was significantly less than that of the F_1 controls, and there was a significant negative exposure concentration trend in the F_1 generation in the ANCOVA analysis with body weight as covariate (Figure 12 and Table H1). However, the maximum difference in mean AGDs between an exposed group and its corresponding control group was approximately 5%, while intergenerational differences exceeding 5% were observed within all of the exposure groups. The effect of genistein exposure on mean AGD in females varied across generations, with a significant negative linear exposure concentration trend and smaller AGD (absolute, 7%; relative to body weight, 4%) relative to the controls in the 500 ppm group of the F_1 generation (Figure 13 and Table H1). There was also a negative linear exposure concentration trend for AGD in the F_3 generation of females, but other significant exposure-related differences were dependent on the statistical model used, with the 500 ppm F_2 and the 100 ppm F_3 groups of females having significantly smaller AGDs than their respective control groups only in the ANCOVA model with body weight as covariate. Similar to observations in males, the differences between mean AGDs across generations within exposure groups were greater than the differences between exposed and control groups within a generation.

Survival of pups between the time of litter standardization and the time of weaning was not significantly affected by genistein in any consistent manner (analysis not presented). Among female pups, there was decreased survival in the control group relative to exposed groups in the F_4 generation and in the 5 ppm lineage group relative to other groups in the F_5 generation. These results seem likely to be chance observations.

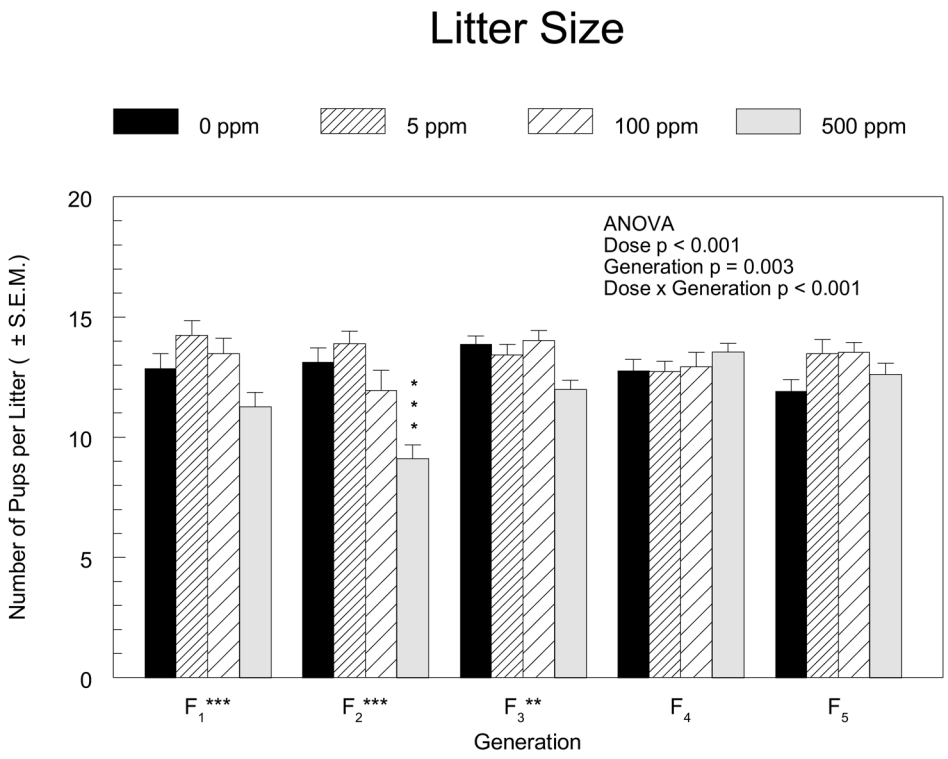


FIGURE 11
Effect of Dietary Genistein on Total Litter Size
The mean number of pups per litter ± standard error are shown along with the results of a two-way ANOVA with dose and generation as factors (inset). Asterisks (*) on the x-axis indicate a significant linear exposure concentration trend within the marked generation. Asterisks above the data bar 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 H1. **, P≤0.01; ***, P≤0.001.

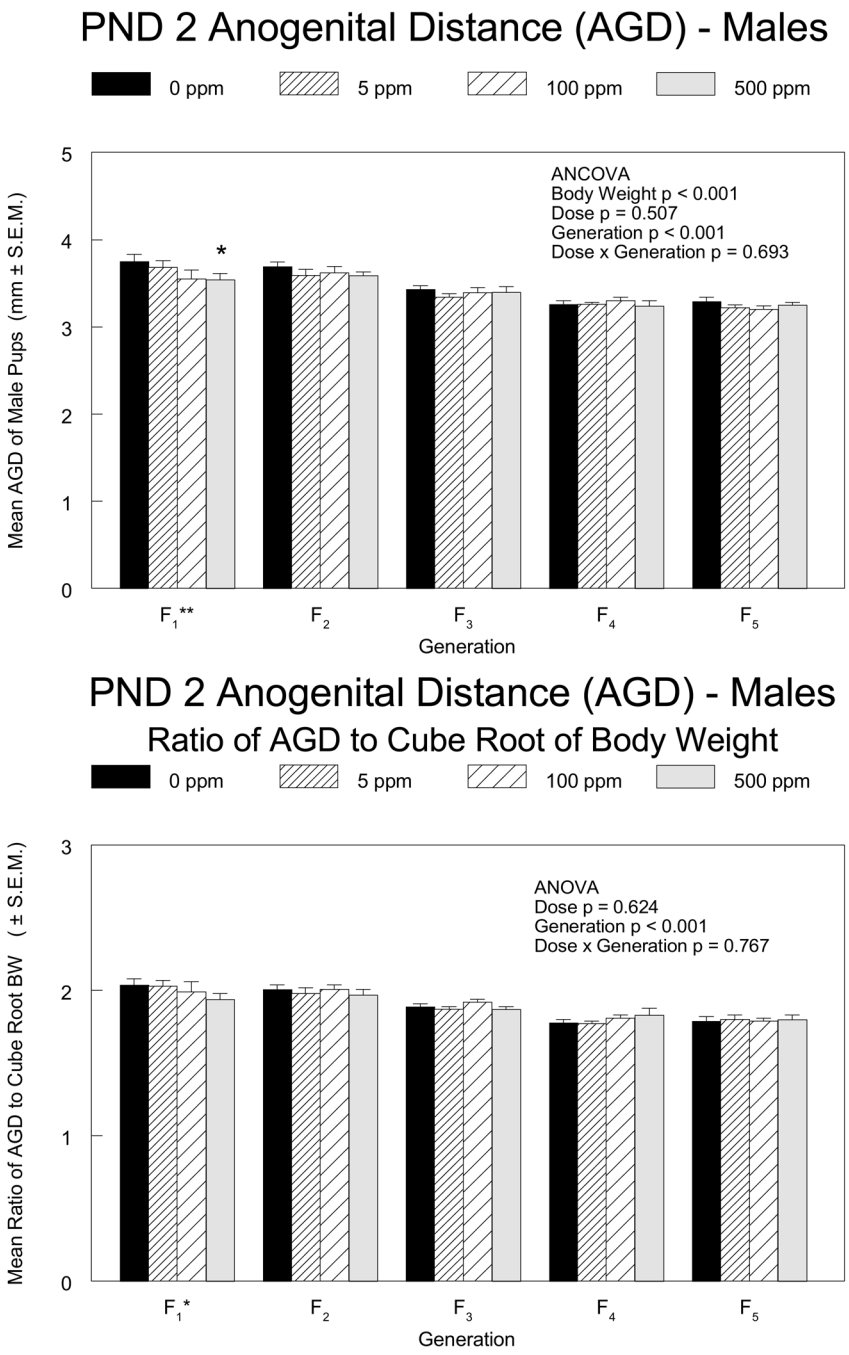


FIGURE 12

Effect of Dietary Genistein on Anogenital Distance of Male Pups Measured on Postnatal Day 2

Measurements were made on four pups (after standardization) from 10 litters in each exposure group. The top panel is mean AGD (mm) ± standard error. Results shown are from an analysis of covariance with body weight as the covariate. The bottom panel is mean ratio of AGD to the cube root of body weight ± standard error. Results shown are from an analysis of variance. An asterisk (*) above the data bar indicates a significant difference between the means of the marked group and the control group in that generation (Dunnett's test). Asterisks on the x-axis indicate a significant linear exposure concentration trend within the marked generation. These data are tabulated in Table H1. * , $P \leq 0.05$.

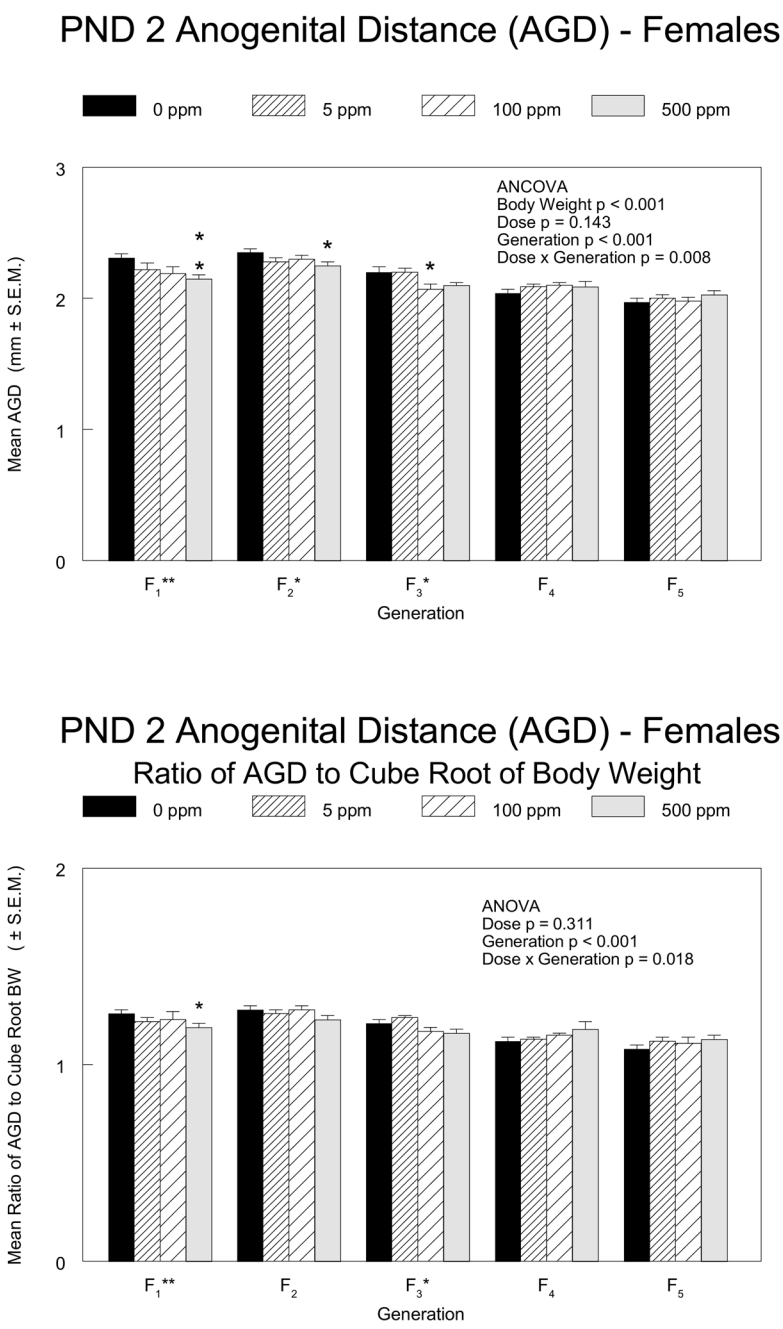


FIGURE 13
Effect of Dietary Genistein on Anogenital Distance of Female Pups Measured on Postnatal Day 2
Measurements were made on four pups (after standardization) from 10 litters in each exposure group. The top panel is mean AGD (mm) ± standard error. Results shown are from an analysis of covariance with body weight as the covariate. The bottom panel is mean ratio of AGD to the cube root of body weight ± standard error. Results shown are from an analysis of variance. 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). Asterisks on the x-axis indicate a significant linear exposure concentration trend within the marked generation. These data are tabulated in Table H1. * , P≤0.05; **, P≤0.01.

Markers of Sexual Development

The age and body weight at vaginal opening are shown in Figure 14 and Table I1. For age at vaginal opening, statistically significant overall effects of exposure concentration were found in the F_1 through F_3 generations, with vaginal opening occurring approximately 3 days earlier in 500 ppm F_1 and F_2 females than in their respective control groups. The age at vaginal opening in 5 ppm F_3 females differed from that in the controls by approximately 1 day, which was significant. When the body weight at vaginal opening was examined, significant negative linear exposure concentration trends were observed in the F_1 through F_4 generations, with vaginal opening occurring when the body weights of 500 ppm F_1 through F_3 animals were 73% to 85% of the body weights of the controls. Body weight at vaginal opening in 5 ppm F_1 females was significantly lower (10%) than that in the F_1 controls. Within the control groups across generations, the day of vaginal opening differed by up to 2 days, and the body weight at vaginal opening varied by up to 9%.

Genistein did not significantly affect the markers of male sexual maturation that were monitored, preputial separation and testicular descent (Tables I2 and I3, respectively), except for a significant positive linear exposure concentration trend for age at testicular descent in the F_3 generation and a significant delay of testicular descent in 500 ppm F_3 males compared to that in the F_3 controls.

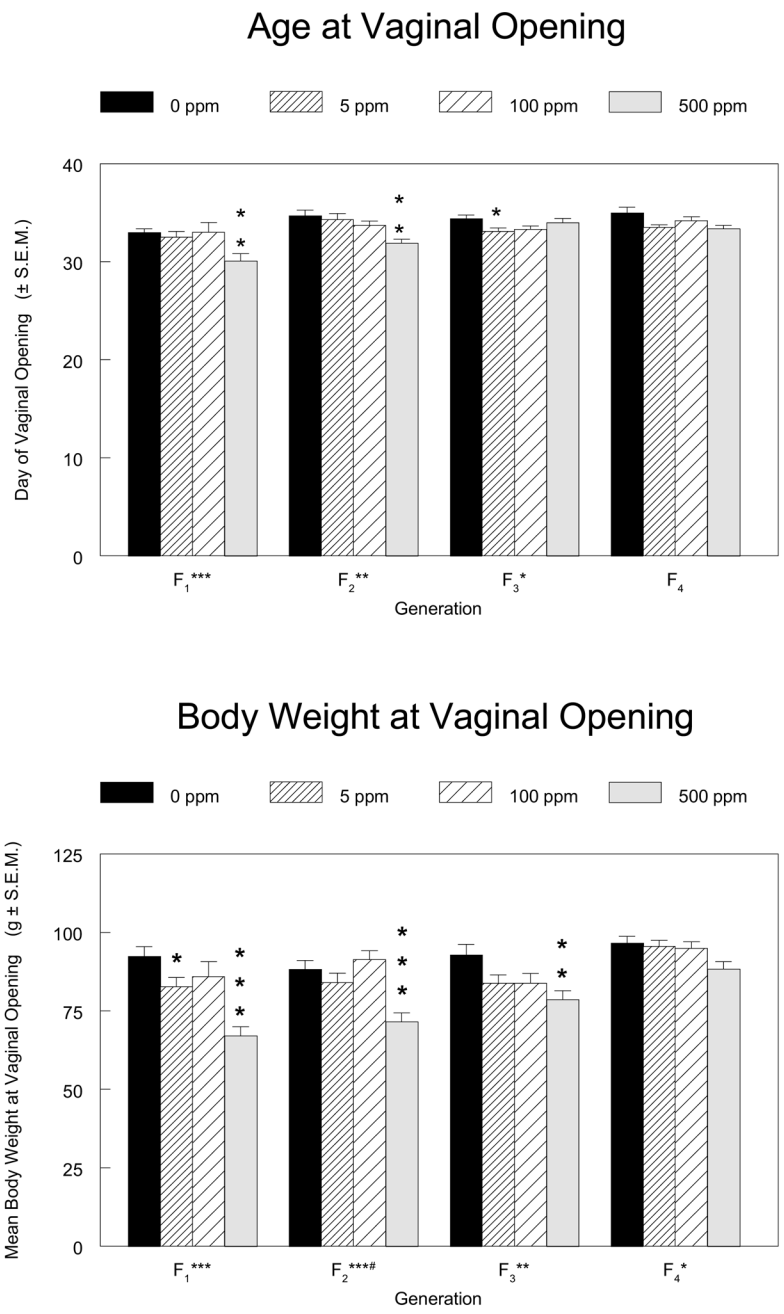


FIGURE 14
Effects of Dietary Genistein on Age (top panel) and Body Weights (bottom panel) at Vaginal Opening
Results of nonparametric analyses within generations are presented for the age at vaginal opening (mean ± standard error). Asterisks (*) on the x-axis indicate a significant overall Kruskal-Wallis' 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, while a pound sign (#) indicates a significant quadratic exposure concentration trend. 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. * or #, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

The Estrous Cycle

Starting 3 days after the observation of vaginal opening, vaginal smears were taken for 14 consecutive days from 25 females per exposure group that were littermates of the breeding females in generations F_1 through F_4. 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 3 or more consecutive days in estrus or 4 or more consecutive days in diestrus, and length of cycles. These data are tabulated in Table J1. In the F_1 generation, a statistically significant reduction in the mean percentage of time in proestrus and significant increases in the mean number and percentage of abnormal cycles were observed in the 500 ppm group relative to the controls (Figure 15 and Table J1). For these two endpoints, the only significant effects of genistein exposure that occurred outside of the F_1 generation were a decrease in the mean percentage of time in diestrus in the 100 ppm group of the F_4 generation and a significant overall dose effect on the mean number of abnormal cycles due to extended estrus in the F_2 generation. Length of cycle was analyzed by two nonparametric methods, a Kruskal-Wallis' ANOVA on ranks followed by pairwise comparisons of exposed groups to controls by Wilcoxon's tests, and a more powerful Jonckheere-Terpstra exposure concentration trend test and Shirley's tests to compare exposed groups to controls. Mean lengths of cycle were significantly increased in 500 ppm females in the continuously exposed F_1 (60% increase) and F_2 (15% increase) generations; the effect in 500 ppm F_2 females was significant only by the trend test (Figure 16 and Table J1). In the F_4 generation, although the overall ANOVA was significant and the mean length of cycle was 27% greater in the 500 ppm group than in the F_4 control group, neither the pairwise comparison to the controls nor the test for increasing exposure concentration trend was statistically significant.

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). While the mean lengths of cycles in several exposed groups in the F_0 through F_4 generations differed from their respective controls (longer, except for a 3% shorter cycle in the 100 ppm F_3 females), none of the differences were statistically significant (Figure 17 and Table J2). The mean numbers and percentages of abnormal cycles due to prolonged diestrus or diestrus and estrus combined showed significant overall exposure concentration effects in the F_3 generation and had elevated means in the 500 ppm group versus F_3 controls, but only the mean number of abnormal cycles due to prolonged diestrus and estrus combined was found to be significantly increased after correction for multiple comparisons (Figure 18 and Table J2). Significant overall exposure concentration effects for mean percentages of time in the cycle stages were found in the F_2 generation for diestrus, F_1 and F_2 generations for estrus, and the F_3 generation for proestrus. The percentage of time in diestrus was significantly increased (16%), and the percentage of time in estrus was significantly decreased (19%) in 5 ppm F_2 females compared to controls.

The ovary, uterus, and vagina taken from each animal 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 genistein on estrous cycle synchrony were found in these organs.

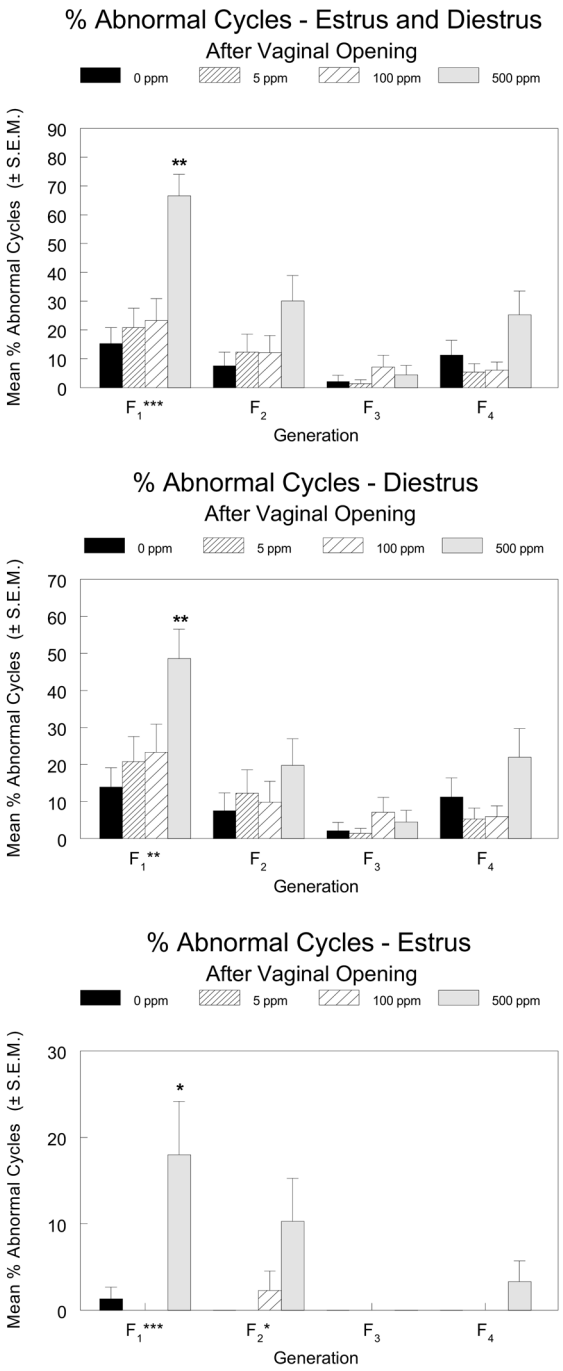


FIGURE 15
Effect of Dietary Genistein on the Percentage of Abnormal Cycles in Females Monitored Shortly after Vaginal Opening

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 mean percentage of abnormal cycles due to either prolonged diestrus or estrus; the middle panel gives the mean percentage of abnormal cycles due to prolonged diestrus, and the bottom panel gives the mean percentage of abnormal cycles due to prolonged estrus. Data were analyzed within generations by Kruskal-Wallis' nonparametric ANOVA and the Holm's adjusted Wilcoxon's test for pairwise comparisons with the controls. 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. These data are tabulated in Table J1. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

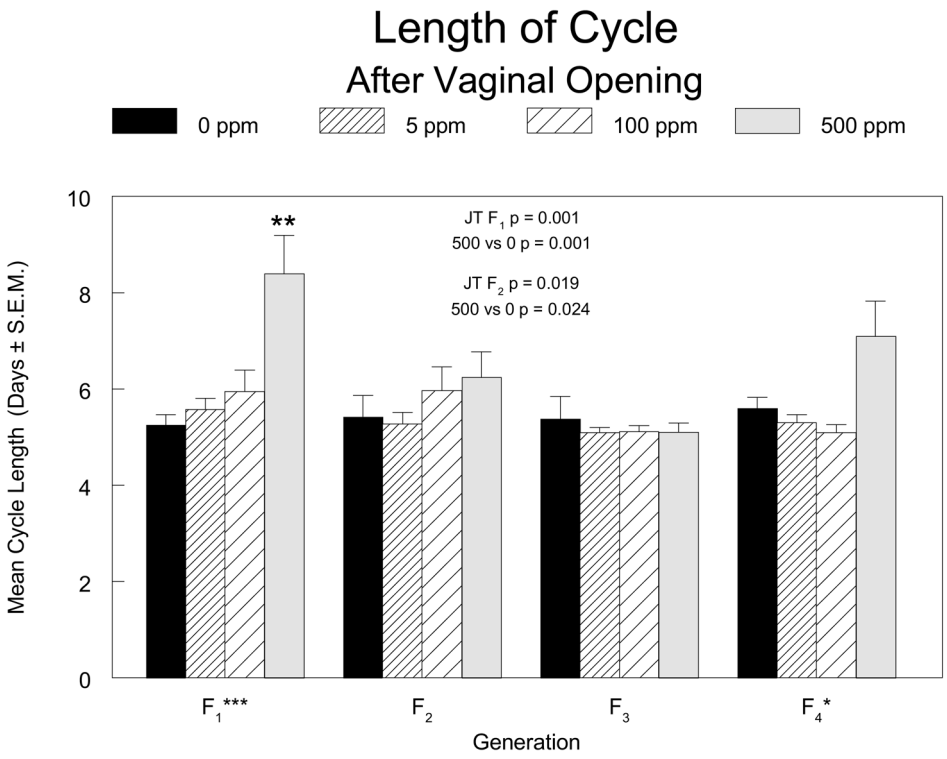


FIGURE 16
Mean Cycle Length for Females Examined for 14 Days Beginning 3 Days after Vaginal Opening
Significant results from the Jonckheere-Terpstra (JT) exposure concentration trend test and Shirley's test for the comparison of exposed groups to controls in the same generation are shown in the inset. Asterisks (*) on the x-axis indicate a significant overall Kruskal-Wallis' test for the marked generation. Asterisks above the data bar indicate a significant difference between the means of the marked group and the control group in that generation. These data are tabulated in Table J1. *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

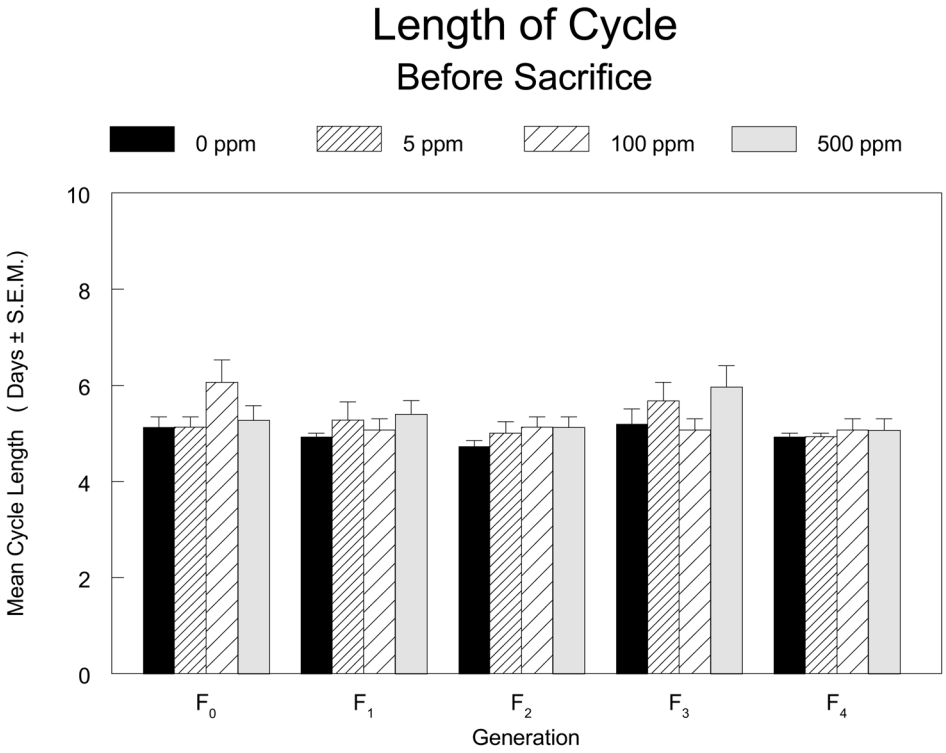


FIGURE 17
Mean Cycle Length for Females during the Last 10 Days prior to Termination
These data are tabulated in Table J2.

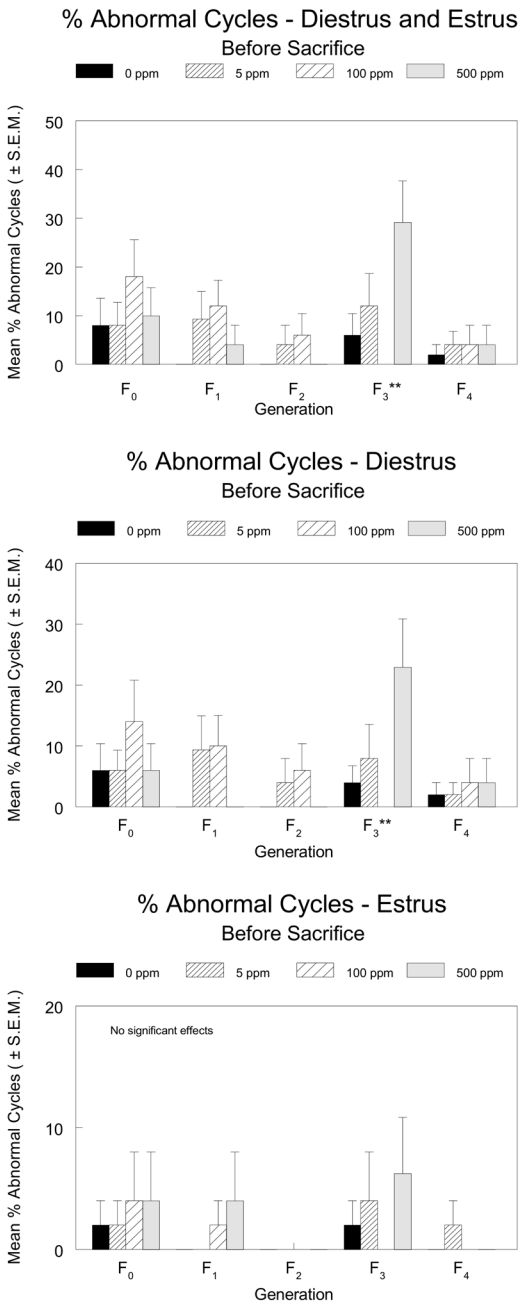


FIGURE 18
Effect of Dietary Genistein on the Percentage of Abnormal Cycles in Females Monitored for 10 Days prior to Termination
Abnormal cycles were defined as 3 or more consecutive days of estrus or 4 or more consecutive days of diestrus. The top panel gives the mean percentage of abnormal cycles due to either prolonged diestrus or estrus; the middle panel gives the mean percentage of abnormal cycles due to prolonged diestrus, and the bottom panel gives the mean percentage of abnormal cycles due to prolonged estrus. Data were analyzed within generations by Kruskal-Wallis' nonparametric ANOVA and the Holm's adjusted Wilcoxon's test for pairwise comparisons with the controls. Asterisks (*) on the x-axis indicate a significant overall Kruskal-Wallis' test for the marked generation. These data are tabulated in Table J2. **, $P \leq 0.01$.

Organ Weights

Dietary genistein had minimal effects on organ weights, and the statistically significant effects that were found were often confined to a single generation or did not follow a consistent pattern across genistein-exposed generations (Appendix K). In males, 2-way ANOVAs found no statistically significant dose main effects or dose × generation interactions for any of the reproductive organs measured (dorsolateral and ventral prostate gland; Tables K7 and K8, respectively), epididymis (Table K3), seminal vesicle with coagulating gland (Table K9), and testis (Table K11). In 500 ppm F_0 males, absolute and relative testis weights were significantly increased by 8% to 9% relative to the F_0 controls, and there was a significant positive linear exposure concentration trend in the F_0 generation. The increased absolute testis weight was within the range of control testis weights observed across the generations. Relative ventral prostate gland weights of 500 ppm males in the F_0, F_1, and F_2 generations were 6%, 16%, and 9% greater than those of their respective controls, but none of these increases were statistically significant.

For the weights of the nonreproductive organs in males, statistically significant effects of genistein exposure on absolute and/or relative weights were found for the brain, kidney, liver, pituitary gland, spleen, and thymus. Of these, only the pituitary gland (Table K6), thymus (Table K12), and spleen (Table K10) had exposed groups that differed from controls by more than 10%. Absolute and relative pituitary gland weights of 500 ppm F_2 males were significantly greater (18% and 17%, respectively) than those of the F_2 controls. Absolute and relative thymus weights of 100 ppm F_2 males were significantly less (21% and 20%, respectively) than those of the F_2 controls. Although absolute and relative thymus weights of all exposed groups of F_4 males were less than those in the F_4 controls, only the absolute thymus weight of the 100 ppm group was significantly decreased (19%). Absolute and relative spleen weights of 5 ppm F_0 males were significantly greater than those of the F_0 controls, and absolute spleen weight of the 5 ppm F_2 males was significantly increased.

In females, significant effects of genistein exposure were observed for the absolute and/or relative weights of the brain, kidney, liver, pituitary gland, spleen, thymus, and thyroid gland. As for the males, organ weight effects were generally small and did not occur in a consistent pattern that clearly indicated biological or toxicologic significance. Effects of the greatest magnitude (>10%) that could not be directly attributed to the body weight effects of genistein in 500 ppm females were found in the pituitary gland (Table K19), thymus (Table K21), and spleen (Table K20). Absolute and relative pituitary gland weights of 100 ppm F_0 females and the relative pituitary gland weight of 500 ppm F_1 females were significantly greater than those of the F_0 and F_1 controls, respectively. Absolute and relative thymus weights of 100 ppm F_3 and F_4 females were significantly less than those of the same-generation control groups. Absolute and relative spleen weights of 5 ppm F_1 females were significantly greater than those of the F_1 controls. Relative liver weights of 5, 100, and 500 ppm F_1 females were greater (10%, 8%, and 8%, respectively) than that of the F_1 controls; the increases in the 5 and 500 ppm groups were statistically significant (Table K17). Significantly decreased absolute thyroid gland weight in 500 ppm F_2 females and significantly increased relative thyroid gland weight in 500 ppm F_1 females appeared to be related to an unusually high control value in the F_2 generation and to body weight depression in the F_1 generation (Table K22).

Sperm Parameters

Results of the sperm analyses are presented in Tables L1 through L4. There were no statistically significant differences between exposed and control groups for any of the measured sperm parameters in any generation. Statistical comparisons of sperm parameters across generations were not conducted, although it is evident that epididymal sperm counts were generally lower in all exposure groups of the F_4 generation (Table L2) and the percent abnormal sperm was generally higher in the F_4 generation (Table L4). The fact that sperm analyses for the F_0 through F_3 generations were conducted in a different laboratory than those for the F_4 generation may in part account for the apparent differences in values for the F_4 generation.

Ovarian Follicle Counts

H&E-stained step sections from the ovaries of eight selected animals in each exposure group of each generation were used for enumeration of small, growing, and antral follicles, and the mean values and results of statistical evaluations are shown in Table M1. Significant differences were observed among the generations for small, growing, small and growing combined, and total follicle counts, with the most pronounced fluctuations in the small (and small and growing) follicles in the control and 100 ppm groups. For antral follicle counts, there was a significant negative linear exposure

concentration trend in the F_2 generation, and the count was significantly decreased in 500 ppm F_2 females compared to that in the F_2 controls; the slightly increased antral follicle count in the control group may account for this apparent decrease. A significant positive quadratic exposure concentration trend in the F_1 generation may have been due to increased counts in the 100 ppm group. These statistically significant exposure effects are of doubtful biological significance.

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 in male rats. 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_1 and F_2 generation males, and the incidences of this lesion in 100 and 500 ppm F_1 and F_2 males were significantly greater than those in the same-generation controls (Tables 3 and A2a through A2e). These generations were continuously exposed to genistein from conception through termination. In the F_0 generation that was exposed from PND 42 to termination and the F_3 generation that was exposed to genistein only until weaning, the mammary gland effect in males was much weaker, with a significant increase in the incidence of alveolar/ductal hyperplasia in the 500 ppm F_0 group and a significant positive linear exposure concentration trend in the incidences of this lesion in the F_3 generation. Incidences of mammary gland hyperplasia were not exposure related in females, which were necropsied after they had delivered and nursed litters.

Hyperplasia of mammary gland alveoli and/or ducts was defined as a relative increase in the tubuloalveolar patterns of growth and/or branching ducts per unit area of hypodermis; this increased density correlated positively with the severity of hyperplasia. The tubuloalveolar growth was characterized by alveoli attached to or in close approximation to branched, linear arrays of hypertrophied ducts. Vacuolization of alveolar and ductal epithelium was frequently noted. Lumina of glands were usually not patent, while ductal lumina sometimes were patent and contained secretory material. Varying amounts of fibrous connective tissue surrounded the ducts and alveoli.

Kidney: Renal tubule mineralization (nephrocalcinosis) was the primary effect noted in the kidney of genistein-exposed males (Tables 4 and A2a through A2e). Incidences of nephrocalcinosis were not exposure

TABLE 3
Incidences and Severities of Alveolar/ductal Hyperplasia of the Mammary Gland in Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

Generation	0 ppm	5 ppm	100 ppm	500 ppm
F_0	1/23 (1.0)	3/24 (1.3)	2/23 (1.5)	5/24 (1.6)*
F_1***, ###	1/26 (1.0)	1/24 (1.0)	5/25 (1.0)*	15/25 (1.9)***, ###
F_2***, ###	2/24 (2.0)	0/25 (0.0)	8/25 (1.2)*	18/25 (1.6)***, ###
F_3*	4/24 (1.2)	2/25 (1.0)	6/25 (1.0)	8/23 (1.5)
F_4	4/25 (1.5)	4/25 (1.2)	6/25 (1.5)	6/24 (1.7)

[a] The severity of hyperplasia was graded on an ordinal scale as follows: no hyperplasia, 0; minimal, 1; mild, 2; moderate, 3; marked, 4. All mammary glands for males received in pathology were examined microscopically except in cases where this was precluded by autolysis or insufficient glandular tissue in the section. The number of animals with alveolar/ductal hyperplasia 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 (*): *, $P \leq 0.05$; ***, $P \leq 0.001$. 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 superscripted 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: # # #, $P \leq 0.001$.

related in females (Tables B2a through B2e). Incidences of renal tubule mineralization occurred with positive linear exposure concentration trends in the continuously exposed F_1 and F_2 generations of males, and the incidences of this lesion were significantly increased in 100 and 500 ppm F_1 and F_2 males compared to those in the same-generation controls (Tables 4 and A2a through A2e). Renal tubule mineralization is also described as nephrocalcinosis because it consists of intratubular calcified deposits mainly at the corticomedullary junction, but also in the medulla. The incidences of minimal to mild inflammation and tubule regeneration in F_1 males occurred with positive linear exposure concentration trends, and incidences of these lesions in the 500 ppm group were significantly greater than those in the F_1 controls. Chronic inflammation was characterized by interstitial small aggregates of mononuclear inflammatory cells along with a slight fibrosis. Regeneration was evident by a few scattered foci of tubules displaying an increased number of epithelial cells that had a more intense cytoplasmic basophilia. Occasional mitotic figures and pyknotic cells were also noted.

TABLE 4
Incidences and Severities of Nonneoplastic Lesions of the Kidney in Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

Lesion	Generation	0 ppm	5 ppm	100 ppm	500 ppm
Renal Tubule Mineralization					
	F_0	1/24 (1.0)	—	0/1 (0.0)	0/25 (0.0)
	F_1***, ###	1/26 (1.0)	3/25 (2.0)	8/25 (1.0)***, #	15/25 (1.7)***, ###
	F_2**, #	0/25 (0.0)	1/25 (1.0)	4/25 (1.0)*	6/25 (1.0)**, #
	F_3	0/25 (0.0)	0/1 (0.0)	—	0/25 (0.0)
	F_4	1/25 (1.0)	—	—	0/25 (0.0)
Inflammation					
	F_0	20/24 (1.1)		0/1 (0.0)	18/25 (1.1)
	F_1**, #	16/26 (1.0)	16/25 (1.1)	19/25 (1.0)	22/25 (1.1)**, #
	F_2#	20/25 (1.1)	16/25 (1.0)	16/25 (1.0)	23/25 (1.0)
	F_3	21/25 (1.1)	0/1 (0.0)	—	18/25 (1.0)
	F_4	18/25 (1.1)	—	—	15/25 (1.0)
Renal Tubule Regeneration					
	F_0	10/24 (1.0)	—	0/1 (0.0)	7/25 (1.0)
	F_1***, ###	6/26 (1.0)	6/25 (1.0)	8/25 (1.0)	19/25 (1.2)***, ###
	F_2	8/25 (1.0)	3/25 (1.0)	8/25 (1.0)	9/25 (1.0)
	F_3	8/25 (1.1)	0/1 (0.0)	—	13/25 (1.0)
	F_4	6/25 (1.2)	—	—	10/25 (1.0)

[a] Kidneys from animals in the 500 ppm and control groups were examined microscopically in all generations. In addition, kidneys in the 5 and 100 ppm groups of the F_1 and F_2 generations, one kidney in the 100 ppm F_0 group, and one kidney in the 5 ppm F_3 group were examined; as indicated by dashes, none from the other groups in the F_0, F_3, or F_4 generations were examined. All kidney lesions for which statistically significant exposure-related effects were observed are listed. 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 superscripted 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: #, $P \leq 0.05$; # # #, $P \leq 0.001$.

DISCUSSION

The present study was one of a series designed to evaluate the long-term effects of compounds with various estrogenic potencies over multiple generations. Based on the results of a reproductive dose range-finding feed study (NTP, 2007), the highest dietary genistein exposure concentration chosen for the current study was 500 ppm with the expectation that mild reproductive effects would be produced in the F_1 generation. The lowest exposure concentration of 5 ppm produced no discernible effect in the reproductive dose range-finding study. While the lack of effects at this exposure concentration is compatible with many other reports discussed below, significant adverse effects in both male and female rodents have been reported by others at this dietary exposure concentration (Awoniyi *et al.*, 1998; Wisniewski *et al.*, 2003, 2005).

Genistein was selected as a test compound for these studies due to its weak estrogenic activity and its widespread presence in the human diet, in soy infant formulas, and in dietary supplements taken with the goal of improving health and preventing chronic disease. While benefits of soy consumption have been reported, some of which may be associated with the isoflavone content of soy, there have also been reports of adverse effects following exposure of developing animals and enhancement of carcinogenesis in older animals. There have been inconsistencies in these data, with differences in route and timing of exposure, test species and strain, and dose levels likely accounting for much of the variation. The extent of biological variability among control animals, which may be influenced by variations in dietary components, has also been discussed as a potential contributory factor to differences in observations across studies (Ashby *et al.*, 2004; Thigpen *et al.*, 2004). In the current design, the F_0 animals were exposed from 6 weeks of age until termination at 20 weeks of age, the F_1 and F_2 animals were exposed from conception through termination at 20 weeks of age, and the F_3 animals were exposed from conception through weaning and then maintained on control diet to termination at 20 weeks of age; the F_4 generation, terminated at 20 weeks of age, and their F_5 offspring, terminated at weaning, were not exposed to dosed feed. The study thus provides data on multiple windows of exposure, the reversibility of effects, and the carryover of any effects into unexposed generations. The base diet used in this study was free of soy meal and alfalfa to limit variability in background phytoestrogen levels. A chronic study using various exposure windows was also conducted and is reported elsewhere (NTP, 2008a).

Exposure Assessment

As indicated in Table 1, the average daily compound consumption resulting from the 5, 100, and 500 ppm dietary exposures of the test animals in the F_0 through F_2 generations was approximately 0.3, 7, and 35 mg genistein/kg body weight in males and 0.4 to 0.7, 9 to 15, and 44 to 78 mg/kg body weight in females, depending on the stage of the experiment (i.e., nonlactating versus lactating). The compound consumption of animals exposed to 500 ppm genistein was considerably greater than the doses ingested by even the most heavily exposed human consumers of soy. Setchell *et al.* (1997) reported that infants consuming soy formula as their sole source of nutrition ingested 6 to 9 mg/kg per day of total isoflavones, of which approximately half would be genistein. However, it is known that blood concentrations are generally higher in humans than in rodents for an equivalent oral dose (Holder *et al.*, 1999; Chang *et al.*, 2000; Whitten and Patisaul, 2001), and thus measures of internal dose are more appropriate for comparison with human exposures. In conjunction with the experiments reported here, blood and tissue concentrations of genistein were measured in adult animals (Chang *et al.*, 2000), fetal animals (Doerge *et al.*, 2001), and nursing animals (Doerge *et al.*, 2006). In adult rats consuming 500 ppm dietary genistein, total circulating genistein reached concentrations of approximately 6 to 8 µM, with conjugated forms, primarily glucuronides, accounting for 95% to 99% of the total. Tissue/serum partition coefficients ranged from 0.05 to 0.2 for total and from 0.6 to 26 for aglycone genistein, consistent with significant tissue exposures to the aglycone, which is the predominant active estrogenic form of genistein (Morito *et al.*, 2001; Kinjo *et al.*, 2004).

Genistein was detected in fetal plasma and the brain 2 hours after oral dosing of pregnant rats (Doerge *et al.*, 2001). Total genistein concentrations were lower in fetal plasma than in the plasma of the dams, although the percent present as aglycone was higher (approximately 30%) in the fetus, possibly due to the less efficient glucuronidation capacity of the fetus. This higher aglycone exposure and possible differences in blood-brain barrier resulted in brain levels of aglycone genistein that were approximately fivefold higher than in a correspondingly exposed adult. These findings suggested that significant exposures to the active form of genistein could occur during the fetal period.

Prior to the start of the current study, the data of Fritz *et al.* (1998) were the most relevant to lactational exposure of Sprague-Dawley rats to genistein. Specifically, while lactating dams fed 250 ppm genistein in AIN-76A diet had serum total genistein concentrations of 0.42 µM, their pups were reported to have serum concentrations of 0.73 µM on postnatal day 7 (PND 7). Milk from the dams contained 0.14 µM total genistein, but the pup stomach contents reportedly contained 4.4 µM. Stomach contents and pup serum contained genistein aglycone at percentages greater than 75% and 14%, respectively, and were considerably higher than the aglycone percentages found in the milk (57%) or serum (2%) of dams. Thus, it appeared that genistein aglycone was present in rat milk at high concentrations and was efficiently transferred to the serum of pups. Weber *et al.* (2001) also reported high concentrations of total isoflavones in the plasma of infant rats and concluded that there was efficient transfer of genistein from the mother's plasma to milk, although milk concentrations were not reported. On the other hand, a more recent study by Lewis *et al.* (2003) reported less efficient transfer of genistein to the milk in dams administered a single gavage dose of 50 mg/kg. Peak concentrations of total genistein in milk (0.63 µM) were approximately 9% of those in dam plasma (6.7 µM). Given the conflicting data in the literature, the exposure of 10-day-old pups exposed to genistein solely through their dam's milk was evaluated under the conditions of the current study (Doerge *et al.*, 2006). The results indicated that the exposure of rat pups to genistein through lactation from dams consuming dietary genistein produced modest circulating concentrations of both total and aglycone genistein. Serum collected on PND 10 from control pups consistently had serum genistein concentrations below the detection limits of the analytical methods (0.0005 µM). Serum from pups consuming milk from 500 ppm dams contained total genistein concentrations in the range of 0.022 to 0.053 µM, with associated aglycone concentrations that were 1.2% to 4.6% of the total. The ratio of genistein concentrations in the pups to serum genistein concentrations in the dams ranged between 0.01 and 0.28 for total genistein and between 0.01 and 0.15 for aglycone genistein, with respective means of 0.12 ± 0.12 and 0.07 ± 0.08. Data on serum and urine genistein concentrations from human infants consuming soy infant formula suggest that absorption and excretion of genistein is efficient (Setchell *et al.,* 1997; Irvine *et al.,* 1998). The low internal exposure of nursing pups in the current study apparently results from the minimal amount of genistein secreted into the milk (approximately 0.12 ppm) relative to either the concentration in dam feed (500 ppm) or blood. The higher concentration of aglycone genistein observed in milk relative to dam blood suggests that preferential secretion occurs, but this does not appear to facilitate overall absorption by the pups. Similar to the observations in the current study on genistein concentrations in the dams' milk, concentrations of isoflavones in the breast milk of humans consuming soy are low relative to concentrations found in soy infant formulas (Setchell *et al.,* 1998). Thus, dietary exposure of nursing dams as used in the current study does not mimic developmental exposure to soy infant formula.

Thus, under the conditions of the current study, fetal, weanling, and adult animals were exposed to internal (serum and tissue) concentrations of genistein that are achievable in humans ingesting soy products. The fact that genistein exposure was extremely limited in neonatal pups, and lower than in the fetal animals, is important to the interpretation of the data. On the one hand, effects attributable to early (preweaning) exposure are more likely to result from fetal exposure, because of the higher *in utero* concentrations of the active estrogenic aglycone form of genistein, or from direct exposures after the pups begin directly consuming dosed feed. On the other hand, the low exposure to genistein during the sensitive early postnatal period resulting from dietary exposure of the dams needs to be considered when making safety evaluations based on the results of these studies. The early neonatal period in rodents, which roughly corresponds to the second and third trimester of human gestation (Pryor *et al.*, 2000), is known to be a period of development that is highly sensitive to disruption by exposure to estrogenic agents, including genistein (Faber and Hughes, 1993; Yang *et al.*, 2000; Nagao *et al.*, 2001; Newbold *et al.*, 2001; Jefferson *et al.*, 2002, 2005; Foster *et al.*,

2004). Thus, negative results, particularly with respect to endpoints reported in other studies to be affected by neonatal dosing with genistein, must be interpreted with caution. It should be noted that concentrations of serum genistein in weanling animals at approximately PND 21 were 20% to 55% of the concentrations found in adults (Chang *et al.,* 2000), indicating that exposure increased considerably after pups began directly ingesting dosed feed.

Effects of Genistein Exposure

Few consistent biological effects of genistein were observed across exposed generations in this multigenerational reproductive toxicology study. Many of the significant effects observed within a generation were not greater than the variation seen in control groups across generations. Decreased body weight was observed in exposed females and was most prominent at 500 ppm in the generations that were continuously consuming genistein (F_0 through F_2), although body weight was also depressed in females of the unexposed F_4 generation. In all cases where body weight was decreased in females, except for the 100 ppm group in the F_1 generation, feed consumption was also decreased. Male body weight in the postweaning period was significantly decreased in the 100 and 500 ppm F_1 groups. While pup birth weights were, in general, not lower in exposed groups, body weight gains during the preweaning period were decreased in 500 ppm males and females in all generations, as well as in 5 and 100 ppm males in the F_1 generation. As noted previously, the dose of genistein administered to the pups through the milk was quite low, and the depression of body weight gain in the preweaning pups may reflect other changes in the milk secondary to the decreased body weight gain in the dams. Pup birth weight was significantly decreased in 100 ppm F_1 males and in all exposed groups of males and females in the F_5 generation. Pup body weights in these exposed groups were within the range seen in previous generations, and the significant decreases are likely due to slightly increased control pup body weights rather than to any latent carryover effect of genistein exposure. Estrogens are known to have an anorectic effect and to modulate energy utilization (Wade and Schneider, 1992). Studies with dietary genistein at concentrations of 500 to 1,500 ppm in intact or ovariectomized rodents have shown decreased feed consumption, body weight, and/or adipose deposition (Casanova *et al.*, 1999; Naaz *et al.*, 2003; Kim *et al.*, 2006). The study of Naaz *et al.* (2003) in ovariectomized mice demonstrated that the decrease in adipose deposition was dependent on estrogen receptor α.

While there were no statistically significant effects of genistein on measures of mating success, there was a clear reduction of litter size in the 500 ppm F_2 group. While the differences were not statistically significant, litter sizes in the 500 ppm F_1 and F_3 groups, whose parents were also continuously dosed through adulthood, were also lower than those in the controls. No effects of genistein on follicle counts or ovarian histology were noted, although perturbations of the estrous cycle, including prolonged cycles, were noted in continuously exposed 500 ppm F_1 and F_2 animals soon after the time of vaginal opening, but not in animals near the time of termination at 20 weeks, after these animals had delivered and nursed litters. Prolonged cycles and increased time in estrus or diestrus have been observed in animals treated prepubertally with genistein (Lamartiniere *et al.*, 1995; Murrill *et al.*, 1996; You *et al.*, 2002a; Kouki *et al.*, 2003; Nikaido *et al.*, 2004, 2005; Takagi *et al.*, 2004). Genistein-induced ovarian degeneration and abnormal cycling were observed in 1,250 ppm rats in the earlier reproductive dose range-finding feed study when genistein was administered from gestation day 7 (GD 7) through PND 50 (NTP, 2007), and these effects have also been described following neonatal treatment of rats with high doses of genistein (three subcutaneous doses of 5 mg per animal on PNDs 2, 4, and 6) (Lamartiniere *et al.*, 1995). Similar doses administered to older prepubertal animals and dietary administration of 25 or 250 ppm from conception through weaning did not result in ovarian toxicity, although mammary gland differentiation was affected by both treatments (Fritz *et al.*, 1998). On the other hand, Awoniyi *et al.* (1998) reported that dietary exposure to 5 ppm genistein from GD 17 through weaning or continuing until PND 70 had lasting effects on the ovary of Sprague-Dawley rats, including degeneration of follicles and a persistent interstitial compartment; these effects were persistent in animals that had been removed from dosed feed at weaning, suggesting that *in utero* and/or lactational exposure was responsible for the effects. In a gavage study, Nagao *et al.* (2001) dosed Sprague-Dawley rat pups on PNDs 1 to 5 with 12.5 to 100 mg genistein/kg per day and necropsied the animals at 21 days or 18 weeks of age. In the older animals, all dosed groups showed an increase of abnormal cycles and decreased fertility indexes (number of animals pregnant/number of animals copulated). Some animals in the 50 or 100 mg/kg per day groups showed atrophic ovaries with atretic follicles and no corpora lutea, but lesions were not observed in the lower dose groups. Lamartiniere *et al.* (1998b) detected ovarian toxicity only at high doses of genistein (5 mg/rat) administered subcutaneously to neonatal

rats, and Kang *et al.* (2002) reported no ovarian lesions with maternal gavage exposures of 0.4 or 4 mg/kg body weight per day. Jefferson *et al.* (2002) treated neonatal mice (PNDs 1 to 5) with subcutaneous injections of 1, 10, or 100 µg genistein per day and found induced expression of estrogen receptor α and an increased number of ovulated oocytes at the lowest dose and a decrease in the number of ovulated oocytes at the higher doses. A dose-related increase in multioocyte follicles was also observed, but, unlike the induction of estrogen receptor α, this effect was not observed in estrogen receptor β knockout mice. In a subsequent study, Jefferson *et al.* (2005) reported that female CD-1 mice treated neonatally with subcutaneous doses of 5 mg genistein/kg body weight produced progressively smaller litters over time, while females dosed with 50 mg/kg did not produce litters. This dose is close to the ingested dose in the current multigenerational rat study, and serum concentrations resulting from this dosing regimen in mice had previously been shown to approximate those produced by oral dosing (Doerge *et al.*, 2002). The observations by Jefferson *et al.* (2005) of reduced fertility in mice were accompanied by altered estrous cycles, altered ovarian function, and early reproductive senescence, with some effects seen at doses of 0.5 mg/kg. There is thus some discrepancy in the literature about the extent of the ovarian toxicity of genistein and the exposure windows and dose concentrations that produce this toxicity. In studies that have reported ovarian toxicity, the neonatal period appears to be particularly sensitive. It is conceivable that the limited neonatal exposure achieved in the current multigenerational rat feed study contributed to the limited effects observed on ovarian and fertility endpoints. It is difficult to reconcile the ovarian effects reported by Awoniyi *et al.* (1998) in Sprague-Dawley rats fed 5 ppm genistein with the results of the current study.

Effects of genistein on anogenital distance (AGD) were observed in the current study, although the significance of the effects was dependent for the most part on the statistical model used and the AGDs in exposed groups were not outside the range observed in control groups across the five generations. Male pups in the 500 ppm group of the F_1 generation showed a decreased mean AGD relative to controls in that generation, while females showed decreased AGDs relative to controls in the F_1 (500 ppm), F_2 (500 ppm), and F_3 (100 ppm) generations. There was no evidence of a carryover effect of genistein on AGD in pups of the unexposed F_4 and F_5 generations. AGD in neonates is dependent upon prenatal exposure to androgens, which stimulate the growth of the perineum (Clark, 1999; McIntyre *et al.*, 2001; Zehr *et al.*, 2001; Bowman *et al.*, 2003). A depression of testosterone production, its conversion to dihydrotestosterone, or inhibition of androgen receptor binding could lead to shorter AGDs. As discussed below, studies that have evaluated the effects of genistein exposure on testosterone and dihydrotestosterone concentrations have produced mixed results, and data on prenatal and neonatal hormone levels resulting from gestational exposure of rodents to genistein are not available from the current study or from the literature. Mixed and relatively modest effects of genistein or soy on AGD have also been reported in the literature. The administration of genistein in feed (1,000 ppm) from GD 1 to PND 56 (Casanova *et al.*, 1999) or a soy-containing diet from GDs 0 to 20 (Weber *et al.*, 2001) increased (i.e., masculinized) the AGD in female pups. Casanova *et al.* (1999) also found a significantly increased AGD in female pups of dams fed a soy-containing diet versus a soy-free diet. On the other hand, Levy *et al.* (1995) reported a decreased AGD in female pups born to dams that had been treated with 5 mg genistein by subcutaneous injection on GDs 16 to 20. These investigators found that 5 mg of genistein, but not 25 mg, administered to dams from GDs 16 to 20 by subcutaneous injection decreased the AGD in newborn males. On the other hand, it was reported that male pups of dams receiving a diet containing approximately 600 ppm soy phytoestrogens throughout gestation had increased ratios of AGD to body weight just before birth at GD 20.5, although no significant difference was observed between exposed and control pups on PND 3 (Weber *et al.*, 2001). A diet containing 200 ppm soy phytoestrogens had no effect. The overall results suggest that phytoestrogens may be able to masculinize or hyperfeminize female pups depending on the treatment conditions, but the long-term biological meaning of these small and sometimes transient effects on AGD is not clear.

In the current study, exposure to 500 ppm genistein affected the time of and body weight at vaginal opening in female pups of the F_1 through F_3 generations such that the time of vaginal opening was accelerated and/or shifted to lower body weights. This effect did not carry over into the unexposed F_4 generation, and both the effect and the exposure concentration at which it was observed are generally consistent with other literature reports. Levy *et al.* (1995) administered 5 or 25 mg genistein (about 12 or 60 mg/kg per day) by subcutaneous injection to pregnant dams on GDs 16 to 20 and found a delay in vaginal opening only at the lower dose. Other

studies in rats or mice with injected, gavaged, or dietary exposures to genistein or with dietary soy protein isolate have generally reported an acceleration of vaginal opening or no effect (Lamartiniere *et al.*, 1998a; Casanova *et al.*, 1999; Badger *et al.*, 2001; You *et al.*, 2002a; Lewis *et al.*, 2003; Nikaido *et al.*, 2004, 2005). In two experiments using developmental exposure windows somewhat different from those used in the current study, acceleration of vaginal opening was reported within the exposure concentration range utilized here. You *et al.* (2002a) reported accelerated vaginal opening in females continuously fed 300 or 800 ppm genistein from conception through the end of the study. The 300 ppm diet yielded a genistein dose of between 20 and 40 mg/kg body weight, depending on the stage of the experiment. Lewis *et al.* (2003) reported accelerated vaginal opening in pups injected subcutaneously with 2 mg/kg daily from birth to PND 7 and then gavaged with 40 mg/kg through PND 21. In the same experiment, an identical exposure regimen using 10-fold lower doses had no effect on the timing of vaginal opening. Takagi *et al.* (2004), on the other hand, did not observe an acceleration of vaginal opening after feeding 1,250 ppm genistein to rats in a soy- and alfalfa-free diet from GD 15 through PND 11, and Nagao *et al.* (2001) saw no acceleration of vaginal opening after gavage dosing of rat pups with up to 100 mg/kg on PNDs 1 to 5. In the reproductive dose range-finding feed study of genistein, which utilized only five litters per exposure group, the mean time of vaginal opening in the 1,250 ppm group was earlier than in the controls, but the difference was not statistically significant (NTP, 2007).

With the exception of a significant delay in the time of testicular descent in the F_3 generation exposed to genistein only until weaning, there were no exposure-related effects on male pubertal markers in the current study. Other investigators have reported mixed data on the effects of genistein on the onset of puberty in male rodents. Wisniewski *et al.* (2003, 2005) reported a delayed time of preputial separation along with reduced testosterone concentrations and impaired reproductive behavior in Long-Evans rats exposed to 5 or 300 ppm dietary genistein during gestation and lactation, although a similar exposure in C57BL/6 mice did not lead to altered preputial separation time. The studies of Casanova *et al.* (1999), using dietary genistein at exposure concentrations up to 1,000 ppm from GD 1 to PND 56, and Lewis *et al.* (2003), using the subcutaneous injection and gavage regimen described in the previous paragraph, found no effect of genistein on preputial separation time. Masutomi *et al.* (2003) did not find an effect of 1,000 ppm genistein in feed on the time of preputial separation but did report this landmark event to occur at a lower body weight than in controls when the rats were exposed to genistein from GD 15 to PND 10. Hughes *et al.* (2001) found that genistein accelerated the time of preputial separation in male pups when rat dams were gavaged with 15 mg genistein/kg per day from GD 14 through PND 21.

In the current study, significant effects of genistein exposure on organ weights in both sexes were few and generally confined to single generations so that consistent patterns across the exposed generations were not clearly discernible. For example, pituitary gland weights were increased in 500 ppm F_2 males, and there was a significant positive exposure concentration trend in the F_2 generation. No associated exposure-related microscopic pituitary gland lesions were noted. In the reproductive dose range-finding feed study (NTP, 2007), male rats exposed to 1,250 ppm genistein from GD 7 through PND 50 showed increased pituitary gland weights. While the increased weight of the pituitary gland observed at the lower exposure concentration of 500 ppm in the F_2 generation of the current study could be interpreted as a possible cumulative effect over the genistein-exposed generations, there is a lack of supporting evidence from other endpoints for this conclusion.

In the current study, the sole significant genistein-induced change in reproductive tract weights in either sex occurred in the F_0 generation that was exposed to genistein only during late puberty and adulthood, and it consisted of increased weight of the testis in the 500 ppm group, accompanied by a positive linear exposure concentration trend in the F_0 generation. Reports of the effects of genistein and preparations containing mixtures of soy isoflavones on the testis and male reproductive tract in the literature are mixed, and complex factors including route of exposure, timing of exposure, test species and strain, and dose no doubt contribute to the diversity of observations. In the reproductive dose range-finding feed study of genistein, there was some evidence of decreased or delayed spermatogenesis at the highest exposure concentration (1,250 ppm) in pubertal animals, as well as decreased ventral prostate gland weight (NTP, 2007). In associated studies conducted with excess pups from the F_1 and F_2 generations of the current study, increases in serum testosterone (500 ppm) and dihydrotestosterone (100 and 500 ppm) concentrations were observed, although they were seen only in the F_1 generation, and they were not accompanied by changes in the weights of reproductive tract organs

(Dalu *et al.*, 2002). Similarly, no consistent pattern of exposure concentration-related reproductive tract organ weight changes, histopathology, or alterations of sperm parameters were observed in males in the current study. Other studies have also reported increased serum testosterone concentrations and/or male reproductive organ weights following exposure to genistein or soy isoflavones. Fritz *et al.* (2002a) found increased serum testosterone and dihydrotestosterone concentrations in Sprague-Dawley rats fed 25 or 250 ppm genistein from conception through PND 70. Fisher *et al.* (1999) reported increased testis weights at PND 75 in Wistar rats given subcutaneous injections of 4 mg genistein/kg body weight between PNDs 2 and 12; a transient reduction in efferent duct epithelial cell height was observed in early life and was abolished by PND 25 in this study. McVey *et al.* (2004a,b) reported alterations of testicular steroidogenic enzymes and increases in serum testosterone, serum dihydrotestosterone, and testicular testosterone concentrations on PND 120 in animals exposed to diets containing 236 or 1,047 ppm soy isoflavones (approximately 50% genistein) continuously from conception. Robertson *et al.* (2002) found that soy-containing feed could partially reverse the deficient spermatogenesis observed in aromatase knockout mice. Kang *et al.* (2002) found no effects on sperm number or the distribution of cell types or their numbers in adult rats whose mothers were exposed by gavage to 0.4 or 4 mg/kg body weight per day from GD 6 to parturition and then from PNDs 2 to 20. In the current study, the weight of the prostate gland was significantly increased in the 4 mg/kg group at PND 70 but not at PND 100, indicating a transient effect. A similar transient effect on prostate gland weight in mice has also been reported (Kyselova *et al.*, 2004). However, null effects, transient effects, or negative effects on testosterone concentrations and male reproductive organs or function have also been reported after treatment with genistein. Casanova *et al.* (1999) found no significant effects of genistein on weights of the testis or ventral prostate gland in Sprague-Dawley rat pups exposed to 200 or 1,000 ppm dietary genistein from gestation through termination at puberty. Lewis *et al.* (2003) reported no effects of genistein on the weights of male reproductive organs in Sprague-Dawley rats treated from birth to PND 21 with 4 or 40 mg/kg per day (subcutaneous injections on PNDs 1 to 7, gavage treatment thereafter). Nagao *et al.* (2001) found no effect of genistein on sperm counts, serum testosterone concentrations, or ventral prostate gland weights in adult animals after neonatal (PNDs 1 through 5) gavage doses ranging from 12.5 to 100 mg/kg per day. In a multigenerational study, Kyselova *et al.* (2004) found that 2.5 and 25 mg genistein/kg body weight administered in drinking water reduced the weights of the testis, prostate gland, and seminal vesicle in 30 day old male CD-1 pups, but the effect was not seen in adults at 90 days of age. Decreased acrosomal staining was reported in sperm from these mice, but there was no dose-response for this effect, and there was no impact on fertility. A recent report indicated that rats treated with a mixture of soy-derived isoflavones (45% genistein; 200 and 2,000 ppm in the diet) during adulthood (treatment for a year starting at 10 weeks of age) resulted in no effects on testicular or epididymal weights or on sperm count, motility, or morphology (Faqi *et al.*, 2004). Likewise, feeding genistein at 250 or 1,000 ppm from PNDs 21 to 35 had no effect on testis weight or histology, in contrast to the decreased weight and altered morphology induced by 75 ppb diethylstilbestrol (DES) (Fritz *et al.*, 2003). Adachi *et al.* (2004) treated ICR mice subcutaneously with 1 mg genistein or 50 µg DES per day on PNDs 1 to 5 and showed that both compounds had similar effects on gene expression in the testis, including downregulation of androgen receptors and estrogen receptor α. However, while DES showed histologic effects and induced an increase in apoptotic cells in the testis, genistein was without effect. Fielden *et al.* (2003) found no effects of lower gavage doses of genistein, ranging from 0.1 to 10 mg/kg, administered during gestation and lactation, on gene expression or spermatogenesis in mice. Naciff *et al.* (2005) examined changes in gene expression on GD 20 in the testis and epididymis of Sprague-Dawley rats exposed *in utero* from GD 11 by subcutaneous injection of the dams with up to 100 mg/kg per day. In genistein-exposed fetuses, dose-related gene expression changes that overlapped those seen with ethinyl estradiol treatment were observed, although no gross or microscopic testicular lesions were observed.

Lund *et al.* (2001) reported that Long-Evans rats exposed to a soy diet containing 600 ppm total phyto-estrogens (approximately 50% genistein) through gestation and throughout life had decreased prostate gland weights relative to controls fed a phytoestrogen-free diet, although testosterone concentrations were not altered. This same group found that Sprague-Dawley rats fed the same phytoestrogen-containing diet for 5 weeks from PND 70 had reduced body and prostate gland weights, as well as decreased testosterone concentrations (Weber *et al.*, 2001). Strauss *et al.* (1998) found that while ventral prostate gland weight was reduced in NMRI mice sub-

cutaneously injected with 50 or 500 mg/kg per day on PNDs 1 to 3, hyperplasia and abnormal prostate gland histology, similar to that produced by neonatal treatment with potent estrogens, was observed only in the high dose animals as adults. Fritz *et al.* (2002b) administered dietary genistein at 250 or 1,000 ppm for 2 weeks after weaning to male Sprague-Dawley rats and found a reduction in the growth of lateral prostate type 1 buds in the 1,000 ppm group but no evidence of toxicity.

Atanassova *et al.* (2000) found that both a soy-containing diet and subcutaneous injection of 4 mg genistein/kg per day from PNDs 2 to 18 retarded spermatogenesis in neonatal Wistar rats. In male dogs treated orally with genistein capsules for 13 or 52 weeks starting near sexual maturity, McClain *et al.* (2005) found evidence of male reproductive toxicity at 500 mg/kg per day, but not at lower doses, with reduced organ weights, atrophy of the testis and prostate gland, and reduced epididymal sperm counts. These effects were judged to be reversible and typical of the effects of a weak estrogen. Finally, in a primate study by Sharpe *et al.* (2002), male marmosets fed soy formula had a depressed neonatal testosterone surge and Leydig cell number increase in the testis. In a follow-up study of these animals, no effects of soy formula feeding on the timing or progression of puberty, fertility, or development or length of the penis were observed (Tan *et al.,* 2006). Testis weight and Sertoli and Leydig cell numbers were increased in the marmosets exposed to soy formula as infants.

Thus, the data available to this point show mixed evidence for a biological effect of soy and genistein on the reproductive tract of male rodents and primates. With the exception of the study of Wisniewski *et al.* (2003) mentioned previously, which reported adverse reproductive effects in male Long-Evans rats fed 5 or 300 ppm genistein through gestation and lactation, clear adverse reproductive outcomes or other long-term testicular toxicities have not been demonstrated. Roberts *et al.* (2000) found no effect of 5 ppm genistein fed to Sprague-Dawley rats from GD 17 through PNDs 21, 70, or 130, with all animals necropsied at PNDs 70 or 130. The current study did not indicate male reproductive toxicity at dietary exposures up to 500 ppm under treatment conditions similar to those used by Wisniewski *et al.* (2003). Whether rat strain sensitivity differences can account for these discrepant results remains to be determined.

Histopathology

No exposure-related lesions were found in microscopic evaluations of tissues from female rats, and exposure-related lesions in male rats were confined to the mammary gland and kidney. Consistent with our observations in the reproductive dose range-finding feed study (NTP, 2007), induction of hyperplasia in the mammary gland of males was among the most sensitive of the endpoints affected by genistein in the current study. The pattern of induction of hyperplasia across generations, with the strongest effects seen in the 100 and 500 ppm groups of the continuously exposed F_1 and F_2 generations, indicates that both developmental and postweaning exposures contribute to this effect. Late pubertal and adult exposure, as in the F_0 generation, or purely developmental exposure, as in the F_3 generation, produced lesser effects. The incidence of mammary gland alveolar/ductal hyperplasia was slightly increased in 500 ppm males in the F_4 generation, but this was not a statistically significant increase. In contrast to the reproductive dose range-finding study (NTP, 2007), where hyperplasia of the mammary gland was observed in both sexes, only the mammary gland of males was affected in the current study. In the previous study, young virgin rats were examined, while in the current study, parous rats were examined shortly after lactation. You *et al.* (2002b) have also reported that the mammary gland of male rat pups is more sensitive to genistein than that of female rats. In this feed study, increased ductal branching was seen on PND 22 in the mammary gland of male rats exposed to 800 ppm genistein *in utero* and throughout the nursing period; this effect was not seen in males exposed to 300 ppm genistein. The different exposure periods and evaluation times used likely contribute to the differences in effective doses (100 ppm in the current study versus 800 ppm in the study by You *et al.*, 2002b). The literature on normal development and xenobiotic or estrogenic effects on male mammary tissue is sparse. In 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 gland in adult males indicated feminization. Cardy (1991) reported that treatment of male rats with a dopamine antagonist resulted in mammary gland 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 investigator also suggested that the mammary gland of males may be a valuable marker tissue for endocrine-

active compounds. Genistein has been reported to increase prolactin concentrations in ovariectomized female rats fed 750 ppm genistein (Santell *et al.*, 1997) and stimulate prolactin production in pituitary gland cells in culture (Stahl *et al.*, 1998). The hormonal status of the animals in the current study was not evaluated, and pituitary gland weights of F_2 males were affected by genistein with the occurrence of a positive linear exposure concentration trend and a significant increase in the 500 ppm group in this generation. The current results, together with the results of You *et al.* (2002b), underline the potential utility of the male mammary gland in screening for endocrine-active compounds.

Also consistent with the results of the reproductive dose range-finding study (NTP, 2007), increased incidences of mineralization of renal tubules, or nephrocalcinosis, occurred in the current study in males exposed to 100 or 500 ppm genistein, with the increase confined to the continuously exposed F_1 and F_2 generations. Nephrocalcinosis is reported to be a sex-related lesion common in untreated female rats and influenced by diet composition (Ritskes-Hoitinga and Beynen, 1992). These investigators also reported that nephrocalcinosis can be induced by estrogen treatment in males, and thus the occurrence of this lesion could be related to the estrogenic activity of genistein. On the other hand, a treatment-related increase in the incidence of nephrocalcinosis in males has not been noted after dietary administration of 17β-estradiol or 17α-ethinyl estradiol to rats (Schardein, 1980; Biegel *et al.*, 1998). While the nephrocalcinosis induced by genistein in males was of minimal to mild severity and would not be expected to impact longevity or fertility, it is of interest that this endpoint, together with the stimulation of the male mammary gland, seems to be consistently affected by genistein exposures of 100 ppm or greater.

Relevant Results from Associated Studies

Extra animals from the current study, or animals treated in an identical manner, were used for various measurements that have been reported elsewhere; publications resulting from these associated studies are listed in Appendix P. Some of the results of these studies have been discussed previously, and the remainder will be briefly reviewed here.

Chang and Doerge (2000) examined thyroid peroxidase activity in male and female rats treated identically to those in the F_1 generation of the current study, that is, continuous dietary exposure to 0, 5, 100, or 500 ppm genistein from conception through PND 140. These investigators found that thyroid peroxidase activity was decreased in an exposure concentration-dependent manner with statistically significant reductions in all exposed groups relative to that in the controls; however, there were no corresponding effects on thyroid hormone levels, thyroid gland weights, or thyroid gland histology. Iodine deficiency has been shown to play an important role in soy-related thyroid gland toxicity, and components of soy in addition to the isoflavones have been implicated as contributing to this toxicity (Ikeda *et al.*, 2000; Son *et al.*, 2001; reviewed in Doerge and Sheehan, 2002).

Limited behavioral and neurochemical evaluations of rats treated under the conditions of the current study were also conducted. No significant effects of genistein on nursing behavior in dams of the F_0 through F_3 generations were found (Flynn *et al.*, 2000). Genistein significantly potentiated the amphetamine-stimulated release of dopamine in the striatum of 500 ppm F_1 and F_2 male rats; in this study, these were the only generations evaluated (Ferguson *et al.*, 2002). While dopamine release was increased in ovariectomized 500 ppm F_1 and F_2 female rats, the increases were not statistically significant. This effect is independent of intracellular estrogen receptors and is dependent rather on membrane actions, and this study thus indicates that dietary genistein at 500 ppm can affect this membrane pathway. Finally, Scallet *et al.* (2004) measured the volume of the calbindin D28k-labeled sexually dimorphic nucleus in the hypothalamus of F_1 male and female rat brains at PND 140. While the volume of this nucleus was not affected in females, all exposed groups of males showed statistically significant increases in the volume of this brain region. While these data suggest hypermasculinization of male rats even at the low 5 ppm exposure concentration, there was no functional evidence for this effect in the data resulting from the multigenerational reproductive toxicology study.

Summary

Under the conditions of this study, dietary exposure to 500 ppm genistein (approximately 35 mg genistein/kg body weight per day in males and 51 mg/kg per day in females) decreased body weights, accelerated vaginal opening, decreased anogenital distance, and altered estrous cyclicity in females continuously ingesting genistein. Significant decreases in postweaning body weight and decreases in anogenital distance in males were confined to the F_1 generation and were not seen in the similarly exposed F_2 generation. In animals exposed to 500 ppm, there was some evidence for reduced litter size in the F_1 and F_2 generations that were continuously exposed to the test chemical. No other impacts on fertility and no histopathologic lesions were observed in females. The male reproductive tract did not show significant alterations, but increased incidences of hyperplasia of the mammary gland and calcification of renal tubules were observed in continuously exposed 100 and 500 ppm males examined at 20 weeks of age. Weaker effects on the incidences of male mammary gland hyperplasia were observed in 500 ppm males exposed only as adults or exposed only *in utero* and through lactation. Other than decreased body weight gains in preweaning pups, there was no evidence for a carryover of genistein effects into unexposed generations.

A summary of the Technical Reports Review Subcommittee comments and the public discussion on this Technical Report appears on page 14.

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

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary[a]				
Animals initially in study	35	35	35	35
Early deaths				
Dead	1	0	0	0
Retired	4	5	4	7
Surplus	5	5	5	3
Survey/sentinel	1	0	1	0
Survivors				
Terminal sacrifice	24	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(24)	(6)		(25)
Lymphoma malignant				1 (4%)
Hematopoietic System				
Spleen	(24)			(25)
Lymphoma malignant				1 (4%)

Systems Examined with No Neoplasms Observed
Cardiovascular System
Endocrine System
General Body System
Genital 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. All other pups were allocated to addenda studies or euthanized (Discard, Issue other, Not allocated, Reallocated, Retired, Surplus). In some cases, young pups that died were likely cannibalized by the dam and were thus indicated as Missing. Survey/sentinel animals were microbiological sentinels. Animals designated Dead no CID (carcass identification number) were animals that were not selected for continuation on study but died prior to weaning. Only animals processed by pathology received CIDs.

TABLE A1b
Summary of the Incidence of Neoplasms in F_1 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	124	119	116	125
Early deaths				
Dead no CID	0	0	0	1
Discard	10	1	2	9
Issue other	18	29	30	29
Not allocated	0	0	1	0
Reallocate	20	20	20	20
Retired	7	8	9	9
Surplus	41	34	28	31
Survey/sentinel	2	2	1	1
Survivors				
Terminal sacrifice	26	25	25	25
Animals examined microscopically	26	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

The footnote for this table is defined in Table A1a.

TABLE A1c
Summary of the Incidence of Neoplasms in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	113	109	109	96
Early deaths				
Dead no CID	8	10	13	7
Issue other	36	33	32	28
Missing	6	4	3	1
Moribund	0	1	0	0
Reallocate	20	20	20	20
Retired	12	15	15	15
Surplus	1	0	1	0
Survey/sentinel	5	1	0	0
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

The footnote for this table is defined in Table A1a.

TABLE A1d
Summary of the Incidence of Neoplasms in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	107	123	136	143
Early deaths				
Dead	0	1	0	0
Dead no CID	3	3	2	4
Discard	0	2	0	0
Missing	3	2	2	2
Not allocated	0	0	1	0
Reallocate	66	70	83	73
Retired	7	10	10	10
Surplus	0	6	13	29
Survey/sentinel	3	3	0	0
Wrong sex	0	1	0	0
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

The footnote for this table is defined in Table A1a.

TABLE A1e
Summary of the Incidence of Neoplasms in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	121	105	124	109
Early deaths				
Dead no CID	6	1	0	2
Discard	2	1	0	0
Missing	3	1	1	4
Reallocate	20	20	20	24
Retired	4	5	5	6
Surplus	55	52	73	48
Survey/sentinel	6	0	0	0
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

The footnote for this table is defined in Table A1a.

TABLE A2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	35	35	35	35
Early deaths				
Dead	1	0	0	0
Retired	4	5	4	7
Surplus	5	5	5	3
Survey/sentinel	1	0	1	0
Survivors				
Terminal sacrifice	24	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(24)	(6)		(25)
Fibrosis, mild	1 (4%)			
Fibrosis, mild, perivascular		1 (17%)		
Fibrosis, moderate		1 (17%)		
Hemorrhage, minimal				1 (4%)
Hepatodiaphragmatic nodule	3 (13%)	1 (17%)		2 (8%)
Inflammation, chronic, mild	1 (4%)			
Inflammation, chronic, minimal	17 (71%)			19 (76%)
Malformation		3 (50%)		2 (8%)
Necrosis, mild				1 (4%)
Necrosis, minimal	2 (8%)			3 (12%)
Vacuolization cytoplasmic, mild	1 (4%)			
Vacuolization cytoplasmic, minimal	6 (25%)			8 (32%)
Mesentery				1
Inflammation, chronic, marked, fat				1 (100%)
Endocrine System				
Adrenal cortex	(23)			(25)
Accessory adrenal cortical nodule	1 (4%)			1 (4%)
Pituitary gland	(24)			(25)
Cyst, multiple, pars distalis				2 (8%)
Thyroid gland	(24)			(25)
Inflammation, chronic, minimal	1 (4%)			2 (8%)
Genital System				
Epididymis	(24)	(25)	(25)	(25)
Hypospermia, marked				1 (4%)
Hypospermia, mild	1 (4%)			
Hypospermia, moderate	1 (4%)			
Infiltration cellular, lymphocyte, minimal	1 (4%)	2 (8%)	2 (8%)	
Preputial gland				(1)
Dilitation, marked, duct				1 (100%)
Inflammation, suppurative, marked				1 (100%)

[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 Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Genital System (continued)				
Prostate, dorsal	(23)	(25)	(25)	(25)
Inflammation, chronic, mild		1 (4%)	2 (8%)	3 (12%)
Inflammation, chronic, minimal	6 (26%)	3 (12%)	4 (16%)	7 (28%)
Inflammation, suppurative, mild	2 (9%)	7 (28%)	2 (8%)	4 (16%)
Inflammation, suppurative, minimal	6 (26%)	2 (8%)	4 (16%)	11 (44%)
Inflammation, suppurative, moderate			1 (4%)	1 (4%)
Prostate, ventral	(23)	(25)	(25)	(25)
Inflammation, chronic, mild	10 (43%)	5 (20%)	6 (24%)	4 (16%)
Inflammation, chronic, minimal	7 (30%)	11 (44%)	13 (52%)	13 (52%)
Inflammation, suppurative, mild	1 (4%)	1 (4%)	1 (4%)	2 (8%)
Inflammation, suppurative, minimal	1 (4%)	1 (4%)	1 (4%)	
Seminal vesicle	(24)	(23)	(25)	(24)
Degeneration, mild, epithelium	1 (4%)			
Dilatation, mild	3 (13%)	1 (4%)		1 (4%)
Malformation		1 (4%)		
Testes	(25)	(25)	(25)	(25)
Autolysis	1 (4%)			
Degeneration, marked, seminiferous tubule	2 (8%)			1 (4%)
Degeneration, minimal, seminiferous tubule	1 (4%)	1 (4%)	2 (8%)	1 (4%)
Degereration, moderate, seminiferous tubule		1 (4%)		
Edema, mild				1 (4%)
Testes, rete testes	(24)	(25)	(24)	(25)
Dilatation, mild	1 (4%)			
Dilatation, minimal	1 (4%)			
Hematopoietic System				
Spleen	(24)			(25)
Fibrosis, mild, capsule				1 (4%)
Inflammation, chronic, minimal, adventitia				2 (8%)
Inflammation, chronic, minimal, capsule				1 (4%)
Pigmentation, mild				3 (12%)
Pigmentation, minimal	5 (21%)			6 (24%)
Integumentary System				
Mammary gland	(23)	(24)	(23)	(24)
Hyperplasia, mild, alveolus		1 (4%)	1 (4%)	3 (13%)
Hyperplasia, minimal, alveolus	1 (4%)	2 (8%)	1 (4%)	2 (8%)
Hyperplasia, minimal, duct				1 (4%)
Urinary System				
Kidney	(24)		(1)	(25)
Casts protein, minimal	1 (4%)			1 (4%)
Cyst, cortex			1 (100%)	
Dilatation, mild, renal tubule	6 (25%)			
Dilatation, minimal, renal tubule	2 (8%)			8 (32%)
Fibrosis, mild, interstitium	2 (8%)			
Hyaline droplet, minimal	2 (8%)			
Inflammation, chronic, mild	2 (8%)			1 (4%)
Inflammation, chronic, minimal	18 (75%)			17 (68%)
Mineralization, minimal, renal tubule	1 (4%)			
Regeneration, minimal, renal tubule	10 (42%)			7 (28%)

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

	0 ppm	5 ppm	100 ppm	500 ppm
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	124	119	116	125
Early deaths				
Dead no CID	0	0	0	1
Discard	10	1	2	9
Issue other	18	29	30	29
Not allocated	0	0	1	0
Reallocate	20	20	20	20
Retired	7	8	9	9
Surplus	41	34	28	31
Survey/sentinel	2	2	1	1
Survivors				
Terminal sacrifice	26	25	25	25
Animals examined microscopically	26	25	25	25
Alimentary System				
Liver	(26)			(25)
Hemorrhage, minimal	1 (4%)			
Hepatodiaphragmatic nodule	1 (4%)			
Inflammation, chronic, mild	1 (4%)			
Inflammation, chronic, minimal	21 (81%)			22 (88%)
Necrosis, mild	1 (4%)			
Necrosis, minimal	4 (15%)			2 (8%)
Vacuolization cytoplasmic, mild	8 (31%)			3 (12%)
Endocrine System				
Adrenal cortex	(26)			(24)
Accessory adrenal cortical nodule	2 (8%)			
Infiltration cellular, lymphocyte, minimal				1 (4%)
Pituitary gland	(26)			(25)
Cyst, multiple, pars distalis				1 (4%)
Mineralization, minimal	1 (4%)			
Thyroid gland	(26)			(25)
Inflammation, chronic, minimal				1 (4%)
Genital System				
Epididymis	(26)	(25)	(25)	(25)
Hypospermia, marked	1 (4%)	1 (4%)		
Infiltration cellular, lymphocyte, minimal	2 (8%)	1 (4%)	1 (4%)	2 (8%)
Preputial gland				(1)
Inflammation, chronic, mild				1 (100%)
Prostate, dorsal	(26)	(25)	(25)	(24)
Hyperplasia, minimal, epithelium			1 (4%)	
Inflammation, chronic, mild	2 (8%)			3 (13%)
Inflammation, chronic, minimal	6 (23%)	4 (16%)	5 (20%)	6 (25%)
Inflammation, suppurative, marked				1 (4%)
Inflammation, suppurative, mild	6 (23%)	2 (8%)	6 (24%)	7 (29%)
Inflammation, suppurative, minimal	1 (4%)	5 (20%)	6 (24%)	5 (21%)
Inflammation, suppurative, moderate	1 (4%)			

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

	0 ppm		5 ppm		100 ppm		500 ppm	
Genital System (continued)								
Prostate, ventral	(26)		(25)		(25)		(24)	
Inflammation, chronic, mild	9	(35%)	5	(20%)	5	(20%)	2	(8%)
Inflammation, chronic, minimal	10	(38%)	10	(40%)	11	(44%)	11	(46%)
Inflammation, chronic, moderate	1	(4%)			2	(8%)		
Inflammation, suppurative, mild			2	(8%)	1	(4%)	1	(4%)
Inflammation, suppurative, minimal			3	(12%)	1	(4%)	2	(8%)
Seminal vesicle	(26)		(25)		(25)		(25)	
Dilatation, mild			2	(8%)			3	(12%)
Testes	(26)		(25)		(25)		(25)	
Degeneration, marked, seminiferous tubule	1	(4%)	1	(4%)				
Degeneration, minimal, seminiferous tubule			1	(4%)	1	(4%)	2	(8%)
Hematopoietic System								
Spleen	(26)						(25)	
Fibrosis, mild, red pulp	1	(4%)						
Hyperplasia, stromal, minimal							2	(8%)
Inflammation, chronic, minimal, adventitia	1	(4%)						
Pigmentation, minimal	6	(23%)					5	(20%)
Thymus	(26)						(25)	
Hemorrhage, mild							1	(4%)
Hemorrhage, moderate	1	(4%)						
Integumentary System								
Mammary gland	(26)		(24)		(25)		(25)	
Hyperplasia, mild, alveolus							5	(20%)
Hyperplasia, mild, duct							5	(20%)
Hyperplasia, minimal, alveolus	1	(4%)			3	(12%)	3	(12%)
Hyperplasia, minimal, duct			1	(4%)	2	(8%)	3	(12%)
Hyperplasia, moderate, alveolus							2	(8%)
Urinary System								
Kidney	(26)		(25)		(25)		(25)	
Casts protein, minimal	1	(4%)					1	(4%)
Cyst, capsule, fat			1	(4%)			1	(4%)
Cyst, cortex			2	(8%)			1	(4%)
Cyst, multiple, cortex							1	(4%)
Dilatation, mild, pelvis					1	(4%)		
Dilatation, mild, renal tubule	1	(4%)	4	(16%)	1	(4%)	4	(16%)
Dilatation, minimal, renal tubule	4	(15%)	2	(8%)			3	(12%)
Dilatation, moderate, renal tubule					1	(4%)	1	(4%)
Hyperplasia, minimal, epithelium							1	(4%)
Hyperplasia, minimal, pelvis, epithelium			1	(4%)				
Infiltration cellular, lymphocyte, minimal					1	(4%)		
Inflammation, chronic, mild			1	(4%)			3	(12%)
Inflammation, chronic, minimal	16	(62%)	15	(60%)	19	(76%)	19	(76%)
Mineralization, marked, renal tubule			1	(4%)				
Mineralization, mild, renal tubule							8	(32%)
Mineralization, minimal, renal tubule	1	(4%)	2	(8%)	8	(32%)	6	(24%)
Mineralization, moderate, renal tubule							1	(4%)
Regeneration, mild, renal tubule							3	(12%)
Regeneration, minimal, renal tubule	6	(23%)	6	(24%)	8	(32%)	16	(64%)
Vacuolization cytoplasmic, minimal, renal tubule							1	(4%)

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

	0 ppm	5 ppm	100 ppm	500 ppm
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	113	109	109	96
Early deaths				
Dead no CID	8	10	13	7
Issue other	36	33	32	28
Missing	6	4	3	1
Moribund	0	1	0	0
Reallocate	20	20	20	20
Retired	12	15	15	15
Surplus	1	0	1	0
Survey/sentinel	5	1	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)			(25)
Inflammation, chronic, minimal	18 (72%)			22 (88%)
Malformation	1 (4%)			
Necrosis, minimal	1 (4%)			1 (4%)
Vacuolization cytoplasmic, minimal	3 (12%)			2 (8%)
Endocrine System				
Adrenal cortex	(25)			(25)
Accessory adrenal cortical nodule	2 (8%)			
Vacuolization cytoplasmic, mild				1 (4%)
Pituitary gland	(25)			(25)
Cyst, multiple, pars distalis	2 (8%)			1 (4%)
Cyst, pars distalis	1 (4%)			
Vacuolization cytoplasmic, mild, pars distalis	2 (8%)			
Vacuolization cytoplasmic, minimal, pars distalis	1 (4%)			
Genital System				
Epididymis	(25)	(25)	(25)	(25)
Degeneration, moderate				1 (4%)
Hypospermia, marked				1 (4%)
Hypospermia, moderate			1 (4%)	
Infiltration cellular, lymphocyte, minimal	1 (4%)			
Prostate, dorsal	(25)	(25)	(25)	(25)
Hyperplasia, minimal, epithelium			1 (4%)	
Inflammation, chronic, mild	2 (8%)	1 (4%)	1 (4%)	1 (4%)
Inflammation, chronic, minimal	5 (20%)	3 (12%)	5 (20%)	5 (20%)
Inflammation, suppurative, mild	1 (4%)	3 (12%)	5 (20%)	2 (8%)
Inflammation, suppurative, minimal	3 (12%)	3 (12%)	3 (12%)	5 (20%)
Prostate, ventral	(25)	(25)	(25)	(25)
Inflammation, chronic, mild	7 (28%)	9 (36%)	8 (32%)	7 (28%)
Inflammation, chronic, minimal	11 (44%)	6 (24%)	7 (28%)	10 (40%)
Inflammation, chronic, moderate	1 (4%)			
Inflammation, suppurative, mild	4 (16%)	2 (8%)	2 (8%)	1 (4%)
Inflammation, suppurative, minimal	2 (8%)		4 (16%)	4 (16%)

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm		5 ppm		100 ppm		500 ppm	
Genital System (continued)								
Seminal vesicle	(25)		(25)		(25)		(25)	
Dilatation, mild			1	(4%)			2	(8%)
Dilatation, minimal	1	(4%)						
Hyperplasia, mild, epithelium			1	(4%)				
Inflammation, chronic, minimal			1	(4%)	1	(4%)		
Testes	(25)		(25)		(25)		(24)	
Degeneration, marked, seminiferous tubule					1	(4%)		
Degeneration, mild, seminiferous tubule					1	(4%)		
Degeneration, minimal, seminiferous tubule	2	(8%)					1	(4%)
Degeneration, moderate, seminiferous tubule	1	(4%)						
Testes, rete testes	(25)				(1)		(25)	
Dilatation, mild					1	(100%)		
Hematopoietic System								
Spleen	(25)						(25)	
Hyperplasia, stromal, minimal							1	(4%)
Inflammation, chronic, minimal, adventitia	1	(4%)						
Pigmentation, mild	2	(8%)						
Pigmentation, minimal	3	(12%)					4	(16%)
Thymus	(25)				(1)		(25)	
Atrophy, moderate					1	(100%)		
Integumentary System								
Mammary gland	(24)		(25)		(25)		(25)	
Hyperplasia, mild, alveolus	2	(8%)			2	(8%)	4	(16%)
Hyperplasia, mild, duct							4	(16%)
Hyperplasia, minimal, alveolus					5	(20%)	12	(48%)
Hyperplasia, minimal, duct					1	(4%)	6	(24%)
Hyperplasia, moderate, alveolus							2	(8%)
Urinary System								
Kidney	(25)		(25)		(25)		(25)	
Casts protein, minimal			2	(8%)				
Cyst, capsule, fat			1	(4%)	1	(4%)	1	(4%)
Cyst, cortex			1	(4%)			2	(8%)
Dilatation, mild, pelvis							1	(4%)
Dilatation, mild, renal tubule	4	(16%)			2	(8%)	2	(8%)
Dilatation, minimal, renal tubule	3	(12%)	1	(4%)	2	(8%)	4	(16%)
Dilatation, moderate, renal tubule	1	(4%)			1	(4%)		
Inflammation, chronic, mild	2	(8%)						
Inflammation, chronic, minimal	18	(72%)	16	(64%)	16	(64%)	23	(92%)
Mineralization, minimal, renal tubule			1	(4%)	4	(16%)	6	(24%)
Regeneration, minimal, renal tubule	8	(32%)	3	(12%)	8	(32%)	9	(36%)

TABLE A2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Musculoskeletal System				
Nervous System				
Respiratory System				
Special Senses System				

TABLE A2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	107	123	136	143
Early deaths				
Dead	0	1	0	0
Dead no CID	3	3	2	4
Discard	0	2	0	0
Missing	3	2	2	2
Not allocated	0	0	1	0
Reallocate	66	70	83	73
Retired	7	10	10	10
Surplus	0	6	13	29
Survey/sentinel	3	3	0	0
Wrong sex	0	1	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)			(24)
Fibrosis, mild	1 (4%)			
Hepatodiaphragmatic nodule	1 (4%)			
Inflammation, chronic, minimal	22 (88%)			23 (96%)
Necrosis, mild				1 (4%)
Necrosis, minimal	2 (8%)			
Vacuolization cytoplasmic, minimal	3 (12%)			
Endocrine System				
Adrenal cortex	(25)			(25)
Accessory adrenal cortical nodule	3 (12%)			1 (4%)
Pituitary gland	(25)			(25)
Cyst, multiple, pars distalis	3 (12%)			1 (4%)
Cyst, pars distalis	1 (4%)			
Thyroid gland	(25)			(25)
Inflammation, chronic, minimal	1 (4%)			
Genital System				
Ductus deferens		(1)		
Ectasia, marked		1 (100%)		
Epididymis	(25)	(25)	(25)	(25)
Degeneration, moderate	1 (4%)			
Hypospermia, marked	1 (4%)	1 (4%)		
Infiltration cellular, lymphocyte, minimal	3 (12%)			1 (4%)
Prostate, dorsal	(25)	(25)	(25)	(25)
Ectasia, marked, lymphatic		1 (4%)		
Hyperplasia, minimal, epithelium	1 (4%)			
Inflammation, chronic, mild	1 (4%)		3 (12%)	
Inflammation, chronic, minimal	7 (28%)	1 (4%)	3 (12%)	7 (28%)
Inflammation, suppurative, mild	3 (12%)	1 (4%)	4 (16%)	
Inflammation, suppurative, minimal	2 (8%)	7 (28%)	5 (20%)	2 (8%)
Inflammation, suppurative, moderate			1 (4%)	

TABLE A2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm		5 ppm		100 ppm		500 ppm	
Genital System (continued)								
Prostate, ventral	(25)		(25)		(25)		(25)	
Inflammation, chronic, mild	7	(28%)	3	(12%)	8	(32%)	6	(24%)
Inflammation, chronic, minimal	11	(44%)	16	(64%)	9	(36%)	13	(52%)
Inflammation, suppurative, mild					2	(8%)		
Inflammation, suppurative, minimal	3	(12%)	4	(16%)	3	(12%)	3	(12%)
Seminal vesicle	(25)		(25)		(25)		(25)	
Dilatation, mild	3	(12%)					1	(4%)
Ectasia, marked, lymphatic			1	(4%)				
Testes	(25)		(25)		(25)		(25)	
Degeneration, marked, seminiferous tubule	1	(4%)	1	(4%)	1	(4%)		
Degeneration, minimal, seminiferous tubule	1	(4%)			1	(4%)	3	(12%)
Testes, rete testes	(24)		(25)		(25)		(24)	
Dilatation, marked	1	(4%)	1	(4%)				
Dilatation, mild			1	(4%)				
Hematopoietic System								
Spleen	(25)						(25)	
Hyperplasia, stromal, minimal							2	(8%)
Inflammation, chronic, minimal, adventitia	1	(4%)						
Pigmentation, mild							1	(4%)
Pigmentation, minimal	2	(8%)					3	(12%)
Thymus	(25)						(25)	
Hyperplasia, mild, epithelial cell	1	(4%)						
Integumentary System								
Mammary gland	(24)		(25)		(25)		(23)	
Hyperplasia, mild, alveolus	1	(4%)					1	(4%)
Hyperplasia, mild, duct							1	(4%)
Hyperplasia, minimal, alveolus	3	(13%)	2	(8%)	5	(20%)	2	(9%)
Hyperplasia, minimal, duct					1	(4%)	3	(13%)
Hyperplasia, moderate, alveolus							1	(4%)
Musculoskeletal System								
Bone, vertebra			(1)					
Malformation			1	(100%)				
Urinary System								
Kidney	(25)		(1)				(25)	
Casts protein, minimal							1	(4%)
Cyst, capsule, fat			1	(100%)			1	(4%)
Dilatation, mild, pelvis							1	(4%)
Dilatation, mild, renal tubule	5	(20%)					2	(8%)
Dilatation, minimal, renal tubule	2	(8%)					2	(8%)
Dilatation, moderate, renal tubule	1	(4%)						
Inflammation, chronic, mild	2	(8%)						
Inflammation, chronic, minimal	19	(76%)					18	(72%)
Regeneration, mild, renal tubule	1	(4%)						
Regeneration, minimal, renal tubule	7	(28%)					13	(52%)

TABLE A2d
Summary of the Incidence of Nonneoplastic Lesions in F_3 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Systems Examined with No Lesions Observed				
Cardiovascular System				
General Body System				
Nervous System				
Respiratory System				
Special Senses System				

TABLE A2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	121	105	124	109
Early deaths				
Dead no CID	6	1	0	2
Discard	2	1	0	0
Missing	3	1	1	4
Reallocate	20	20	20	24
Retired	4	5	5	6
Surplus	55	52	73	48
Survey/sentinel	6	0	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)			(25)
Inflammation, chronic, mild				1 (4%)
Inflammation, chronic, minimal	18 (72%)			17 (68%)
Malformation				1 (4%)
Necrosis, mild				1 (4%)
Necrosis, minimal	3 (12%			1 (4%)
Vacuolization cytoplasmic, mild	1 (4%)			
Vacuolization cytoplasmic, minimal	2 (8%)			1 (4%)
Mesentery		(1)		
Inflammation, chronic active, marked		1 (100%)		
Endocrine System				
Adrenal cortex	(25)			(25)
Accessory adrenal cortical nodule	1 (4%)			2 (8%)
Pituitary gland	(25)			(25)
Cyst, multiple, pars distalis				1 (4%)
Cyst, pars distalis				1 (4%)
Genital System				
Epididymis	(25)	(25)	(25)	(25)
Hypospermia, marked	1 (4%)			
Infiltration cellular, lymphocyte, minimal	1 (4%)	2 (8%)	1 (4%)	1 (4%)
Prostate, dorsal	(25)	(25)	(25)	(25)
Inflammation, chronic, mild	2 (8%)	1 (4%)	1 (4%)	
Inflammation, chronic, minimal	6 (24%)	4 (16%)	5 (20%)	6 (24%)
Inflammation, suppurative, mild	2 (8%)	3 (12%)	2 (8%)	2 (8%)
Inflammation, suppurative, minimal	2 (8%)	3 (12%)	3 (12%)	4 (16%)
Prostate, ventral	(25)	(25)	(25)	(25)
Inflammation, chronic, mild	6 (24%)	9 (36%)	7 (28%)	9 (36%)
Inflammation, chronic, minimal	11 (44%)	8 (32%)	16 (64%)	14 (56%)
Inflammation, chronic, moderate	1 (4%)			
Inflammation, suppurative, mild		1 (4%)	2 (8%)	1 (4%)
Inflammation, suppurative, minimal	3 (12%)	2 (8%)	3 (12%)	1 (4%)
Inflammation, suppurative, moderate	1 (4%)			

TABLE A2e
Summary of the Incidence of Nonneoplastic Lesions in F_4 Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm		5 ppm		100 ppm		500 ppm	
Genital System (continued)								
Seminal vesicle	(25)		(25)		(24)		(25)	
Dilatation, mild							1	(4%)
Testes	(25)		(25)		(25)		(25)	
Degeneration, marked, seminiferous tubule	1	(4%)						
Degeneration, minimal, seminiferous tubule	2	(8%)	1	(4%)	4	(16%)	3	(12%)
Testes, rete testes	(25)		(20)		(24)		(22)	
Dilatation, moderate	1	(4%)						
Hematopoietic System								
Spleen	(25)						(25)	
Inflammation, chronic, minimal, adventitia	2	(8%)						
Pigmentation, minimal	6	(24%)					10	(40%)
Integumentary System								
Mammary gland	(25)		(25)		(25)		(24)	
Hyperplasia, mild, alveolus	2	(8%)	1	(4%)	1	(4%)	4	(17%)
Hyperplasia, minimal, alveolus	2	(8%)	3	(12%)	4	(16%)	2	(8%)
Hyperplasia, moderate, alveolus					1	(4%)		
Urinary System								
Kidney	(25)						(25)	
Casts protein, minimal							1	(4%)
Cyst, cortex							2	(8%)
Dilatation, mild, pelvis	1	(4%)					1	(4%)
Dilatation, mild, renal tubule	5	(20%)						
Dilatation, minimal, renal tubule	8	(32%)					3	(12%)
Dilatation, moderate, pelvis							1	(4%)
Inflammation, chronic, mild	1	(4%)						
Inflammation, chronic, minimal	17	(68%)					15	(60%)
Mineralization, minimal, renal tubule	1	(4%)						
Regeneration, mild, renal tubule	1	(4%)						
Regeneration, minimal, renal tubule	5	(20%)					10	(40%)

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

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

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary[a]				
Animals initially in study	35	35	35	35
Early deaths				
Discard	0	1	0	2
Harvest	4	5	3	3
Moribund	1	0	1	0
Retired	4	4	5	5
Survey/sentinel	1	0	1	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	26	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

[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. All other pups were allocated to addenda studies or euthanized (Discard, Issue other, Not allocated, Reallocated, Retired, Surplus). In some cases, young pups that died were likely cannibalized by the dam and were thus indicated as Missing. Survey/sentinel animals were microbiological sentinels. Animals designated Dead no CID (carcass identification number) were animals that were not selected for continuation on study but died prior to weaning. Only animals processed by pathology received CIDs.

TABLE B1b
Summary of the Incidence of Neoplasms in F_1 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	125	120	120	125
Early deaths				
Dead	1	0	0	0
Discard	10	2	2	9
Harvest	7	6	7	9
Issue other	6	5	6	6
Reallocate	45	44	45	45
Retired	0	3	2	0
Surplus	29	34	32	30
Survey/sentinel	3	1	1	1
Survivors				
Terminal sacrifice	24	25	25	25
Animals examined microscopically	25	25	25	25
Urinary System				
Kidney	(25)	(25)	(24)	(25)
Nephroblastoma		1 (4%)		

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

The footnote for this table is defined in Table B1a.

TABLE B1c
Summary of the Incidence of Neoplasms in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	112	115	107	93
Early deaths				
Dead no CID	2	14	11	6
Harvest	13	9	4	4
Issue other	8	9	9	5
Missing	3	5	1	0
Reallocate	45	43	42	41
Retired	0	5	11	11
Surplus	11	4	4	1
Survey/sentinel	5	1	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Endocrine System				
Adrenal cortex	(25)			(25)
Adenoma				1 (4%)

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

The footnote for this table is defined in Table B1a.

TABLE B1d
Summary of the Incidence of Neoplasms in F_3 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	101	125	138	144
Early deaths				
Dead no CID	2	2	1	2
Harvest	5	6	4	5
Missing	3	0	5	3
Reallocate	61	73	75	78
Retired	2	4	6	4
Surplus	0	15	19	27
Survey/sentinel	3	0	3	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Urinary System				
Kidney	(25)	(25)	(25)	(25)
Adenoma, tubular	1 (4%)			

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

The footnote for this table is defined in Table B1a.

TABLE B1e
Summary of the Incidence of Neoplasms in F_4 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	119	103	124	107
Early deaths				
Dead	1	0	0	0
Dead no CID	9	0	2	2
Harvest	3	3	3	5
Issue other	0	0	1	2
Missing	2	7	2	1
Reallocate	46	46	45	46
Retired	2	5	5	3
Surplus	25	17	41	23
Survey/sentinel	6	0	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	26	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

The footnote for this table is defined in Table B1a.

TABLE B2a
Summary of the Incidence of Nonneoplastic Lesions in F_0 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	35	35	35	35
Early deaths				
Discard	0	1	0	2
Harvest	4	5	3	3
Moribund	1	0	1	0
Retired	4	4	5	5
Survey/sentinel	1	0	1	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	26	25	26	25
Alimentary System				
Esophagus	(1)		(1)	
Dilatation, moderate	1 (100%)			
Liver	(26)	(1)	(1)	(25)
Crystals, marked			1 (100%)	
Developmental malformation	3 (12%)	1 (100%)		
Developmental malformation, left lateral lobe				2 (8%)
Developmental malformation, median lobe				1 (4%)
Inflammation, chronic active, mild	1 (4%)			1 (4%)
Inflammation, chronic active, minimal				1 (4%)
Cardiovascular System				
Heart	(1)		(1)	
Cardiomyopathy, mild	1 (100%)			
Endocrine System				
Adrenal medulla	(26)		(1)	(25)
Vacuolization cytoplasmic, mild, bilateral	1 (4%)			
Genital System				
Clitoral gland		(1)	(1)	(1)
Abscess				1 (100%)
Inflammation, chronic active, mild		1 (100%)		
Keratin cyst				1 (100%)
Ovary	(26)	(25)	(26)	(25)
Diestrus	10 (38%)	9 (36%)	7 (27%)	6 (24%)
Estrus	2 (8%)	2 (8%)	1 (4%)	
Hypertrophy, moderate, corpus luteum			1 (4%)	
Metestrus	6 (23%)	6 (24%)	8 (31%)	13 (52%)
Proestrus	8 (31%)	8 (32%)	9 (35%)	6 (24%)
Oviduct	(26)	(25)	(26)	(25)
Estrus				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 Genistein

	0 ppm		5 ppm		100 ppm		500 ppm	
Genital System (continued)								
Uterus	(26)		(25)		(26)		(25)	
Decidual reaction	24	(92%)	24	(96%)	24	(92%)	23	(92%)
Diestrus	10	(38%)	9	(36%)	7	(27%)	6	(24%)
Estrus	2	(8%)	2	(8%)	1	(4%)		
Infiltration cellular, eosinophilic, moderate	1	(4%)						
Metestrus	7	(27%)	5	(20%)	8	(31%)	13	(53%)
Pregnancy					1	(4%)		
Proestrus	7	(27%)	8	(32%)	9	(35%)	6	(24%)
Vagina	(26)		(25)		(26)		(25)	
Diestrus	10	(38%)	9	(36%)	8	(31%)	7	(28%)
Estrus	3	(12%)	6	(24%)	2	(8%)	1	(4%)
Hyperplasia, moderate, mucocyte					1	(4%)		
Hypertrophy, mild, mucocyte	1	(4%)						
Hypertrophy, moderate, mucocyte					1	(4%)		
Infiltration cellular, eosinophilic, marked	1	(4%)						
Keratin cyst							1	(4%)
Metestrus	7	(27%)	6	(24%)	8	(31%)	12	(48%)
Proestrus	6	(23%)	4	(16%)	7	(27%)	5	(20%)
Hematopoietic System								
Spleen	(25)				(1)		(24)	
Pigmentation, mild	1	(4%)						
Thymus	(26)				(1)		(25)	
Atrophy, moderate	1	(4%)						
Integumentary System								
Mammary gland	(25)		(25)		(26)		(25)	
Hyperplasia, marked, alveolus	1	(4%)			1	(4%)		
Hyperplasia, marked, lobules	1	(4%)						
Hyperplasia, mild, alveolus	3	(12%)	5	(20%)	4	(15%)	1	(4%)
Hyperplasia, minimal, alveolus	5	(20%)	3	(12%)	2	(8%)	6	(24%)
Hyperplasia, minimal, lobules	2	(8%)	1	(4%)	3	(12%)	1	(4%)
Hyperplasia, moderate, alveolus	1	(4%)	4	(16%)	1	(4%)	1	(4%)
Hyperplasia, moderate, duct					1	(4%)		
Musculoskeletal System								
Bone	(1)							
Necrosis, focal, moderate	1	(100%)						
Respiratory System								
Lung	(1)				(1)			
Inflammation, multifocal, chronic active, moderate	1	(100%)						
Special Senses System								
Eye	(1)							
Edema, moderate, cornea	1	(100%)						

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

	0 ppm	5 ppm	100 ppm	500 ppm
Urinary System				
Kidney	(26)	(25)	(26	(25)
Apoptosis, marked			1 (4%)	
Cyst, mild, left				1 (4%)
Dilatation, mild, bilateral, pelvis		1 (4%)		
Dilatation, mild, left, pelvis	1 (4%)			
Dilatation, mild, pelvis	1 (4%)			
Inflammation, chronic active, minimal		1 (4%)	2 (8%)	
Mineralization, marked	1 (4%)			3 (12%)
Mineralization, mild	4 (15%)	5 (20%)	4 (15%)	3 (12%)
Mineralization, minimal	9 (35%)	15 (60%)	15 (58%)	7 (28%)
Mineralization, moderate	3 (12%)	1 (4%)	3 (12%)	1 (4%)
Nephropathy, mild, bilateral	1 (4%)			
Proliferation, moderate, bilateral, parenchymal cell			1 (4%)	

Systems Examined with No Lesions Observed
General Body System
Nervous System

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	125	120	120	125
Early deaths				
Dead	1	0	0	0
Discard	10	2	2	9
Harvest	7	6	7	9
Issue other	6	5	6	6
Reallocate	45	44	45	45
Retired	0	3	2	0
Surplus	29	34	32	30
Survey/sentinel	3	1	1	1
Survivors				
Terminal sacrifice	24	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)	(1)		(25)
Autolysis, mild	1 (4%)			
Developmental malformation				1 (4%)
Inflammation, focal, chronic active, mild	1 (4%)			
Inflammation, focal, chronic active, minimal	3 (12%)			5 (20%)
Necrosis, focal, minimal				1 (4%)
Salivary glands	(1)			
Autolysis, moderate	1 (100%)			
Stomach, forestomach	(1)			
Autolysis, marked	1 (100%)			
Cardiovascular System				
Heart	(1)			
Cardiomyopathy, focal, mild	1 (100%)			
Endocrine System				
Adrenal cortex	(25)			(25)
Autolysis, mild	1 (4%)			
Hyperplasia, focal, minimal				1 (4%)
Adrenal medulla	(25)			(25)
Autolysis, mild	1 (4%)			
Pituitary gland	(25)			(25)
Autolysis, mild	1 (4%)			
Cyst, Rathke's cleft	1 (4%)			2 (8%)
Thyroid gland	(25)			(25)
Autolysis, mild	1 (4%)			
Infiltration cellular, lymphocyte, focal, minimal, bilateral				1 (4%)
Ultimobranchial cyst				1 (4%)
Ultimobranchial cyst, bilateral	1 (4%)			

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

	0 ppm	5 ppm	100 ppm	500 ppm
Genital System				
Ovary	(25)	(25)	(25)	(25)
Autolysis, minimal	1 (4%)			
Cyst		1 (4%)		1 (4%)
Diestrus	8 (32%)	2 (8%)	4 (16%)	8 (32%)
Estrus	7 (28%)	8 (32%)	6 (24%)	5 (20%)
Metestrus	1 (4%)	6 (24%)	7 (28%)	5 (20%)
Proestrus	8 (32%)	9 (36%)	7 (28%)	7 (28%)
Uterus	(25)	(25)	(25)	(25)
Angiectasis, focal, moderate		1 (4%)		
Autolysis, mild	1 (4%)			
Decidual reaction	22 (88%)	20 (80%)	21 (84%)	20 (80%)
Diestrus	8 (32%)	2 (8%)	4 (16%)	7 (28%)
Dilatation, minimal, endometrium				1 (4%)
Estrus	6 (24%)	8 (32%)	7 (28%)	5 (20%)
Inflammation, diffuse, mild, endometrium		1 (4%)		
Inflammation, focal, chronic active, moderate		1 (4%)		
Metestrus	2 (8%)	5 (20%)	7 (28%)	5 (20%)
Proestrus	8 (32%)	9 (36%)	7 (28%)	8 (32%)
Vagina	(25)	(25)	(25)	(25)
Autolysis, mild	1 (4%)			
Diestrus	8 (32%)	1 (4%)	4 (16%)	7 (28%)
Estrus	9 (36%)	10 (40%)	7 (28%)	5 (20%)
Infiltration cellular, polymorphonuclear, mild		1 (4%)		
Metestrus	2 (8%)	6 (24%)	7 (28%)	6 (24%)
Proestrus	5 (20%)	7 (28%)	7 (28%)	7 (28%)
Hematopoietic System				
Lymph node, mandibular	(1)			
Autolysis, moderate	1 (100%)			
Lymph node, mesenteric	(1)			
Autolysis, mild	1 (100%)			
Spleen	(25)			(25)
Autolysis mild	1 (4%)			
Integumentary System				
Mammary gland	(24)	(23)	(25)	(25)
Galactocele	1 (4%)		1 (4%)	
Hyperplasia, marked, alveolus	1 (4%)	2 (9%)		
Hyperplasia, marked, lobules	1 (4%)	2 (9%)		
Hyperplasia, mild, alveolus	2 (8%)	1 (4%)	4 (16%)	3 (12%)
Hyperplasia, mild, lobules			1 (4%)	
Hyperplasia, minimal, alveolus	4 (17%)	3 (13%)	7 (28%)	5 (20%)
Hyperplasia, minimal, lobules			1 (4%)	
Hyperplasia, moderate, alveolus	1 (4%)		3 (12%)	
Skin	(25)		(1)	(25)
Abscess, lip			1 (100%)	

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

	0 ppm	5 ppm	100 ppm	500 ppm
Nervous System				
Brain, brain stem	(1)			
Autolysis, mild	1 (100%)			
Brain, cerebellum	(1)			
Autolysis, mild	1 (100%)			
Brain, cerebrum	(1)			
Autolysis, mild	1 (100%)			
Respiratory System				
Lung	(1)			
Congestion, diffuse, marked	1 (100%)			
Nose	(1)			
Congestion, diffuse, marked	1 (100%)			
Urinary System				
Kidney	(25)	(25)	(24)	(25)
Autolysis, moderate	1 (4%)			
Cyst	1 (4%)			
Cyst, multiple		1 (4%)		1 (4%)
Dilatation, minimal, pelvis		1 (4%)		
Dilatation, moderate, pelvis				1 (4%)
Infarct	1 (4%)			
Inflammation, chronic active, minimal			1 (4%)	
Mineralization, marked	2 (8%)	3 (12%)	1 (4%)	2 (8%)
Mineralization, mild	8 (32%)	3 (12%)	5 (21%)	5 (20%)
Mineralization, minimal	9 (36%)	13 (52%)	11 (46%)	12 (48%)
Mineralization, moderate	2 (8%)	4 (16%)	5 (21%)	5 (20%)
Nephropathy, mild				1 (4%)
Nephropathy, minimal				1 (4%)
Nephropathy, minimal, bilateral	2 (8%)			

Systems Examined with No Lesions Observed
General Body System
Musculoskeletal System
Special Senses System

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	112	115	107	93
Early deaths				
Dead no CID	2	14	11	6
Harvest	13	9	4	4
Issue other	8	9	9	5
Missing	3	5	1	0
Reallocate	45	43	42	41
Retired	0	5	11	11
Surplus	11	4	4	1
Survey/sentinel	5	1	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)	(2)		(25)
Developmental malformation		2 (100%)		1 (4%)
Developmental malformation, median lobe	1 (4%)			1 (4%)
Inflammation, bile duct				1 (4%)
Inflammation, focal, chronic active, minimal				1 (4%)
Necrosis, focal, minimal				1 (4%)
Endocrine System				
Thyroid gland	(25)			(25)
Cyst				1 (4%)
Genital System				
Ovary	(25)	(25)	(25)	(25)
Cyst				1 (4%)
Diestrus	7 (28%)	6 (24%)	6 (24%)	6 (24%)
Estrus	5 (20%)	7 (28%)	6 (24%)	2 (8%)
Metestrus	6 (24%)	4 (16%)	6 (24%)	7 (28%)
Proestrus	7 (28%)	8 (32%)	7 (28%)	10 (40%)
Uterus	(25)	(25)	(25)	(25)
Decidual reaction	24 (96%)	24 (96%)	24 (96%)	22 (88%)
Diestrus	7 (28%)	6 (24%)	6 (24%)	6 (24%)
Estrus	5 (20%)	7 (28%)	6 (24%)	2 (8%)
Metestrus	6 (24%)	4 (16%)	6 (24%)	7 (28%)
Proestrus	7 (28%)	8 (32%)	7 (28%)	10 (40%)
Vagina	(24)	(23)	(24)	(23)
Diestrus	7 (29%)	6 (26%)	6 (25%)	6 (26%)
Estrus	6 (25%)	8 (35%)	8 (33%)	2 (9%)
Metestrus	6 (25%)	4 (17%)	6 (25%)	7 (30%)
Proestrus	5 (21%)	5 (22%)	4 (17%)	8 (35%)

TABLE B2c
Summary of the Incidence of Nonneoplastic Lesions in F_2 Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

	0 ppm	5 ppm	100 ppm	500 ppm
Integumentary System				
Mammary gland	(24)	(25)	(25)	(25)
Hyperplasia, mild, alveolus	11 (46%)	12 (48%)	5 (20%)	5 (20%)
Hyperplasia, minimal, alveolus	7 (29%)	2 (8%)	8 (32%)	12 (48%)
Hyperplasia, moderate, alveolus		1 (4%)	3 (12%)	4 (16%)
Urinary System				
Kidney	(25)	(25)	(24)	(25)
Cyst		2 (8%)	2 (8%)	2 (8%)
Cyst, bilateral		1 (4%)		
Dilatation, focal, mild, renal tubule			1 (4%)	
Dilatation, moderate, pelvis		1 (4%)		
Infarct				1 (4%)
Inflammation, chronic active, mild		1 (4%)	1 (4%)	
Inflammation, chronic active, minimal		1 (4%)	1 (4%)	
Mineralization, marked		2 (8%)	2 (8%)	1 (4%)
Mineralization, mild	9 (36%)	3 (12%)	2 (8%)	5 (20%)
Mineralization, minimal	9 (36%)	13 (52%)	17 (68%)	10 (40%)
Mineralization, moderate	2 (8%)	3 (12%)	2 (8%)	3 (12%)
Proliferation, focal, minimal, parenchymal cell		1 (4%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	101	125	138	144
Early deaths				
Dead no CID	2	2	1	2
Harvest	5	6	4	5
Missing	3	0	5	3
Reallocate	61	73	75	78
Retired	2	4	6	4
Surplus	0	15	19	27
Survey/sentinel	3	0	3	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	25	25	25	25
Alimentary System				
Liver	(25)	(2)		(24)
Developmental malformation	1 (4%)			
Hepatodiaphragmatic nodule		1 (50%)		
Inflammation, chronic active, mild				1 (4%)
Endocrine System				
Adrenal cortex	(25)	(1)		(25)
Hyperplasia, focal, minimal	1 (4%)			
Genital System				
Ovary	(25)	(25)	(25)	(25)
Diestrus	7 (28%)	2 (8%)	9 (36%)	6 (24%)
Estrus	9 (36%)	8 (32%)	3 (12%)	6 (24%)
Metestrus	3 (12%)	10 (40%)	4 (16%)	7 (28%)
Proestrus	6 (24%)	5 (20%)	9 (36%)	6 (24%)
Uterus	(25)	(25)	(25)	(25)
Cyst, endometrium				1 (4%)
Decidual reaction	23 (92%)	24 (96%)	22 (88%)	25 (100%)
Diestrus	7 (28%)	2 (8%)	9 (36%)	8 (32%)
Estrus	10 (40%)	6 (24%)	3 (12%)	3 (12%)
Hyperplasia, cystic, marked, endometrium	1 (4%)			
Metestrus	3 (12%)	10 (40%)	4 (16%)	8 (32%)
Proestrus	5 (20%)	7 (28%)	9 (36%)	6 (24%)
Vagina	(24)	(25)	(25)	(25)
Diestrus	7 (29%)	2 (8%)	9 (36%)	7 (28%)
Estrus	7 (29%)	8 (32%)	7 (28%)	5 (20%)
Keratin cyst				1 (4%)
Metestrus	4 (17%)	10 (40%)	4 (16%)	8 (32%)
Proestrus	6 (25%)	5 (20%)	5 (20%)	5 (20%)

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

	0 ppm	5 ppm	100 ppm	500 ppm
Integumentary System				
Mammary gland	(25)	(25)	(25)	(25)
Hyperplasia, marked, alveolus		1 (4%)		2 (8%)
Hyperplasia, marked, lobules		1 (4%)		1 (4%)
Hyperplasia, mild, alveolus	6 (24%)	1 (4%)	5 (20%)	3 (12%)
Hyperplasia, mild, lobules		1 (4%)		1 (4%)
Hyperplasia, minimal, alveolus	3 (12%)	5 (20%)	5 (20%)	7 (28%)
Hyperplasia, minimal, lobules		2 (8%)		
Hyperplasia, moderate, alveolus	2 (8%)	5 (20%)	1 (4%)	
Hyperplasia, moderate, lobules				1 (4%)
Urinary System				
Kidney	(25)	(25)	(25)	(25)
Cyst		2 (8%)		
Cyst, multiple			1 (4%)	
Cyst, renal tubule	1 (4%)			1 (4%)
Dilatation, mild, bilateral, pelvis				1 (4%)
Dilatation, mild, unilateral, pelvis	1 (4%)			
Inflammation, chronic active, minimal				1 (4%)
Inflammation, focal, chronic active, minimal				1 (4%)
Mineralization, marked	4 (16%)	1 (4%)	4 (16%)	
Mineralization, mild	6 (24%)	6 (24%)	7 (28%)	3 (12%)
Mineralization, minimal	10 (40%)	13 (52%)	7 (28%)	12 (48%)
Mineralization, moderate	1 (4%)	2 (8%)	4 (16%)	
Regeneration, minimal, renal tubule		1 (4%)		

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Hematopoietic System
Musculoskeletal System
Nervous System
Respiratory System
Special Senses System

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

	0 ppm	5 ppm	100 ppm	500 ppm
Disposition Summary				
Animals initially in study	119	103	124	107
Early deaths				
Dead	1	0	0	0
Dead no CID	9	0	2	2
Harvest	3	3	3	5
Issue other	0	0	1	2
Missing	2	7	2	1
Reallocate	46	46	45	46
Retired	2	5	5	3
Surplus	25	17	41	23
Survey/sentinel	6	0	0	0
Survivors				
Terminal sacrifice	25	25	25	25
Animals examined microscopically	26	25	25	25
Alimentary System				
Intestine large, cecum	(1)			
Autolysis, mild, epithelium	1 (100%)			
Intestine small, duodenum	(1)			
Autolysis, moderate, epithelium	1 (100%)			
Hyperplasia, mild	1 (100%)			
Intestine small, ileum	(1)			
Autolysis, moderate, epithelium	1 (100%)			
Intestine small, jejunum	(1)			
Autolysis, moderate, epithelium	1 (100%)			
Liver	(26)		(1)	(25)
Congestion, moderate			1 (100%)	
Developmental malformation			1 (100%)	
Pancreas	(1)			
Autolysis, moderate	1 (100%)			
Salivary glands	(1)			
Autolysis, mild	1 (100%)			
Endocrine System				
Adrenal cortex	(26)			(25)
Hyperplasia, focal, multifocal, mild	1 (4%)			
Genital System				
Ovary	(25)	(24)	(24)	(25)
Diestrus	6 (24%)	8 (33%)	7 (29%)	8 (32%)
Estrus	3 (12%)	4 (17%)	9 (38%)	2 (8%)
Metestrus	2 (8%)	2 (8%)		4 (16%)
Proestrus	14 (56%)	10 (42%)	8 (33%)	11 (44%)
Oviduct	(25)	(24)	(24)	(25)
Proestrus	2 (8%)			3 (12%)

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

	0 ppm	5 ppm	100 ppm	500 ppm
Genital System (continued)				
Uterus	(26)	(25)	(25)	(25)
Autolysis, mild	1 (4%)			
Decidual reaction	25 (96%)	24 (96%)	23 (92%)	23 (92%)
Diestrus	6 (23%)	9 (36%)	8 (32%)	8 (32%)
Estrus	3 (12%)	4 (16%)	9 (36%)	2 (8%)
Metestrus	3 (12%)	2 (8%)		5 (20%)
Proestrus	14 (54%)	10 (40%)	8 (32%)	10 (40%)
Vagina	(26)	(25)	(25)	(25)
Autolysis, mild	1 (4%)			
Diestrus	6 (23%)	9 (36%)	8 (32%)	8 (32%)
Estrus	9 (35%)	6 (24%)	14 (56%)	4 (16%)
Metestrus	3 (12%)	2 (8%)		5 (20%)
Proestrus	8 (31%)	8 (32%)	3 (12%)	8 (32%)
Hematopoietic System				
Bone marrow	(26)			(25)
Autolysis, mild	1 (4%)			
Lymph node, mandibular	(1)			
Autolysis, mild	1 (100%)			
Lymph node, mesenteric	(1)			
Hyperplasia, reticulum cell, mild	1 (100%)			
Integumentary System				
Mammary gland	(26)	(25)	(25)	(25)
Hyperplasia, marked, alveolus		1 (4%)	2 (8%)	1 (4%)
Hyperplasia, marked, lobules			1 (4%)	1 (4%)
Hyperplasia, mild, alveolus	5 (19%)	5 (20%)	6 (24%)	3 (12%)
Hyperplasia, mild, lobules	3 (12%)	2 (8%)	1 (4%)	
Hyperplasia, minimal, alveolus	2 (8%)	5 (20%)		3 (12%)
Hyperplasia, minimal, lobules	3 (12%)	2 (8%)	3 (12%)	2 (8%)
Hyperplasia, moderate, alveolus	7 (27%)	4 (16%)	3 (12%)	4 (16%)
Hyperplasia, moderate, lobules	1 (4%)	2 (8%)	1 (4%)	1 (4%)
Nervous System				
Brain, cerebrum	(1)			
Hydrocephalus, moderate, unilateral	1 (100%)			
Respiratory System				
Lung	(1)			
Autolysis, mild	1 (100%)			
Congestion, marked	1 (100%)			
Infiltration cellular, macrophage, moderate	1 (100%)			
Nose	(1)			
Autolysis, mild	1 (100%)			

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

	0 ppm	5 ppm	100 ppm	500 ppm
Urinary System				
Kidney	(26)	(25)	(25)	(25)
Cyst		2 (8%)	1 (4%)	
Dilatation, moderate, unilateral, pelvis				1 (4%)
Mineralization, marked		2 (8%)		
Mineralization, mild	2 (8%)	5 (20%)	6 (24%)	1 (4%)
Mineralization, minimal	17 (65%)	16 (64%)	9 (36%)	15 (60%)
Mineralization, moderate	4 (15%)		7 (28%)	

Systems Examined with No Lesions Observed
Cardiovascular System
General Body System
Musculoskeletal System
Special Senses System

APPENDIX C
CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

CHEMICAL CHARACTERIZATION AND DOSE FORMULATION STUDIES

PROCUREMENT AND CHARACTERIZATION OF GENISTEIN

Genistein was obtained from Toronto Research Chemicals, Inc. (North York, Ontario, Canada), in one lot (2-BP-136-6). 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 genistein study are on file at the NCTR.

Lot 2-BP-136-6 of the chemical, a pale-yellow crystalline solid, was identified as genistein by proton nuclear magnetic resonance (NMR) spectroscopy. NMR spectra were consistent with the structure of genistein. A representative proton NMR spectrum is presented in Figure C1.

The purity of lot 2-BP-136-6 was determined by high-performance liquid chromatography (HPLC) with ultraviolet (UV) and mass spectrophotometric (MS) detection, by gas chromatography (GC) with MS detection, and by Probe/MS methods. The detailed methods for these analyses are as follows:

HPLC/UV: The system utilized a Waters HPLC system and photodiode array (PDA) detector (Waters Corp., Milford, MA). The analytical column was a Phenomenex ODS(3) (250 mm × 4.6 mm, 5 µm particle size) column (Phenomenex, Torrance, CA). The isocratic mobile phase was 30:70 acetonitrile:0.1% formic acid (pH 3.0) delivered at a flow rate of 1 mL per minute. PDA detection (230 to 400 nm scanned) utilized extraction of the 260 nm wavelength (the UV maximum for genistein) for quantitation.

HPLC/MS System 1: The system consisted of a Hewlett-Packard HPLC (Hewlett-Packard, Palo Alto, CA) coupled to a Hewlett-Packard mass spectrometer operated in electrospray ionization mode with a Prodigy ODS(3) column (Phenomenex). The column parameters were 250 mm × 2.0 mm, 5 µm particle size, 100 Å. The mobile phase (flow rate of 0.2 mL per minute) consisted of A) acetonitrile and B) 3mM ammonium formate, changing linearly from 20%A:80%B to 80%A:20%B in 40 minutes, then held for 20 minutes. The single quadrupole was operated in a full scan mode from *m/z* 50 to *m/z* 450 in 0.5 seconds.

HPLC/MS System 2: The system 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 was scanned from *m/z* 150 to *m/z* 600 in 1 second.

GC/MS: Most of the samples were analyzed on a ThermoFinnigan mass spectrometer coupled to a Varian gas chromatograph (Varian Inc., Sunnyvale, CA) equipped with a DB-5ms capillary column (J&W Scientific, Folsom, CA). The column was 30 m × 0.25 mm, with a 0.25 µm film thickness. The oven was heated linearly from 80° C to 280° C at 20° C per minute, and the carrier gas helium was delivered at a constant pressure of 15 psi. The first quadrupole was scanned from *m/z* 50 to *m/z* 550 with a 0.5-second cycle time. Electron ionization at 70 eV was used.

Probe/MS: The analyses were performed on a ThermoFinnigan mass spectrometer. The samples, in methanol, were applied to the wire of the direct exposure probe. The solvent was allowed to evaporate in air, and the probe was inserted into the mass spectrometer for analysis. The probe was heated at 5 mA per second while the first quadrupole was scanned from *m/z* 50 to *m/z* 650 with a 0.5-second cycle time. Electron ionization at 70 eV was used.

HPLC/UV and HPLC/MS spectra agreed with the structure of genistein and matched the spectrum obtained from a purchased standard of genistein, indicating a purity of essentially 100%. GC/MS spectra indicated one major peak and minor impurities with a purity greater than 99%. Probe/MS testing indicated one major component with two minor components, suggesting little impurity. The overall purity of lot 2-BP-136-6 was determined to be greater than 99%.

To ensure stability, the bulk chemical was stored at –70° C, protected from light, in the original shipping containers. Purity was periodically measured during the study using the methods described above; no degradation of the bulk chemical was detected.

Background Isoflavone Content of Base Diet

The base diet used for the current study was an irradiated soy- and alfalfa-free rodent feed, designated 5K96, obtained from Purina Mills, Inc. (Richmond, IN), in an attempt to maintain consistently low background exposure to phytoestrogens. In some associated publications resulting from this study (Appendix P), this feed is referred to as NIH-31C because it maintains the nutritional specifications of the NIH-31 feed and contains casein. The composition of this diet and the results of the routine monitoring of the diet conducted throughout the study are presented in Appendix N. The control feed was routinely assayed for total isoflavone content after acid hydrolysis by the study laboratory using the two HPLC/MS methods described previously for bulk chemical purity analyses except that the first quadrupole of HPLC/MS System 1 was operated in specific ion monitoring mode using *m/z* 253 for daidzein and *m/z* 269 for genistein. The first quadrupole HPLC/MS System 2 was scanned from *m/z* 140 to *m/z* 450 in 1 second. Analyses of 10 consecutive lots of 5K96 feed by these methods indicated 0.417 ± 0.213 ppm genistein and 0.271 ± 0.161 ppm daidzein. These results were consistent with an earlier study of four lots of 5K96 feed assayed at the study laboratory using liquid chromatography/tandem mass spectrometry that yielded concentrations of 0.54 ± 0.31 ppm genistein and 0.48 ± 0.21 ppm daidzein (Doerge *et al.*, 2000). Animals consuming control feed were ingesting a concentration of genistein approximately 10-fold lower than that of the groups exposed to the lowest experimental exposure concentration, a concentration consistent with the isoflavone intake of individuals consuming typical Western diets.

Preparation and Analysis of Dose Formulations

The dose formulations were prepared every 5 weeks or as needed by mixing genistein with feed (Table C1). A premix of genistein and feed ground to a fine white powder using a mortar and pestle was layered with preweighed diet in a neoprene jar. The jar was capped and shaken, and the contents were dry mixed for 45 minutes with the remainder of the preweighed feed in a Patterson-Kelley twin-shell blender using an intensifier bar. Formulations were stored in stainless steel cans at 4° ± 2° C for up to 8.5 weeks.

Homogeneity (analysis of three samples each from the bottom, middle, and top of blends) and stability studies of a 5 ppm dose formulation using lot 1-BP-118-3 were conducted by the study laboratory as part of the reproductive dose range-finding study (NTP, 2007) using the HPLC/UV system described previously. Homogeneity was confirmed, and stability in stainless steel cans was confirmed for up to 17 days at ambient temperature and for up to 32 weeks at 4° ± 2° C.

Periodic analyses of the dose formulations of genistein (analysis of one sample each from the top, middle, and bottom of blends) were conducted by the study laboratory using the HPLC/UV system described previously. The dose formulations were analyzed at intervals of 1 to 4 weeks (Table C2). All 50 of the dose formulations analyzed and used in the study were within 10% of the target concentrations. Animal room samples of these dose formulations were analyzed at the end of the study; the 5 ppm formulation was within 10% of the target concentration, but the 100 and 500 ppm formulations were 16% and 11% below the target concentrations, respectively.

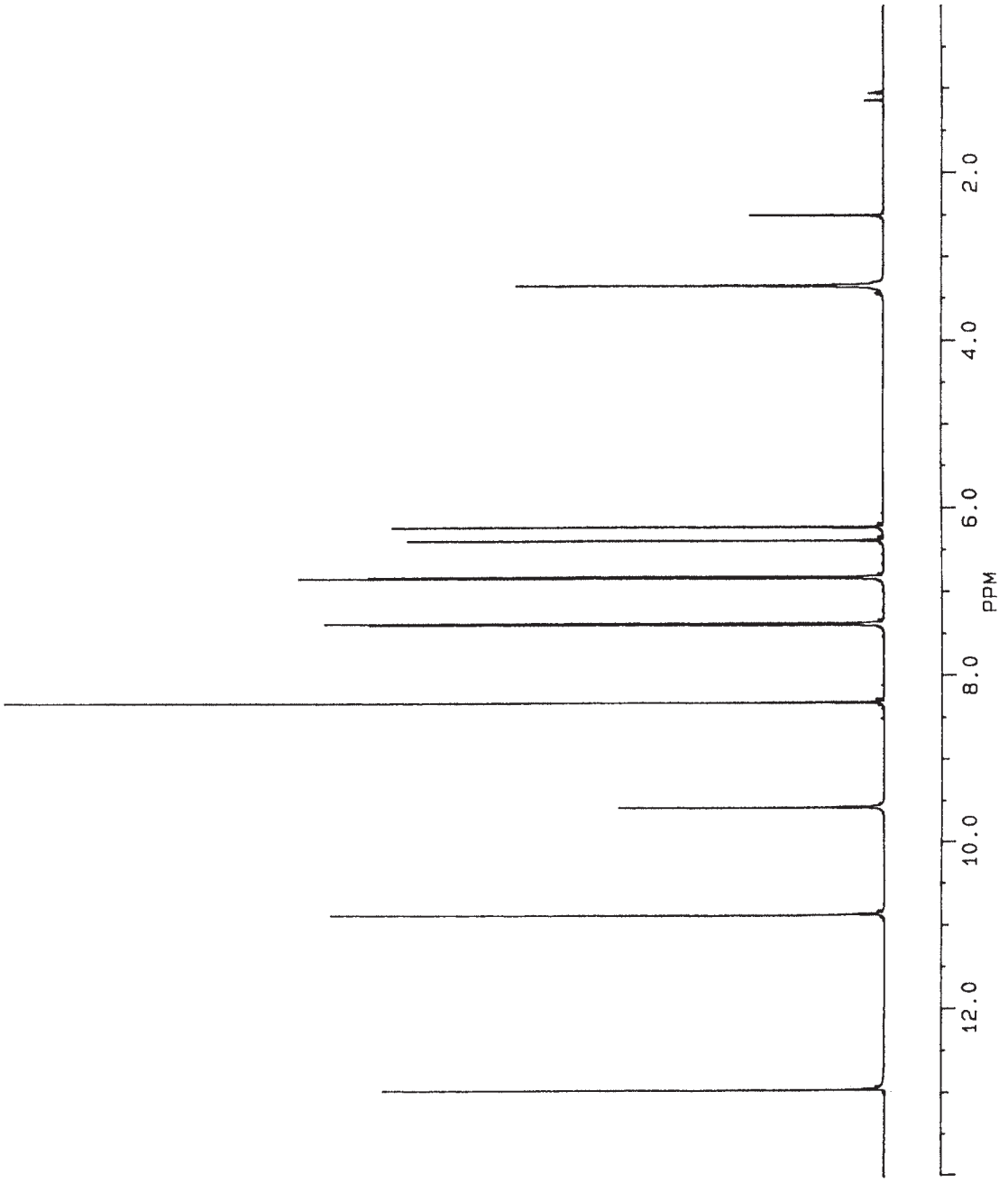

Figure C1
Proton Nuclear Magnetic Resonance Spectrum of Genistein

TABLE C1
Preparation and Storage of Dose Formulations in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Preparation
A premix of feed and genistein ground to a fine powder using a mortar and pestle was layered with preweighed feed in a neoprene jar. The jar was capped and shaken for 2 minutes, and the contents were dry mixed with the remainder of the preweighed feed in a Patterson-Kelley twin-shell blender with the intensifier bar on for 45 minutes. The dose formulations were prepared every 5 weeks or as needed.

Chemical Lot Number
2-BP-136-6

Maximum Storage Time
8.5 weeks

Storage Conditions
Dose formulations were stored in stainless steel cans secured with tie-downs at 4° ± 2° C.

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

TABLE C2
Results of Analyses of Dose Formulations Administered to Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Date Prepared	Target Concentration (ppm)	Determined Concentration[a] (ppm)	Difference from Target (%)
August 4, 1998	5	4.72 ± 0.10	–6
	100	91.7 ± 1.65	–8
	500	488.0 ± 14.0	–2
August 31, 1998	5	5.02 ± 0.23	0
September 18, 1998	5	4.96 ± 0.41	–1
	100	94.3 ± 2.90	–6
	500	498.8 ± 15.5	0
October 15, 1998	5	4.90 ± 0.07	–2
October 26, 1998	5	4.61 ± 0.13	–8
November 12, 1998	5	4.90 ± 0.21	–2
	100	90.0 ± 2.62	–10
	500	469.0 ± 10.5	–6
November 19, 1998	5	4.58 ± 0.19	–8
December 4, 1998	5	4.73 ± 0.10[b]	–5
	100	95.5 ± 1.7[b]	–5
	500	425.0 ± 8.0[c]	–15
December 7, 1998	5	4.75 ± 0.15[d]	–5
	100	91.4 ± 1.90[d]	–9
December 11, 1998	500	501 ± 13[d]	0
December 30, 1998	5	4.89 ± 0.17	–2
	5	4.98 ± 0.32	0
January 29, 1999	5	4.70 ± 0.07	–6
	5	4.94 ± 0.16	–1
	100	98.5 ± 1.4	–2
	500	479.0 ± 14.2	–4
February 12, 1999	5	4.99 ± 0.29	0
	5	5.34 ± 0.50	+7
	500	494.0 ± 4.5	–1
February 19, 1999	5	4.95 ± 0.17	–1
	5	5.17 ± 0.08	+3
February 25, 1999	100	91.0 ± 2.4	–9
	100	90.9 ± 3.0	–9
March 18, 1999	5	5.12 ± 0.32	+2
	500	498.0 ± 5.5	0
March 24, 1999	5	5.09 ± 0.06	+2
	100	91.1 ± 2.1	–9

TABLE C2
Results of Analyses of Dose Formulations Administered to Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Date Prepared	Target Concentration (ppm)	Determined Concentration (ppm)	Difference from Target (%)
April 7, 1999	5	4.67 ± 0.13	–7
	5	4.84 ± 0.07	–3
	5	4.80 ± 0.09	–4
	100	102.0 ± 0.2	+2
	500	479.0 ± 1.0	–4
April 16, 1999	5	4.83 ± 0.14	–3
April 27, 1999	5	4.66 ± 0.05	–7
	5	4.62 ± 0.04	–8
May 11, 1999	5	5.44 ± 0.16	+9
	500	465.0 ± 1.7	–7
May 20, 1999	5	4.61 ± 0.40	–8
	100	94.8 ± 0.20	–5
	100	104.6 ± 1.30	+5
June 4, 1999	5	4.84 ± 0.26	–3
	500	493.0 ± 14.7	–1
Animal room samples[e]	5	5.20 ± 0.33	+4
	100	83.9 ± 6.1	–16
	500	446 ± 14.6	–11

[a] Results of triplicate analyses (mean ± standard deviation)
[b] Remixed; used in study
[c] Remixed; not used in study
[d] Results of remix of dose formulations from December 4, 1998
[e] Results of quadruplicate analyses (mean ± standard deviation). Animal room samples were sampled on June 1, 1999.

APPENDIX D
BODY WEIGHTS

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
6*	152.6 ± 2.7	155.4 ± 2.8	154.4 ± 2.9	147.4 ± 2.8
7**	184.2 ± 2.9	180.4 ± 3.6 (24)	183.0 ± 3.4	172.8 ± 3.3*
8***	206.9 ± 2.9	207.1 ± 3.1	204.1 ± 3.7	193.1 ± 3.3**
9***	224.4 ± 3.1	228.9 ± 3.5	220.6 ± 3.2	207.0 ± 3.5***
10***	244.8 ± 3.7	247.6 ± 3.6	239.6 ± 3.5	224.2 ± 3.5***
11***	264.3 ± 3.5	270.9 ± 4.5	260.2 ± 4.9	237.9 ± 4.0***
12***	304.0 ± 4.0	309.4 ± 4.3	297.2 ± 3.6	270.1 ± 4.5***
13***	357.9 ± 4.4	369.1 ± 6.5	352.9 ± 5.6	319.3 ± 5.0***
16***	290.7 ± 4.0 (23)	305.6 ± 4.7 (22)	290.6 ± 5.0	271.8 ± 4.0 (22)
17***	280.2 ± 4.0	291.4 ± 3.8	277.3 ± 4.0	259.2 ± 4.6**
18***	272.1 ± 2.9	275.1 ± 3.0 (24)	265.5 ± 2.7	251.8 ± 3.1**
19***	278.4 ± 3.0 (24)	282.9 ± 3.1	271.8 ± 2.7	255.4 ± 3.4***

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

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the age column indicate significant linear and quadratic exposure concentration trends, respectively, at that week in that generation as determined by contrasts; 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: * or #, $P \leq 0.05$; ** or ##, $P \leq 0.01$; ***, $P \leq 0.001$.

[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 exposure 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 and the interaction between the F_0 breed mother and F_0 breed father were significant at $P<0.50$ but could not be included in the model due to computational unfeasibility.
2) Dam predelivery (birth to week 13) body weights, F_1 to F_4: dose, $P<0.001$; generation, $P=0.028$; dose × generation, $P=0.002$; 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 breed parents were included in the statistical model.
3) Dam postdelivery (weeks 16 to 19) body weights, F_0 to F_4: dose, $P<0.001$; generation, $P=0.002$; dose × generation, $P<0.001$; weeks, $P<0.001$; weeks × dose, $P=0.039$; weeks × generation, $P<0.001$; weeks × dose × generation, $P=0.595$. Random effects of the F_0 breed father 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.

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3***	40.6 ± 1.0 (23)	41.1 ± 0.8 (23)	38.8 ± 1.0	35 ± 1.2***
4	70.8 ± 1.7 (22)	70 ± 1.4	66.5 ± 1.6	65.1 ± 2.4
5***	108.9 ± 2.2 (24)	108.6 ± 2.0	103.7 ± 2.5	97.3 ± 2.6**
6***	146.9 ± 3.1 (24)	144.9 ± 2.3	138.6 ± 3.2	128.8 ± 2.4***
7***	170.0 ± 3.6 (24)	169.0 ± 2.4	160.3 ± 3.5	152.6 ± 2.8***
8***	199.5 ± 3.9 (23)	197.6 ± 3.1	186.8 ± 3.8*	168.2 ± 2.4***
9***	220.0 ± 3.7 (24)	214.1 ± 2.8	207.0 ± 4.0*	184.5 ± 2.6***
10***	234.5 ± 4.5 (24)	231.3 ± 3.6	223.3 ± 4.5	197.2 ± 2.7***
11***	252.0 ± 4.7 (24)	246.2 ± 4.2 (24)	238.7 ± 4.7*	206.4 ± 3.0*** (24)
12***	265.8 ± 5.4 (24)	255.9 ± 4.5	250.9 ± 5.1*	216.7 ± 3.6***
13***	301.1 ± 7.1 (24)	289.1 ± 6.2	281.2 ± 6.8*	239.4 ± 4.5***
16***	312.7 ± 6.2 (23)	307.4 ± 5.4 (24)	298 ± 7.3	252.4 ± 3.2*** (24)
17***	299.8 ± 5.4 (23)	300.8 ± 5.9	295.4 ± 5.3	255.2 ± 3.3*** (23)
18***	293.2 ± 4.7 (24)	290 ± 4.6	278.9 ± 5.0 (24)	251.3 ± 3.5***
19***	287 ± 4.3 (24)	284.7 ± 4.5	278.5 ± 4.2	243.2 ± 3.3*** (24)

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3*	37.1 ± 1.1	38.5 ± 1.3	39.3 ± 0.8	35.6 ± 0.9
4	60.6 ± 2.3	60.0 ± 2.3	62.6 ± 2.1	61.4 ± 2.6
5	97.0 ± 3.1	95.4 ± 3.1	100.0 ± 2.6	96.9 ± 3.2
6	133.8 ± 3.1	131.7 ± 3.5	137.0 ± 2.7	130.0 ± 3.4
7	161.4 ± 3.5	159.0 ± 3.7	164.5 ± 2.8	156.1 ± 3.6
8	187.3 ± 3.5	184.5 ± 4.0	188.5 ± 3.1	179.7 ± 3.4
9**	210.5 ± 3.8 (24)	206.6 ± 4.1	209.2 ± 3.5	197.3 ± 3.1*
10***	228.7 ± 3.8	224.0 ± 4.2	225.9 ± 3.7 (24)	210.3 ± 3.3*** (24)
11***	240.5 ± 3.2	235.1 ± 4.2 (24)	237.1 ± 3.6 (24)	219.3 ± 3.3** (22)
12***	254.3 ± 4.0	248.6 ± 4.3	254.5 ± 4.0 (24)	227 ± 3.4***
13***	277.9 ± 4.4	275.7 ± 4.9	279.0 ± 3.9	250.6 ± 3.5***
16**	300.1 ± 5.1 (22)	301.2 ± 5.9 (21)	294.2 ± 4.2 (18)	279.9 ± 5.1* (22)
17***	296.0 ± 4.8	296.9 ± 4.7	287.6 ± 5.4	274.8 ± 5.4** (24)
18**	293.2 ± 5.5 (24)	286.7 ± 5.2 (24)	282.3 ± 4.7	271.6 ± 4.2**
19***	270.3 ± 3.6	269.6 ± 3.2	267.4 ± 3.0	252.1 ± 3.4**

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3**	37.9 ± 0.9	39.0 ± 1.0	37.7 ± 0.7	35.1 ± 1.0
4	65.1 ± 1.5	63.8 ± 1.9	64.1 ± 1.1	61.6 ± 1.6
5	101.6 ± 1.9	99.8 ± 2.3	99.1 ± 1.6	95.9 ± 2.3
6	140.3 ± 2.2	137.1 ± 2.6	135.5 ± 2.2	131.9 ± 2.5
7	167.8 ± 2.7	164.5 ± 2.8	162.5 ± 2.5	159.1 ± 2.7
8*	190.5 ± 3.0	192.4 ± 3.0	187.6 ± 2.7	183.0 ± 3.1
9	210.6 ± 3.2	212.7 ± 3.0	207.1 ± 2.9	203.9 ± 3.7
10	226.9 ± 3.3	230.0 ± 3.3	221.4 ± 3.0	219.6 ± 3.9
11	244.1 ± 3.7	248.3 ± 3.4	241.7 ± 3.6	237.5 ± 3.9
12	251.9 ± 3.6	254.6 ± 3.3	248.6 ± 4.0	245.1 ± 4.3
13*	275.9 ± 3.4	280.2 ± 4.3	268.5 ± 5.0	264.3 ± 4.8
16	303.6 ± 4.4	311.4 ± 3.9 (24)	297.1 ± 5.2 (24)	295.7 ± 5.5 (23)
17	305.2 ± 5.5	317.0 ± 4.9 (23)	303.1 ± 5.6	296.8 ± 4.6 (24)
18	288.2 ± 5.8	293.4 ± 6.4	286.8 ± 5.6	281.2 ± 4.9
19 # #	270.9 ± 3.2	275.7 ± 3.6	260.2 ± 3.1	265.9 ± 4.8

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3***	38.8 ± 0.9	40.8 ± 1.2	38.0 ± 1.0	35.4 ± 1.1
4**	61.8 ± 2.2	66.2 ± 3.3	58.9 ± 1.3	55.8 ± 1.4
5**	97.3 ± 2.6	102.7 ± 4.2	94.0 ± 1.4	89.9 ± 1.8
6**	134.9 ± 2.7	138.3 ± 4.1	131.7 ± 1.8	125.6 ± 2.1
7**	163.4 ± 2.7	165.9 ± 3.9	161.2 ± 2.2	153.2 ± 2.5
8**	189.5 ± 3.0 (24)	189.7 ± 3.9	186.3 ± 2.6	177.3 ± 3.0*
9***	210.4 ± 3.3	209.7 ± 3.9	204.4 ± 3.0	196.3 ± 3.2**
10**	225.7 ± 3.7	221.7 ± 3.0	221.3 ± 3.3	212.0 ± 3.8*
11**	228.8 ± 4.6	226.1 ± 4.1 (24)	217.9 ± 3.3 (24)	214.7 ± 3.9* (24)
12	253.4 ± 4.7 (24)	245.8 ± 4.8	240.4 ± 3.7	239.6 ± 4.0 (24)
13	280.1 ± 4.7	276.6 ± 4.8	265.3 ± 3.3	265.7 ± 4.9 (24)
16**, #	299.9 ± 5.0	298.9 ± 4.5	286.3 ± 3.7*	279.9 ± 4.6**
17**	300.6 ± 5.8 (23)	300.2 ± 5.1 (24)	290.4 ± 4.3 (24)	283.7 ± 4.9* (24)
18**	300.7 ± 5.9 (23)	295.9 ± 3.6	287.4 ± 4.7*	279.9 ± 4.6** (22)
19*	279.8 ± 4.4	273.9 ± 3.1	271.1 ± 3.3	266.6 ± 4.4*

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 Genistein[a,b]

Generation	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_1	PND 2	6.5 ± 0.3 (24)	5.9 ± 0.1	6.1 ± 0.3	6.0 ± 0.2
	PND 4	8.2 ± 0.3 (23)	8.0 ± 0.2	7.8 ± 0.2	7.8 ± 0.3
	PND 7	13.4 ± 0.5 (24)	12.8 ± 0.3	12.4 ± 0.4	11.8 ± 0.4
	PND 14***	27.1 ± 0.6 (24) [2]	27.3 ± 0.6	25.6 ± 0.7	23.9 ± 0.7**
	PND 21***	40.6 ± 1.0 (23) [2, 3, 4]	41.1 ± 0.8 (23) [2]	38.8 ± 1.0	34.8 ± 1.0***
F_2	PND 2	6.0 ± 0.1	6.2 ± 0.1	6.2 ± 0.2 (24)	6.2 ± 0.2 (24)
	PND 4	7.5 ± 0.2	7.7 ± 0.3	8.0 ± 0.2	7.8 ± 0.2
	PND 7	11.6 ± 0.5	12.0 ± 0.6	12.6 ± 0.4	11.6 ± 0.4 (24)
	PND 14##	24.5 ± 0.9 [1]	25.1 ± 1.0	26.6 ± 0.6*	23.6 ± 0.6
	PND 21***, #	36.8 ± 1.1 [1]	38.2 ± 1.2 [1]	38.3 ± 0.8	34.6 ± 0.8*

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

Generation	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_3	PND 2	5.9 ± 0.1	6.0 ± 0.1	5.8 ± 0.1	6.3 ± 0.1
	PND 4	7.7 ± 0.2	7.8 ± 0.2	7.6 ± 0.2	8.1 ± 0.2
	PND 7	11.7 ± 0.4	12.3 ± 0.4	12.4 ± 0.3	12.4 ± 0.4
	PND 14	25.2 ± 0.7	26.1 ± 0.7	25.6 ± 0.5	24.2 ± 0.6
	PND 21***	37.6 ± 0.9 [1]	38.9 ± 1.0	37.7 ± 0.7	34.2 ± 0.8 ** (23)
F_4	PND 2	6.2 ± 0.1	6.6 ± 0.2	6.0 ± 0.2	5.9 ± 0.2 (24)
	PND 4	7.9 ± 0.2	8.2 ± 0.2	8.0 ± 0.2	7.2 ± 0.2
	PND 7	12.3 ± 0.4	12.8 ± 0.4	13.1 ± 0.4 (24)	11.3 ± 0.3
	PND 14**	25.5 ± 0.5	26.3 ± 0.6	26.2 ± 0.7	23.7 ± 0.6
	PND 21***	38.1 ± 0.7 [1]	39.6 ± 0.9	37.9 ± 1.0	35.4 ± 1.1**

ANOVA results (P values for main effects and their interactions): dose, P=0.015; generation, P=0.266; dose × generation, P=0.775; days, P<0.001; days × dose, P<0.001; days × generation, P=0.005; days × dose × generation, P=0.120.

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the age column indicate significant linear and quadratic, respectively, at that age in that exposure concentration trends within a generation as determined by contrasts; 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: * or #, P≤0.05; ** or ##, P≤0.01; ***, P≤0.001. Significant differences (P≤0.05) between generations within an exposure group on a given day are indicated by generation numbers in brackets.

[b] There was a significant (P<0.50) random F_0 breed mother effect determined by a log-likelihood ratio test that was incorporated into the statistical model.

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
6	203.4 ± 4.8	201.3 ± 4.5	199.1 ± 3.9	201.9 ± 5.0
7	263.8 ± 5.4	258.7 ± 5.0	257.2 ± 4.0	264.4 ± 5.7
8	306.3 ± 5.6	295.9 ± 4.8	298.1 ± 3.6	298.6 ± 4.5
9	333.0 ± 6.5	324.5 ± 5.6	328.0 ± 4.5	335.6 ± 5.5 (24)
10	375.3 ± 6.1	365.9 ± 7.1	367.2 ± 4.8 (24)	392.8 ± 5.2 (15)
11	361.0 ± 6.9	347.8 ± 5.7	347.2 ± 5.5	362.1 ± 5.6
12	380.3 ± 7.0 (24)	367.8 ± 5.2	363.8 ± 5.4	382.6 ± 6.4
13	384.0 ± 6.5 (24)	372.1 ± 5.9	368.2 ± 4.3	381.5 ± 5.0
14 #	400.3 ± 7.2 (24)	393.6 ± 6.2	381.0 ± 4.8	398.0 ± 5.3
15	404.2 ± 6.7 (24)	399.2 ± 6.3	393.5 ± 4.5	401.7 ± 4.7
16 #	432.3 ± 6.9 (24)	415.6 ± 5.9	407.7 ± 4.8**	420.7 ± 4.5
17	434.1 ± 6.9 (24)	422.8 ± 6.3	418.5 ± 5.2	428.4 ± 5.0
18	429.3 ± 6.6 (24)	418.3 ± 6.4	415.2 ± 5.6	426.5 ± 5.1
19	434.9 ± 7.4 (24)	428.2 ± 6.5	426.2 ± 4.5	430.7 ± 5.1

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

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

[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 exposure 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. The ANOVA results for each analysis were as follows:

1) Male body weights, weeks 6 to 19, F_0 to F_4: dose, P=0.004; generation, P=0.009; dose × generation, P=0.156; weeks, P<0.001; weeks × dose, P<0.001; weeks × generation, P<0.001; weeks × dose × generation, P<0.001. The random effect of the F_0 breed father was significant at P<0.50 and was included in the model.
2) Male body weights, birth to week 19, F_1 to F_4: dose, P=0.004; generation, P=0.012; dose × generation, P=0.258; 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 breed parents were included in the statistical model.

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3	44.5 ± 0.7 (26)	42.6 ± 0.9	39.7 ± 0.8	37.8 ± 1.3 (24)
4	78.9 ± 1.9 (26)	74.6 ± 1.9 (24)	70.5 ± 1.6	69.5 ± 2.8
5	126.4 ± 2.6 (26)	122.6 ± 3.7	116.7 ± 2.4	117.9 ± 4.0
6	182.7 ± 3.4	174.8 ± 3.8 (24)	170.6 ± 2.9*	168.9 ± 4.7*
7	233.5 ± 3.5	224.1 ± 4.4	218.7 ± 3.4*	216.8 ± 5.2*
8*, #	288.7 ± 3.8 (26)	273.6 ± 5.2	268.1 ± 3.4**	263.9 ± 5.6**
9*	331.2 ± 3.9 (26)	317.2 ± 5.8	313.5 ± 3.8*	309.1 ± 5.9*
10	362.0 ± 3.7 (26)	351.2 ± 6.0	346.8 ± 4.3	340.1 ± 6.0* (24)
11**	398.6 ± 4.1 (26)	388.6 ± 7.0	382.8 ± 5.6	372.2 ± 5.9**
12**	410.0 ± 5.1 (26)	396.3 ± 7.7	387.4 ± 5.2*	379.0 ± 6.0**
13*	399.3 ± 4.8 (26)	391.9 ± 8.6	383.1 ± 5.8	375.6 ± 7.1*
14*	407.9 ± 4.2 (26)	396.4 ± 8.0	388.2 ± 5.7*	384.0 ± 6.1*
15# #	417.9 ± 4.2 (26)	410.0 ± 7.2	390.9 ± 5.2** (17)	398.9 ± 6.2
16 #	421.4 ± 5.4	423.5 ± 7.9 (24)	405.6 ± 5.9	411.3 ± 5.3
17 # #	435.1 ± 4.9 (26)	434.0 ± 8.6 (20)	415.8 ± 5.1*	419.9 ± 6.0 (21)
18**, #	458.0 ± 4.8	451.7 ± 7.6	435.7 ± 5.7*	429.7 ± 5.8**
19*, #	455.4 ± 4.5 (26)	444.8 ± 7.6	431.4 ± 5.0**	429.4 ± 5.6**

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3	41.9 ± 0.8	41.3 ± 0.7	40.1 ± 0.7	36.5 ± 0.9
4	80.2 ± 2.0	77.7 ± 1.4	70.8 ± 2.5	73.9 ± 2.8
5	124.3 ± 3.5	126.3 ± 2.4	116.9 ± 3.1	123.0 ± 3.8
6 # #	182.3 ± 3.6	183.4 ± 3.4	169.4 ± 4.1*	174.4 ± 4.4
7 #	234.1 ± 4.2	237.8 ± 4.0	223.8 ± 3.9 (24)	229.3 ± 5.2
8 # #	286.3 ± 4.4	293.0 ± 4.8	273.1 ± 5.3	283.4 ± 5.8
9 #	326.4 ± 3.9	333.3 ± 5.6	316.0 ± 5.4	323.0 ± 5.6
10	359.5 ± 5.1	371.3 ± 6.7 (24)	354.2 ± 5.4	357.6 ± 5.6
11#	396.5 ± 5.2 (24)	403.7 ± 6.9	384.4 ± 6.1	390.1 ± 6.2 (24)
12	407.3 ± 4.8	415.8 ± 7.4	398.3 ± 7.0	402.0 ± 5.7
13	405.1 ± 5.1	416.6 ± 6.2	404.1 ± 6.8	408.9 ± 5.3
14	391.3 ± 4.7	408.5 ± 8.3	396.2 ± 7.8	389.1 ± 6.3
15 #	428.9 ± 5.1	442.0 ± 6.8	420.2 ± 6.8	424.2 ± 5.5
16	414.2 ± 4.2	431.3 ± 6.1	410.5 ± 6.6	417.0 ± 5.1
17 # #	435.7 ± 4.7	446.9 ± 6.1	421.2 ± 7.8	433.5 ± 4.2
18 #	442.3 ± 4.1	456.6 ± 5.6	432.9 ± 8.2	447.2 ± 4.7
19 #	452.3 ± 5.0	464.4 ± 5.8	442.0 ± 8.0 (24)	456.1 ± 4.7 (24)

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3	41.0 ± 0.8	40.1 ± 1.0a	38.3 ± 0.8	37.5 ± 1.1
4	75.3 ± 1.4	73.9 ± 2.3	75.4 ± 1.4	74.7 ± 1.9
5	123.3 ± 2.1	121.0 ± 3.5	123.1 ± 2.4	122.0 ± 2.9
6	178.4 ± 2.4	174.3 ± 4.3 (24)	178.2 ± 3.2	178.4 ± 3.2
7	235.0 ± 3.3 (24)	222.7 ± 5.7	230.8 ± 3.6	227.1 ± 3.7 (23)
8	296.4 ± 3.5	285.2 ± 6.7	293.2 ± 3.7	288.1 ± 5.4 (18)
9	334.6 ± 4.2 (22)	317.4 ± 9.0 (17)	331.1 ± 5.1 (21)	314.3 ± 5.3*
10	364.0 ± 5.0	342.2 ± 7.0*	356.2 ± 6.0	348.5 ± 6.2
11	388.0 ± 4.4	370.4 ± 7.7	383.5 ± 6.6	368.6 ± 7.7
12	397.5 ± 4.7 (24)	381.7 ± 7.6	397.2 ± 5.6	390.0 ± 6.6
13	397.3 ± 5.1	392.7 ± 7.7	400.1 ± 5.0	397.3 ± 7.1
14	410.8 ± 5.1	401.8 ± 8.0	405.6 ± 4.9	405.9 ± 6.2
15	411.3 ± 4.3	404.1 ± 7.6 (24)	421.1 ± 5.2	414.3 ± 5.2
16	415.5 ± 4.9	404.1 ± 7.8	414.6 ± 4.1	413.8 ± 6.2
17	410.4 ± 4.1	402.2 ± 6.8	402.5 ± 3.9	405.7 ± 6.2
18	443.7 ± 4.8	432.2 ± 7.5	439.6 ± 5.2 (24)	431.5 ± 6.7 (24)
19*	443.6 ± 4.9	434.5 ± 7.2	437.5 ± 5.2	423.6 ± 5.5*

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
3	39.7 ± 1.1	42.9 ± 1.0	39.0 ± 0.9	37.1 ± 1.1
4	85.0 ± 2.2 (18)	86.5 ± 3.1 (17)	80.0 ± 2.3 (14)	76.0 ± 3.0 (15)
5	120.9 ± 4.8	124.5 ± 4.8	112.5 ± 4.6	110.3 ± 4.5
6*	174.6 ± 5.8	176.4 ± 5.4	165.9 ± 5.1	162.9 ± 5.0
7	228.2 ± 6.4	223.9 ± 7.0	217.8 ± 6.0	215.1 ± 5.7
8*	282.5 ± 6.8	281.7 ± 6.9	272.6 ± 6.8	268.2 ± 6.3
9	327.5 ± 6.5	325.3 ± 6.9	318.9 ± 6.8	314.3 ± 6.0
10	362.1 ± 6.9	360.8 ± 7.4	353.3 ± 7.7	348.0 ± 7.0 (24)
11	387.1 ± 6.8	388.9 ± 7.3	381.9 ± 7.6	375.0 ± 6.6
12	398.3 ± 7.0	398.3 ± 6.8	397.5 ± 7.5	388.1 ± 7.6
13	387.6 ± 7.5	391.2 ± 6.8	380.7 ± 7.2	383.7 ± 6.1
14*	401.7 ± 7.6	399.7 ± 6.5	386.7 ± 7.3	381.6 ± 7.0
15	399.3 ± 7.5	404.1 ± 5.5	397.1 ± 6.4	395.8 ± 6.1
16	411.1 ± 7.6	414.5 ± 6.8	406.1 ± 6.5	404.7 ± 6.9
17	428.0 ± 8.1	422.3 ± 5.6	417.3 ± 6.5	415.7 ± 6.2
18	440.2 ± 7.8	431.5 ± 6.5	426.9 ± 7.1	424.5 ± 6.4
19	434.3 ± 7.2	427.1 ± 6.2	425.1 ± 6.8	427.2 ± 5.8

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 Genistein[a,b]

Generation	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_1	PND 2	6.8 ± 0.1 (26)	6.1 ± 0.1	6.2 ± 0.1 (24)	6.7 ± 0.2
	PND 4	9.2 ± 0.2 (24)	8.5 ± 0.2	8.2 ± 0.2 (24)	9.0 ± 0.3
	PND 7	15.1 ± 0.3 (26)	14.0 ± 0.3	13.5 ± 0.3	13.3 ± 0.3
	PND 14***, # #	30.1 ± 0.5 (26) [2, 3, 4]	28.6 ± 0.6	26.5 ± 0.6***	25.7 ± 0.7***
	PND 21***, # # #	44.5 ± 0.7 (26) [2, 3, 4]	42.3 ± 0.8* [3]	39.6 ± 0.8***	38.8 ± 1.0*** (22)
F_2	PND 2	6.4 ± 0.2	6.5 ± 0.1	6.6 ± 0.2 (22)	6.8 ± 0.1 (24)
	PND 4	8.5 ± 0.3	8.7 ± 0.3	8.4 ± 0.3	9.0 ± 0.2
	PND 7	13.2 ± 0.3	13.9 ± 0.4	13.2 ± 0.5 (23)	13.2 ± 0.3 (24)
	PND 14***	27.4 ± 0.5 [1]	28.2 ± 0.7	27.4 ± 0.6	25.3 ± 0.6*
	PND 21***	41.2 ± 0.7 [1]	41.3 ± 0.7	39.9 ± 0.7	36.5 ± 0.9***

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

Generation	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_3	PND 2	6.4 ± 0.1	6.4 ± 0.1	6.3 ± 0.2	6.9 ± 0.2
	PND 4	8.5 ± 0.2	8.4 ± 0.2	8.3 ± 0.2	8.9 ± 0.3
	PND 7	13.2 ± 0.4	13.1 ± 0.4	13.2 ± 0.4	13.4 ± 0.4
	PND 14	27.2 ± 0.5 [1]	27.1 ± 0.8	26.5 ± 0.6	25.5 ± 0.6
	PND 21***	40.9 ± 0.8 [1]	39.9 ± 1.0 (24) [1, 4]	38.3 ± 0.8**	36.6 ± 0.9*** (23)
F_4	PND 2	6.8 ± 0.1	6.7 ± 0.2	6.3 ± 0.1	6.3 ± 0.2 (24)
	PND 4	8.9 ± 0.3	8.9 ± 0.3	8.2 ± 0.2	8.0 ± 0.2
	PND 7	13.8 ± 0.5	14.2 ± 0.4	13.1 ± 0.4 (23)	12.5 ± 0.3
	PND 14**	26.8 ± 0.7 [1]	28.8 ± 0.6	26.4 ± 0.5	25.5 ± 0.5
	PND 21***, #	39.7 ± 1.1 [1]	42.9 ± 1.0** [3]	39 ± 0.9	37.1 ± 1.0**

ANOVA results (P values for main effects and their interactions): dose, P<0.001; generation, P=0.109; dose × generation, P=0.434; days, P< 0.001; days × dose, P<0.001; days × generation, P<0.001; days × dose × generation, P=0.046.

[a] Mean body weight (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the age column indicate significant linear and quadratic exposure concentration trends, respectively, at that age in that generation as determined by contrasts; 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: * or #, P≤0.05; ** or ##, P≤0.01; *** or ###, P≤0.001. Significant differences (P≤0.05) between generations within an exposure group on a given day are indicated by generation numbers in brackets.

[b] There was a significant (P<0.50) random F_0 breed mother effect determined by a log-likelihood ratio test that was incorporated into the statistical model.

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

Generations covered[b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
$F_0 - F_4$ Dose P<0.001 Gen P<0.001 DxG P<0.001	F_0***	205.2 ± 4.6 [1, 2, 3, 4]	213.7 ± 5.5 [1, 2, 3, 4]	198.5 ± 5.0 [1, 2, 3, 4]	171.9 ± 5.0*** [1, 2, 3, 4]
	F_1***	154.2 ± 6.0 (24) [0, 3]	144.2 ± 5.9 [0]	142.6 ± 5.0 [0]	110.7 ± 4.5*** [0]
	F_2***	144.1 ± 3.4 [0]	144.0 ± 3.2 [0]	142.0 ± 3.1 [0]	120.6 ± 2.2*** [0]
	F_3	135.6 ± 2.7 [0, 1]	143.1 ± 3.7 [0]	133.1 ± 3.6 [0]	132.5 ± 3.6 [0]
	F_4	145.3 ± 3.1 [0]	138.3 ± 2.6 [0]	133.6 ± 2.3 [0]	139.5 ± 4.2 (24) [0]
$F_1 - F_4$ Dose P<0.001 Gen P=0.063 DxG P<0.001	F_1***	294.6 ± 7.1 (24) [2, 3, 4]	283.2 ± 6.2	275.1 ± 6.7*	233.4 ± 4.4*** [3, 4]
	F_2***	272.0 ± 4.4 [1]	269.5 ± 4.9	272.7 ± 3.8	244.4 ± 3.4***
	F_3	270.0 ± 3.4 [1]	274.1 ± 4.2	262.8 ± 5.0	258.0 ± 4.8 [1]
	F_4	273.9 ± 4.7 [1]	269.9 ± 4.8	259.2 ± 3.2	256.8 ± 4.0 (23) [1]

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

[b] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G). 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 the F_0 breed mother and the F_0 breed father were included in the statistical model.

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

ANOVA Results[b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
F_0 - F_4 Dose P<0.001 Gen P<0.001 DxG P=0.392	F_0	-12.9 ± 3.5 (22) [2, 3]	-21.6 ± 3.3 (22) [3]	-18.8 ± 4.2 [3]	-18.1 ± 2.5 (22) [3]
	F_1***	-25.5 ± 5.0 (23)	-24.1 ± 3.4 (24)	-19.5 ± 4.4 [3]	-9.7 ± 3.7** (23) [2, 3]
	F_2	-28.4 ± 4.4 (22) [0]	-32.5 ± 4.1 (21)	-25.3 ± 4.9 (18)	-27.2 ± 2.9 (22) [1, 4]
	F_3	-32.7 ± 3.0 [0]	-37.5 ± 2.9 (24) [0]	-37.5 ± 3.9 (24) [0, 1, 4]	-33.7 ± 3.5 (23) [0, 1, 4]
	F_4*	-20.1 ± 2.2	-24.9 ± 3.4	-15.2 ± 2.6 [3]	-13.3 ± 3.0 [2, 3]

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

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

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

Sex[b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Female Dose P<0.001 Gen P=0.014 DxG P=0.885	F_1***	34.1 ± 1.0 (23)	35.2 ± 0.7 (23)	32.7 ± 1.0	28.8 ± 0.9***
	F_2*	30.9 ± 1.0	32.0 ± 1.1	32.0 ± 0.8 (24)	28.4 ± 0.8 (24)
	F_3***	31.7 ± 0.8	32.9 ± 0.9	32.0 ± 0.7	28.0 ± 0.8* (23)
	F_4**	32.0 ± 0.7	33.0 ± 0.8	31.8 ± 1.0	29.3 ± 1.0* (24)
Male Dose P<0.001 Gen P<0.001 DxG P=0.264	F_1***, # #	37.6 ± 0.7 (26) [2, 3, 4]	36.2 ± 0.8 [3]	33.5 ± 0.8*** (24)	32.0 ± 0.9*** (22)
	F_2***	34.8 ± 0.7 [1]	34.8 ± 0.7	33.3 ± 0.7 (22)	29.6 ± 0.8*** (24)
	F_3**	34.6 ± 0.8 [1]	33.5 ± 0.9 (24) [1, 4]	31.9 ± 0.7	29.7 ± 0.9* (23)
	F_4***	32.9 ± 1.1 [1]	36.2 ± 0.8 [3]	32.7 ± 0.9	30.6 ± 1.0* (24)

[a] Mean body weight gain (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the generation column indicate significant linear and quadratic exposure concentration trends, respectively, within that generation as determined by contrasts; asterisks in shaded cells in the exposed group columns indicate significant difference from controls in the same generation as determined by Dunnett's test: *, P≤0.05; ** or ## P≤0.001 ***, P≤0.001. Significant differences (P≤0.05) between generations within an exposure group are indicated by generation numbers in brackets.

[b] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G). For both males and females, there was a significant (P<0.50) random F_0 breed mother effect that was incorporated into the statistical model.

TABLE D8
Total Body Weight Gains of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

Generations covered[b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
$F_0 - F_4$ Dose P=0.570 Gen P<0.001 DxG P=0.126	F_0	231.5 ± 5.8 (24)	226.9 ± 5.0	227.0 ± 3.9	228.8 ± 4.0
	F_1	273.5 ± 4.3	270.7 ± 6.4 (24)	260.7 ± 5.3	260.5 ± 5.5
	F_2	270.0 ± 5.0	281.0 ± 4.8	271.9 ± 7.7 (24)	281.8 ± 5.1 (24)
	F_3**	265.2 ± 5.1	262.1 ± 6.0 (24)	259.3 ± 4.6	245.2 ± 4.7*
	F_4	259.6 ± 7.3	250.7 ± 5.7	259.2 ± 5.7	264.3 ± 5.8
$F_1 - F_4$ Dose P=0.004 Gen P<0.001 DxG P=0.135	F_1**, #	448.6 ± 4.5 (26)	438.6 ± 7.5	424.7 ± 5.1* (24)	422.8 ± 5.6**
	F_2#	445.9 ± 5.0	457.8 ± 5.7	435.2 ± 7.9 (24)	449.2 ± 4.7 (24)
	F_3*	437.3 ± 4.8	428.0 ± 7.2	431.2 ± 5.2	416.8 ± 5.4*
	F_4	427.5 ± 7.2	420.3 ± 6.1	418.8 ± 6.8	420.1 ± 6.0 (24)

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

[b] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G). 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. No random effects for the F_0 breed parents were included in the statistical models.

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

Sex[b]	Generation	Dietary Genistein (ppm)				Trends	
		0	5	100	500	Linear	Quad
Female Dose P<0.001 Gen P=0.090 DxG P<0.001	F_0	268.7 ± 3.1	275.3 ± 3.1 [2, 3, 4][c]	266.3 ± 3.1	245.6 ± 3.7***	***	-
	F_1	272.6 ± 4.4 (24)	269.5 ± 4.0	262.7 ± 4.5	234.8 ± 3.0*** [2, 3, 4]	***	-
	F_2	265.7 ± 2.6	263.8 ± 3.2 [0]	264.0 ± 2.8	250.4 ± 3.7* [1]	*	-
	F_3	262.1 ± 3.2	264.2 ± 2.6 [0]	256.0 ± 2.8	255.2 ± 3.4 [1]	-	-
	F_4	262.1 ± 3.9	261.4 ± 2.9 [0]	255.9 ± 2.8	251.2 ± 3.3 [1]	-	-
Male Dose P<0.166 Gen P<0.001 DxG P<0.108	F_0	425.7 ± 6.7 (24)	422.5 ± 6.6 [2]	427.4 ± 5.6	429.0 ± 5.2 [2]	-	-
	F_1	437.7 ± 4.4 (26)	435.5 ± 7.7 [2]	419.9 ± 5.6	413.2 ± 5.1* [2]	**	-
	F_2	445.3 ± 4.9 (24)	459.2 ± 6.0 [0, 1, 3, 4]	437.0 ± 8.3	449.3 ± 5.0 [0, 1, 3, 4]	-	-
	F_3	432.4 ± 4.5	424.4 ± 8.0 [2]	429.9 ± 4.9	423.9 ± 6.1 [2]	-	-
	F_4	433.1 ± 7.7	427.2 ± 6.1 [2]	424.1 ± 6.6	419.8 ± 5.4 [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 in the same generation as determined by Dunnett's test; asterisks in the Trends columns indicate significant linear or quadratic (Quad) exposure concentration trends within that generation as determined by contrasts: *, P≤0.05; **, P≤0.01; ***, P≤0.001. A dash in the Trends columns indicates that the exposure concentration trend test was not significant (P>0.05).

[b] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the 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. Random effects for the F_0 breed mother and the interaction between the F_0 breed mother and F_0 breed father were incorporated into the covariance structure of the model for the females. Random effects for 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 for the males.

[c] Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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 Genistein[a,b]

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	NSD	NA	NSD	NA	NSD
4	NA	1v2** ↓14% 1v4* ↓13%	NA	1v2** ↓14%	NA	1v4*↓11%	NA	1v4** ↓14%
5	NA	1v2** ↓11% 1v4** ↓11%	NA	1v2** ↓12%	NA	1v4* ↓9%	NA	NSD
6	**0v2*** ↓12%** **0v3* ↓8%** **0v4*** ↓12%** 1v2** ↓9% 1v4* ↓8%	1v2** ↓9% 1v4* ↓8%	**0v1* ↓7%** **0v2*** ↓15%** **0v3*** ↓12%** **0v4*** ↓11%** 1v2** ↓9%	1v2** ↓9%	**0v1*** ↓10%** **0v2*** ↓11%** **0v3*** ↓12%** **0v4*** ↓15%**	NSD	**0v1*** ↓13%** **0v2*** ↓12%** **0v3*** ↓11%** **0v4*** ↓15%**	NSD
7	**0v1** ↓8%** **0v2*** ↓12%** **0v3*** ↓9%** **0v4*** ↓11%**	NSD	**0v1* ↓6%** **0v2*** ↓12%** **0v3*** ↓9%** **0v4** ↓8%**	NSD	**0v1*** ↓12%** **0v2*** ↓10%** **0v3*** ↓11%** **0v4*** ↓12%**	NSD	**0v1*** ↓12%** **0v2*** ↓10%** **0v3* ↓8%** **0v4*** ↓11%**	NSD
8	**0v2*** ↓9%** **0v3** ↓8%** **0v4*** ↓8%** 1v2* ↓6%	1v2* ↓6%	**0v2*** ↓11%** **0v3* ↓8%** **0v4*** ↓7%** 1v2* ↓7%	1v2* ↓7%	**0v1** ↓8%** **0v2** ↓8%** **0v3** ↓8%** **0v4*** ↓9%**	NSD	**0v1*** ↓13%** **0v2* ↓7%** **0v4** ↓8%** 1v3** ↑9%	1v3** ↑9%

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	**0v2* ↓6%** **0v3* ↓6%** **0v4* ↓6%**	NSD	**0v1* ↓6%** **0v2***↓10%** **0v3** ↓7%** **0v4***↓8%**	NSD	**0v1* ↓6%** **0v3* ↓6%** **0v4** ↓7%**	NSD	**0v1***↓11%** 1v3***↑11%	1v2* ↑7% 1v3***↑11%
10	**0v2* ↓7%** **0v3** ↓7%** **0v4** ↓8%**	NSD	**0v1** ↓7%** **0v2***↓10%** **0v3** ↓7%** **0v4***↓10%**	NSD	**0v1* ↓7%** **0v3** ↓8%** **0v4** ↓8%**	NSD	**0v1***↓12%** **0v2* ↓6%** 1v3***↑11% 1v4* ↑8%	1v2* ↑7% 1v3***↑11% 1v4* ↑8%
11	**0v2***↓9%** **0v3** ↓8%** **0v4***↓13%** **1v4***↓9%** **3v4* ↓6%**	1v4***↓9% 3v4* ↓6%	**0v1***↓9%** **0v2***↓13%** **0v3***↓8%** **0v4***↓17%** 1v4** ↓8% 3v4***↓9%	1v4** ↓8% 3v4***↓9%	**0v1***↓8%** **0v2***↓9%** **0v3** ↓7%** **0v4***↓16%** 1v4***↓9% 2v4***↓8% 3v4***↓10%	1v4***↓9% 2v4***↓8% 3v4***↓10%	**0v1***↓13%** **0v2* ↓8%** **0v4***↓10%** 1v2* ↑6% 1v3***↑15% 2v3* ↑8% 3v4***↓10%	1v2* ↑6% 1v3***↑15% 2v3* ↑8% 3v4***↓10%
12	**0v1***↓13%** **0v2***↓16%** **0v3***↓17%** **0v4***↓17%**	NSD	**0v1***↓17%** **0v2***↓20%** **0v3***↓18%** **0v4***↓21%**	NSD	**0v1***↓16%** **0v2***↓14%** **0v3***↓16%** **0v4***↓19%**	NSD	**0v1***↓20%** **0v2***↓16%** **0v3***↓9%** **0v4***↓11%** 1v3***↑13% 1v4***↑11% 2v3** ↑8%	1v3***↑13% 1v4***↑11% 2v3** ↑8%
13	**0v1***↓16%** **0v2***↓22%** **0v3***↓23%** **0v4***↓22%** 1v2** ↓8% 1v3** ↓8% 1v4* ↓7%	1v2** ↓8% 1v3** ↓8% 1v4* ↓7%	**0v1***↓22%** **0v2***↓25%** **0v3***↓24%** **0v4***↓25%**	NSD	**0v1***↓20%** **0v2***↓21%** **0v3***↓24%** **0v4***↓25%**	NSD	**0v1***↓25%** **0v2***↓22%** **0v3***↓17%** **0v4***↓17%** 1v3** ↑10% 1v4** ↑11%	1v3** ↑10% 1v4***↑11%
16	NSD	NA	NSD	NA	NSD	NA	**0v1** ↓7%** **0v3* ↑9%** 1v2***↑11% 1v3***↑17% 1v4***↑11% 2v3* ↑6% 3v4* ↓5%	NA
17	**0v1* ↑7%** **0v3* ↑9%**	NA	**0v3* ↑9%**	NA	**0v3** ↑9%**	NA	**0v3***↑15%** **0v4* ↑9%** 1v2* ↑8% 1v3***↑16% 1v4***↑11% 2v3** ↑8%	NA

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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
18	**0v1* ↑8%** **0v2* ↑8%** **0v4***↑11%**	NA	**0v4* ↑7%**	NA	**0v3* ↑8%** **0v4* ↑8%**	NA	**0v2* ↑8%** **0v3***↑12%** **0v4***↑11%** 1v2** ↑8% 1v3***↑12% 1v4***↑11%	NA
19	1v2** ↓6% 1v3** ↓6%	NA	NSD	NA	1v2* ↓4% 1v3***↓7%	NA	**0v1* ↓5%** 1v3***↑9% 1v4***↑10%	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 "0v1" means F_0 versus F_1. Asterisks (*) indicate the level of significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. 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 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) are tabulated separately (Table D2).

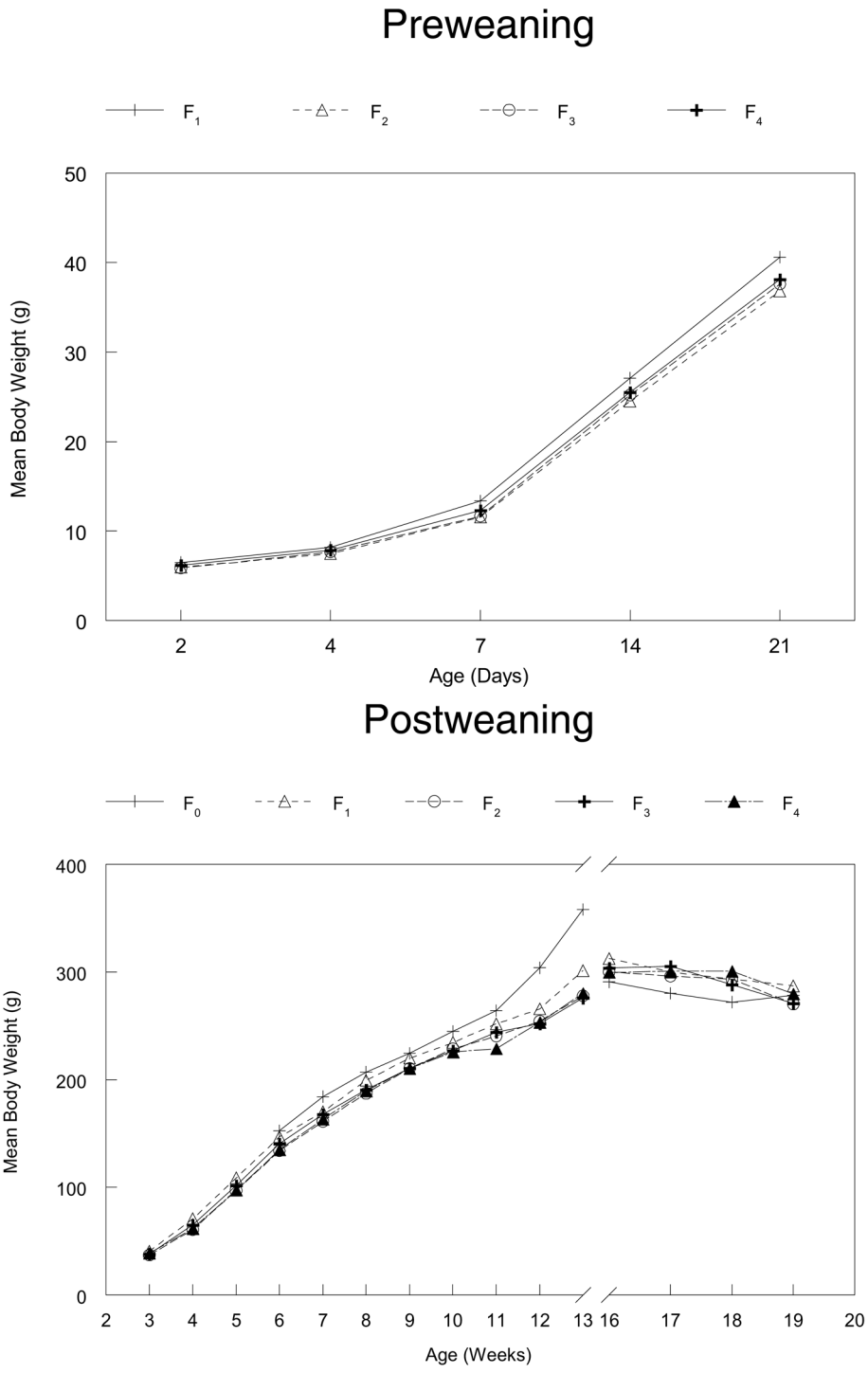

FIGURE D1
Body Weights of 0 ppm Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

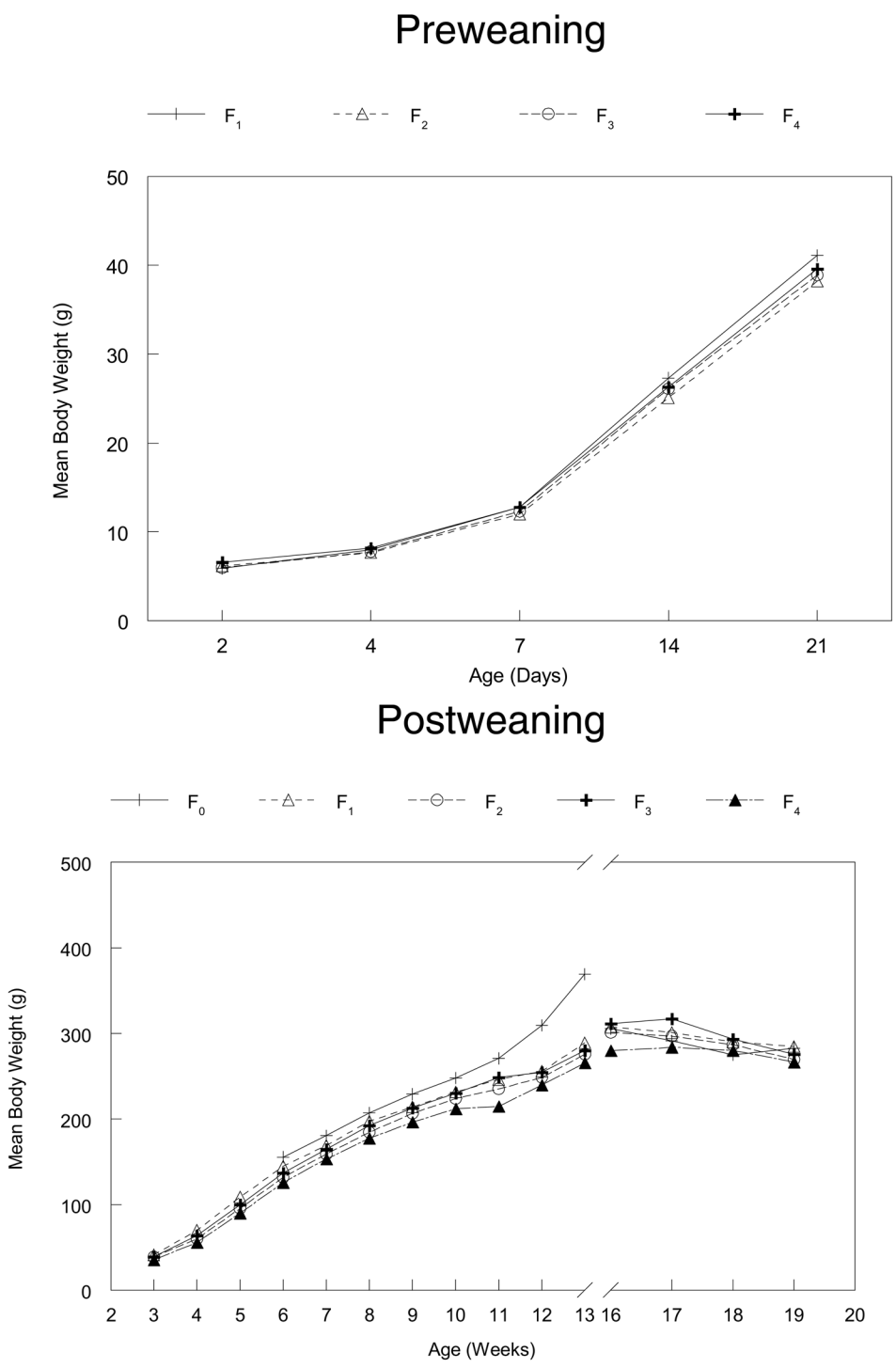

FIGURE D2
Body Weights of 5 ppm Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

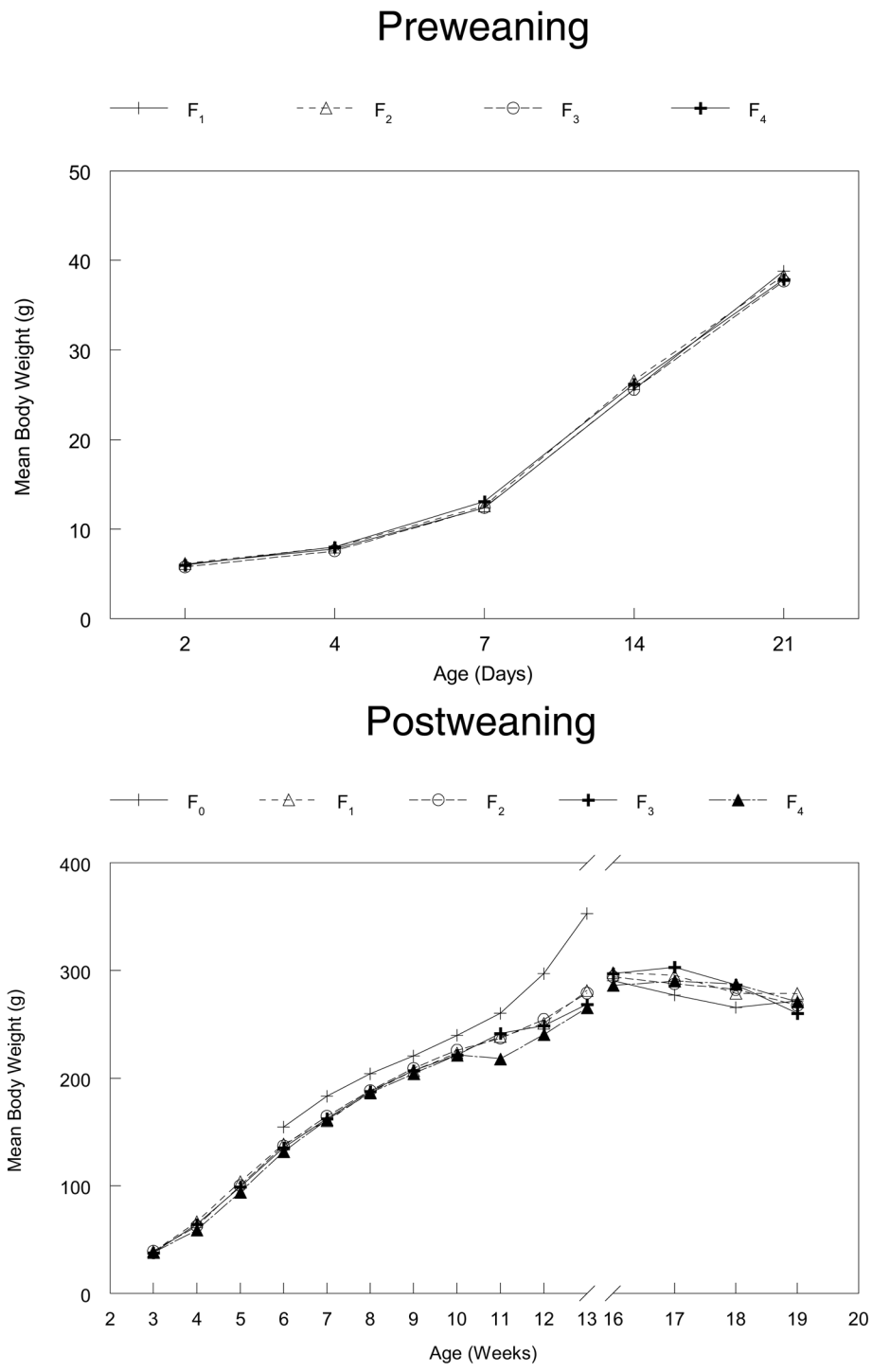

FIGURE D3
Body Weights of 100 ppm Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

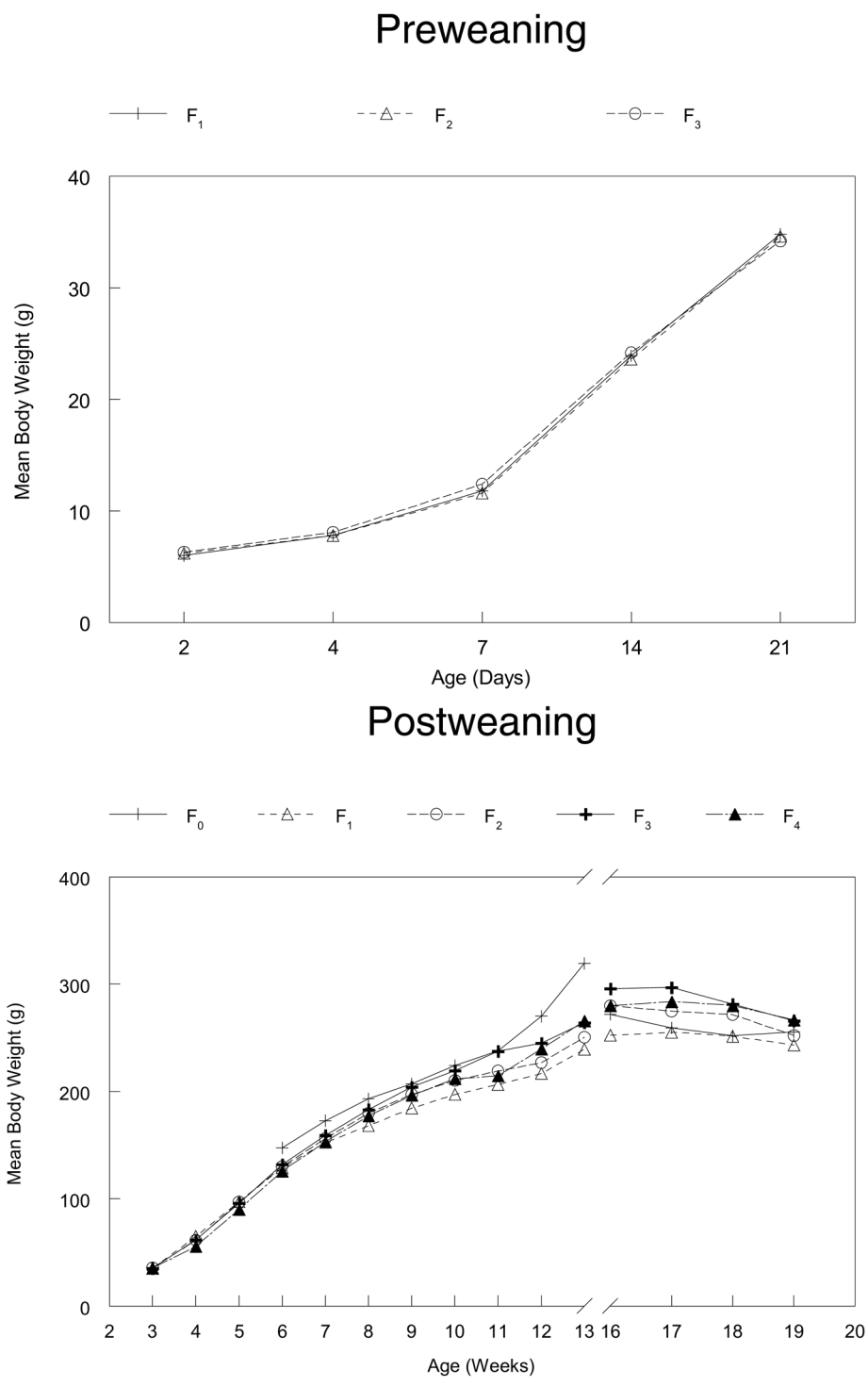

FIGURE D4
Body Weights of 500 ppm Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	NSD	NA	NSD	NA	NSD
4	NA	NSD	NA	NSD	NA	NSD	NA	NSD
5	NA	NSD	NA	NSD	NA	NSD	NA	NSD
6	**0v1** ↓10% **0v2***↓10% **0v3***↓12% **0v4***↓14%	NSD	**0v1***↓13% **0v2** ↓9% **0v3***↓13% **0v4***↓12%	NSD	**0v1***↓14% **0v2***↓15% **0v3***↓10% **0v4***↓17%	NSD	**0v1***↓16% **0v2***↓14% **0v3***↓12% **0v4***↓19% 3v4* ↓9%	NSD
7	**0v1***↓11% **0v2***↓11% **0v3***↓11% **0v4***↓13%	NSD	**0v1***↓13% **0v2** ↓8% **0v3***↓14% **0v4***↓13%	NSD	**0v1***↓15% **0v2***↓13% **0v3***↓10% **0v4***↓15%	NSD	**0v1***↓18% **0v2***↓13% **0v3***↓14% **0v4***↓19%	NSD
8	**0v2*** ↓7% **0v4*** ↓8%	NSD	**0v1*** ↓8% 1v2* ↑7%	1v2* ↑7%	**0v1***↓10% **0v2**↓8% **0v4**↓9% 1v3** ↑9% 2v3* ↑7% 3v4* ↓7%	1v3** ↑9% 2v3* ↑7% 3v4* ↓7%	**0v1***↓12% **0v4***↓10%	1v2* ↑7% 1v3* ↑9%
9		NSD	NSD	NSD	NSD	NSD	**0v1**↓8% **0v3*** ↓6% **0v4*** ↓6%	NSD

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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
10	NSD	NSD	**0v3* ↓6%** 2v3** ↓8%	1v2* ↑6% 2v3***↓8% 3v4* ↑5%	NSD	NSD	**0v1***↓13%** **0v3** ↓11%** **0v4** ↓11%**	NSD
11	**0v1***↑10%** **0v2***↑10%** **0v3* ↑7%** **0v4* ↑7%**	NSD	**0v1***↑12%** **0v2***↑16%** **0v4***↑12%** 2v3***↓8%	1v3* ↓5% 2v3***↓8% 3v4* ↑5%	**0v1***↑10%** **0v2***↑11%** **0v3***↑10%** **0v4***↑10%**	NSD	**0v2* ↑8%**	2v3* ↓6%
12	**0v1** ↑8%** **0v2* ↑7%**	NSD	**0v1* ↑8%** **0v2***↑13%** **0v4** ↑8%** 2v3***↓8%	1v2* ↑5% 2v3***↓8% 2v4* ↓4%	**0v2***↑9%** **0v3** ↑9%** **0v4** ↑9%**	NSD	NSD	1v2** ↑6%
13	NSD	NSD	**0v2***↑12%** 1v2* ↑6% 2v3* ↓6% 2v4* ↓6%	1v2** ↑6% 2v3** ↓6% 2v4** ↓6%	**0v2***↑10%** **0v3** ↑9%**	1v2* ↑5% 1v3* ↑4% 2v4** ↓6% 3v4* ↓5%	**0v2* ↑7%** 1v2***↑9% 2v4* ↓6%	1v2*** ↑9% 1v3** ↑6% 2v4** ↓6%
14	NSD	2v3* ↑5%	NSD	NSD	NSD	3v4* ↓5%	NSD	1v3** ↑6% 3v4** ↓6%
15	**0v2* ↑6%** 2v4** ↓7%	1v4* ↓4% 2v3* ↓4% 2v4***↓7%	**0v2***↑11%** 1v2***↑8% 2v3***↓9% 2v4***↓9%	1v2***↑8% 2v3***↓9% 2v4***↓9%	**0v2* ↑7%** **0v3* ↑7%** 1v2* ↑7% 1v3* ↑8% 2v4* ↓5% 3v4* ↓6%	1v2** ↑7% 1v3** ↑8% 2v4** ↓5% 3v4** ↓6%	**0v2* ↑6%** 1v2* ↑6% 2v4** ↓7%	1v2***↑6% 2v4***↓7% 3v4* ↓4%

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	NSD	NSD	2v3** ↓6%	1v3* ↓5% 2v3***↓6%	NSD	NSD	NSD	NSD
17	**0v3* ↓5%** 1v3* ↓6% 2v3* ↓6%	1v3** ↓6% 2v3** ↓6% 3v4* ↑4%	**0v2* ↑6%** 1v3***↓7% 2v3***↓10% 2v4* ↓6%	1v3***↓7% 2v3***↓10% 2v4** ↓6% 3v4* ↑5%	NSD	2v3* ↓4%	**0v3* ↓5%** 2v3** ↓6%	2v3***↓6%
18	**0v1** ↑7%**	NSD	**0v1***↑8%** **0v2***↑9%** 2v3* ↓5% 2v4* ↓5%	1v3* ↓4% 1v4* ↓4% 2v3** ↓5% 2v4** ↓5%	NSD	NSD	NSD	2v4** ↓5%
19	NSD	1v4* ↓5% 2v4* ↓4%	**0v2***↑8%** 2v3** ↓6% 2v4***↓8%	1v2* ↑4% 1v4* ↓4% 2v3***↓6% 2v4***↓8%	NSD	NSD	**0v2* ↑6%** 1v2** ↑6% 2v3***↓7% 2v4** ↓6%	1v2***↑6% 2v3***↓7% 2v4***↓6%

[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 "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 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).

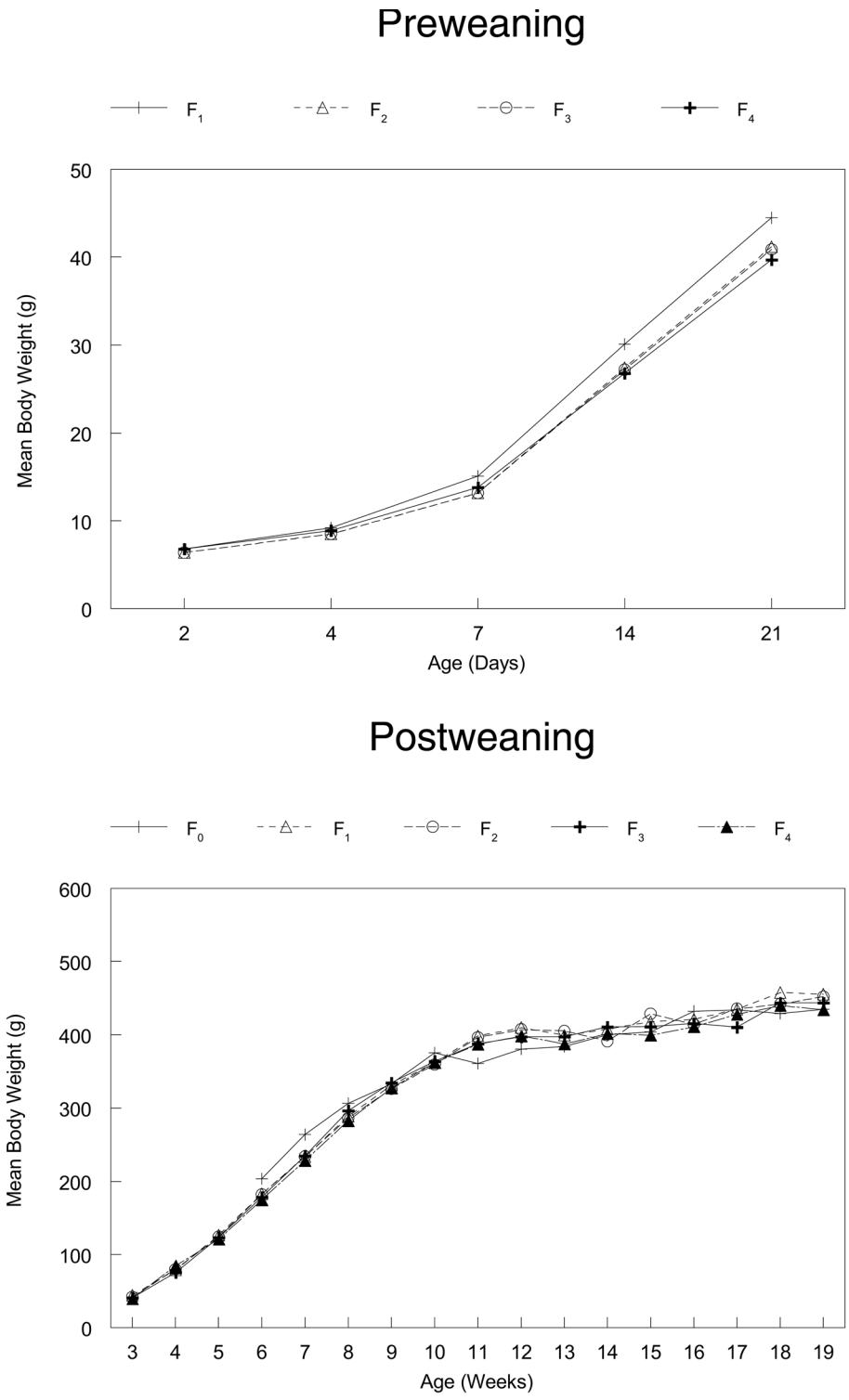

FIGURE D5
Body Weights of 0 ppm Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

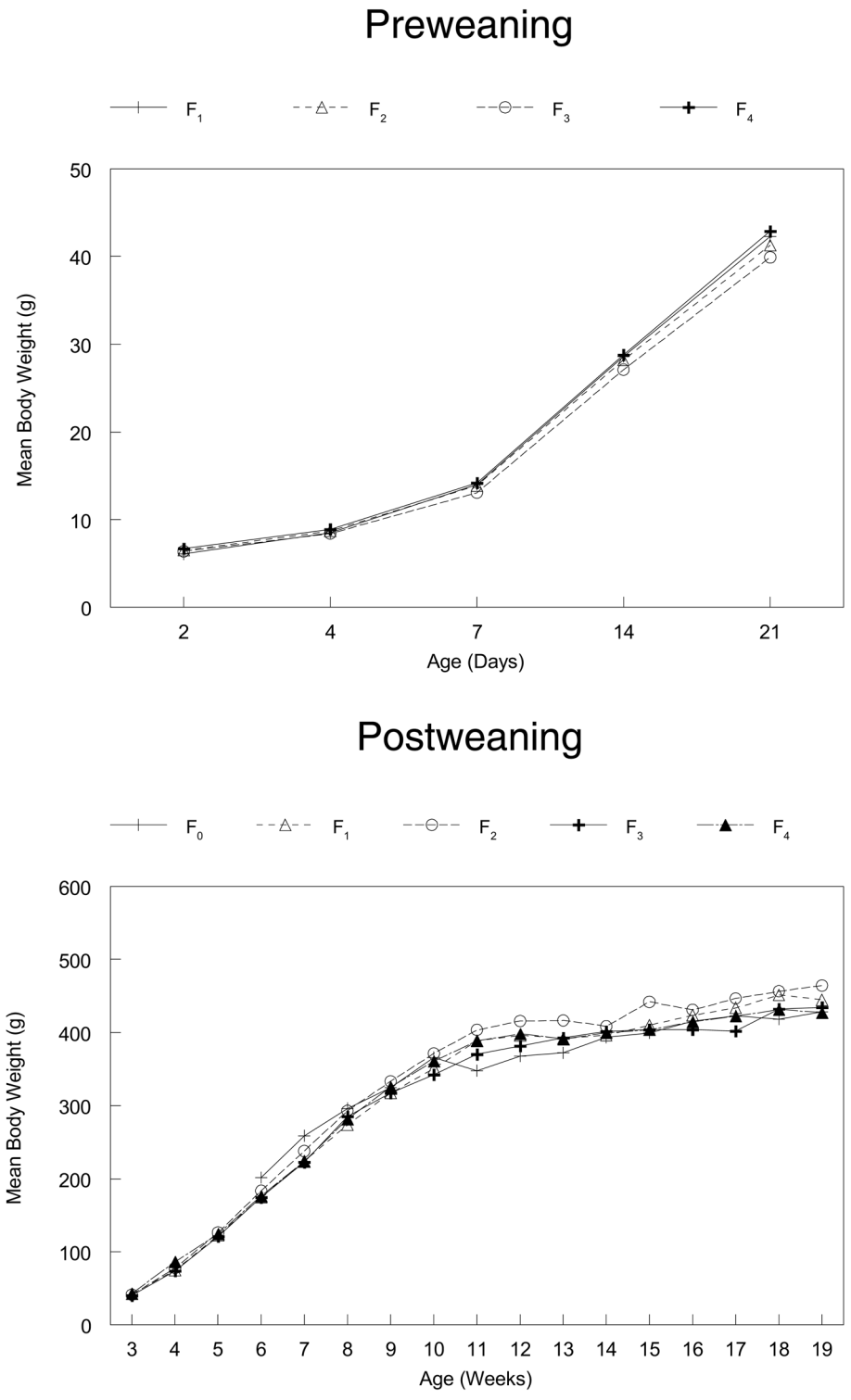

FIGURE D6
Body Weights of 5 ppm Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

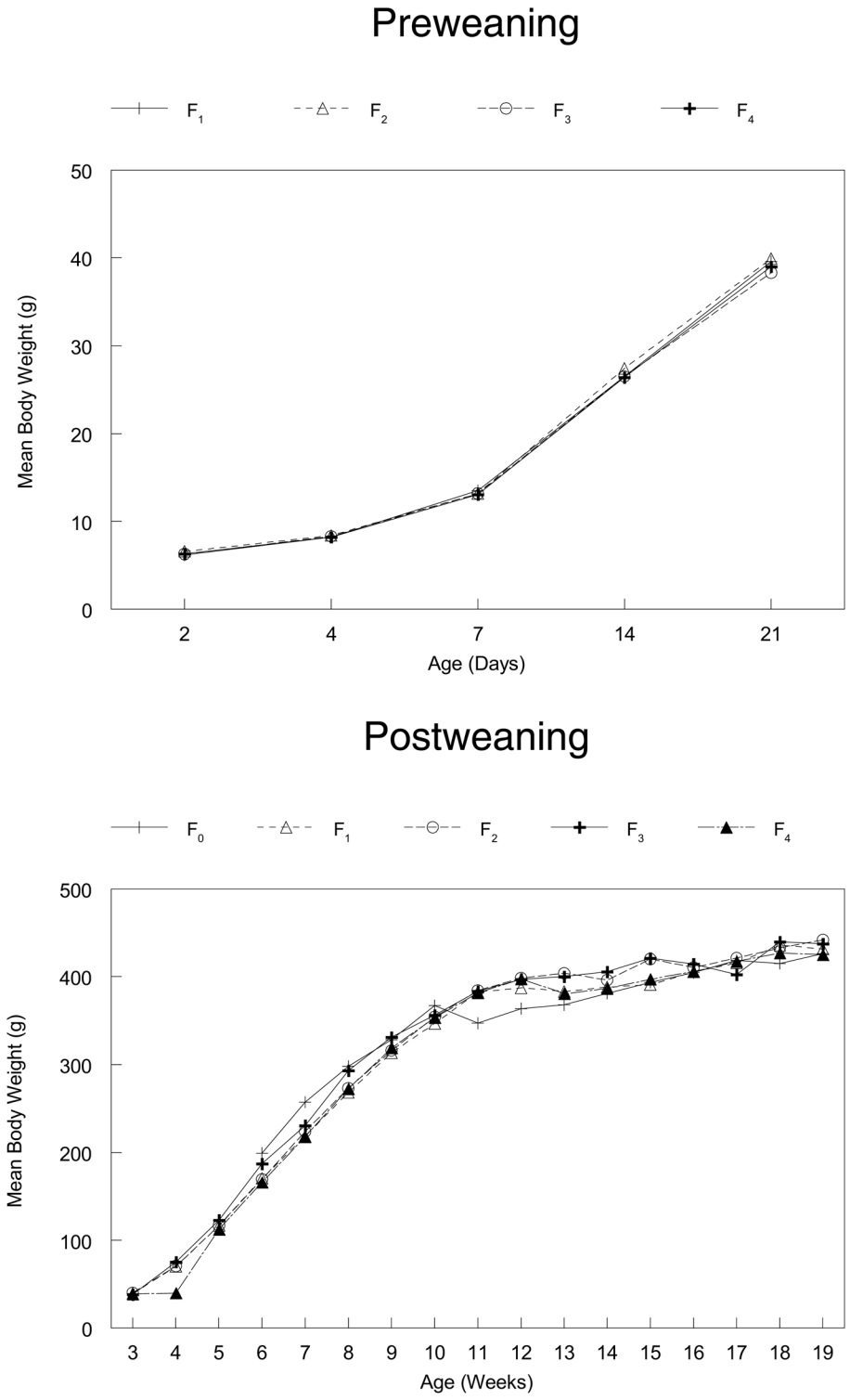

FIGURE D7
Body Weights of 100 ppm Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

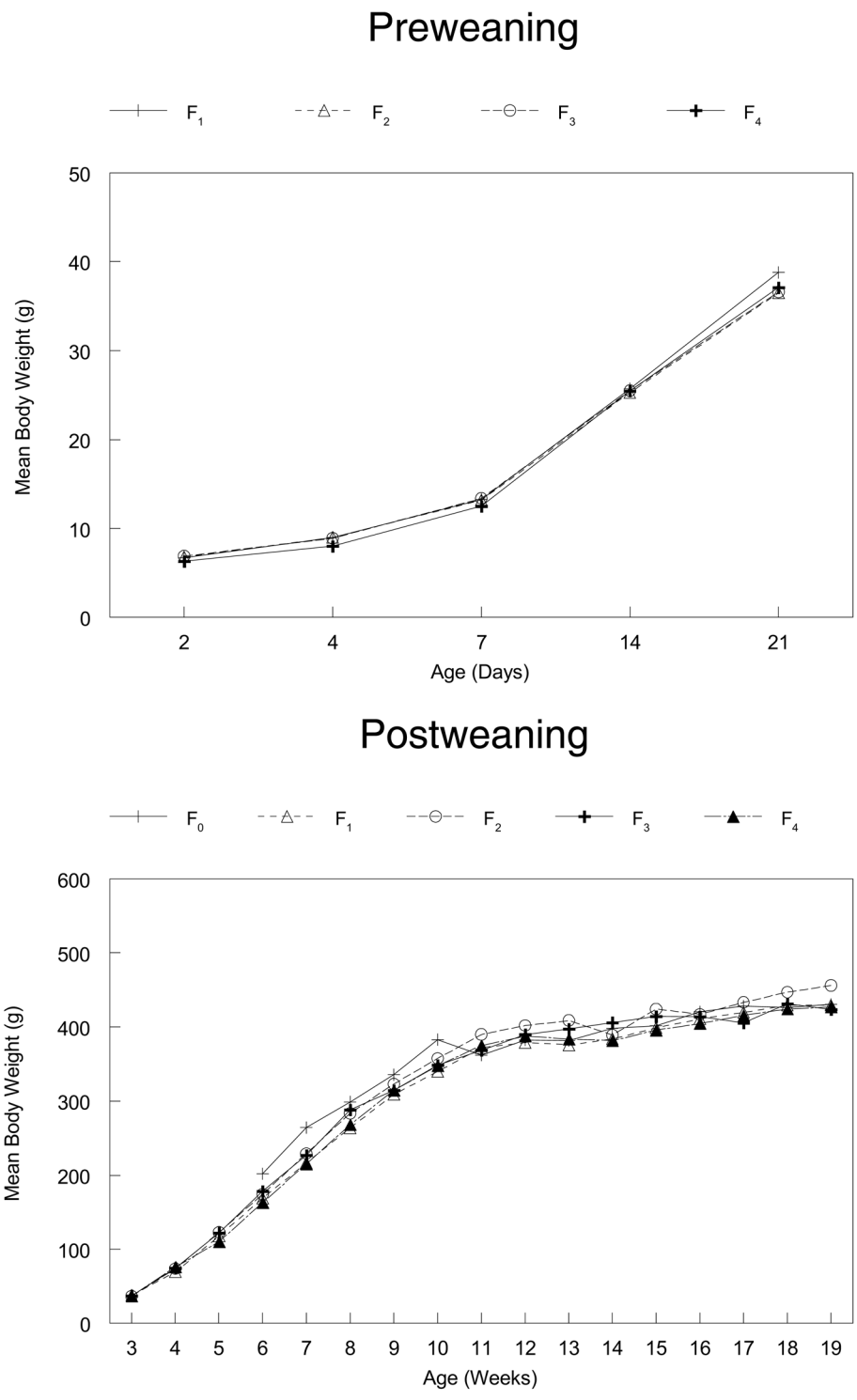

FIGURE D8
Body Weights of 500 ppm Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

APPENDIX E
FEED CONSUMPTION

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
7***	19.4 ± 0.5	19.2 ± 0.4	19.6 ± 0.7	17.2 ± 0.5**
8	19.4 ± 0.7	18.4 ± 0.7	18.4 ± 0.4	18.0 ± 0.4*
9	19.1 ± 0.4	19.3 ± 0.3	18.3 ± 0.5	18.0 ± 0.3
10***	20.7 ± 0.5	20.0 ± 0.4	20.2 ± 0.7	18.3 ± 0.3***
13*	23.9 ± 0.6	23.1 ± 0.8	22.1 ± 0.6	20.7 ± 0.7*
14***, # # #	40.8 ± 0.9	40.0 ± 1.1	35.9 ± 1.1***	34.6 ± 1.1***
15	48.5 ± 1.2	49.8 ± 1.0	48.3 ± 1.6	47.1 ± 1.2
16*	53.2 ± 1.1	54.8 ± 2.3	52.7 ± 1.2	49.5 ± 1.2

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

[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 exposure groups (results shown in Table E6), two sets of statistical analyses were conducted for feed consumption for females: the first included data from week 7 to the start of litter delivery for all generations (F_0 to F_4), and the second included all data from 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, and 6 are from the latter analysis, while results from weeks 7 to 16 are from the former analysis. In both analyses, data from the weeks during which males and females were paired for mating (weeks 11 and 12) were not included. Data from weeks 17 to 20 are presented separately (Table E2).

[c] Feed consumption data were analyzed using a repeated measures approach to a mixed model ANOVA. 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 ANOVA results for each analysis were as follows:

1) Female feed consumption, weeks 8 to 17, F_0 to F_4: dose, $P<0.001$; generation, $P<0.001$; dose × generation, $P=0.225$; 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 and the interaction between the F_0 breed mother and F_0 breed father were significant at $P<0.50$ and were incorporated into the model.
2) Female feed consumption, weeks 5 to 19, F_1 to F_4: dose, $P<0.001$; generation, $P=0.219$; dose × generation, $P=0.233$; 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 incorporated into the model.

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

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	11.2 ± 0.6 (23)	11.4 ± 0.6 (24)	10.4 ± 0.4	10.5 ± 0.4
5**	13.7 ± 0.5 (23)	14.5 ± 0.4	14.5 ± 0.4	12.6 ± 0.7
6***	17.0 ± 0.3 (24)	16.9 ± 0.3	16.2 ± 0.3	14.7 ± 0.3**
7***	17.7 ± 0.4 (24)	18.3 ± 0.5	17.7 ± 0.6	14.8 ± 0.3***
8***	18.3 ± 0.3 (24)	17.7 ± 0.3	17.3 ± 0.3	14.8 ± 0.3***
9**	18.1 ± 0.4 (24)	18.2 ± 0.3	17.9 ± 0.4	16.6 ± 0.4
10***	18.6 ± 0.5 (24)	18.4 ± 0.4	18.4 ± 0.4	16.7 ± 0.4**
13	23.2 ± 0.6 (24)	22.4 ± 0.8 (24)	21.7 ± 0.6	21.0 ± 1.4 (24)
14*	21.8 ± 0.9 (24)	22.3 ± 0.7 (24)	21.2 ± 0.8	19.9 ± 0.6
15	31.0 ± 1.6 (24)	30.1 ± 1.4	29.9 ± 1.3	29.5 ± 1.4
16	42.2 ± 1.5 (24)	40.4 ± 1.6	41.8 ± 1.7	39.7 ± 1.2

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	10.4 ± 0.3	11.0 ± 0.3	10.9 ± 0.3	10.5 ± 0.3
5*	14.3 ± 0.4	14.3 ± 0.3	14.9 ± 0.3	13.1 ± 0.3
6*	16.1 ± 0.3	16.3 ± 0.4	16.7 ± 0.3	15.2 ± 0.3
7*	19.1 ± 0.5	18.9 ± 0.3	19.1 ± 0.4	17.8 ± 0.3
8***	19.5 ± 0.4 (24)	18.6 ± 0.3	18.6 ± 0.4	17.5 ± 0.3***
9*	19.3 ± 0.4 (24)	18.4 ± 0.3	18.8 ± 0.4	17.7 ± 0.4*
10	17.6 ± 0.3	17.6 ± 0.4	17.7 ± 0.4	16.8 ± 0.2
13***	25.2 ± 2.2	21.0 ± 0.8**	21.6 ± 1.4** (24)	19.6 ± 0.7*** (23)
14*	24.0 ± 0.8	23.4 ± 1.0 (24)	25.0 ± 1.0	21.4 ± 0.9 (24)
15#	24.7 ± 1.2	28.2 ± 1.7	30.4 ± 1.2**	27.5 ± 1.1
16#	39.1 ± 1.1	40.5 ± 1.2	44.1 ± 2.2	39.7 ± 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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	12.5 ± 0.7	11.4 ± 0.5	11.8 ± 0.6	11.0 ± 0.7
5	14.4 ± 0.6	15.5 ± 0.4	15.3 ± 0.4	15.1 ± 0.5
6*	17.1 ± 0.4	16.8 ± 0.3	16.8 ± 0.3	15.5 ± 0.3
7	17.8 ± 0.5	17.4 ± 0.3	17.8 ± 0.4	17.1 ± 0.4
8**	17.8 ± 0.4	17.9 ± 0.3	17.2 ± 0.4	16.5 ± 0.4*
9	19.4 ± 0.5	18.8 ± 0.3	19.0 ± 0.6	19.4 ± 0.6
10*	18.7 ± 0.4	18.4 ± 0.4	17.7 ± 0.4	17.3 ± 0.4*
13	22.3 ± 1.0 (19)	22.3 ± 0.9 (19)	22.7 ± 0.8 (23)	22.0 ± 0.6 (22)
14	25.4 ± 0.6	25.4 ± 0.7 (24)	25.1 ± 0.6	24.0 ± 0.7 (23)
15	29.4 ± 1.3 (24)	27.6 ± 1.0	26.2 ± 1.1	25.8 ± 0.9
16*	40.4 ± 1.2	39.0 ± 1.5	39.4 ± 1.3	36.1 ± 1.6

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4**	12.3 ± 0.4	12.1 ± 0.5	11.5 ± 0.4	10.4 ± 0.5* (24)
5	15.1 ± 0.3	15.4 ± 0.3	14.8 ± 0.3	14.0 ± 0.7
6	17.9 ± 0.4	17.3 ± 0.3	17.6 ± 0.4	17.4 ± 1.4
7	17.0 ± 0.2	17.0 ± 0.5	17.5 ± 0.3	16.6 ± 0.4 (24)
8	18.9 ± 0.4 (24)	18.5 ± 0.3	18.9 ± 0.4	18.2 ± 0.3 (24)
9	17.7 ± 0.4	17.6 ± 0.3	17.5 ± 0.3	18.5 ± 1.1
10###	15.6 ± 0.4	16.3 ± 0.6	14.1 ± 0.5*	16.3 ± 0.6
13	23.9 ± 0.6	23.3 ± 0.6	22.9 ± 0.5	21.9 ± 0.6 (23)
14	25.9 ± 0.8	24.5 ± 0.8	23.8 ± 0.8	23.3 ± 0.9 (24)
15	29.8 ± 1.7	28.9 ± 1.2	27.7 ± 1.3	27.5 ± 1.1
16	44.3 ± 2.2	42.9 ± 0.9	43.1 ± 0.9	41.0 ± 1.4

The footnotes for this table are defined in Table E1a.

TABLE E2
Feed Consumption by Female Rats during Postnatal Weeks 17 to 20 in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a]

Gen[b]	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_0	17	23.6 ± 0.9 (21)	22.6 ± 1.0 (22)	24.3 ± 1.4 (25)	24.5 ± 1.5 (22)
	18	17.1 ± 0.8 (25)	16.6 ± 0.4 (25)	17.3 ± 0.4 (25)	19.6 ± 0.6 (25)
	19[c]	21.5 ± 0.6 (25)	21.8 ± 0.9 (25) [4]	22.6 ± 0.5 (25) [4]	22.9 ± 0.8 (24) [4]
	20	23.9 ± 0.9 (12)	20.4 ± 1.6 (12)	21.4 ± 0.5 (13)	22.0 ± 1.2 (13)
F_1	17	45.2 ± 1.4 (24)	42.8 ± 1.5 (25)	43.6 ± 1.2 (25)	44.0 ± 1.5 (25)
	18	30.9 ± 2.3 (24)	29.4 ± 2.3 (25)	28.6 ± 2.1 (25)	27.1 ± 1.7 (25)
	19[c]	22.7 ± 0.9 (24) [4]	24.1 ± 1.1 (25) [4]	24.2 ± 0.7 (25) [3, 4]	22.6 ± 0.5 (25)
	20	25.4 ± 2.0 (6)	23.5 ± 2.3 (6)	24.6 ± 1.2 (6)	No data
F_2	17	48.8 ± 1.0 (25)	46.8 ± 1.2 (24)	45.2 ± 2.0 (25)	45.7 ± 1.3 (25)
	18	45.1 ± 3.1 (21)	38.1 ± 3.4 (18)	31.5 ± 2.7 (23)	38.0 ± 2.9 (18)
	19[c]	21.8 ± 0.8 (24)	21.3 ± 0.6 (25) [4]	23.1 ± 0.6 (25) [4]	21.5 ± 0.6 (25)
	20	22.6 ± 1.6 (7)	19.4 ± 3.1 (4)	21.9 ± 1.4 (3)	23.4 ± 1.2 (4)
F_3	17	45.8 ± 1.7 (25)	46.6 ± 1.6 (25)	45.9 ± 1.5 (25)	43.4 ± 1.7 (25)
	18	34.7 ± 2.6 (25)	33.5 ± 2.2 (25)	35.4 ± 2.2 (25)	34.7 ± 2.6 (25)
	19[c]	20.6 ± 0.5 (25)	22.9 ± 1.4 (25) [4]	20.4 ± 0.6 (25) [1, 4]	22.0 ± 0.9 (25)
	20	22.3 ± 2.1 (6)	20.7 ± 1.5 (9)	21.5 ± 0.9 (4)	23.7 (1)

TABLE E2
Feed Consumption by Female Rats during Postnatal Weeks 17 to 20 in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Gen	Age	Dietary Genistein (ppm)			
		0	5	100	500
F_4	17	51.4 ± 1.5 (25)	47.9 ± 1.0 (25)	48.1 ± 1.2 (25)	47.0 ± 1.7 (25)
	18	41.2 ± 3.2 (25)	35.9 ± 3.3 (24)	39.9 ± 2.8 (25)	39.7 ± 3.1 (25)
	19[c]	19.1 ± 0.7 (25) [1]	17.9 ± 0.9 (25) [0, 1, 2, 3]	17.2 ± 0.6 (24) [0, 1, 2, 3]	19.4 ± 1.1 (25) [0]
	20	18.3 ± 0.7 (3)	17.8 ± 1.3 (5)	18.7 ± 0.8 (5)	16.7 ± 0.6 (8)

[a] Mean daily feed consumption ± standard error for the weeks indicated. The number in parentheses indicates the number of animals for which data were available. Because the F_0 animals were mated at earlier ages than the subsequent generations, pregnancy, litter delivery, and lactation were shifted in time for these generations. Thus, postnatal weeks 17 and 18 were after litter delivery for the F_0 generation but generally not for the F_1 to F_4 generations. During week 20, scheduled removals and necropsies occurred, and the data are limited, indicated by the numbers in parentheses, or missing, indicated by "No data." Therefore, only week 19 was a shared postdelivery week with complete data, and only this week was included in the statistical analysis of dose and generation effects for postnatal weeks 17 to 20.

[b] Gen=generation

[c] Data from postnatal week 19, the only postdelivery week with complete data for all generations, were analyzed by two-way ANOVA with dose and generation as factors. The random effect for the F_0 breed mother was significant in a log-likelihood test at $P<0.50$ and was incorporated into the statistical model. The overall ANOVA results were as follows: dose, $P=0.549$; generation, $P<0.001$; and dose × generation, $P=0.102$. Significant differences between generations within an exposure group for week 19 were determined by Holm's adjusted t-tests; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$. There were no significant exposure concentration-related trends within generations and no significant differences between exposed groups and controls indicated by Dunnett's test.

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

Generations covered [b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
$F_0 - F_4$[c] Dose P<0.001 Gen P<0.001 DxG P=0.402	F_0***	1714.9 ± 21.2 [1, 2, 3, 4]	1712.9 ± 22.2 [1, 2, 3, 4]	1648.5 ± 26.4 [1, 2, 3, 4]	1563.7 ±25.5*** [1, 2, 3, 4]
	F_1***	1336.2 ± 28.0 (24) [0]	1302.1 ± 32.0 [0]	1300.9 ± 28.4 [0]	1205.2 ± 22.8** [0]
	F_2**, #	1308.4 ± 21.8 [0]	1299.4 ± 28.5 [0]	1359.9 ± 28.1 [0]	1228.9 ± 24.5 [0]
	F_3	1292.3 ± 24.7 [0]	1263.5 ± 29.8 [0]	1283.3 ± 26.1 [0]	1215.0 ± 32.3 [0]
	F_4*	1347.0 ± 26.2 [0]	1323.2 ± 20.3 [0]	1298.7 ± 17.9 [0]	1254.5 ± 28.0* [0]
$F_1 - F_4$[d] Dose P<0.001 Gen P<0.109 DxG P=0.718	F_1***	1622.0 ± 30.3 (24)	1598.4 ± 34.0	1588.8 ± 32.0	1470.0 ± 26.9**
	F_2**, #	1594.0 ± 23.3	1590.6 ± 33.6	1657.1 ± 30.3	1500.7 ± 27.2
	F_3*	1599.9 ± 25.9	1569.6 ± 36.5	1590.6 ± 31.6	1506.2 ± 38.3
	F_4**	1663.9 ± 30.4	1636.8 ± 25.6	1606.0 ± 22.9	1544.8 ± 33.5*

[a] Total feed consumed per animal (g) ± standard error in the period before litters were delivered. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the generation column indicate significant linear and quadratic exposure concentration trends, respectively, within that generation as determined by contrasts; asterisks in shaded cells in the exposed group columns indicate significant difference from controls in the same generation as determined by Dunnett's test. * or #, 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; generation 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; 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 a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G).

[c] ANOVA results for the F_0 to F_4 analysis are indicated. Random effects for the F_0 breed father and the interaction between the F_0 breed mother and F_0 breed father were 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 were 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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
7	25 ± 0.5	24.8 ± 0.5	25.7 ± 0.9	25.6 ± 0.5
8*	23.6 ± 0.4	22.0 ± 0.4	23.0 ± 0.4	21.7 ± 0.4*
9	24.1 ± 0.4	24.1 ± 0.5	24.3 ± 0.4	25.2 ± 0.4
10	28.8 ± 0.4	29.7 ± 1.2	29.0 ± 0.7 (24)	30.7 ± 0.8 (15)
11	20.6 ± 0.6 (24)	20.1 ± 0.6	19.2 ± 0.5	19.9 ± 0.4
12	23.6 ± 0.8 (24)	25.3 ± 0.6	22.5 ± 0.6	23.4 ± 0.8
15	20.8 ± 0.8 (24)	21.7 ± 0.5	21.8 ± 0.5	20.9 ± 0.4
16*, # # #	30.5 ± 0.7 (24)	28.0 ± 0.9	25.7 ± 0.5***	26.8 ± 0.7**
17	23.6 ± 0.8 (24)	25.5 ± 0.6	24.8 ± 0.6	25.3 ± 0.7
18	21.7 ± 0.7 (24)	19.8 ± 0.4	21.3 ± 0.4	19.7 ± 0.4

[a] Mean daily feed consumption (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) and pound signs (#) in shaded cells in the age column indicate significant linear and quadratic exposure concentration trends, respectively, at that week in that generation; asterisks in shaded cells in the exposed group columns indicate significant difference from controls at the same week in the same generation as determined by Dunnett's test: * or #, P≤0.05; ** or # #, P≤0.01; *** or ###, 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 exposure groups (results shown in Table E7), 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 18 are from the former analysis. In both analyses, data from the weeks during which males and females were paired for mating (weeks 11 and 12) were not included.

[c] Feed consumption data were analyzed using a repeated measures approach to a mixed model ANOVA. 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. In the case of feed consumption of the males, both analyses incorporated significant random effects of the F_0 breed mother and an F_0 breed mother by F_0 breed father interaction. The ANOVA results for each analysis were as follows:

1) Male feed consumption, weeks 8 to 19, F_0 to F_4: dose, P=0.029; generation, P<0.001; dose × generation, P=0.332; weeks, P<0.001; weeks × dose, P<0.001; weeks × generation, P<0.001; weeks × dose × generation, P<0.001.
2) Male feed consumption, weeks 5 to 19, F_1 to F_4: dose, P=0.031; generation, P<0.001; dose × generation, P=0.361; 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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	12.3 ± 0.5 (26)	11.0 ± 0.4	11.9 ± 0.6	11.8 ± 0.3 (24)
5	16.9 ± 0.5	16.9 ± 0.7	15.8 ± 0.5	15.5 ± 0.4
6	20.1 ± 0.5	18.1 ± 0.8*	20.5 ± 0.5	19.8 ± 0.3
7	22.6 ± 0.6 (26)	22.2 ± 0.3	21.6 ± 0.5	21.3 ± 0.5
8	25.3 ± 0.3 (26)	24.3 ± 0.5 (24)	24.2 ± 0.4	24.3 ± 0.7
9	25.3 ± 0.4 (26)	25.5 ± 0.6	25.3 ± 0.4	25.3 ± 0.4
10	26.8 ± 0.3 (26)	25.8 ± 0.5	27.7 ± 0.8	27.2 ± 0.7
13	25.6 ± 0.7 (26)	23.9 ± 0.8	23.1 ± 0.8 (24)	25.3 ± 0.8
14*	21.8 ± 0.6 (26)	22.1 ± 0.7	22.8 ± 0.6	24.5 ± 1.0
15	22.9 ± 1.1 (26)	25.4 ± 0.7	22.8 ± 0.9	22.0 ± 0.5
16***	24.9 ± 1.1 (20)	27.0 ± 0.8 (19)	25.7 ± 1.1 (20)	30.8 ± 1.2*** (16)
17	30.2 ± 1.0 (26)	29.8 ± 1.0	28.0 ± 0.7	28.8 ± 0.9
18	25.3 ± 1.0 (26)	23.1 ± 1.2	23.7 ± 0.9	25.4 ± 1.0

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	12 ± 0.3	11.1 ± 0.4	12.1 ± 0.5	11.2 ± 0.6
5	15.4 ± 0.4 (24)	16.1 ± 0.6 (24)	17.3 ± 0.8	17.3 ± 1.8
6	22.8 ± 0.5	23.1 ± 0.5	22.5 ± 0.9	21.8 ± 0.4
7# #	22.7 ± 0.3	23.4 ± 0.4	21.6 ± 1.0 (24)	23.1 ± 0.4
8	24.7 ± 0.5	25.8 ± 0.4	24.3 ± 0.7	25.4 ± 0.5
9	25.0 ± 0.3	24.7 ± 0.5	25.9 ± 0.5	25.2 ± 0.5
10	26.1 ± 0.6	27.6 ± 0.4	27.0 ± 0.4	27.4 ± 0.3
13# #	17.4 ± 1.0 (21)	19.7 ± 1.1 (21)	22.3 ± 1.3* (19)	18.9 ± 1.1 (21)
14**	29.6 ± 1.6	30.5 ± 1.1	28.4 ± 1.3 (24)	26.7 ± 1.6 (24)
15*	23.4 ± 1.3	21.6 ± 1.2	21.9 ± 1.2	24.5 ± 1.4
16#	24.4 ± 0.9	24.0 ± 0.6	21.9 ± 0.7	23.4 ± 0.9
17	22.6 ± 0.8	22.8 ± 0.7	22.3 ± 0.6	24.5 ± 0.4
18	23.9 ± 1.0	24.4 ± 0.6	23.9 ± 0.7	24.7 ± 0.8

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	11.3 ± 0.6	10.8 ± 0.5	10.6 ± 0.5	11.2 ± 0.6
5	17.2 ± 0.4	16.6 ± 0.5	16.4 ± 0.5	16.7 ± 0.6
6	20.9 ± 0.4	19.6 ± 0.5	21.0 ± 0.6	20.7 ± 0.4
7	22.6 ± 0.4 (19)	21.2 ± 0.4 (18)	21.5 ± 0.3 (21)	21.4 ± 0.4 (21)
8	24.3 ± 0.3	24.2 ± 0.5	24.7 ± 0.3	24.3 ± 0.4
9	23.9 ± 0.5	22.9 ± 0.5	23.3 ± 0.4	23.3 ± 0.5
10	26.6 ± 0.4	24.7 ± 0.4	25.5 ± 0.7	25.1 ± 0.6
13*	22.1 ± 1.6 (21)	21.2 ± 1.2 (19)	21.7 ± 0.9 (23)	24.0 ± 1.6 (23)
14	25.4 ± 1.2	24.3 ± 0.9	25.4 ± 0.6	26.9 ± 0.7
15**, #	20.4 ± 0.6	19.7 ± 0.6	23.1 ± 0.8	23.6 ± 1.0*
16	18.5 ± 0.8	18.8 ± 0.8	17.5 ± 0.6	19.5 ± 0.7
17# # #	23.4 ± 1.2	23.3 ± 1.0	20.0 ± 0.7**	22.2 ± 0.9
18	28.9 ± 1.3	27.1 ± 1.2	27.3 ± 0.9 (24)	26.3 ± 1.1

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 Genistein[a,b,c]

Age (Weeks)	Dietary Genistein (ppm)			
	0	5	100	500
4	14.6 ± 0.4	14.2 ± 0.5	12.1 ± 0.3*** (24)	12.2 ± 0.4** (24)
5	16.7 ± 0.4	17.0 ± 0.4	16.6 ± 0.5	16.9 ± 0.6
6	21.0 ± 0.4	20.3 ± 0.5	20.7 ± 0.4	20.0 ± 0.6
7	23.6 ± 0.5	23.4 ± 0.5	24.0 ± 0.5	23.5 ± 0.4
8	26.6 ± 0.4	25.8 ± 1.0	27.0 ± 0.5	25.7 ± 0.4
9	26.6 ± 0.5	26.5 ± 0.8	26.1 ± 0.6	26.3 ± 0.5
10	29.2 ± 0.7	28.7 ± 0.6 (24)	29.4 ± 0.9	28.4 ± 0.4 (24)
13**	24.9 ± 2.2	19.5 ± 1.3**	19.2 ± 1.9**	18.4 ± 1.4***
14	20.4 ± 0.9	21.3 ± 0.7	20.9 ± 1.0	20.3 ± 0.4 (24)
15***	17.4 ± 0.9	18.8 ± 0.7	20.1 ± 0.7	22.9 ± 0.9***
16	23.2 ± 0.8	21.6 ± 0.8	21.0 ± 0.9	22.0 ± 0.6
17	22.9 ± 1.0	21.4 ± 0.9	21.4 ± 0.7	22.9 ± 0.7
18	22.9 ± 0.7	23.1 ± 0.7	21.7 ± 0.7	23.7 ± 0.5

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 Genistein[a]

Generations covered[b]	Generation	Dietary Genistein (ppm)			
		0	5	100	500
$F_0 - F_4$[c] Dose P=0.483 Gen P<0.001 DxG P=0.017	F_0**	1655.6 ± 46.0	1686.9 ± 24.1 [3]	1653.1 ± 13.0	1587.5 ± 30.0 [1]
	F_1	1715.3 ± 23.2 (26) [3]	1691.8 ± 25.0 [3]	1671.5 ± 21.5 [3]	1707.5 ± 24.8 [0]
	F_2	1659.8 ± 18.5	1689.7 ± 23.5 [3]	1624.3 ± 27.3	1677.7 ± 21.6
	F_3*	1591.3 ± 18.0 [1]	1515.2 ± 21.5 [0, 1, 2]	1564.9 ± 21.6 [1]	1619.0 ± 22.3
	F_4	1664.3 ± 29.4	1602.9 ± 28.0	1616.3 ± 25.9	1624.4 ± 17.6
$F_1 - F_4$[d] Dose P=0.040 Gen P<0.001 DxG P=0.147	F_1	2050.5 ± 27.2 (26) [3]	2013.7 ± 26.8 [3]	2008.3 ± 27.0 [3]	2034.2 ± 28.4
	F_2	2006.9 ± 23.6	2037.0 ± 27.7 [3]	1987.7 ± 31.7	2029.4 ± 30.0
	F_3*	1937.4 ± 22.1 [1, 4]	1844.1 ± 22.9 [1, 2, 4]	1900.8 ± 20.6 [1]	1959.1 ± 24.6 [1]
	F_4	2030.6 ± 34.7 [3]	1963.4 ± 33.9 [3]	1958.7 ± 28.7	1965.1 ± 19.9

[a] Total feed consumed per animal (g) ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) in shaded cells in the generation column indicate significant linear exposure concentration trends within that generation as determined by contrasts: *, $P \leq 0.05$; **, $P \leq 0.01$. There were no significant differences between exposed groups and controls within generations indicated by Dunnett's test. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$. 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 a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G).

[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 were 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 father and the interaction between the F_0 breed mother and F_0 breed father were 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 Genistein[a,b]

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	2v3* ↑20% 2v4* ↑18%	NA	NSD	NA	NSD	NA	NSD
5	NA	NSD	NA	NSD	NA	NSD	NA	1v3***↑20% 2v3***↑15%
6	NA	2v4* ↑11%	NA	NSD	NA	NSD	NA	1v4***↑18% 2v4***↑14% 3v4* ↑12%
7	**0v4** ↓**12%** 2v4** ↓11%	1v2* ↑8% 2v3* ↓7% 2v4***↓11%	**0v3* ↓9%** **0v4***↓11%** 2v4** ↓10%	2v3* ↓8% 2v4** ↓10%	**0v1* ↓10%** **0v3* ↓9%** **0v4**↓11%**	2v4** ↓8%	**0v1***↓14%** 1v2***↑20% 1v3** ↑16% 1v4* ↑12%	1v2***↑20% 1v3***↑16% 1v4***↑12%
8	**0v3* ↓8%** 2v3* ↓9%	2v3*** ↓9% 3v4* ↑6%	NSD	NSD	3v4* ↑10%	1v4* ↑9% 2v3** ↓8% 3v4** ↑10%	**0v1***↓18%** **0v3* ↓8%** 1v2***↑18% 1v3* ↑11% 1v4***↑23% 3v4***↑10%	1v2***↑18% 1v3***↑11% 1v4***↑23% 2v4* ↑4% 3v4***↑10%
9	NSD	NSD	**0v4* ↓9%**	NSD	NSD	NSD	1v3*** ↑17%	1v3***↑17% 1v4* ↑11% 2v3* ↑10%

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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
10	**0v1** ↓10% **0v2***↓15% **0v3** ↓10% **0v4*** ↓25% 1v4*** ↓16% 2v4** ↓11% 3v4*** ↓17%	1v4***↓16% 2v4** ↓11% 3v4***↓17%	**0v1*** ↓8% **0v2***↓12% **0v3*** ↓8% **0v4***↓19% 1v4** ↓11% 3v4** ↓11%	1v4** ↓11% 3v4** ↓11%	**0v1*** ↓9% **0v2***↓12% **0v3***↓12% **0v4***↓30% 1v4***↓23% 2v4***↓20% 3v4***↓20%	1v4***↓23% 2v4***↓20% 3v4***↓20%	**0v4**** ↓11%	NSD
13	NSD	NSD	NSD	NSD	NSD	NSD	NSD	NSD
14	**0v1***↓47% **0v2***↓41% **0v3***↓38% **0v4***↓37% 1v3* ↑17% 1v4** ↑19%	1v3* ↑17% 1v4***↑19%	**0v1***↓44% **0v2***↓42% **0v3***↓37% **0v4***↓39%	NSD	**0v1***↓41% **0v2***↓30% **0v3***↓30% **0v4***↓34% 1v2** ↑18% 1v3** ↑18%	1v2** ↑18% 1v3** ↑18%	**0v1***↓42% **0v2***↓38% **0v3***↓31% **0v4***↓33% 1v3** ↑21% 1v4* ↑17%	1v3** ↑21% 1v4** ↑17%
15	**0v1***↓36% **0v2***↓49% **0v3***↓39% **0v4***↓39% 1v2** ↓20% 2v3* ↑19% 2v4* ↑21%	1v2** ↓20% 2v4* ↑21%	**0v1***↓40% **0v2***↓43% **0v3***↓45% **0v4***↓42%	NSD	**0v1***↓38% **0v2***↓37% **0v3***↓46% **0v4***↓43%	NSD	**0v1***↓37% **0v2***↓42% **0v3***↓45% **0v4***↓42%	NSD
16	**0v1***↓21% **0v2***↓27% **0v3***↓24% **0v4***↓17%	NSD	**0v1***↓26% **0v2***↓26% **0v3***↓29% **0v4***↓22%	NSD	**0v1***↓21% **0v2***↓16% **0v3***↓25% **0v4***↓18%	NSD	**0v1***↓20% **0v2***↓20% **0v3***↓27% **0v4***↓17%	NSD

[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 "0v1" means F_0 versus F_1. Asterisks (*) indicate the level of significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. 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 week 7 to the start of littering for all generations (F_0 to F_4), and the second included all data from week 4 to the start of littering 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 16 are from the former analysis. In both analyses, data from the weeks during which males and females were paired for mating (weeks 11 and 12) were not included.

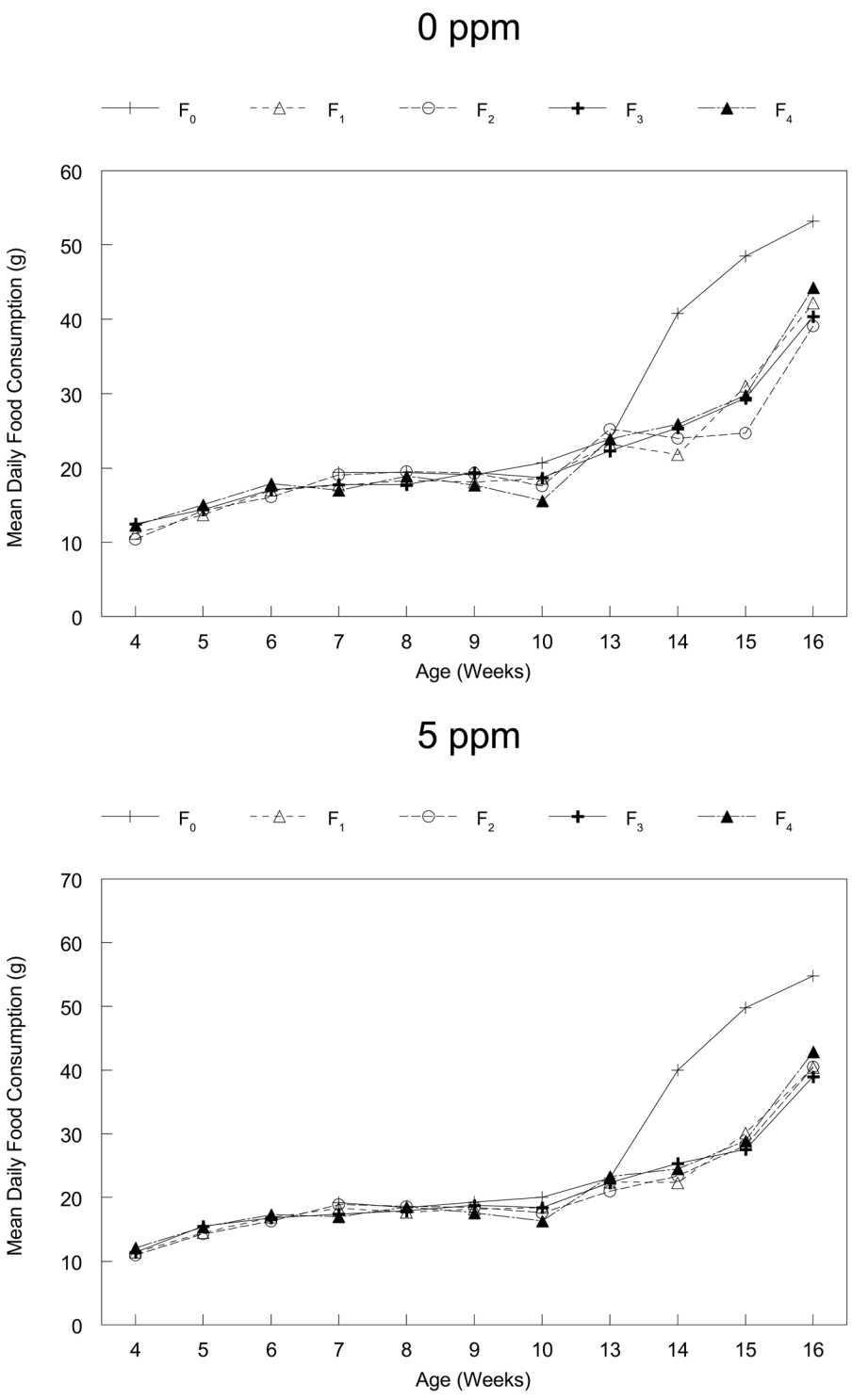

FIGURE E1
Feed Consumption by 0 and 5 ppm Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

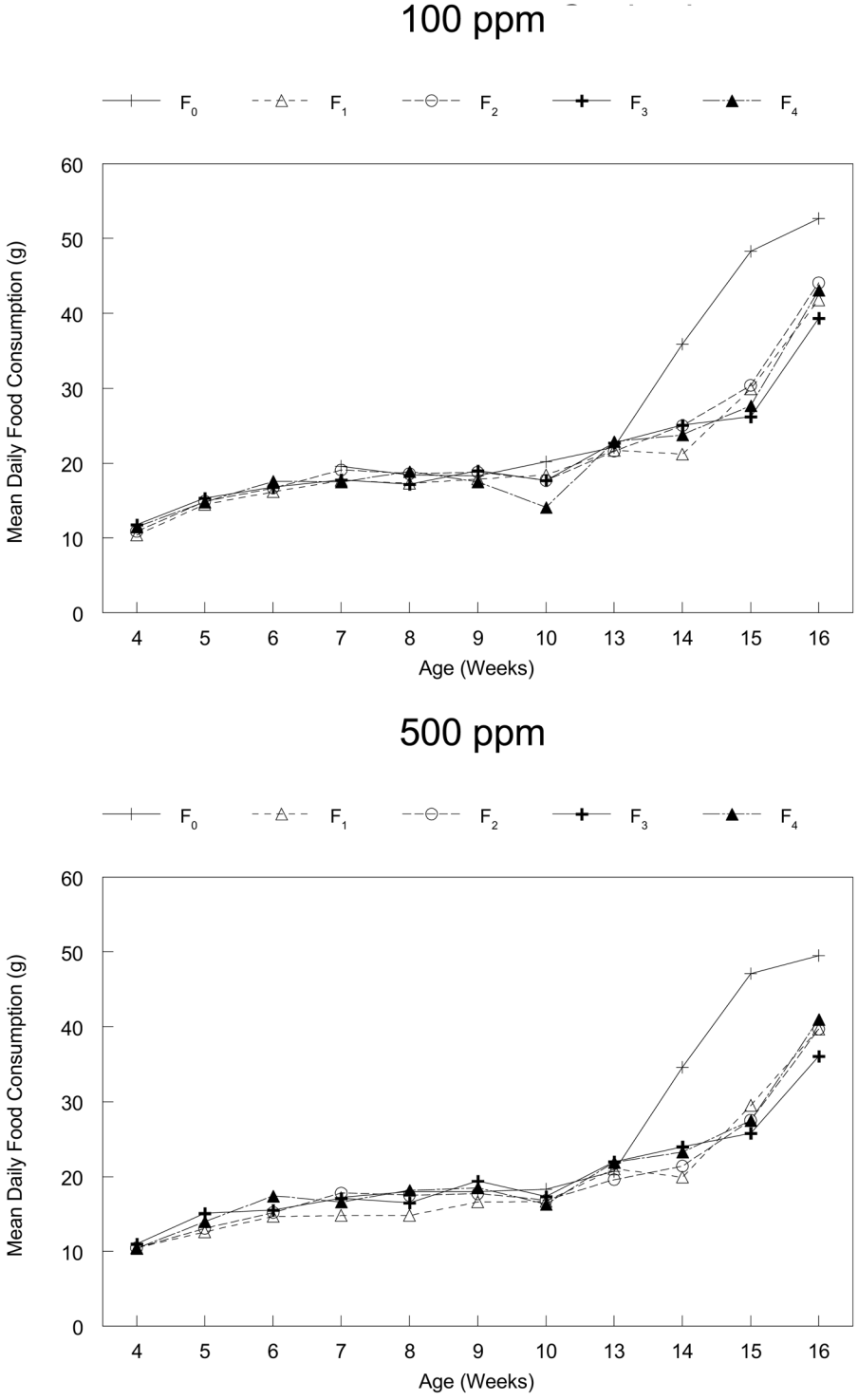

FIGURE E2
Feed Consumption by 100 and 500 ppm Female Rats
in the Multigenerational Reproductive Toxicology Feed Study of Genistein

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

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	1v4***↑19% 2v4***↑22% 3v4***↑29%	NA	1v4***↑29% 2v4***↑28% 3v4***↑31%	NA	NSD	NA	NSD
5	NA	NSD	NA	NSD	NA	NSD	NA	NSD
6	NA	1v2** ↑13%	NA	1v2***↑28% 1v4* ↑12% 2v3***↓15% 2v4***↓12%	NA	1v2* ↑10%	NA	1v2* ↑10%
7	**0v1* ↓10%** **0v2* ↓9%** **0v3* ↓10%**	NSD	**0v1** ↓10%** **0v3***↓15%**	NSD	**0v1***↓16%** **0v2***↓16%** **0v3***↓16%** 1v4** ↑11% 2v4** ↑11% 3v4** ↑12%	1v4***↑11% 2v4***↑11% 3v4***↑12%	**0v1***↓17%** **0v2** ↓10%** **0v3***↓16%** **0v4* ↓8%** 1v4* ↑10%	1v2* ↑8% 1v4** ↑10% 3v4* ↑10%
8	**0v4***↑13%** 2v4* ↑8% 3v4** ↑9%	2v4* ↑8% 3v4** ↑9%	**0v1** ↑10%** **0v2***↑17%** **0v3* ↑10%** **0v4***↑17%**	NSD	**0v4***↑17%** 1v4***↑12% 2v4***↑11% 3v4** ↑9%	1v4***↑12% 2v4***↑11% 3v4** ↑9%	**0v1***↑12%** **0v2***↑17%** **0v3***↑12%** **0v4***↑18%**	NSD
9	**0v4** ↑10%** 3v4***↑11%	3v4***↑11%	**0v4** ↑10%** 1v3***↓10% 3v4***↑16%	1v3***↓10% 3v4***↑16%	2v3** ↓10% 3v4***↑12%	1v3* ↓8% 2v3***↓10% 3v4***↑12%	1v3* ↓8% 3v4***↑13%	1v3* ↓8% 2v3* ↓8% 3v4***↑13%
10	**0v2* ↓9%** 1v4* ↑9% 2v4** ↑12% 3v4* ↑10%	1v4** ↑9% 2v4***↑12% 3v4** ↑10%	**0v1***↓13%** **0v3***↓17%** 1v4* ↑11% 2v3** ↓11% 3v4***↑16%	1v4** ↑11% 2v3** ↓11% 3v4***↑16%	**0v3***↓12%** 2v4* ↑9% 3v4***↑15%	1v3* ↓8% 2v4* ↑9% 3v4***↑15%	**0v1* ↓11%** **0v2* ↓11%** **0v3***↓18%** 3v4** ↑13%	1v3* ↓8% 2v3* ↓8% 3v4***↑13%

TABLE E7
Generational Effects in Feed Consumption by Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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	**0v1* ↑24%** 1v2***↓32% 2v4***↑43%	1v2***↓32% 2v4***↑43%	NSD	NSD	NSD	NSD	**0v1** ↑27%** **0v3* ↑21%** 1v2***↓25% 1v4***↓27% 2v3* ↑27% 3v4** ↓23%	1v2** ↓25% 1v4***↓27% 2v3* ↑27% 3v4** ↓23%
14	**0v2***↑25%** 1v2***↑36% 1v3* ↑17% 2v3** ↓14% 2v4***↓31% 3v4** ↓20%	1v2***↑36% 1v3* ↑17% 2v3** ↓14% 2v4***↓31% 3v4** ↓20%	**0v2***↑21%** **0v4* ↓16%** 1v2***↑38% 2v3***↓20% 2v4***↓30%	1v2***↑38% 2v3***↓20% 2v4***↓30%	**0v2***↑26%** 1v2***↑25% 2v4***↓26% 3v4** ↓18%	1v2***↑25% 2v4***↓26% 3v4** ↓18%	**0v3* ↑15%** 1v4** ↓17% 2v4***↓24% 3v4***↓25%	1v4** ↓17% 2v4***↓24% 3v4***↓25%
15	1v4***↓24% 2v4***↓26%	1v4***↓24% 2v4***↓26%	**0v1* ↑17%** 1v2* ↓15% 1v3***↓22% 1v4***↓26%	1v2* ↓15% 1v3***↓22% 1v4***↓26%	NSD	NSD	**0v2* ↑17%**	NSD
16	**0v1***↓18%** **0v2***↓20%** **0v3***↓39%** **0v4***↓24%** 1v3***↓26% 2v3***↓24% 3v4***↑25%	1v3***↓26% 2v3***↓24% 3v4***↑25%	**0v2***↓14%** **0v3***↓33%** **0v4***↓23%** 1v2* ↓11% 1v3***↓30% 1v4***↓20% 2v3***↓22% 2v4* ↓10% 3v4* ↑15%	1v2* ↓11% 1v3***↓30% 1v4***↓20% 2v3***↓22% 2v4* ↓10% 3v4* ↑15%	**0v2** ↓15%** **0v3***↓32%** **0v4***↓18%** 1v2** ↑15% 1v3***↓32% 1v4***↓18% 2v3***↓20% 3v4** ↑20%	1v2** ↓15% 1v3***↓32% 1v4***↓18% 2v3***↓20% 3v4** ↑20%	**0v1** ↑15%** **0v2** ↓13%** **0v3***↓27%** **0v4***↓18%** 1v2***↓24% 1v3***↓37% 1v4***↓29% 2v3** ↓17%	1v2***↓24% 1v3***↓37% 1v4***↓29% 2v3** ↓17%

TABLE E7
Generational Effects in Feed Consumption by Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein

Age (Weeks)	Dietary Genistein (ppm)							
	0		5		100		500	
	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
17	**0v1*** ↑28%** 1v2***↓25% 1v3***↓23% 1v4***↓24%	1v2***↓25% 1v3***↓23% 1v4***↓24%	**0v1** ↑17%** **0v4** ↑16%** 1v2***↓23% 1v3***↓22% 1v4***↓28%	1v2***↓23% 1v3***↓22% 1v4***↓28%	**0v1* ↑13%** **0v3*** ↑19%** **0v4* ↑14%** 1v2***↓20% 1v3***↓29% 1v4***↓24%	1v2***↓20% 1v3***↓29% 1v4***↓24%	**0v1* ↑14%** 1v2** ↓15% 1v3***↓23% 1v4***↓20%	1v2** ↓15% 1v3***↓23% 1v4***↓20%
18	**0v1* ↑17%** **0v3*** ↑33%** 1v3* ↑14% 2v3***↑21% 3v4***↓21%	1v3* ↑14% 2v3***↑21% 3v4***↓21%	**0v1* ↑17%** **0v2*** ↑23%** **0v3*** ↑37%** **0v4* ↑17%** 1v3** ↑17% 3v4** ↓15%	1v3* ↑17% 3v4** ↓15%	**0v3*** ↑28%** 1v3* ↑15% 2v3* ↑14% 3v4***↓21%	1v3* ↑15% 2v3* ↑14% 3v4***↓21%	**0v1*** ↑29%** **0v2*** ↑25%** **0v3*** ↑34%** **0v4** ↑20%**	NSD

[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 "0v1" means F_0 versus F_1. Asterisks (*) indicate the level of significance: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. 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 18 are from the former analysis. In both analyses, data from the weeks during which males and females were paired for mating (weeks 11 and 12) were not included.

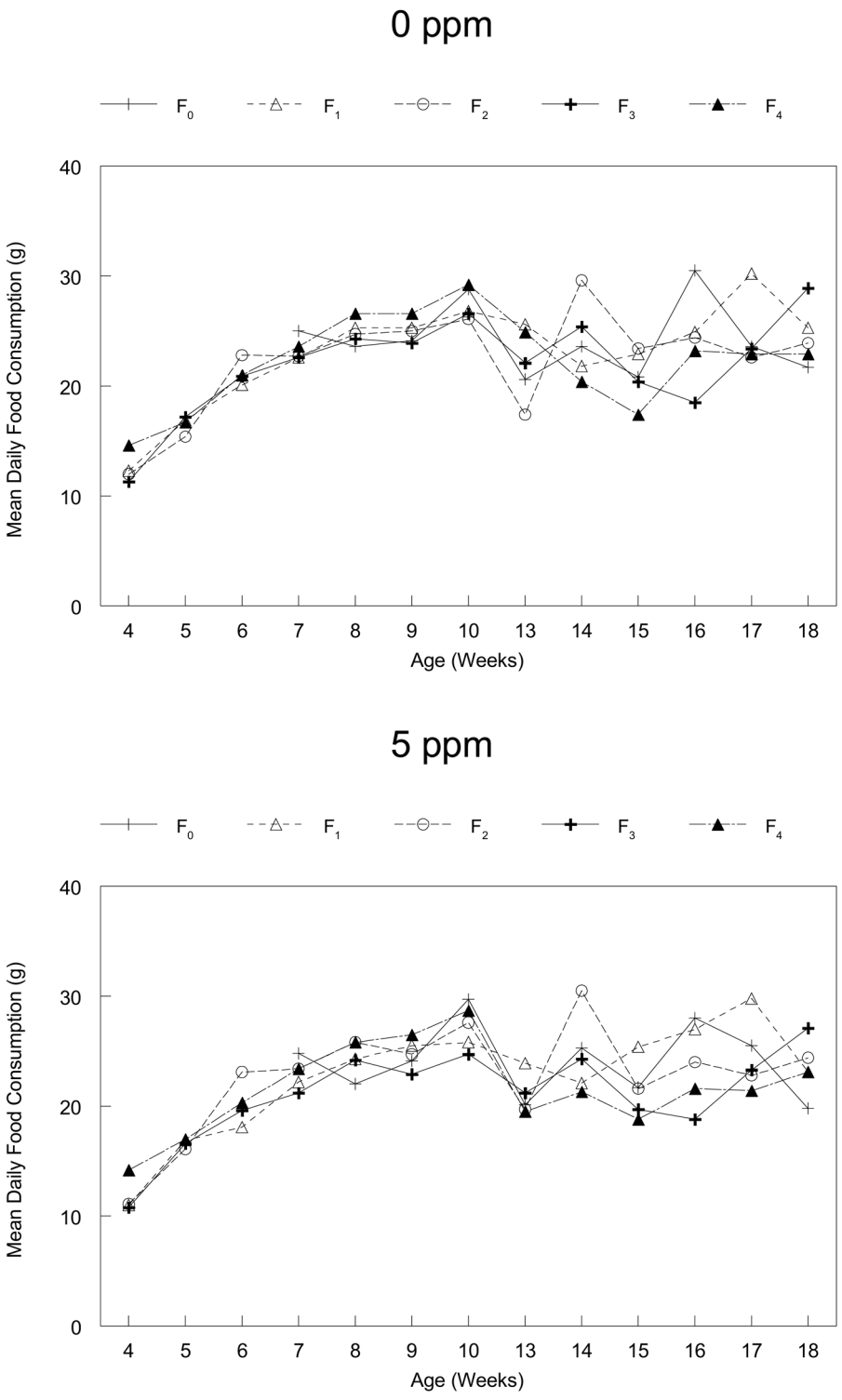

FIGURE E3
Feed Consumption by 0 and 5 ppm Male Rats
in the Multigenerational Reproductive Toxicology Feed Study of Genistein

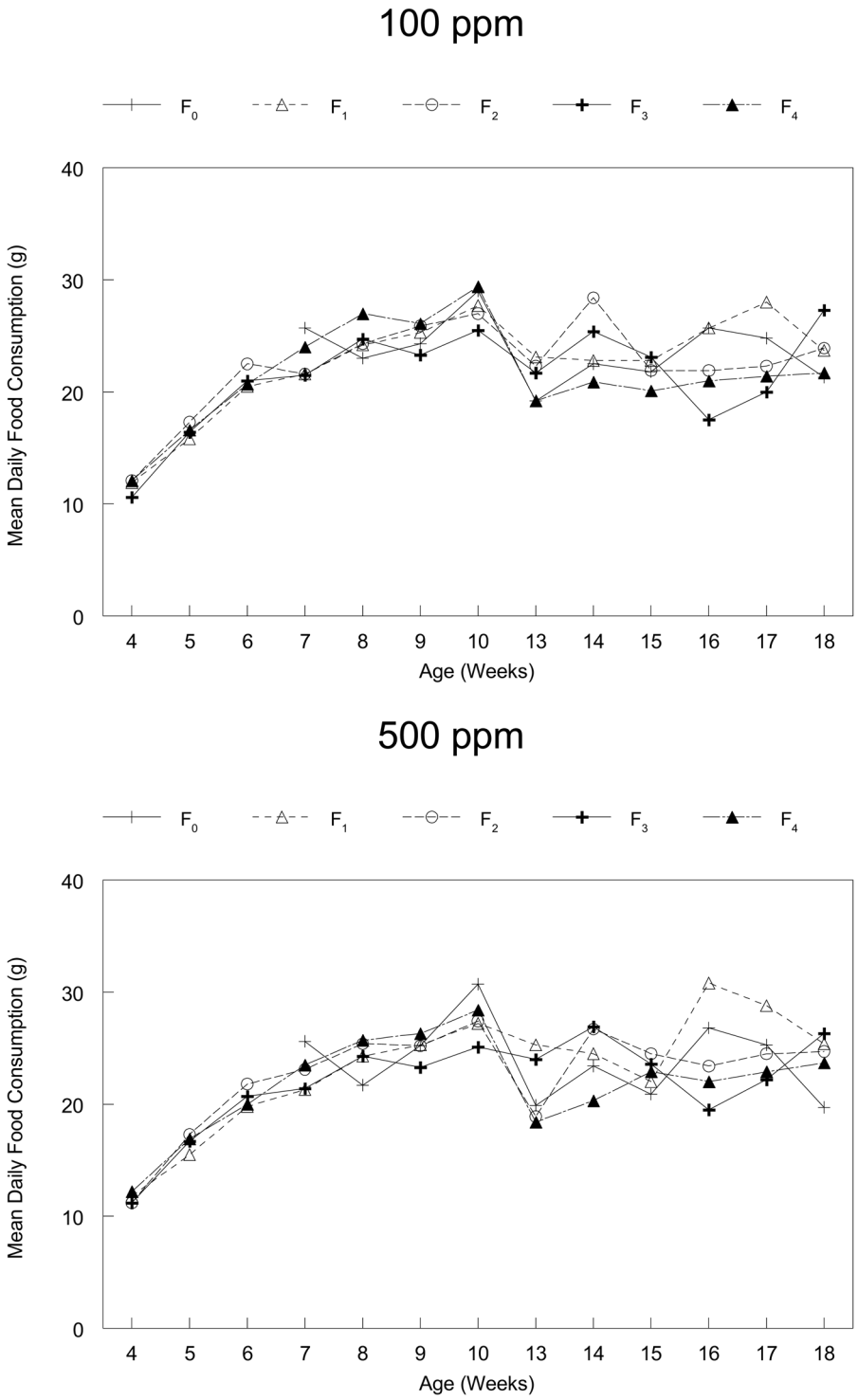

FIGURE E4
Feed Consumption by 100 and 500 ppm Male Rats
in the Multigenerational Reproductive Toxicology Feed Study of Genistein

APPENDIX F
WATER CONSUMPTION

TABLE F1a
Water Consumption by F_0 Female Rats during Lactation in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Postnatal Day	Dietary Genistein (ppm)			
	0	5	100	500
3	58.9 ± 4.9 (17) [3, 4]	64.5 ± 3.8 (18) [1, 2, 3, 4]	70.1 ± 3.5 (17) [1, 2, 3, 4]	65.4 ± 12.6 (14) [2, 3, 4]
4	51.8 ± 2.7 (23)	49.8 ± 2.4	49.7 ± 1.7 (24)	50.8 ± 2.6 (21)
5	53.9 ± 1.6	53.2 ± 1.5	53.0 ± 2.0	51.3 ± 1.6 (24)
6	55.4 ± 1.8	56.8 ± 1.5	58.7 ± 1.4	50.4 ± 2.4
7	58.1 ± 1.7 (24)	55.9 ± 1.5	56.5 ± 1.6	58.3 ± 2.8 (24)
8	60.0 ± 2.9	58.4 ± 1.5	58.6 ± 2.3	62.8 ± 2.6 (23)
9	60.7 ± 2.2	58.9 ± 1.9	57.3 ± 2.5	61.2 ± 3.0 (24)
10*	60.5 ± 2.2	61.6 ± 2.0	57.8 ± 2.6	64.6 ± 2.8 [3]
11*	62.8 ± 2.2	62.2 ± 2.7 (24)	65.6 ± 3.7	67.7 ± 2.7 [1, 3, 4]
12	64.6 ± 2.0	65.6 ± 2.3	66.4 ± 2.8	68.8 ± 2.0 [1, 3, 4]
13*	65.2 ± 2.1 [3]	69.1 ± 2.3	72.0 ± 2.7 [3]	72.5 ± 3.7* [1, 3, 4]
14	74.4 ± 3.7 (24) [1, 3]	71.2 ± 3.1	76.2 ± 3.2 [3]	74.7 ± 3.4 (24) [3]
15**	73.8 ± 3.1	73.8 ± 2.3	85.2 ± 5.8* (23) [1, 3, 4]	84.1 ± 5.4** (21) [1, 2, 3, 4]
16	72.7 ± 3.3 (24)	71.0 ± 2.5	75.1 ± 3.6 (24) [3]	71.0 ± 4.2 (22)
17	76.5 ± 3.4 (24) [3]	72.7 ± 2.6 [4]	74.0 ± 2.9 [3]	77.5 ± 3.3 [1, 3, 4]
18***	69.4 ± 2.6	72.0 ± 2.2	73.4 ± 3.1	81.3 ± 4.5** (24) [3, 4]
19*	82.8 ± 3.6	86.2 ± 6.9 [1, 2, 3, 4]	78.1 ± 2.9 (24)	74.5 ± 3.7 (24)
20	87.0 ± 4.3 (16) [1, 3]	82.5 ± 6.1 (16)	85.1 ± 3.9 (20) [1, 3]	82.7 ± 3.1 (20)

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

[a] Mean mL of water consumed per day ± standard error. Twenty-five animals in each group except where indicated by number in parentheses. Asterisks (*) in shaded cells in the Postnatal Day column indicate significant linear exposure concentration trends on that day in that generation as determined by contrasts; asterisks in the exposed group columns indicate significant differences from controls on the same day in the same generation as determined by Dunnett's test: *, P≤0.05; **, P≤0.01; ***, P≤0.001. 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.

[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=0.001; generation, P<0.001; dose × generation, P=0.443; days, P<0.001; days × dose, P=0.267; days × generation, P<0.001; days × dose × generation, P=0.474.

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

Postnatal Day	Dietary Genistein (ppm)			
	0	5	100	500
3	54.4 ± 1.9 (22)	49.2 ± 2.7 (22) [0]	48.0 ± 3.0 (21) [0]	55.7 ± 2.7 (21)
4	56.0 ± 2.2 (24)	53.2 ± 1.5	54.9 ± 2.4 (24)	53.3 ± 2.4 (23)
5	54.9 ± 3.9 (24)	51.2 ± 1.7	57.9 ± 3.9	54.5 ± 2.4 (23)
6	53.9 ± 2.1 (24)	56.4 ± 3.3	55.5 ± 1.7	52.0 ± 1.1 (23)
7	54.9 ± 1.4 (24)	55.0 ± 2.2	56.8 ± 2.5 (24)	52.1 ± 1.7 (22)
8	59.8 ± 4.1 (24)	56.3 ± 1.5	61.4 ± 1.9	59.6 ± 1.5 (22)
9	58.0 ± 1.8 (24)	55.5 ± 2.9	58.4 ± 2.3	55.2 ± 1.4 (23)
10**	55.8 ± 2.7 (24)	56.3 ± 2.1	59.3 ± 1.9	63.8 ± 5.7 (23)
11	58.4 ± 2.6 (22)	57.2 ± 2.4	59.6 ± 2.4	57.1 ± 1.9 (23) [0]
12	60.9 ± 2.0 (24)	59.8 ± 3.0	62.1 ± 2.7	56.7 ± 2.2 (23) [0]
13	61.6 ± 2.2 (23)	63.6 ± 3.2	61.9 ± 1.9	57.7 ± 2.3 (23) [0]
14	61.4 ± 3.6 [0] (23)	68.5 ± 5.0 (24)	69.1 ± 4.4 [3]	64.9 ± 2.6 (23)
15	67.1 ± 2.8 (24)	67.7 ± 2.6	62.5 ± 2.2 [0, 2]	67.6 ± 3.6 (23) [0]
16	77.6 ± 7.3 (24) [3]	66.3 ± 3.2*	71.1 ± 2.9 (23) [3]	70.2 ± 4.3 (23)
17	71.1 ± 3.6 (24)	72.6 ± 3.8 [4]	66.0 ± 2.8	64.7 ± 3.1 (23) [0]
18	73.1 ± 2.9 (24) [3]	70.8 ± 3.1	73.1 ± 2.6	72.3 ± 3.6 (23)
19	72.8 ± 2.6 (24)	71.1 ± 3.5 [0]	69.8 ± 2.0	75.1 ± 4.3 (23)
20	72.6 ± 2.7 (21) [0, 4]	73.9 ± 3.3 (23)	72.8 ± 3.0 (22) [0, 4]	75.7 ± 7.6 (19)

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 Genistein[a,b]

Postnatal Day	Dietary Genistein (ppm)			
	0	5	100	500
3	47.4 ± 2.4 (24)	43.6 ± 1.8 (24) [0]	49.5 ± 1.6 [0]	45.2 ± 2.8 (24) [0]
4	53.6 ± 2.1 (23)	49.3 ± 1.7 (23)	52.9 ± 2.1 (24)	51.3 ± 1.2
5	46.9 ± 1.3 (22)	50.9 ± 1.3 (22)	56.6 ± 1.6 (22)	50.9 ± 1.5
6	55.1 ± 1.9	51.4 ± 1.3	57.7 ± 1.7	53.7 ± 1.8
7	59.4 ± 1.8	55.4 ± 1.5	60.8 ± 2.1 (24)	60.6 ± 3.5 [3]
8**	57.6 ± 1.6 (22)	56.7 ± 1.6	60.4 ± 1.4	64.8 ± 2.7*
9	59.2 ± 2.1	57.3 ± 1.4	59.8 ± 1.8	61.2 ± 1.9
10	58.7 ± 2.1	55.2 ± 1.5	58.0 ± 1.8	55.6 ± 2.2 (23)
11	56.0 ± 1.5	55.8 ± 1.4	56.7 ± 2.3	58.6 ± 1.9
12	63.7 ± 2.6	59.1 ± 1.4	58.3 ± 2.1	58.6 ± 2.1 (24)
13	62.7 ± 2.5	61.7 ± 2.7	62.8 ± 2.8	62.9 ± 3.2
14	69.6 ± 3.0	66.6 ± 2.4	66.8 ± 3.2 (24)	67.3 ± 3.5
15	69.9 ± 2.3	63.9 ± 2.0	76.8 ± 3.0 [1, 3]	69.8 ± 3.1 (24) [0]
16	69.2 ± 4.1	66.6 ± 2.2	67.4 ± 2.1	68.8 ± 2.9 (24)
17	69.9 ± 2.8	64.6 ± 1.8	69.3 ± 2.0	67.9 ± 3.2 (24)
18	72.3 ± 3.8 [3]	64.4 ± 2.9	73.8 ± 2.0	73.5 ± 2.6 (24)
19	80.6 ± 5.2	69.6 ± 2.9* [0]	70.8 ± 2.2* (24)	72.6 ± 3.2 (24)
20	75.9 ± 3.7 (24) [4]	72.5 ± 2.7	75.2 ± 2.4 [4]	72.6 ± 3.2 (24)

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 Genistein[a,b]

Postnatal Day	Dietary Genistein (ppm)			
	0	5	100	500
3	45.6 ± 2.9 (23) [0]	44.4 ± 2.9 (24) [0]	45.1 ± 1.9 (22) [0]	47.1 ± 2.2 [0]
4	49.5 ± 2.1	53.5 ± 4.5 (23)	55.1 ± 4.7	48.8 ± 2.0 [4]
5	52.8 ± 5.5	47.7 ± 1.5 (24)	57.8 ± 4.5 (24)	48.7 ± 1.6
6	49.8 ± 2.6	48.6 ± 2.6	60.8 ± 6.0*	50.0 ± 1.8
7	55.9 ± 3.9 (24)	54.0 ± 2.1	49.9 ± 1.6 (23)	50.3 ± 1.9 [2]
8	59.3 ± 1.9	55.2 ± 2.3	57.1 ± 2.1 (24)	61.4 ± 4.7
9	55.0 ± 1.0	53.3 ± 1.4	54.4 ± 1.8	54.2 ± 1.8
10	53.6 ± 1.1	53.8 ± 1.4	54.4 ± 1.4	54.1 ± 1.7 [0]
11	55.8 ± 1.8 (24)	56.9 ± 2.0	60.6 ± 6.6	56.8 ± 2.2 [0]
12	57.4 ± 1.2	56.3 ± 1.6	59.9 ± 3.4	57.1 ± 2.1 [0]
13	54.8 ± 1.5 [0]	57.2 ± 2.0	55.4 ± 2.6 [0, 4]	55.9 ± 1.9 [0]
14	64.0 ± 8.2 (24) [0]	60.6 ± 2.3	57.2 ± 2.7 [0, 1]	61.4 ± 3.1 [0]
15	67.2 ± 5.5	71.1 ± 7.8	61.6 ± 2.6 [0, 2]	65.7 ± 5.2 [0]
16	63.2 ± 2.7 [1]	60.2 ± 2.0	59.8 ± 2.0 [0, 1]	62.1 ± 3.4
17	62.8 ± 2.5 [0]	65.7 ± 4.4	63.3 ± 3.2 [0]	62.1 ± 3.2 (24) [0]
18	61.0 ± 2.4 [1, 2]	70.2 ± 4.4* (24)	65.4 ± 2.6	67.9 ± 3.8 (24) [0]
19	76.6 ± 3.8	64.3 ± 3.0* (24) [0]	71.6 ± 3.4	64.6 ± 3.3* (24)
20	71.2 ± 3.5 [0, 4]	73.7 ± 4.2	70.4 ± 3.8 [0, 4]	73.6 ± 3.9

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 Genistein[a,b]

Postnatal Day	Dietary Genistein (ppm)			
	0	5	100	500
3	47.0 ± 1.7 (22) [0]	49.8 ± 1.7 (24) [0]	52.1 ± 2.0 (24) [0]	52.3 ± 2.6 (22) [0]
4*	53.4 ± 1.9	54.1 ± 1.6	54.9 ± 2.1	62.6 ± 7.1 [3]
5	54.9 ± 1.9 (24)	54.9 ± 3.7	57.5 ± 1.8	54.4 ± 2.1
6	51.1 ± 2.2	51.9 ± 2.1	53.6 ± 1.7	55.6 ± 4.5
7	56.7 ± 1.5	55.2 ± 3.1	58.4 ± 1.9	59.2 ± 4.5 (24)
8	58.4 ± 1.4	55.9 ± 1.5	59.0 ± 2.0	61.6 ± 2.4
9	58.8 ± 1.9	56.6 ± 1.7	61.1 ± 1.7	58.6 ± 2.7
10	62.2 ± 1.5	57.1 ± 1.4	64.2 ± 1.7	62.0 ± 2.2
11	59.6 ± 1.9	59.4 ± 1.7	64.1 ± 1.9	59.6 ± 1.6 [0]
12	61.9 ± 3.3	63.7 ± 2.4	62.4 ± 1.8	60.8 ± 1.5 [0]
13	59.8 ± 2.1 (24)	62.9 ± 1.8	68.9 ± 2.5 [3]	63.1 ± 2.2 [0]
14	68.5 ± 2.0	62.9 ± 2.0	68.8 ± 3.3	67.7 ± 2.7
15	66.2 ± 2.4	64.4 ± 1.9	69.6 ± 2.2 [0]	66.9 ± 2.1 [0]
16	67.9 ± 2.1	63.8 ± 1.9	68.4 ± 2.0	68.3 ± 2.4
17	70.6 ± 2.8	59.9 ± 2.6* [0, 1]	75.1 ± 3.0	69.7 ± 2.3 [0]
18	69.0 ± 2.2	66.5 ± 2.5	72.4 ± 2.7	66.4 ± 2.0 [0]
19	77.5 ± 3.0	69.9 ± 2.5 [0]	79.4 ± 2.9	74.2 ± 2.6
20	88.0 ± 3.1 [1, 2, 3]	75.4 ± 3.1**	88.0 ± 2.4 [1, 2, 3]	85.1 ± 3.5 (24)

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 Genistein

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Mating Index[a]	F_0	1.00 (35)	1.00 (35)	1.00 (35)	1.00 (35)
	F_1	1.00 (35)	1.00 (35)	1.00 (35)	1.00 (35)
	F_2	1.00 (40)	1.00 (40)	1.00 (40)	1.00 (40)
	F_3	1.00 (35)	1.00 (35)	1.00 (35)	1.00 (35)
	F_4	1.00 (35)	1.00 (35)	1.00 (35)	1.00 (35)
Mating Time[b]	F_0	3.1 ± 0.5 (26)	4.0 ± 0.7 (27)	4.6 ± 0.7 (27)	3.8 ± 0.5 (27)
	F_1	3.5 ± 0.4 (33)	4.2 ± 0.6 (33)	4.3 ± 0.5 (33)	3.0 ± 0.4 (33)
Dose P=0.726	F_2	3.6 ± 0.4 (34)	3.3 ± 0.4 (30)	3.1 ± 0.3 (31)	3.3 ± 0.4 (32)
Gen P=0.266	F_3*	3.4 ± 0.4 (29)	3.4 ± 0.6 (22)	3.4 ± 0.4 (28)	4.7 ± 0.7 (28)
DxG P=0.127	F_4	3.6 ± 0.4 (29)	2.5 ± 0.3 (30)	3.1 ± 0.3 (33)	3.7 ± 0.6 (30)
Fertility Index[c]	F_0	0.85 (26)	0.85 (27)	0.89 (27)	0.89 (27)
	F_1	0.79 (33)	0.82 (33)	0.79 (33)	0.73 (33)
Dose P=0.674	F_2	0.71 (34)	0.77 (30)	0.90 (31)	0.91 (32)
Gen P=0.268	F_3	0.83 (29)	0.73 (22)	0.86 (28)	0.82 (28)
DxG P=0.687	F_4	0.90 (29)	0.90 (30)	0.88 (33)	0.80 (30)
Pregnancy Index[d]	F_0	0.89 (35)	0.86 (35)	0.89 (35)	0.91 (35)
	F_1	0.80 (35)	0.80 (35)	0.77 (35)	0.71 (35)
Dose P=0.604	F_2	0.68 (40)	0.78 (40)	0.90 (40)	0.90 (40)
Gen P=0.161	F_3	0.86 (35)	0.77 (35)	0.89 (35)	0.77 (35)
DxG P=0.414	F_4	0.86 (35)	0.86 (35)	0.86 (35)	0.77 (35)

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Gestation Time[e] Dose P=0.964 Gen P=0.041 DxG P=0.280	F_0**	22.3 ± 0.2 (22)	22.4 ± 0.1 (23)	22.3 ± 0.1 (24)	22.0 ± 0.2 (24)
	F_1	22.3 ± 0.1 (26)	22.3 ± 0.1 (27)	22.4 ± 0.1 (26)	22.3 ± 0.1 (24)
	F_2	22.5 ± 0.1 (23)	22.4 ± 0.1 (23)	22.4 ± 0.1 (26)	22.3 ± 0.1 (30)
	F_3*	22.2 ± 0.1 (24)	22.2 ± 0.1 (16)	22.3 ± 0.1 (24)	22.5 ± 0.1 (23)
	F_4	22.3 ± 0.1 (25)	22.5 ± 0.1 (27)	22.5 ± 0.1 (29)	22.5 ± 0.1 (24)

[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. All dams were found to be vaginal plug-positive and/or delivered litters.

[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. Only the F_0 breed mother effect was significant and was incorporated into the analysis model. There were no significant exposure concentration effect ($P \leq 0.05$) within any generation as determined by Dunnett's tests and no significant generation effect ($P \leq 0.05$) within exposure groups as determined by Holm's adjusted t-tests. A significant linear exposure concentration trend in the F_3 generation is indicated by an asterisk (*) in the shaded cells in the generation column: *, $P \leq 0.05$.

[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 the vaginal plug was detected or not. The results of a logistic ANOVA are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There were no significant exposure concentration effects ($P \leq 0.05$) within any generation as determined by Holm's adjusted Chi-square tests. There were no significant generation effects ($P \leq 0.05$) within exposure groups indicated by Holm's adjusted t-tests.

[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 ANOVA are given for the factors dose, generation (Gen), and dose × generation interaction (D×G). There were no significant exposure concentration effects ($P \leq 0.05$) within any generations as determined by Holm's adjusted Chi-square tests. There were no significant generation effects ($P \leq 0.05$) within exposure groups indicated by Holm's adjusted t-tests.

[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 two-way ANOVA are indicated for dose and generation (Gen) main effects and the 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. All three random effects were significant, but it was only computationally feasible to include the F_0 breed mother and the F_0 breed father effects in the model. There were no significant exposure concentration effects ($P \leq 0.05$) within any generation as determined by Dunnett's tests and no significant generation effects ($P \leq 0.05$) within exposure groups as determined by Holm's adjusted t-tests. Significant linear exposure concentration trends within a generation are indicated by asterisks (*) in shaded cells in the generation column: *, $P \leq 0.05$; **, $P \leq 0.01$.

APPENDIX H
LITTER AND PERINATAL PUP PARAMETERS

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Total Pups Born[b,c,d] Dose P<0.001 Gen P=0.003 DxG P<0.001	F_1***	12.87 ± 0.60 (31)	14.23 ± 0.62 (30)	13.48 ± 0.64 (31)	11.28 ± 0.60 (32) [2, 4]
	F_2***	13.14 ± 0.58 (28)	13.89 ± 0.52 (28)	11.96 ± 0.82 (27) [3]	9.12 ± 0.56*** (25) [1, 3, 4, 5]
	F_3**	13.88 ± 0.33 (26)	13.42 ± 0.45 (31)	14.03 ± 0.40 (34) [2]	12.00 ± 0.38 (36) [2]
	F_4	12.77 ± 0.48 (30)	12.73 ± 0.44 (26)	12.94 ± 0.59 (31)	13.56 ± 0.35 (27) [2]
	F_5	11.93 ± 0.47 (29)	13.47 ± 0.60 (30)	13.53 ± 0.41 (30)	12.63 ± 0.45 (27) [2]
Live Pups Born[b,c,e] Dose P<0.001 Gen P=0.007 DxG P<0.001	F_1***	12.84 ± 0.61 (31)	14.23 ± 0.62 (30)	13.42 ± 0.64 (31)	11.22 ± 0.59 (32) [2, 4]
	F_2***	13.11 ± 0.58 (28)	13.89 ± 0.52 (28)	11.96 ± 0.82 (27) [3]	9.12 ± 0.56*** (25) [1, 3, 4, 5]
	F_3*	13.58 ± 0.39 (26)	13.42 ± 0.45 (31)	14.00 ± 0.40 (34) [2]	11.89 ± 0.39 (36)
	F_4	12.77 ± 0.48 (30)	12.73 ± 0.44 (26)	12.94 ± 0.59 (31)	13.52 ± 0.35 (27)
	F_5	11.93 ± 0.47 (29)	13.47 ± 0.60 (30)	13.53 ± 0.41 (30)	12.59 ± 0.46 (27)

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Female Live Births[b,c,e] Dose P<0.001 Gen P=0.093 DxG P=0.052	F_1**	6.48 ± 0.36 (31)	7.10 ± 0.47 (30)	7.26 ± 0.49 (31) [2]	5.44 ± 0.44 (32)
	F_2***, #	6.43 ± 0.45 (28)	7.39 ± 0.40 (28)	5.30 ± 0.42 (27) [1, 3, 4]	4.32 ± 0.45** (25) [4]
	F_3	6.31 ± 0.46 (26)	6.65 ± 0.40 (31)	7.21 ± 0.42 (34) [2]	5.67 ± 0.37 (36)
	F_4	6.13 ± 0.40 (30)	6.58 ± 0.36 (26)	7.03 ± 0.40 (31) [2]	6.22 ± 0.40 (27) [2]
	F_5	6.48 ± 0.44 (29)	6.90 ± 0.52 (30)	6.83 ± 0.36 (30)	5.63 ± 0.37 (27)
Male Live Births[b,c,d] Dose P=0.578 Gen P=0.232 DxG P=0.007	F_1	6.35 ± 0.43 (31)	7.13 ± 0.51 (30)	6.16 ± 0.53 (31)	5.78 ± 0.37 (32)
	F_2**	6.68 ± 0.41 (28)	6.50 ± 0.42 (28)	6.67 ± 0.56 (27)	4.80 ± 0.37* (25) [4, 5]
	F_3	7.27 ± 0.39 (26)	6.77 ± 0.41 (31)	6.79 ± 0.40 (34)	6.22 ± 0.42 (36)
	F_4	6.63 ± 0.48 (30)	6.15 ± 0.43 (26)	5.90 ± 0.39 (31)	7.30 ± 0.39 (27) [2]
	F_5	5.45 ± 0.39 (29)	6.57 ± 0.43 (30)	6.70 ± 0.41 (30)	6.96 ± 0.47 (27) [2]

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Pups Born Dead[c,f] Dose P=0.182 Gen P=0.075 DxG P=0.832	F_1	0.03 ± 0.03 (31)	0.00 ± 0.00 (30)	0.06 ± 0.04 (31)	0.06 ± 0.04 (32)
	F_2	0.00 ± 0.00 (28)	0.00 ± 0.00 (28)	0.00 ± 0.00 (27)	0.00 ± 0.00 (25)
	F_3	0.00 ± 0.00 (26)	0.00 ± 0.00 (31)	0.03 ± 0.03 (34)	0.00 ± 0.00 (36)
	F_4	0.00 ± 0.00 (30)	0.00 ± 0.00 (26)	0.00 ± 0.00 (31)	0.04 ± 0.04 (27)
	F_5	0.00 ± 0.00 (29)	0.00 ± 0.00 (30)	0.00 ± 0.00 (30)	0.04 ± 0.04 (27)
Male Pup Weight[c,d] Litter Size P<0.001 Dose P=0.001 Gen P=0.999 DxG P=0.123	F_1[#]	6.3 ± 0.1 (28)	6.1 ± 0.1 (30)	5.9 ± 0.1* (28)	6.2 ± 0.2 (30)
	F_2	6.2 ± 0.1 (27)	6.0 ± 0.1 (26)	6.3 ± 0.1 (27)	6.3 ± 0.1 (25)
	F_3[# #]	6.1 ± 0.1 (26)	6.2 ± 0.1 (30)	5.9 ± 0.1 (35)	6.2 ± 0.1 (33)
	F_4	6.2 ± 0.1 (30)	6.2 ± 0.1 (27)	6.1 ± 0.1 (30)	6.1 ± 0.1 (27)
	F_5[#]	6.5 ± 0.1 (27)	6.1 ± 0.1** (30)	5.9 ± 0.1** (30)	6.1 ± 0.1* (27)

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Female Pup Weight[c,g] Litter Size P<0.001 Dose P=0.124 Gen P=0.917 DxG P=0.474	F_1	5.8 ± 0.1 (29)	5.7 ± 0.1 (30)	5.7 ± 0.1 (30)	5.9 ± 0.2 (29)
	F_2	5.8 ± 0.1 (27)	5.8 ± 0.2 (27)	5.8 ± 0.1 (27)	6.0 ± 0.1 (24)
	F_3	5.9 ± 0.1 (26)	5.8 ± 0.1 (31)	5.5 ± 0.1 (34)	5.8 ± 0.1 (34)
	F_4	5.8 ± 0.1 (30)	5.8 ± 0.1 (27)	5.8 ± 0.1 (31)	5.7 ± 0.1 (25)
	F_5	6.1 ± 0.1 (27)	5.6 ± 0.1* (30)	5.6 ± 0.1* (30)	5.7 ± 0.1* (27)
Sex Ratio[b,c,h] Dose P<0.001 Gen P=0.713 DxG P=0.573	F_1*	1.06 ± 0.11 (29)	1.12 ± 0.13 (30)	1.01 ± 0.16 (30)	1.81 ± 0.46 (31)
	F_2	1.27 ± 0.15 (28)	0.93 ± 0.10 (27)	1.40 ± 0.17 (27)	1.52 ± 0.27 (25)
	F_3	1.43 ± 0.17 (26)	1.26 ± 0.16 (31)	1.14 ± 0.12 (35)	1.65 ± 0.33 (36)
	F_4*, #	1.38 ± 0.23 (30)	1.18 ± 0.14 (27)	0.88 ± 0.07 (31)	2.57 ± 0.78 (27)
	F_5	1.63 ± 0.46 (27)	1.23 ± 0.17 (30)	1.17 ± 0.14 (30)	1.57 ± 0.24 (27)

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Male Anogenital Distance[b,c,g,i] Body Weight P<0.001 Dose P=0.507 Gen P<0.001 DxG P=0.693	F_1**	3.75 ± 0.08 [3, 4, 5]	3.68 ± 0.08 [3, 4, 5]	3.55 ± 0.10 [3,4, 5]	3.54 ± 0.07* [4, 5]
	F_2	3.69 ± 0.05 [3, 4, 5]	3.59 ± 0.07 [3, 4, 5]	3.62 ± 0.07 [3, 4, 5]	3.59 ± 0.04 [4, 5]
	F_3	3.43 ± 0.04 [1, 2]	3.34 ± 0.04 [1, 2]	3.39 ± 0.06 [1, 2]	3.40 ± 0.06
	F_4	3.26 ± 0.04 [1, 2]	3.26 ± 0.02 [1, 2]	3.30 ± 0.04 [1, 2]	3.24 ± 0.06 [1, 2]
	F_5	3.29 ± 0.05 [1, 2]	3.22 ± 0.03 [1, 2]	3.20 ± 0.04 [1, 2]	3.25 ± 0.03 [1, 2]
Male Anogenital Distance Ratio[b,c,g,i] Dose P=0.624 Gen P<0.001 DxG P=0.767	F_1*	1.97 ± 0.04 [3, 4, 5]	1.98 ± 0.03 [3, 4, 5]	1.97 ± 0.05 [3, 4, 5]	1.89 ± 0.04 [4]
	F_2	1.96 ± 0.02 [3, 4, 5]	1.96 ± 0.03 [3, 4, 5]	1.98 ± 0.02 [3, 4, 5]	1.94 ± 0.03 [4, 5]
	F_3	1.84 ± 0.02 [1, 2]	1.83 ± 0.01 [1, 2]	1.85 ± 0.02 [1, 2]	1.85 ± 0.02
	F_4	1.77 ± 0.02 [1, 2]	1.74 ± 0.02 [1, 2]	1.78 ± 0.02 [1, 2]	1.77 ± 0.03 [1, 2]
	F_5	1.75 ± 0.03 [1, 2]	1.75 ± 0.04 [1, 2]	1.76 ± 0.03 [1, 2]	1.79 ± 0.02 [2]

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Female Anogenital Distance[b,c,i,j] Body Weight P=0.001 Dose P=0.143 Gen P<0.001 DxG P=0.008	F_1**	2.31 ± 0.03 [4, 5]	2.22 ± 0.05 [4, 5]	2.19 ± 0.05 [3,5]	2.15 ± 0.03**
	F_2*	2.35 ± 0.03 [3, 4, 5]	2.28 ± 0.03 [4, 5]	2.30 ± 0.03 [3, 4, 5]	2.25 ± 0.03* [3, 4, 5]
	F_3*	2.20 ± 0.04 [2, 4, 5]	2.20 ± 0.03 [4, 5]	2.07 ± 0.04* [1,2]	2.10 ± 0.02 [2]
	F_4	2.04 ± 0.03 [1, 2, 3]	2.09 ± 0.02 [1, 2, 3]	2.10 ± 0.02 [2]	2.09 ± 0.04 [2]
	F_5	1.97 ± 0.03 [1, 2, 3]	2.00 ± 0.03 [1, 2, 3]	1.98 ± 0.03 [1, 2]	2.03 ± 0.03 [2]
Female Anogenital Distance Ratio[b,c,i,j] Dose P=0.311 Gen P<0.001 DxG P=0.018	F_1**	1.25 ± 0.02 [4, 5]	1.23 ± 0.02 [4, 5]	1.23 ± 0.03 [4,5]	1.18 ± 0.02*
	F_2	1.21 ± 0.02 [3,4, 5]	1.26 ± 0.02 [4, 5]	1.30 ± 0.01 [3, 4, 5]	1.24 ± 0.02 [3,4,5]
	F_3*	1.21 ± 0.02 [2,4, 5]	1.23 ± 0.01 [4, 5]	1.16 ± 0.02 [2]	1.16 ± 0.01 [2]
	F_4	1.13 ± 0.02 [1, 2, 3]	1.14 ± 0.02 [1, 2, 3]	1.16 ± 0.01 [1,2]	1.16 ± 0.03
	F_5	1.07 ± 0.02 [1, 2, 3]	1.11 ± 0.02 [1, 2, 3]	1.11 ± 0.02 [1, 2]	1.13 ± 0.01 [2]

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

[a] Asterisks (*) and pound signs (#) in shaded cells in the generation column indicate significant linear and quadratic exposure concentration trends, respectively, in that 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 #, $P \leq 0.05$; ** or # #, $P \leq 0.01$; ***, $P \leq 0.001$.

[b] Mean ± standard error reported. Statistical analyses were run on square root transformations, or, for the sex ratio, natural log transformations, of the raw data to stabilize variance. Numbers in parentheses are the numbers of litters. Generation numbers in brackets indicate significant differences ($P \leq 0.05$) between the indicated generations within an exposure group as determined by Holm's adjusted t-tests.

[c] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the dose × generation interaction (D×G); for ANCOVA, the covariates were litter size (for pup weights) or terminal 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 effects of the F_0 breed mother and the F_0 breed mother × F_0 breed father interaction were incorporated into the statistical model.

[e] A significant random effect of the F_0 breed mother was incorporated into the statistical model.

[f] Non-transformed data, rather than square root transformations, were analyzed since response variables had values of only 0 and 1. There were no significant random effects incorporated into the statistical model.

[g] 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.

[h] The sex ratio is the ratio of males to females per litter. A significant random effect of the F_0 breed mother × F_0 breed father interaction was incorporated into the statistical model.

[i] All anogenital distance measurements were made on the pups in 10 litters after culling to four pups per sex. Data were analyzed by ANCOVA with pup body weight as covariate or as the ratio of measured anogenital distance to the cube root of body weight.

[j] Significant random effects of the F_0 breed mother and the F_0 breed father were incorporated into the statistical model.

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 Genistein[a]

	Dietary Genistein (ppm)				Trends	
	0	5	100	500	Linear	Quad
Age (Postnatal Day) at vaginal opening[b]						
F_1***	33.0 ± 0.34 [3,4]	32.5 ± 0.60	33.0 ± 1.0 (34)	30.1 ± 0.72** [2, 3, 4]	NA	NA
F_2**	34.7 ± 0.58 (40)	34.3 ± 0.61 (40)	33.7 ± 0.47 (40)	31.9 ± 0.41** (40) [1, 3, 4]	NA	NA
F_3*	34.4 ± 0.35 [1]	33.1 ± 0.36*	33.3 ± 0.35 (34)	34.0 ± 0.40 (34) [1, 2]	NA	NA
F_4	35.0 ± 0.56 (34) [1]	33.5 ± 0.28	34.2 ± 0.38 (34)	33.4 ± 0.29 [1, 2]	NA	NA
Body Weight (g) at vaginal opening[c]						
F_1	92.4 ± 3.1	82.7 ± 3.0* [4]	85.9 ± 4.8 (34)	67.1 ± 2.8*** [3, 4]	***	-
F_2	88.3 ± 2.8 (40)	84.0 ± 3.0 (40) [4]	91.3 ± 2.8 (40)	71.6 ± 2.7*** (40) [4]	***	*
F_3	92.9 ± 3.3	83.8 ± 2.6 [4]	83.8 ± 3.1 (34)	78.6 ± 2.8** (34) [1, 4]	**	-
F_4	96.7 ± 2.1 (34)	95.6 ± 1.8 [1, 2, 3]	94.9 ± 2.1 (34)	88.4 ± 2.3 [1, 2, 3]	*	-

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

[b] For age at vaginal opening, a two-way nonparametric ANOVA was conducted. The overall dose effect was significant at P=0.001; the overall generation effect was also significant at P=0.001, and the overall dose × generation interaction was significant at P=0.049. *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 (*) in shaded cells adjacent to generation designations indicate a significant overall Kruskal-Wallis' test; asterisks in shaded cells in the exposed group columns indicate significant difference from controls in the same generation: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$.

[c] For body weight at vaginal opening, a two-way ANOVA was conducted. Significant random effects of the F_0 breed father, the F_0 breed father × F_0 breed mother interaction, and unique female lineage were included in the statistical model. The overall dose effect and the overall generation effect were also significant at P<0.001, and the overall dose × generation interaction was not significant at P=0.064. 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. Asterisks (*) in shaded cells in the exposed group columns indicate a significant difference from controls in the same generation, and asterisks in shaded cells in the Trends columns indicate significant exposure concentration trends in that generation: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. A dash in the Trends 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 Genistein[a]

	Dietary Genistein (ppm)				Trends	
	0	5	100	500	Linear	Quad
Age[b]						
F_1	43.2 ± 0.33 [3]	43.0 ± 0.32	43.7 ± 0.40 [2, 3]	44.6 ± 0.46 [2, 3]	NA	NA
F_2	42.3 ± 0.33 (39) [4]	42.4 ± 0.46 (40) [4]	42.0 ± 0.26 (40) [1, 4]	43.1 ± 0.38 (39) [1, 4]	NA	NA
F_3	41.6 ± 0.31 [1, 4]	42.8 ± 0.64 [4]	41.5 ± 0.22 [1, 4]	42.3 ± 0.30 (33) [1, 4]	NA	NA
F_4	44.1 ± 0.36 (34) [2, 3]	43.7 ± 0.35 [2, 3]	43.7 ± 0.30 [2, 3]	44.6 ± 0.41 [2, 3]	NA	NA
Body Weight[c]						
F_1	179.1 ± 3.0 (34) [3]	177.5 ± 2.9 (34)	174.1 ± 3.3	168.0 ± 3.4 [4]	-	-
F_2	173.1 ± 3.7 (39)	164.5 ± 4.2 (40)	166.3 ± 2.9 (40)	167.1 ± 3.4 (39) [4]	-	-
F_3	159.9 ± 3.3 [1, 4]	166.5 ± 3.9	164.6 ± 4.5	168.0 ± 3.5 (33)	-	-
F_4	179.7 ± 3.8 (34) [3]	174.6 ± 3.3	172.8 ± 4.4	181.6 ± 3.5 [1, 2]	-	-

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

[b] For age at preputial separation, a two-way nonparametric ANOVA was conducted. The overall dose effect and the overall generation effect were significant at P=0.001, and the overall dose × generation interaction was not significant at P=0.675. *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). There were no significant exposure concentration-related ($P \leq 0.05$) differences within any generation.

[c] For body weight at preputial separation, a two-way ANOVA was conducted. No random effects for the F_0 parents were included in the statistical model. The overall dose effect was not significant at P<0.475; the overall generation effect was significant at P<0.001; and the overall dose × generation interaction was not significant at P=0.176. Contrasts were used to test for linear and quadratic exposure concentration trends, and Dunnett's tests were used to compare exposed group means to control means within a generation. A dash in the Trends columns indicates that the exposure concentration trend test was not significant (P>0.05). There were no significant exposure concentration-related differences ($P \leq 0.05$) within any generation.

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

	Dietary Genistein (ppm)			
	0	5	100	500
F_1	23.4 ± 0.2 (31) [2, 4]	23.2 ± 0.3 (32) [4]	23.8 ± 0.5 (33) [2, 4]	23.8 ± 0.5 (32) [2, 3, 4]
F_2	22.3 ± 0.5 (36) [1, 3]	22.6 ± 0.4 (40) [3]	21.4 ± 0.3 (40) [1, 3]	22.1 ± 0.3 (40) [1, 3]
F_3**	23.6 ± 0.3 (32) [2, 4]	24.3 ± 0.5 [2, 4]	23.9 ± 0.4 [2, 4]	25.5 ± 0.4** (34) [1, 2, 4]
F_4	21.4 ± 0.2 (33) [1, 3]	21.3 ± 0.2 [1, 3]	21.9 ± 0.2 (33) [1, 3]	21.9 ± 0.2 [1, 3]

[a] Mean day of testicular descent ± standard error. Thirty-five animals in each group except where indicated by numbers in parentheses. Generation numbers in brackets indicate significant differences ($P \leq 0.05$) between the indicated generations within an exposure group. A two-way nonparametric ANOVA was conducted. The overall dose effect was not significant at P=0.122; the overall generation effect was significant at P=0.001, and the overall dose × generation interaction was not significant at P=0.104. *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 (*) in shaded cells adjacent to generation designations indicate a significant overall Kruskal-Wallis's test; asterisks in shaded cells in the exposed group columns indicate significant difference controls in the same generation: **, $P \leq 0.01$.

APPENDIX J
ESTROUS CYCLE CHARACTERIZATION

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

Endpoint	Generation	Dietary Genistein (ppm) 0	5	100	500
Number of animals (n)	F_1	25	24	25	25
	F_2	24	23	22	21
	F_3	23	24	21	22
	F_4	25	25	25	25
% Time in cycle stages[b]					
% Time in Diestrus	F_1	56.80 ± 1.39	60.71 ± 1.81 [4]	60.80 ± 2.15 [4]	60.53 ± 3.12
	F_2	57.50 ± 1.83	54.18 ± 2.12	56.53 ± 1.97	55.74 ± 2.17
	F_3	57.58 ± 1.12	58.21 ± 0.83 [4]	57.78 ± 1.41 [4]	54.55 ± 1.68
	F_4	58.75 ± 1.51	54.00 ± 1.31 [1, 3]	52.57 ± 1.48* [1, 3]	57.77 ± 2.49
% Time in Estrus	F_1	25.33 ± 1.22	23.97 ± 1.71	21.60 ± 1.65 [4]	26.59 ± 3.06
	F_2	24.72 ± 1.47	26.60 ± 1.82	26.36 ± 2.08	27.05 ± 1.97
	F_3	23.83 ± 1.31	22.84 ± 0.68	23.49 ± 0.99 [4]	26.36 ± 1.42
	F_4	22.52 ± 1.27	24.00 ± 1.08	26.86 ± 1.26 [1, 3]	24.56 ± 1.91
% Time in Proestrus	F_1*	17.87 ± 1.14	15.32 ± 1.23 [4]	17.60 ± 1.15 [4]	12.88 ± 1.22* [3, 4]
	F_2	17.78 ± 1.11	19.21 ± 1.03 [4]	17.11 ± 0.98 [4]	17.21 ± 0.75
	F_3	18.59 ± 1.17	18.95 ± 0.77 [4]	18.73 ± 1.18 [4]	19.09 ± 1.10 [1]
	F_4	18.73 ± 1.08	22.00 ± 0.91 [1, 2, 3]	20.57 ± 1.04 [1, 2, 3]	17.68 ± 1.55 [1]

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Number of Abnormal Cycles[b]					
# of Abnormal Cycles - Diestrus	F_1***	0.32 ± 0.11	0.46 ± 0.15	0.48 ± 0.15	1.32 ± 0.21** [2, 3, 4]
	F_2	0.17± 0.10	0.26 ± 0.13	0.18 ± 0.11	0.48 ± 0.18 [1]
	F_3	0.04 ± 0.04	0.04 ± 0.04	0.19 ± 0.11	0.09 ± 0.06 [1]
	F_4	0.24 ± 0.10	0.12 ± 0.07	0.16 ± 0.07	0.32 ± 0.11 [1]
# of Abnormal Cycles - Estrus	F_1***	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00	0.48 ± 0.16* [3]
	F_2*	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.05	0.29 ± 0.14
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1]
	F_4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.08 ± 0.06
# of Abnormal Cycles – Diestrus and Estrus	F_1***	0.36 ± 0.13	0.46 ± 0.15	0.48 ± 0.15	1.80 ± 0.22** [2, 3, 4]
	F_2	0.17 ± 0.10	0.26 ± 0.13	0.23 ± 0.11	0.76 ± 0.23 [1, 3]
	F_3	0.04 ± 0.04	0.04 ± 0.04	0.19 ± 0.11	0.09 ± 0.06 [1, 2]
	F_4	0.24 ± 0.10	0.12 ± 0.07	0.16 ± 0.07	0.40 ± 0.13 [1]

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Percentage of Abnormal Cycles[b]					
% of Abnormal Cycles - Diestrus	F_1**	14.00 ± 5.15	20.83 ± 6.75	23.33 ± 7.64	48.67 ± 7.87** [3]
	F_2	7.64 ± 4.70	12.32 ± 6.31	9.85 ± 5.66	19.84 ± 7.15
	F_3	2.17 ± 2.17	1.39 ± 1.39	7.14 ± 4.08	4.55 ± 3.14 [1]
	F_4	11.33 ± 5.07	5.33 ± 3.00	6.00 ± 2.87	22.00 ± 7.68
% of Abnormal Cycles - Estrus	F_1***	1.33 ± 1.33	0.00 ± 0.00	0.00 ± 0.00	18.00 ± 6.16* [3]
	F_2*	0.00 ± 0.00	0.00 ± 0.00	2.27 ± 2.27	10.32 ± 4.94
	F_3	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00 [1]
	F_4	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	3.33 ± 2.36
% of Abnormal Cycles – Diestrus and Estrus	F_1***	15.33 ± 5.52	20.83 ± 6.75	23.33 ± 7.64	66.67 ± 7.33** [2, 3, 4]
	F_2	7.64 ± 4.70	12.32 ± 6.31	12.12 ± 5.92	30.16 ± 8.81 [1, 3]
	F_3	2.17 ± 2.17	1.39 ± 1.39	7.14 ± 4.08	4.55 ± 3.14 [1, 2]
	F_4	11.33 ± 5.07	5.33 ± 3.00	6.00 ± 2.87	25.33 ± 8.17 [1]

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Length of Cycle[b,c]					
Length of Cycle (Days)	F_1***, # # #	5.25 ± 0.22	5.57 ± 0.24	5.95 ± 0.44	8.40 ± 0.79**, # # # [3]
	F_2#	5.42 ± 0.44	5.27 ± 0.24	5.97 ± 0.49	6.25 ± 0.52#
	F_3	5.38 ± 0.46	5.10 ± 0.10	5.12 ± 0.12	5.11 ± 0.18 [1]
	F_4*	5.60 ± 0.22	5.30 ± 0.17	5.10 ± 0.16	7.10 ± 0.73

[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 dose within each generation and on generation within each dose group. Holm's adjusted pairwise Wilcoxon's tests were run to compare exposed groups to controls within generations or to compare all generations within an exposure group. For the analysis of dose 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: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Significant differences ($P \leq 0.05$) between generations within an exposure group are indicated by generation 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, and exposed groups were compared to the controls in the same generation by Williams' modification of Shirley's test if the trend test was significant. Significant exposure concentration trend tests are indicated by pound signs (#) in shaded cells in the generation column, and exposed groups that differ significantly from the controls in the same generation are indicated by pound signs in shaded cells in the exposed group columns: #, $P \leq 0.05$; # # #, $P \leq 0.001$.

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Number of Animals (n)	F_0	25	25	25	25
	F_1	24	25	25	25
	F_2	25	25	25	25
	F_3	25	25	25	24
	F_4	25	25	25	25
% Time in cycle stages[b]					
% Time in Diestrus	F_0	50.80 ± 1.72	51.82 ± 1.80	53.20 ± 2.93	51.60 ± 2.92
	F_1	52.50 ± 1.62	55.60 ± 2.24	53.60 ± 1.81	48.80 ± 2.47
	F_2**	47.20 ± 1.47	54.80 ± 1.65**	51.20 ± 1.85	46.63 ± 1.97
	F_3	52.00 ± 1.63	51.20 ± 2.60	50.00 ± 1.63	51.67 ± 3.93
	F_4	49.20 ± 1.72	52.40 ± 1.56	50.00 ± 1.53	52.40 ± 1.56
% Time in Estrus	F_0	30.40 ± 1.58	28.89 ± 1.75	30.40 ± 2.74	30.22 ± 3.55
	F_1*	28.75 ± 1.51	23.47 ± 1.64	27.60 ± 1.56	31.20 ± 2.73
	F_2***	31.20 ± 1.33	25.20 ± 1.17*	28.40 ± 1.70	35.66 ± 1.77 [4]
	F_3	25.60 ± 1.54	30.40 ± 2.48	28.80 ± 1.56	31.67 ± 3.79
	F_4	29.60 ± 1.58	27.20 ± 1.47	26.80 ± 1.60	26.00 ± 1.41 [2]
% Time in Proestrus	F_0	18.80 ± 0.88	19.29 ± 1.29	16.40 ± 1.81	18.18 ± 1.43
	F_1	18.75 ± 1.51	20.93 ± 1.76	18.80 ± 1.67	20.00 ± 1.41
	F_2	21.60 ± 1.11	20.00 ± 1.41	20.40 ± 1.58	17.71 ± 1.62
	F_3*	22.40 ± 1.33	18.40 ± 1.60	21.20 ± 1.45	16.67 ± 1.67
	F_4	21.20 ± 0.88	20.40 ± 1.47	23.20 ± 1.25	21.60 ± 1.38

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Number of Abnormal Cycles[b]					
# of Abnormal Cycles - Diestrus	F_0	0.08 ± 0.06	0.12 ± 0.07	0.20 ± 0.10	0.08 ± 0.06
	F_1	0.00 ± 0.00	0.16 ± 0.09	0.16 ± 0.07	0.00 ± 0.00
	F_2	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.06	0.00 ± 0.00
	F_3*	0.08 ± 0.06	0.08 ± 0.06	0.00 ± 0.00	0.38 ± 0.13
	F_4	0.04 ± 0.04	0.04 ± 0.04	0.04 ± 0.04	0.04 ± 0.04
# of Abnormal Cycles - Estrus	F_0	0.04 ± 0.04	0.04 ± 0.04	0.08 ± 0.08	0.08 ± 0.08
	F_1	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.08
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_3	0.04 ± 0.04	0.08 ± 0.08	0.00 ± 0.00	0.17 ± 0.13
	F_4	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.00 ± 0.00
# of Abnormal Cycles – Diestrus and Estrus	F_0	0.12 ± 0.09	0.16 ± 0.09	0.28 ± 0.12	0.16 ± 0.09
	F_1	0.00 ± 0.00	0.16 ± 0.09	0.20 ± 0.08	0.08 ± 0.08
	F_2	0.00 ± 0.00	0.04 ± 0.04	0.08 ± 0.06	0.00 ± 0.00 [3]
	F_3**	0.12 ± 0.09	0.16 ± 0.09	0.00 ± 0.00	0.54 ± 0.17* [2]
	F_4	0.04 ± 0.04	0.08 ± 0.06	0.04 ± 0.04	0.04 ± 0.04

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Percentage of Abnormal Cycles[b]					
% of Abnormal Cycles - Diestrus	F_0	6.00 ± 4.40	6.00 ± 3.32	14.00 ± 6.78	6.00 ± 4.40
	F_1	0.00 ± 0.00	9.33 ± 5.62	10.00 ± 5.00	0.00 ± 0.00
	F_2	0.00 ± 0.00	4.00 ± 4.00	6.00 ± 4.40	0.00 ± 0.00
	F_3**	4.00 ± 2.77	8.00 ± 5.54	0.00 ± 0.00	22.92 ± 7.95
	F_4	2.00 ± 2.00	2.00 ± 2.00	4.00 ± 4.00	4.00 ± 4.00
% of Abnormal Cycles - Estrus	F_0	2.00 ± 2.00	2.00 ± 2.00	4.00 ± 4.00	4.00 ± 4.00
	F_1	0.00 ± 0.00	0.00 ± 0.00	2.00 ± 2.00	4.00 ± 4.00
	F_2	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
	F_3	2.00 ± 2.00	4.00 ± 4.00	0.00 ± 0.00	6.25 ± 4.58
	F_4	0.00 ± 0.00	2.00 ± 2.00	0.00 ± 0.00	0.00 ± 0.00
% of Abnormal Cycles – Diestrus and Estrus	F_0	8.00 ± 5.54	8.00 ± 4.73	18.00 ± 7.57	10.00 ± 5.77
	F_1	0.00 ± 0.00	9.33 ± 5.62	12.00 ± 5.23	4.00 ± 4.00
	F_2	0.00 ± 0.00	4.00 ± 4.00	6.00 ± 4.40	0.00 ± 0.00 [3]
	F_3**	6.00 ± 4.40	12.00 ± 6.63	0.00 ± 0.00	29.17 ± 8.47 [2]
	F_4	2.00 ± 2.00	4.00 ± 2.77	4.00 ± 4.00	4.00 ± 4.00

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

Endpoint	Generation	Dietary Genistein (ppm)			
		0	5	100	500
Length of Cycle[b,c]					
Length of Cycle (Days)	F_0	5.13 ± 0.21	5.13 ± 0.21	6.07 ± 0.46	5.27 ± 0.30
	F_1	4.93 ± 0.07	5.27 ± 0.38	5.07 ± 0.23	5.40 ± 0.28
	F_2	4.73 ± 0.12	5.00 ± 0.24	5.13 ± 0.21	5.13 ± 0.21
	F_3	5.20 ± 0.31	5.67 ± 0.40	5.07 ± 0.23	5.97 ± 0.44
	F_4	4.93 ± 0.07	4.93 ± 0.07	5.07 ± 0.23	5.07 ± 0.23

[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 dose within each generation and on generation within each dose 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 dose 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: *, $P \leq 0.05$; **, $P \leq 0.01$; ***, $P \leq 0.001$. Significant differences ($P \leq 0.05$) between generations within an exposure group are indicated by generation 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, and exposed groups were compared to the controls in the same generation by Williams' modification of Shirley's test if the trend test was significant. There were no significant exposure concentration trend tests.

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

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.340 Gen P=0.013 DxG P=0.152	F_0	55.2 ± 1.9 (23)	79.7 ± 20.9** [1,2,3,4]	55.8 ± 1.7	57.0 ± 1.3	-	-
	F_1	53.5 ± 1.5 (26)	52.3 ± 1.0 [1]	49.0 ± 1.6	51.1 ± 1.1	-	-
	F_2	55.0 ± 1.9 (24)	51.6 ± 1.2 [1]	50.9 ± 2.1	53.7 ± 1.6	-	-
	F_3	49.2 ± 1.4	46.0 ± 1.4 [1]	49.6 ± 2.0	47.8 ± 1.0	-	-
	F_4	60.8 ± 11.6	48.4 ± 2.1 [1]	45.8 ± 1.3	47.1 ± 1.6	-	-
Relative[e] Dose P=0.520 Gen P=0.004 DxG P=0.157	F_0	129.5 ± 4.0 (23)	186.3 ± 46.6**	131.1 ± 4.3	133.5 ± 3.6	-	-
	F_1	122.4 ± 3.5 (26)	120.8 ± 2.9	116.9 ± 3.7	124.0 ± 2.9	-	-
	F_2	123.5 ± 3.9 (24)	112.8 ± 3.0	117.9 ± 6.5	119.8 ± 3.6	-	-
	F_3	113.5 ± 2.9	109.1 ± 3.5	115.3 ± 4.5	112.9 ± 2.1	-	-
	F_4	141.4 ± 27.1	113.5 ± 5.4	108.2 ± 2.6	112.4 ± 3.6	-	-
ANCOVA[f] Dose P=0.452 Gen P=0.010 DxG P=0.137 BW P=0.059	F_0	- (23)	**	-	-	-	-
	F_1	- (26)	-	-	-	-	-
	F_2	- (24)	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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 × F_0 breed father interaction (P=0.268) random effect incorporated into the analysis model.
[e] F_0 breed mother × F_0 breed father interaction (P=0.319) random effect incorporated into the analysis model.
[f] F_0 breed mother × F_0 breed father interaction (P=0.336) 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. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.038 Gen P=0.156 DxG P=0.499	F_0	2078.5 ± 26.4 (23)	2096.8 ± 20.8	2076.0 ± 15.3 (24)	2099.5 ± 21.6 (24)	-	-
	F_1	2104.5 ± 17.6 (26)	2057.2 ± 22.9	2055.2 ± 16.4	2035.5 ± 24.7	-	-
	F_2	2138.0 ± 19.0 (24)	2090.2 ± 20.2	2109.6 ± 28.0	2065.7 ± 30.0*	-	-
	F_3	2081.3 ± 16.3 (24)	2072.7 ± 23.8	2096.6 ± 20.7	2090.9 ± 19.9	-	-
	F_4	2097.4 ± 18.8 (23)	2075.3 ± 17.8	2081.2 ± 21.8	2027.2 ± 21.1*	*	-
Relative[e] Dose P=0.489 Gen P=0.001 DxG P=0.206	F_0	4904.2 ± 94.6 (23)	4993.1 ± 93.1 [2]	4877.2 ± 61.0 (24)	4910.5 ± 76.5 (24) (2)	-	-
	F_1	4820.8 ± 65.3 (26)	4757.5 ± 95.7	4912.0 ± 67.3	4931.5 ± 46.8 (2)	-	-
	F_2	4811.6 ± 56.7 (24)	4567.0 ± 64.5* [0, 3, 4]	4871.8 ± 117.9	4609.8 ± 80.3 (0, 1, 3)	-	*
	F_3	4825.8 ± 62.4 (24)	4917.3 ± 92.3 [2]	4894.6 ± 77.9	4951.1 ± 68.9 (2)	-	-
	F_4	4906.6 ± 76.7 (23)	4876.4 ± 64.8 [2]	4927.2 ± 70.2	4843.0 ± 66.0	-	-
ANCOVA[f] Dose P=0.096 Gen P=0.544 DxG P=0.461 BW P<0.001	F_0	- (23)	-	- (24)	- (24)	-	-
	F_1	- (26)	-	-	-	-	-
	F_2	- (24)	-	-	*	-	-
	F_3	- (24)	-	-	-	-	-
	F_4	- (23)	-	-	-	*	-

[a] Mean ± 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=0.301) and F_0 breed mother × F_0 breed father interaction (P=0.007) random effects incorporated into the analysis model. F_0 breed father (P=0.001) random effect could not be incorporated due to computational unfeasibility.
[e] F_0 breed mother × F_0 breed father interaction (P=0.010) random effect incorporated into the analysis model.
[f] F_0 breed father (P=0.015) and F_0 breed mother × F_0 breed father interaction (P=0.010) 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; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.793 Gen P<0.001 DxG P=0.936	F_0	1179.8 ± 24.0 (24)	1259.4 ± 32.5 [3]	1202.0 ± 31.6 (24)	1223.4 ± 35.4	-	-
	F_1	1248.2 ± 24.6	1229.2 ± 21.7	1228.1 ± 23.5	1222.2 ± 20.6	-	-
	F_2	1222.0 ± 30.2 (24)	1254.2 ± 16.9 [3]	1208.2 ± 29.7	1220.7 ± 16.3	-	-
	F_3	1147.0 ± 23.2 (22)	1147.0 ± 29.8 [0, 2]	1161.1 ± 19.0 (24)	1170.4 ± 16.3	-	-
	F_4	1168.0 ± 25.8	1165.0 ± 36.1	1150.9 ± 24.4	1141.6 ± 20.3	-	-
Relative[e] Dose P=0.728 Gen P<0.001 DxG P=0.774	F_0	2779.6 ± 57.3 (24)	2989.4 ± 75.7 [2, 3, 4]	2829.7 ± 77.2 (24)	2860.7 ± 88.3	-	-
	F_1	2853.9 ± 67.0	2833.4 ± 49.9	2933.2 ± 62.1	2960.5 ± 42.4 [2]	-	-
	F_2	2747.1 ± 69.9	2740.6 ± 45.5 [0]	2787.7 ± 83.4	2723.5 ± 43.6 [1]	-	-
	F_3	2652.9 ± 48.4	2708.6 ± 66.5 [0]	2709.3 ± 48.5 (24)	2756.2 ± 42.7 (22)	-	-
	F_4	2713.5 ± 70.8	2725.4 ± 78.3 [0]	2721.0 ± 56.1	2722.7 ± 42.3	-	-
ANCOVA[f] Dose P=0.893 Gen P<0.001 DxG P=0.938 BW P<0.001	F_0	- (24)	- [3, 4]	- (24)	-	-	-
	F_1	-	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	- [0]	- (24)	-	-	-
	F_4	-	- [0]	-	-	-	-

[a] Mean ± 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=0.374), F_0 breed father (P=0.226), and F_0 breed mother × F_0 breed father interaction (P=0.028) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.360), F_0 breed father (P=0.219), and F_0 breed mother × F_0 breed father interaction (P=0.129) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.219) and F_0 breed mother × F_0 breed father interaction (P=0.265) 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; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P =0.451 Gen P<0.001 DxG P=0.143	F_0	2911.2 ± 58.1 (24)	2920.8 ± 64.5 [3]	2924.4 ± 66.3	2999.9 ± 59.5 [3, 4]	-	-
	F_1	2951.5 ± 64.8 (26)	2954.0 ± 57.5 [3]	2894.3 ± 54.0	2982.9 ± 67.6 [3, 4]	-	-
	F_2	2812.0 ± 48.2 (24)	2946.1 ± 74.6 [3]	2929.7 ± 69.7 [3]	2952.1 ± 62.6	-	-
	F_3	2806.7 ± 70.3	2642.7 ± 65.1 [0, 1, 2]	2699.4 ± 40.4 [2]	2747.4 ± 57.4 [0, 1]	-	-
	F_4	2898.1 ± 60.7	2786.3 ± 65.7	2742.4 ± 53.6	2733.6 ± 42.5 [0, 1]	-	-
Relative[e] Dose P=0.083 Gen P<0.001 DxG P=0.026	F_0	6837.8 ± 79.1 (24) [2]	6911.1 ± 101.4 [2, 3]	6866.5 ± 163.3 [3]	6992.7 ± 108.6 [2, 3, 4]		
	F_1	6744.3 ± 139.5 (26)	6786.9 ± 79.2 [3]	6891.8 ± 87.6 [3, 4]	7217.3 ± 133.1** [2, 3, 4]	***	
	F_2	6321.2 ± 100.3 (24) [0, 4]	6405.9 ± 118.3 [0]	6712.3 ± 119.6 [3]	6575.5 ± 127.5 [0, 1]		*
	F_3	6492.7 ± 156.0	6228.4 ± 104.6 [0, 1]	6284.4 ± 78.0 [0, 1, 2]	6482.4 ± 98.4 [0, 1]		
	F_4	6693.9 ± 78.9 [2]	6509.2 ± 89.0	6466.3 ± 73.2 [1]	6521.8 ± 94.0 [0, 1]		
ANCOVA[f] Dose P=0.125 Gen P<0.001 DxG P=0.040 BW P<0.001	F_0	- (24) [2]	- [2, 3]	- [3]	-	-	-
	F_1	- (26)	- [3]	- [3]	*	**	-
	F_2	- (24) [0]	- [0]	- [3]	-	-	*
	F_3	-	- [0, 1]	- [0, 1, 2]	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.013), F_0 breed father (P=0.005), 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=0.058), F_0 breed father (P=0.168), 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=0.066), F_0 breed father (P=0.200), and F_0 breed mother × F_0 breed father interaction (P=0.009) 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; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.843 Gen P<0.001 DxG P=0.093	F_0	12214 ± 382.9 (24)	11932 ± 307.6 [2]	12673 ± 279.8	12484 ± 262.0 [4]	-	-
	F_1	12622 ± 190.0 (26)	12689 ± 329.5 [3]	12564 ± 290.5	12683 ± 300.2 [4]	-	-
	F_2	12632 ± 240.0 (24)	13329 ± 331.9 [0, 3, 4]	12917 ± 421.0	13005 ± 323.0 [3, 4]	-	-
	F_3	11879 ± 230.5	11415 ± 234.0 [1, 2]	12045 ± 201.8	11689 ± 339.9 [2]	-	-
	F_4	12556 ± 329.1 (23)	12063 ± 289.2	12190 ± 270.0	11175 ± 219.7* [0, 1, 2]	**	-
Relative[e] Dose P=0.172 Gen P<0.001 DxG P=0.026	F_0	28599 ± 605.1 (24)	28217 ± 522.9	29628 ± 462.7	29090 ± 464.9 [4]	-	-
	F_1	28867 ± 421.7 (26)	29092 ± 412.8 [3]	29872 ± 460.2 [3]	30726 ± 682.3** [2, 3, 4]	**	-
	F_2	28346 ± 364.1 (24)	28986 ± 527.8 [3]	29448 ± 630.0	28911 ± 567.0 [1, 4]	-	-
	F_3	27460 ± 425.4	26934 ± 388.6 [1, 2]	28017 ± 329.2 [1]	27500 ± 539.4 [1]	-	-
	F_4	28635 ± 352.7 (23)	28188 ± 406.7	28721 ± 393.5	26625 ± 409.1* [0, 1, 2]	**	-
ANCOVA[f] Dose P=0.104 Gen P<0.001 DxG P=0.026 BW P<0.001	F_0	- (24)	-	-	- [1, 4]	-	-
	F_1	- (26)	- [3]	- [3]	** [0, 2, 3, 4]	***	-
	F_2	- (24)	-	-	- [1, 4]	-	-
	F_3	-	- [1]	- [1]	- [1]	-	-
	F_4	- (23)	-	-	* [0, 1, 2]	**	-

[a] Mean ± 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=0.002) and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model. F_0 breed mother (P=0.031) random effect not included due to computational unfeasibility.

[e] F_0 breed mother (P=0.007), F_0 breed father (P=0.007), 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=0.005), F_0 breed father (P=0.010), and F_0 breed mother × F_0 breed father interaction (P=0.007) 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; generation 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; ***, 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 Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.021 Gen P<0.001 DxG P=0.071	F_0	13.9 ± 0.4 (24)	13.9 ± 0.5	14.0 ± 0.4	14.7 ± 0.3 [2]	-	-
	F_1	15.3 ± 0.6 (26) [4]	15.0 ± 0.6 [3]	14.9 ± 0.5	15.3 ± 0.6 [3]	-	-
	F_2	13.8 ± 0.3 (23)	14.0 ± 0.4	14.6 ± 0.4	16.3 ± 0.5*** [0, 3, 4]	***	-
	F_3	14.0 ± 0.3	12.7 ± 0.3 [1]	13.4 ± 0.3	13.2 ± 0.4 [1, 2]	-	-
	F_4	13.3 ± 0.4 [1]	13.7 ± 0.5	13.6 ± 0.4	13.8 ± 0.4 [2]	-	-
Relative[e] Dose P=0.001 Gen P<0.001 DxG P=0.122	F_0	32.6 ± 0.7 (24)	32.9 ± 1.1	32.8 ± 0.9	34.2 ± 0.6	-	-
	F_1	34.9 ± 1.2 (26) [4]	34.5 ± 1.3 [2, 3]	35.4 ± 0.8 [3]	36.9 ± 1.2 [3, 4]	*	-
	F_2	31.0 ± 0.8 (23)	30.5 ± 0.7 [1]	33.5 ± 1.0	36.3 ± 1.2*** [3]	***	-
	F_3	32.5 ± 0.6	30.0 ± 0.7 [1]	31.2 ± 0.7 [1]	31.3 ± 0.9 [1, 2]	-	-
	F_4	30.8 ± 1.0 [1]	32.0 ± 0.9	32.1 ± 0.9	32.8 ± 1.0 [1]	-	-
ANCOVA[f] Dose P=0.001 Gen P<0.001 DxG P=0.077 BW P<0.001	F_0	- (24)	-	-	-	-	-
	F_1	- (26) [4]	- [3]	-	- [3, 4]	-	-
	F_2	- (23)	-	-	*** [3, 4]	***	-
	F_3	-	- [1]	-	- [1, 2]	-	-
	F_4	- [1]	-	-	- [1, 2]	-	-

[a] Mean ± 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=0.002) and F_0 breed mother × F_0 breed father interaction (P<0.001) random effects incorporated into the analysis model. F_0 breed mother (P=0.418) random effect not included due to computational unfeasibility.

[e] F_0 breed father (P=0.006) and F_0 breed mother × F_0 breed father interaction (P=0.004) random effects incorporated into the analysis model.

[f] F_0 breed father (P=0.006) 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≤0.001. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.293 Gen P<0.001 DxG P=0.990	F_0	381.8 ± 24.3 (23)	388.9 ± 30.5 [4]	425.9 ± 20.9	379.9 ± 21.6	-	-
	F_1	430.7 ± 28.9	435.2 ± 27.2	449.4 ± 19.8	439.4 ± 20.6 (23)	-	-
	F_2	376.1 ± 17.2 (24)	392.1 ± 21.4	425.7 ± 27.5	419.8 ± 25.3	-	-
	F_3	390.6 ± 26.1	396.3 ± 24.0 (23)	412.2 ± 22.4	429.6 ± 26.9	-	-
	F_4	464.2 ± 19.9	485.6 ± 26.8 (24) (0)	473.8 ± 24.0	477.8 ± 23.3	-	-
Relative[e] Dose P=0.075 Gen P<0.001 DxG P=0.978	F_0	900.9 ± 61.9 (23)	918.7 ± 69.3	1000.4 ± 50.2	885.2 ± 48.9 [4]	-	-
	F_1	987.1 ± 66.2	999.1 ± 60.3	1077.0 ± 51.2	1069.1 ± 52.6 (23)	-	-
	F_2	849.1 ± 42.3 (24)	852.5 ± 43.2 [4]	976.7 ± 62.0	936.6 ± 56.7	-	-
	F_3	904.6 ± 61.3	939.6 ± 61.2 (23)	961.9 ± 53.1	1016.5 ± 63.8	-	-
	F_4	1072.2 ± 41.9	1136.9 ± 61.4 (24) [2]	1126.3 ± 62.4	1140.3 ± 56.2 [0]	-	-
ANCOVA[f] Dose P=0.166 DxG P=0.987 Gen P<0.001 BW P=0.013	F_0	- (23)	-	-	- [4]	-	-
	F_1	-	-	-	- (23)	-	-
	F_2	- (24)	- [4]	-	-	-	-
	F_3	-	- (23)	-	-	-	-
	F_4	-	- (24) [2]	-	- [0]	-	-

[a] Mean ± 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=0.396) random effect incorporated into the analysis model.
[e] No significant random effects for F_0 parents incorporated into the analysis model.
[f] No significant random effects for F_0 parents 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; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.180 Gen P<0.001 DxG P=0.167	F_0	489.0 ± 26.0 (23)	467.4 ± 26.6 [2, 4]	501.6 ± 26.1 (24)	523.0 ± 25.1	-	-
	F_1	463.7 ± 17.3	462.4 ± 21.1 [2, 4]	431.5 ± 19.5	507.8 ± 26.5 (23)	-	-
	F_2	519.5 ± 20.7 (24)	584.0 ± 29.8 [0, 1]	521.7 ± 24.6 (24)	573.6 ± 19.3	-	-
	F_3	534.8 ± 23.4	543.0 ± 23.1	498.6 ± 22.5	515.4 ± 21.2	-	-
	F_4	536.8 ± 25.0	592.2 ± 22.4 [0, 1]	558.2 ± 26.5	518.0 ± 26.6	-	-
Relative[e] Dose P=0.148 Gen P<0.001 DxG P=0.197	F_0	1152.5 ± 66.2 (23)	1105.8 ± 61.7 [4]	1168.3 ± 53.7 (24)	1221.0 ± 58.6	-	-
	F_1	1061.2 ± 38.1	1066.6 ± 49.3 [3, 4]	1027.4 ± 44.4 [4]	1229.7 ± 60.8 (23)	**	-
	F_2	1169.3 ± 47.8 (24)	1270.0 ± 62.1	1198.6 ± 51.2 (24)	1279.8 ± 44.3	-	-
	F_3	1237.7 ± 52.6	1284.1 ± 50.5 [1]	1166.9 ± 56.1	1215.7 ± 44.9	-	-
	F_4	1240.8 ± 55.2	1384.8 ± 48.1 [0, 1]	1319.1 ± 62.8 [1]	1235.0 ± 62.3	-	-
ANCOVA[f] Dose P=0.144 Gen P<0.001 DxG P=0.177 BW P<0.001	F_0	- (23)	- [4]	- (24)	-	-	-
	F_1	-	- [2, 3, 4]	- [4]	- (23)	*	-
	F_2	- (24)	- [1]	- (24)	-	-	-
	F_3	-	- [1]	-	-	-	-
	F_4	-	- [0, 1]	- [1]	-	-	-

[a] Mean ± 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=0.332), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.146) random effects incorporated into the analysis model.

[e] F_0 breed mother (P=0.356), F_0 breed father (P=0.002), and F_0 breed mother × F_0 breed father interaction (P=0.272) random effects incorporated into the analysis model.

[f] F_0 breed mother (P=0.438), F_0 breed father (P=0.001), and F_0 breed mother × F_0 breed father interaction (P=0.267) 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; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.

[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: *, $P \leq 0.05$; **, $P \leq 0.01$.

TABLE K9
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 Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.328 Gen P=0.001 DxG P=0.683	F_0	1057.5 ± 56.9 (24)	1065.6 ± 47.0	1070.7 ± 45.1 (24)	1027.2 ± 58.7	-	-
	F_1	1058.1 ± 55.8	905.3 ± 38.4	983.2 ± 44.5	907.6 ± 45.2	-	-
	F_2	1027.4 ± 51.7 (24)	1008.4 ± 62.4	1048.0 ± 53.7	1072.0 ± 62.7	-	-
	F_3	1137.8 ± 69.9	1000.8 ± 56.9	1131.0 ± 64.3	1086.3 ± 70.9	-	-
	F_4	954.0 ± 53.5	1003.6 ± 44.5	952.6 ± 43.4	916.8 ± 42.2	-	-
Relative[e] Dose P=0.343 Gen P=0.001 DxG P=0.836	F_0	2500.7 ± 138.3 (24)	2526.7 ± 110.6	2519.9 ± 122.8 (24)	2397.0 ± 134.2	-	-
	F_1	2413.4 ± 125.7	2101.5 ± 101.7	2344.6 ± 107.4	2186.4 ± 95.8	-	-
	F_2	2316.2 ± 122.3 (24)	2196.4 ± 133.6	2407.3 ± 119.7	2402.2 ± 149.9	-	-
	F_3	2624.6 ± 156.9	2351.4 ± 122.7	2640.8 ± 157.9	2580.2 ± 173.3	-	-
	F_4	2217.3 ± 131.8	2345.0 ± 90.9	2259.4 ± 108.4	2182.3 ± 92.0	-	-
ANCOVA[f] Dose P=0.376 Gen P=0.001 DxG P=0.753 BW P=0.013	F_0	- (24)	-	- (24)	-	-	-
	F_1	-	-	-	-	-	-
	F_2	- (24)	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.169) random effect was not included in the analysis model due to computational unfeasibility.
[e] No significant random effects for F_0 parents incorporated into the analysis model.
[f] F_0 breed father (P=0.387) random effect was not included in 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. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; there were no significant generation effects in pairwise comparisons for the seminal vesicle/coagulating gland in male rats.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells.

TABLE K10
Spleen Weights and Spleen Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.001 Gen P<0.001 DxG P=0.832	F_0	657.8 ± 16.3 (24)	725.2 ± 31.1*	673.8 ± 15.7	681.1 ± 20.2 (24)	-	-
	F_1	691.1 ± 19.1 (26)	712.7 ± 22.5	663.3 ± 18.9	671.6 ± 33.1	-	-
	F_2	712.5 ± 14.3 (24)	785.3 ± 21.1* [3]	701.3 ± 21.7	699.5 ± 18.2	-	-
	F_3	669.2 ± 13.2	675.2 ± 16.5 [2]	651.2 ± 18.2	641.0 ± 25.2	-	-
	F_4	684.4 ± 17.2	740.8 ± 24.2	676.1 ± 19.1	652.6 ± 21.4	-	-
Relative[e] Dose P<0.001 Gen P=0.066 DxG P=0.852	F_0	1545.7 ± 29.7 (24)	1719.7 ± 74.5*	1576.7 ± 30.2	1588.2 ± 39.7	-	-
	F_1	1577.6 ± 39.9 (26)	1636.0 ± 41.4	1581.2 ± 42.8	1628.7 ± 77.9	-	-
	F_2	1600.7 ± 28.4 (24)	1714.9 ± 47.9	1611.4 ± 49.9	1559.3 ± 41.4	-	-
	F_3	1546.8 ± 24.5	1592.6 ± 30.0	1514.3 ± 38.0	1506.1 ± 46.1	-	-
	F_4	1578.1 ± 22.2	1736.3 ± 55.1	1595.8 ± 40.4	1551.0 ± 41.3	-	-
ANCOVA[f] Dose P<0.001 Gen P=0.068 DxG P=0.882 BW P<0.001	F_0	- (24)	*	-	- (24)	-	-
	F_1	- (26)	-	-	-	-	-
	F_2	- (24)	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.009), 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=0.101), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.015) random effects could not be incorporated into the analysis model due to computational unfeasibility.

[f] F_0 breed mother (P=0.056), F_0 breed father (P<0.001), and F_0 breed mother × F_0 breed father interaction (P=0.005) 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. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

TABLE K11
Left and Right Testis Weights and Testis Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.364 Gen P=0.363 DxG P=0.239	F_0	3123.3 ± 76.2 (24) [1]	3256.3 ± 94.0 (24)	3307.4 ± 45.9	3394.7 ± 75.1*	*	-
	F_1	3416.6 ± 67.6 [0]	3385.9 ± 68.2	3285.2 ± 50.3	3305.6 ± 51.1	-	-
	F_2	3174.7 ± 66.5 (24)	3408.3 ± 59.1	3338.2 ± 86.9	3330.9 ± 59.7	-	-
	F_3	3256.0 ± 42.2	3245.5 ± 87.2	3257.5 ± 48.9	3297.8 ± 54.4	-	-
	F_4	3252.0 ± 81.6	3365.9 ± 56.6	3273.1 ± 45.2	3265.3 ± 45.3	-	-
Relative[e] Dose P=0.086 Gen P=0.002 DxG P=0.803	F_0	7351.0 ± 170.0 (24)	7745.6 ± 247.2 (24)	7769.7 ± 147.6	7930.6 ± 180.2*	*	-
	F_1	7816.1 ± 190.7	7796.6 ± 144.5	7840.7 ± 121.5	8016.5 ± 129.6	-	-
	F_2	7141.3 ± 161.3 (24)	7444.7 ± 140.6	7715.2 ± 260.3	7431.7 ± 147.6	-	*
	F_3	7539.4 ± 97.8	7669.4 ± 210.6	7591.4 ± 122.5	7797.8 ± 124.3	-	-
	F_4	7555.7 ± 213.8	7897.6 ± 132.3	7750.7 ± 135.9	7798.3 ± 119.4	-	-
ANCOVA[f] Dose P=0.207 Gen P=0.170 DxG P=0.524 BW P<0.001	F_0	- (24)	- (24)	-	*	*	-
	F_1	- [2]	-	-	-	-	-
	F_2	- (24) [1]	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.013) and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model. F_0 breed mother (P=0.235) random effect was not incorporated into the model due to computational unfeasibility.

[e] F_0 breed mother (P=0.081), F_0 breed father (P=0.004), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.

[f] F_0 breed mother (P=0.107), F_0 breed father (P=0.004), 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: *, $P \leq 0.05$. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.

[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: *, $P \leq 0.05$.

TABLE K12
Thymus Weights and Thymus Weight-to-Body-Weight Ratios for Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.002 Gen P<0.001 DxG P=0.175	F_0	336.2 ± 15.0 (23)	325.5 ± 16.6 (24)	304.2 ± 12.2 (24)	322.0 ± 15.0	-	-
	F_1	333.3 ± 13.2 [2]	354.9 ± 13.3	333.4 ± 25.0	319.8 ± 10.7 (24)	-	-
	F_2	403.1 ± 16.6 (24) [0, 1]	404.9 ± 17.8 [0, 3, 4]	318.2 ± 15.6**	370.2 ± 14.6	-	***
	F_3	360.9 ± 12.3	340.8 ± 15.1 [2]	321.7 ± 17.0	315.9 ± 15.3	-	-
	F_4	346.4 ± 17.7	303.3 ± 9.5 [2]	281.8 ± 13.3*	318.7 ± 15.8	-	-
Relative[e] Dose P=0.016 Gen P=0.001 DxG P=0.249	F_0	787.8 ± 32.0 (23)	769.0 ± 33.9 (24)	711.8 ± 24.9 (24)	752.5 ± 35.2	-	-
	F_1	765.2 ± 29.5	818.8 ± 31.0	795.6 ± 60.0	778.2 ± 29.1 (24)	-	-
	F_2	906.6 ± 36.3 (24)	884.0 ± 39.6 [4]	724.7 ± 32.7**	824.8 ± 30.9	-	***
	F_3	834.5 ± 27.5	805.9 ± 35.1	749.5 ± 39.5	744.4 ± 31.4	*	-
	F_4	793.7 ± 31.2	713.8 ± 25.1 [2]	665.4 ± 30.9	760.6 ± 37.7	-	-
ANCOVA[f] Dose P=0.012 Gen P=0.001 DxG P=0.234 BW P<0.001	F_0	- (23)	-	- (24)	-	-	-
	F_1	-	-	-	- (24)	-	-
	F_2	- (24)	- [4]	**	-	-	***
	F_3	-	-	-	-	-	-
	F_4	-	- [2]	-	-	-	-

[a] Mean ± 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=0.187), F_0 breed father (P=0.049), 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=0.081), F_0 breed father (P=0.090), and F_0 breed mother × F_0 breed father interaction (P=0.003) random effects incorporated into the analysis model.

[f] F_0 breed mother (P=0.094), F_0 breed father (P=0.050), and F_0 breed mother × F_0 breed father interaction (P=0.003) 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; generation 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≤0.001.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.875 Gen P<0.001 DxG P=0.234	F_0	28.8 ± 1.2 (24) [4]	28.4 ± 1.1	27.8 ± 1.0 [2]	28.9 ± 1.1 [2, 4]	-	-
	F_1	31.9 ± 1.2 (26) [3, 4]	29.4 ± 1.2	29.2 ± 1.3	30.8 ± 1.2 [4]	-	-
	F_2	32.6 ± 1.3 (24) [3, 4]	33.6 ± 1.9 [4]	33.6 ± 1.2 [0, 3, 4]	35.1 ± 1.1 [0, 3, 4]	-	-
	F_3	26.2 ± 1.1 [1, 2]	29.1 ± 1.3	27.3 ± 1.3 [2]	27.7 ± 1.1 [2, 4]	-	-
	F_4	23.5 ± 1.1 [0, 1, 2]	25.6 ± 1.4 [2]	27.2 ± 2.2 [2]	22.5 ± 1.1 [0, 1, 2, 3]	-	*
Relative[e] Dose P=0.580 Gen P<0.001 DxG P=0.190	F_0	67.8 ± 2.8 (24) [4]	67.2 ± 2.5	65.0 ± 2.2 [2]	67.5 ± 2.6 [4]	-	-
	F_1	73.1 ± 3.0 (26) [3, 4]	68.1 ± 3.1	69.4 ± 2.8	74.3 ± 2.6 [4]	-	-
	F_2	73.3 ± 3.0 (24) [3, 4]	73.4 ± 4.3 [4]	77.7 ± 3.4 [0, 3, 4]	78.2 ± 2.3 [3, 4]	-	-
	F_3	60.6 ± 2.7 [1, 2]	69.3 ± 3.5	63.7 ± 3.2 [2]	65.7 ± 2.5 [2]	-	-
	F_4	54.4 ± 2.4 [0, 1, 2]	59.7 ± 2.9 [2]	64.0 ± 4.8 [2]	53.9 ± 2.9 [0, 1, 2]	-	*
ANCOVA[f] Dose P=0.789 Gen P<0.001 DxG P=0.221 BW P=0.002	F_0	- (24) [4]	-	- [2]	- [2, 4]	-	-
	F_1	- (26) [3, 4]	-	-	- [4]	-	-
	F_2	- (24) [3, 4]	- [4]	- [0, 3, 4]	- [0, 3, 4]	-	-
	F_3	- [1,2]	-	- [2]	- [2, 4]	-	-
	F_4	- [0, 1, 2]	- [2]	- [2]	- [0, 1, 2, 3]	-	*

[a] Mean ± 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=0.063) and F_0 breed mother × F_0 breed father interaction (P=0.029) random effects incorporated into the analysis model.

[e] F_0 breed father (P=0.010) and F_0 breed mother × F_0 breed father interaction (P=0.054) random effects incorporated into the analysis model.

[f] F_0 breed father (P=0.032) and F_0 breed mother × F_0 breed father interaction (P=0.039) 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; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.

[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: *, $P \leq 0.05$.

TABLE K14
Adrenal Gland Weights and Adrenal Gland Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[e]				Trends[f]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.656 Gen P=0.394 DxG P=0.580	F_0	69.0 ± 1.6	70.7 ± 1.6	77.7 ± 10.1	68.4 ± 1.7	-	-
	F_1	64.9 ± 1.9 (24)	64.2 ± 1.6	66.6 ± 1.5	62.4 ± 1.7	-	-
	F_2	81.9 ± 17.0	62.9 ± 2.1*	63.2 ± 1.7	63.6 ± 2.0	-	-
	F_3	65.3 ± 2.0	66.3 ± 2.0	63.3 ± 1.6	64.6 ± 1.9	-	-
	F_4	63.8 ± 1.5	72.2 ± 14.4	62.7 ± 1.7	63.2 ± 2.0	-	-
Relative[d] Dose P=0.933 Gen P=0.617 DxG P=0.537	F_0	256.8 ± 4.7	257.1 ± 5.2	292.6 ± 38.5	278.8 ± 6.3	-	-
	F_1	238.4 ± 6.8 (24)	238.1 ± 4.7	254.9 ± 6.5	266.2 ± 7.4	-	-
	F_2	307.7 ± 63.3	239.2 ± 8.5	239.9 ± 6.5	254.3 ± 7.9	-	-
	F_3	249.1 ± 6.6	251.5 ± 8.0	247.6 ± 6.3	254.7 ± 8.6	-	-
	F_4	244.7 ± 7.2	278.2 ± 57.0	244.4 ± 4.8	252.0 ± 7.4	-	-
ANCOVA[d] Dose P=0.937 Gen P=0.495 DxG P=0.571 BW P=0.118	F_0	-	-	-	-	-	-
	F_1	- (24)	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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 random effects for F_0 parents incorporated into the analysis model.
[e] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: *, $P \leq 0.05$. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests There were no significant generation effects in pairwise comparisons for the adrenal gland of female rats.
[f] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.003 Gen P=0.001 DxG P=0.939	F_0	1939.6 ± 18.4	1988.7 ± 22.8 (24)	1985.3 ± 19.1 (24)	1918.5 ± 20.0	-	-
	F_1	1907.1 ± 25.2 (24)	1915.2 ± 23.7	1908.4 ± 22.8	1848.1 ± 23.9	*	-
	F_2	1969.2 ± 18.0	1939.6 ± 25.2	1958.9 ± 20.2	1909.0 ± 27.4	-	-
	F_3	1944.6 ± 20.5	1965.5 ± 19.2	1935.0 ± 22.0	1920.4 ± 21.9	-	-
	F_4	1940.5 ± 16.2	1958.8 ± 20.9	1944.1 ± 20.1	1901.7 ± 27.9	-	-
Relative[e] Dose P<0.001 Gen P=0.088 DxG P=0.005	F_0	7236.3 ± 92.6	7227.2 ± 92.6 (24)	7468.8 ± 95.4 (24)	7846.9 ± 124.8***	***	-
	F_1	7028.7 ± 125.4 (24) [2,3]	7135.6 ± 114.9	7293.2 ± 102.2	7894.2 ± 123.9***	***	-
	F_2	7430.8 ± 107.1 [1]	7367.0 ± 96.3	7441.9 ± 112.6	7637.4 ± 93.5	-	-
	F_3	7441.8 ± 106.6 [1]	7448.3 ± 75.7	7574.7 ± 107.6	7546.4 ± 100.9	-	-
	F_4	7428.5 ± 88.9	7512.5 ± 105.5	7607.8 ± 79.3	7585.6 ± 112.3	-	-
ANCOVA[f] Dose P=0.486 Gen P=0.001 DxG P=0.941 BW P<0.001	F_0	-	-	- (24)	-	-	-
	F_1	- (24)	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.241), F_0 breed father (P=0.303), and F_0 breed mother × F_0 breed father interaction (P=0.365) random effects incorporated into the analysis model.

[e] F_0 breed mother (P=0.003) and F_0 breed mother × F_0 breed father interaction (P=0.172) random effects incorporated into the analysis model.

[f] No significant random effects for F_0 parents 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≤0.001. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P<0.001 Gen P<0.001 DxG P=0.015	F_0	1861.4 ± 29.5	1995.1 ± 46.7 [4]	1892.0 ± 29.2 [4]	1782.5 ± 47.0	**	-
	F_1	1933.0 ± 47.3 (24)	1999.3 ± 40.7 [4]	1907.3 ± 40.3 [3,4]	1709.1 ± 36.2***	***	-
	F_2	1871.0 ± 28.1	1903.0 ± 40.4	1890.0 ± 42.3 [4]	1759.6 ± 31.6	*	-
	F_3	1814.1 ± 29.2	1881.2 ± 33.1	1803.6 ± 33.0 [1]	1788.8 ± 25.2	-	-
	F_4	1798.3 ± 25.9	1842.7 ± 26.9 [1,2]	1738.2 ± 35.8 [0,1,2]	1757.8 ± 25.7	-	*
Relative[e] Dose P=0.095 Gen P<0.001 DxG P=0.648	F_0	6930.2 ± 86.4	7238.4 ± 128.4	7109.6 ± 88.8	7247.4 ± 120.2	-	-
	F_1	7081.6 ± 97.4 (24)	7418.4 ± 94.9	7262.0 ± 98.8 [4]	7288.4 ± 145.9	-	-
	F_2	7041.5 ± 78.3	7201.1 ± 90.6	7154.2 ± 126.7 [4]	7032.3 ± 92.8	-	-
	F_3	6924.4 ± 82.4	7115.3 ± 93.8	7045.1 ± 104.5	7023.4 ± 92.9	-	-
	F_4	6871.3 ± 77.9	7054.6 ± 87.4	6788.4 ± 108.7 [1,2]	7002.9 ± 72.2	-	*
ANCOVA[f] Dose P=0.113 Gen P<0.001 DxG P=0.694 BW P<0.001	F_0	-	-	-	-	-	-
	F_1	- (24)	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	*

[a] Mean ± 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=0.055), 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=0.001), 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<0.001), 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.

[g] Significant differences between exposed groups and controls within a generation given by Dunnett's tests are indicated in shaded cells as follows: ***, P≤0.001. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.022 Gen P<0.001 DxG P=0.116	F_0	8129.7 ± 144.9	8536.2 ± 204.3	8367.8 ± 188.1	7863.1 ± 175.8	-	-
	F_1	8463.5 ± 229.8 (24)	9209.9 ± 251.4 [2,3,4]	8804.9 ± 264.3 [3,4]	7889.3 ± 128.0	***	-
	F_2	8128.4 ± 176.9	8332.3 ± 205.2 [1]	8589.0 ± 226.5 [3,4]	7834.5 ± 195.5	-	*
	F_3	7788.7 ± 176.5	8117.1 ± 216.7 [1]	7856.3 ± 161.4 [1,2]	7795.3 ± 209.7	-	-
	F_4	7960.4 ± 226.0	7891.3 ± 177.6 [1]	7794.1 ± 185.4 [1,2]	7614.5 ± 187.9 [a]	-	-
Relative[e] Dose P=0.029 Gen P<0.001 DxG P=0.345	F_0	30285 ± 487.7	30962 ± 559.3	31393 ± 547.0	32004 ± 510.0	-	-
	F_1	31042 ± 640.4 (24)	34119 ± 649.3**	33444 ± 644.5	33650 ± 509.5*	-	-
	F_2	30541 ± 487.5	31540 ± 591.8	32499 ± 708.4*	31321 ± 687.4	-	*
	F_3	29722 ± 551.7	30674 ± 657.4	30690 ± 539.4	30497 ± 617.8	-	-
	F_4	30358 ± 722.4	30232 ± 682.8	30434 ± 608.2	30318 ± 561.5	-	-
ANCOVA[f] Dose P=0.008 Gen P<0.001 DxG P=0.245 BW P<0.001	F_0	-	-	-	-	*	-
	F_1	- (24)	** [0,2,3,4]	* [3,4]	** [2,3,4]	-	-
	F_2	-	- [1]	*	- [1]	-	*
	F_3	-	- [1]	- [1]	- [1]	-	-
	F_4	-	- [1]	- [1]	- [1]	-	-

[a] Mean ± 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) 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=0.048), F_0 breed father (P=0.013), and F_0 breed mother × F_0 breed father interaction (P=0.002) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.042), F_0 breed father (P=0.006), 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≤0.01. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation 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.

TABLE K18
Left and Right Ovary Weights and Ovary Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.249 Gen P=0.164 DxG P=0.489	F_0	147.6 ± 4.0	147.9 ± 4.1	138.7 ± 4.8	136.1 ± 3.6	-	*
	F_1	154.6 ± 3.6 (24)	233.2 ± 76.1 (24)	154.9 ± 4.9	143.9 ± 4.7	-	-
	F_2	155.4 ± 5.0	166.6 ± 6.6	157.2 ± 4.6	181.3 ± 38.9	**	-
	F_3	150.5 ± 5.2	155.8 ± 5.1	146.1 ± 3.9	144.8 ± 3.9	-	-
	F_4	152.3 ± 3.9	149.1 ± 3.8	149.6 ± 3.5	146.5 ± 4.9	-	-
Relative[e] Dose P=0.389 Gen P=0.135 DxG P=0.578	F_0	548.9 ± 13.1	536.1 ± 11.8	520.7 ± 17.3	556.8 ± 16.7	-	*
	F_1	569.5 ± 14.0 (24)	864.9 ± 281.2 (24)	591.4 ± 17.6	614.4 ± 20.0	-	-
	F_2	584.1 ± 16.9	631.8 ± 23.2 [3,4]	595.2 ± 16.2	736.0 ± 165.7	*	-
	F_3	574.2 ± 19.3	591.5 ± 20.9 [2]	571.8 ± 15.8	567.5 ± 12.9	-	-
	F_4	582.9 ± 15.5	572.5 ± 16.2 [2]	584.7 ± 12.1	584.6 ± 19.7	-	-
ANCOVA[f] Dose P=0.350 Gen P=0.154 DxG P=0.532 BW P=0.282	F_0	-	-	-	-	-	*
	F_1	-	-	-	-	-	-
	F_2	-	-	-	-	**	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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 ($P<0.001$) 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.
[e] 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.
[f] F_0 breed father ($P<0.001$) 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. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.
[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: *, $P \leq 0.05$; **, $P \leq 0.01$.

TABLE K19
Pituitary Gland Weights and Pituitary Gland Weight-to-Body-Weight Ratios for Female Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.340 Gen P<0.001 DxG P=0.343	F_0	15.6 ± 0.5	16.4 ± 0.7	17.6 ± 0.6* [3,4]	15.4 ± 0.5	-	**
	F_1	16.7 ± 0.6 (24)	17.0 ± 0.4	17.8 ± 0.5 [3,4]	16.8 ± 0.4 (24)	-	-
	F_2	17.5 ± 0.7	17.0 ± 0.6	17.7 ± 0.6 [3,4]	16.6 ± 0.6	-	-
	F_3	15.6 ± 0.5	15.5 ± 0.6	15.0 ± 0.5 [0,1,2]	15.2 ± 0.4	-	-
	F_4	15.7 ± 0.5	15.9 ± 0.4	15.1 ± 0.4 [0,1,2]	15.6 ± 0.4	-	-
Relative[e] Dose P=0.021 Gen P<0.001 DxG P=0.196	F_0	57.9 ± 1.6	59.7 ± 2.5	66.3 ± 2.2* [1,2]	62.7 ± 1.9	-	**
	F_1	61.4 ± 2.3 (24)	63.2 ± 1.4	68.0 ± 1.6 [3,4]	71.4 ± 1.6** (24)	***	-
	F_2	66.1 ± 2.6	64.5 ± 2.0	67.3 ± 2.5 [3,4]	66.4 ± 2.0	-	-
	F_3	59.5 ± 1.8	58.6 ± 2.0	58.5 ± 1.9 [0,1,2]	60.0 ± 1.8	-	-
	F_4	59.9 ± 1.6	60.8 ± 1.5	59.0 ± 1.5 [3]	62.3 ± 1.5	-	-
ANCOVA[f] Dose P=0.130 Gen P<0.001 DxG P=0.347 BW P<0.001	F_0	- [2]	-	* [3]	- [1]	-	**
	F_1	- (24)	-	- [3,4]	- (24) [0, 3, 4]	*	-
	F_2	- [0]	-	- [3,4]	-	-	-
	F_3	-	-	- [0,1,2]	- [1]	-	-
	F_4	-	-	- [1,2]	- [1]	-	-

[a] Mean ± 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=0.071), and F_0 breed mother × F_0 breed father interaction (P=0.159) random effects incorporated into the analysis model.
[e] F_0 breed father (P=0.045), and F_0 breed mother × F_0 breed father interaction (P=0.196) random effects incorporated into the analysis model.
[f] F_0 breed father (P=0.056), and F_0 breed mother × F_0 breed father interaction (P=0.195) 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; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P<0.001 Gen P=0.048 DxG P=0.086	F_0	559.2 ± 18.4 (23)	569.0 ± 14.6	550.8 ± 14.9	509.2 ± 14.4	*	-
	F_1	517.2 ± 10.6 (24)	586.7 ± 22.2*	549.0 ± 15.9	473.7 ± 11.1	***	-
	F_2	550.7 ± 11.6	585.9 ± 12.8	544.4 ± 15.4	524.3 ± 16.1 (24)	*	-
	F_3	524.6 ± 10.5	573.3 ± 17.3	506.2 ± 14.2	507.1 ± 11.4	-	-
	F_4	547.0 ± 15.0	562.6 ± 12.3	516.6 ± 11.8	518.3 ± 11.2	-	-
Relative[e] Dose P=0.045 Gen P=0.082 DxG P=0.085	F_0	2088.4 ± 62.0	2067.8 ± 49.0	2062.6 ± 39.6	2073.6 ± 50.8	-	-
	F_1	1904.3 ± 42.3 (24)	2171.3 ± 65.3**	2085.1 ± 37.9	2019.0 ± 41.6	-	-
	F_2	2074.9 ± 43.4	2221.2 ± 40.5	2060.7 ± 50.7	2093.4 ± 54.9 (24)	-	-
	F_3	2003.2 ± 34.8	2169.4 ± 60.8	1979.6 ± 55.0	1989.0 ± 40.5	-	-
	F_4	2085.6 ± 45.9	2156.4 ± 47.9	2017.8 ± 37.7	2065.3 ± 38.8	-	-
ANCOVA[f] Dose P=0.051 Gen P=0.081 DxG P=0.057 BW P<0.001	F_0	-	-	-	-	-	-
	F_1	- (24)	***	-	-	-	-
	F_2	-	-	-	- (24)	-	-
	F_3	-	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.006), 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<0.001), 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<0.001), 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.

[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; 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≤0.001.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P<0.001 Gen P=0.031 DxG P=0.881	F_0	321.6 ± 10.4	315.9 ± 10.6	301.4 ± 11.4 (24)	288.9 ± 9.8	*	-
	F_1	336.9 ± 10.6 (24)	332.1 ± 14.2	304.9 ± 11.0	297.1 ± 12.4	*	-
	F_2	353.6 ± 12.6	337.6 ± 15.8	311.2 ± 13.0	327.3 ± 14.0	-	-
	F_3	342.2 ± 16.0	324.1 ± 14.9	280.8 ± 11.3**	306.4 ± 15.4	-	**
	F_4	334.0 ± 11.9	305.5 ± 11.5	281.3 ± 8.2*	310.0 ± 13.0	-	*
Relative[e] Dose P=0.001 Gen P=0.022 DxG P=0.870	F_0	1199.9 ± 40.0	1147.0 ± 35.9	1135.6 ± 43.9	1175.7 ± 34.8	-	-
	F_1	1239.0 ± 37.6 (24)	1230.2 ± 48.3	1160.8 ± 38.4	1260.8 ± 44.4	-	-
	F_2	1333.9 ± 49.5	1279.7 ± 58.2	1178.6 ± 47.4	1304.4 ± 49.6	-	*
	F_3	1305.6 ± 59.6	1230.0 ± 57.8	1095.8 ± 41.7**	1199.1 ± 55.0	-	**
	F_4	1275.2 ± 41.3	1170.2 ± 43.9	1099.9 ± 30.8*	1237.8 ± 53.9	-	*
ANCOVA[f] Dose P=0.001 Gen P=0.019 DxG P=0.908 BW P<0.001	F_0	-	-	- (24)	-	-	-
	F_1	- (24)	-	-	-	-	-
	F_2	-	-	-	-	-	*
	F_3	-	-	**	-	-	**
	F_4	-	-	*	-	-	*

[a] Mean ± 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) and F_0 breed mother × F_0 breed father interaction (P=0.016) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.012) 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=0.010) 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≤0.01. 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 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g] 0	5	100	500	Trends[h] Linear	Quad
Absolute[d] Dose P=0.002 Gen P<0.001 DxG P=0.578	F_0	24.2 ± 1.0 [2, 4]	25.4 ± 1.1 [2, 3]	24.4 ± 1.0 [2]	24.3 ± 1.0 [2, 4]	-	-
	F_1	25.4 ± 0.7 [2, 4]	26.6 ± 0.7 [2, 4]	25.5 ± 0.9 [2, 4]	25.4 ± 0.9 [2, 4]	-	-
	F_2	36.8 ± 2.0 (24) [0, 1, 3, 4]	35.9 ± 1.3 [0, 1, 3, 4]	32.9 ± 1.3 [0, 1, 3, 4]	31.5 ± 1.4** [0, 1, 3, 4]	**	-
	F_3	27.4 ± 1.1 [0,1,2,3]	29.5 ± 1.4 [1,2,3]	25.6 ± 0.7 [1,2,3]	25.8 ± 1.1 [0,1,2,3]	-	-
	F_4	20.4 ± 1.0 [2, 4]	21.9 ± 0.7 [0, 2, 4]	20.9 ± 0.9 [2, 4]	19.4 ± 0.9 [2, 4]	-	-
Relative[e] Dose P=0.255 Gen P<0.001 DxG P=0.074	F_0	90.6 ± 4.1 [2]	91.9 ± 3.6 [2, 3]	91.9 ± 3.9 [2]	99.2 ± 4.0 [2,4]	-	-
	F_1	92.0 ± 3.5 [2]	98.7 ± 2.2 [2]	97.0 ± 3.0 [2]	108.2 ± 4.0* [2, 4]	**	-
	F_2	139.1 ± 7.5 (24) [0, 1, 3, 4]	136.0 ± 4.4 [0, 1, 3, 4]	125.2 ± 5.0 [0, 1, 3, 4]	126.0 ± 5.9 [0, 1, 3, 4]	-	-
	F_3	104.7 ± 4.1 [2, 3]	111.5 ± 5.0 [2, 3]	100.3 ± 2.7 [2, 3]	101.7 ± 4.9 [0, 1, 2, 3]	-	-
	F_4	78.2 ± 4.0 [2, 4]	83.6 ± 2.5 [0, 2, 4]	82.0 ± 3.8 [2, 4]	77.3 ± 3.4 [2, 4]	-	-
ANCOVA[f] Dose P=0.025 Gen P<0.001 DxG P=0.375 BW P=0.011	F_0	- [2]	- [2, 3]	- [2]	- [2, 4]	-	-
	F_1	- [2, 4]	- [2, 4]	- [2, 4]	- [2, 4]	-	-
	F_2	- (24) [0, 1, 3, 4]	- [0, 1, 3, 4]	- [0, 1, 3, 4]	* [0, 1, 3, 4]	**	-
	F_3	- [2,4]	- [0, 2, 4]	- [2, 4]	- [1, 2, 4]	-	-
	F_4	- [1, 2, 3]	- [1, 2, 3]	- [1, 2, 3]	- [0, 2, 3]	-	-

[a] Mean ± 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=0.334) 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=0.051), F_0 breed father (P=0.248), 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=0.248) and F_0 breed mother × F_0 breed father interaction (P=0.003) 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; generation 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.

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

Organ Weight/ Analysis Type[c]	Generation	Dietary Genistein (ppm)[g]				Trends[h]	
		0	5	100	500	Linear	Quad
Absolute[d] Dose P=0.878 Gen P=0.002 DxG P=0.584	F_0	464.5 ± 19.5	483.2 ± 26.8	481.3 ± 24.0	473.6 ± 22.6	-	-
	F_1	593.7 ± 50.1 (24)	554.4 ± 32.5	539.0 ± 36.4	555.1 ± 32.5	-	-
	F_2	490.6 ± 24.1	520.2 ± 35.2	537.0 ± 37.9	559.3 ± 48.2	-	-
	F_3	495.3 ± 23.8 (24)	504.4 ± 20.1	544.4 ± 37.2	495.7 ± 24.4	-	-
	F_4	601.9 ± 44.6	512.6 ± 23.0	598.6 ± 49.5	537.0 ± 39.4	-	-
Relative[e] Dose P=0.368 Gen P<0.001 DxG P=0.491	F_0	1728.6 ± 69.9 [0]	1757.8 ± 95.7	1812.5 ± 94.5	1941.7 ± 98.3	-	-
	F_1	2177.9 ± 176.8 (24)	2068.5 ± 126.9	2049.1 ± 136.3	2369.5 ± 141.9	-	-
	F_2	1860.7 ± 106.9	1967.1 ± 127.3	2033.2 ± 141.0	2225.7 ± 181.7	-	-
	F_3	1905.1 ± 103.9 (24)	1914.0 ± 78.6	2137.3 ± 155.0	1954.7 ± 103.9	-	-
	F_4	2311.6 ± 176.7 [4]	1965.5 ± 89.7	2336.7 ± 191.7	2146.0 ± 162.4	-	-
ANCOVA[f] Dose P=0.831 Gen P=0.001 DxG P=0.574 BW P=0.105	F_0	-	-	-	-	-	-
	F_1	- (24)	-	-	-	-	-
	F_2	-	-	-	-	-	-
	F_3	- (24)	-	-	-	-	-
	F_4	-	-	-	-	-	-

[a] Mean ± 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=0.002), F_0 breed father (P=0.400), and F_0 breed mother × F_0 breed father interaction (P=0.051) random effects incorporated into the analysis model.
[e] F_0 breed mother (P=0.002), F_0 breed father (P=0.248), and F_0 breed mother × F_0 breed father interaction (P=0.003) random effects incorporated into the analysis model.
[f] F_0 breed mother (P=0.002) and F_0 breed mother × F_0 breed father interaction (P=0.112) 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; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$.
[h] Contrasts were used to test for linear and quadratic (Quad) exposure concentration trends within a generation. Significance is indicated in shaded cells.

APPENDIX L
SPERM PARAMETERS

TABLE L1
Sperm Motility of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Generation	Dietary Genistein (ppm)			
	0	5	100	500
F_0	93 ± 6 (23)	89 ± 9 (25)	88 ± 12 (25)	93 ± 5 (23)
F_1	94 ± 5 (25)	95 ± 5 (24)	90 ± 8 (25)	94 ± 5 (25)
F_2	91 ± 12 (25)	88 ± 13 (25)	90 ± 9 (24)	91 ± 8 (25)
F_3	92 ± 5 (18)	92 ± 4 (20)	89 ± 7 (21)	90 ± 7 (21)
F_4	95 ± 6 (23)	95 ± 5 (25)	95 ± 5 (25)	96 ± 4 (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 Genistein[a,b]

Generation	Dietary Genistein (ppm)			
	0	5	100	500
F_0	688 ±287 (24)	785 ± 245 (24)	743 ± 218 (25)	704 ± 195 (22)
F_1	840 ± 199 (26)	754 ± 221 (25)	840 ± 208 (25)	812 ± 237 (25)
F_2	793 ± 187 (25)	856 ± 190 (25)	844 ± 145 (25)	781 ± 176 (25)
F_3	712 ± 197 (25)	703 ± 209 (25)	740 ± 237 (25)	774 ± 187 (25)
F_4	507 ± 304 (24)	483 ± 439 (25)	464 ± 227 (25)	497 ± 240 (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. No significant effects were observed.

TABLE L3
Testicular Spermatid Head Count of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b]

Generation	Dietary Genistein (ppm)			
	0	5	100	500
F_0	103 ± 32 (24)	112 ± 27 (24)	109 ± 19 (25)	121 ± 25 (22)
F_1	106 ± 15 (26)	108 ± 15 (25)	108 ± 14 (25)	100 ± 11 (25)
F_2	118 ± 19 (25)	117 ± 17 (25)	112 ± 25 (25)	114 ± 20 (25)
F_3	98 ± 27 (25)	101 ± 18 (25)	109 ± 13 (25)	101 ± 13 (25)
F_4	96 ± 24 (24)	91 ± 25 (25)	110 ± 30 (25)	99 ± 26 (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. No significant effects were observed.

TABLE L4
Sperm Morphology of Male Rats in the Multigenerational Reproductive Toxicology Feed Study of Genistein[a,b,c]

Generation	Dietary Genistein (ppm)			
	0 ppm	5 ppm	100 ppm	500 ppm
F_0	0.4 ± 0.7 (23)	0.4 ± 0.6 (24)	0.2 ± 0.4 (25)	0.1 ± 0.3 (22)
F_1	0.2 ± 0.4 (26)	0.3 ± 0.4 (25)	0.3 ± 0.5 (25)	0.1 ± 0.3 (25)
F_2	0.1 ± 0.2 (25)	0.1 ± 0.3 (25)	0.1 ± 0.2 (25)	0.1 ± 0.3 (25)
F_3	0.2 ± 0.3 (25)	0.2 ± 0.3 (25)	0.1 ± 0.2 (25)	0.2 ± 0.3 (25)
F_4	0.7 ± 0.7 (25)	0.5 ± 0.7 (25)	0.5 ± 0.5 (25)	0.6 ± 0.7 (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.
[c] A minimum of 200 caudal epididymal sperm per animal were microscopically evaluated for head (amorphous, small, enlarged, double) or tail (coiled, bent, double) abnormalities, and the percentage of sperm containing abnormalities was calculated.

APPENDIX M
OVARIAN FOLLICLE COUNTS

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

Follicle Class	Generation	Dietary Genistein (ppm)				Trends	
		0	5	100	500	Linear	Quad
Small[b]	F_0	19.7 ± 0.9 [2]	21.9 ± 2.9	22.6 ± 2.4	18.8 ± 2.4	-	-
	F_1	25.0 ± 3.5	22.9 ± 3.0	31.1 ± 4.0	28.6 ± 4.8	-	-
Dose P=0.209	F_2	37.2 ± 5.2 [0, 3, 4]	26.7 ± 3.0	36.7 ± 3.6 [4]	26.7 ± 3.6	-	-
Gen P<0.001	F_3	21.9 ± 2.0 [2]	26.1 ± 1.9	29.2 ± 3.8	32.4 ± 4.0	-	-
DxG P=0.396	F_4	21.8 ± 3.3 [2]	18.5 ± 2.6	20.5 ± 2.9 [2]	24.0 ± 4.5	-	-
Growing[b]	F_0	1.8 ± 0.3	2.2 ± 0.2	2.2 ± 0.2	1.9 ± 0.4	-	-
	F_1	2.2 ± 0.5	2.5 ± 0.3	2.2 ± 0.3	2.7 ± 0.5	-	-
Dose P=0.875	F_2	2.2 ± 0.3	1.8 ± 0.2	2.0 ± 0.3	1.4 ± 0.2	-	-
Gen P=0.028	F_3	1.9 ± 0.2	1.7 ± 0.2	1.9 ± 0.3	2.5 ± 0.2	-	-
DxG P=0.523	F_4	1.5 ± 0.2	1.6 ± 0.3	1.7 ± 0.2	1.8 ± 0.3	-	-

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

Follicle Class	Generation	Dietary Genistein (ppm)				Trends	
		0	5	100	500	Linear	Quad
Small & Growing Combined[b]	F_0	21.5 ± 0.9 [2]	24.1 ± 2.9	24.8 ± 2.5	20.7 ± 2.7 [3]	-	-
	F_1	27.1 ± 3.6	25.4 ± 3.1	33.2 ± 4.1	31.4 ± 5.2	-	-
	F_2	39.4 ± 5.3 [0, 3, 4]	28.5 ± 3.1	38.7 ± 3.7 [4]	28.1 ± 3.7	-	-
Dose P=0.234	F_3	23.8 ± 2.2 [2]	27.8 ± 1.9	31.1 ± 3.9	34.9 ± 4.1 [0]	-	-
Gen P<0.001	F_4	23.4 ± 3.5 [2]	20.1 ± 2.7	22.1 ± 3.0 [2]	25.9 ± 4.7	-	-
DxG P=0.364							
Antral[b]	F_0	1.6 ± 0.4	1.2 ± 0.3	1.4 ± 0.2	1.5 ± 0.3	-	-
	F_1	1.5 ± 0.3	1.7 ± 0.2	2.4 ± 0.3	1.6 ± 0.3	-	*
	F_2	2.5 ± 0.3	1.7 ± 0.2	1.8 ± 0.3	1.3 ± 0.2*	*	-
Dose P=0.441	F_3	1.8 ± 0.2	1.7 ± 0.3	1.4 ± 0.1	2.1 ± 0.3	-	-
Gen P=0.134	F_4	1.6 ± 0.2	1.6 ± 0.3	1.7 ± 0.4	1.5 ± 0.4	-	-
DxG P=0.186							

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

Follicle Class	Generation	Dietary Genistein (ppm)				Trends	
		0	5	100	500	Linear	Quad
All[b] Dose P=0.252 Gen P<0.001 DxG P=0.365	F_0	23.1 ± 1.1 [2]	25.4 ± 3.1	26.2 ± 2.6	22.2 ± 3.0 [3]	-	-
	F_1	28.6 ± 3.7	27.2 ± 3.2	35.6 ± 4.3	32.9 ± 5.4	-	-
	F_2	41.9 ± 5.4 [0, 3, 4]	30.1 ± 3.3	40.5 ± 4.0 [4]	29.4 ± 3.7	-	-
	F_3	25.6 ± 2.4 [2]	29.5 ± 1.9	32.5 ± 3.9	37.0 ± 4.2 [0]	-	-
	F_4	24.9 ± 3.5 [2]	21.7 ± 2.9	23.8 ± 3.1 [2]	27.3 ± 4.9	-	-

[a] Mean ± standard error. Eight animals were in each group. Five step sections of both ovaries were evaluated by two independent reviewers (counters). Asterisks (*) in shaded cells in the exposed group columns indicate significant differences from controls in the same generation as determined by Dunnett's test; asterisks in the Trends columns indicate significant linear or quadratic (Quad) exposure concentration trends within that generation as determined by contrasts: *, $P \leq 0.05$. Significant differences between generations within an exposure group were determined by Holm's adjusted t-tests; generation numbers in brackets indicate the generations whose means are significantly different from the given mean value at $P \leq 0.05$. A dash in the Trends columns indicates that the exposure concentration trend test was not significant ($P > 0.05$).

[b] Results of a two-way ANOVA are indicated for dose and generation (Gen) main effects and the 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. Only in the case of Growing Follicles were any random terms significant via the log-likelihood ratio test; the F_0 breed mother and the interaction between the F_0 breed mother and F_0 breed father were significant and were included in the model.

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

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

	Amount	Source
Vitamins		
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 hydrochloride
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		
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, %	18.2 ± 1.0	37
Total Fat, %	5.1 ± 1.0	39
Volatiles, %	6.7 ± 1.9	39
Vitamin A, ppm	8.5 ± 1.7	39
Vitamin B_1, mg/gm	0.05 ± 0.07	39
Vitamin E, ppm	87.7 ± 1.9	39
Selenium, ppm	0.39 ± 0.11	39

TABLE N3
Contaminant Levels in Purina 5K96 Rat Ration

Contaminant	Mean ± Standard Deviation	# Lots/# Lots Positive
Arsenic, ppm	0.125 ± 0.06	39/30
Cadmium, ppb	0.085 ± 0.03	39/16
Lead, ppm	0.37 ± 0.18	39/38
Fumonisin B_1, ppb	36.1 ± 19.6	27/27**
Total Fumonisin, ppb	53.5 ± 26.4	39/39
Aflatoxin B_1, ppb	<MDL	39/0
Aflatoxin B_2, ppb	<MDL	39/0
Aflatoxin G_1, ppb	<MDL	39/0
Aflatoxin G_2, ppb	<MDL	39/0

** Fumonisin B_1 routine analysis discontinued after initial 27 feed lots.

APPENDIX O
SENTINEL ANIMAL PROGRAM

SENTINEL ANIMAL PROGRAM

METHODS

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

Serum samples were collected from 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.

Method and Test	Time of Analysis
RATS	
ELISA	
H-1 (Toolan's H-1 virus)	15, 17, 18, and 20 weeks, study termination
KRV (Kilham Rat Virus)	15, 17, 18, and 20 weeks, study termination
Mycoplasma pulmonis	15, 17, 18, and 20 weeks, study termination
PVM (pneumonia virus of mice)	15, 17, 18, and 20 weeks, study termination
RCV/SDA (rat coronavirus/sialodacryoadenitis virus)	15, 17, 18, and 20 weeks, study termination
Sendai	15. 17, 18, and 20 weeks, study termination

RESULTS

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

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.

Chang, H.C., and Doerge, D.R. (2000). Dietary genistein inactivates rat thyroid peroxidase *in vivo* without an apparent hypothyroid effect. *Toxicol. Appl. Pharmacol.* **168**, 244-252.

Chang, H.C., Churchwell, M.I., Delclos, K.B., Newbold, R.R., and Doerge, D.R. (2000). Mass spectrometric determination of genistein tissue distribution in diet-exposed Sprague-Dawley rats. *J. Nutr.* **130**, 1963-1970.

Dalu, A., Blaydes, B.S., Bryant, C.W., Latendresse, J.R., Weis, C.C., and Delclos, K.B. (2002). Estrogen receptor expression in the prostate of rats treated with dietary genistein. *J. Chromatogr. B Analyt. Technol. Biomed. Life Sci.* **777**, 249-260.

Doerge, D.R., Churchwell, M.I., Chang, H.C., Newbold, R.R., and Delclos, K.B. (2001). Placental transfer of the soy isoflavone genistein following dietary and gavage administration to Sprague Dawley rats. *Reprod. Toxicol.* **15**, 105-110.

Doerge, D.R., Twaddle, N.C., Churchwell, M.I., Newbold, R.R., and Delclos, K.B. (2006). Lactational transfer of the soy isoflavone, genistein, in Sprague-Dawley rats consuming dietary genistein. *Reprod. Toxicol.* **21**, 307-312.

Ferguson, S.A., Flynn, K.M., Delclos, K.B., Newbold, R.R., and Gough, B.J. (2002). Effects of lifelong dietary exposure to genistein or nonylphenol on amphetamine-stimulated striatal dopamine release in male and female rats. *Neurotoxicol. Teratol.* **24**, 37-45.

Flynn, K.M., Ferguson, S.A., Delclos, K.B., and Newbold, R.R. (2000). Multigenerational exposure to dietary genistein has no severe effects on nursing behavior in rats. *Neurotoxicology* **21**, 997-1001.

Scallet, A.C., Divine, R.L., Newbold, R.R., and Delclos, K.B. (2004). Increased volume of the calbindin D28k-labeled sexually dimorphic hypothalamus in genistein and nonylphenol-treated male rats. *Toxicol. Sci.* **82**, 570-576.