NTP
National Toxicology Program
U.S. Department of Health and Human Services

NTP Research Report on The CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats

NTP RR 9

SEPTEMBER 2018

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Research Report 9

National Toxicology Program

September 2018

National Toxicology Program
Public Health Service
U.S. Department of Health and Human Services
ISSN: 2473-4756

Foreword

This study was carried out under the auspices of the National Toxicology Program (NTP) as part of the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA), a consortium-based research program between the National Institute of Environmental Health Sciences (NIEHS) of the National Institutes of Health (NIH) and the National Center for Toxicological Research (NCTR) of the US Food and Drug Administration (FDA).

The aim of the CLARITY-BPA program was to bridge guideline-compliant research conducted at the FDA with hypothesis-based research investigations conducted by academia on the toxicity of bisphenol A (BPA). A detailed description of the CLARITY-BPA program is covered in Heindel and co-authors (https://www.ncbi.nlm.nih.gov/pubmed/26232693).

The CLARITY-BPA research program has two components: 1) A "core" guideline-compliant chronic study conducted at NCTR according to FDA Good Laboratory Practice (GLP) regulations (two-year perinatal only or chronic BPA exposure, including perinatal), and 2) CLARITY-BPA grantee studies of various health endpoints, conducted by NIEHS-funded researchers at academic institutions using animals born to the same exposed pregnant rats as the core GLP study.

This NTP Research Report only covers the core study and includes the narrative, tables, and figures reported in the GLP report. The core study GLP report had 34 appendices, which are listed in Appendix A and referred to as Supplemental Appendices throughout this report. The original GLP report for the core study is on file at NCTR.

The interpretation of biological and toxicological responses described in this report is based only on the results of the core GLP study. Integration of these data with other data from the grantee studies conducted as part of the CLARITY-BPA research program or extrapolation of the results to other species, including characterization of hazards and risks to humans, is outside of the scope of this report.

The core GLP study was designed to characterize and evaluate the toxicologic potential of BPA following perinatal only or chronic exposure in rats under the conditions of a chronic, extended-dose response design. The core GLP study was designed by NCTR and NIEHS scientists with substantial input from the CLARITY-BPA consortium members.

This study was funded via an interagency agreement between FDA and NIEHS. The study's conduct and progress were monitored by the Toxicology Study Selection and Review Committee (composed of representatives from NCTR, other FDA product centers, and NIEHS, ad hoc members of other federal government agencies, and academia), the CLARITY-BPA Steering Committee, and the CLARITY-BPA External Scientific Panel.

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals. The core GLP study is subjected to retrospective quality assurance audits before being presented for public review.

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Peer Review

NTP convened an expert panel on April 26, 2018, to peer review the *NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats*. The panel members are listed below. These reviewers served as independent scientists, not as representatives of any institution, company, or governmental agency. In this capacity, the panel provided input on the scientific and technical elements of the CLARITY-BPA Core Study and ensured that this NTP Research Report presented the experimental results and conclusions fully and clearly.

Meeting materials, the peer review report, and presentation recordings can be found on the NTP website: https://ntp.niehs.nih.gov/events/past/index.html.

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Publication Details

Publisher: National Toxicology Program

Publishing Location: Research Triangle Park, NC

ISSN: 2473-4756

DOI: https://doi.org/10.22427/NTP-RR-9

Report Series: NTP Research Report Series

Report Series Number: 9

Official citation: National Toxicology Program. 2018. *NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats*. NTP RR 9. Research Triangle Park, NC. National Toxicology Program (9): 1-221.

Abstract

Bisphenol A (BPA, CAS #80-05-7) is a high-production-volume industrial chemical used as a monomer for polycarbonate plastics and epoxy resins that have broad applications in consumer products, including storage containers for foods and beverages and medical devices. The potential toxicity resulting from chronic exposure to BPA as an indirect food additive is the concern addressed in this study.

This study is part of the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA), a research program between the National Institute of Environmental Health Sciences (NIEHS) and the National Center for Toxicological Research (NCTR) of the Food and Drug Administration (FDA), developed to bridge guideline-compliant research conducted at the FDA with hypothesis-based research investigations conducted by academia on the toxicity of BPA. The CLARITY-BPA research program has two components: 1) A "core" guideline-compliant chronic study conducted at NCTR according to FDA Good Laboratory Practice (GLP) regulations and 2) studies of various endpoints, conducted by NIEHS-funded researchers at academic institutions using animals born to the same exposed pregnant rats as the core GLP study. The purpose of this research program was to evaluate chronic exposure to BPA over a broad dose range using traditional and non-traditional endpoints. It aimed to determine if non-traditional endpoints reveal toxicity not detected by traditional guideline study endpoints and provide mechanistic support for observations made in the guideline study. The current research report covers only data from the "core" guideline-compliant chronic study.

The toxicity of BPA administered by oral gavage from gestation day (GD) 6 through the start of labor and then by oral gavage to pups from postnatal day (PND) 1 (day of birth = PND 0) until termination at one year or two years was examined in Sprague-Dawley rats from the NCTR breeding colony (Sprague-Dawley/CD23/NctrBR). BPA doses were 2.5, 25, 250, 2,500, and 25,000 μg/kg body weight (bw)/day. A vehicle (0.3% carboxymethylcellulose (CMC)) control group was also included. In addition to animals that were dosed daily throughout the study, a stop-dose study arm was included with animals dosed daily until PND 21 and then held without further treatment until termination to assess any effects that were due to early exposure only. Because many of the effects of BPA reported in the literature are associated with estrogen signaling pathways, two doses (0.05 and 0.5 μg/kg bw/day) of the orally active estrogen ethinyl estradiol (EE_2) were also included in the continuous-dose arm to assess the sensitivity of the test system to low doses of an estrogen. Reference estrogen groups were not included in the stop-dose study arm of the core study due to resource constraints, primarily lack of animal facility space. Rats were obtained as weanlings from the NCTR breeding colony and placed under study conditions (soy- and alfalfa-free diet (5K96, LabDiet, Purina Mills), polysulfone cages, hardwood chip bedding, glass water bottles, and food-grade silicone stoppers) until mating. Study materials were monitored for background BPA levels; the only material with detectable levels of BPA was the diet, which had less than 3 ppb BPA. Prior to mating to males that were not siblings or first cousins, female rats were stratified by body weight and were randomized to treatment groups to give approximately equivalent mean starting body weights in each group. Each morning after pairing, females were examined for evidence of mating (presence of an in situ vaginal plug or sperm-positive vaginal smear). Upon evidence of mating, the females were separated from the males and individually housed; this day was considered GD 0. On GD 6, daily dosing of the dam with BPA, EE_2, or vehicle began and was based on the body weight

measured immediately prior to the administration of these compounds. Direct gavage dosing of the pups was started on PND 1, with the same dose and agent that was administered to their dams. At weaning on PND 21, no more than one animal per sex per litter was assigned to the following study arms: 1) continuous dosing to sacrifice at two years (terminal sacrifice, 46–50 animals per sex per vehicle control or BPA treatment group and 26 animals per sex per EE_2 group); 2) continuous dosing to sacrifice at one year (interim sacrifice, 20–26 animals per sex for all groups); 3) no further treatment after PND 21 until sacrifice at two years (stop-dose terminal sacrifice, 46–50 animals per sex per preweaning vehicle control or BPA group); and 4) no further treatment after PND 21 until sacrifice at one year (stop-dose interim sacrifice, 20–26 animals per sex for preweaning vehicle control and BPA groups). The stop-dose study arms for which gavage dosing was not continued beyond weaning were included to assess the potential of permanent effects induced by exposure to hormonally active compounds during developmental stages. The interim (one-year) sacrifice group was included to allow evaluation of long-term exposure effects with less confounding due to background lesions of aging than would be expected at two years, and to allow assessment of any precursors of any treatment-related lesions observed at two years.

Data collected included body weights, litter parameters, age at vaginal opening, vaginal cytology, clinical pathology (interim sacrifice only), sperm parameters (interim sacrifice only), organ weights (interim sacrifice only), and histopathology (both interim and terminal sacrifices). Vaginal cytology data were collected for 14 consecutive days at approximately 16 weeks of age from the same subset of females in the terminal sacrifice arm that had been monitored for vaginal opening; these same animals were then monitored for five consecutive days monthly to estimate the time at which they began having aberrant estrous cycles. In addition to the summary tables provided in this report and appendices, all individual animal data are available online (https://doi.org/10.22427/NTP-DATA-018-00015-0001-000-6).

Table 1 lists all non-histopathology endpoints analyzed and associated statistical findings. For histopathology data, Table 1 only lists the endpoints where a statistically significant difference was found by the primary statistical tests applied (Cochran-Armitage/Fisher's Exact Test for interim sacrifice animals; survival-adjusted Poly-3 test for terminal sacrifice animals). Results from all statistical tests applied to the histopathology data, which further included Jonckheere-Terpstra/Shirley-Williams (JT/SW) and relative treatment effect (RTE) tests for non-neoplastic lesions assigned severity scores, are included in the text of this abstract and in the report text and tables. Statistically significant results are indicated regardless of biological significance.

There were few significant effects of BPA treatment in the in-life data collected. In the late stages of the study (weeks 96–104), mean female body weights in the 250 µg BPA/kg bw/day continuous-dose group were significantly higher than the mean vehicle control body weights. For clinical pathology endpoints and organ weights, some statistically significant effects of continuous- or stop-dose BPA treatments were observed. These effects were of questionable relevance to BPA toxicity given that they were seen only in single-dose groups, in several cases differed from the vehicle control by less than 10%, and, in the case of organ weights, were not significant when adjusted for body weight.

In the stop-dose BPA study arm at two years, there was a statistically significant increase in the incidence of female mammary gland adenocarcinoma (22% versus 6%; $p = 0.016$) and the combination of adenoma and adenocarcinoma (24% versus 8%; $p = 0.018$) in the 2.5 µg BPA/kg bw/day dose group. No increase in female mammary gland neoplasms was observed in the

continuous BPA dose arm at two years. There were no significant treatment-related non-neoplastic lesions in the mammary gland of interim or terminal sacrifice stop-dose BPA groups. In the interim and terminal BPA continuous dosing arm, there was an increase, significant by the secondary RTE test only, in female mammary gland atypical foci at 2.5 μg BPA/kg bw/day (14% versus 0% and 15% versus 4% for the interim and terminal dose group animals, respectively). Increased adenoma/adenocarcinoma incidence observed only in the stop-dose animals, lack of a dose response, absence of non-neoplastic lesions in interim or terminal sacrifice stop-dose animals, and comparison to limited historical control data for this strain of rats in experiments conducted at NCTR bring into question the biological plausibility of this lesion as a BPA treatment-related effect. In addition to mammary gland neoplasms, a significant trend ($p = 0.037$) for uterine stromal polyps in the interim sacrifice animals in the continuous BPA dose arm was observed; this was not observed in the terminal sacrifice animals.

In the histopathological evaluations, there were many non-neoplastic lesions associated with aging in this strain of rats in both males and females that were variable across control and BPA treatment levels. In the interim stop-dose sacrifice BPA females, there was a significant dose trend with a significant increase in follicular cysts in the ovary at 25,000 μg BPA/kg bw/day dose group. The secondary statistical tests, which incorporated both incidence and severity scores, indicated an increase in cystic endometrial hyperplasia and squamous metaplasia in the uterus at 25,000 μg BPA/kg bw/day in the interim stop-dose females. In the terminal stop-dose animals, secondary tests indicated an increase in cystic endometrial hyperplasia at 2,500 and 25,000 μg BPA/kg bw/day, although severity was similar in the vehicle control and the BPA-treated groups. Cardiomyopathy was increased in the terminal stop-dose females at 2.5, 250, 2,500, and 25,000 μg BPA/kg bw/day, as assessed by statistical tests that incorporated incidence and severity scores, although background incidence was high at this age and severity score differences across dose groups were similar. In interim continuous-dose females, uterine apoptosis and vaginal epithelial hyperplasia were elevated at 25,000 μg BPA/kg bw/day. Vaginal epithelial hyperplasia was also increased in terminal continuous-dose animals at doses from 25 to 25,000 μg BPA/kg bw/day, with a similar response across each of those dose levels.

There were no significant differences between treatment groups and vehicle controls in the incidences of neoplastic lesions in stop-dose or continuous-dose interim or terminal sacrifice males. There were also no apparent treatment-related non-neoplastic effects in interim stop-dose males; in terminal stop-dose BPA males, an increase of hyperplasia in the pars distalis of the pituitary at 25,000 μg BPA/kg bw/day was noted. In interim, but not terminal, continuous-dose males there was an increase in exfoliated germ cells and an increase in lymphocyte infiltration in the epididymis at 25,000 μg BPA/kg bw/day. No significant effects on sperm parameters or testicular histopathology were noted in the BPA dose groups. In the terminal continuous-dose males, hyperplasia of the pars distalis of the pituitary was increased at 25 and 25,000 μg BPA/kg bw/day. Increases in dorsal/lateral prostate inflammation in most BPA dose groups were variable across a high background in both interim and terminal sacrifice animals.

In the EE_2 reference estrogen dose groups, there were multiple significant treatment-related effects at the 0.5 μg/kg bw/day exposure level in females. At the time of estrous cycle evaluation at 16 weeks, more than 90% of the females in the 0.5 μg EE_2/kg bw/day dose group were exhibiting prolonged estrus. At the interim sacrifice, mean weights of the adrenal glands, heart, kidney, liver, and pituitary gland were higher in the 0.5 μg EE_2/kg bw/day dose group than the vehicle control means. Ovarian/parametrial fat pad and ovary weights were significantly lower

than mean vehicle control weights in the high EE_2 dose group. At the interim sacrifice, lobular hyperplasia and ductal dilatation were elevated in the mammary glands of the 0.5 μg EE_2/kg bw/day dose group. Increases in apoptosis, cystic endometrial hyperplasia, and squamous metaplasia were observed in the uterus of the interim high dose EE_2 females. Atrophy and cystic follicles were increased in the ovaries, the incidence of vaginal hyperplasia was increased, and increases in hyperplasia of the pars distalis and angiectasis were observed in the pituitary at 0.5 μg EE_2/kg bw/day. The incidences of cardiomyopathy and nephropathy were also increased in the high dose EE_2 females at one year. At terminal sacrifice, there were significant increases in the incidence of mammary gland adenocarcinomas and combined adenomas/carcinomas of the pituitary pars distalis in the 0.5 μg EE_2/kg bw/day dose group. There was a trend toward increasing uterine metaplasia at two years, and the incidence of nephropathy was increased in both the 0.05 and 0.5 μg EE_2/kg bw/day dose groups.

Few statistically significant effects of EE_2 in males were observed. In the high dose EE_2 group, there was an elevated incidence of lymphocyte infiltration observed in the epididymis in interim sacrifice animals and an increase in hyperplasia in the pars distalis of the pituitary at two years.

In conclusion, in the CLARITY-BPA core study, statistical differences between BPA treatment groups, particularly below 25,000 μg/kg bw/day, and the vehicle control group detected by the low-stringency statistical tests applied to histopathology lesions, were not dose responsive, sometimes occurring in only one low or intermediate dose group, and did not demonstrate a clear pattern of consistent responses within or across organs within the stop- and continuous-dose arms and sacrifice times. In contrast, the high EE_2-dose elicited several estrogenic effects in females in a clearly interpretable and biologically plausible manner. Several observations at 25,000 μg BPA/kg bw/day may be treatment related, including effects mentioned above in the female reproductive tract (ovary, uterus, and vagina) and in the male pituitary.

Table 1. Summary of Endpoints Evaluated and Statistically Significant Treatment Effects of BPA and EE_2 Relative to Vehicle Controls[a,b]

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	Continuous-Dose EE_2
Gestational weight	NA	-	-
Implantation sites	NA	-	-
Litter size	NA	-	-
Sex ratio	NA	-	-
Litter weight	NA	-	-
Male group pup weight, PND 1	NA	-	-
Female group pup weight, PND 1	NA	-	-
Preweaning pup survival, male	NA	-	-
Preweaning pup survival, female	NA	-	↓ (0.05)
Preweaning pup body weight, male	NA	-	-
Preweaning pup body weight, female	NA	-	↓ (0.05) PND 4 & 7
Age at vaginal opening	-	-	-
Body weight at vaginal opening	Not analyzed[c]	-	-
Postweaning survival, male, 1 year	-	-	-
Postweaning survival, female, 1 year	-	-	-
Postweaning body weight, male, 1 year	-	-	-
Postweaning body weight, female, 1 year	-	-	-
Postweaning survival, male, 2 years	-	-	-
Postweaning survival, female, 2 years	-	-	-
Postweaning body weight, male, 2 years	↓ (T), wk 4	-	-
Postweaning body weight, female, 2 years	↓ (T), wk 4	↑ (250) wks 96–104	↑ (0.5) wks 4 and 8
Abnormal estrous cycles at 16 weeks of age	-	-	↑ (0.5)
Early onset of aberrant estrous cycles	↓ (2,500)	-	↑ (0.5)
Female organ weights, 1 year[d]			
Adrenal gland	-	-	↑ (0.5)[e]
Fat pad, ovarian/parametrial	-	-	↓ (0.5)
Fat pad, retroperitoneal	-	-[f]	-
Heart	-	-	↑ (0.5)
Kidney	-	-	↑ (0.5)[e]
Liver	-	↑ (T)	↑ (0.5)[e]
Ovary	↓ (T)[g]	-	↓ (0.5)[e]
Pituitary gland	-	-	↑ (0.5)[e]
Spleen	-	-	-
Thymus	-	-	-
Thyroid gland	-	-	-
Uterus	-	-	-
Male organ weights, 1 year[d]			
Adrenal gland	-	-	-
Epididymis	-	-	-
Fat pad, epididymal	-	-	-
Fat pad, retroperitoneal	-	-	-
Heart	-	-	-
Kidney	-	-	-
Liver	↑ (T)	↓ (2.5)	-
Pituitary gland	-	-	-

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	Continuous-Dose EE_2
Spleen	-	-	-
Testes	-	-	-
Thymus	-	-	-
Thyroid gland	-	-	-
Female hematology and clinical chemistry, 1 year			
Hematocrit	-	-	-
Hemoglobin concentration	-	↑ (T)	-
Red blood cells	↑ (T)	-	-
% Reticulocytes	-	-	-
Packed cell volume	-	-	-
Mean corpuscular volume	-	-	-
Mean corpuscular hemoglobin	↓ (T)	-	-
Mean corpuscular hemoglobin concentration	-	↑ (25)	-
Platelets	-	↓ (25,000, T)	↓ (0.5)
White blood cells	-	-	-
Neutrophils	-	-	-
% Neutrophils	-	-	-
Lymphocytes	-	-	-
% Lymphocytes	-	-	-
Monocytes	-	↑ (T)	-
% Monocytes	-	-	-
Basophils	-	-	-
% Basophils	↑ (T)	-	-
Eosinophils	-	↓ (250)	↓ (0.5)
% Eosinophils	-	-	↓ (0.5)
Blood urea nitrogen (BUN)	-	-	-
Creatinine	-	-	-
Total protein	-	-	-
Albumin	(T)[h]	-	-
Alkaline phosphatase	-	↑ (250)	↑ (0.05)
Alanine aminotransferase	-	-	-
Aspartate aminotransferase	-	-	-
Sorbitol dehydrogenase	-	-	-
Gamma-glutamyl transferase	-	-	-
Total bile acids	-	-	-
Cholesterol	-	-	-
Glucose	-	-	-
Triglycerides	-	-	-
Insulin	-	-	-
Leptin	-	-	-
Triiodothyronine (T3)	-	-	-
Thyroxine (T4)	-	-	-
Thyroid-stimulating hormone (TSH)	-	-	↑ (0.5)
Male hematology, clinical chemistry, and sperm analyses, 1 year			
Hematocrit	-	↑ (T)	-
Hemoglobin concentration	-	↑ (25,000, T)	↑ (0.05)
Red blood cells	-	-	-
% Reticulocytes	-	-	-
Packed cell volume	-	↑ (T)	-
Mean corpuscular volume	-	↑ (T)	-

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	Continuous-Dose EE_2
Mean corpuscular hemoglobin	-	↑ (T)	-
Mean corpuscular hemoglobin concentration		-	-
Platelets		↓ (T)	
White blood cells	-	-	-
Neutrophils	-	-	-
% Neutrophils	↓ (T)	-	-
Lymphocytes	-	-	-
% Lymphocytes	-	-	-
Monocytes	-	-	-
% Monocytes	-	-	-
Basophils	-	-	-
% Basophils	-	-	-
Eosinophils	-	-	-
% Eosinophils	-	↓ (250)	-
Blood urea nitrogen (BUN)	-	-	-
Creatinine	-	-	-
Total protein	↓ (25)	-	-
Albumin	-	(T)[h]	-
Alkaline phosphatase	-	-	-
Alanine aminotransferase	-	-	-
Aspartate aminotransferase	-	-	-
Sorbitol dehydrogenase	-	-	-
Gamma-glutamyl transferase	-	-	-
Total bile acids	↓ (25, T)	↓ (T)	-
Cholesterol	-	-	-
Glucose	-	-	-
Triglycerides	-	-	↑ (0.5)
Insulin	-	-	↓ (0.05)
Leptin	-	-	-
Troponin T		↑ (T)	
T3	-	-	-
T4	↓ (T)	(T)[h]	-
TSH	-	-	-
Testicular spermatid head counts	-	-	-
Cauda sperm counts	-	-	-
Cauda sperm, % motility	-	-	-
Cauda sperm, abnormal	-	-	-
Females, neoplastic lesions, 1 year			
Uterus, stromal polyps	-	↑ (T)	-
Females, neoplastic lesions, 2 years			
Mammary gland, adenocarcinoma	↑ (2.5)	-	↑ (0.5, T)
Adrenal, medulla, pheochromocytoma, benign	-	-	↑ (T)
Thyroid gland, adenoma, C-cell	-	-	↑ (T)
Females, non-neoplastic lesions, 1 year[i, j]			
Mammary gland, dilatation, duct	-	-	↑ (0.5, T)
Mammary gland, hyperplasia, lobular	-	-	↑ (0.5, T)
Mammary gland, galactocele	-	-	↑(T)
Uterus, apoptosis	-	↑ (25,000, T)	↑ (0.5, T)

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	Continuous-Dose EE_2
Uterus, hyperplasia, cystic, endometrium	-	-	↑ (0.5, T)
Uterus, metaplasia, squamous	-	↑ (T)	↑ (0.5, T)
Ovary, atrophy	-	-	↑ (0.5, T)
Ovary, cyst, follicle	↑ (25,000, T)	-	↑ (0.5, T)
Ovary, cyst, bursa	-	-	↑(T)
Ovary, cyst, corpora lutea	-	↑ (T)	-
Ovary, depletion, corpora lutea	-	↑ (T)	↑ (0.5, T)
Ovary, hypertrophy, interstitial cell	-	↑ (T)	↑ (0.5, T)
Vagina, hyperplasia, epithelium	-	↑ (T)	↑ (0.5, T)
Vagina, mucification, epithelium	-	-	↑ (T)
Pituitary, angiectasis	-	-	↑ (T)
Heart, cardiomyopathy	-	-	↑ (0.5, T)
Kidney, mineralization	-	↑ (T)	-
Kidney, cyst, renal tubule	-	↑ (2.5)	↑ (0.05)
Kidney, nephropathy	-	-	↑ (0.5, T)
Liver, infiltration, mononuclear cells	↑ (2.5, 25,000)	-	-
Females, non-neoplastic lesions, 2 years[i, k]			
Mammary gland, dilatation, duct	-	-	↑ (0.5, T)
Mammary gland, dilatation, alveolus	-	-	↑ (0.5, T)
Mammary gland, galoctocele	-	-	↑ (T)
Ovary, cyst	-	-	↑ (T)
Ovary, cyst, bursa	-	-	↑ (T)
Uterus, dilatation, lumen	-	↑ (T)	-
Uterus, cyst, endometrium	-	-	↑ (T)
Uterus, hyperplasia, endometrium	-	-	↑ (0.05)
Uterus, metaplasia, squamous	-	-	↑(T)
Uterus, atrophy	-	-	↑ (0.5, T)
Vagina, hyperplasia, epithelium	-	↑ (25, 2,500, 25,000, T)	-
Vagina, degeneration, epithelium	-	↑ (2,500)	-
Pituitary, angiectasis	-	-	↑ (0.5, T)
Pituitary, hemorrhage	-	-	↑ (0.5, T)
Kidney, nephropathy	-	↑ (2.5)	↑ (T)
Kidney, cyst, renal tubule	↑ (2.5)	-	-
Kidney, cyst, cortex	-	-	↑ (0.05)
Liver, cystic degeneration	↑ (T)	-	-
Liver, basophilic focus	-	-	↑ (T)
Liver, vacuolization, cytoplasmic	-	-	↑ (0.05)
Thyroid, hyperplasia, follicular cells	-	-	↑ (0.05)
Thyroid, ultimobranchial cyst	↑ (250, 2,500)	-	↑ (T)
Thymus, atrophy	-	↑ (T)	-
Pancreas, hyperplasia, acinar cell	-	-	↑ (T)
Adrenal cortex, degeneration, cystic	-	-	↑ (0.5, T)
Adrenal cortex, pigmentation	-	-	↑ (T)
Bone marrow, hyperplasia, myeloid cell	-	-	↑ (T)
Spleen, pigmentation	-	-	↑ (0.5, T)
Brain stem, compression	-	-	↑ (0.5, T)
Brain stem, hemorrhage	-	-	↑ (0.5, T)

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	Continuous-Dose EE_2
Males, neoplastic lesions, 1 year			
None	-	-	-
Males, neoplastic lesions, 2 years			
Malignant lymphoma, systemic	↑ (T)[l]	-	-
Males, non-neoplastic lesions, 1 year[i, j]			
Epididymis, exfoliated germ cells	-	↑ (25,000, T)	-
Epididymis, infiltration cellular, lymphocyte	-	↑ (25,000, T)	-
Liver, hepatodiaphragmatic nodule	-	↑ (2,500)	-
Liver, infiltration, mononuclear cells	-	↑ (250, 2,500)	↑ (0.05)
Liver, fatty change	-	-	↑ (T)
Spleen, hematopoietic cell proliferation		↑ (T)	
Spleen, pigmentation	↑ (250, T)	-	-
Males, non-neoplastic, 2 years[i, k]			
Dorsal/lateral prostate, suppurative inflammation	-	↑ (2.5)	-
Mammary gland, dilatation, alveolus	-	↑ (2.5)	-
Kidney, hyperplasia, transitional epithelium	↑ (T)	↑ (25)	-
Kidney, cyst, renal tubule	↑ (T)	↑ (250, 2,500)	↑ (0.05)
Pituitary gland, hyperplasia, pars distalis	↑ (25,000, T)	↑ (25,000, T)	↑ (0.5, T)
Pituitary, cyst, pars distalis	↑(250)	↑ (T) (multilocular)	-
Thyroid gland, hyperplasia, C-cell	-	↑ (2,500, T)	↑ (0.05)
Parathyroid gland, hyperplasia	↑ (T)	↑ (25)	-
Pancreas, pigmentation	↑ (2.5)	-	-
Pancreas, polyarteritis	↑ (2,500, T)	-	-
Heart, cardiomyopathy	↑ (T)	-	-
Heart, metaplasia, osseus	-	-	↑ (T)
Adrenal medulla, hyperplasia	↑ (2,500, T)	-	-
Adrenal cortex, hypertrophy	-	-	↑ (0.05)
Testes, polyarteritis	↑ (2,500, T)	-	-
Bone marrow, hypocellularity	↑ (250, 25,000)	-	-
Spleen, hyperplasia, lymphoid	↑ (250, T)	-	-
Liver, angiectasis	-	↑ (2.5)	-
Liver, vacuolization, cytoplasmic	-	-	↑ (0.5, T)

[a]Statistically significant results are summarized without consideration of potential biological relevance, which is further discussed in the text. Results for the sensitivity analyses that excluded a subset of animals (see text) are not included in this summary table. NA, not applicable; ↑ or ↓, significant increase or decrease relative to controls at the exposure concentration (µg/kg bw/day) indicated in parentheses; "-" = no significant treatment effect; "T" = trend.

[b]For EE_2 dose groups, trend analyses were only conducted for the histopathology data.

[c]Endpoint was not analyzed since body weight at vaginal opening was not recorded for many pups.

[d]Results for organ weights adjusted for body weights are summarized in this table.

[e]Absolute organ weight and organ weight adjusted for brain weight were also significantly different from vehicle control.

[f]Absolute organ weight and organ weight adjusted for brain weight in the 2.5 µg BPA/kg bw/day dose group were significantly higher than the vehicle control.

[g]There were also significant dose trends for the absolute organ weight and organ weight adjusted for brain weight, and the 25,000 µg BPA/kg bw/day dose group was significantly lower than the vehicle control weight in both cases.

[h]Statistical analysis indicated a significant trend, but the nature of the trend is not evident from inspection of the data.

[i]For histopathology data, statistically significant effects in a negative, or potentially beneficial, direction relative to controls are not included in this summary table, but are presented in the Supplemental Appendices and, in some cases, in the body of the report. Some diagnoses included in the statistical tables (Supplemental Appendices XXXIII and XXXIV), such as stages of the

estrous cycle in females or changes noted that would not be considered of pathological significance (e.g., tension lipidosis), are not tabulated in this summary table.
[j]For interim non-neoplastic lesions, only Cochran-Armitage with Fisher's exact (CAFE) test results are summarized in this table. Results of additional statistical tests are presented and discussed in the text.
[k]For terminal non-neoplastic lesions, only Poly-3 test results are summarized in this table. Results of additional statistical tests are presented and discussed in the text.
[l]A significant trend for systemic lymphoma was noted in liver, bone marrow, spleen, kidney, and dorsal/lateral prostate. In addition, the incidence in the dorsal/lateral prostate at 25,000 µg BPA/kg bw/day was significantly higher than the incidence in vehicle control.

Background

Bisphenol A (BPA) is a high-production-volume industrial chemical that is used as a monomer in the production of polycarbonate plastics and epoxy resins that have broad applications in consumer products, including storage containers for foods and beverages and in medical devices. BPA has undergone extensive toxicological evaluations in laboratories around the world, but the conclusions derived from the aggregate results of the studies remain under debate. The current safety assessments by the preponderance of international regulatory agencies conclude that BPA at current exposure levels (upper 95th percentile of typical daily aggregate exposure <0.5 μg/kg body weight (bw)/day) does not pose a risk to humans via dietary exposure[1-4]. In contrast, others have concluded that the overall body of evidence from BPA investigations indicates that BPA is likely to be a human health hazard[5-7]. France has banned the use of BPA in food contact materials based on the assessment of the French Agency for Food, Environmental Health and Safety[8]. The Office of Environmental Health Hazard Assessment of the California Environmental Protection Agency has listed BPA as a reproductive toxicant under California's Proposition 65[9].

The National Toxicology Program (NTP) previously assessed BPA in two-year dietary administration studies in both sexes of F344 rats at feed concentrations of 1,000 and 2,000 ppm (approximately 50,000 and 100,000 μg BPA/kg bw/day), in male B6C3F$_1$ mice at feed concentrations of 1,000 and 5,000 ppm (approximately 150,000 and 750,000 μg BPA/kg bw/day), and in female B6C3F$_1$ mice at feed concentrations of 5,000 and 10,000 ppm (approximately 750,000 and 1,500,000 μg BPA/kg bw/day)[10]. The NTP conclusions from these studies were that there was no convincing evidence of carcinogenesis. The earlier NTP studies did not address the current issues of toxicities resulting from developmental exposures at levels closer to human exposure levels. Given the current public controversy, the NTP and the National Institute of Environmental Health Sciences (NIEHS) Division of Extramural Research and Training (DERT) agreed to sponsor a study that would involve exposures to a broad range of BPA doses, include developmental exposure, and, in addition to evaluating endpoints typically used for regulatory decision-making, provide animals or tissues from this study to a group of NIEHS-funded academic scientists to pursue hypothesis-driven studies in various organ systems. The purpose of this research program was to evaluate chronic exposure to BPA over a broad dose range using traditional and non-traditional endpoints. It aimed to determine if the non-traditional endpoints could reveal toxicity not detected by traditional guideline study endpoints and provide mechanistic support for observations made in the guideline study. For most of the hypothesis-driven studies, the academic scientists were provided by the Food and Drug Administration (FDA)'s National Center for Toxicological Research (NCTR) with tissues or serum from animals of various ages. The exceptions were behavioral and erectile dysfunction studies, where staff from the academic laboratories conducted the studies with animals at NCTR with the assistance of NCTR staff. Detailed descriptions of the general plan of this project, termed the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA), have been published[11; 12]. This report focuses only on the description of the conduct of and results from the core chronic study, which focused on standard toxicological endpoints.

Animal Model

The animal model used in these studies was the Sprague-Dawley rat maintained at NCTR. This colony had its origins in the late 1970s from Charles River Sprague-Dawley founders and has been used in toxicology studies with hormonally active agents for over a decade at NCTR[13-20]. A comprehensive evaluation of the pharmacokinetics of BPA in this rat strain across life stages, including upon oral exposure by gavage, has been conducted[21-23]. Dietary ethinyl estradiol (EE_2) exposure studies in this strain[15; 16; 18; 20] resulted in peak blood levels of EE_2 in adult animals below 10 pg/mL (30 pM) at the highest dose tested (50-ppb feed concentration, or approximately 4 and 5-6 μg/kg bw/day in males and females, respectively)[24]. Stimulation of male mammary hyperplasia was observed at 2, 10, and 50 ppb dietary EE_2 at postnatal day (PND) 140, but not in older animals (two years of age). Effects on vaginal opening, body weight gain, food efficiency, and estrous cyclicity were observed at the higher doses of 10 and 50 ppb, approximately 1 and 6 μg/kg bw/day, respectively. Similar, but not identical, effects were observed with dietary genistein over a dose range of 5 to 500 ppm (approximately 300 to 50,000 μg/kg bw/day)[18]. Taken together with the more recent gavage BPA and EE_2 studies[19; 25], these data demonstrate the relative sensitivity of this rat strain to estrogenic compounds.

Rationale for Dose Selection for BPA and the Reference Estrogen, EE_2

Prior to the design of this chronic toxicity study and the conception of the CLARITY-BPA project, a subchronic study was conducted at NCTR using the same animal model and dosing regimen used in the current chronic study[19]. BPA in 0.3% carboxymethylcellulose (CMC) was administered by oral gavage to pregnant female Sprague-Dawley rats from gestation day (GD) 6 through the start of parturition, with seven doses between 2.5 and 2,700 μg BPA/kg bw/day spaced at half-log intervals. Two high doses of BPA (100,000 and 300,000 μg/kg bw/day) were added as doses expected to produce adverse effects based on published guideline studies[26; 27]. Vehicle and naïve (not dosed by gavage) controls were included. Dams were not dosed after their litters were born, but pups were directly dosed by oral gavage from PND 1 until termination (PND 90 ± 5). Pups were directly dosed because of the demonstrated low transmission of BPA to pups via milk[28]. BPA showed clearly adverse effects in F_1 females at the highest doses of 100,000 and 300,000 μg BPA/kg bw/day. Statistically significant differences at lower BPA doses were few and sporadic and were judged not to provide evidence of BPA-induced toxicity[19]. There were no BPA effects observed in the males.

The design of the CLARITY-BPA core study was discussed and finalized at a series of meetings in late 2011 and early 2012. These meetings included NTP, NCTR, NIEHS/DERT, and FDA product center representatives, as well as NIEHS-funded CLARITY-BPA grantees. It was proposed that, because of the literature concerning permanent adverse effects resulting from developmental exposures to hormonally active agents, the study would include groups of animals for which exposure would terminate at weaning. For discussion of dose selection, a summary of data obtained from the NCTR BPA subchronic study[19] was presented and discussed. It was initially agreed that a vehicle control and six log-spaced doses between 2.5 and 250,000 μg BPA/kg bw/day would provide an adequate dose range from reasonably close to human exposure on the low end to a dose expected to produce clear adverse effects at the high end. Serum measurements of BPA across the postnatal life span of animals in the subchronic study[25] showed

that the high dose of 300,000 μg BPA/kg bw/day gave rise to approximately 50 μM active unconjugated BPA in PND 4 animals and approximately 1 μM in PND 80 animals, which were clearly out of the range of attainable human internal exposure from dietary sources, estimated to be in the low to sub-pM range[29]. There was general agreement that the current concern was restricted to a lower dose range, below the previously reported no-observed-adverse-effect level in guideline-compliant regulatory toxicity assays, which was 5,000 μg/kg bw/day[26; 27]. The 250,000 μg BPA/kg bw/day dose group would provide little additional information to influence regulatory action. A high dose of 25,000 μg BPA/kg bw/day was viewed as providing an adequate margin of human exposure, greater than 25,000-fold based on the aggregate human exposure estimates of <0.5 μg BPA/kg bw/day mentioned above. The low dose selected, 2.5 μg BPA/kg bw/day, provided a margin of exposure at least 10-fold higher than the maximum allowed background dietary exposure (see below).

Much of the research on BPA, particularly early in the investigations of the potential toxicity of low doses of BPA, focused on its estrogenic activity, although the involvement of mechanisms other than classical estrogen receptors have been increasingly implicated in BPA actions[30-33]. Both doses of EE_2 used in the NCTR BPA subchronic study (0.5 and 5 μg EE_2/kg bw/day) produced strong effects in female reproductive organs and on estrous cyclicity. In males, the low dose of 0.5 μg/kg bw/day had little effect, with an increase in male mammary hyperplasia as the only apparent effect at PND 90[19]. The 5 μg EE_2/kg bw/day dose also stimulated male mammary gland hyperplasia at PND 90, and increased hyperplasia in the coagulating gland, increased degeneration in the testicular germinal epithelium, and increased exfoliated germ cells and hypospermia in the epididymis. Based on the observed effects in the NCTR BPA subchronic study, two levels of EE_2, one of which was lower than the doses used in the subchronic study, were selected for use in the current study to expand information on the sensitivity of the animal model to EE_2. In the absence of data from this exposure model to guide the selection of the EE_2 lower dose, a 10-fold lower dose was chosen. The CLARITY-BPA consortium consensus for the two doses of EE_2 used in the current study was 0.05 and 0.5 μg/kg bw/day. Resource limitations did not allow for the inclusion of stop-dose EE_2 groups. Likewise, although the NCTR BPA subchronic study had included a naïve control group that was not dosed by gavage, this group could not be included in the chronic study. The responses of the naïve and vehicle control groups in the NCTR BPA subchronic study were similar[19].

Internal Dosimetry of BPA and EE_2

In the previous NCTR BPA subchronic study, blood levels of conjugated (biologically inactive) and unconjugated (biologically active) BPA (dose levels of 2.5, 8, 25, 80, 260, 840, 2,700, 100,000, and 300,000 μg/kg bw/day) and EE_2 (0.5 and 5.0 μg/kg bw/day) were measured. The previous study used identical conditions to the current chronic study, including the same animal model, feed, housing materials, dosing formulation, and dosing regimen. Blood was collected at approximate maximal concentration time (C_{max}, 15–30 minutes after dosing) at PND 4, 21, and 80[10]. For both BPA and EE_2, percentages of unconjugated compound were highest on PND 4 and decreased with age, reflective of increasing capacity for metabolism with age[17]. Although the percentage of unconjugated BPA at doses lower than 100,000 μg/kg bw/day were approximately 4% at PND 4 and 1–2% at PNDs 21 and 80, EE_2 was 100%, 50%, and 10% unconjugated on PNDs 4, 21, and 80, respectively. For BPA, there was evidence of saturation of metabolism at the higher doses, with ≥10% unconjugated at 100,000 and 300,000 μg/kg bw/day. Based on these

data and an earlier single-dose pharmacokinetic study in this rat strain[21], peak serum levels of unconjugated BPA across the 2.5–25,000 μg/kg bw/day dose span tested in the present study were anticipated to range from <0.5 nM to approximately 2.5–3 μM at PND 4 and from approximately 0.01 nM (below detectable limits) to approximately 30 nM after weaning. In the case of 0.5 μg EE_2/kg/day, equivalent to the high EE_2 dose of in this chronic study, peak measured serum levels were approximately 500 and 10 pM on PND 4 and 21, respectively, and below the limit of detection of 5 pM in adults (PND 80)[25]. Estimates of the potency of BPA relative to EE_2 from in vitro transcriptional and in vivo uterotrophic assays are in the range of 10^{-4}–10^{-5} (see, for example, Conley et al.[34]).

Materials and Methods

Procurement and Characterization of Bisphenol A

Bisphenol A (BPA, CAS #80-05-7, synonyms: 2,2-bis(4-hydroxyphenyl)propane; 4,4'-isopropylidenediphenol) was supplied by TCI America (Portland, OR; product #B0494) as lot #6052012, with a purity assessment on the Certificate of Analysis by Battelle, Inc. (Columbus, OH) of 99.9%. This lot of BPA (original lot designation AOHOK) was purchased by the NTP in 2009 and was characterized for identity and purity by proton nuclear magnetic resonance (NMR) and high performance liquid chromatography with photodiode array detection (HPLC-PDA) at NCTR prior to the start of the BPA subchronic study (NCTR E0217601). Battelle Laboratories extensively characterized this lot of BPA and reported the analysis to be consistent with the manufacturer's stated purity of 99.9% (Bulk Chemical Limited Analysis Report: Bisphenol A, Battelle Project No. G005430-DSU, October 29, 2010, which was included as part of the NCTR Technical Report for E0217601, July 15, 2013). For the present study, Battelle air milled this same lot of BPA prior to shipment to NCTR and provided a current statement of purity. Purity assessment was conducted at NCTR prior to the study start, at intervals during the study, and after study completion using the technique of HPLC-PDA (spectral purity). A sample of the test article was subjected to HPLC separation at least in triplicate and the PDA was scanned from 200 to 400 nm. A single major peak was obtained at the expected HPLC retention time for BPA and showed 99–100% purity (Supplemental Appendix VII).[a]

The reference estrogen test article ethinyl estradiol (EE_2, CAS #57-63-6, product #E4876, lot #071M1492V) was purchased from Sigma-Aldrich Corporation (St. Louis, MO). The stated purity of the EE_2 was >98%. A sample of this test article was evaluated by HPLC with PDA and electrospray mass spectrometry and found to contain a single peak that contained fragment ions consistent with and matching a reference sample of EE_2 from Steraloids, Inc. (Newport, RI). Analysis in the same manner as described for BPA showed purity >99% (Supplemental Appendix VII).

CMC (sodium salt; product #C5013, lot #041M0105V) was obtained from Sigma-Aldrich (St. Louis, MO). Aqueous CMC solutions were prepared for use as the vehicle for BPA and EE_2 by the Diet Preparation Support Group at NCTR as described in Supplemental Appendix IX.

Preparation and Analysis of Dose Formulations

Dosing solutions or suspensions were prepared by the Diet Preparation Support Group at NCTR (Supplemental Appendix IX). For BPA, the target concentrations of the dose preparations were 0.5, 5, 50, 500, and 5000 μg/mL 0.3% CMC for the 2.5, 25, 250, 2,500, and 25,000 μg/kg bw/day dose groups, respectively. For EE_2, the target concentrations were 0.01 and 0.1 μg/mL 0.3% CMC for the 0.05 and 0.5 μg/kg bw/day dose groups, respectively. The two high BPA dosing suspensions were mixed by directly adding BPA solid to the vehicle with sonication, and the suspensions were stirred constantly. The three low BPA and the two EE_2 dosing solutions were mixed by serial dilutions of stock solutions prepared and certified by the NCTR Chemistry Support Group. Stabilities (± 10% of the target) of the low and high dose levels of both BPA and

[a]Supplemental Appendices at: https://doi.org/10.22427/NTP-DATA-018-00015-0001-000-6.

EE_2 were established for up to 50 days. Homogeneity (± 10% of the target) of the high BPA suspension and both EE_2 dosing solutions was established (Supplemental Appendix VII).

At the start of the study and approximately every 8–10 weeks over the course of the study, all dose level preparations were assayed by the Chemistry Support Group prior to delivery to the animal rooms and certified to be within 10% of the target concentration with a % CV of ≤10%. In addition, at intervals spaced 4-7 months apart, BPA and EE_2 dosing preparations from the animal rooms were assayed at the end of their use to verify the dose concentrations. One batch of 0.3% CMC vehicle was found to have a detectable concentration of BPA (0.11 μg/mL) that was approximately 20% of the low BPA dose level (Table 13 in Supplemental Appendix VII). One container of this batch of vehicle was used to dose 16 cages (31 animals; 6 males and 6 females continuous-dose one-year sacrifice and 9 males and 10 females continuous-dose two-year sacrifice) of vehicle control animals ranging in age from PND 139 to PND 141 on a single day before this batch of vehicle was discarded (Supplemental Appendix III, note to study file October 13, 2017). Given the short half-live of BPA and age of the animals, the impact of this event was considered minimal. All affected animals were from the first mating group. Thus, these animals were excluded from the sensitivity analysis described in the Statistical Methods section below. Following the detection of this contaminated batch of vehicle, all batches of vehicle that had been previously used in the study and all subsequent batches of vehicle were assayed and none contained BPA detectable above the analytical background.

Administration of Dose Formulations and Vehicle

Doses were administered by gavage with modified Hamilton Microlab® ML511C programmable 115 V pumps (Hamilton Co., Reno, NV). Dosing containers were constantly stirred and dose volume calculation and dispensing were automated[35]. Four separate dosing stations were used in each animal room: (1) vehicle control; (2) 2.5, 25, and 250 μg BPA/kg bw/day; (3) 2,500 and 25,000 μg BPA/kg bw/day; and (4) 0.05 and 0.5 μg EE_2/kg bw/day. Dosing was conducted from the lowest to highest dose on any given pump and the Teflon tubing was flushed between dose levels. At the end of each day, each pump was flushed sequentially with water, 70% ethanol, and water. Verification of the accuracy of dose delivery from these pumps had been demonstrated for BPA dosing solutions ranging from 0.5 to 60,000 μg/mL 0.3% CMC and for 0.1 and 1 μg EE_2/mL 0.3% CMC for the prior NCTR BPA subchronic study (NCTR E0217601); accuracy was verified before the start of this study by chemical analysis for the low dose of EE_2, which was 10-fold lower than the lowest dose used in the previous experiment (Table 8C in Supplemental Appendix VII). Subsequently, the accuracy of liquid delivery from the pumps was assessed every three months and established to be within 10% of the target volume (Supplemental Appendix XI).

Diet Assessment: Nutrients and Contaminants, Including Background BPA

Pelleted rodent chow, verified casein diet 10 IF, irradiated, 5K96 (product #1810069, TestDiet, Purina Mills, Richmond, IN) was the diet used in the study. The manufacturer provided analyses for selected nutritive quality attributes (including protein, fat, crude fiber, calcium, phosphorous, and vitamins A, B_1, and E) and contaminants (including nitrosamines, fumonisins, arsenic, cadmium, lead, mercury, aflatoxins, organochlorine and organophosphate pesticides, butylated

hydroxyanisole, butylated hydroxytoluene, and tert-butyl hydroquinone). These analysis reports are found in Supplemental Appendix XII.

On arrival at NCTR, each lot of diet used in the present study was sampled and assayed by the Chemistry Support Group for background BPA and selected phyto- and myco-estrogens (genistein, daidzein, coumestrol, and zearalenone). These results are found in Supplemental Appendix VII. Low (ppb) background levels of BPA above the limit of blank (LOB) had previously been reported in all lots of 5K96 diet used in the prior NCTR BPA subchronic study and in other rodent diets[19; 36] and a rejection limit of 5 ppb BPA in feed was set for both that and the present study. Ten of the 11 lots of diet used in the present study contained detectable levels of BPA, with an average (using 0 for the lot with no BPA detectable above the LOB) of 1.3 ± 0.9 (standard deviation, S.D.) ng/g diet (range 0-3.0). Based on estimates of food consumption (Supplemental Appendix XIIIa and XIIIb) for interim and terminal sacrifice animals, respectively, this resulted in an average consumption over the course of the study of approximately 0.05-0.06 μg BPA/kg bw/day (2-2.5% of the lowest BPA dose tested) at the mean dietary concentration of BPA and approximately 0.12-0.15 μg BPA/kg bw/day (5–6% of the lowest BPA dose tested) at the maximum measured dietary concentration (calculations summarized in Supplemental Appendix XIV). Since younger animals consume higher quantities of food per unit of body weight, younger animals consumed higher background levels of dietary BPA. For example, estimates of food consumption for the week after weaning (week 4) indicate that the amount of BPA consumed per kg bw was approximately 2- to 3-fold higher than the mean value calculated over the entire study (Supplemental Appendix XIV).

The 5K96 diet, which is low in soy-derived phytoestrogens, was selected to ensure a consistent and low level of these phytoestrogens to minimize any impact on the endpoints measured in this study. A goal of 2 ppm total genistein and daidzein was stated in the protocol based on our prior experience with the isoflavone levels attainable in this diet. Although the diet manufacturer indicated that the levels of isoflavones were less than 10 ppm in this diet (page 6 of Supplemental Appendix IX), less than 2 ppm combined genistein and daidzein (measured after hydrolysis of glucosides in the diet) was typical in studies conducted with this diet at NCTR[13-16; 25;36]. Samples of each lot of diet were acid hydrolyzed to convert the glucosides of genistein and daidzein to aglycones that were quantified using HPLC-electrospray ionization-multiple reaction monitoring mass spectrometry. There was variation from lot to lot (Supplemental Appendix VII), but values of genistein and daidzein reported over the course of the study were consistent with the expectation of combined levels less than 2 ppm. After the study concluded and during the preparation of the Chemistry Support Report, however, it was discovered that a calculation error was made and that values of genistein and daidzein were 10-fold higher than those reported during the study. The values reported during the study are reflected in the Diet Preparation Report, Supplemental Appendix IX and the corrected values are found in the Chemistry Support Report, Supplemental Appendix VII. Mean values for genistein and daidzein in the 11 diet lots tested were 1.79 ± 1.94 (S.D.) and 1.66 ± 2.06 (S.D.) ppm, respectively. Although the values of isoflavones in the diet were higher than anticipated based on prior experience with this diet[13-16; 25; 36], the measured levels are low relative to other commonly used chow diets that have been reported to have values ranging from 100 to greater than 600 ppm[37-40]. The phytoestrogen coumestrol and the mycoestrogen zearalenone were also measured in the diets used in the present study. No rejection level was set for these compounds, although any lot of diet with greater than 0.5 ppm of coumestrol or zearelonone required approval prior to use. Two of the 11 diet lots

tested were positive for coumestrol (0.05 and 0.08 ppm) and two lots were positive for zearalenone (0.01 and 0.05 ppm) (Supplemental Appendix VII).

Assessment of Background BPA in Study Materials Other than Diet

In addition to the dosing vehicle and diet, the rodent drinking water and extracts of animal cages and bedding (hardwood chip and cellulose) were assayed for background BPA levels. These results are reported in Supplemental Appendix VII. For the polysulfone cages (Ancare Corporation, Bellmore, NY) used in this study, previous results from NCTR BPA subchronic study (NCTR E0217601) indicated no BPA detectable above the analytical background (NCTR Technical Report for E0217601, July 15, 2013). For the present study, three previously used polysulfone cages and three newly purchased cages were extracted and the extracts confirmed to have no detectable BPA above the analytical background. Similarly, all drinking water samples and bedding extracts had BPA levels that were less than or close to the analytical limits of the BPA assay.

Animal Source and Microbiological Surveillance

The NCTR Multigeneration Support System (MGSS), an operator-prompted database system, was used to track the genealogy of all animals in the current study and to collect in-life animal data. For the parental (F_0) generation, 600 male and 600 female weanling (circa PND 21) Sprague-Dawley/CD23/NctrBR rats were obtained from the NCTR breeding colony in five groups of 120 of each sex. While in the breeding colony, these F_0 breeders were maintained with their dams under the standard conditions used in the NCTR colony (fed NIH-41 irradiated pellets and housed in polycarbonate cages with hardwood chip bedding and water from polycarbonate bottles). Once assigned to the study at weaning on PND 21, the F_0 breeders were fed pelleted irradiated TestDiet 5K96 feed, housed two to three per cage in polysulfone cages with hardwood chip bedding, and given water from glass water bottles with silicone stoppers. The rats were held under these conditions until they were individually housed prior to mating for one to two weeks for females, or for 48 hours for males. Animals were 10–15 weeks of age at mating. The animal rooms, water, feed, and health of the animals were monitored during the study in accordance with NCTR's Sentinel Animal Program; the results are reported in Supplemental Appendix VIII. As noted in a protocol deviation (Supplemental Appendix II), one room was without a sentinel for approximately the last month of the study as the sentinel animal had to be removed as moribund. All 46 sentinel animals evaluated periodically over the course of the study were determined to be free of pathogenic organisms.

Animal Welfare

Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals (https://grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf). The study was conducted in an animal facility accredited by the Association for the Assessment and Accreditation of Laboratory Animal Care International. The study was approved by the NCTR Animal Care and Use Committee and conducted in accordance with all relevant NIH and NTP animal care and use policies and applicable federal, state, and local regulations and guidelines. NCTR Veterinary Services Staff monitored the health of the animals throughout the study and made recommendations as to the timing of animal removal from the study based on evident

distress or discomfort, decreased mobility, or inability to eat or drink. The Veterinary Staff also recommended the transfer of a subset of animals with pododermatitis or ventral masses from hardwood chip bedding that was irritating these lesions to Alpha-Dri cellulose bedding (Shepherd Specialty Papers, Watertown, TN; Supplemental Appendix II and Supplemental Appendix XV, Animals transferred to Alpha-Dri bedding).

Study Design

A sacrifice was conducted at one year of age (PND 365 ± 20) and the terminal sacrifice was conducted at two years of age (PND 730 ± 20). The interim (one-year) sacrifice group was included to allow evaluation of long-term exposure effects with less confounding due to background lesions of aging than would be expected at two years. Organ weights and clinical pathology (clinical chemistry and hematology) data were also collected from interim, but not terminal, sacrifice animals given the expected increased moribund removal later in the study and the level of variability in older animals due to age-related disease. A summary of the experimental design and data collected is found in Table 2**,** and a scheme is shown in Figure 1. The study protocol and all amendments are found in Supplemental Appendix I. Protocol deviations are included in Supplemental Appendix II. Many of these deviations document missing data collections or missed or under- or over-dosing of specific animals on specific days. Cases where missing data affected data analyses are noted in the data summary tables and in the statistical report appendices (Supplemental Appendices XVII-XXXI, XXXIII, and XXXIV).

An important component of this study, shown in Figure 1, was the provision of animals from the core study to academic investigators for studies funded by NIEHS as part of the CLARITY-BPA consortium[11; 12]. Results from these academic grantee-conducted studies are not reported here, but are or will be reported in the open literature. All the data collected by the academic grantees as part of the CLARITY-BPA-funded studies will be made available to the public via the NTP website (https://ntp.niehs.nih.gov) and in the NTP's Chemical Effects in Biological Systems (CEBS) database[b]. The allocation of animals to these studies did impact the allocation of animals to the study reported here, particularly regarding adjustment of the sex distribution for PND 1 culling and the number of animals assigned to the interim sacrifice.

Animal Maintenance, Breeding, Randomized Allocation to Study, and Dosing

Animals were housed in rooms with a 12-hour light cycle (lights on at 6 AM and off at 6 PM) and 10 room air changes per hour. For all animals, cages were changed twice weekly. Glass water bottles were changed weekly or as often as necessary to maintain a constant supply of drinking water. Throughout the study, cage racks were changed every two weeks and cage locations on those racks were rotated every two weeks. The study definition documents and startup memos that describe the cage and rack arrangements in the animal rooms are included as Supplemental Appendices IV and V. Animal rooms were maintained at 23 ± 3°C and 50 ± 20% humidity and were monitored so that corrective action could be taken if values went outside these limits. Summary temperature and humidity reports for all animal rooms are in

[b] https://doi.org/10.22427/NTP-DATA-018-00015-0001-000-6.

Supplemental Appendix X. In one case, a malfunction of a humidity sensor required movement of animals to another animal room for approximately 11 weeks (Supplemental Appendix II).

Approximately two weeks prior to mating, female breeders were randomized to treatment groups, stratified by body weight to produce approximately equivalent mean body weights in each group. The weight ranking and pairing information provided by the NCTR Statistical Support Group for each mating are in Supplemental Appendix VI. Male breeders were assigned to breeding pairs with the stipulation that no sibling or first cousin mating was permitted. Rats were mated at 10–14 weeks of age for females and 11–15 weeks of age for males. Females were placed in solid-bottomed polysulfone cages with hardwood chip bedding with the assigned males and were assessed daily for up to 10 days for sperm-positive vaginal smears or a copulation plug that precluded a vaginal smear. In three cases, males intended for mating with females in BPA or EE_2 dose groups died prior to mating and, in two of these cases, males from the vehicle control group that had already been mated with control females were re-mated with these females (Table 3). Dams were separated from the males on the day of sperm/plug detection, which was designated GD 0. If no sperm-positive vaginal smear or copulation plug was detected after 10 days, the pair was removed from the study and euthanized. Mating was conducted in five cohorts spaced four weeks apart. The number of pairs assigned to treatment groups in each mating group is shown in Table 3. While equivalent numbers of breeding pairs were assigned to the vehicle control and all BPA dose groups in the first three cohorts, adjustments were made in the fourth and fifth mating assignments based on the number of pups that had been allocated to the study from the earlier matings. The number of litters produced in each dose group in each mating and the number of litters contributing pups to the study are shown in Table 4 and Table 5, respectively. Mating was conducted prior to the start of dosing on GD 6, and thus breeding success was not an endpoint for analysis in this study.

Daily gavage dosing of the dams began on GD 6 (GD 0 = sperm- or plug-positive day) and continued until the initiation of parturition. Pups were not dosed on the day of birth (PND 0). Pups without evident malformations were randomly culled to a maximum of five males and five females on PND 1. While the post-culling sex ratio was generally balanced, the sex distribution was skewed toward males later in the study because the hypothesis-driven studies conducted by academic investigators required a culling strategy to provide more males than females. Litters with fewer than three pups/sex and live litters born to dams earlier than GD 20 were excluded from the core study, except in the cases of three females in the 25,000 μg/kg bw/day continuous BPA dose group that came from litters that did not have three pups/sex (Supplemental Appendix II). Direct gavage dosing of the pups started on PND 1 after the litter was culled. For animals younger than PND 5, the gavage needle was inserted to the opening of the esophagus, but did not enter the esophagus[41]. This dosing method for young pups had also been used in the BPA subchronic study conducted at NCTR, where serum levels were measured after dosing in PND 4 animals demonstrating the effectiveness of this dosing procedure[19; 25]. Pups were weighed and dosed daily until weaning at PND 21. After weaning, same-sex pups were housed two per cage and either dosed by gavage with vehicle, BPA, or EE_2 daily until termination (continuous-dose arm) or maintained without further dosing (stop-dose arm).

At weaning, up to a maximum of three pups/sex/litter were assigned to the chronic study. Same-sex littermates were not assigned to the same combination of study dose arm and time of sacrifice so that litter of origin was not a factor to be considered in the statistical analysis of endpoints collected after weaning. Twenty to 26 pups/sex/dose group were assigned to the one-

year interim continuous-dose assessment (Table 6); 19 to 22 pups/sex/dose group were assigned to the one-year interim stop-dose assessment (Table 7); 46 to 50 pups/sex/BPA dose group/dose arm and 26 pups/sex/EE_2 dose group were assigned to the two-year study continuous-dose assessment (Table 8); and 46 to 50 pups/sex/BPA dose group/dose arm were assigned to the two-year study stop-dose assessment (Table 9). The remaining pups from those litters with more than three same-sex pups were assigned to the hypothesis-driven studies of academic investigators. The reason that stop-dose EE_2 groups were not included in the study was solely an animal facility space and resource consideration, given the number of animals that needed to be provided and housed for both this study and the NIEHS-funded academic CLARITY-BPA grantee studies.

Animal Identification

Prior to mating, all F_0 females were identified with their unique cage number by tail tattoo (Animal Identification and Marking Systems, Inc., Hornell, NY). F_1 pups were initially numbered on their backs with an indelible marker after culling on PND 1 and then shortly thereafter with a standard 4-paw tattoo pattern. The paw tattoo pattern and dam identification number (cage number) provided unique identification for preweaning pups. Retained F_1 pups were marked by tail tattoo with their unique identification number (cage number and an additional digit to distinguish cage mates) after weaning on PND 21.

Data Collected in Interim and Terminal Sacrifice Animals

In-Life Data Collection

All activities conducted by animal care technicians in the animal rooms were monitored by the MGSS. Morbidity/mortality checks were performed twice daily and clinical observations were recorded weekly or when a significant clinical observation was noted. Starting at six months of age, animals were palpated weekly to detect the presence and progress of tissue masses. Body weights were obtained prior to dosing for dams from GD 6 through parturition and similarly for the pups from PND 1, as described above. Feed consumption was measured weekly from the start of dosing for approximately the next 13 weeks and monthly afterward. These data were not analyzed beyond summary statistics that were used to estimate consumption of background dietary BPA as a result of the low (<5 ppb) level of BPA in the feed (Supplemental Appendices XIII a and b, XIV). On the day of birth, PND 0, the number of pups alive and dead was recorded. On PND 1, the number of pups alive and dead, sex ratio, and live litter weight by sex were determined prior to culling. Individual body weights for all retained pups were recorded daily from PND 1 until weaning at PND 21. Animals in the continuous-dose arm were weighed daily through PND 90 ± 3 and weekly thereafter. Weights of animals in the stop-dose arm were recorded weekly after weaning.

All dams that were sperm- or plug-positive were euthanized after litters were weaned, on the litter's day of birth if the litter had less than three pups/sex, or on GD 26 if no litter was produced. The uterus was removed and stained with 10% ammonium sulfide for enumeration of implantation sites.

Females (26 animals from 13 randomly selected cages per dose group per dose arm from the two-year study arm) were monitored daily for vaginal opening from PND 22. Vaginal smears were collected for 14 consecutive days from these same animals beginning at 16 ± 2 weeks of

age. One month after these vaginal smears were completed, the same animals had vaginal smears collected for five consecutive days monthly until the animal did not show evidence of cycling, that is, that showed three or more consecutive days of estrus (including estrus, estrus/diestrus, or proestrus/estrus intermediate stages) or five consecutive days that did not include an estrus smear for two consecutive months.

Clinical Chemistry and Hematology, Interim Sacrifice Animals

For the one-year interim sacrifice, food, but not water, was removed from cages on the evening before the scheduled necropsy. Animals were anesthetized with gaseous carbon dioxide and blood was collected from the retro-orbital sinus. Hematology and clinical chemistry endpoints evaluated are listed in Table 2. All blood was collected into serum and ethylenediaminetetraacetic acid (EDTA) tubes between 7:00 AM and 12:00 PM. Blood in the serum tubes was allowed to clot and centrifuged at 1000 × *g* for 10 minutes at room temperature. The serum was removed and aliquoted into two tubes. One tube was used for immediate testing (see below) and the other was frozen at -60˚C until additional testing was performed. The EDTA tube was used for hematology testing performed the same day as collection.

Clinical chemistry analyses were conducted on an Alfa Wassermann ALERA (West Caldwell, NJ). Alfa Wassermann reagents were used to quantify glucose, alkaline phosphatase, alanine aminotransferase, aspartate aminotransferase, γ-glutamyl transpeptidase, total protein, albumin, cholesterol, triglycerides, blood urea nitrogen, and creatinine. Catachem (Bridgeport, CT) reagents were used to quantify sorbitol dehydrogenase and total bile acids. All testing was completed on the day of collection. The instrument was calibrated daily and two levels of assayed controls were included in daily analyses as internal controls.

Rat-specific troponin I, troponin T (Life Diagnostics, West Chester, PA), and leptin (Millipore, St. Charles, MO) were quantified using ELISA methods. The plates were read on an ELx800 Universal Microplate Reader (Bio-Tek, Winooski, VT). A standard curve was run with each batch of samples and results were calculated by the instrument's software. Insulin, triiodothyronine (T3), and thyroxine (T4) were quantified using Siemens (Los Angeles, CA) RIA "Coat-a-Count" method and thyroid-stimulating hormone (TSH) with rat-specific TSH radioimmune assay (Alpco, Salem, NH). The tubes were then counted on a Wizard2 gamma counter (PerkinElmer, Shelton, CT). A standard curve was run with each batch of samples and results were calculated by the instrument's software. Two levels of assayed controls were included in daily analyses as internal controls.

Complete blood counts were determined on an Pentra 60 C+ analyzer (ABX, Irvine, CA). Maintenance and calibration were done per the manufacturer's recommendations. Three levels of assayed controls were included in daily analyses as internal controls. Packed cell volume (PCV) analysis was performed by centrifugation in a CritSpin centrifuge (Beckman Coulter, Inc, Indianapolis, IN) and PCV determined by manual read. For determining the percentage of reticulocytes, 1,000 cells per animal were counted on slides prepared from blood collected in EDTA tubes and stained with New Methylene Blue Reticulocyte Stain (Volu-Sol, Salt Lake City, UT).

Sperm Evaluations, Interim Sacrifice Animals

For sperm motility assessment, the left epididymis was dissected from the testis and weighed. If gross lesions or abnormalities were noted at necropsy with either the left testis or epididymis, the right organ was used for motility studies and the left was sent for histology. The cauda section was dissected and immediately placed in a petri dish containing 40 mL of a solution consisting of 1% bovine serum albumin dissolved in phosphate buffered saline. The solution was prewarmed to a temperature of approximately 37°C. A minimum 2-minute period was allowed for the sperm to swim out. Following the swim-out period, a sperm sample was obtained using an 80 µm deep slide. The slide was immediately loaded into the prewarmed stage of the Hamilton Thorne IVOS automated sperm analyzer. Five fields were automatically selected by the analyzer, and each motion image was recorded and stored on an optical disk. The images were subsequently analyzed and the percent motility determined for each animal. Two eosin-stained slides were also prepared for each animal from the caudal epididymis suspension for evaluation of morphological development; a minimum of 200 sperm cells/animal was examined.

After the motility and morphology samples were collected, the cauda was minced with scissors and mixed. Approximately 1 mL of the suspension was placed in a prelabeled tube and frozen on dry ice for subsequent determination of the total caudal sperm count. The left testis was weighed, placed on dry ice, and stored frozen until evaluation for testicular spermatid head counts. Each frozen epididymis suspension and testis was thawed. The tunic was removed from the testis, and each testis was weighed and homogenized. The suspension was transferred to a vial containing a dye (IDENT, bis-benzimide trihydrochloride, Hamilton Thorne, Inc., Beverly, MA) that uniquely stains the head of sperm. A sample of the stained sperm was placed into a 20 µm deep glass slide that was loaded into the analyzer. Twenty fields were automatically selected by the instrument for each animal, and total sperm counts were determined. The counts were reported adjusted for testis weight (million sperm/g tissue).

Organ Weights and Histopathology

All animals maintained on study after weaning that survived to the scheduled sacrifice dates or were removed early as dead or moribund were subjected to a full necropsy. All gross lesions were processed for histological evaluation. Selected organs were collected and weighed at the interim sacrifice (Table 2). Weights of the dorsal/lateral and ventral prostate lobes were not collected, because the dissection and trimming procedure necessary to obtain these weights would interfere with the processing of the organ for histopathology (Supplementary Appendix II, Protocol Amendment #7). Organs were not weighed at the terminal sacrifice. Tissues not specified for microscopic evaluation, which are listed in Table 2, were processed to paraffin block and held for potential later evaluation. For tissues specified for evaluation by the study pathologist (Table 2), tissues from all dose groups were evaluated for both the interim and terminal sacrifice animals. Tissues were processed in accordance with NTP specifications (https://ntp.niehs.nih.gov/ntp/test_info/finalntp_reprospecsmay2011_508.pdf, https://ntp.niehs.nih.gov/ntp/test_info/finalntp_toxcarspecsjan2011.pdf), except that six step sections of each prostate were examined. The International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidelines (https://www.toxpath.org/inhand.asp) were used as diagnostic criteria for the microscopic evaluations. For the female reproductive tissues, mammary gland, and male reproductive tissues, the diagnostic criteria outlined in Dixon et al. (2014), Rudmann et al. (2012), and Creasy et al. (2012)[42-44], respectively, were used.

Approximately 9% of the animals in the study, represented in all dose groups, were observed to have seizures over the course of the study, mostly during handling procedures (e.g., dosing, cage changes). The brains, spinal cords, and peripheral nerves of these animals were examined by the study pathologist for any histological abnormalities (Supplemental Appendix XXXII). For the two-year sacrifice, animals were not fasted, and no clinical chemistry, hematology, organ weights, or sperm evaluations were conducted.

Upon completion of the microscopic evaluations, the data were entered into the NTP's Toxicology Data Management System Enterprise. Slides, paraffin blocks, and residual wet tissues were sent to the Block and Slide Laboratory for inventory, slide/block match, and wet tissue audit. Individual animal data records and pathology tables were evaluated by an independent quality assessment (QA) group, and QA pathologists evaluated selected histopathology slides. The reviewed slides, along with the diagnoses made by the study pathologist and QA pathologists, were reviewed by the Pathology Working Group (PWG). The QA pathologists served as coordinators of the PWG. Representative histopathology slides containing examples of lesions potentially related to chemical administration, examples of disagreements in diagnoses between the laboratory and QA pathologists, or lesions of general interest were presented by the coordinator to the PWG for review. While tissues from multiple organ systems were selected by the QA pathologists for review by the PWG to allow evaluation and confirmation of the broad spectrum of lesions observed in control and treatment groups, female and male reproductive tissues were emphasized in the review. The PWG consisted of the QA pathologists, the study pathologist, and other pathologists experienced in rodent toxicological pathology. This group examined the tissues with no knowledge of dose groups. When the PWG consensus differed from the opinion of the study pathologist, the diagnosis was changed. Final diagnoses for reviewed lesions represent a consensus of the PWG.

Statistical Methods

The full statistical analysis reports for all protocol-specified endpoints, including detailed methods and results for each analysis, including the omnibus tests, are found in Supplemental Appendices XVII-XXXI, XXXIII, and XXXIV. The pairwise comparisons to the vehicle control and trend tests are the comparisons of interest that are presented in the tables in this report. The statistical methodology for each endpoint is summarized below. Statistical comparisons were conducted within sex and, for data collected after weaning, within dosing arm (continuous-dose or stop-dose). For pairwise comparisons, the five BPA dose groups were compared to the vehicle control group. Similarly, the two EE_2 reference estrogen dose groups were compared to the vehicle control. Tests were conducted at the 0.05 significance level and, in most cases, were two-sided. Exceptions were one-sided tests for the pairwise comparisons of histopathology lesion incidence and severity to vehicle controls and trend tests for abnormal estrous cycles. Although a *p*-value of <0.05 was used to flag a result as significant, the actual *p*-values are included in some of the tables in this report and in all the statistical report appendices (Supplemental Appendices XVII-XXXI, XXXIII, and XXXIV) to aid in the further evaluation of the statistical and biological significance of each result. Trend tests for treatment effect (either increased or decreased relative to vehicle control) with increasing dose were conducted only for vehicle control and BPA treatment groups, except for non-neoplastic and neoplastic lesions, where trend tests were also conducted within the vehicle control and EE_2 groups. Because pups within litter

and sex were assigned at weaning to different dosing arms and sacrifice times, litter correlation was not a consideration for endpoints evaluated after weaning in this study.

Survival Analyses (Supplemental Appendices XX, XXI, and XXII)

Animals with a disposition of dead or moribund were treated as uncensored observations, while those reaching PND 21, one year, or two years were considered censored for the preweaning, interim, and terminal survival analyses, respectively. To compare survival of treatment groups to the control group, Cox proportional hazards regression analysis was performed. For the interim sacrifice survival analysis, several groups had 100% survival. For this situation, a modified Cox proportional hazards regression analysis was performed after adjusting the number of uncensored observations by adding one for each treatment group and sex to allow estimability. Multiple comparisons of treatments to the vehicle control group were adjusted using Holm's (step-down Bonferroni) method.

Body Weight Analyses (Supplemental Appendices XVII, XXIII, XXIV, and XXV)

Gestational weight at parturition was analyzed using analysis of covariance (ANOCOVA) with terms for treatment group, dam weight at baseline, and litter size as covariates, and the interaction between treatment and litter size. Data were collected at baseline on GD 0 or GD 1 prior to dosing and daily from GD 6 to parturition. Gestational weight at parturition was defined as the last dam body weight prior to delivery. For preweaning pup body weight data, the analysis was performed using contrasts within sex and PND. The experimental unit was the litter, and a stratified one-way repeated measures, mixed model analysis of variance (ANOVA) was used to test for treatment effect and to account for litter correlation assuming a compound symmetric correlation structure. The cross-sectional analysis was performed on selected PNDs (1, 4, 7, 14, and 21) so that the intra-litter correlation could be accounted for accurately.

For the interim sacrifice and terminal sacrifice postweaning analyses, there were no littermates among the males or females in any dose group within each dosing arm and sacrifice time, so intra-litter correlation was not considered. Body weight data collected from four to 52 weeks (interim sacrifice animals) or four to 104 weeks (terminal sacrifice animals) were analyzed using the last weekly observation for each animal, with PND 21 defined as the first day of week four. Although no formal comparisons were made between the continuous- and stop-dose study arms, for females and males in both the interim and terminal sacrifice phases, the week 4 body weights in the continuous-dose groups appeared to be higher than the week 4 body weights in the stop-dose groups (compare Table 24 and Table 25 with Table 26 and Table 27 for females; compare Table 28 and Table 29 with Table 30 and Table 31 for males). This apparent difference was an artifact of the experimental design and the statistical analysis. After weaning, the continuous-dose groups were weighed daily until PND 90, so that the last body weight of week 4 was PND 27. For the stop-dose groups, weekly body weights were taken after weaning, so the last body weight recorded in week 4 was generally earlier than PND 27. Outliers were identified by comparing observed body weight to predicted body weight using a five-point running median smoother and nearest neighbor interpolation. A threshold for outlier exclusion was set at a difference between observed and predicted weights greater than 35 g for both sexes in the interim analysis, and 60 and 65 g for females and males, respectively, in the terminal analysis. Lists of the outliers are found in Supplemental Appendices XXIV and XXV. Pairwise

comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA for females and males separately. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure that allows for correlated differences in variability across time points. Pairwise comparisons of each treatment mean to the vehicle control group mean were performed using contrasts with Dunnett's method of adjustment for multiple comparisons.

Implantation Sites and Litter Parameters (Supplemental Appendices XVIII and XIX)

Implantation site counts and litter weight data were analyzed using a one-way ANOVA and litter mean pup weights were analyzed using contrasts within an ANOCOVA, with litter size as a covariate, to test for treatment effect. Dunnett's test was used for comparisons to the vehicle control group to adjust for multiple comparisons. Sex ratios of pups within litters were analyzed for treatment effects using logistic regression. Pup counts (number alive, males, females, number unsexed (i.e., pups that could not be definitively assigned as male or female), and number born dead) were analyzed using Poisson regression. For analyses of sex proportions and female and male counts, unsexed pups were assigned either as male sex or female sex in separate runs with comparable results (Supplemental Appendix XIX).

Analyses of Vaginal Opening, Vaginal Cytology, and Onset of Aberrant Estrous Cycles (Supplemental Appendices XXVI-XXVIII)

Analyses of age and body weight at occurrence of vaginal opening were performed using contrasts within a one-way ANOVA to test for treatment effect. Comparisons of dosed groups to vehicle control for age and body weight were performed with Dunnett's method to adjust for multiple comparisons.

Summary statistics were calculated for proportions of days spent in estrus, diestrus, and proestrus for each animal and for estrous cycle length. Cycle length in days was defined from the first day of estrus in one sequence of contiguous days to the first day of estrus in the following sequence of stages. Cycles were considered censored if the last stage of data collection was either diestrus or proestrus.

Analyses were conducted on proportions of animals with abnormal cycles. The endpoints evaluated were any abnormal cycling, extended estrus, extended diestrus, and excessive proestrus. Extended estrus was defined as three or more consecutive days of estrus; extended diestrus was defined as four or more consecutive days of diestrus; and excessive proestrus was defined as two or more consecutive days of proestrus in a cycle. For abnormal cycling defined by animal, the Cochran-Armitage method for binomial proportions was used to evaluate the pairwise differences in proportions. The two-sided *p*-value for the Fisher's exact test is reported for comparisons of dosed groups to control, and the one-sided Cochran-Armitage trend test was performed. Holm's (step-down Bonferroni) method was used to adjust for multiple pairwise comparisons of dosed groups to control.

An accelerated failure time model assuming a lognormal distribution was used for onset of aberrant cycling, which was defined as occurring at first swab date of two consecutive months of aberrant estrous cycle data. An aberrant estrous cycle was defined as three or more consecutive

days of estrus or five consecutive days without estrus. The data for this endpoint contained left-, right-, and interval-censored data, which can all be accommodated by the accelerated failure time model used. Left censoring occurred because some animals had begun to show aberrant cycles prior to the start of monitoring, while right censoring occurred because some animals died or reached the end of the study without showing aberrant cycles. The intermittent nature of the data collection, one 5-day period every month, makes it impossible to determine the exact time of onset of aberrant cycles, so the data are interval censored. Multiple comparisons were adjusted using Holm's (step-down Bonferroni) method for treatment group comparisons to the control group.

Organ Weight Analyses (Supplemental Appendix XXX)

Statistical analyses were performed separately for the BPA study arms, stop-dose and continuous-dose, and for the EE_2 continuous-dose arm in one-year interim sacrifice rats. Weights of paired organs were analyzed as combined weight. ANOVA was performed for each sex and organ to determine the effect of treatment on organ weight. ANOCOVA was performed to determine the effect of treatment on organ weight adjusted for body weight at necropsy or brain weight. Separate analyses were performed with each covariate. Comparisons of dosed groups versus vehicle control were performed using Dunnett's method for adjusted contrasts. Tests of trend, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Organ weight exclusions based on the observation of gross cysts or consideration of statistical distributions are listed in Supplemental Appendix XXX.

Clinical Chemistry and Hematology Analyses (Supplemental Appendix XXIX)

A non-parametric ANOVA method based on mid-ranks was used to evaluate the effect of treatment on clinical chemistry and hematology assessments assuming an unstructured covariance[45]. The average of the left and right ranks was used for ties. Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trends over increasing BPA dose concentrations. Any measurements below the limit of detection (LOD) were evaluated at half the LOD level.

Sperm Parameter Analyses (Supplemental Appendix XXXI)

Analysis of sperm morphology data was performed using a generalized linear model with a Poisson distribution and a log link function. Each treatment was compared to the vehicle control group, and adjustment for multiple comparisons was performed using Dunnett's method. Percent sperm motility, testes sperm counts, and caudal sperm counts were analyzed using an ANOVA model with Kenward-Roger estimated degrees of freedom[46]. Each treatment was compared to the control group, and adjustment for multiple comparisons was performed using Dunnett's method. Tests of trends, increasing treatment effect with increasing dose, were performed for each compound and dosing arm.

Analyses of Non-Neoplastic and Neoplastic Lesions, Interim and Terminal Sacrifice (Supplemental Appendices XXXIII and XXXIV)

Microscopic findings were recorded in NTP's automated Toxicology Data Management System Enterprise. For the statistical analyses of groups with 20−26 animals (all interim sacrifice groups

and EE_2 terminal sacrifice groups), any lesion present in at least two animals in any dose group was included in the analyses. In groups with 46−50 animals (all terminal sacrifice vehicle control and BPA groups), any lesion present in at least four animals in any dose group was included in the analyses.

The NTP-preferred approach to assess neoplastic and non-neoplastic lesion prevalence is a survival-adjusted quantal-response procedure (see description of Poly-3 test below) that modifies the Cochran-Armitage linear trend test to take survival differences into account (https://ntp.niehs.nih.gov/testing/types/stats/index.html). For neoplasm and non-neoplasm incidence for interim sacrifice animals, where early removals or deaths were few, the Cochran-Armitage test, without survival adjustment, was used to test for a linear dose trend, with the Fisher's exact test used to compare dosed groups to the vehicle control. This combination of tests is referred to as CAFE. For neoplasm and non-neoplasm incidence for terminal sacrifice animals, the Poly-3 method of Bailer and Portier[47], as modified by Bieler and Williams[48], and the NIEHS continuity-correction, discussed in Peddada and Kissling[49], was used to analyze age-adjusted incidence for linear dose trend and for pairwise comparisons to the vehicle control. For both the analysis of interim and terminal neoplasm and non-neoplasm incidences, tests were one-sided for treatment comparisons to the vehicle control group with no adjustment for multiple comparisons to the vehicle control, while the trend test was two-sided.

Lesion severities, ordinal scores provided by the Study Pathologist, were available for many of the non-neoplastic lesions. Although these scores are more subjective than the lesion incidences, statistical tests were run to include this additional information with the incidence data. The Jonckheere-Terpstra test[50; 51] was run to test for monotonic dose trends, followed by Shirley's test[52; 53] to compare to controls. This combination of tests is referred to as JT/SW, and it presumes a monotonic dose response. One aspect of this study was to consider potential non-monotonic effects, which would be detected by the pairwise comparisons. JT/SW is blind to this effect. Therefore, a test not typically used in NTP studies, the non-parametric relative treatment effect (RTE) method[45] was used to test for non-monotonic dose effects. While the results of the JT/SW and RTE tests are presented in the summary tables and in the statistical appendices (Supplemental Appendices XXXIII and XXXIV), the CAFE and Poly-3 tests are the primary tests to be considered both because of the nature of the severity scores and, in the case of terminal sacrifice, because the JT/SW and RTE tests are not mortality adjusted.

A subset of lesions is discussed and tabulated in the body of this report. Comprehensive tabulations of all statistical results, including the JT/SW and RTE test results, for all lesions diagnosed in the study are found in Supplemental Appendices XXXIII and XXXIV.

Historical Control Data

The concurrent control group represents the most valid comparison to the treated groups and is the only control group analyzed statistically in NTP bioassays. Historical control data, however, are often helpful in interpreting potential treatment-related effects. Although these historical control data are generally most useful for uncommon or rare neoplasms, they can be useful for assessing the range of background lesions in the animal strain used as part of the weight-of-evidence consideration of whether an observed lesion is treatment related. For meaningful comparisons, the conditions for studies used for historical control comparisons generally should be similar. In the case of the present study, two two-year studies conducted with the Sprague-Dawley/CD23/Nctr BR rat using the same diet used in the present study have been conducted[14;

[16]. These were multigenerational studies in which animals in the F_1 and F_3 generations were evaluated at two years of age. Thus, each study had two sets of control animals of both sexes. These were dietary administration studies, rather than gavage studies, and were conducted outside the five-year period generally used for such comparisons, although drift in neoplasm incidence over time can vary among strain and neoplasm type[54; 55]. Historical incidences of neoplasms (mean percentages and ranges) from these studies are referenced in the Results and Discussion sections.

Sensitivity Analyses

In the present study, approximately 20% of the animals were housed for a short period early in the study in the same room as animals dosed with 250,000 μg BPA/kg bw/day exclusively for a CLARITY-BPA grantee study. These animals were potentially exposed to low levels of BPA, leading to blood levels of BPA metabolites above the LOD and similar to those resulting from the 2.5 μg BPA/kg bw/day dose[12] (Supplemental Appendix XVI). Animals co-housed only with the high dose from the two-year study (25,000 μg BPA/kg bw/day) had no detectable BPA metabolites in their blood[12]. As a conservative approach to determine if this low-level exposure to a subset of animals impacted the interpretation of study results, a sensitivity analysis was conducted for each endpoint. In the sensitivity analysis, all animals that for any portion of their lives were co-housed in the same room as the subset of animals treated with the 250,000 μg BPA/kg bw/day dose were excluded. Any significant effects found in the sensitivity analysis that were not found in the analysis that included all animals are noted in data tables and in the statistical appendices (Supplemental Appendices XVII-XXXI, XXXIII, and XXXIV). Only those animals involved in or resulting from the first mating were co-housed at any point in their lives with the animals dosed with 250,000 μg BPA/kg bw/day. Thus, the sensitivity analyses that were conducted after exclusion of all animals in the first mating removed all animals that potentially had exposure to BPA above that present in the diet. This included the subset of animals that had received a single dose of contaminated vehicle as adults (see above under "Preparation and Analysis of Dose Formulations" and Supplemental Appendix III, note to study file October 13, 2017).

Quality Assurance

This study was conducted in compliance with the Food and Drug Administration (FDA) Good Laboratory Practice (GLP) for the conduct of nonclinical laboratory studies (United States Code of Federal Regulations Title 21, Part 58). The Quality Assurance Unit at NCTR performed audits and inspections of the protocols, procedures, data, and reports throughout the course of the study. Separate audits covering completeness and accuracy of the pathology data, pathology specimens, and final pathology tables, and a draft of this technical report were conducted. Audit procedures and findings are on file at NCTR. The audit findings were reviewed and assessed by the NCTR staff, and all comments were resolved or otherwise addressed either before or during the preparation of the technical report. Raw data sheets from the study are archived by NCTR's Record Management Unit. Histopathology samples collected during the study are stored in the archives of Toxicologic Pathology Associates at NCTR. Backup computer data are maintained by the Computer Support Group at the NCTR. All records and samples are stored in accordance with FDA GLP Regulations.

Results

Gestational Body Weight, Fetal Implantation, and Litter Parameters

Dam body weights during pregnancy were not affected by BPA or EE_2 treatment (Table 10). The number of implantation sites in mated dams did not differ across BPA or EE_2 treatment groups and control groups as expected given that treatment did not begin until after implantation. There were no treatment effects on litter size, sex ratio, litter weight by sex, or mean pup weight at birth by sex (Table 11).

Survival, Preweaning and Postweaning Study Phases

Preweaning Survival

Females

The survival of female pups between PND 1 and PND 21 ranged from 91–95% in vehicle control and BPA dose groups (Table 12). There were no significant BPA treatment effects. The survival of female pups in the 0.05 and 0.5 μg EE_2/kg bw/day groups was 85% and 91%, respectively. Survival in the lower EE_2 group was significantly lower than that in the vehicle control group. The study protocol did not call for detailed evaluation of the cause of morbidity/death in pups removed from the study prior to weaning.

Males

The survival of male pups between PND 1 and PND 21 ranged from 91–95% in vehicle control and BPA dose groups (Table 13). There were no significant BPA treatment effects. The survival of male pups in the 0.05 and 0.5 μg EE_2/kg bw/day groups was 85% and 91%, respectively, and did not differ significantly from the vehicle control. The study protocol did not call for detailed evaluation of the cause of morbidity/death in pups removed from the study prior to weaning.

Postweaning Survival in Interim Sacrifice Animals

Females, Continuous-Dose Arm

The survival of females dosed daily with vehicle or BPA until the scheduled one-year sacrifice ranged from 91–100% and there were no treatment effects (Table 14). Over the same period, the survival of females in the low and high EE_2 dose groups was 92 and 100%, respectively, and did not differ from vehicle controls. Causes of morbidity/death in animals that did not survive to the interim sacrifice, when known, are noted in footnotes to Table 14.

Females, Stop-Dose Arm

In the stop-dose study arm, there was 100% survival in all dose groups of females scheduled for sacrifice at one year, except for the 25,000 μg BPA/kg bw/day dose group where survival was 91% (Table 15). There was no treatment effect. Causes of morbidity/death in animals that did not survive to the interim sacrifice, when known, are noted in footnotes to Table 15.

Males, Continuous-Dose Arm

Survival of males dosed daily with vehicle or various BPA doses until the scheduled one-year sacrifice ranged from 82–100%, with the lowest percent survival seen in the vehicle control group (Table 16). There were no significant treatment effects on survival after this one-year exposure. Over the same period, the survival of males in the low and high EE_2 dose groups was 85 and 88%, respectively, and did not differ significantly from vehicle controls. Causes of morbidity/death in animals that did not survive to the interim sacrifice, when known, are noted in footnotes to Table 16.

Males, Stop-Dose Arm

In the stop-dose study arm, there was 100% survival in males of all BPA dose groups sacrificed at one year, except for the 25 μg BPA/kg bw/day dose group where survival was 95% (Table 17). There was no treatment effect. The cause of death in the one animal in the 25 μg BPA/kg bw/day dose group that died early was uncertain (Supplemental Appendix XXXII, Subappendix VI).

Postweaning Survival in Terminal Sacrifice Animals

Females, Continuous-Dose Arm

The Kaplan-Meier survival curves for females dosed daily with the vehicle or BPA, or with the vehicle or EE_2, until sacrifice at two years are shown in Figure 2 and Figure 3, respectively. Data and analysis results are shown in Table 18. Survival at the end of the study in the BPA dose groups ranged from 17–40%. Survival in the low and high EE_2 dose groups was 27 and 15%, respectively. No significant treatment effects were seen for this chronic exposure to BPA or EE_2 at the doses administered. Most of the animals that did not survive until the terminal sacrifice were removed as moribund between one and two years of age. Causes of death/morbidity are listed in an appendix to the pathology report (Supplemental Appendix XXXII, Subappendix VI); mammary gland fibroadenomas and pituitary adenomas accounted for most of the early removals.

Females, Stop-Dose Arm

The Kaplan-Meier survival curves for females in the vehicle control or BPA two-year stop-dose arm are shown in Figure 4. Data and analysis results are shown in Table 19. Survival at the end of the study in the BPA dose groups ranged from 24-34%; survival in the vehicle control group was 22%. There were no significant treatment effects in female rats after only gestational and preweaning exposure to BPA. Most animals that did not survive until the terminal sacrifice were removed as moribund between one and two years of age. Causes of death/morbidity are listed in an appendix to the pathology report (Supplemental Appendix XXXII, Subappendix VI); as was the case in the two-year continuous-dose arm females, mammary gland fibroadenomas and pituitary adenomas accounted for most of the early removals.

Males, Continuous-Dose Arm

The Kaplan-Meier survival curves for males dosed daily with vehicle or BPA, or with vehicle or EE_2, until sacrifice at two years are shown in Figure 5 and Figure 6, respectively. Data and analysis results are shown in Table 20. Survival at the end of the study in the BPA dose groups ranged from 24–35%. Survival in the low and high EE_2 dose groups was 35 and 46%, respectively. There were no significant treatment effects. Most animals that did not survive until the terminal sacrifice were removed as moribund between one and two years of age. Causes of

death/morbidity are listed in an appendix to the pathology report (Supplemental Appendix XXXII, Subappendix VI). Many primary and contributory conditions in various organs were diagnosed, with pituitary adenomas, nephropathy, preputial gland carcinoma, and malignant lymphoma indicated as primary causes of death/morbidity in multiple animals across all dose groups.

Males, Stop-Dose Arm

The Kaplan-Meier survival curves for males in the vehicle control or BPA two-year stop-dose arm are shown in Figure 7. Data and analysis results are shown in Table 21. Survival at the end of the study in the BPA dose groups ranged from 20–33% in comparison to the 34% survival seen in the vehicle controls. There were no significant treatment effects. Most animals that did not survive until the terminal sacrifice were removed as moribund between one and two years of age. Causes of death/morbidity are listed in an appendix to the pathology report (Supplemental Appendix XXXII, Subappendix VI) and were similar to those diagnosed for the continuous-dose arm males.

Body Weights

Body Weights in Preweaning Animals

Females

Body weights on PNDs 1, 4, 7, 14, and 21 in female pups treated daily with vehicle, BPA, or EE_2 are shown in Table 22. There were no BPA treatment-related effects on female pup body weights at these time points. On PNDs 4 and 7, the low EE_2 dose group females had significantly lower mean body weight than vehicle controls, with body weights in the treated animals approximately 5% lower than controls on both days. At PND 4, both the 2.5 μg BPA/kg bw/day and the 0.05 μg EE_2/bw/day dose groups had identical means and standard errors, but only the latter was statistically significant, suggesting that the smaller sample size in that group contributed to this marginal difference.

Males

Body weights on PNDs 1, 4, 7, 14, and 21 in male pups treated daily with vehicle, BPA, or EE_2 are shown in Table 23. There were no BPA or EE_2 treatment-related effects on male pup body weights at these time points.

Body Weights in Interim and Terminal Sacrifice Animals

Females, Continuous-Dose Arm

Body weights of females in the continuous vehicle control and BPA groups scheduled for the interim sacrifice are shown in Figure 8 and Table 24. The data for the EE_2 groups are also tabulated in Table 24 and are shown graphically in Figure 9. There were no significant differences from the vehicle controls in any of the BPA or EE_2 dose groups. The mean body weights in the 2.5 μg BPA/kg bw/day group in weeks 36–52 were 10–13% higher than vehicle control means; however, these differences were not statistically significant.

Body weights of females in the continuous vehicle control, BPA, and EE_2 dose groups scheduled for the terminal sacrifice are shown in Table 25 and in Figure 10 and Figure 11 for BPA and EE_2,

respectively. Mean body weights of females in the 250 μg BPA/kg bw/day dose group were significantly higher by 16−18% than those of the vehicle control group for weeks 96–104. In this same period, the mean body weights of females in the 2.5 and 25 μg BPA/kg bw/day dose groups were 11–16% higher than those of vehicle controls, but these differences were not significant. Animals in the 2.5 μg BPA/kg bw/day dose group did not have higher mean body weights than vehicle controls in the earlier weeks noted above for interim sacrifice animals. In the terminal sacrifice high EE_2 dose group, transiently higher mean body weights were observed at 4 and 8 weeks (7% and 8%, respectively). The same tendency in the high EE_2 dose group was seen in the interim sacrifice animals at 4 and 8 weeks, although the differences in mean body weights were not statistically significant.

Females, Stop-Dose Arm

Body weights of females in the vehicle control and BPA stop-dose groups scheduled for the interim sacrifice are shown in Table 26 and Figure 12. There were no significant treatment effects. In the stop-dose females scheduled for terminal sacrifice (Table 27 and Figure 13), there was a significant decreasing trend at week 4, but no other treatment effects. Although statistical comparisons were not conducted between continuous- and stop-dose arms, the stop-dose arm females had higher mean weights over the course of the study (vehicle controls, mean stop-dose compared to continuous-dose: interim, 104%, range 98–109% from weeks 8–52; terminal, 107%, range 97–114% from weeks 8–104).

Males, Continuous-Dose Arm

Body weights of the continuously dosed males in the vehicle control, BPA, and EE_2 groups for interim and terminal sacrifices are shown in Table 28 and Table 29, respectively. The growth curves for interim BPA and EE_2 dose groups are shown in Figure 14 and Figure 15, respectively, and the growth curves for terminal BPA and EE_2 dose groups are shown in Figure 16 and Figure 17, respectively. There were no significant treatment effects for either compound, although means were 10–16% higher than vehicle control means in the 250 μg BPA/kg bw/day dose group from weeks 92 through 104.

Males, Stop-Dose Arm

Mean body weights of the stop-dose arm vehicle control and BPA groups of male rats for interim and terminal sacrifices are shown in Table 30 and Table 31 and graphically depicted in Figure 18 and Figure 19, respectively. The sole statistically significant treatment effect was a decreasing dose trend at week 4 in the terminal sacrifice animals (Table 31). Although statistical comparisons were not conducted between continuous- and stop-dose arms, the stop-dose arm males had higher mean weights over the course of the study (vehicle controls, mean stop-dose compared to continuous-dose: interim, 102%, range 92–105% from weeks 8–52; terminal, 107%, range 92–113% from weeks 8–104).

Vaginal Opening

Female pups were evaluated for vaginal opening starting on PND 22. Mean age and body weight at vaginal opening for continuous-dose vehicle control, BPA, and EE_2 dose groups are shown in Table 32. There were no treatment-related effects on these endpoints for any dose of either compound. For stop-dose vehicle control and BPA groups, mean age at vaginal opening is shown in Table 33. No treatment effects were observed in the stop-dose BPA groups. Body weight at

vaginal opening could not be analyzed for the stop-dose animals because weight at vaginal opening was not recorded for many of the animals due to a technical error (Supplemental Appendix II, deviations 72–74). While no formal analysis was conducted comparing vehicle controls in the continuous- and stop-dose arms, the mean vaginal opening date appears to be later regardless of BPA treatment in the stop-dose groups.

Vaginal Cytology – Estrous Cycle Analysis at Approximately 16 Weeks of Age

Continuous-Dose Arm

Data and results of analysis of the 14 consecutive daily vaginal smears collected from animals in the continuous-dose arm at 16 ± 2 weeks of age are summarized in Table 34. The individual animal data are found in the statistical report in Supplemental Appendix XXVII. There were no significant differences from the vehicle control among the continuous BPA dose groups. The high EE_2 dose had a highly significant effect on the estrous cycle, with 96% of the animals showing extended estrus as compared to 12% of the vehicle controls (Table 34). When all types of abnormal cycles were considered, 100% of the high EE_2 dose group animals showed abnormal cycles compared to 27% of the vehicle controls.

Stop-Dose Arm

There were no BPA treatment-related effects on the estrous cycle in the stop-dose animals (Table 35). The individual animal data are found in the statistical report in Supplemental Appendix XXVII.

Vaginal Cytology – Onset of Aberrant Estrous Cycles in Aging Animals

Continuous-Dose Arm

The time of onset of aberrant estrous cycles in aging females was estimated by evaluating five consecutive vaginal smears every month (see Materials and Methods and legend to Table 36). The data for the animals in the continuous-dose arm are summarized in Table 36, and the complete statistical report is found in Supplemental Appendix XXVIII. The Kaplan-Meier survival curves related to the onset of aberrant cycles for the continuous vehicle control and BPA and vehicle control and EE_2 groups are shown in Figure 20 and Figure 21, respectively. There was no treatment effect of BPA in the continuous-dose arm. None of the dose groups differed significantly in the median onset time of 56.8 weeks in the vehicle control group. As expected based on the previously mentioned analysis of estrous cycle data at 16 weeks, the onset of aberrant cycles occurred significantly earlier in the 0.5 μg EE_2/kg bw/day dose group.

Stop-Dose Arm

The time of onset of aberrant estrous cycles in the stop-dose BPA females is shown in Table 37. The Kaplan-Meier survival curves related to the onset of aberrant cycles for the stop-dose vehicle control and BPA groups are shown in Figure 22, and the complete statistical report is found in Supplemental Appendix XXVIII. The sole significant effect was a delay in the median

time of onset in the 2,500 μg BPA/kg bw/day dose group (57 weeks versus 42 weeks in vehicle controls). While no formal analysis was conducted to compare the continuous-dose vehicle control group with the stop-dose vehicle control group, the estimated median time of onset of aberrant cycling appeared shorter in the stop-dose vehicle control group.

Hematology Endpoints in Interim Sacrifice Animals

Females, Continuous-Dose Arm

Hematology endpoints examined at the interim sacrifice in females dosed continuously with BPA or EE_2 are shown in Table 38. Platelet counts were significantly lower (~10%) than vehicle controls in the 25,000 μg BPA/kg bw/day dose group. Eosinophils were decreased (~25%) in the 250 μg BPA/kg bw/day dose group relative to the vehicle control group, and mean corpuscular hemoglobin concentration was significantly higher (~1.4%) than vehicle controls in the 25 μg BPA/kg bw/day dose group. There were significant trends over increasing levels of BPA dose concentrations for hemoglobin concentration ($p = 0.023$), monocytes ($p = 0.045$), and platelet counts ($p = 0.008$). In female rats continuously dosed with 0.5 μg EE_2/kg bw/day, lower eosinophil counts (~25%), % eosinophils (~ 21%), and platelet counts (~8%) were observed.

Females, Stop-Dose Arm

Hematology endpoints examined at the interim sacrifice in stop-dose BPA female rats are shown in Table 39. No statistically significant differences in values were observed in pairwise comparisons between BPA dose groups and vehicle controls. Significant trends were noted for % basophils (p = 0.031), mean corpuscular hemoglobin ($p = 0.013$), and red blood cells ($p = 0.044$).

Males, Continuous-Dose Arm

Hematology endpoints examined at the interim sacrifice in males dosed continuously with BPA or EE_2 are shown in Table 40. Hemoglobin levels were significantly higher (~4%) in the 25,000 μg BPA/kg bw/day group and the percentage of eosinophils lower (~28%) in the 250 μg BPA/kg bw/day group relative to vehicle controls. Significant trends were observed for hematocrit ($p = 0.006$), hemoglobin concentration ($p = 0.016$), PCV ($p = 0.008$), mean corpuscular hemoglobin ($p = 0.018$), mean corpuscular hemoglobin volume ($p = 0.016$), and platelet counts ($p = 0.011$). The sole observed statistically significant effect in the EE_2 groups was an elevated hemoglobin concentration (~3%) relative to the vehicle control level in the 0.05 μg EE_2/kg bw/day group.

Males, Stop-Dose Arm

Hematology endpoints examined at the interim sacrifice in stop-dose BPA male rats are shown in Table 41. No statistically significant effects were observed in pairwise comparisons between BPA dose groups and vehicle controls. A significant trend for % neutrophils ($p = 0.045$) was noted over the levels of BPA dose concentrations in the stop-dose arm.

Serum Clinical Chemistry Endpoints in Interim Sacrifice Animals

Females, Continuous-Dose Arm

Clinical chemistry endpoints examined at the interim sacrifice in females dosed continuously with BPA or EE_2 are shown in Table 42. Alkaline phosphatase levels were significantly higher (~31%) in the 250 μg BPA/kg bw/day group than levels in the vehicle control group. Although mean levels of alkaline phosphatase were higher than controls in most of the BPA groups, none of the others were statistically significant. There were no other statistically significant treatment effects on clinical chemistry endpoints in any continuous BPA dose group. The female rats in the continuous 0.5 μg EE_2/kg bw/day dose group had higher (~38%) mean levels of TSH, while rats in the 0.05 μg EE_2/kg bw/day dose group had higher (~24%) mean levels of alkaline phosphatase than the vehicle control group. There were no EE_2 treatment effects on T3 or T4.

Females, Stop-Dose Arm

Clinical chemistry endpoints examined at the interim sacrifice in stop-dose BPA female rats are shown in Table 43. No statistically significant differences were noted in pairwise comparisons between stop-dose BPA-treated female rats and the vehicle controls. There was a significant trend over levels of BPA dose concentrations for albumin ($p = 0.004$).

Males, Continuous-Dose Arm

Clinical chemistry endpoints examined at the interim sacrifice in males dosed continuously with BPA or EE_2 are shown in Table 44. No statistically significant differences were noted in pairwise comparisons between any BPA treatment group and vehicle controls. Significant trends were noted for albumin ($p = 0.007$), T4 ($p = 0.002$), total bile acids ($p = 0.026$), and troponin T ($p = 0.003$). For EE_2, mean insulin levels were significantly lower (~35%, $p = 0.047$) in the 0.05 μg EE_2/kg bw/day dose group and mean triglyceride levels were significantly higher (~26%) in the 0.5 μg EE_2/kg bw/day dose group than those in vehicle controls.

Males, Stop-Dose Arm

Clinical chemistry endpoints examined at the interim sacrifice in stop-dose BPA male rats are shown in Table 45. In the 25 μg BPA/kg bw/day dose group, decreases in the mean levels of total protein (~4%) and total bile acids (~31%) relative to vehicle controls were observed. Significant trends were noted over the levels of BPA dose concentrations for T4 ($p = 0.046$) and for total bile acids ($p = 0.024$).

Organ Weights in Interim Sacrifice Animals

Females, Continuous-Dose Arm

Summary statistics for organ weights collected from females in the continuous-dose BPA and EE_2 dose groups are shown in Table 46. Organ weights were analyzed as absolute weights, and relative weights with brain and body weight at necropsy as covariates. There were few sporadic significant differences between BPA groups and the vehicle control group. In the 2.5 μg BPA/kg bw/day dose group, the mean absolute retroperitoneal fat pad weight was significantly higher (40%) than the mean weight in the vehicle control group. The brain weight-adjusted

retroperitoneal fat pad weight was similarly significantly greater than the vehicle control. The mean retroperitoneal fat pad weight adjusted for body weight was higher (23%) than the vehicle control group, but this was not a statistically significant difference. The only other significant BPA treatment effect was a dose trend for liver adjusted for body weight.

Multiple organ weights were significantly affected by the 0.5 μg EE_2/kg bw/day treatment. Mean absolute adrenal weights, as well as adrenal weights adjusted for brain and body weights, were increased by 27%, 28%, and 22%, respectively. Mean ovarian/parametrial fat pad weight was decreased, with mean weight adjusted for body weight significantly decreased (~17%) relative to the vehicle control group mean. Mean heart weight was also increased in the high EE_2 dose group, with mean heart weight adjusted for body weight significantly increased (~6%) relative to the vehicle control group mean. Mean absolute kidney weights, as well as kidney weights adjusted for brain and body weights, were increased relative to the vehicle control mean by 15%, 16%, and 13%, respectively. Mean absolute liver weights, as well as liver weights adjusted for brain and body weights, were increased relative to the vehicle control mean by 20%, 20%, and 18%, respectively. Mean absolute ovary weights, as well as ovary weights adjusted for brain and body weights, were decreased relative to the vehicle control mean by 18%, 16%, and 15%, respectively. Mean absolute pituitary weights, as well as pituitary weights adjusted for brain and body weights, were increased by 31%, 32%, and 20%, respectively.

Females, Stop-Dose Arm

Summary statistics for organ weights collected from females in the BPA stop-dose groups are shown in Table 47. There were significant dose trends for absolute ovary weight and ovary weight adjusted for brain and body weight. The mean absolute ovary weight, as well as ovary weight adjusted for brain weight, in the 25,000 μg BPA/kg bw/day dose group were significantly lower than the vehicle control group by approximately 13% and 12%, respectively. Mean ovary weight adjusted for body weight was also 9% lower than the controls, but this difference was not statistically different.

Males, Continuous-Dose Arm

Summary statistics for organ weights collected from males in the continuous-dose BPA and EE_2 dose groups are shown in Table 48. The sole significant BPA treatment effect was a lower (~7%) mean liver weight adjusted for body weight relative to the vehicle control in the 2.5 μg BPA/kg bw/day dose group. There were no significant treatment effects of either EE_2 dose.

Males, Stop-Dose Arm

Summary statistics for organ weights collected from males in the BPA stop-dose groups are shown in Table 49. The sole significant treatment effect was a significant dose trend for liver weight adjusted for body weight.

Sperm Analysis, Interim Sacrifice Animals

Testicular spermatid head counts, caudal sperm counts, and caudal sperm motility and morphology data are shown in Table 50 for continuous BPA and EE_2 dose groups and in Table 51 for BPA stop-dose groups. There were no significant treatment effects observed for either compound.

Histopathology

The pathology report, which has tabulations of organs assessed and all lesions noted in all animals in both the interim (one-year) and terminal (two-year) phases of the study, along with the Study Pathologist's narrative report, is found in Supplemental Appendix XXXII. Neoplastic lesions that showed any statistically significant increased incidence for either BPA or the reference estrogen, EE_2, are discussed below. As noted in the pathology narrative, many non-neoplastic lesions common to aging animals in this strain of rat were found in both sexes at one and two years of age. Incidences were highly variable across dose groups and BPA treatment effects were not evident to the Study Pathologist. After the finalization of the pathology report, statistical analyses, described in Materials and Methods, were conducted for any lesion that had an incidence of two or more in the interim sacrifice animals or in the EE_2 terminal sacrifice animals and four or more in the BPA-treated terminal sacrifice animals. All statistical results are shown in Supplemental Appendix XXXIII for interim sacrifice animals and Supplemental Appendix XXXIV for terminal sacrifice animals and selected lesions with statistically significant increased incidences in BPA or EE_2 dose groups are discussed below. Lesions for which there was significantly lower incidence in treatment groups relative to vehicle controls are generally not discussed, although a few of these cases are presented. For statistical analyses of microscopic lesions, one-sided *p*-values with no correction for multiple comparisons are reported for pairwise comparisons with the vehicle control, while two-sided results are reported for trends. In the text, the unadjusted incidence (i.e., lesions observed/animals examined) is reported, while in the tables both the unadjusted and survival-adjusted (Poly-3) incidences are shown for terminal sacrifice animals.

Females

Neoplastic lesions showing statistically significant differences in the BPA dose groups versus the vehicle control group were limited to the female mammary gland and uterus. For EE_2, there were significant trends and significant differences between the high dose group and vehicle control for adenocarcinomas in the mammary gland and combined adenoma/carcinoma in the pituitary pars distalis. Significant dose trends were also noted in terminal sacrifice animals for benign pheochromocytoma in the adrenal medulla and C-cell adenomas in the thyroid gland with incidences in the high dose EE_2 group of 8% versus 0% in controls for both cases (Supplemental Appendix XXXII and XXXIV).

Mammary gland, neoplastic lesions

Neoplastic lesions in the mammary glands of interim and terminal sacrifice females for continuous BPA, continuous EE_2 and stop-dose BPA treatments are shown in Table 52, Table 53, and Table 54, respectively. Fibroadenomas are a common high-incidence lesion in this strain of rats, with a historical control incidence at two years of 132/210 (63%, range 59–69%). Fibroadenomas were observed in 4–25% of interim sacrifice females and 54–90% of terminal sacrifice animals with no significant treatment effects. Fibroadenoma counts in each affected animal were recorded and are found in an appendix to the pathology report (Supplemental Appendix XXXII, Subappendix VII).

Adenocarcinomas and/or adenomas were observed in the continuous-dose BPA-treated animals (Table 52). In interim sacrifice animals, there were no treatment-related increases in neoplasm incidence, but both the 2.5 and 25 μg BPA/kg bw/day continuous-dose groups had single

adenocarcinomas in 22 animals examined (4% incidence). In the terminal sacrifice continuous-dose vehicle control animals, 8% had adenocarcinoma and 12% had adenoma or adenocarcinoma. In the continuous BPA dose groups, the incidence of adenocarcinoma varied between 6 and 18%, and the incidence of adenocarcinoma or adenoma varied between 9 and 20%. None of these incidences were significant compared to the vehicle control group. The historical control incidence of combined adenoma/adenocarcinomas was 32/210 (15.2%, range 12–17%).

In interim sacrifice animals, 2 of 26 animals (8% incidence) from the 0.05 μg EE_2/kg bw/day dose group had adenocarcinomas (Table 53). For the terminal sacrifice EE_2 treatment groups, there was a significant dose trend ($p < 0.001$) and a significant increase in adenocarcinomas in the 0.5 μg EE_2/kg bw/day dose group (38% versus 8%, $p < 0.001$).

Adenocarcinomas and/or adenomas were also observed in the terminal stop-dose females (Table 54). In the terminal sacrifice stop-dose females, vehicle controls had 6% animals with adenocarcinomas and 8% with adenomas or adenocarcinomas. The 2.5 μg BPA/kg bw/day stop-dose group had a significantly higher incidence of adenocarcinomas (22% versus 6%, $p = 0.016$) or adenomas and adenocarcinomas combined (24% versus 8%, $p = 0.018$).

Mammary gland, non-neoplastic lesions

Non-neoplastic lesions in the mammary glands of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA treatments are shown in Table 55, Table 56, and Table 57, respectively.

In the continuous-dose BPA groups, in both the interim and terminal sacrifice females, the incidences of atypical foci were higher in some treatment groups than in vehicle controls, and this was significant (by the RTE test only) for the 2.5 μg BPA/kg bw/day dose group in both the interim (14% versus 0%) and terminal (15% versus 4%) females (Table 55). In the interim sacrifice animals, there was a significantly increased incidence (RTE test only) of ductal dilatation (32% versus 9%) in the 25 μg BPA/kg bw/day dose group, but this was not the case in the terminal sacrifice females, where the incidence in the dose group was decreased relative to the vehicle controls (15% versus 30%).

In the continuous-dose EE_2 treatments, there were several significant trends and high dose treatment effects observed by all statistical tests applied (Table 56). In both interim and terminal animals, there was a significant trend and a significant pairwise comparison of the 0.5 μg EE_2/kg bw/day dose group and vehicle control for ductal dilatation (85% versus 9%, interim; 81% versus 30%, terminal). In interim sacrifice animals, there was a significant trend and a significant pairwise comparison of the 0.5 μg EE_2/kg bw/day dose group and vehicle control for lobular hyperplasia (88% versus 44%). In terminal sacrifice animals, there was a significant trend and a significant pairwise comparison of the 0.5 μg EE_2/kg bw/day dose group and vehicle control for alveolar dilatation (85% versus 18%).

In the stop-dose BPA treatments, there were no statistically significant increased mammary gland non-neoplastic lesion incidences in BPA dose groups relative to vehicle controls, although multiple cases of decreased incidences in BPA groups relative to vehicle were observed (Table 57).

Uterus, neoplastic lesions

Stromal polyps were found in interim and terminal females and their incidences are shown for continuous BPA and EE2 and stop-dose BPA in Table 58, Table 59, and Table 60, respectively.

There was a significant dose trend in the interim sacrifice females treated continuously with BPA (Table 58). The incidence for the vehicle control group was 1/23 (4%) compared to 3/20 (15%) for the 2,500 μg BPA/kg bw/day group and 3/24 (12%) for the 25,000 μg BPA/kg bw/day group, but these differences were not statistically significant. This trend toward increased incidence at the higher BPA dose groups was not observed in the terminal sacrifice animals, where the control unadjusted incidence was 5/50 (10%) and incidences in BPA treatment groups varied from 4–16%, with 4/48 (8%) and 3/46 (6%) in the 2,500 and 25,000 μg BPA/kg bw/day groups, respectively.

There were no significant effects of continuous EE_2 treatment (Table 59).

In the stop-dose terminal females, the vehicle control incidence of stromal polyps was 14%, and a negative trend was observed along with a reduced incidence in the 25,000 μg BPA/kg bw/day dose group relative to the vehicle control (14% versus 2%, Table 60). There were no other statistically significant neoplastic effects observed in interim or terminal sacrifice BPA continuous or stop-dose treatments in the uterus.

Uterus, non-neoplastic lesions

Non-neoplastic lesions in the uteri of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA treatments are shown in Table 61, Table 62, and Table 63, respectively.

In interim sacrifice, continuous-dose BPA females, there was a significant dose trend for apoptosis in the luminal epithelial cells of the endometrium for all statistical analyses applied, with the incidence in the 25,000 μg BPA/kg bw/day dose group significantly higher than the vehicle controls (38% versus 9%, Table 61). Endometrial hyperplasia was significantly increased in the interim continuous-dose 2.5 and 250 μg BPA/kg bw/day dose groups (RTE test only, 32% versus 9% and 29% versus 9%, respectively). There were also significant dose trends for squamous metaplasia and dilatation of the lumen in the interim and terminal sacrifice continuous-dose BPA females, respectively, with no significant pairwise comparisons for any dose group to vehicle controls (Table 61).

In the continuous EE_2-treated interim sacrifice females, there were increased trends and significant pairwise comparisons of the 0.5 μg EE_2/kg bw/day dose group to the vehicle control detected in all statistical tests applied for uterine apoptosis (69% versus 9%), cystic endometrial hyperplasia (54% versus 22%), and squamous metaplasia (54% versus 4%) (Table 62). In the terminal animals, a trend was detected for squamous metaplasia in both the Poly-3 and JT/SW tests (incidences of 4%, 8%, and 15% in the vehicle control, low and high EE_2 dose groups, respectively).

In the stop-dose females, the incidence of apoptosis in the 25,000 μg BPA/kg bw/day dose group was higher than that in vehicle controls (27% versus 10%), but this difference was not statistically significant (Table 63). In stop-dose BPA-treated females, there was a significant increase in cystic endometrial hyperplasia relative to vehicle controls in the 25,000 μg BPA/kg bw/day dose group (32% versus 10%, JT/SW and RTE tests) at interim sacrifice, while in the terminal sacrifice females there was a significant dose trend and the incidences in the 2,500 and

25,000 μg BPA/kg bw/day dose groups were significantly higher than that in the vehicle control (57% and 52%, respectively, versus 37%). Additional statistically significant differences in BPA stop-dose interim sacrifice animals versus controls were an increased incidence of squamous metaplasia in the 25,000 μg BPA/kg bw/day dose group (JT/SW and RTE tests, 18% versus 0%) and an increased incidence of dilatation of the lumen in the 250 μg BPA/kg bw/day dose group (RTE test only, 18% versus 5%) (Table 63).

Ovary, non-neoplastic lesions

Non-neoplastic lesions in the interim and terminal sacrifice animals for continuous BPA and EE_2 and stop-dose BPA are shown in Table 64, Table 65, and Table 66, respectively.

There were no statistically significant BPA treatment-related effects in the continuous-dose terminal sacrifice females. Females in the interim sacrifice continuous BPA dose arm showed significant dose trends for depletion of corpora lutea and interstitial cell hypertrophy (Table 64). For interstitial cell hypertrophy, the 2,500 μg BPA/kg bw/day dose group was significantly different from the vehicle control incidence (RTE test only, 40% versus 17%). The 25,000 μg BPA/kg bw/day dose group had an incidence of 38% ($p = 0.068$).

In the ovaries of continuous EE_2-treated interim sacrifice females, there were significant dose trends and significant pairwise comparisons of the 0.5 μg EE_2/kg bw/day dose group to the vehicle control detected in all statistical tests applied for atrophy (100% versus 44%), follicular cysts (100% versus 35%), depleted corpora lutea (100% versus 17%), and interstitial cell hypertrophy (100% versus 17%) (Table 65). In the terminal sacrifice females, there was a 94% incidence of ovarian atrophy in vehicle controls and 100% atrophy in the high EE_2 dose group, with greater severity in the EE_2-treated group that was significantly greater than controls by both tests that incorporate severity scores (JT/SW and RTE, $p < 0.001$ for both).

There were no statistically significant BPA treatment-related effects in the stop-dose terminal sacrifice females. In the stop-dose BPA-treated interim sacrifice animals, there was a significant dose trend ($p < 0.001$) for follicular cysts and the 25,000 μg BPA/kg bw/day dose group had a higher incidence than vehicle controls (82% versus 25%) (Table 66). The 2,500 μg BPA/kg bw/day dose group had an incidence of 55% ($p = 0.053$). There were no continuous-dose BPA groups with significantly higher follicular cyst incidence than the vehicle controls (Table 64).

Vagina, non-neoplastic lesions

Non-neoplastic lesions in the vaginas of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 67, Table 68, and Table 69, respectively.

Statistically significant effects were observed in both the interim and terminal sacrifice BPA-treated animals for epithelial hyperplasia for the continuous-dose arm (Table 67). For the interim sacrifice animals, there was a significant dose trend (all statistical tests) and the 25,000 μg BPA/kg bw/day dose group had a significantly higher incidence of hyperplasia than the vehicle controls (JT/SW and RTE tests, 33% versus 13%). The incidence in the 2,500 μg BPA/kg bw/day continuous-dose group was 30% ($p = 0.074$ and 0.067 for the JT/SW and RTE tests, respectively). In the terminal sacrifice females, there was a significant dose trend (all statistical tests) and significant pairwise comparisons to control for 25–25,000 μg BPA/kg bw/day dose groups, although the response was similar across dose groups (incidences of 8% in vehicle controls and 27%, 20%, 22%, and 26% for the 25, 250, 2,500, and 25,000 μg BPA/kg bw/day

dose groups, respectively). The Poly-3 and RTE tests were not significant for the 250 μg BPA/kg bw/day dose group.

In the vaginas of females treated continuously with EE_2, statistically significant effects were observed in the interim sacrifice animals, but not in terminal sacrifice animals. For all statistical tests applied, there was a dose trend for epithelial hyperplasia and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (77% versus 13%) (Table 68). There was also a trend toward increased epithelial mucification (all statistical tests) and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (69% versus 44%, JT/SW and RTE tests).

In the stop-dose arm, there were no statistically significant BPA effects in the interim or terminal sacrifice females, although the incidence of epithelial hyperplasia was 27% in the 25,000 μg BPA/kg bw/day dose group versus 10% in the vehicle control ($p = 0.064$) in the interim sacrifice animals (Table 69).

Pituitary, neoplastic and non-neoplastic lesions

Neoplastic lesions in the pituitaries of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 70, Table 71, and Table 72, respectively. Adenomas or carcinomas of the pars distalis were observed at low incidence in interim animals, and higher incidences, primarily of adenomas, were observed in terminal animals. There were no statistically significant effects in BPA continuous- or stop-dose interim or terminal sacrifice animals (Table 70 and Table 72). In the terminal EE_2 females, the incidence of combined adenomas and carcinomas of the pars distalis was increased in the 0.5 μg/kg bw/day dose group (77% versus 44%, Table 71).

Non-neoplastic lesions in the pituitaries of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 73, Table 74, and Table 75, respectively.

There were no statistically significant treatment effects in the pituitaries of continuous BPA dose arm interim or terminal sacrifice females (Table 73).

In the pituitaries of females treated continuously with EE_2, increased lesion incidences relative to vehicle controls were observed in the interim and terminal sacrifice animals (Table 74). In the interim sacrifice animals, there was a significant increasing trend for hyperplasia in the pars distalis and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to the high incidence in the vehicle control (96% versus 78%, JT/SW and RTE tests). There was also a significant trend for angiectasis in the interim sacrifice females (all statistical tests) and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (23% versus 4%, JT/SW and RTE tests). Similar trends and pairwise comparisons of the high EE_2 dose group to vehicle control were seen in the terminal sacrifice females (65% versus 20%, all statistical tests).

There were few statistically significant increased incidences over vehicle control in the stop-dose BPA arm, all indicated only by the RTE test (Table 75). In the interim sacrifice females, there was an increase in angiectasis (dilated vasculature) in the 2.5 μg BPA/kg bw/day stop-dose group (9% versus 0%). In the stop-dose terminal sacrifice females, the incidence of hyperplasia in the pars distalis was increased in the 2.5 and 25 μg BPA/kg bw/day stop-dose groups (64% and 71%, respectively, versus 51%) (Table 75). In the terminal sacrifice continuous-dose females, the incidences of the same lesion in the vehicle control, 2.5 and 25 μg BPA/kg bw/day stop-dose

groups were 54%, 46%, and 70%, respectively, with no statistically significant comparisons (Table 73).

Heart, non-neoplastic lesions

Cardiomyopathy is a high-incidence background lesion in this rat strain, which increases with aging in both sexes. The lower incidences in females than in males allowed for a better evaluation of any potential treatment effects. Incidences and severity scores for cardiomyopathy in interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 76, Table 77, and Table 78, respectively.

There were no statistically significant positive effects observed in female rats dosed continuously with BPA (Table 76). In the terminal sacrifice continuous-dose females, the vehicle control incidence of cardiomyopathy was 70%, with a reduced incidence (52%) in the 25 μg BPA/kg bw/day dose group.

In interim sacrifice females dosed continuously with EE_2, all three statistical tests applied indicated a significant dose trend and a significant increase in the incidence of cardiomyopathy in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (65% versus 30%) (Table 77). The JT/SW and RTE tests also detected an increase in the terminal sacrifice females (85% in the 0.5 μg EE_2/kg bw/day dose group versus 70% in the vehicle control group). The RTE test also indicated a significant increasing trend in terminal sacrifice EE_2 females.

In stop-dose BPA animals, no effects were observed in interim sacrifice females (Table 78). In terminal sacrifice stop-dose females, the statistical tests that incorporate severity scores along with incidence (JT/SW and RTE) detected a dose trend, and there were significant pairwise comparisons for 2.5, 250, 2,500, and 25,000 μg BPA/kg bw/day dose groups relative to the vehicle control (incidences of 74%, 74%, 70%, and 76%, respectively, versus 64%). The mean severity scores varied from 1.5 to 1.7 in the significantly different BPA groups versus 1.3 in the vehicle control, mostly due to an increased percentage of lesions diagnosed as mild rather than minimal in those groups.

Kidney, non-neoplastic lesions

Non-neoplastic lesions in the kidneys of interim and terminal sacrifice females for continuous BPA, EE_2, and stop-dose BPA are shown in Table 79, Table 80, and Table 81, respectively.

The incidence of nephropathy was high and highly variable between continuous- and stop-dose vehicle controls (continuous interim and terminal controls, 26% and 38% (Table 79), respectively; stop-dose interim and terminal controls, 50% and 57% (Table 81), respectively).

For interim sacrifice females dosed continuously with BPA, the RTE test indicated an increased incidence of nephropathy in the 25 and 2,500 μg BPA/kg bw/day dose groups relative to the vehicle control (50% and 55%, respectively, versus 26%) (Table 79). In the terminal sacrifice females in the continuous BPA dose arm, both the Poly-3 and RTE tests detected increased incidences of nephropathy in the 2.5 μg BPA/kg bw/day group (58% versus 38%). In addition, the JT/SW and RTE tests detected an increased incidence in the 25,000 μg BPA/kg bw/day dose group relative to vehicle control (54% versus 38%). Additional statistically significant effects in the continuous BPA dose groups in the interim sacrifice animals were an increase in renal tubular cysts in the 2.5 μg BPA/kg bw/day group relative to vehicle controls (32% versus 0%, CAFE test; the control incidence in stop-dose group was 20% (Table 81) and an increased dose

trend (all statistical tests applied) of mineralization, with a greater incidence in the 25,000 μg BPA/kg bw/day group relative to the vehicle control group (67% versus 48%, JT/SW and RTE tests only).

In females dosed continuously with EE_2, statistically significant increased incidences of kidney lesions relative to vehicle controls were also observed (Table 80). In interim sacrifice females, all statistical tests applied indicated a dose trend and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control for nephropathy (58% versus 26%). This trend was also evident in the terminal sacrifice females (all statistical tests applied), and the JT/SW and/or RTE tests indicated significantly increased incidences in both the low and high EE_2 dose groups (54% and 58%, respectively, versus 38%). In the interim female 0.05 μg EE_2/kg bw/day dose group, there was an increase in renal tubule cysts (19% versus 0%) and mineralization (RTE test only, 65% versus 48%).

In terminal stop-dose females, renal tubular cysts were increased in the 2.5 μg BPA/kg bw/day group relative to the vehicle control group (43% versus 21%) (Table 81).

Liver, non-neoplastic lesions

Non-neoplastic lesions in the livers of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 82, Table 83, and Table 84, respectively.

No statistically significant positive treatment-related effects were observed in continuous BPA or EE_2 treatment groups (Table 82 and Table 83).

In the stop-dose BPA treatments in interim sacrifice females, mononuclear cell infiltration showed an increased incidence in several dose groups that was statistically significant for the 2.5 (CAFE and RTE tests) and 25,000 μg BPA/kg bw/day stop-dose groups (all statistical tests applied; 46% and 36%, respectively, versus 10%) (Table 84). In the terminal sacrifice females, there was a trend toward an increased incidence of cystic degeneration (all statistical tests applied) and significant pairwise comparisons (JT/SW and RTE tests) for 2,500 and 25,000 μg BPA/kg bw/day stop-dose groups (16% and 15%, respectively, versus 4%).

Thyroid, non-neoplastic lesions

Non-neoplastic lesions in the thyroid glands of interim and terminal sacrifice females for continuous BPA and EE_2 and stop-dose BPA are shown in Table 85, Table 86, and Table 87, respectively.

In the continuous-dose BPA terminal sacrifice females, the sole significant effect was an elevated incidence (RTE test only) of follicular cell hyperplasia in the 2.5 μg BPA/kg bw/day dose group relative to the vehicle control (12% versus 2%) (Table 85). The vehicle control incidence of follicular cell hyperplasia in the terminal sacrifice stop-dose vehicle control was 8% (Table 87).

The only statistically significant effects in females treated continuously with EE_2 were an elevation (Poly-3 and RTE tests) of the incidence of follicular cell hyperplasia in the 0.05 μg EE_2/kg bw/day dose group relative to vehicle control (15% versus 2%) and an increasing trend for ultimobranchial cysts in terminal sacrifice animals (Table 86).

In stop-dose BPA interim sacrifice females, the 2.5 μg BPA/kg bw/day stop-dose group had a higher incidence of C-cell hyperplasia than controls (RTE test only; 73% versus 50%) (Table 87). In stop-dose BPA terminal sacrifice females, the incidence of ultimobranchial cysts

was elevated in the 250 and 2,500 μg BPA/kg bw/day stop-dose groups relative to vehicle controls (19% and 22%, respectively, versus 4%) (Table 87).

Males

No statistically significant differences versus control were found regarding organ-specific neoplasms in males in any BPA treatment group. There was an increased trend (*p*-values ranging from 0.002 to 0.009) for systemic lymphoma that presented in multiple organs (liver, dorsal/lateral prostate, bone marrow, spleen, and kidney) in terminal sacrifice animals of the stop-dose BPA arm (Supplemental Appendix XXXII and XXXIV). The incidence of lymphoma in the dorsal/lateral prostate was increased in the 25,000 μg BPA/kg bw/day stop-dose group (9% versus 0%, Supplemental Appendix XXXIV). Selected neoplastic and non-neoplastic lesions are presented below.

Epididymis, non-neoplastic lesions

Non-neoplastic lesions in the epididymides of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 88, Table 89, and Table 90, respectively.

In the continuous-dose BPA interim sacrifice males, there were significant trends (all statistical tests applied) for exfoliated germ cells and lymphocyte infiltration (Table 88). With both lesions, the incidence in the 25,000 μg BPA/kg bw/day dose group was significantly higher than that in the vehicle control group (all statistical tests applied; 27% versus 4% for exfoliated germ cells, 23% versus 0% for lymphocyte infiltration). There were no significant BPA treatment effects in the terminal sacrifice males in the continuous BPA dose groups.

In males dosed continuously with EE_2, there were increased trends for lymphocyte infiltration in interim (JT/SW and RTE tests) and terminal (RTE test only) sacrifice animals and significant pairwise comparisons for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (12% versus 0% for interim animals, 38% versus 20% for terminal animals) (Table 89).

The sole statistically significant effect in the stop BPA dose males was an increase in exfoliated germ cells in the 2.5 μg BPA/kg bw/day dose group (RTE test only; 15% versus 0%) (Table 90).

Dorsal/Lateral and ventral prostate, neoplastic and non-neoplastic lesions

Non-neoplastic lesions in the dorsal/lateral prostates of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 91, Table 92, and Table 93, respectively.

There were statistically significant increased incidences relative to vehicle controls of lymphocyte infiltration and suppurative inflammation in continuous-dose BPA groups, primarily in the interim sacrifice males (Table 91). Lymphocyte infiltration was increased (RTE test only) in the 2.5 μg BPA/kg bw/day dose group (46% versus 18%). The incidence of suppurative inflammation was increased (RTE and/or JT/SW tests) over a high background (82% in vehicle control) in the 2.5, 250, 2,500, and 25,000 μg BPA/kg bw/day dose groups (91%, 92%, 90%, and 86%, respectively) in interim sacrifice continuous-dose males (Table 91). The incidence of suppurative inflammation was increased (Poly-3 test) in the 2.5 μg BPA/kg bw/day dose group in terminal sacrifice animals (96% versus 82%; Table 91).

There were no statistically significant differences in lesion incidences in continuous-dose EE_2 (Table 92) or stop-dose BPA (Table 93) groups relative to their respective vehicle control group.

The incidences of ventral prostate adenomas in terminal sacrifice males were discussed in the narrative pathology report (Supplemental Appendix XXXII) and are shown for continuous BPA and EE_2 and stop-dose BPA in Table 94, Table 95, and Table 96, respectively. The continuous- and stop-dose vehicle control incidences were 12% and 8%, respectively, and there were no treatment-related differences in any exposure group relative to vehicle controls. Regarding non-neoplastic lesions in the ventral prostate, lower incidences of suppurative inflammation were seen in some treatment groups in continuous BPA (Table 97) or EE_2 (Table 98), but not stop-dose BPA (Table 99), relative to the vehicle control group.

Pituitary, non-neoplastic lesions

Non-neoplastic lesions in the pituitaries of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 100, Table 101, and Table 102, respectively.

There were no significant treatment effects in interim sacrifice animals in the continuous (Table 100) or stop-dose (Table 102) BPA arms or in the continuous EE_2 treatments (Table 101).

In terminal sacrifice males treated continuously with BPA, there was a significant dose trend (all statistical tests applied) for hyperplasia in the pars distalis and a significant increase relative to vehicle controls in the 25 μg BPA/kg bw/day dose group (RTE test only, 40% versus 23%) and the 25,000 μg BPA/kg bw/day dose group (all statistical tests applied, incidence 42% versus 23%) (Table 100).

In males continuously dosed with EE_2, there was an increased trend (all statistical tests applied) for pars distalis hyperplasia in terminal sacrifice animals and a significant pairwise comparison for the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (50% versus 23%) (Table 101).

In stop-dose BPA terminal sacrifice males, the Poly-3 test indicated a significant dose trend for pars distalis hyperplasia and a significant pairwise comparison between the 25,000 μg BPA/kg bw/day dose group and vehicle controls (incidences, 44% versus 26%) (Table 102).

Thyroid, non-neoplastic lesions

Non-neoplastic lesions in the thyroid glands of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 103, Table 104, and Table 105, respectively.

There were no statistically significant treatment effects in the thyroid gland of continuous-dose interim sacrifice males (Table 103). In terminal sacrifice males continuously dosed with BPA, there was a significant dose trend (Poly-3 test), and the 2,500 μg BPA/kg bw/day dose group had a higher hyperplasia incidence than vehicle controls (Poly-3 and RTE tests, 46% versus 20%). Follicular cell hyperplasia was increased at 25 μg BPA/kg bw/day relative to the vehicle control group (19% versus 6%, RTE test only).

In males continuously dosed with EE_2, the incidence of C-cell hyperplasia was significantly higher in the 0.05 μg EE_2/kg bw/day dose group relative to vehicle control (Poly-3 and RTE tests, 48% versus 20%) (Table 104).

There were no statistically significant effects in the thyroid of stop-dose BPA interim or terminal sacrifice males (Table 105).

Parathyroid, non-neoplastic lesions

Non-neoplastic lesions in the parathyroid glands of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 106, Table 107, and Table 108, respectively.

Statistically significant treatment effects were seen in the terminal sacrifice animals, but not in the interim sacrifice animals. In the males dosed continuously with BPA, the incidence of hyperplasia in the parathyroid gland was increased at 25 μg BPA/kg bw/day (Poly-3 and RTE tests, 49% versus 22%) and at 250 μg BPA/kg bw/day (RTE test only, 36% versus 22%) (Table 106).

In males continuously dosed with EE_2, there was a significant dose trend (JT/SW and RTE tests) in parathyroid gland hyperplasia, and the incidence in the 0.5 μg EE_2/kg bw/day dose group was significantly higher than in the vehicle control group (44% versus 22%) (Table 107).

In terminal sacrifice BPA stop-dose males, there was a significant dose trend (Poly-3 test) in the incidence of parathyroid gland hyperplasia (Table 108).

Kidney, non-neoplastic lesions

Non-neoplastic lesions in the kidneys of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 109, Table 110, and Table 111, respectively.

Hyperplasia of the transitional epithelium of the kidney was significantly increased in terminal sacrifice males relative to vehicle controls in the 25 μg BPA/kg bw/day dose group of the continuous BPA dose arm (Poly-3 and RTE tests, 25% versus 6%) (Table 109).

In males continuously dosed with EE_2, there were no observed statistically significant effects in the kidney (Table 110).

In the BPA stop-dose males in the terminal sacrifice arm, there was a significant dose trend for hyperplasia of the transitional epithelium of the kidney (Poly-3 test) (Table 111). The only dose group that had an apparent, although not statistically significant, increased incidence relative to controls was the 2,500 μg BPA/kg bw/day dose group (40% versus 24%).

Liver, non-neoplastic lesions

Non-neoplastic lesions in the livers of interim and terminal sacrifice males for continuous BPA and EE_2 and stop-dose BPA are shown in Table 112, Table 113, and Table 114, respectively.

In the livers of continuous BPA dose males, statistically significant pairwise comparisons to vehicle controls were noted for fatty change, hepatodiaphragmatic nodules, and mononuclear cell infiltration (Table 112). Two of 20 animals in the 25 μg BPA/kg bw/day interim sacrifice dose group were diagnosed with fatty change; these were the only animals in the interim sacrifice continuous BPA dose group diagnosed with this change, which was statistically significant by the RTE test only. The diagnosis occurred in 4–17% of the terminal sacrifice continuous BPA dose males (vehicle control incidence, 8%), but there was no significant treatment effect.

Stop-dose BPA interim sacrifice males had variable incidences (4–10%) of fatty liver diagnosed in some dose groups, but none were significant and there were similar findings in the terminal sacrifice stop-dose males (Table 114).

Fatty liver was also diagnosed in the interim sacrifice males treated continuously with EE_2, and there was a significant dose trend (all tests) and a significantly higher incidence in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (15% versus 0 %, JT/SW and RTE tests only) (Table 113).

Hepatodiaphragmatic nodule, growth of the median lobe into the diaphragm, is a congenital background lesion. As with fatty liver, there were variable incidences of this lesion diagnosed across treatment groups in both interim and terminal sacrifice males for continuous- and stop-dose BPA and continuous-dose EE_2. In one case, there was a significantly higher incidence (CAFE test) in the interim sacrifice 2,500 μg BPA/kg bw/day continuous-dose group relative to control (21% versus 0%) (Table 112).

Mononuclear cell infiltration was diagnosed in all dose groups in both interim and terminal sacrifice groups for continuous- and stop-dose BPA and continuous-dose EE_2. Significant increases over control were noted in multiple continuous BPA dose treatment groups (2.5, 250, 2,500, and 25,000 μg BPA /kg bw/day) relative to vehicle control in interim sacrifice males (Table 112). There were no statistically significant treatment effects in terminal sacrifice BPA-treated males. Mononuclear cell infiltration was increased relative to the vehicle controls in the 0.05 μg EE_2/kg bw/day terminal sacrifice males (85% versus 70%, Table 113).

Discussion

BPA is a high-production-volume chemical to which there is ubiquitous sub-microgram/kg bw/day human exposure, primarily through the diet. A large body of data has been published on the effects of BPA in in vitro and in vivo systems, including epidemiological studies investigating the association of early life exposures to BPA with a variety of diseases[7; 56-59]. After considering these data, the levels of exposure, and the extent of metabolic inactivation of BPA upon ingestion, most international regulatory agencies have concluded that current non-occupational BPA exposures do not pose a credible risk to humans. However, this conclusion is not without controversy. Given the large body of data available on BPA and the level of human exposure that is prompting the debate, the present study was designed not as a high dose hazard identification study, but rather to examine a broad dose range from reasonably close to human dietary exposure levels to levels >25,000-fold higher than current estimated aggregate non-occupational exposure. The dose spacing of 10-fold used over the broad dose range in the study is typically the maximum dose range covered in a bioassay.

Yoshida et al.[60] originally raised the issue of the presence of BPA in commercial rodent diets when they reported approximately 40 ppb in the diet as measured by an HPLC-fluorescence method. The background diet analyses that were conducted for this study and for previous studies using a more specific HPLC-MS/MS method[19; 36] confirmed that commercial rodent diets contain trace levels (low ppb) of BPA. The ingested levels resulting from this dietary background did not lead to measurable levels of BPA or its metabolites in blood or tissues, but the presence of this background places a lower limit on the dose of BPA that can be tested. For the present study, a rejection level of 5 ppb BPA in the diet was established. This level of dietary BPA would have resulted in ingestion of approximately 0.25 μg/kg bw/day, a 10-fold lower exposure, on a μg/kg bw/day basis, over the course of the study than the exposure in the lowest chosen BPA dose group of 2.5 μg/kg bw/day. None of the lots of diet used in the study had a background level of BPA >3 ppb.

Survival of the interim sacrifice animals was 82–100%, and there were no effects of BPA on survival of the interim or terminal sacrifice animals. However, ≥60% of the terminal sacrifice animals did not survive to the scheduled sacrifice. Most were removed as moribund for animal welfare considerations and provided a full set of data for evaluation. The Poly-3 test applied for the analysis of the two-year pathology data is adjusted for mortality.

Female body weights in the continuous 250 μg BPA/kg bw/day dose group were significantly higher than the mean vehicle control body weights in the final weeks of the study (weeks 96–104). There were no other statistically significant body weight differences in pairwise comparisons of BPA dose groups to the vehicle controls. There were few statistically significant effects of BPA treatment, in either the continuous- or stop-dose arms, on clinical pathology endpoints or organ weights, and these effects could not be clearly defined as treatment-related as they were observed only in single-dose groups, in several cases differed from the vehicle control by less than 10%, and, in the case of organ weights, were not significant when adjusted for body weight.

The approach used by the NTP to determine if neoplasms are likely related to treatment has always focused on a weight-of-evidence approach, encompassing a range of factors, including statistical analysis, consideration of consistency of responses within the study, and historical

controls[61; 62]. This approach was followed in the present study to evaluate the histopathology data for the neoplastic and non-neoplastic lesions. There were relatively few neoplastic lesions that showed potential treatment effects. There were many non-neoplastic lesions in both males and females that were variable across control and BPA treatment levels. One issue that contributed to the challenge of clearly establishing effects as treatment related was that most diagnosed lesions, whether neoplastic or non-neoplastic, were not rare lesions, but rather common lesions associated with aging. To assess these lesions for any potential treatment effects, the CAFE test was applied for lesion incidence in the one-year study, and the survival-adjusted Poly-3 test was used for lesion incidence in the two-year study. The pairwise comparisons conducted as part of these analyses do not assume a monotonic dose response, but do not incorporate the severity scores assigned to many of the non-neoplastic lesions during the microscopic evaluation. The JT/SW test, which assumes monotonicity across dose levels, was applied to incorporate severity scores along with the incidence data. Because non-monotonic dose responses have been central to the discussion of the effects of BPA and other potential hormonally active agents, another test was applied that incorporates both incidence and severity scores and does not assume monotonicity[45]. This test (RTE) has not been widely used in toxicology studies, although it was used in the previous BPA subchronic study conducted at NCTR[19]. Neither the JT/SW nor the RTE tests adjust for survival. Although the use of multiple statistical tests coupled with the multitude of endpoints examined and lack of correction for multiple comparisons can lead to erroneous inferences in the interpretation of histopathology data based on *p*-values alone, the results of all statistical tests are presented in the Results section and Supplemental Appendices XXXIII and XXXIV, primarily to guide the selection of histopathological lesions that required evaluation.

At the terminal sacrifice of females in the stop-dose BPA study arm, there was a statistically significant increase in the incidence of mammary gland adenocarcinoma (22% versus 6%, $p = 0.016$) and the combination of adenoma/adenocarcinoma (24% versus 8%, $p = 0.018$) in the 2.5 μg BPA/kg bw/day dose group. This incidence is higher than the limited data available for historical controls of this rat strain at NCTR utilizing the same diet,[14; 15] which indicate a background rate of 11–16% for mammary gland adenocarcinoma in two-year-old control females. In the continuous-dose BPA study arm, the incidence of female mammary gland adenocarcinoma and the combination of adenoma/adenocarcinoma was 8% and 12%, respectively, in the control group. The incidence in each of the BPA continuous-dose groups varied between 6 and 18% for adenocarcinoma and between 9 and 20% for combined adenoma/adenocarcinoma, none of which was significant.

There were no treatment-related non-neoplastic changes in the mammary gland of interim or terminal sacrifice female stop-dose animals. In the interim and terminal continuous-dose arm, there were some differences in incidence and severity between BPA groups and the vehicle control group indicated by the secondary RTE statistical test. There was an increase in atypical foci in the mammary gland at 2.5 μg BPA/kg bw/day (14% versus 0% and 15% versus 4% for the interim and terminal dose group animals, respectively). There was also an increase in ductal dilatation at the interim sacrifice in animals continuously administered 25 μg BPA/kg bw/day (32% versus 9%).

Evaluation of the totality of the evidence regarding the elevated incidence of mammary adenocarcinomas or combined adenomas and adenocarcinomas in the stop-dose females exposed to 2.5 μg BPA/kg bw/day makes it unlikely that this is a plausible BPA treatment-related lesion.

The elevated incidence occurs in a single dose group of the stop-dose arm and was not observed in the continuous-dose arm. In addition, there were no treatment-related non-neoplastic lesions in the mammary gland of interim or terminal sacrifice stop-dose animals. Comparison of the incidences of mammary gland adenoma/adenocarcinoma in control and treatment groups to the limited historical control data for this strain of rats further questions the biological plausibility of this lesion as a treatment-related effect. In the continuous-dose, but not stop-dose, arm animals, there was an elevation of atypical foci in the 2.5 μg BPA/kg bw/day group in interim and terminal sacrifice animals, but no increase in adenomas or adenocarcinomas. It is difficult to envision a mechanism whereby the stop-dose animals would develop mammary adenocarcinomas, while continuous-dose animals would not. Yoshizawa et al.[63] reported a gavage study of 2, 3′, 4, 4′, 5-pentachlorobiphenyl (PCB 118) in rats where the highest dose group was either exposed from 8 weeks of age through two years (continuous exposure group), or from 8 weeks of age through 32 weeks and then dosed with vehicle on the same schedule as the continuously exposed animals through two years (stop exposure group). Uterine carcinoma was elevated only in the stop exposure group. A plausible hypothesis proposed by the authors was that the body weight depression of greater than 10% in the continuously exposed group may have suppressed development of the uterine carcinomas, and cited that mammary fibroadenomas, a spontaneous neoplasm known to be suppressed by decreased body weight gain, was also lower in the continuously dosed animals. In the present study, although there were no statistical comparisons conducted across dosing arms, there was an apparently higher body weight in the stop-dose females (average 7% and 5% higher than continuous-dose females for vehicle control and 2.5 μg BPA/kg bw/day groups, respectively). The development of spontaneous mammary gland fibroadenomas, however, was not suppressed in the BPA continuous-dose females nor was the development of mammary gland adenocarcinomas in high dose EE_2 animals (discussed below).

In contrast to BPA treatments, the reference estrogen EE_2 had a clearly interpretable impact on the female mammary gland. As indicated earlier, although targets for BPA other than classical estrogen receptors have been proposed[30-33], many of the reported effects of BPA involve estrogen signaling pathways. Thus, sensitivity of the animal model to exogenous estrogen was considered important, and EE_2 was included for this purpose rather than necessarily to compare effects of the two agents. The high dose (0.5 μg EE_2/kg bw/day) induced an increased incidence of adenocarcinoma (38% versus 8% in controls) and dilatation of ducts and alveoli in the mammary glands of terminal animals. In the mammary glands of interim females, the incidences of lobular hyperplasia and dilatation of ducts were increased in the high EE_2 dose group. In addition, increased incidence of combined adenomas and carcinomas in the pituitary pars distalis at 0.5 μg EE_2/kg bw/day may be related to increased mammary neoplasm incidence. Exogenous estrogen exposure in the rat can lead to decreased activity of dopaminergic neurons in the hypothalamus leading to prolactinomas in the pituitary that contribute to mammary adenocarcinoma development[64; 65]. The production of prolactin from the pituitary neoplasms was not investigated in this study, although serum prolactin was reported to be elevated in adult rats at 0.5 μg EE_2/kg bw/day in the previous NCTR subchronic BPA study[19].

The only other statistically significant neoplastic lesion in BPA-treated females was a significant trend ($p = 0.037$) for uterine stromal polyps in the interim continuous-dose arm animals. This trend was driven by incidences of 3/20 (15%) and 3/24 (12%) in the 2,500 and 25,000 μg BPA/kg bw/day dose groups, respectively, which were not statistically different in pairwise comparisons with the control incidence of 1/23 (4%). No trend toward higher

incidences in the higher BPA dose groups was evident in the terminal sacrifice continuous-dose animals, nor were there any significant pairwise comparisons between any BPA dose group and controls in the terminal sacrifice animals. Likewise, stromal polyps were not induced in the stop-dose BPA or in the interim or terminal sacrifice EE_2 animals. The relatively small increased incidences leading to the significant trend in the interim sacrifice animals together with the lack of any effects in the terminal sacrifice animals indicate that uterine stromal polyps are not likely to be a consequence of BPA treatment.

In the uterus, cystic endometrial hyperplasia was elevated in interim and terminal sacrifice animals in the stop-dose study arm at 25,000 μg BPA/kg bw/day (interim) and at 2,500 and 25,000 μg BPA/kg bw/day (terminal). The incidence in the terminal stop-dose vehicle control was low compared to all other terminal sacrifice groups. An increase in this lesion was not observed in the continuous-dose BPA animals, but did occur at the interim sacrifice with the high dose EE_2 animals. Uterine squamous metaplasia was also increased in interim stop-dose animals at 25,000 μg BPA/kg bw/day. None of these uterine BPA effects were significant using the CAFE or Poly-3 tests that were considered the primary statistical tests in the study and, in the case of the cystic endometrial hyperplasia, severity was not increased in the treated groups relative to the vehicle control. An increase in squamous metaplasia of the uterus also occurred at the interim sacrifice with the high dose EE_2 animals. In interim continuous-dose females, there were significant trends for increased apoptosis in luminal epithelial cells of the endometrium and squamous metaplasia, with a statistically significant elevation of the apoptosis at 25,000 μg BPA/kg bw/day. Apoptosis of the endometrial luminal epithelial cells also was significantly increased by the high EE_2 dose in interim sacrifice animals. Taken together, these data, particularly at the interim sacrifice, suggest that the 25,000 μg BPA/kg bw/day dose may exert weak estrogen-like effects on the uterus.

In the ovary, there were trends in interim sacrifice animals for depletion of corpora lutea and interstitial cell hypertrophy in the continuous-dose arm and a trend and increase in follicular cysts at 25,000 μg BPA/kg bw/day in stop-dose BPA groups. The magnitude of the increase in follicular cysts at the high BPA dose and the trend evident in the two highest BPA groups suggests that this is a treatment-related effect, although the lack of effect on this endpoint in the continuous-dose animals cannot be readily explained. In the ovaries of interim sacrifice high dose EE_2 females, there was a 100% incidence of cystic follicles.

There was an increasing trend for vaginal epithelial hyperplasia in interim sacrifice continuous BPA dose females with an increased incidence at 25,000 μg BPA/kg bw/day, although not in the primary CAFE test pairwise comparison. Vaginal epithelial hyperplasia was increased to nearly the same magnitude at all doses from 25–25,000 μg BPA/kg bw/day in terminal continuous-dose BPA animals. There were no significant BPA effects on vaginal epithelial hyperplasia in stop-dose animals. A more pronounced increased incidence of vaginal epithelial hyperplasia was also indicated by primary and secondary statistical tests with the interim sacrifice 0.5 μg EE_2/kg bw/day dose group. This was accompanied by an increased incidence of epithelial mucification in the EE_2 animals. When considered together with the observations mentioned above on the uterus in continuous-dose animals, the increased vaginal epithelial hyperplasia observed at 25,000 μg BPA/kg bw/day may be a plausible estrogenic effect. The sporadic and inconsistent responses observed at lower BPA doses in the female reproductive tract suggest that they are not of toxicological relevance.

BPA treatment did not have adverse effects on the estrous cycle. In the high dose EE_2 females, the estrous cycle was disrupted by the time of evaluation at approximately 16-weeks of age and the one-year sacrifice animals showed multiple organ weight changes and histological changes expected of estrogenic stimulation in the ovary, uterus, and vagina. We did not observe an effect of EE_2 on the time of vaginal opening in the present study. In the previous BPA subchronic study conducted at NCTR[19], a delay in the time of vaginal opening was observed with 0.5 μg EE_2/kg bw/day, with a more pronounced effect at 5 μg EE_2/kg bw/day. Variable effects on this endpoint depending on timing and dose have been reported in the literature[66; 67]. Disruption of the estrous cycle, as observed in the present study, may be a more sensitive response to early estrogen treatment, as has been reported with a different exposure regimen. Shirota et al.[68] administered five consecutive daily subcutaneous doses of EE_2 (0.4 and 2.0 μg/kg bw/day) to female Sprague-Dawley rat pups starting on PND 1 and found no effect on vaginal opening at either dose level, but a disruption of the estrous cycle at later times.

By the secondary statistical tests that include severity scores and incidence, but do not adjust for survival, cardiomyopathy was increased in the stop-dose BPA terminal sacrifice females at 2.5, 250, 2,500, and 25,000 μg BPA/kg bw/day, although background incidence was high at this age. Mean severity scores in the higher treated groups varied from 1.5–1.7, while the vehicle control severity score was 1.3. The percentage of lesions evaluated as mild rather than minimal in these BPA stop-dose groups relative to the controls appears to account for the modest difference in scores, and the percentage of mild diagnoses in the terminal stop-dose controls is lower than the continuous-dose controls or any other terminal sacrifice group. Cardiomyopathy was also increased in the high dose EE_2 females at both the interim and terminal sacrifices, with all statistical tests indicating a significant increase in the interim sacrifice animals (65% versus 30%, with mean severity of 1.2 versus 1.1). The high background incidence of this lesion at terminal sacrifice and variability in incidence and severity scores across groups make the toxicological significance of these results questionable, with the possible exception of the acceleration of development of the lesion by high dose EE_2.

For males, there were no significant differences in the incidences of neoplasms in treatment groups versus vehicle controls in any organ in stop-dose or continuous-dose interim or terminal sacrifice males. There were also no treatment-related non-neoplastic effects in stop-dose interim or terminal sacrifice males. An increase in exfoliated germ cells and lymphocyte cellular infiltration in the epididymis at one year in the continuous-dose study arm and hyperplasia in the pars distalis of the pituitary at two years in both continuous- and stop-dose study arms at 25,000 μg BPA/kg bw/day were notable effects in males. Hyperplasia of the pituitary pars distalis was also observed in 0.5 μg EE_2/kg bw/day terminal sacrifice males. For the epididymal lesions, there were no potentially related lesions noted in the testes to increase confidence in this observation as an effect of toxicological significance.

Other non-neoplastic lesions in males showed variable increases in some dose groups without a pattern in dose response. In the thyroid gland of continuous-dose BPA males at terminal sacrifice, an increase in C-cell hyperplasia was noted at 2,500 μg BPA/kg bw/day, with high variability across dose groups for this endpoint. In addition, TSH levels were not elevated in any BPA dose group. Multiple step sections of the prostate lobes were examined in this study, since prostate has been a target organ identified in the BPA literature. Increases in dorsal/lateral prostate inflammation were variable across a high background in both interim and terminal sacrifice animals. Inflammation in the aging rat prostate is common and has been associated with

increasing prolactin levels[44] and there were no differences between treated groups and vehicle control for hyperplasia or other non-neoplastic lesions observed in the prostate lobes.

As expected from the previous NCTR BPA subchronic study conducted under identical conditions to those reported here[19], males were less responsive to EE_2 than the females. This is consistent with other rat studies (e.g., Howdeshell et al., 2008; Ryan et al., 2010, and references therein)[69; 70]. The only significant effect observed in males in the NCTR BPA subchronic study at 0.5 μg EE_2/kg bw/day was an increase in mammary gland hyperplasia at 90 days of age[19]. Our previous work with EE_2 administered in the diet indicated increased mammary gland hyperplasia in young male animals (140 days of age) that ingested a dose approximating the low dose in this study (estimated between 0.5 and 1.0 μg/kg bw/day). An attenuated response was observed in two-year-old animals[20]. Hence, the lack of an increase of male mammary hyperplasia by EE_2 in the current study is likely due to the advanced age of animals at examination. In addition, measurements of serum EE_2 levels at approximate maximum concentration (C_{max}) were made at various ages in animals from the NCTR BPA subchronic study[25]. Like BPA, serum levels of EE_2 at a given dose level declined with age; that is, peak serum levels after an oral dose of 0.5 μg EE_2/kg bw were ~500 pM, ~10 pM, and below the LOD of 5 pM on PND 4, 21, and 80, respectively[25]. Thus, in the present study, although the EE_2 dosing was continuous throughout the study, the highest internal exposures occurred in preweaning animals. In the case of the low dose, 0.05 μg EE_2/kg bw/day, serum levels would be expected to be less than 1 pM by the time of weaning.

After the present study began, analysis of samples from a separate study conducted under identical conditions (NCTR BPA subchronic study)[19] indicated that vehicle controls housed in the same room as animals dosed with ≥100,000 μg BPA/kg bw/day had blood levels of BPA-glucuronide consistent with the lowest exposure dose in the study, which was 2.5 μg/kg bw/day[25]. Thus, evaluation of treatment effects observed in the subchronic study needed to consider the background exposure to BPA above the dietary exposure of ≤0.25 μg/kg bw/day. Since clear adverse effects occurred only at BPA doses ≥100,000 μg /kg bw/day and there were robust effects of the reference estrogen EE_2 at doses of 0.5 and 5 μg/kg bw/day, the background BPA exposure was considered to have no impact on the study[19; 71]. It should also be noted that given the presence of variable levels of BPA in commercial diets as discussed earlier, all BPA studies should account for the margin of exposure between the BPA dose levels showing effects and the background level of BPA exposure from the diet.

In the present study, a subset of the animals was housed for a short period early in the study in the same room as animals dosed with 250,000 μg BPA/kg bw/day for a CLARITY-BPA academic grantee study. As discussed in the Statistical Methods section, a conservative approach was taken in this study that assumed all animals that were housed for any period of their lives in the same rooms as the animals dosed with 250,000 μg BPA/kg bw/day were potentially exposed to low levels of BPA above the dietary exposure. An additional statistical analysis excluding these animals, referred to throughout the text as a sensitivity analysis, was conducted for each endpoint. It was reasoned that if inadvertent exposure of vehicle controls to environmental BPA approximately 10-fold higher (i.e., 2.5 μg BPA/kg bw/day) than had been anticipated was masking robust effects of BPA at the lower end of the dose range, this would be detected with the sensitivity analysis. The results of the sensitivity analyses did not show any consistency with respect to tissues or doses in revealing treatment effects not evident in the inclusive analysis and

indicated that any inadvertent BPA exposure early in the study had minimal impact on the conclusions derived from the statistical tests.

In conclusion, in the CLARITY-BPA core study, differences between BPA treatment groups, particularly below 25,000 μg BPA/kg bw/day, and the vehicle control group detected by the low-stringency statistical tests applied to histopathology lesions, were not dose responsive, sometimes occurring in only one low or intermediate dose group, and did not demonstrate a clear pattern of consistent responses within or across organs within the stop- and continuous-dose arms and the interim and terminal sacrifices. In contrast, the high EE_2 dose elicited several strong effects in females in a clearly interpretable and biologically plausible as estrogenic effects. At 25,000 μg BPA/kg bw/day, several observations may be treatment related, including the effects mentioned above in the female reproductive tract (ovary, uterus, and vagina) and in the male pituitary.

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Table 2. Experimental Design and Materials and Methods in the Two-Year Chronic Gavage Toxicology Study of Bisphenol A (NCTR E0219001), Including Interim (One-Year) Assessment

	Experimental Design and Materials and Methods
Study Laboratory	National Center for Toxicological Research (NCTR); Jefferson, AR
Test Article	Bisphenol A (BPA), >99% (CAS #80-05-7), TCI America, Portland, OR [Catalogue #B0494, Lot #6052012, ground to a fine powder by Batelle, Inc., Columbus, OH]
Control Article, Reference Estrogen	Ethinyl estradiol (EE_2), >98% (CAS #57–63-6), Sigma-Aldrich Corporation, St. Louis, MO [Catalogue #E4876, Lot #071M1492V]
Control Article, Vehicle	Carboxymethylcellulose, sodium salt, Sigma-Aldrich Corporation, St. Louis, MO, [Catalogue #C-5013, Lot #041M0105V] used as a 0.3% (w/w) aqueous solution
Strain and Species	Rats: Sprague-Dawley/CD23/Nctr BR
Animal Source	NCTR breeding colony (Jefferson, AR)
Time Held Before Study	Breeder animals for the study were obtained from the breeding colony at weaning (approximately PND 21) and placed under study conditions (TestDiet low phytoestrogen 5K96 diet, polysulfone rat cages, hardwood chip bedding, glass water bottles with silicone stoppers) until mated at PND 70–100 for females and PND 77– 105 for males.
Age When Exposure Began	Sperm- or in situ vaginal plug-positive females were dosed from GD 6 (sperm or plug detection = GD 0)
Date of First Exposure for F_0 (GD 6)	Mating #1 09/08/2012 Mating #2 10/06/2012 Mating #3 11/03/2012 Mating #4 12/01/2012 Mating #5 12/29/2012
Route of Exposure	Oral gavage (for pups <PND 5, gavage needle did not enter esophagus)
Duration of Exposure	Dams were dosed daily until start of parturition. There was no dosing on the day of birth (PND 0), and pups were dosed directly from PND 1 until PND 21 for stop-dose group animals and from PND 1 until the day prior to termination for continuous-dose group animals
Date of Last Exposure	01/14/2015
Age at Necropsy	Scheduled interim necropsy at 1 year (365 ± 20 days of age) Scheduled terminal necropsy at 2 years (104 ± 3 weeks of age) Moribund and dead animals necropsied on removal
Size of Study Groups	For vehicle and BPA dose groups: 20-26 litters in the interim necropsy groups, and 46–50 litters in the terminal necropsy groups For EE_2 dose groups: 26 litters (interim and terminal necropsy groups)
Method of Animal Allocation	F_0 females from the NCTR breeding colony were randomly allocated to dose groups prior to mating to give approximately equal mean body weights per dose group. Sires were selected randomly with the specification that there would be no brother/sister or first cousin matings. After the first mating, the numbers of mating pairs assigned to dose groups were adjusted to meet any deficits in pups available in a particular dose group. Pups were randomly culled to a maximum of 5 males and 5 females on PND 1, no fostering was conducted. The minimum litter size for keeping a litter on study was 3 males and 3 females. Up to 3 pups per sex per litter were assigned to the study at weaning; additional pups were assigned to studies conducted by CLARITY-BPA academic investigators and reported elsewhere. The rotating order of assignment of pups of a given sex to the study was as follows: 1) continuous-dose, 2-year sacrifice; 2) stop-dose, 2-year sacrifice; 3) continuous-dose, 1-year sacrifice; 4) continuous-dose, 2-year sacrifice; 5) stop-dose, 2-year sacrifice; and 6) stop-dose, 1-year sacrifice.
Animals per Cage	Pregnant females were housed singly and litters were kept with their dams until weaning at PND 21. Animals were pair housed after weaning in same-sex pairs within dose groups. If an animal died or was removed as moribund, the cage mate remained single housed.

	Experimental Design and Materials and Methods
Method of Animal Identification	Tail tattoo; newborns identified by paw tattoo until tail tattoo identification, which occurred within one week of weaning at PND 21
Microbiological Surveillance	Sentinel animals were maintained in each of the animal rooms and animals were removed for surveillance approximately every three months during room occupancy. Animal room supplies (food, water, and bedding) and swabs from the animal rooms were also evaluated.
Diet	Rodent chow, verified casein diet 10 IF, round pellets, irradiated, 5K96 (TestDiet, Purina Mills, Richmond, IN) [Catalogue #1810069] Fed *ad libitum*
Water	Millipore-filtered tap water (NCTR well water) via water bottle, available *ad libitum.* Water samples were screened by the Chemistry Support Group, Division of Biochemical Toxicology, NCTR, as part of the normal surveillance procedures of the NCTR.
Cages	Polysulfone (Ancare Corporation, Bellmore, NY) Changed twice weekly, rotated every two weeks.
Bedding	Hardwood chips (P.J. Murphy, Montville, NJ) Changed weekly Alpha-Dri® (Shepherd Specialty Papers, Watertown, TN) was used for animals that developed lower body lesions as recommended by Veterinary Services
Cage Bonnets	Microisolator tops (Ancare Corporation, Bellmore, NY)
Racks	Metal animal cage racks (Allentown Caging Equipment Co., Allentown, NJ). Changed every two weeks and rotated every two weeks.
Animal Room Environment	Temperature: 23°± 3°C Relative humidity: 50% ± 20% Room fluorescent light: 12 hours/day (on 6 AM, off 6 PM) Room air changes: at least 10/hour
Exposure Concentrations	Vehicle control (0.3% CMC) BPA: 2.5, 25, 250, 2,500, and 25,000 μg/kg bw/day EE_2: 0.05 and 0.5 μg/kg bw/day
Dose administration volume	BPA, EE_2, and vehicle were administered at a rate of 5 mL/kg bw/day.
Type and Frequency of Observation	Twice daily morbidity/mortality checks, abnormal clinical observations recorded weekly or when a significant clinical observation was noted. Daily body weights for dams from GD 6 through parturition. Pups weighed daily from PND 1 to PND 21. Pups in continuous-dose arm were weighed daily until PND 90 ± 3 and weekly thereafter. Pups in the stop-dose arm were weighed weekly after weaning. Feed consumption was measured weekly for approximately the first 13 weeks and monthly afterwards. Litter parameters: number of pups alive and dead on day of birth (PND 0); number of pups alive and dead and live litter weight by sex on PND 1. Females (26 animals from 13 randomly selected cages per dose group) were monitored daily for vaginal opening from PND 22. Vaginal smears were collected for 14 consecutive days from these same animals beginning at 16 ± 2 weeks of age. One month after these vaginal smears were completed, the same animals had vaginal smears collected for 5 consecutive days monthly until the animal did not show evidence of cycling (three or more consecutive days of estrus (E, E/D or P/E) or five consecutive days that did not include an E) for two consecutive months.
Method of Sacrifice	Asphyxiation with carbon dioxide
Necropsy (F_0 Dams)	F_0 dams that were observed to be sperm-positive or had an in situ vaginal plug observed during mating were removed after litters were weaned, after litters that did not meet study criteria were euthanized, or after GD 26 if no litter was delivered. The uterus was removed and implantation sites were counted.

	Experimental Design and Materials and Methods
Necropsy (Dead or moribund F_0 dams and all postweaning F_1 animals)	Animals underwent a gross examination and complete necropsy as described for interim and terminal sacrifice animals (see below). In addition to tissues designated for processing and/or histopathology in the protocol, the following tissues were examined microscopically to assess possible cause of death in all dead or moribund animals: esophagus, colon, ileum, lung, nose, stomach, trachea, and any gross lesions.
Necropsy (Interim sacrifice, PND 365 ± 20)	Animals were fasted overnight. Animals were anesthetized with carbon dioxide and blood was collected from the retro-orbital sinus prior to euthanasia and complete necropsy. Weighed organs (males): adrenals, brain, epididymides, heart, kidneys, liver, pituitary (after 48-hour fixation), seminal vesicles with coagulating gland, spleen, testes, thymus, thyroid with parathyroid (after 48-hour fixation), epididymal, and retroperitoneal fat pads. Weighed organs (females): adrenals, brain, heart, kidneys, liver, ovaries (with oviducts), pituitary (after 48-hour fixation), spleen, thymus, thyroid with parathyroid (after 48-hour fixation), uterus (blotted), ovarian and parametrial (combined), and retroperitoneal fat pads.
Hematology (Interim sacrifice, PND 365 ± 20)	The following endpoints were evaluated in an aliquot of whole blood: hematocrit, hemoglobin concentration, erythrocyte, leukocyte, reticulocyte, and platelet counts, leukocyte differential count, mean corpuscular volume, and mean corpuscular hemoglobin.
Clinical Chemistry (Interim sacrifice, PND 365 ± 20)	The following endpoints were measured in serum: total protein, albumin, urea nitrogen, creatinine, alanine aminotransferase, gamma glutamyl transpeptidase, sorbitol dehydrogenase, aspartate aminotransferase, alkaline phosphatase, total bile acids, glucose, cholesterol, triglycerides, insulin, leptin, cardiac troponins T and I, T3, T4, and TSH.
Sperm analysis (Interim sacrifice, PND 365 ± 20)	Testicular spermatid head counts (left testis); epididymal sperm counts, morphology, and motility evaluations (left epididymis).
Necropsy and histopathology (Terminal sacrifice, 104 ± 3 weeks)	Procedures were similar to those used for the interim sacrifice, except that the animals were not fasted, blood was not collected, and there were no hematology, clinical chemistry, sperm, or organ weight data collected.
Histopathology (Interim sacrifice, PND 365 ± 20 and terminal sacrifice, 104 ± 3 weeks)	The following organs, as well as all gross lesions, were evaluated microscopically: *Males*—adrenals, aorta (thoracic), bone marrow (femur), brain, right epididymis, heart, kidneys, liver, 5th left mammary gland (inguinal), pancreas, parathyroid, pituitary, prostate (dorsal/lateral and ventral), seminal vesicles with coagulating gland, spleen, right testis, thymus, and thyroid. For the dorsal/lateral prostate, 6 step sections cut at 100 µm intervals were evaluated. Subsets of intermediate sections were collected and stored unstained for potential additional evaluation. *Females*—adrenals, aorta (thoracic), bone marrow (femur), brain, heart, kidneys, liver, 5th left mammary gland (inguinal), ovaries, oviduct, pancreas, parathyroid, pituitary, spleen, thymus, thyroid, uterus, and vagina. All tissues, except testes and eyes, were fixed in 10 % NBF and stained with H&E for microscopic evaluation. Testes and eyes were fixed in modified Davidson's fixative and testes were stained with periodic acid-Schiff (PAS) stain. Fixation times for the tissues listed for histopathology, except the brain, were limited to 96–120 hours. Brain remained in fixative until processing.
Tissues removed, fixed in 10% NBF, processed to block and stored (Interim sacrifice, PND 365 ± 20 and terminal sacrifice, 104 ± 3 weeks)	Clitoral gland, esophagus, epididymal fat pad, ovarian/parametrial fat pad, retroperitoneal fat pad, Harderian gland, cecum, colon, rectum, duodenum, ileum, jejunum, lung with bronchi, lymph nodes (mandibular and mesenteric), nose, penis, preputial gland, salivary glands, skin, forestomach, glandular stomach, trachea, and urinary bladder.

Table 3. Numbers of F_0 Breeding Pairs Assigned to Study[a]

Matings	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2	Total
Mating 1	16	16	16[b]	16	16	16	10	14	**120**
Mating 2	17	17	17	17	17	17	9	9	**120**
Mating 3	17	17	17	17	17	17	9	9	**120**
Mating 4	15	15	17	16	14[c]	14	13[c]	14	**118**
Mating 5	15	16	20	18	15	11	10	15	**120**
Total, Matings 1-5[d]	**80**	**81**	**87**	**84**	**79**	**75**	**51**	**61**	**598**

[a]Doses of BPA and EE_2 are µg/kg bw/day.
[b]Only 15 pairs were mated due to the death of a male prior to breeding.
[c] Due to the deaths of male breeders, one female in each of these groups was mated with a male that had previously mated with a control female.
[d]The numbers of resulting sperm-positive dams were as follows: vehicle, 78; 2.5 BPA, 74; 25 BPA, 76; 250 BPA, 78; 2500 BPA, 76; 25000 BPA, 71; 0.05 EE_2, 51; 0.50 EE_2, 60.

Table 4. Number of Litters Produced Per Mating[a]

Matings	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2	Total
Mating 1	16	15	8	9	13	12	9	12	**94**
Mating 2	14	13	10	14	15	16	8	8	**98**
Mating 3	15	13	13	13	15	16	6	8	**99**
Mating 4	13	13	13	12	12	11	10	10	**94**
Mating 5	15	11	17	16	9	10	8	13	**99**
Total, Matings 1-5	**73**	**65**	**61**	**64**	**64**	**65**	**41**	**51**	**484**

[a]Doses of BPA and EE_2 are µg/kg bw/day.

Table 5. Number of Litters Contributing Pups to Interim and/or Terminal Assessments[a]

Matings	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2	Total
Mating 1	16	14	8	7	13	12	6	10	**86**
Mating 2	13	13	10	14	15	14	7	8	**94**
Mating 3	15	12	12	11	14	14	4	6	**88**
Mating 4	12	12	13	11	11	11	8	4	**82**
Mating 5	15	11	16	16	8	9	2	3	**80**
Total, Matings 1-5	**71**	**62**	**59**	**59**	**61**	**60**	**27**	**31**	**430**

[a]Doses of BPA and EE_2 are µg/kg bw/day.

Table 6. Number of Male and Female Pups Represented in Interim (1-Year) Sacrifice from Each Mating, Continuous-Dose[a]

Continuous-Dose	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2	Total
Males, Mating 1	6	6	4	2	4	4	6	8	**40**
Males, Mating 2	4	4	4	6	6	6	6	8	**44**
Males, Mating 3	4	6	4	4	6	6	4	4	**38**
Males, Mating 4	6	4	4	4	2	4	8	4	**36**
Males, Mating 5	2	2	4	8	2	2	2	2	**24**
Total males, Matings 1-5	**22**	**22**	**20**	**24**	**20**	**22**	**26**	**26**	**182**
Females, Mating 1	8	6	4	4	6	4	6	8	**46**
Females, Mating 2	5	4	4	6	6	6	6	8	**45**
Females, Mating 3	6	4	4	4	4	6	4	6	**38**
Females, Mating 4	2	2	4	4	4	4	8	2	**30**
Females, Mating 5	2	6	6	6	0	4	2	2	**28**
Total females, Matings 1-5	**23**	**22**	**22**	**24**	**20**	**24**	**26**	**26**	**187**

[a]Doses of BPA and EE_2 are µg/kg bw/day.

Table 7. Number of Male and Female Pups Represented in Interim (1 Year) Sacrifice from Each Mating, Stop-Dose[a]

Stop-Dose	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	Total
Males, Mating 1	4	4	2	2	6	6	**24**
Males, Mating 2	6	4	2	6	6	4	**28**
Males, Mating 3	6	4	6	4	6	6	**32**
Males, Mating 4	2	4	6	4	2	4	**22**
Males, Mating 5	2	4	4	4	0	2	**16**
Total males, Matings 1-5	**20**	**20**	**20**	**20**	**20**	**22**	**122**
Females, Mating 1	4	6	2	2	4	6	**24**
Females, Mating 2	4	6	4	4	6	6	**30**
Females, Mating 3	6	6	2	4	6	6	**30**
Females, Mating 4	4	4	6	4	2	4	**24**
Females, Mating 5	2	0	6	8	2	0	**18**
Total females, Matings 1-5	**20**	**22**	**20**	**22**	**20**	**22**	**126**

[a]Doses of BPA are µg/kg bw/day.

Table 8. Number of Male and Female Pups Represented in Terminal (2 Year) Sacrifice from Each Mating, Continuous-Dose[a]

Continuous-Dose	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2	Total
Males, Mating 1	13	12	6	6	10	10	6	10	**73**
Males, Mating 2	11	10	8	12	12	12	6	8	**79**
Males, Mating 3	12	10	10	8	12	12	4	4	**72**
Males, Mating 4	12	8	12	10	8	8	8	2	**68**
Males, Mating 5	2	8	12	14	8	4	2	2	**52**
Total males, Matings 1-5	**50**	**48**	**48**	**50**	**50**	**46**	**26**	**26**	**344**
Females, Mating 1	14	12	6	6	10	10	6	10	**74**
Females, Mating 2	10	10	8	12	12	10	6	8	**76**
Females, Mating 3	12	10	10	8	12	10	4	4	**70**
Females, Mating 4	10	8	10	10	8	8	8	2	**64**
Females, Mating 5	4	8	12	14	8	8	2	2	**58**
Total females, Matings 1-5	**50**	**48**	**46**	**50**	**50**	**46**	**26**	**26**	**342**

[a]Doses of BPA and EE_2 are µg/kg bw/day.

Table 9. Number of Male and Female Pups Represented in Terminal (2 Year) Sacrifice from Each Mating, Stop-Dose[a]

Stop-Dose	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	Total
Males, Mating 1	14	12	6	6	10	10	**58**
Males, Mating 2	10	10	8	12	12	12	**64**
Males, Mating 3	12	10	10	8	12	12	**64**
Males, Mating 4	12	8	12	10	10	8	**60**
Males, Mating 5	2	8	12	14	6	4	**46**
Total males, Matings 1-5	**50**	**48**	**48**	**50**	**50**	**46**	**292**
Females, Mating 1	14	12	6	6	10	10	**58**
Females, Mating 2	10	10	8	12	12	12	**64**
Females, Mating 3	12	10	10	8	12	12	**64**
Females, Mating 4	10	10	12	10	10	8	**60**
Females, Mating 5	4	8	12	14	6	4	**48**
Total females, Matings 1-5	**50**	**50**	**48**	**50**	**50**	**46**	**294**

[a]Doses of BPA are µg/kg bw/day.

Table 10. Dam Body Weights from Time of Mating to Parturition in Vehicle, BPA, and EE_2 Dose Groups (Mean ± S.E.M.)[a]

Body Weight	Vehicle n = 72	2.5 BPA n = 65	25 BPA n = 61	250 BPA n = 63	2500 BPA n = 63	25000 BPA n = 64	0.05 EE_2 n = 41	0.5 EE_2 n = 51
Baseline (GD 0/ GD 1), g	244 ± 3	248 ± 3	248 ± 4	244 ± 3	246 ± 3	252 ± 4	247 ± 4	253 ± 4
GD 6, g	275 ± 3	281 ± 4	278 ± 4	274 ± 4	278 ± 3	283 ± 4	278 ± 5	284 ± 4
Parturition, g	393 ± 4	406 ± 5	397 ± 6	394 ± 5	402 ± 5	396 ± 5	398 ± 6	402 ± 6

[a]Number of dams producing litters given under dose group column headings. Doses of BPA and EE_2 are µg/kg bw/day.
Gestational weight at parturition was analyzed separately for the BPA and EE_2 dose groups using ANOCOVA, with terms for treatment group, dam weight at baseline as a covariate, litter size as a covariate, and the interaction between treatment and litter size. Data were collected at baseline on GD 0 or GD 1 and daily from GD 6 to parturition. Gestational weight at parturition was defined as the last dam body weight prior to delivery. Pairwise comparisons of treatment means to the control group were performed using contrasts with Dunnett's adjustment for multiple comparisons. Tests of trend, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups only. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant results. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). Full results of the statistical analyses are found in Supplemental Appendix XVII.

Table 11. Implantation Sites And Litter Parameters For Vehicle, BPA, And EE_2 Dose Groups (Mean ± S.E.M)[a]

Endpoint	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
Implantation sites	12.8 ± 0.5	12.4 ± 0.6	11.2 ± 0.7	11.2 ± 0.6	11.6 ± 0.6	12.2 ± 0.6	11.7 ± 0.8	12.1 ± 0.7
	(78)	(74)	(74)	(78)	(74)	(70)	(49)	(59)
Litter size[b]	11.8 ± 0.4	12.6 ± 0.3	11.9 ± 0.5	11.6 ± 0.5	12.3 ± 0.4	11.5 ± 0.4	11.8 ± 0.6	12.2 ± 0.4
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
Males[b]	5.8 ± 0.2	6.4 ± 0.3	6.2 ± 0.3	5.7 ± 0.2	6.2 ± 0.3	5.5 ± 0.3	6.1 ± 0.4	5.8 ± 0.3
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
Females[b]	5.8 ± 0.2	6.0 ± 0.3	5.6 ± 0.3	5.7 ± 0.4	5.8 ± 0.3	5.8 ± 0.3	5.4 ± 0.4	6.1 ± 0.3
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
Unsexed[c]	0.2 ± 0.1	0.2 ± 0.1	0.1 ± 0	0.2 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.3 ± 0.2	0.3 ± 0.1
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
Born dead	0 ± 0	0.08 ± 0.05	0.02 ± 0.02	0 ± 0	0.02 ± 0.02	0.02 ± 0.02	0 ± 0	0.04 ± 0.04
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
% Males[d]	49.4 ± 1.5	51.5 ± 2.3	52.2 ± 2.2	50.6 ± 2.0	50.9 ± 1.8	47.3 ± 2.3	53.2 ± 2.4	48.0 ± 2.1
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
% Females[d]	49.5 ± 1.5	47.0 ± 2.3	47.0 ± 2.2	47.9 ± 2.1	46.7 ± 1.7	51.5 ± 2.3	44.8 ± 2.2	49.6 ± 2.1
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
% Unsexed	1.1 ± 0.4	1.6 ± 0.6	0.8 ± 0.3	1.5 ± 0.7	2.3 ± 0.9	1.1 ± 0.6	1.9 ± 1.1	2.4 ± 1.0
	(73)	(65)	(61)	(64)	(64)	(64)	(41)	(51)
Litter weight, total[e]	78.6 ± 2.3	80.7 ± 2.6	80.2 ± 2.8	75.6 ± 2.9	78.7 ± 2.4	75.3 ± 2.6	76.7 ± 3.9	78.6 ± 2.9
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)
Litter weight, males[e]	40.1 ± 1.7	43.6 ± 2.3	43.5 ± 2.2	39.1 ± 1.6	41.6 ± 1.8	38.1 ± 2.0	41.8 ± 2.9	38.6 ± 2.0
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)
Litter weight, females[e]	38.5 ± 1.6	37.2 ± 2.3	36.7 ± 2.0	36.4 ± 2.2	37.0 ± 1.7	37.2 ± 1.7	34.9 ± 2.4	40 ± 2.3
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)
Mean pup weight[f]	7.0 ± 0.1	6.8 ± 0.1	7.0 ± 0.1	6.9 ± 0.1	6.9 ± 0.1	7.0 ± 0.1	6.8 ± 0.1	7.0 ± 0.1
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)
Mean male pup weight[f]	7.1 ± 0.1	7.0 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.0 ± 0.1	7.2 ± 0.1	7.1 ± 0.1	7.2 ± 0.1
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)
Mean female pup weight[f]	6.8 ± 0.1	6.6 ± 0.1	6.8 ± 0.1	6.8 ± 0.1	6.7 ± 0.1	6.9 ± 0.1	6.6 ± 0.1	6.9 ± 0.1
	(73)	(62)	(61)	(64)	(63)	(64)	(39)	(50)

[a]BPA and EE_2 doses are µg/kg bw/day. Numbers in parentheses are numbers of dams (for implantation sites) or litters from which data were collected. All analyses and adjustments for multiple comparisons were performed separately for the BPA and EE_2 treatments. Dunnett's method was used to adjust for multiple comparisons. Tests of trend, for increasing treatment effect with increasing dose, were performed for the vehicle and BPA groups. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects for any endpoint. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). Full results of the analyses are found in Supplemental Appendices XVIII (implantation sites) and XIX (all other endpoints).

[b]Litter size (number alive) and numbers of males and females were analyzed using Poisson regression.

[c]Unsexed pups (i.e., pups that could not be definitively assigned as male or female) were assigned as males for analysis of sex proportions and of female and male counts.

[d]Sex proportions were analyzed using logistic regression.

[e]Litter weight data (g), across and by sex, were analyzed using contrasts within a one-way ANOVA to test for treatment effects.
[f]Litter mean pup weights (g) were analyzed using ANOCOVA, with litter size as a covariate, to test for treatment effects.

Table 12. Survival of Female Pups from PND 1 to Weaning in the Vehicle, BPA, and EE_2 Dose Groups[a]

Preweaning Females	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
Number of female pups after PND 1 culling	311	266	259	250	260	244	153	180
Dead	5	11	7	6	2	3	8	5
Missing	3	5	2	5	13	6	5	3
Moribund	1	1	5	4	1	2	2	0
Reallocated[b]	8	8	8	8	8	8	8	8
Pups surviving to weaning	294	241	237	227	236	225	130	164
Percent survival at weaning	95	91	92	91	91	92	85	91
Survival analysis, *p*-value	0.361[c]	0.245	0.280	0.245	0.245	0.313	0.005**	0.369

[a]BPA and EE_2 doses are μg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups. Female pups removed as dead or moribund were considered uncensored, while pups surviving to weaning were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. Statistically significant effects are indicated by asterisks (**, $p < 0.01$). There were no additional significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XX.

[b]The reallocated pups were removed by design on PND 15 for the associated CLARITY-BPA study that will be reported separately.

[c]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 13. Survival of Male Pups from PND 1 to Weaning in the Vehicle, BPA, and EE_2 Dose Groups[a]

Preweaning Males	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
Number of male pups after PND 1 culling	338	300	281	292	292	275	156	208
Dead	5	1	4	8	4	2	8	5
Missing	9	14	5	9	6	1	5	6
Moribund	1	3	1	0	1	4	2	0
Reallocated[b]	8	8	8	8	8	8	8	8
Pups surviving to weaning	315	274	263	267	273	260	133	189
Percent survival at weaning	93	91	94	91	93	95	85	91
Survival analysis, *p*-value	0.143[c]	1.000	1.000	1.000	1.000	1.000	0.062	0.656

[a]BPA and EE_2 doses are µg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups. Male pups removed as dead or moribund were considered uncensored, while pups surviving to weaning were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XX.

[b]The reallocated pups were removed by design on PND 15 for the associated CLARITY-BPA study that will be reported separately.

[c]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 14. Survival of Female Pups from Weaning to Interim (1 Year) Sacrifice in the Continuous Vehicle, BPA, and EE_2 Dose Groups[a]

Interim Sacrifice Females	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2
Females initially allocated for interim evaluation	23	22	22	24	20	24	26	26
Moribund	1[b]	0	0	2[d]	0	0	1[e]	0
Natural deaths	1[b]	0	1[c]	0	0	0	1[e]	0
Animals surviving to scheduled termination	21	22	21	22	20	24	24	26
Percent survival at end of study	91	100	95	92	100	100	92	100
Survival analysis, *p*-value	0.470[f]	1.000	1.000	1.000	1.000	1.000	0.921	0.605

[a]BPA and EE_2 doses are µg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups for all female pups assigned to the continuously dosed interim sacrifice group at weaning. Female pups removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Since there was 100% survival in both BPA and EE_2 dose groups, a modified analysis in which one was added to the number of all uncensored observations was conducted to allow estimability. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXI.

[b]One animal had nephropathy, while the cause of death/morbidity of the second animal was uncertain (Subappendix VI in Supplemental Appendix XXXII).

[c]Nephropathy was the cause of death (Subappendix VI in Supplemental Appendix XXXII).

[d]Nephropathy was the cause of death/morbidity in one animal and a mammary fibroadenoma in the second animal (Subappendix VI in Supplemental Appendix XXXII).

[e]One animal had a mammary adenocarcinoma, while the cause of death/morbidity in the second animal was uncertain (Subappendix VI in Supplemental Appendix XXXII).

[f]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 15. Survival of Female Pups from Weaning to Interim (1 Year) Sacrifice in the Stop-Dose Vehicle and BPA Groups[a]

Interim Sacrifice Females	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Females initially allocated for interim evaluation	20	22	20	22	20	22
Moribund	0	0	0	0	0	2[b]
Natural deaths	0	0	0	0	0	0
Animals surviving to scheduled termination	20	22	20	22	20	20
Percent survival at end of study	100	100	100	100	100	91
Survival analysis, *p*-value	0.455[c]	1.000	1.000	1.000	1.000	1.000

[a]BPA doses are µg/kg bw/day. Cox proportional hazard analyses were performed for the BPA dose groups for all female pups assigned to the stop-dose interim sacrifice group at weaning. Female pups removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Since there was 100% survival in most dose groups, a modified analysis in which one was added to the number of all uncensored observations was conducted to allow estimability. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXI.

[b]One animal had nephropathy and the second animal had a malignant meningioma of the cerebellum as the primary cause of morbidity/death (Subappendix VI in Supplemental Appendix XXXII).

[c]A test of dose trend, increasing treatment effect with increasing dose, was performed and the *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA columns are for pairwise comparisons to the vehicle control.

Table 16. Survival of Male Pups from Weaning to Interim (1 Year) Sacrifice in the Continuous Vehicle, BPA, and EE_2 Dose Groups[a]

Interim Sacrifice Males	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
Males initially allocated for interim evaluation	22	22	20	24	20	22	26	26
Moribund	4[b]	0	1[c]	0	0	0	2[e]	0
Natural deaths	0	0	1[c]	0	2[d]	1[d]	2[e]	3[d]
Animals surviving to scheduled termination	18	22	18	24	18	21	22	23
Percent survival at end of study	82	100	90	100	90	95	85	88
Survival analysis, *p*-value	0.666[f]	0.597	1.000	0.597	1.000	0.789	1.000	1.000

[a]BPA and EE_2 doses are µg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups for all male pups assigned to the continuously dosed interim sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Since there was 100% survival in some of the BPA dose groups, a modified analysis in which one was added to the number of all uncensored observations was conducted to allow estimability. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXI.

[b]Two animals had malignant lymphomas in their spleens, while the cause of death could not be determined for 2 animals (Subappendix VI in Supplemental Appendix XXXII).

[c]One animal had a perforated esophagus and another had a hemorrhaged lung (Subappendix VI in Supplemental Appendix XXXII).

[d]Cause of death/morbidity was uncertain (Subappendix VI in Supplemental Appendix XXXII).

[e]One animal had nephropathy, one animal was removed due to an abscessed skin wound, and the cause of death/morbidity for two animals was uncertain (Subappendix VI in Supplemental Appendix XXXII).

[f]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 17. Survival of Male Pups from Weaning to Interim (1 Year) Sacrifice in the Stop-Dose Vehicle and BPA Groups[a]

Interim Sacrifice Males	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Males initially allocated for interim evaluation	20	20	20	19	20	22
Moribund	0	0	0	0	0	0
Natural deaths	0	0	1[b]	0	0	0
Animals surviving to scheduled termination	20	20	19	19	20	22
Percent survival at end of study	100	100	95	100	100	100
Survival analysis, *p*-value	0.927[c]	1.000	1.000	1.000	1.000	1.000

[a]BPA doses are µg/kg bw/day. Cox proportional hazard analyses were performed for the BPA dose groups for all male pups assigned to the stop-dose interim sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Since there was 100% survival in some dose groups, a modified analysis in which one was added to the number of all uncensored observations was conducted to allow estimability. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXI.

[b]Cause of death/morbidity was uncertain (Subappendix VI in Supplemental Appendix XXXII).

[c]A test of dose trend, increasing treatment effect with increasing dose, was performed and the *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA columns are for pairwise comparisons to the vehicle control.

Table 18. Survival of Female Pups from Weaning to Terminal (2 Year) Sacrifice in the Continuous Vehicle, BPA, and EE_2 Dose Groups[a]

Terminal Sacrifice Females	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2
Females initially allocated for terminal evaluation	50	48	46	49	50	46	26	26
Moribund	28	28	31	31	33	35	18	18
Natural deaths	6	1	1	5	7	3	1	4
Animals surviving to scheduled termination	16	19	14	13	10	8	7	4
Percent survival at end of study	32	40	30	27	20	17	27	15
Survival analysis, *p*-value	0.071[b]	1.000	1.000	1.000	0.502	1.000	0.396	0.188

[a]BPA and EE_2 doses are µg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups for all female pups assigned to the continuously dosed terminal sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXII.

[b]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 19. Survival of Female Pups from Weaning to Terminal (2 Year) Sacrifice in the Stop-Dose Vehicle and BPA Groups[a]

Terminal Sacrifice Females	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Females initially allocated for terminal evaluation	50	50	48	50	50	46
Moribund	36	32	32	35	30	31
Natural deaths	3	6	3	2	3	2
Animals surviving to scheduled termination	11	12	13	13	17	13
Percent survival at end of study	22	24	27	26	34	28
Survival analysis, *p*-value	0.203[b]	1.000	1.000	1.000	1.000	1.000

[a]BPA doses are µg/kg bw/day. Cox proportional hazard analyses were performed for the BPA dose groups for all female pups assigned to the stop-dose interim sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXII.
[b]A test of dose trend, increasing treatment effect with increasing dose, was performed and the *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA columns are for pairwise comparisons to the vehicle control.

Table 20. Survival of Male Pups from Weaning to Terminal (2 Year) Sacrifice in the Continuous Vehicle, BPA, and EE_2 Dose Groups[a]

Terminal Sacrifice Males	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.50 EE_2
Males initially allocated for terminal evaluation	50	48	48	50	50	46	26	26
Moribund	24	16	27	21	24	27	14	10
Natural deaths	11	16	4	15	10	8	3	4
Animals surviving to scheduled termination	15	16	17	14	16	11	9	12
Percent survival at end of study	30	33	35	28	32	24	35	46
Survival analysis, *p*-value	0.327[b]	1.000	1.000	1.000	1.000	1.000	0.879	0.419

[a]BPA and EE_2 doses are µg/kg bw/day. Cox proportional hazard analyses were performed separately for the BPA and EE_2 dose groups for all male pups assigned to the continuously dosed terminal sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint, including is found in Supplemental Appendix XXII.

[b]A test of dose trend, increasing treatment effect with increasing dose, was performed for vehicle and BPA groups only. The *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA and EE_2 columns are for pairwise comparisons to the vehicle control.

Table 21. Survival of Male Pups from Weaning to Terminal (2 Year) Sacrifice in the Stop-Dose Vehicle and BPA Groups[a]

Terminal Sacrifice Males	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Males initially allocated for terminal evaluation	50	48	48	50	50	46
Moribund	20	20	24	29	27	29
Natural deaths	13	12	8	8	8	8
Animals surviving to scheduled termination	17	16	16	13	15	9
Percent survival at end of study	34	33	33	26	30	20
Survival analysis, *p*-value	0.053[b]	1.000	1.000	0.424	1.000	0.209

[a]BPA doses are µg/kg bw/day. Cox proportional hazard analyses were performed for the BPA dose groups for all male pups assigned to the stop-dose interim sacrifice group at weaning. Animals removed as dead or moribund were considered uncensored, while animals surviving to scheduled removal were considered censored. Multiple comparisons of treatments to the vehicle control were adjusted using Holm's method. All tests were performed as two-sided tests at the 0.05 significance level. There were no statistically significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods). The full statistical report for this endpoint is found in Supplemental Appendix XXII.
[b]A test of dose trend, increasing treatment effect with increasing dose, was performed and the *p*-value for the trend analysis is given in the vehicle column; *p*-values in BPA columns are for pairwise comparisons to the vehicle control.

Table 22. Prewean Body Weights (g) of Female Pups in the Vehicle, BPA, and EE_2 Dose Groups (Mean ± S.E.M.)[a]

Postnatal Day	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
1	6.8 ± 0.1 (71)	6.7 ± 0.1 (60)	6.8 ± 0.1 (57)	6.8 ± 0.1 (58)	6.7 ± 0.1 (59)	6.8 ± 0.1 (59)	6.6 ± 0.1 (34)	6.9 ± 0.1 (47)
4	10.6 ± 0.1 (71)	10.1 ± 0.2 (59)	10.5 ± 0.2 (57)	10.4 ± 0.1 (58)	10.3 ± 0.2 (58)	10.4 ± 0.2 (59)	10.1 ± 0.2* (34)	10.9 ± 0.2 (47)
7	15.8 ± 0.2 (71)	15.2 ± 0.2 (59)	15.6 ± 0.2 (57)	15.3 ± 0.2 (58)	15.1 ± 0.3 (58)	15.4 ± 0.2 (59)	15.0 ± 0.3* (34)	16.0 ± 0.3 (47)
14	30.6 ± 0.4 (71)	30.1 ± 0.4 (59)	30.9 ± 0.4 (57)	30.0 ± 0.4 (58)	29.6 ± 0.4 (58)	29.9 ± 0.4 (59)	29.9 ± 0.6 (34)	31.4 ± 0.4 (47)
21	50.1 ± 0.6 (70)	49.5 ± 0.7 (56)	50.6 ± 0.6 (55)	49.2 ± 0.7 (56)	48.6 ± 0.7 (56)	49.1 ± 0.6 (57)	50.2 ± 0.9 (33)	51.9 ± 0.7 (47)

[a]BPA and EE_2 doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Analysis was performed using contrasts within sex- and PND-stratified one-way repeated measures, mixed model ANOVA to test for treatment effects accounting for litter correlation assuming a compound symmetric correlation structure. Pairwise comparisons of treatment group means to the vehicle control group mean were performed using contrasts with Dunnett's method of adjustment for multiple comparisons separately for BPA and EE_2 groups. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and control groups. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIII. Values that are significantly different from the vehicle control are indicated with an asterisk (*, $p < 0.05$). There were no additional significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 23. Prewean Body Weights (g) of Male Pups in the Vehicle, BPA, and EE_2 Dose Groups (Mean ± S.E.M.)[a]

Postnatal Day	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
1	7.2 ± 0.1 (71)	7.1 ± 0.1 (60)	7.3 ± 0.1 (57)	7.2 ± 0.1 (59)	7.1 ± 0.1 (60)	7.3 ± 0.1 (60)	7.0 ± 0.1 (32)	7.2 ± 0.1 (49)
4	11.4 ± 0.2 (71)	11 ± 0.1 (59)	11.4 ± 0.2 (57)	11.2 ± 0.2 (58)	10.9 ± 0.2 (59)	11.4 ± 0.2 (60)	10.8 ± 0.2 (32)	11.3 ± 0.2 (48)
7	16.9 ± 0.2 (71)	16.4 ± 0.2 (59)	16.8 ± 0.3 (57)	16.4 ± 0.2 (58)	16.2 ± 0.3 (59)	16.7 ± 0.2 (60)	16.1 ± 0.4 (32)	16.6 ± 0.3 (48)
14	32.3 ± 0.4 (71)	31.7 ± 0.4 (59)	32.4 ± 0.4 (57)	31.5 ± 0.4 (58)	31.3 ± 0.4 (59)	31.7 ± 0.4 (60)	31.8 ± 0.6 (32)	32.5 ±0.5 (48)
21	53.0 ± 0.6 (70)	52.2 ± 0.6 (58)	53.6 ± 0.6 (56)	51.8 ± 0.7 (58)	51.5 ± 0.7 (59)	52.4 ± 0.7 (59)	53.0 ± 1.0 (32)	54.0 ± 0.8 (47)

[a]BPA and EE_2 doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Analysis was performed using contrasts within sex- and PND-stratified one-way repeated measures, mixed model ANOVA to test for treatment effects accounting for litter correlation assuming a compound symmetric correlation structure. Pairwise comparisons of treatment group means to the vehicle control group mean were performed using contrasts with Dunnett's method of adjustment for multiple comparisons separately for BPA and EE_2 groups. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and control groups. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIII. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 24. Female Postwean Body Weights (g), Vehicle, BPA, and EE_2 Continuous-Dose, Interim (1 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
4	76 ± 1 (23)	77 ± 2 (22)	76 ± 2 (22)	74 ± 2 (24)	73 ± 1 (20)	75 ± 2 (24)	75 ± 2 (26)	81 ± 2 (26)
8	196 ± 5 (23)	203 ± 4 (22)	205 ± 4 (22)	196 ± 4 (24)	196 ± 4 (20)	197 ± 4 (24)	201 ± 4 (26)	209 ± 4 (26)
12	268 ± 6 (22)	277 ± 6 (22)	278 ± 6 (22)	266 ± 6 (24)	262 ± 5 (20)	262 ± 6 (24)	273 ± 7 (26)	262 ± 5 (26)
16	302 ± 8 (22)	314 ± 7 (22)	317 ± 7 (22)	299 ± 7 (24)	293 ± 5 (20)	293 ± 7 (24)	308 ± 7 (26)	291 ± 5 (26)
20	322 ± 8 (22)	342 ± 9 (22)	345 ± 8 (22)	316 ± 8 (24)	311 ± 6 (20)	316 ± 10 (24)	332 ± 8 (26)	312 ± 6 (26)
24	340 ± 10 (22)	363 ± 12 (22)	362 ± 9 (22)	331 ± 9 (24)	331 ± 6 (20)	332 ± 11 (24)	352 ± 9 (26)	330 ± 8 (26)
28	354 ± 10 (22)	382 ± 13 (22)	380 ± 11 (22)	344 ± 10 (24)	346 ± 7 (20)	351 ± 12 (24)	365 ± 9 (26)	348 ± 8 (26)
32	370 ± 13 (22)	403 ± 14 (22)	395 ± 11 (22)	360 ± 11 (24)	362 ± 8 (20)	366 ± 13 (24)	384 ± 10 (26)	367 ± 10 (26)
36	384 ± 14 (22)	422 ± 16b (22)	410 ± 12 (22)	374 ± 12 (24)	376 ± 9 (20)	378 ± 14 (24)	398 ± 10 (26)	384 ± 10 (26)
40	396 ± 15 (22)	439 ± 18[b] (22)	422 ± 12 (22)	377 ± 10 (23)	394 ± 10 (20)	396 ± 14 (24)	417 ± 11 (25)	397 ± 10 (26)
44	410 ± 16 (22)	455 ± 19[b] (22)	434 ± 13 (22)	389 ± 11 (23)	409 ± 12 (20)	409 ± 16 (24)	434 ± 11 (25)	412 ± 11 (26)
48	421 ± 18 (21)	476 ± 20[b] (22)	447 ± 14 (21)	408 ± 13 (22)	424 ± 12 (20)	428 ± 17 (24)	449 ± 13 (24)	427 ± 12 (26)
52	436 ± 19 (21)	494 ± 22[b] (22)	460 ± 15 (21)	427 ± 14 (22)	436 ± 13 (20)	441 ± 18 (24)	468 ± 14 (24)	440 ± 12 (26)

[a]BPA and EE_2 doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXIV. Analyses were conducted separately for BPA and EE_2 dose groups. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIV. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

[b]Mean body weights at the later time points, weeks 36 to 52, were 10–13% higher than control means in the 2.5 μg BPA/kg bw/day. The Dunnett-corrected *p*-values were 0.080, 0.058, 0.071, 0.065, and 0.062, for weeks 36, 40, 44, 48, and 52, respectively.

Table 25. Female Postwean Body Weights (g), Vehicle, BPA, and EE_2 Continuous-Dose, Terminal (2 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
4	75 ± 1	76 ± 1	78 ± 1	76 ± 1	74 ± 1	76 ± 1	76 ± 1	80 ± 2*
	(50)	(48)	(46)	(49)	(50)	(46)	(26)	(26)
8	196 ± 3	201 ± 3	202 ± 2	199 ± 2	198 ± 3	204 ± 3[b]	200 ± 4	212 ± 4*
	(50)	(48)	(46)	(49)	(50)	(45)	(26)	(26)
12	266 ± 4	269 ± 4	270 ± 4	267 ± 3	266 ± 4	269 ± 3	269 ± 6	268 ± 6
	(50)	(48)	(46)	(49)	(50)	(45)	(26)	(26)
16	302 ± 5	305 ± 5	303 ± 5	299 ± 3	300 ± 4	302 ± 4	299 ± 6	299 ± 7
	(50)	(47)	(46)	(49)	(50)	(45)	(26)	(26)
20	324 ± 6	327 ± 6	325 ± 6	319 ± 4	319 ± 5	321 ± 4	320 ± 7	320 ± 9
	(50)	(47)	(46)	(49)	(50)	(45)	(26)	(26)
24	342 ± 6	345 ± 7	346 ± 6	336 ± 5	336 ± 5	338 ± 5	339 ± 8	338 ± 10
	(50)	(46)	(45)	(49)	(50)	(45)	(26)	(26)
28	359 ± 7	360 ± 7	362 ± 7	354 ± 5	352 ± 6	356 ± 5	351 ± 8	354 ± 11
	(50)	(46)	(45)	(49)	(50)	(45)	(26)	(26)
32	372 ± 8	376 ± 8	376 ± 8	366 ± 6	366 ± 6	369 ± 6	366 ± 9	373 ± 12
	(50)	(46)	(45)	(49)	(50)	(45)	(26)	(26)
36	387 ± 8	392 ± 9	393 ± 8	384 ± 6	375 ± 7	379 ± 6	375 ± 9	390 ± 13
	(50)	(46)	(45)	(49)	(50)	(45)	(26)	(26)
40	402 ± 9	407 ± 10	407 ± 10	396 ± 7	388 ± 8	394 ± 7	392 ± 10	406 ± 13
	(50)	(46)	(45)	(48)	(49)	(45)	(26)	(26)
44	417 ± 10	421 ± 11	419 ± 10	410 ± 8	403 ± 8	410 ± 8	403 ± 11	421 ± 14
	(50)	(45)	(45)	(47)	(48)	(45)	(26)	(26)
48	431 ± 10	438 ± 12	437 ± 12	426 ± 8	419 ± 8	424 ± 8	418 ± 11	439 ± 15
	(50)	(44)	(44)	(48)	(48)	(45)	(26)	(26)
52	447 ± 10	456 ± 13	451 ± 12	448 ± 8	434 ± 9	442 ± 9	433 ± 12	450 ± 15
	(50)	(44)	(44)	(48)	(48)	(45)	(26)	(26)
56	463 ± 11	474 ± 14	463 ± 13	466 ± 9	449 ± 10	457 ± 9	450 ± 13	459 ± 16
	(50)	(44)	(44)	(47)	(48)	(45)	(26)	(25)
60	478 ± 12	492 ± 15	484 ± 14	487 ± 10	468 ± 11	474 ± 10	462 ± 13	475 ± 18
	(50)	(44)	(43)	(47)	(48)	(45)	(26)	(25)
64	495 ± 12	510 ± 16	502 ± 15	504 ± 10	477 ± 11	491 ± 10	477 ± 14	485 ± 20
	(49)	(44)	(42)	(47)	(44)	(45)	(26)	(25)
68	506 ± 12	532 ± 17	519 ± 16	528 ± 11	489 ± 13	506 ± 11	497 ± 15	492 ± 23
	(47)	(44)	(41)	(46)	(39)	(44)	(24)	(24)
72	520 ± 14	544 ± 18	544 ± 18	547 ± 11	497 ± 12	520 ± 12	521 ± 16	506 ± 24
	(44)	(43)	(38)	(44)	(36)	(42)	(21)	(22)
76	531 ± 16	549 ± 19	564 ± 21	564 ± 13	500 ± 12	522 ± 12	530 ± 19	521 ± 26
	(40)	(39)	(34)	(41)	(33)	(38)	(17)	(21)
80	542 ± 16	562 ± 22	560 ± 19	571 ± 13	514 ± 13	537 ± 12	544 ± 18	531 ± 34
	(39)	(34)	(26)	(40)	(31)	(33)	(15)	(17)
84	533 ± 15	577 ± 24	574 ± 20	579 ± 15	521 ± 14	542 ± 14	552 ± 20	551 ± 40
	(35)	(33)	(26)	(38)	(27)	(29)	(14)	(15)
88	534 ± 18	584 ± 26	585 ± 22	588 ± 20	527 ± 17	556 ± 15	539 ± 23	564 ± 51
	(32)	(31)	(24)	(27)	(24)	(28)	(13)	(11)
92	530 ± 15	584 ± 22	565 ± 21	608 ± 23	526 ± 19	564 ± 16	561 ± 22	512 ± 27
	(26)	(26)	(18)	(22)	(21)	(27)	(12)	(8)
96	531 ± 17	604 ± 23	594 ± 20	616 ± 25*	540 ± 23	569 ± 19	563 ± 26	525 ± 34
	(23)	(22)	(16)	(19)	(18)	(21)	(10)	(7)
100	537 ± 21	621 ± 26	594 ± 16	634 ± 32*	528 ± 17	584 ± 18	597 ± 34	542 ± 36
	(17)	(21)	(14)	(16)	(13)	(17)	(7)	(6)
104	534 ± 22	619 ± 28	607 ± 18	622 ± 36*	524 ± 22	597 ± 34	602 ± 35	562 ± 50
	(17)	(18)	(13)	(12)	(11)	(9)	(7)	(4)

[a]BPA and EE_2 doses are µg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the

vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXV. Significant effects are indicated with asterisks (*, $p < 0.05$).

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods), the mean for week 8, 25,000 μg BPA/kg bw/day was significantly different ($p = 0.031$) from the vehicle control mean.

Table 26. Female Postwean Body Weights (g), Vehicle and BPA Stop-Dose, Interim (1 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
4	58 ± 3 (20)	54 ± 3 (22)	55 ± 3 (20)	54 ± 2 (22)	55 ± 3 (20)	51 ± 2 (22)
8	192 ± 5 (20)	184 ± 3 (22)	190 ± 4 (20)	190 ± 5 (22)	188 ± 5 (20)	184 ± 4 (21)
12	270 ± 7 (20)	263 ± 5 (20)	270 ± 7 (20)	266 ± 6 (22)	272 ± 8 (20)	262 ± 6 (21)
16	304 ± 11 (18)	302 ± 5 (22)	305 ± 9 (20)	305 ± 7 (22)	310 ± 8 (20)	303 ± 8 (21)
20	331 ± 11 (20)	329 ± 7 (22)	328 ± 10 (20)	330 ± 8 (22)	338 ± 10 (20)	326 ± 8 (21)
24	351 ± 12 (20)	350 ± 8 (22)	344 ± 11 (20)	348 ± 8 (22)	358 ± 12 (20)	344 ± 9 (21)
28	369 ± 12 (20)	366 ± 9 (22)	360 ± 12 (20)	368 ± 10 (22)	373 ± 14 (20)	361 ± 9 (21)
32	386 ± 14 (20)	391 ± 10 (20)	374 ± 13 (20)	381 ± 11 (22)	392 ± 15 (20)	374 ± 10 (21)
36	400 ± 14 (20)	399 ± 10 (22)	389 ± 15 (20)	399 ± 13 (22)	404 ± 15 (20)	386 ± 10 (21)
40	410 ± 16 (18)	419 ± 12 (22)	404 ± 16 (20)	418 ± 15 (20)	421 ± 17 (20)	407 ± 12 (20)
44	439 ± 17 (20)	433 ± 12 (22)	419 ± 16 (20)	431 ± 16 (22)	438 ± 18 (20)	420 ± 12 (20)
48	459 ± 18 (20)	453 ± 14 (22)	436 ± 17 (20)	449 ± 18 (22)	452 ± 18 (20)	440 ± 13 (20)
52	477 ± 19 (20)	468 ± 14 (22)	450 ± 19 (20)	468 ± 20 (22)	466 ± 19 (20)	455 ± 14 (20)

[a]BPA doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXIV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIV. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 27. Female Postwean Body Weights (g), Vehicle and BPA Stop-Dose, Terminal (2 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
4	57 ± 2[*]	56 ± 2	54 ± 2	53 ± 1	52 ± 2[b]	54 ± 1
	(50)	(50)	(48)	(50)	(50)	(46)
8	190 ± 4	190 ± 2	189 ± 2	193 ± 2	187 ± 4	190 ± 2
	(50)	(50)	(48)	(50)	(50)	(46)
12	270 ± 5	270 ± 3	268 ± 3	271 ± 4	267 ± 5	270 ± 4
	(50)	(50)	(48)	(50)	(50)	(46)
16	312 ± 7	312 ± 4	307 ± 5	310 ± 5	305 ± 6	311 ± 5
	(50)	(50)	(48)	(50)	(50)	(46)
20	338 ± 8	339 ± 5	330 ± 5	334 ± 5	330 ± 7	338 ± 5
	(50)	(50)	(46)	(50)	(50)	(46)
24	359 ± 8	361 ± 5	349 ± 6	353 ± 6	350 ± 7	358 ± 6
	(50)	(50)	(48)	(50)	(49)	(46)
28	379 ± 10	375 ± 6	365 ± 7	370 ± 7	366 ± 8	376 ± 6
	(50)	(50)	(48)	(50)	(49)	(46)
32	393 ± 10	393 ± 6	380 ± 7	384 ± 8	381 ± 8	392 ± 7
	(50)	(48)	(48)	(50)	(49)	(46)
36	409 ± 10	409 ± 7	397 ± 8	403 ± 9	394 ± 9	409 ± 8
	(50)	(50)	(48)	(50)	(49)	(46)
40	420 ± 11	428 ± 8	414 ± 9	418 ± 10	411 ± 10	427 ± 9
	(43)	(47)	(44)	(44)	(47)	(44)
44	436 ± 11	446 ± 9	426 ± 9	435 ± 10	426 ± 10	445 ± 9
	(47)	(50)	(48)	(50)	(48)	(46)
48	457 ± 12	466 ± 10	446 ± 10	451 ± 10	444 ± 11	463 ± 10
	(49)	(50)	(48)	(50)	(49)	(46)
52	479 ± 12	489 ± 10	462 ± 10	474 ± 11	459 ± 11	477 ± 11
	(49)	(49)	(47)	(50)	(49)	(46)
56	497 ± 13	506 ± 10	482 ± 11	496 ± 12	480 ± 12	495 ± 12
	(49)	(49)	(47)	(50)	(49)	(45)
60	516 ± 14	524 ± 11	502 ± 11	515 ± 12	496 ± 12	513 ± 14
	(48)	(46)	(47)	(50)	(48)	(44)
64	536 ± 15	549 ± 13	520 ± 12	537 ± 12	517 ± 13	533 ± 15
	(48)	(45)	(47)	(50)	(48)	(43)
68	558 ± 16	573 ± 13	540 ± 13	546 ± 13	537 ± 14	550 ± 15
	(47)	(44)	(43)	(46)	(47)	(39)
72	571 ± 18	580 ± 13	552 ± 14	561 ± 14	556 ± 16	552 ± 14
	(44)	(39)	(41)	(44)	(45)	(35)
76	570 ± 19	598 ± 14	568 ± 15	574 ± 15	568 ± 16	575 ± 16
	(36)	(40)	(39)	(41)	(41)	(35)
80	576 ± 18	619 ± 15	575 ± 15	586 ± 18	580 ± 17	589 ± 16
	(34)	(39)	(35)	(36)	(38)	(35)
84	588 ± 21	633 ± 18	601 ± 18	605 ± 19	591 ± 19	604 ± 19
	(31)	(32)	(30)	(34)	(36)	(31)
88	595 ± 22	624 ± 20	599 ± 18	614 ± 22	606 ± 21	613 ± 20
	(28)	(26)	(26)	(31)	(33)	(29)
92	591 ± 28	626 ± 23	590 ± 21	626 ± 24	609 ± 24	625 ± 22
	(23)	(20)	(20)	(28)	(31)	(26)
96	600 ± 33	608 ± 20	595 ± 25	651 ± 29	633 ± 28	616 ± 24
	(20)	(16)	(16)	(23)	(24)	(22)
100	600 ± 30	625 ± 21	614 ± 28	650 ± 37	614 ± 34	610 ± 29
	(17)	(16)	(15)	(18)	(19)	(17)
104	608 ± 45	631 ± 23	595 ± 25	630 ± 39	641 ± 35	623 ± 35
	(11)	(13)	(13)	(13)	(17)	(13)

[a]BPA doses are µg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control

group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXV. The only significant effect is indicated with an asterisk (dose trend, week 4, $p = 0.037$).

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods), the mean for week 4, 2,500 μg BPA/kg bw/day was significantly different (approximately 12% lower, $p = 0.016$) from the vehicle control mean.

Table 28. Male Postwean Body Weights (g), Vehicle, BPA, and EE_2 Continuous-Dose, Interim (1 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
4	84 ± 2 (22)	83 ± 2 (22)	85 ± 2 (20)	81 ± 2 (24)	83 ± 2 (20)	80 ± 1 (22)	84 ± 2 (26)	88 ± 2 (26)
8	298 ± 7 (22)	299 ± 7 (22)	296 ± 7 (20)	288 ± 7 (24)	296 ± 8 (20)	290 ± 5 (21)	298 ± 7 (26)	304 ± 5 (26)
12	424 ± 9 (22)	429 ± 8 (22)	429 ± 10 (20)	416 ± 8 (24)	426 ± 11 (20)	417 ± 8 (21)	431 ± 9 (26)	437 ± 6 (26)
16	494 ± 10 (22)	505 ± 9 (22)	500 ± 11 (20)	490 ± 8 (24)	496 ± 12 (20)	484 ± 9 (21)	504 ± 10 (26)	505 ± 8 (26)
20	540 ± 10 (22)	554 ± 11 (22)	553 ± 13 (19)	541 ± 10 (24)	542 ± 13 (20)	532 ± 10 (21)	551 ± 12 (25)	550 ± 10 (24)
24	578 ± 12 (22)	590 ± 12 (22)	586 ± 14 (19)	576 ± 12 (24)	579 ± 14 (20)	568 ± 12 (21)	588 ± 13 (25)	584 ± 9 (26)
28	604 ± 13 (21)	621 ± 12 (22)	612 ± 15 (19)	607 ± 12 (24)	610 ± 16 (20)	596 ± 13 (21)	613 ± 14 (25)	611 ± 10 (26)
32	627 ± 14 (21)	643 ± 13 (22)	636 ± 16 (19)	633 ± 12 (24)	635 ± 18 (20)	620 ± 14 (21)	636 ± 14 (25)	634 ± 10 (26)
36	645 ± 15 (21)	667 ± 14 (22)	653 ± 17 (19)	653 ± 13 (24)	656 ± 19 (20)	639 ± 14 (21)	660 ± 15 (25)	652 ± 12 (25)
40	664 ± 17 (20)	690 ± 16 (22)	673 ± 18 (19)	674 ± 14 (24)	675 ± 21 (19)	659 ± 15 (21)	681 ± 16 (24)	673 ± 13 (25)
44	688 ± 16 (19)	707 ± 17 (22)	689 ± 19 (19)	691 ± 14 (24)	693 ± 21 (19)	676 ± 17 (21)	697 ± 17 (25)	687 ± 13 (25)
48	702 ± 18 (19)	724 ± 20 (22)	715 ± 20 (18)	712 ± 15 (24)	706 ± 24 (18)	693 ± 17 (21)	712 ± 19 (24)	715 ± 14 (23)
52	720 ± 20 (18)	742 ± 21 (22)	729 ± 22 (18)	732 ± 16 (24)	724 ± 26 (18)	714 ± 19 (21)	730 ± 21 (22)	724 ± 16 (23)

[a]BPA and EE_2 doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXIV. Analyses were conducted separately for BPA and EE_2 dose groups. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIV. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 29. Male Postwean Body Weights (g), Vehicle, BPA, And EE_2 Continuous-Dose, Terminal (2 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE_2	0.5 EE_2
4	84 ± 1	84 ± 1	86 ± 1	84 ± 1	82 ± 1	85 ± 1	82 ± 2	88 ± 2
	(50)	(48)	(48)	(50)	(50)	(46)	(26)	(26)
8	295 ± 4	302 ± 4	297 ± 4	290 ± 4	292 ± 4	305 ± 4	297 ± 5	306 ± 5
	(50)	(48)	(48)	(50)	(50)	(46)	(26)	(26)
12	429 ± 7	434 ± 5	425 ± 5	419 ± 5	420 ± 5	435 ± 5	428 ± 8	431 ± 8
	(49)	(47)	(48)	(50)	(50)	(46)	(26)	(26)
16	503 ± 10	506 ± 6	496 ± 6	492 ± 6	496 ± 6	505 ± 6	504 ± 10	502 ± 10
	(49)	(47)	(48)	(50)	(50)	(46)	(26)	(26)
20	550 ± 11	554 ± 7	546 ± 8	543 ± 6	543 ± 7	552 ± 6	553 ± 11	549 ± 12
	(49)	(45)	(48)	(50)	(50)	(46)	(26)	(26)
24	586 ± 12	591 ± 7	583 ± 8	581 ± 7	577 ± 8	586 ± 7	586 ± 13	581 ± 11
	(49)	(47)	(48)	(50)	(50)	(46)	(26)	(26)
28	614 ± 14	620 ± 8	609 ± 9	612 ± 7	603 ± 9	614 ± 8	614 ± 13	608 ± 13
	(49)	(46)	(48)	(50)	(50)	(46)	(26)	(24)
32	639 ± 16	643 ± 9	631 ± 10	641 ± 8	625 ± 9	637 ± 8	637 ± 14	632 ± 13
	(49)	(46)	(48)	(50)	(50)	(46)	(26)	(26)
36	647 ± 10	663 ± 10	654 ± 10	661 ± 9	643 ± 9	656 ± 9	658 ± 16	655 ± 14
	(48)	(46)	(47)	(50)	(50)	(46)	(26)	(26)
40	667 ± 10	685 ± 10	672 ± 11	682 ± 10	660 ± 9	674 ± 9	678 ± 16	673 ± 15
	(48)	(46)	(48)	(49)	(50)	(46)	(26)	(26)
44	684 ± 11	700 ± 10	691 ± 12	703 ± 11	678 ± 10	687 ± 10	696 ± 17	692 ± 16
	(47)	(45)	(47)	(49)	(49)	(46)	(26)	(26)
48	700 ± 12	717 ± 11	707 ± 13	729 ± 11	696 ± 10	707 ± 10	712 ± 18	710 ± 17
	(47)	(45)	(47)	(48)	(49)	(44)	(26)	(26)
52	719 ± 13	732 ± 12	719 ± 12	746 ± 10	712 ± 11	727 ± 11	726 ± 20	726 ± 18
	(47)	(45)	(44)	(47)	(48)	(43)	(26)	(26)
56	736 ± 13	750 ± 13	745 ± 14	765 ± 11	726 ± 12	750 ± 12	754 ± 20	742 ± 18
	(47)	(45)	(46)	(46)	(47)	(41)	(24)	(26)
60	748 ± 13	768 ± 13	756 ± 16	784 ± 12	743 ± 12	770 ± 13	783 ± 19	760 ± 18
	(47)	(45)	(45)	(45)	(46)	(40)	(23)	(26)
64	765 ± 14	791 ± 14	779 ± 17	803 ± 13	755 ± 14	791 ± 14	803 ± 21	771 ± 20
	(46)	(44)	(44)	(45)	(46)	(40)	(23)	(25)
68	783 ± 15	805 ± 14	800 ± 19	818 ± 14	784 ± 15	815 ± 16	828 ± 22	788 ± 26
	(45)	(42)	(44)	(43)	(43)	(37)	(22)	(24)
72	792 ± 17	821 ± 15	822 ± 20	838 ± 15	799 ± 16	832 ± 17	848 ± 24	806 ± 24
	(42)	(41)	(40)	(40)	(42)	(37)	(22)	(22)
76	801 ± 19	840 ± 16	842 ± 20	858 ± 15	817 ± 17	848 ± 19	864 ± 24	832 ± 24
	(41)	(40)	(39)	(39)	(40)	(34)	(22)	(21)
80	819 ± 19	858 ± 18	846 ± 22	868 ± 17	828 ± 17	862 ± 20	873 ± 27	855 ± 25
	(38)	(38)	(36)	(39)	(37)	(31)	(20)	(20)
84	827 ± 21	867 ± 19	858 ± 25	890 ± 19	833 ± 16	875 ± 21	889 ± 29	854 ± 25
	(34)	(37)	(29)	(36)	(33)	(29)	(18)	(19)
88	826 ± 23	865 ± 19	867 ± 25	894 ± 20	829 ± 18	871 ± 21	917 ± 28	870 ± 27
	(30)	(35)	(27)	(31)	(30)	(25)	(15)	(16)
92	823 ± 27	858 ± 22	873 ± 26	905 ± 24	819 ± 17	868 ± 22	925 ± 31	872 ± 27
	(26)	(31)	(25)	(24)	(27)	(24)	(14)	(17)
96	831 ± 30	864 ± 24	883 ± 23	914 ± 24	834 ± 19	860 ± 27	925 ± 34	873 ± 28
	(23)	(25)	(22)	(24)	(24)	(16)	(13)	(16)
100	823 ± 36	854 ± 26	856 ± 25	904 ± 29	846 ± 22	894 ± 23	926 ± 42	888 ± 30
	(20)	(21)	(19)	(22)	(20)	(14)	(10)	(15)
104	818 ± 40	877 ± 25	847 ± 30	946 ± 36	842 ± 22	864 ± 12	901 ± 53	853 ± 23
	(18)	(16)	(17)	(14)	(16)	(12)	(9)	(13)

[a]BPA and EE_2 doses are µg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the

vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXV. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 30. Male Postwean Body Weights (g), Vehicle and BPA Stop-Dose, Interim (1 Year) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
4	61 ± 2	58 ± 3	59 ± 3	60 ± 3	57 ± 3	61 ± 2
	(20)	(20)	(20)	(19)	(20)	(22)
8	274 ± 7	269 ± 8	271 ± 6	270 ± 9	261 ± 6	277 ± 4
	(20)	(20)	(20)	(19)	(20)	(22)
12	424 ± 11	432 ± 9	426 ± 7	430 ± 12	413 ± 8	436 ± 8
	(20)	(20)	(20)	(19)	(20)	(22)
16	505 ± 14	529 ± 12	512 ± 9	515 ± 14	503 ± 11	522 ± 9
	(20)	(20)	(20)	(19)	(20)	(22)
20	561 ± 15	587 ± 14	572 ± 11	566 ± 15	560 ± 12	581 ± 10
	(20)	(20)	(19)	(19)	(20)	(22)
24	597 ± 16	628 ± 16	610 ± 12	603 ± 16	593 ± 11	618 ± 11
	(20)	(20)	(19)	(19)	(20)	(22)
28	627 ± 18	658 ± 17	642 ± 14	630 ± 17	623 ± 14	645 ± 12
	(20)	(20)	(18)	(19)	(20)	(22)
32	652 ± 19	685 ± 18	671 ± 15	655 ± 18	651 ± 15	667 ± 12
	(20)	(20)	(19)	(19)	(20)	(22)
36	675 ± 21	710 ± 19	701 ± 18	677 ± 19	674 ± 17	685 ± 13
	(20)	(20)	(17)	(19)	(20)	(22)
40	684 ± 22	737 ± 21	717 ± 18	687 ± 18	692 ± 18	709 ± 14
	(18)	(19)	(19)	(18)	(20)	(22)
44	717 ± 23	761 ± 21	738 ± 19	716 ± 20	712 ± 19	726 ± 15
	(20)	(20)	(19)	(19)	(20)	(22)
48	731 ± 25	783 ± 23	758 ± 22	735 ± 22	735 ± 20	743 ± 16
	(20)	(19)	(19)	(19)	(20)	(22)
52	753 ± 26	803 ± 23	775 ± 23	743 ± 23	751 ± 22	759 ± 17
	(20)	(20)	(19)	(18)	(20)	(22)

[a]BPA doses are μg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXIV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXIV. There were no statistically significant trends or pairwise comparisons to controls. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 31. Male Postwean Body Weights (g), Vehicle and BPA Stop-Dose, Terminal (2 Years) Sacrifice (Mean ± S.E.M.)[a]

Week	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
4	61 ± 2*	60 ± 2	59 ± 2	56 ± 2	58 ± 2	57 ± 2
	(50)	(48)	(48)	(50)	(50)	(46)
8	270 ± 4	273 ± 5	281 ± 4	279 ± 4	271 ± 4	268 ± 5
	(49)	(48)	(48)	(50)	(50)	(46)
12	428 ± 5	425 ± 6	437 ± 6	433 ± 5	426 ± 6	428 ± 7
	(49)	(48)	(48)	(50)	(50)	(46)
16	509 ± 6	508 ± 7	522 ± 8	515 ± 7	511 ± 6	515 ± 8
	(49)	(48)	(48)	(50)	(50)	(46)
20	567 ± 6	564 ± 8	582 ± 10	572 ± 8	569 ± 7	574 ± 9
	(49)	(48)	(48)	(50)	(50)	(43)
24	606 ± 7	602 ± 9	619 ± 10	613 ± 9	608 ± 8	612 ± 10
	(49)	(48)	(48)	(50)	(49)	(45)
28	637 ± 8	630 ± 10	648 ± 11	642 ± 10	635 ± 8	639 ± 10
	(48)	(48)	(45)	(50)	(49)	(45)
32	664 ± 9	655 ± 10	673 ± 12	665 ± 10	661 ± 9	664 ± 11
	(47)	(48)	(48)	(50)	(49)	(45)
36	688 ± 10	681 ± 11	698 ± 12	687 ± 11	683 ± 9	688 ± 12
	(49)	(48)	(48)	(50)	(49)	(45)
40	704 ± 10	695 ± 11	724 ± 14	705 ± 14	700 ± 10	710 ± 14
	(47)	(44)	(44)	(44)	(46)	(42)
44	732 ± 12	724 ± 12	746 ± 14	731 ± 14	723 ± 10	732 ± 15
	(46)	(48)	(47)	(50)	(48)	(43)
48	752 ± 12	745 ± 13	763 ± 15	749 ± 15	744 ± 11	755 ± 16
	(49)	(48)	(48)	(50)	(49)	(43)
52	772 ± 13	763 ± 14	781 ± 15	757 ± 14	756 ± 12	770 ± 17
	(49)	(47)	(48)	(49)	(49)	(43)
56	789 ± 14	778 ± 15	800 ± 16	773 ± 15	778 ± 13	792 ± 18
	(49)	(47)	(48)	(49)	(49)	(42)
60	811 ± 15	799 ± 17	813 ± 17	798 ± 16	793 ± 14	810 ± 19
	(48)	(46)	(46)	(46)	(49)	(42)
64	831 ± 16	815 ± 16	832 ± 18	816 ± 17	815 ± 15	826 ± 20
	(48)	(44)	(46)	(45)	(47)	(42)
68	855 ± 16	834 ± 18	846 ± 19	837 ± 17	836 ± 16	848 ± 22
	(46)	(43)	(46)	(45)	(45)	(39)
72	874 ± 17	851 ± 19	861 ± 22	837 ± 19	858 ± 18	866 ± 22
	(46)	(42)	(43)	(42)	(44)	(35)
76	884 ± 17	870 ± 21	896 ± 22	874 ± 19	869 ± 18	878 ± 24
	(44)	(39)	(40)	(37)	(41)	(34)
80	888 ± 18	883 ± 23	913 ± 25	892 ± 22	875 ± 20	893 ± 30
	(43)	(39)	(38)	(33)	(40)	(29)
84	896 ± 17	873 ± 22	920 ± 25	895 ± 24	879 ± 18	915 ± 32
	(39)	(35)	(36)	(30)	(37)	(26)
88	906 ± 18	885 ± 26	932 ± 23	908 ± 30	874 ± 21	918 ± 34
	(37)	(31)	(33)	(26)	(34)	(23)
92	903 ± 16	879 ± 25	911 ± 22	935 ± 35	887 ± 23	955 ± 28
	(32)	(27)	(31)	(21)	(30)	(20)
96	903 ± 19	874 ± 28	901 ± 26	941 ± 44	884 ± 27	925 ± 30
	(29)	(25)	(27)	(17)	(27)	(16)
100	928 ± 20	876 ± 29	893 ± 30	953 ± 49	878 ± 26	932 ± 31
	(21)	(18)	(21)	(13)	(25)	(14)
104	908 ± 23	845 ± 30	908 ± 33	941 ± 45	863 ± 33	894 ± 37
	(16)	(16)	(16)	(13)	(16)	(10)

[a]BPA doses are µg/kg bw/day. Numbers of litters are shown in parentheses. Outlier identification and exclusion are described in Materials and Methods and Supplemental Appendix XXV. Pairwise comparisons of means were performed using contrasts within a two-way repeated measures, mixed model ANOVA. Model terms were treatment group, weeks, and the interaction. Within-group correlations were modeled using a heterogeneous first-order autoregressive (ARH(1)) correlation structure, which allows for correlated differences in variability across time points. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Pairwise comparisons of treatment groups to the vehicle control

group were conducted with Dunnett's method to adjust for multiple comparisons. All tests were performed as two-sided tests at the 0.05 significance level. Full results of the analyses are presented in Supplemental Appendix XXV. The only significant effect is indicated with an asterisk (dose trend, week 4, $p = 0.043$). There were no additional significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 32. Vaginal Opening, Age and Body Weight (Means ± S.E.M.) at Occurrence, Vehicle, BPA, and EE_2 Continuous-Dose[a]

Endpoint	Vehicle (26)[b]	2.5 BPA (25)	25 BPA (24)	250 BPA (25)	2500 BPA (25)	25000 BPA (24)	0.05 EE_2 (25)	0.5 EE_2 (21)
Age, days	35.9 ± 1.1	35.2 ± 0.7	36.5 ± 0.8	37.8 ± 1.4	34.1 ± 0.5	35.4 ± 0.6	35.5 ± 0.7	34.8 ± 2.8
Body weight, g	120.5 ± 4.6	117.1 ± 5.0	128.6 ± 4.6	131.1 ± 5.8	109.6 ± 2.6	121.0 ± 4.3	123.0 ± 4.8	117.1 ± 11.8

[a]BPA and EE_2 doses are μg/kg bw/day. Twenty-six females (13 cages) from the 2-year continuous-dose groups were scheduled to be assessed for age and weight at vaginal opening and undergo vaginal cytology later in the study to evaluate estrous cyclicity. There were no litter mates among these animals. Several dose groups have less than 26 animals due to either failure to record the information or delayed start of monitoring for vaginal opening. These incidents were documented in protocol deviations. BPA and EE_2 dose groups were analyzed separately. Analyses were performed using contrasts within a one-way ANOVA to test for treatment effect. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Comparisons of dosed groups to vehicle control for age and body weight were performed with Dunnett's method for adjusted contrasts. All tests were performed as two-sided tests at the 0.05 significance level. The full statistical report is found in Supplemental Appendix XXVI. There were no statistically significant treatment effects. There were no additional significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).
[b]Numbers in parentheses are the number of animals examined.

Table 33. Vaginal Opening, Age and Body Weight (Means ± S.E.M.) at Occurrence, Vehicle and BPA Stop-Dose[a]

Endpoint	Vehicle (26)[b]	2.5 BPA (26)	25 BPA (25)	250 BPA (26)	2500 BPA (26)	25000 BPA (26)
Age, days	41.1 ± 1.8	42.1 ± 2.5	40.0 ± 1.5	39.6 ± 1.2	42.4 ± 1.2	38.0 ± 1.3
Body weight, g	–	–	–	–	–	–

[a]BPA doses are μg/kg bw/day. Twenty-six females (13 cages) from the 2-year stop-dose groups were scheduled to be assessed for age and weight at vaginal opening and undergo vaginal cytology later in the study to evaluate estrous cyclicity. There were no litter mates among these animals. The 25 μg BPA/kg bw/day dose group had one animal for which the date of vaginal opening was not recorded. Due to a technical error, 31 animals in the stop-dose arm did not have body weights recorded on the day of vaginal opening and thus this endpoint was not analyzed (Supplemental Appendix II, protocol deviations #72–74). Analysis was performed using contrasts within a one-way ANOVA to test for treatment effect. Tests of trends, increasing treatment effect with increasing dose, were performed for the BPA and vehicle control groups. Comparisons of dosed groups to vehicle control for age and body weight were performed with Dunnett's method for adjusted contrasts. All tests were performed as two-sided tests at the 0.05 significance level. The full statistical report is found in Supplemental Appendix XXVI. There were no statistically significant treatment effects. There were no additional significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).
[b]Numbers in parentheses are the number of animals examined.

Table 34. Estrous Cycle Analysis, Vehicle, BPA, and EE_2 Continuous-Dose[a]

Endpoint	Vehicle (26)[b]	2.5 BPA (25)	25 BPA (26)	250 BPA (25)	2500 BPA (26)	25000 BPA (25)	0.05 EE_2 (26)	0.5 EE_2 (26)
# readable smears	362	348	359	348	362	348	363	361
% Diestrus	57.7	54.0	55.2	49.4	59.9	55.7	54.3	15.5
% Proestrus	13.8	14.9	11.1	17.2	13.8	12.6	11.0	0.8
% Estrus	28.5	31.0	33.7	33.3	26.2	31.6	34.7	83.7
Cycle length, days[c]	4.37 ± 0.18[d]	4.56 ± 0.29[d]	4.47 ± 0.21[e]	5.20 ± 0.51[e]	4.33 ± 0.15[e]	4.84 ± 0.31[f]	4.72 ± 0.33[d]	5.58 ± 0.64[g]
Abnormal Diestrus[h]								
Abnormal	4	2	4	1	4	5	6	2
Normal	22	23	22	24	22	20	20	24
% Abnormal	15.4	8.0	15.4	4.0	15.4	20.0	23.1	7.7
Abnormal Estrus[h]								
Abnormal	3	3	4	4	2	4	4	25
Normal	23	22	22	21	24	21	22	1
% Abnormal	11.5	12.0	15.4	16.0	7.7	16.0	15.4	96.2***
Abnormal Proestrus[h]								
Abnormal	0	0	0	1	1	1	0	0
Normal	26	25	26	24	25	24	26	26
% Abnormal	0	0	0	4.0	3.8	4.0	0	0
Combined Abnormal								
Abnormal	7	5	8	5	7	9	10	26
Normal	19	20	18	20	19	16	16	0
% Abnormal	26.9	20.0	30.8	20.0	26.9	36.0	38.5	100***

[a]BPA and EE_2 doses are μg/kg bw/day. At 16 ± 2 weeks, daily vaginal smears were collected for 14 consecutive days from 26 animals (13 cages) assigned to the continuous-dose study arm, two-year sacrifice. There were no litter mates among the animals.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]Cycle length: first day of estrus in one sequence of contiguous days to the first day of estrus in the following sequence of stages. For cycle length, cycle days were defined from the first day of estrus in one sequence of contiguous days to the first day of estrus in the following sequence of stages. Cycles were considered censored if the last stage of data collection was either diestrus or proestrus. The number of animals, n, for this endpoint includes all animals with at least one uncensored cycle.

[d]n = 23.

[e]n = 24.

[f]n = 22.

[g]n = 12.

[h]Abnormal (extended) diestrus was defined as four or more consecutive days of diestrus; abnormal estrus was defined as three or more consecutive days of estrus; and extended proestrus was defined as two or more consecutive days of proestrus. The Cochran-Armitage trend test (one-sided) was performed, and Fisher's exact test (two-sided) was conducted for comparisons of dosed groups to control. *P*-values for pairwise comparisons were corrected using Holm's method, and unadjusted *p*-values are also presented in the full statistical report (Supplemental Appendix XXVII). BPA and EE_2 dose groups were analyzed separately. Statistically significant treatment effects are shown with asterisks and were confined to the high EE_2 dose group; ***, $p < 0.001$. There were no additional significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

Table 35. Estrous Cycle Analysis, Vehicle and BPA Stop-Dose[a]

Endpoint	Vehicle (26)[b]	2.5 BPA (26)	25 BPA (26)	250 BPA (26)	2500 BPA (26)	25000 BPA (26)
# readable smears	360	360	361	363	362	359
% Diestrus	56.4	60.3	52.4	51.2	58.3	58.8
% Proestrus	13.1	9.2	14.4	15.2	13.8	10.3
% Estrus	30.6	30.6	33.2	33.6	27.9	30.9
Cycle length, days[c]	4.08 ± 0.12[d]	4.23 ± 0.13[e]	4.17 ± 0.12[f]	4.47 ± 0.23[g]	4.42 ± 0.15	4.38 ± 0.17[h]
Abnormal Diestrus[i]						
Abnormal	5	5	2	1	4	5
Normal	21	21	24	25	22	21
% Abnormal	19.2	19.2	7.7	3.8	15.4	19.2
Abnormal Estrus[i]						
Abnormal	5	2	3	5	2	3
Normal	21	24	23	21	24	23
% Abnormal	19.2	7.7	11.5	19.2	7.7	11.5
Abnormal Proestrus[i]						
Abnormal	2	1	1	0	0	0
Normal	24	25	25	26	26	26
% Abnormal	7.7	3.8	3.8	0	0	0
Combined Abnormal						
Abnormal	10	7	5	6	6	8
Normal	16	19	21	20	20	18
% Abnormal	38.5	26.9	19.2[j]	23.1	23.1	30.8

[a]BPA doses are µg/kg bw/day. At 16 ± 2 weeks, daily vaginal smears were collected for 14 consecutive days from 26 animals (13 cages) assigned to the stop-dose study arm, two-year sacrifice. There were no litter mates among the animals.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]Cycle length: first day of estrus in one sequence of contiguous days to the first day of estrus in the following sequence of stages. For cycle length, cycle days were defined from the first day of estrus in one sequence of contiguous days to the first day of estrus in the following sequence of stages. Cycles were considered censored if the last stage of data collection was either diestrus or proestrus. The number of animals, n, for this endpoint includes all animals with at least one uncensored cycle.

[d]n = 20.

[e]n = 22.

[f]n = 23.

[g]n = 25.

[h]n = 24.

[i]Abnormal (extended) diestrus was defined as four or more consecutive days of diestrus; abnormal estrus was defined as three or more consecutive days of estrus; and extended proestrus was defined as two or more consecutive days of proestrus. The Cochran-Armitage trend test (one-sided) was performed, and Fisher's exact test (two-sided) was conducted for comparisons of dosed groups to control. *P*-values for pairwise comparisons were corrected using Holm's method, and unadjusted *p*-values are also presented in the full statistical report (Supplemental Appendix XXVII). There were no statistically significant treatment effects.

[j]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods), there was a significant difference for combined abnormal for BPA stop-dose 25 µg/kg bw/day compared to the vehicle control group ($p = 0.038$). The proportion of total % abnormal in the 25 µg BPA/kg bw/day stop-dose group was lower than in the vehicle control (5.0% abnormal in the dosed group compared to 33.3% abnormal in the control).

Table 36. Time to Onset of Aberrant Estrous Cycles in Vehicle, BPA, and EE_2 Continuous-Dose Groups[a]

Statistic	Vehicle (26)[b]	2.5 BPA (25)	25 BPA (25)	250 BPA (25)	2500 BPA (26)	25000 BPA (25)	0.05 EE_2 (26)	0.5 EE_2 (26)
% uncensored	88	96	100	84	81	96	88	23
% aberrant at start (left censored)	4	0	0	8	4	4	4	77
% with normal cycles at removal (right censored)	8	4	0	8	15	0	8	0
Median onset of aberrant cycles, weeks (lower and upper 95% confidence intervals)	56.8 (42.0, 66.9)	47.0 (36.9, 52.0)	51.9 (42.1, 56.9)	56.9 (46.9, 61.9)	52.0 (46.9, 56.7)	46.9 (41.7, 56.9)	51.8 (37, 62.1)	21.9*** (21.7, 22)
p-value	-	0.74	0.80	0.79	0.80	0.79	0.36	<0.001***

[a]BPA and EE_2 doses are µg/kg bw/day. One month after the collection of the 14 consecutive vaginal smears to evaluate the estrous cycle (Table 34), the same animals from the continuous-dose, two-year study arm were monitored monthly for cycling status with five consecutive daily vaginal smears. The criteria for declaring an animal as having an aberrant estrous cycle were 3 or more consecutive days of estrus (E, E/D, or P/E) or five consecutive days that did not include an estrus. The animal was no longer monitored after two consecutive months with an aberrant cycle, and the time of onset of aberrant cycling was defined as having occurred at the first swab date of two consecutive months of aberrant estrous cycle data. Separate analyses were conducted for the BPA and EE_2 dose groups. An accelerated failure time model assuming a lognormal distribution was used for analysis, and multiple comparisons were adjusted using Holm's method for treatment comparisons to the control. All tests were performed as two-sided tests at the 0.05 significance level. The full statistical report is found in Supplemental Appendix XXVIII. Statistically significant results are marked with asterisks (***, $p < 0.001$). There were no additional significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods).

[b]Numbers in parentheses are the number of animals examined.

Table 37. Time to Onset of Aberrant Estrous Cycles in Vehicle and BPA Stop-Dose Groups[a]

Statistic	Vehicle (26)[b]	2.5 BPA (26)	25 BPA (26)	250 BPA (26)	2500 BPA (25)	25000 BPA (26)
% uncensored	88	77	96	88	80	92
% aberrant at start (left censored)	0	8	0	8	4	0
% with normal cycles at removal (right censored)	12	15	4	4	16	8
Median onset of aberrant cycles, weeks (lower and upper 95% confidence intervals)	41.9 (41.3, 51.7)	51.7 (36.9, 57.0)	46.8 (41.9, 56.9)	51.9 (41.9, 56.9)	56.9* (51.7, 66.6)	52.1 (41.9, 61.9)
p-value	-	1.00	0.83	1.00	0.03*	0.52

[a]BPA doses are µg/kg bw/day. One month after the collection of the 14 consecutive vaginal smears to evaluate the estrous cycle (Table 35), the same animals from the stop-dose two-year study arm were monitored monthly for cycling status with five consecutive daily vaginal smears. The criteria for declaring an animal as having an aberrant estrous cycle were 3 or more consecutive days of estrus (E, E/D, or P/E) or five consecutive days that did not include an estrus. The animal was no longer monitored after two consecutive months with an aberrant cycle, and the time of onset of aberrant cycling was defined as having occurred at the first swab date of two consecutive months of aberrant estrous cycle data. An accelerated failure time model assuming a lognormal distribution was used for analysis, and multiple comparisons were adjusted using Holm's method for treatment comparisons to the control. All tests were performed as two-sided tests at the 0.05 significance level. The full statistical report is found in Supplemental Appendix XXVIII. Statistically significant results are marked with an asterisk (*, $p < 0.05$). There were no additional significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Materials and Methods, Statistical Methods).
[b]Number in parentheses are the number of animals examined.

Table 38. Female Hematology, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (21)[b]	2.5 BPA (22)	25 BPA (21)	250 BPA (22)	2500 BPA (20)	25000 BPA (24)	0.05 EE_2 (24)	0.5 EE_2 (26)
Hematocrit, %	47.3 ± 0.4	46.0 ± 0.4	45.7 ± 0.5	47.4 ± 0.5	46.8 ± 0.5	47.5 ± 0.3	47.1 ± 0.5	46.7 ± 0.8
Hemoglobin, g/dL	16.4 ± 0.1*	16.0 ± 0.1	16.0 ± 0.2	16.5 ± 0.2	16.3 ± 0.2	16.5 ± 0.1	16.4 ± 0.2	16.3 ± 0.2
Red Blood Cells, $10^6/mm^3$	8.3 ± 0.1	8.2 ± 0.1	8.1 ± 0.1	8.5 ± 0.1	8.3 ± 0.1	8.4 ± 0.1	8.3 ± 0.1	8.2 ± 0.1
% Reticulocytes	1.3 ± 0.1	1.3 ± 0	1.2 ± 0	1.4 ± 0.1	1.3 ± 0.1	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Packed Cell Volume, %	47.3 ± 0.4	46.0 ± 0.4	45.9 ± 0.5	47.4 ± 0.5	46.9 ± 0.5	47.5 ± 0.3	47.2 ± 0.5	46.9 ± 0.7
Mean Corpuscular Volume, μm^3	57.0 ± 0.4	56.5 ± 0.3	56.9 ± 0.4	56.0 ± 0.5	56.4 ± 0.4	56.6 ± 0.3	56.5 ± 0.3	56.8 ± 0.3
Mean Corpuscular Hemoglobin, pg	19.7 ± 0.2	19.6 ± 0.1	19.9 ± 0.2	19.4 ± 0.2	19.6 ± 0.1	19.7 ± 0.1	19.7 ± 0.1	19.8 ± 0.1
Mean Corpuscular Hemoglobin Concentration, g/dL	34.6 ± 0.1	34.8 ± 0.1	35.1 ± 0.2**	34.8 ± 0.1	34.7 ± 0.1	34.9 ± 0.1	34.8 ± 0.1	34.9 ± 0.1
Platelets, $10^3/mm^3$	650.4 ± 21.3**	651.5 ± 28.5	651.0 ± 22.8	635.1 ± 21.5	633.1 ± 16.2	585.9 ± 20.5*	598.1 ± 17.4[c]	597.7 ± 18.9*
White Blood Cells, $10^3/mm^3$	8.4 ± 0.3	7.5 ± 0.3	7.9 ± 0.4	7.8 ± 0.4	8.1 ± 0.4	7.6 ± 0.3	8.0 ± 0.4	7.8 ± 0.4
Neutrophils, $10^3/mm^3$	2.1 ± 0.2	2.1 ± 0.1	2.1 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	1.8 ± 0.2	2.1 ± 0.2	2.0 ± 0.1
% Neutrophils	24.9 ± 2.3	29.6 ± 2.6	26.0 ± 1.4	25.6 ± 1.7	26.1 ± 1.6	23.8 ± 1.2	25.7 ± 1.5	25.6 ± 1.0
Lymphocytes, $10^3/mm^3$	5.5 ± 0.3	4.6 ± 0.3	4.9 ± 0.3	5.0 ± 0.3	5.2 ± 0.3	5.1 ± 0.2	5.2 ± 0.3	5.1 ± 0.2
% Lymphocytes	66.1 ± 2.2	61.0 ± 2.5	62.9 ± 1.8	64.7 ± 1.8	64.6 ± 1.5	67.6 ± 1.5	65.4 ± 1.6	66.5 ± 1.0
Monocytes, $10^3/mm^3$	0.6 ± 0.1*	0.6 ± 0.1	0.8 ± 0.1	0.7 ± 0.1	0.7 ± 0.1	0.6 ± 0.1	0.6 ± 0	0.5 ± 0
% Monocytes	7.4 ± 0.4	7.9 ± 0.6	9.5 ± 0.9[d]	8.4 ± 0.8	8.0 ± 0.6	7.1 ± 0.5	7.2 ± 0.4	6.6 ± 0.4
Basophils, $10^3/mm^3$	0.01 ± 0	0.01 ± 0	0.02 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0	0.01 ± 0
% Basophils	0.2 ± 0	0.2 ± 0	0.3 ± 0.1	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
Eosinophils, $10^3/mm^3$	0.12 ± 01	0.09 ± 0.01	0.11 ± 0.01	0.09 ± 0.01*	0.10 ± 0.01	0.10 ± 0.01	0.12 ± 0.01	0.09 ± 0.01*
% Eosinophils	1.4 ± 0.1	1.3 ± 0.1	1.4 ± 0.1	1.1 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.5 ± 0.2	1.1 ± 0.1*

[a]BPA and EE_2 doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The average of the left and right ranks was used for ties. The five BPA treatments were compared to the vehicle control within each sex and dosing regimen. Similarly, the EE_2 reference estrogen control treatments were compared to the vehicle control. Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$). Asterisks in the vehicle column indicate a significant trend in the BPA dose groups versus the vehicle control group. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

[c]Platelet numbers in 0.05 EE_2 dose group significantly different from vehicle control ($p = 0.002$) in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

[d]% monocytes in 25 BPA dose group significantly different from vehicle control ($p = 0.033$) in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

Table 39. Female Hematology, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (22)	25 BPA (20)	250 BPA (22)	2500 BPA (20)	25000 BPA (19)
Hematocrit, %	46.9 ± 0.4	47.1 ± 0.4	47.3 ± 0.6	46.9 ± 0.5	46.6 ± 0.7	47.4 ± 0.4
Hemoglobin, g/dL	16.2 ± 0.1	16.4 ± 0.1	16.4 ± 0.2	16.2 ± 0.2	16.1 ± 0.2	16.3 ± 0.2
Red Blood Cells, $10^6/mm^3$	8.3 ± 0.1*	8.3 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.4 ± 0.1	8.5 ± 0.1
% Reticulocytes	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.4 ± 0.1
Packed Cell Volume, %	47.0 ± 0.4	47.2 ± 0.4	47.3 ± 0.6	47.0 ± 0.5	46.6 ± 0.7	47.4 ± 0.4
Mean Corpuscular Volume, μm^3	56.2 ± 0.3	57.0 ± 0.4	56.3 ± 0.4	55.7 ± 0.5	55.7 ± 0.4	55.6 ± 0.3
Mean Corpuscular Hemoglobin, pg	19.5 ± 0.1*	19.9 ± 0.1	19.5 ± 0.2	19.3 ± 0.2	19.2 ± 0.2	19.1 ± 0.1
Mean Corpuscular Hemoglobin Concentration, g/dL	34.6 ± 0.1	34.8 ± 0.1	34.6 ± 0.1	34.6 ± 0.1	34.5 ± 0.1	34.4 ± 0.1
Platelets, $10^3/mm^3$	645.1 ± 32.6	586.7 ± 21.6	592.6 ± 29.1	594.0 ± 26.2	646.1 ± 36.2	621.1 ± 19.5
White Blood Cells, $10^3/mm^3$	8.0 ± 0.4	7.3 ± 0.4	8.2 ± 0.5	8.3 ± 0.4	8.7 ± 1.2	7.1 ± 0.4
Neutrophils, $10^3/mm^3$	2.0 ± 0.1	1.7 ± 0.2	1.9 ± 0.2	2.1 ± 0.2	2.5 ± 0.7	1.7 ± 0.1
% Neutrophils	24.8 ± 1.1	23.5 ± 1.3	23.4 ± 1.2	25.1 ± 1.8	25.5 ± 1.7	24.1 ± 1.3
Lymphocytes, $10^3/mm^3$	5.3 ± 0.3	4.9 ± 0.3	5.4 ± 0.3	5.3 ± 0.3	5.4 ± 0.4	4.6 ± 0.3
% Lymphocytes	66.3 ± 1.3	67.0 ± 1.6	66.3 ± 1.3	64.9 ± 1.8	65.6 ± 2.1	66.1 ± 1.7
Monocytes, $10^3/mm^3$	0.6 ± 0	0.6 ± 0	0.7 ± 0.1	0.7 ± 0.1	0.8 ± 0.2	0.6 ± 0.1
% Monocytes	7.4 ± 0.5	8.0 ± 0.6	8.8 ± 0.8	8.4 ± 0.8	7.6 ± 0.8	8.5 ± 0.7
Basophils, $10^3/mm^3$	0.01 ± 0	0.01 ± 0	0.02 ± 0	0.02 ± 0.01	0.01 ± 0	0.01 ± 0
% Basophils	0.1 ± 0*	0.2 ± 0	0.2 ± 0	0.3 ± 0.1	0.1 ± 0	0.1 ± 0
Eosinophils, $10^3/mm^3$	0.11 ± 0.01	0.1 ± 0.01	0.1 ± 0.01	0.12 ± 0.01	0.09 ± 0.01	0.09 ± 0.01
% Eosinophils	1.35 ± 0.11	1.34 ± 0.13	1.28 ± 0.08	1.37 ± 0.09	1.17 ± 0.12	1.27 ± 0.1

[a]BPA doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The five BPA treatments were compared to the vehicle control, and Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Asterisks in the vehicle column indicate a significant trend (*, $p < 0.05$). Statistical significance was assessed at the 0.05 level. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

Table 40. Male Hematology, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (18)[b]	2.5 BPA (22)	25 BPA (18)	250 BPA (24)	2500 BPA (18)	25000 BPA (21)	0.05 EE_2 (22)	0.5 EE_2 (23)
Hematocrit, %	47.7 ± 0.4**	47.7 ± 0.5	46.9 ± 0.6	47.9 ± 0.4	47.8 ± 0.5	49.0 ± 0.5	48.8 ± 0.4	48.6 ± 0.3
Hemoglobin, g/dL	16.1 ± 0.1*	16.3 ± 0.2	16.0 ± 0.2	16.3 ± 0.1	16.3 ± 0.2	16.7 ± 0.2*	16.6 ± 0.1*	16.5 ± 0.1
Red Blood Cells, $10^6/mm^3$	9.3 ± 0.1	9.3 ± 0.1	9.3 ± 0.1	9.4 ± 0.1	9.4 ± 0.1	9.4 ± 0.1	9.4 ± 0.1	9.5 ± 0.1
% Reticulocytes	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0	1.3 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Packed Cell Volume, %	47.7 ± 0.4**	47.7 ± 0.5	47.1 ± 0.6	47.9 ± 0.4	47.8 ± 0.5	49.0 ± 0.5	48.7 ± 0.4	48.6 ± 0.3
Mean Corpuscular Volume, μm^3	51.2 ± 0.2*	51.5 ± 0.3	50.5 ± 0.3	51.1 ± 0.3	51.0 ± 0.4	52.3 ± 0.5	51.7 ± 0.3	51.4 ± 0.3
Mean Corpuscular Hemoglobin, pg	17.3 ± 0.1*	17.6 ± 0.1	17.2 ± 0.1	17.4 ± 0.1	17.5 ± 0.2	17.8 ± 0.2	17.6 ± 0.1	17.4 ± 0.1
Mean Corpuscular Hemoglobin Concentration, g/dL	33.9 ± 0.1	34.1 ± 0.1	34.1 ± 0.1	34.1 ± 0.1	34.2 ± 0.1	34.0 ± 0.1	34.0 ± 0.1	34.0 ± 0.1
Platelets, $10^3/mm^3$	751.3 ± 32.6*	716.2 ± 25.1[c]	749.1 ± 45.3[d]	702.3 ± 37.3	754.3 ± 18.1	671 ± 28.4	709.7 ± 26.7	740.6 ± 22.3
White Blood Cells, $10^3/mm^3$	9.8 ± 0.5	10.3 ± 0.3	9.8 ± 0.4	10.5 ± 0.3	9.9 ± 0.6	9.4 ± 0.5	10.5 ± 0.5	11.1 ± 0.5
Neutrophils, $10^3/mm^3$	2.3 ± 0.2	2.4 ± 0.2	2.2 ± 0.2	2.5 ± 0.2	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.5 ± 0.1
% Neutrophils	22.9 ± 0.8	23.4 ± 1.4	22.5 ± 1.2	23.5 ± 1.0	22.1 ± 1.8	23.3 ± 1.2	21.4 ± 1.2	22.4 ± 0.9
Lymphocytes, $10^3/mm^3$	6.6 ± 0.4	6.8 ± 0.3	6.5 ± 0.3	6.9 ± 0.2	6.9 ± 0.5	6.2 ± 0.4	7.1 ± 0.4	7.5 ± 0.4
% Lymphocytes	67.0 ± 1.1	66.0 ± 1.5	66.4 ± 1.8	66.0 ± 1.5	69.0 ± 1.8	65.9 ± 1.3	67.8 ± 1.4	67.5 ± 1.3
Monocytes, $10^3/mm^3$	0.8 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	0.7 ± 0.1	0.9 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
% Monocytes	8.2 ± 0.9	8.6 ± 0.7	9.5 ± 0.9	9.0 ± 0.8	7.5 ± 0.7	9.1 ± 0.6	9.3 ± 0.7	8.6 ± 0.6
Basophils, $10^3/mm^3$	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.01 ± 0	0.02 ± 0	0.02 ± 0	0.03 ± 0.01
% Basophils	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.1 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0.1
Eosinophils, $10^3/mm^3$	0.17 ± 0.02	0.18 ± 0.02	0.15 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.14 ± 0.02	0.14 ± 0.01	0.15 ± 0.01
% Eosinophils	1.71 ± 0.14	1.74 ± 0.23	1.53 ± 0.13	1.23 ± 0.1*	1.29 ± 0.09	1.44 ± 0.16	1.37 ± 0.11	1.33 ± 0.1

[a]BPA and EE_2 doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The average of the left and right ranks was used for ties. The five BPA treatments were compared to the vehicle control within each sex and dosing regimen. Similarly, the EE_2 reference estrogen control treatments were compared to the vehicle control. Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$). Asterisks in the vehicle column indicate a significant trend. Asterisks in the BPA or EE_2 dose group columns indicate significant differences in pairwise comparisons to the vehicle group. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions or missing data are indicated by footnotes.

[c]n = 21 in 2.5 µg BPA/kg bw/day platelet count due to missing data (no result reported) for one animal.

[d]n = 17 in 25 µg BPA/kg bw/day platelet count due to missing data (no result reported) for one animal.

Table 41. Male Hematology, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (19)[b]	2.5 BPA (20)	25 BPA (19)	250 BPA (19)	2500 BPA (20)	25000 BPA (22)
Hematocrit, %	48.5 ± 0.5	48.2 ± 0.4	47.5 ± 0.6	48.0 ± 0.6	47.0 ± 0.8	46.8 ± 1.0
Hemoglobin, g/dL	16.4 ± 0.2	16.3 ± 0.1	16.1 ± 0.2	16.2 ± 0.2	15.8 ± 0.3	15.9 ± 0.3
Red Blood Cells, $10^6/mm^3$	9.5 ± 0.1	9.4 ± 0.1	9.3 ± 0.13	9.5 ± 0.1	9.2 ± 0.2	9.1 ± 0.2
% Reticulocytes	1.3 ± 0.1	1.2 ± 0.1	1.3 ± 0.1	1.2 ± 0.1	1.2 ± 0.1	1.2 ± 0.1
Packed Cell Volume, %	48.5 ± 0.5	48.2 ± 0.4	47.6 ± 0.6	48.0 ± 0.6	46.9 ± 0.8	46.8 ± 1.0
Mean Corpuscular Volume, μm^3	51.3 ± 0.3	51.2 ± 0.4	51.2 ± 0.3	50.7 ± 0.4	51.7 ± 1.0	51.4 ± 0.5
Mean Corpuscular Hemoglobin, pg	17.3 ± 0.1	17.3 ± 0.1	17.4 ± 0.1	17.1 ± 0.2	17.4 ± 0.3	17.5 ± 0.2
Mean Corpuscular Hemoglobin Concentration, g/dL	33.7 ± 0.1[c]	33.8 ± 0.1	33.9 ± 0.1	33.6 ± 0.1	33.7 ± 0.1	33.9 ± 0.1
Platelets, $10^3/mm^3$	753.5 ± 24.6	785.7 ± 24.1	769.0 ± 37.7	712.8 ± 35.2	742.3 ± 12.2	764.3 ± 30.3
White Blood Cells, $10^3/mm^3$	10.5 ± 0.4	11.7 ± 1.0	10.3 ± 0.4	10.9 ± 0.6	10.8 ± 0.5	11.4 ± 0.8
Neutrophils, $10^3/mm^3$	2.4 ± 0.2	3.5 ± 0.6	2.7 ± 0.3	2.8 ± 0.3	2.3 ± 0.1	2.4 ± 0.2
% Neutrophils	23.9 ± 2.0*	28.4 ± 2.3	25.9 ± 2.2	25.2 ± 2.1	21.0 ± 0.8	21.8 ± 1.6
Lymphocytes, $10^3/mm^3$	7.1 ± 0.45	6.9 ± 0.4	6.4 ± 0.3	7.0 ± 0.5	7.5 ± 0.35	7.7 ± 0.6
% Lymphocytes	66.2 ± 2.2	60.9 ± 2.3	62.7 ± 2.2	63.5 ± 2.1	69.5 ± 0.9	67.5 ± 1.7
Monocytes, $10^3/mm^3$	0.9 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.0 ± 0.1	0.9 ± 0.1	1.1 ± 0.2
% Monocytes	8.3 ± 1.0	9.1 ± 0.8	9.6 ± 0.7	9.8 ± 1.0	8.1 ± 0.6	8.9 ± 0.9
Basophils, $10^3/mm^3$	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0
% Basophils	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0	0.2 ± 0
Eosinophils, $10^3/mm^3$	0.15 ± 0.01	0.16 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.17 ± 0.01
% Eosinophils	1.46 ± 0.12	1.42 ± 0.09	1.58 ± 0.13	1.39 ± 0.13	1.23 ± 0.12	1.54 ± 0.11

[a]BPA doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The five BPA treatments were compared to the vehicle control, and Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. An asterisk in the vehicle column indicates a significant trend (*, $p < 0.05$) versus the vehicle control. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

[c]Significant trend ($p = 0.04$) for mean corpuscular hemoglobin concentration in sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

Table 42. Female Clinical Chemistry, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (21)[b]	2.5 BPA (22)	25 BPA (21)	250 BPA (22)	2500 BPA (20)	25000 BPA (24)	0.05 EE_2 (24)	0.5 EE_2 (26)
Urea nitrogen, mg/dL	14.4 ± 0.6	13.5 ± 0.3	14.0 ± 0.4	15.5 ± 0.6	14.5 ± 0.7	14.4 ± 0.4	14.5 ± 0.5	15.6 ± 0.6
Creatinine, mg/dL	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0	0.5 ± 0
Total protein, mg/dL	7.5 ± 0.1	7.6 ± 0.1	7.8 ± 0.1	7.4 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.7 ± 0.1
Albumin, g/dL	4.1 ± 0.1	4.0 ± 0.1	4.1 ± 0.1	4.0 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.1	4.2 ± 0.0
Alkaline phosphatase, U/L	54.3 ± 2.8	62.9 ± 3.6	72.1 ± 7.3	71.0 ± 5.5*	63.9 ± 3.6	58.1 ± 3.6	67.6 ± 3.8*	68.1 ± 5.5
Alanine aminotransferase, U/L	31.0 ± 3.0	37.5 ± 6.8	37.6 ± 4.8	34.2 ± 2.5	32.0 ± 2.3	29.0 ± 1.8	30.5 ± 1.6	37.0 ± 2.6
Aspartate aminotransferase, U/L	90.8 ± 8.0	82.5 ± 4.7	86.7 ± 10.6	76.2 ± 3.7	79.2 ± 4.5	78.9 ± 6.0	78.5 ± 3.7	80.3 ± 3.6
Sorbitol dehydrogenase, U/L	27.4 ± 4.2	30.5 ± 5.7	33.2 ± 5.5	24.1 ± 2.1	22.3 ± 2.1	24.2 ± 3.1	26.8 ± 2.0	19.4 ± 2.0
Gamma-glutamyl transferase, U/L	4.4 ± 0.3	4.1 ± 0.3	4.6 ± 0.3	4.0 ± 0.3	4.2 ± 0.3	4.2 ± 0.3	3.8 ± 0.2[c]	3.9 ± 0.3
Total bile acids, µmol/L	47.0 ± 5.8	53.5 ± 9.9	49.7 ± 5.3	53.0 ± 5.3	51.4 ± 5.7	42.3 ± 5.6	50.6 ± 7.1	72.0 ± 9.1
Cholesterol, mg/dL	109.5 ± 4.4	111.7 ± 7.2	128.9 ± 11.1	107.6 ± 4.6	111.3 ± 7.6	107.7 ± 8.4	127.5 ± 6.6	121.5 ± 4.9
Glucose, mg/dL	130.0 ± 6.0	126.0 ± 4.8	130.2 ± 5	124.8 ± 4.8	122.1 ± 4.3	136.1 ± 6.6	128.8 ± 4.5	120.6 ± 3.3
Triglycerides, ng/mL	266 ± 24.3	258.6 ± 24.3	299.6 ± 41.5	237.5 ± 30.3	330.7 ± 40.3	331.6 ± 67.7	282.9 ± 34.5	369.4 ± 39.2
Insulin, mg/mL	1.5 ± 0.2	2.2 ± 0.4	2.0 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	2.1 ± 0.5	2.0 ± 0.3	1.3 ± 0.1
Leptin, ng/mL	19.3 ± 2.4	27.8 ± 3.5	24.5 ± 3.1	18.5 ± 2.5	19.8 ± 2.1	20.7 ± 2.7	23.7 ± 2.9	17.9 ± 1.8
Troponin T, pg/mL[d]	10.3 ± 1.6	8.0 ± 1.7	10.2 ± 2.2	6.6 ± 1.9	5.9 ± 1.5	6.5 ± 1.9	6.3 ± 1.4	6.9 ± 1.9
	18/21 (86%)[e,f]	*13/22 (59%)*	*14/21 (67%)*	*12/22 (55%)*	*12/20 (60%)*	*13/24 (54%)*	*15/24 (62%)*	*13/26 (50%)*
T3, ng/dL	71.6 ± 3.5	72.1 ± 4.1	79.2 ± 4.3	70.8 ± 3	78.2 ± 3.5	73.3 ± 2.5	79.3 ± 2.6	78.0 ± 3.5
T4, µg/dL	3.8 ± 0.2[g]	3.9 ± 0.2	3.8 ± 0.2	3.8 ± 0.2	3.9 ± 0.2	4.1 ± 0.2	4.2 ± 0.2	3.8 ± 0.2
TSH, ng/mL	3.7 ± 0.7	4.4 ± 0.9	3.6 ± 0.4	4.2 ± 0.4	4.9 ± 0.7	4.5 ± 0.4	3.4 ± 0.3	5.1 ± 0.5*

[a]BPA and EE_2 doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The average of the left and right ranks was used for ties. The five BPA treatments were compared to the vehicle control within each sex and dosing regimen. Similarly, the EE_2 reference estrogen control treatments were compared to the vehicle control. Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Statistically significant effects are indicated by asterisks. Asterisks in the BPA or EE_2 dose group columns indicate significant differences in pairwise comparisons to the vehicle group (*, $p < 0.05$). Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions or missing data are indicated by footnotes.

[c]Gamma-glutamyl transferase in 0.05 µg EE_2 /kg bw/day group significantly different from vehicle control ($p = 0.018$) in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

[d]*Means were calculated using ½ the limit of detection (LOD, 1.0 pg/mL) for samples below LOD.* Troponin I was also measured, but the overall percentage of samples above the limit of detection was less than 5%, so no statistical analysis was conducted.

[e]*Number of samples with troponin T levels above LOD/total number of samples. Percentage of detects is given in parenthesis. Because of the high percentage of non-detects, the proportion of samples with detectable troponin T were statistically analyzed rather than means or medians. No significant trends across BPA dose groups or pairwise comparisons to vehicle control were detected.*

[f]ERRATUM: An error was identified in the NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats (RR-9). The method of calculation of mean Troponin T, number of samples with detectable levels of Troponin T, and statistical analysis approach were not originally included in the Table. This error has been corrected; the new information is italicized.

[g]n = 20 in vehicle T4 assay due to insufficient quantity of serum for assay for one animal.

Table 43. Female Clinical Chemistry, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (22)	25 BPA (20)	250 BPA (22)	2500 BPA (20)	25000 BPA (19)
Urea nitrogen, mg/dL	13.9 ± 0.5	14.0 ± 0.5	14.5 ± 0.5	14.1 ± 0.5	14.3 ± 0.5	14.3 ± 0.5
Creatinine, mg/dL	0.5 ± 0.0	0.5 ± 0.0	0.5 ± 0.0	0.4 ± 0.0	0.5 ± 0.0	0.5 ± 0.0
Total protein, mg/dL	7.4 ± 0.1	7.7 ± 0.1	7.6 ± 0.1	7.6 ± 0.1	7.5 ± 0.1	7.7 ± 0.1
Albumin, g/dL	4.1 ± 0.1**	4.2 ± 0.1	4.0 ± 0.0	4.0 ± 0.1	4.0 ± 0.1	4.2 ± 0.0
Alkaline phosphatase, U/L	67.2 ± 3.8	75.4 ± 5.3	72.9 ± 3.8	62.5 ± 3.0	69.0 ± 6.0	83.1 ± 7.2
Alanine aminotransferase, U/L	29.2 ± 2.0	33.3 ± 2.9	35.2 ± 3.8	29.5 ± 2.0	29.7 ± 2.8	35.4 ± 3.6
Aspartate aminotransferase, U/L	77.5 ± 3.1	81.5 ± 5.7	82.8 ± 6.5	72.4 ± 3.3	76.5 ± 4.0	86.5 ± 7.9
Sorbitol dehydrogenase, U/L	24.5 ± 3.0[c]	29.1 ± 3.5	31.8 ± 4.6	28.0 ± 1.9	22.3 ± 2.4	31.2 ± 2.7
Gamma-glutamyl transferase, U/L	4.4 ± 0.3	4.3 ± 0.3	3.6 ± 0.4	3.8 ± 0.3	3.8 ± 0.3	3.9 ± 0.3
Total bile acids, µmol/L	43.3 ± 4.1	48.9 ± 5.8	56.5 ± 6.1	44.8 ± 4.2	46.0 ± 4.7	59.2 ± 7.5
Cholesterol, mg/dL	116.9 ± 6.7	116.0 ± 7.4	108.6 ± 6.7	111.9 ± 5.1	113.4 ± 4.4	125.8 ± 12.5
Glucose, mg/dL	129.8 ± 3.8	137.2 ± 5.9	128.2 ± 4.5	128.4 ± 4.2	125.6 ± 5.4	125.4 ± 5.5
Triglycerides, ng/mL	261.2 ± 21.4	373.7 ± 68.4	253.2 ± 38.4	274.6 ± 55.8	342.2 ± 49.8	315.2 ± 27.7
Insulin, mg/mL	2.0 ± 0.3	2.6 ± 0.4	1.5 ± 0.2	2.3 ± 0.5	2.0 ± 0.3	2.1 ± 0.3
Leptin, ng/mL	24.2 ± 3.1	28.1 ± 3.4	22.2 ± 2.9	22.4 ± 2.5	22.2 ± 2.7	24.2 ± 2.8
T3, ng/dL	68.1 ± 3.3	72.1 ± 3.6	75.8 ± 3.2	71.0 ± 2.8	71.4 ± 4.1	78.7 ± 4.7
Troponin T, pg/mL[d]	7.0 ± 1.8	6.3 ± 1.5	3.6 ± 1.0	5.3 ± 1.0	6.5 ± 2.2	8.7 ± 2.9
	10/20 (50%)[e,f]	*13/22 (59%)*	*10/20 (50%)*	*12/22 (55%)*	*12/20 (60%)*	*10/19 (53%)*
T4, µg/dL	3.7 ± 0.2	3.6 ± 0.2	4.1 ± 0.2	3.7 ± 0.1	3.7 ± 0.2	3.7 ± 0.2
TSH, ng/mL	4.6 ± 0.8	3.5 ± 0.4	3.8 ± 0.5	4.8 ± 0.7	4.3 ± 0.5	4.7 ± 0.5

[a]BPA doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The five BPA treatments were compared to the vehicle control, and Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Asterisks in the vehicle column indicates a significant trend (**, $p < 0.01$) versus the vehicle control. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

[c]Significant trend ($p = 0.002$) for sorbitol dehydrogenase in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

[d]*Means were calculated using ½ the limit of detection (LOD, 1.0 pg/mL) for samples below LOD.* Troponin I was also measured, but the overall percentage of samples above the limit of detection was less than 5%, so no statistical analysis was conducted.

[e]*Number of samples with troponin T levels above LOD/total number of samples. Percentage of detects is given in parenthesis. Because of the high percentage of non-detects, the proportion of samples with detectable troponin T were statistically analyzed rather than means or medians. No significant trends across BPA dose groups or pairwise comparisons to vehicle control were detected.*

[f]ERRATUM: An error was identified in the NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats (RR-9). The method of calculation of mean Troponin T, number of samples with detectable levels of Troponin T, and statistical analysis approach were not originally included in the Table. This error has been corrected; the new information is italicized.

Table 44. Male Clinical Chemistry, Vehicle, BPA, and EE2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (18)[b]	2.5 BPA (22)	25 BPA (18)	250 BPA (24)	2500 BPA (18)	25000 BPA (21)	0.05 EE_2 (22)	0.5 EE_2 (23)
Urea nitrogen, mg/dL	14.2 ± 0.4	14.0 ± 0.3	14.3 ± 0.5	14.5 ± 0.4	14.3 ± 0.6	14.1 ± 0.3	14.4 ± 0.3	14.6 ± 0.4
Creatinine, mg/dL	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0	0.4 ± 0
Total protein, mg/dL	7.4 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.2 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.3 ± 0.1	7.2 ± 0.1
Albumin, g/dL	3.7 ± 0**	3.7 ± 0	3.6 ± 0	3.6 ± 0[c]	3.7 ± 0.1	3.7 ± 0	3.7 ± 0	3.7 ± 0
Alkaline phosphatase, U/L	130.6 ± 24.1	107.2 ± 5.5	99.9 ± 5.4	98.7 ± 3.9	107.6 ± 7.7	99.7 ± 4.9	107.3 ± 4.5	104.2 ± 5.9
Alanine aminotransferase, U/L	30.1 ± 1.6	33.8 ± 2.4	27.3 ± 2.0	29.6 ± 1.3	32.0 ± 2.3	31.0 ± 1.9	30.6 ± 2.7	33.7 ± 2.0
Aspartate aminotransferase, U/L	69.7 ± 3.2	73.4 ± 4.8	73.5 ± 6.7	68.7 ± 2.4	76.7 ± 5.7	76.3 ± 6.4	79.9 ± 8.5	71.2 ± 3.0
Sorbitol dehydrogenase, U/L	24.5 ± 2.4	26.8 ± 2.7	26.6 ± 2.7	27.4 ± 3.0	24.4 ± 2.7	25.9 ± 2.8	31.5 ± 4.8	22.8 ± 2.0
Gamma-glutamyl transferase, U/L	4.1 ± 0.3	4.6 ± 0.3	4.2 ± 0.3	4.4 ± 0.3	4.8 ± 0.4	4.4 ± 0.3	4.2 ± 0.2	3.9 ± 0.2
Total bile acids, µmol/L	32.8 ± 2.7*	33.3 ± 4.1	32.5 ± 2.4	35.8 ± 3.4	42.0 ± 4.7	28.1 ± 2.7	38.6 ± 4.3	35.6 ± 3.2
Cholesterol, mg/dL	118.0 ± 6.4	116.9 ± 4.8	118.3 ± 6.7	107.4 ± 4.4	127.3 ± 6.7	107.2 ± 5.2	120.4 ± 6.4	117.5 ± 7.1
Glucose, mg/dL	126.1 ± 4.8	125.9 ± 3.9	127.1 ± 4.4	127.3 ± 6.1	136.3 ± 5.8	119.0 ± 3.4	125.4 ± 3.8	123.7 ± 4.1
Triglycerides, ng/mL	267.9 ± 23.4	278.2 ± 19.5	276.1 ± 20.6	252.9 ± 18.7	304.6 ± 24.3	283.0 ± 13.5	266.8 ± 19.1	338.4 ± 21.8*
Insulin, mg/mL	2.0 ± 0.2	1.6 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.7 ± 0.3	1.8 ± 0.1	1.3 ± 0.1*	1.6 ± 0.1
Leptin, ng/mL	26 ± 2.5	29.2 ± 3.0	23.8 ± 2.3	26.3 ± 2.6	26.7 ± 3.0	29.4 ± 2.8	25.4 ± 2.6	27.2 ± 1.9
Troponin T, pg/mL[d]	7.4 ± 2.5	5.9 ± 1.4	9.8 ± 2.4	8.5 ± 2.2	6.4 ± 1.5	11.8 ± 2.2	5.1 ± 1.3	7.2 ± 1.6
	*11/18 (61%)[e,f]***	*10/22 (46%)*	*11/18 (61%)*	*14/24 (58%)*	*12/18 (67%)*	*17/20 (85%)*	*9/22 (41%)*	*18/23 (78%)*
T3, ng/dL	62.8 ± 2.7	57.8 ± 3.4	63.3 ± 2.3	60.5 ± 3.0	67.3 ± 4.1	67.0 ± 4.0[g]	70.5 ± 4.1	66.4 ± 3.5
T4, µg/dL	5.0 ± 0.3*	4.3 ± 0.3	5.0 ± 0.2	4.9 ± 0.2	4.7 ± 0.2	5.5 ± 0.2	5.1 ± 0.2	4.7 ± 0.2
TSH, ng/mL	3.6 ± 0.3	3.4 ± 0.3	3.4 ± 0.3	3.5 ± 0.4	3.5 ± 0.4	3.5 ± 0.4	4.1 ± 0.3	3.2 ± 0.4

[a]BPA and EE_2 doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The average of the left and right ranks was used for ties. The five BPA treatments were compared to the vehicle control within each sex and dosing regimen. Similarly, the EE_2 reference estrogen control treatments were compared to the vehicle control. Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$). Asterisks in the vehicle column indicate a significant trend in the BPA dose groups versus the vehicle control. Asterisks in the BPA or EE_2 dose group columns indicate significant differences in pairwise comparisons to the vehicle group. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

[c]Albumin in 250 µg BPA/kg bw/day group significantly different from vehicle control ($p = 0.012$) in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

[d]*Means were calculated using ½ the limit of detection (LOD, 1.0 pg/mL) for samples below LOD*. Troponin I was also measured, but the overall percentage of samples above the limit of detection was less than 5%, so no statistical analysis was conducted.

[e]*Number of samples with troponin T levels above LOD/total number of samples. Percentage of detects is given in parenthesis. Because of the high percentage of non-detects, the proportion of samples with troponin T were statistically analyzed rather than means or medians. The asterisks indicate a significant trend across BPA dose groups for the proportion of samples with detectable troponin T.*

[f]ERRATUM: An error was identified in the NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats (RR-9). The method of calculation of mean Troponin T, number of samples with detectable levels of Troponin T, and statistical analysis approach were not originally included in the Table. This error has been corrected; the new information is italicized.

[g]n = 20 in 2,500 BPA T3 assay due to insufficient quantity of serum for assay for one animal.

Table 45. Male Clinical Chemistry, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (20)	25 BPA (19)	250 BPA (19)	2500 BPA (20)	25000 BPA (22)
Urea nitrogen, mg/dL	14.1 ± 0.4	13.7 ± 0.3	15.0 ± 1.0	13.8 ± 0.6	14.4 ± 0.4	19.6 ± 3.7
Creatinine, mg/dL	0.4 ± 0	0.4 ± 0	0.5 ± 0	0.5 ± 0	0.4 ± 0	0.6 ± 0.1
Total protein, mg/dL	7.3 ± 0.1	7.1 ± 0.1	7.0 ± 0.1*	7.2 ± 0.1	7.2 ± 0.1	7.2 ± 0.1
Albumin, g/dL	3.6 ± 0	3.6 ± 0	3.5 ± 0.1	3.6 ± 0.1	3.6 ± 0.1	3.6 ± 0.1
Alkaline phosphatase, U/L	108.8 ± 7.9	100.4 ± 4.2	104.5 ± 5.7	103.5 ± 11.3	100.5 ± 5.3	102.9 ± 6.0
Alanine aminotransferase, U/L	32.4 ± 3.1	32.3 ± 2.0	28.5 ± 1.5	32.6 ± 5.5	26.7 ± 1.2	29.0 ± 2.6
Aspartate aminotransferase, U/L	84.6 ± 8.8	85.3 ± 4.5	82.7 ± 7.4	77.2 ± 7.1	68.8 ± 3.1	69.9 ± 3.6
Sorbitol dehydrogenase, U/L	25.0 ± 2.8	27.4 ± 2.8	32.0 ± 3.2	28.9 ± 2.8	26.7 ± 2.3	28.6 ± 2.9
Gamma-glutamyl transferase, U/L	4.3 ± 0.4	4.1 ± 0.3	4.2 ± 0.4	3.7 ± 0.3	4.8 ± 0.2	4.3 ± 0.3
Total bile acids, µmol/L	36.4 ± 3.1*	34.6 ± 3.2	25.0 ± 2.7*	32.6 ± 6.5	33.7 ± 4.3	35.0 ± 2.5
Cholesterol, mg/dL	123.5 ± 5.6	115.5 ± 5.9	115.3 ± 6.7	117.8 ± 8.9	132.7 ± 4.7	147.7 ± 14.2
Glucose, mg/dL	128.7 ± 5.3	121.9 ± 3.2	130.8 ± 5.9	120.1 ± 4.1	131.3 ± 4.7	125.2 ± 3.4
Triglycerides, ng/mL	285.2 ± 20.0	252.3 ± 15.4	282.3 ± 26.1	279.9 ± 16.5	355.2 ± 22	329.8 ± 24.2
Insulin, mg/mL	1.5 ± 0.2	1.5 ± 0.2	1.9 ± 0.2	1.5 ± 0.1	1.7 ± 0.2	1.6 ± 0.2
Leptin, ng/mL	26.8 ± 3.5	26.8 ± 1.7	32.9 ± 3.7	25.5 ± 3.4	28.0 ± 3.0	28.1 ± 2.8
Troponin T, pg/mL[d]	8.0 ± 1.9	5.9 ± 1.7	4.2 ± 1.3	7.7 ± 2.3	4.8 ± 1.6	5.1 ± 1.8
	12/20 (60%)[e,f]	*8/20 (40%)*	*7/19 (37%)*	*12/19 (63%)*	*9/20 (45%)*	*8/22 (36%)*
T3, ng/dL	65.8 ± 3.1	63.3 ± 3.3	56.7 ± 3.0	60.6 ± 4.5	69.4 ± 2.9	66.6 ± 2.8
T4, µg/dL	4.9 ± 0.2*	4.8 ± 0.2	4.7 ± 0.2	4.7 ± 0.2	4.7 ± 0.3	4.2 ± 0.2[c]
TSH, ng/mL	4.0 ± 0.4	4.2 ± 0.4	3.8 ± 0.5	4.5 ± 0.5	5.0 ± 0.6	3.9 ± 0.4

[a]BPA doses are µg/kg bw/day. A non-parametric ANOVA based on mid-ranks was used to evaluate the effect of treatment. The five BPA treatments were compared to the vehicle control, and Dunnett's adjustment was used for pairwise multiple comparisons relative to the control. Orthogonal contrasts were used to test for trend over increasing BPA dose concentrations. All statistical tests are two-sided. Statistical significance was assessed at the 0.05 level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$). Asterisks in the vehicle column indicate a significant trend, while asterisks in BPA dose group columns indicate significant differences in pairwise comparisons to the vehicle group. Full results of the analyses are presented in Supplemental Appendix XXIX.

[b]Numbers in parentheses are the number of animals examined.

[c]25,000 µg BPA/kg bw/day T4 significantly different from vehicle control ($p = 0.015$) in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day.

[d]*Means were calculated using ½ the limit of detection (LOD, 1.0 pg/mL) for samples below LOD*. Troponin I was also measured, but the overall percentage of samples above the limit of detection was less than 5%, so no statistical analysis was conducted.

[e]*Number of samples with troponin T levels above LOD/total number of samples. Percentage of detects is given in parenthesis. Because of the high percentage of non-detects, the proportion of samples with detectable troponin T were statistically analyzed rather than means or medians. No significant trends across BPA dose groups or pairwise comparisons to vehicle control were detected.*

[f]ERRATUM: An error was identified in the NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats (RR-9). The method of calculation of mean Troponin T, number of samples with detectable levels of Troponin T, and statistical analysis approach were not originally included in the Table. This error has been corrected; the new information is italicized.

Table 46. Female Organ Weights, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (21)[b]	2.5 BPA (22)	25 BPA (21)	250 BPA (22)	2500 BPA (20)	25000 BPA (24)	0.05 EE_2 (24)	0.5 EE_2 (26)
Body Weight at Necropsy								
g	420 ± 19	477 ± 22	445 ± 15	411 ± 14	422 ± 13	425 ± 17	453 ± 13	423 ± 12
Adrenal								
Absolute, mg	72.4 ± 2.0	76.4 ± 2.8	74.9 ± 3.8	75.4 ± 4.7	76.5 ± 3.8	73.3 ± 3.1	75.3 ± 4.2	92.2 ± 3.8***
Ratio to Brain, mg/g	34.8 ± 1.0	36.2 ± 1.4	35.6 ± 1.7	36.2 ± 2.2	37.4 ± 1.9	35.4 ± 1.5	36.0 ± 1.9	44.7 ± 1.9***
Ratio to bw, mg/g	0.18 ± 0.01	0.16 ± 0.01	0.17 ± 0.01	0.19 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.17 ± 0.01	0.22 ± 0.01***
Brain								
Absolute, g	2.09 ± 0.02	2.11 ± 0.03	2.10 ± 0.02	2.08 ± 0.02	2.05 ± 0.03	2.08 ± 0.03	2.09 ± 0.02	2.07 ± 0.02
Ratio to bw, mg/g	5.16 ± 0.23	4.60 ± 0.19	4.82 ± 0.16	5.18 ± 0.17	4.93 ± 0.13	5.04 ± 0.18	4.71 ± 0.14	4.98 ± 0.13
Ovarian/Parametrial Fat Pad								
Absolute, g	14.1± 1.2	17.1 ± 1.0	14.4 ± 0.8	14.4 ± 0.8	14.8 ± 1.0	14.2 ± 0.9	15.5 ± 0.8	11.4 ± 0.6
Ratio to Brain, g/g	6.72 ± 0.54	8.13 ± 0.47	6.83 ± 0.35	6.93 ± 0.40	7.21 ± 0.44	6.83 ± 0.40	7.42 ± 0.35	5.50 ± 0.30
Ratio to bw, mg/g	32.3 ± 1.8	36.3 ± 1.7	32.2 ± 1.2	34.8 ± 1.2	34.7 ± 1.6	33.2 ± 1.3	34.1 ± 1.0	26.7 ± 1.0***
Retroperitoneal Fat Pad								
Absolute, g	14.1 ± 1.5	19.8 ± 2.0*	16.1 ± 1.4	14.1 ± 1.1	14.1 ± 1.2	14.7 ± 1.2	15.6 ± 1.2	13.0 ± 0.9
Ratio to Brain, g/g	6.72 ± 0.70	9.41 ± 0.97*	7.64 ± 0.67	6.76 ± 0.52	6.84 ± 0.55	7.09 ± 0.56	7.41 ± 0.58	6.26 ± 0.44
Ratio to bw, mg/g	32.4 ± 2.7	40.0 ± 2.8	35.0 ± 2.1	33.5 ± 1.7	32.9 ± 2.5	33.7 ± 1.6	33.7 ± 2.0	30.0 ± 1.5
Heart								
Absolute, g	1.31 ± 0.05	1.44 ± 0.04	1.42 ± 0.04	1.29 ± 0.03	1.31 ± 0.04	1.38 ± 0.04	1.40 ± 0.04	1.42 ± 0.04
Ratio to Brain, g/g	0.63 ± 0.02	0.68 ± 0.02	0.67 ± 0.02	0.62 ± 0.01	0.64 ± 0.02	0.66 ± 0.02	0.67 ± 0.02	0.68 ± 0.02
Ratio to bw, mg/g	3.16 ± 0.08	3.06 ± 0.08	3.21 ± 0.10	3.18 ± 0.09	3.13 ± 0.08	3.30 ± 0.08	3.13 ± 0.09	3.36 ± 0.04*
Kidney								
Absolute, g	2.27 ± 0.07	2.54 ± 0.08	2.51 ± 0.10	2.23 ± 0.06	2.31 ± 0.08	2.30 ± 0.07[c]	2.42 ± 0.08[c]	2.61 ± 0.08**
Ratio to Brain, g/g	1.09 ± 0.03	1.21 ± 0.04	1.20 ± 0.05	1.07 ± 0.03	1.12 ± 0.03	1.11 ± 0.03	1.15 ± 0.04	1.26 ± 0.04**
Ratio to bw, mg/g	5.50 ± 0.17	5.43 ± 0.17	5.69 ± 0.22	5.47 ± 0.12	5.51 ± 0.17	5.59 ± 0.13	5.34 ± 0.10	6.19 ± 0.10***
Liver								
Absolute, g	10.7 ± 0.5	12.4 ± 0.7	12.2 ± 0.5	10.8 ± 0.3	11.3 ± 0.5	11.6 ± 0.7	12.1 ± 0.4	12.8 ± 0.5**
Ratio to Brain, g/g	5.12 ± 0.24	5.90 ± 0.36	5.81 ± 0.25	5.18 ± 0.16	5.49 ± 0.21	5.60 ± 0.30	5.77 ± 0.20	6.17 ± 0.21**
Ratio to bw, mg/g	25.5 ± 0.6*	25.9 ± 0.8	27.3 ± 0.6	26.4 ± 0.6	26.7 ± 0.7	27.2 ± 0.7	26.7 ± 0.7	30.1 ± 0.5***
Ovary								
Absolute, mg	140 ± 8[d]	147 ± 7	142 ± 6[d]	138 ± 5[d]	140 ± 7[d]	140 ± 5[d]	143 ± 4[d]	115 ± 8*, [d]
Ratio to Brain, mg/g	67 ± 3	70 ± 4	68 ± 3	66 ± 2	68 ± 3	68 ± 3	68 ± 2	56 ± 4*
Ratio to bw, mg/g	0.33 ± 0.02	0.32 ± 0.02	0.33 ± 0.01	0.34 ± 0.01	0.33 ± 0.01	0.34 ± 0.02	0.32 ± 0.01	0.28 ± 0.02*
Pituitary								
Absolute, mg	20.6 ± 1.1	21.2 ± 1.3	23.5 ± 1.8	20.4 ± 1.5	20.2 ± 1.4	21.6 ± 1.1	22.7 ± 1.7	26.9 ± 1.2**
Ratio to Brain, mg/g	9.9 ± 0.5	10.0 ± 0.6	11.2 ± 0.9	9.8 ± 0.7	9.9 ± 0.7	10.4 ± 0.5	10.8 ± 0.8	13.1 ± 0.6**
Ratio to bw, mg/g	0.05 ± 0	0.05 ± 0	0.05 ± 0	0.05 ± 0	0.05 ± 0	0.05 ± 0	0.05 ± 0	0.06 ± 0**

Endpoint, Units	Vehicle (21)[b]	2.5 BPA (22)	25 BPA (21)	250 BPA (22)	2500 BPA (20)	25000 BPA (24)	0.05 EE_2 (24)	0.5 EE_2 (26)
Spleen								
Absolute, mg	598 ± 25	648 ± 27	638 ± 17	600 ± 20	642 ± 23	638 ± 26	645 ± 21	646 ± 25
Ratio to Brain, mg/g	286 ± 11	307 ± 13	304 ± 8	288 ± 9	314 ± 10	307 ± 11	308 ± 10	311 ± 11
Ratio to bw, mg/g	1.44 ± 0.05	1.37 ± 0.04	1.45 ± 0.05	1.48 ± 0.05	1.54 ± 0.05	1.52 ± 0.05	1.43 ± 0.04	1.52 ± 0.04
Thymus								
Absolute, mg	150 ± 11	185 ± 16	155 ± 12	135 ± 12	142 ± 9	151 ± 10	137 ± 10	137 ± 9
Ratio to Brain, mg/g	72 ± 5	88 ± 8	74 ± 6	65 ± 6	70 ± 5	73 ± 5	65 ± 5	66 ± 4
Ratio to bw, mg/g	0.37 ± 0.03	0.39 ± 0.03	0.35 ± 0.02	0.33 ± 0.03	0.34 ± 0.02	0.36 ± 0.02	0.30 ± 0.02	0.32 ± 0.02
Thyroid								
Absolute, mg	38.2 ± 1.7	37.4 ± 1.6	37.2 ± 1.5	35.8 ± 1.2	37.8 ± 2.0	38.5 ± 1.5	39.0 ± 1.8	36.5 ± 1.2
Ratio to Brain, mg/g	18.3 ± 0.8	17.8 ± 0.8	17.8 ± 0.7	17.2 ± 0.6	18.5 ± 1.0	18.6 ± 0.7	18.6 ± 0.8	17.7 ± 0.6
Ratio to bw, mg/g	0.09 ± 0	0.08 ± 0	0.08 ± 0	0.09 ± 0	0.09 ± 0	0.09 ± 0	0.09 ± 0	0.09 ± 0
Uterus								
Absolute, mg	773 ± 45	742 ± 52	872 ± 60[e]	757 ± 58	831 ± 51	832 ± 61	777 ± 38	827 ± 37
Ratio to Brain, mg/g	371 ± 22	354 ± 26	414 ± 26	364 ± 28	411 ± 29	403 ± 30	372 ± 19	399 ± 16
Ratio to bw, mg/g	1.92 ± 0.15	1.64 ± 0.14	1.98 ± 0.15	1.89 ± 0.16	2.05 ± 0.17	2.07 ± 0.21	1.76 ± 0.11	1.97 ± 0.09

[a]BPA and EE_2 doses are µg/kg bw/day. The indicated organs were collected from animals at the interim (one-year) necropsy and weights recorded. BPA and EE_2 groups were analyzed separately. Paired organs are presented and were analyzed as combined weights. ANOVA was performed for absolute organ weights to determine the effect of treatment on organ weight. Separate ANOCOVA were performed to determine the effect of treatment on organ weight adjusted for brain weight or receiving weight. Comparisons of dosed groups versus vehicle control were performed using Dunnett's method to adjust for multiple comparisons. Tests of trends, increasing treatment effect with increasing dose, were also performed for vehicle and BPA groups. Tests were conducted as two-sided at the 0.05 significance level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$); asterisks in the vehicle column indicate a significant trend, while asterisks in BPA or EE_2 dose group columns indicate significant differences in pairwise comparisons to the vehicle group. There were no additional statistically significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods). The complete statistical report is found in Supplemental Appendix XXX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]For kidneys, n = 23 for both the 25,000 BPA and 0.05 EE_2 dose groups.

[d]For ovaries, n = 20 for vehicle group, 17 for 25 BPA, 21 for 250 BPA, 18 for 2,500 BPA, 21 for 25,000 BPA, and 23 for both 0.05 and 0.5 EE_2 dose groups.

[e]For uterus, n = 20 for 25 BPA dose group.

Table 47. Female Organ Weights, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (22)	25 BPA (20)	250 BPA (22)	2500 BPA (20)	25000 BPA (20)
Body Weight						
g	465 ± 19	455 ± 14	437 ± 19	453 ± 19	454 ± 19	444 ± 14
Adrenal						
Absolute, mg	73.1 ± 2.9	70.0 ± 1.6	71.6 ± 2.7	71.2 ± 2.4	68.5 ± 2.9	75.6 ± 3.1
Ratio to Brain, mg/g	34.9 ± 1.3	33.4 ± 0.7	34.3 ± 1.3	34.8 ± 1.2	32.8 ± 1.3	36.5 ± 1.4
Ratio to bw, mg/g	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0	0.16 ± 0.01	0.16 ± 0.01	0.17 ± 0.01
Brain						
Absolute, g	2.09 ± 0.02	2.10 ± 0.02	2.09 ± 0.03	2.05 ± 0.02	2.09 ± 0.02	2.07 ± 0.02
Ratio to bw, mg/g	4.60 ± 0.14	4.68 ± 0.13	4.93 ± 0.20	4.68 ± 0.18	4.75 ± 0.20	4.75 ± 0.14
Ovarian/Parametrial Fat Pad						
Absolute, g	17.2 ± 1.2	16.2 ± 0.7	14.3 ± 1.0[c]	15.3 ± 1.1	16.3 ± 1.1	15.0 ± 0.9
Ratio to Brain, g/g	8.17 ± 0.53	7.72 ± 0.33	6.84 ± 0.45[c]	7.47 ± 0.51	7.79 ± 0.53	7.22 ± 0.41
Ratio to bw, mg/g	36.3 ± 1.3	35.4 ± 1.0	32.4 ± 1.1[c]	33.3 ± 1.6	35.6 ± 1.7	33.3 ± 1.1
Retroperitoneal Fat Pad						
Absolute, g	18.2 ± 1.6	15.4 ± 1.5	17.0 ± 1.8	17.1 ± 1.5	16.2 ± 1.3	16.4 ± 1.4
Ratio to Brain, g/g	8.66 ± 0.67	7.36 ± 0.73	8.10 ± 0.87	8.28 ± 0.69	7.71 ± 0.61	7.93 ± 0.68
Ratio to bw, mg/g	38.1 ± 1.8	33.3 ± 2.6	37.0 ± 2.7	36.3 ± 2.1	34.8 ± 2.0	36.0 ± 2.3
Heart						
Absolute, g	1.43 ± 0.05	1.36 ± 0.02	1.35 ± 0.04	1.37 ± 0.04	1.38 ± 0.04	1.35 ± 0.04
Ratio to Brain, g/g	0.68 ± 0.02	0.65 ± 0.01	0.65 ± 0.02	0.67 ± 0.02	0.66 ± 0.02	0.65 ± 0.02
Ratio to bw, mg/g	3.11 ± 0.08	3.02 ± 0.07	3.13 ± 0.09	3.07 ± 0.10	3.06 ± 0.07	3.06 ± 0.08
Kidney						
Absolute, g	2.47 ± 0.11	2.30 ± 0.09[d]	2.28 ± 0.09	2.41 ± 0.1[d]	2.35 ± 0.09	2.38 ± 0.08
Ratio to Brain, g/g	1.18 ± 0.04	1.10 ± 0.04	1.09 ± 0.04	1.17 ± 0.04	1.12 ± 0.04	1.15 ± 0.04
Ratio to bw, mg/g	5.34 ± 0.12	5.17 ± 0.23	5.24 ± 0.12	5.38 ± 0.15	5.22 ± 0.14	5.39 ± 0.15
Liver						
Absolute, g	12.3 ± 0.7	11.5 ± 0.4	11.2 ± 0.6	12.1 ± 0.6	12.0 ± 0.6	11.6 ± 0.4
Ratio to Brain, g/g	5.86 ± 0.28	5.52 ± 0.19	5.38 ± 0.27	5.89 ± 0.26	5.70 ± 0.25	5.63 ± 0.20
Ratio to bw, mg/g	26.4 ± 0.8	25.4 ± 0.6	25.7 ± 0.6	26.8 ± 0.8	26.4 ± 0.9	26.3 ± 0.6
Ovary						
Absolute, mg	157 ± 6[*]	149 ± 4	148 ± 6	147 ± 4	147 ± 6	136 ± 5[*]
Ratio to Brain, mg/g	75 ± 3[*]	71 ± 2	71 ± 3	72 ± 2	70 ± 3	66 ± 3[*]
Ratio to bw, mg/g	0.34 ± 0.02[*]	0.33 ± 0.01	0.35 ± 0.02	0.33 ± 0.02	0.33 ± 0.01	0.31 ± 0.01
Pituitary						
Absolute, mg	21.1 ± 1	20.3 ± 0.8	19.9 ± 1.3	20.1 ± 0.7	19 ± 0.9	20.3 ± 1.1
Ratio to Brain, mg/g	10.0 ± 0.4	9.7 ± 0.4	9.6 ± 0.7	9.8 ± 0.3	9.1 ± 0.4	9.8 ± 0.5
Ratio to bw, mg/g	0.05 ± 0	0.04 ± 0	0.05 ± 0	0.05 ± 0	0.04 ± 0	0.05 ± 0

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (22)	25 BPA (20)	250 BPA (22)	2500 BPA (20)	25000 BPA (20)
Spleen						
Absolute, mg	678 ± 30	618 ± 16	612 ± 24[e]	693 ± 24	659 ± 30	611 ± 17
Ratio to Brain, mg/g	323 ± 11	295 ± 7[e]	293 ± 11[e]	339 ± 12	315 ± 14	295 ± 8
Ratio to bw, mg/g	1.47 ± 0.05	1.37 ± 0.04	1.41 ± 0.04	1.56 ± 0.06	1.48 ± 0.07	1.40 ± 0.05
Thymus						
Absolute, mg	154 ± 7	139 ± 6[f]	142 ± 10	164 ± 14	150 ± 11	167 ± 12
Ratio to Brain, mg/g	74 ± 3	67 ± 3	68 ± 5	80 ± 7	72 ± 5	80 ± 6
Ratio to bw, mg/g	0.34 ± 0.02	0.31 ± 0.02	0.32 ± 0.02	0.37 ± 0.03	0.34 ± 0.03	0.38 ± 0.02
Thyroid						
Absolute, mg	37.8 ± 2.2	36.0 ± 1.6	36.3 ± 1.7	36.3 ± 1.3	38.2 ± 2.3	36.9 ± 1.7
Ratio to Brain, mg/g	18.0 ± 1	17.2 ± 0.8	17.4 ± 0.8	17.7 ± 0.6	18.3 ± 1.1	17.8 ± 0.8
Ratio to bw, mg/g	0.08 ± 0	0.08 ± 0	0.08 ± 0	0.08 ± 0	0.08 ± 0	0.08 ± 0
Uterus						
Absolute, mg	744 ± 46	699 ± 45	795 ± 80	843 ± 76[g]	747 ± 54	789 ± 51
Ratio to Brain, mg/g	356 ± 22	334 ± 22	382 ± 40	410 ± 35	360 ± 28	381 ± 24
Ratio to bw, mg/g	1.67 ± 0.14	1.58 ± 0.12	1.87 ± 0.19	1.93 ± 0.20	1.74 ± 0.16	1.83 ± 0.14

[a]BPA doses are µg/kg bw/day. The indicated organs were collected from animals at the interim (one-year) necropsy and weights recorded. Paired organs are presented and were analyzed as combined weights. ANOVA was performed for absolute organ weights to determine the effect of treatment on organ weight. Separate ANOCOVA were performed to determine the effect of treatment on organ weight adjusted for brain weight or receiving body weight. Comparisons of dosed groups versus vehicle control were performed using Dunnett's method to adjust for multiple comparisons. Tests of trends, increasing treatment effect with increasing dose, were also performed. Tests were conducted as two-sided at the 0.05 significance level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$); asterisks in the vehicle column indicate a significant trend, while asterisks in BPA dose group columns indicate significant differences in pairwise comparisons to the vehicle group. The complete statistical report is found in Supplemental Appendix XXX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]25 BPA ovarian/parametrial fat pad weight significantly different from vehicle control in sensitivity analysis (see Statistical Methods) that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day: absolute weight, 25.7% lower than control ($p = 0.010$); brain as covariate, 25% lower than control ($p = 0.010$); body weight as covariate, 13.7% lower than control ($p = 0.035$).

[d]n = 21 for kidneys in the 2.5 and 250 BPA dose groups. One kidney weight in each indicated dose group was excluded because kidneys had grossly observable cysts.

[e]Spleen weight statistically different from vehicle control in sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods): 2.5 BPA, brain covariate, 13.1% lower than control ($p = 0.039$); 25 BPA absolute and brain covariate, 14.2% and 13.7% lower than control, respectively ($p = 0.023$ for both).

[f]n = 21 for thymus in the 2.5 BPA dose group, one (627 mg) excluded as a statistical outlier.

[g]n = 21 for uterus in the 250 BPA dose group, one (4,680 mg) excluded as it was the only uterus noted to be fluid filled.

Table 48. Male Organ Weights, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (18)[b]	2.5 BPA (22)	25 BPA (18)	250 BPA (24)	2500 BPA (18)	25000 BPA (21)	0.05 EE_2 (22)	0.5 EE_2 (23)
Body Weight at Necropsy								
g	701 ± 19	722 ± 20	709 ± 21	713 ± 15	704 ± 26	695 ± 18	712 ± 21	704 ± 16
Adrenal								
Absolute, mg	60.1 ± 2.8	64.2 ± 2.8	68.0 ± 2.4	62.5 ± 2.1	61.7 ± 2.6	57.4 ± 1.7	62.6 ± 2.2[c]	62.9 ± 1.8
Ratio to Brain, mg/g	26.1 ± 1.2	27.8 ± 1.1	29.7 ± 1.1	27.8 ± 0.8	27.0 ± 1.0	25.7 ± 0.8	27.4 ± 0.9	27.1 ± 0.7
Ratio to bw, mg/g	0.09 ± 0	0.09 ± 0	0.10 ± 0	0.09 ± 0	0.09 ± 0	0.08 ± 0	0.09 ± 0	0.09 ± 0
Brain								
Absolute, g	2.30 ± 0.02	2.31 ± 0.03	2.30 ± 0.04	2.24 ± 0.03	2.28 ± 0.03	2.24 ± 0.02	2.29 ± 0.02	2.32 ± 0.02
Ratio to bw, mg/g	3.32 ± 0.07	3.25 ± 0.10	3.27 ± 0.08	3.18 ± 0.08	3.30 ± 0.10	3.26 ± 0.08	3.27 ± 0.09	3.32 ± 0.07
Epididymides								
Absolute, g	1.28 ± 0.04	1.21 ± 0.04	1.32 ± 0.02	1.27 ± 0.04	1.27 ± 0.04	1.25 ± 0.02	1.24 ± 0.03	1.34 ± 0.03
Ratio to Brain, g/g	0.56 ± 0.02	0.53 ± 0.02	0.58 ± 0.01	0.56 ± 0.02	0.56 ± 0.01	0.56 ± 0.01	0.54 ± 0.01	0.58 ± 0.01
Ratio to bw, mg/g	1.85 ± 0.06	1.70 ± 0.07	1.89 ± 0.06	1.79 ± 0.06	1.83 ± 0.06	1.82 ± 0.06	1.76 ± 0.06	1.90 ± 0.04
Epididymal Fat Pad								
Absolute, g	13.0 ± 0.7	14.5 ± 0.8	13.6 ± 0.6	14.1 ± 0.8	14.3 ± 1.2	14.2 ± 1.0	14.2 ± 0.8	12.8 ± 0.8
Ratio to Brain, g/g	5.62 ± 0.26	6.28 ± 0.37	5.91 ± 0.23	6.29 ± 0.39	6.24 ± 0.50	6.35 ± 0.45	6.18 ± 0.32	5.54 ± 0.33
Ratio to bw, mg/g	18.5 ± 0.8	19.8 ± 0.7	19.2 ± 0.7	19.5 ± 1.0	20.0 ± 1.1	20.2 ± 1.2	19.8 ± 0.7	18.0 ± 0.8
Retroperitoneal Fat Pad								
Absolute, g	22.2 ± 1.8	22.8 ± 1.6	24.5 ± 2.5	22.9 ± 1.9	21.8 ± 2.7	23.6 ± 2.4	22.6 ± 2.3	21.0 ± 2.3
Ratio to Brain, g/g	9.59 ± 0.74	9.88 ± 0.69	10.57 ± 1.02	10.19 ± 0.81	9.56 ± 1.21	10.59 ± 1.09	9.79 ± 0.97	9.04 ± 1.01
Ratio to bw, mg/g	31.3 ± 2.0	30.9 ± 1.5	33.7 ± 2.6	31.5 ± 2.3	29.7 ± 2.7	33.2 ± 2.9	30.9 ± 2.5	29.0 ± 2.7
Heart								
Absolute, g	2.04 ± 0.06	2.14 ± 0.05	2.26 ± 0.08	2.21 ± 0.07	2.19 ± 0.07	1.99 ± 0.04	2.11 ± 0.06	2.12 ± 0.05
Ratio to Brain, g/g	0.89 ± 0.02	0.93 ± 0.02	0.99 ± 0.04	0.98 ± 0.03	0.96 ± 0.03	0.89 ± 0.02	0.92 ± 0.02	0.91 ± 0.02
Ratio to bw, mg/g	2.92 ± 0.07	3.00 ± 0.09	3.20 ± 0.10	3.11 ± 0.10	3.14 ± 0.09	2.88 ± 0.05	2.99 ± 0.08	3.02 ± 0.08
Kidney								
Absolute, g	4.08 ± 0.15[d]	4.20 ± 0.12	4.15 ± 0.17[d]	4.05 ± 0.11	4.00 ± 0.17[d]	3.88 ± 0.08	4.01 ± 0.13[d]	4.17 ± 0.12
Ratio to Brain, g/g	1.77 ± 0.05	1.82 ± 0.05	1.80 ± 0.06	1.80 ± 0.04	1.75 ± 0.07	1.73 ± 0.04	1.75 ± 0.05	1.80 ± 0.05
Ratio to bw, mg/g	5.83 ± 0.14	5.85 ± 0.14	5.87 ± 0.15	5.70 ± 0.12	5.67 ± 0.16	5.62 ± 0.11	5.63 ± 0.10	5.91 ± 0.09
Liver								
Absolute, g	22.1 ± 0.9	21.1 ± 0.7	22.6 ± 1.0	22.4 ± 0.6	22.1 ± 1.0	20.6 ± 0.6	22.2 ± 0.7	23.1 ± 0.6
Ratio to Brain, g/g	9.58 ± 0.34	9.15 ± 0.29	9.84 ± 0.34	9.97 ± 0.26	9.71 ± 0.43	9.21 ± 0.26	9.66 ± 0.29	9.96 ± 0.29
Ratio to bw, mg/g	31.4 ± 0.6	29.3 ± 0.6[*]	31.9 ± 0.8	31.4 ± 0.5	31.4 ± 0.6	29.7 ± 0.5	31.1 ± 0.5	32.7 ± 0.4
Pituitary								
Absolute, mg	14.6 ± 0.7	14.6 ± 0.6	15.2 ± 0.6	16.1 ± 1.2	15.4 ± 0.8	15.1 ± 0.6	15.6 ± 0.6	16.0 ± 0.5
Ratio to Brain, mg/g	6.3 ± 0.2	6.3 ± 0.3	6.7 ± 0.3	7.1 ± 0.5	6.7 ± 0.4	6.7 ± 0.3	6.8 ± 0.3	6.9 ± 0.2
Ratio to bw, mg/g	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0

Endpoint, Units	Vehicle (18)[b]	2.5 BPA (22)	25 BPA (18)	250 BPA (24)	2500 BPA (18)	25000 BPA (21)	0.05 EE_2 (22)	0.5 EE_2 (23)
Seminal Vesicles								
Absolute, g	1.18 ± 0.04	1.11 ± 0.05	1.14 ± 0.05	1.17 ± 0.05	1.10 ± 0.06	1.19 ± 0.04	1.27 ± 0.06	1.12 ± 0.04
Ratio to Brain, g/g	0.51 ± 0.02	0.48 ± 0.02	0.50 ± 0.02	0.52 ± 0.02	0.48 ± 0.02	0.53 ± 0.02	0.56 ± 0.03	0.49 ± 0.02
Ratio to bw, mg/g	1.70 ± 0.06	1.56 ± 0.07	1.64 ± 0.09	1.66 ± 0.08	1.58 ± 0.10	1.73 ± 0.08	1.82 ± 0.10	1.60 ± 0.05
Spleen								
Absolute, g	0.92 ± 0.03	0.96 ± 0.03	0.97 ± 0.04	0.96 ± 0.03	0.96 ± 0.04	0.87 ± 0.03	0.94 ± 0.04	1.01 ± 0.05
Ratio to Brain, g/g	0.40 ± 0.01	0.42 ± 0.01	0.42 ± 0.02	0.43 ± 0.01	0.42 ± 0.02	0.39 ± 0.01	0.41 ± 0.02	0.44 ± 0.02
Ratio to bw, mg/g	1.33 ± 0.05	1.33 ± 0.04	1.39 ± 0.06	1.36 ± 0.04	1.36 ± 0.05	1.26 ± 0.04	1.32 ± 0.04	1.44 ± 0.07
Testes								
Absolute, g	3.59 ± 0.12	3.44 ± 0.13	3.62 ± 0.08	3.38 ± 0.14	3.56 ± 0.08	3.51 ± 0.12	3.48 ± 0.09	3.56 ± 0.09
Ratio to Brain, g/g	1.56 ± 0.05	1.50 ± 0.06	1.58 ± 0.04	1.50 ± 0.06	1.56± 0.03	1.57 ± 0.05	1.52 ± 0.04	1.54 ± 0.04
Ratio to bw, mg/g	5.16 ± 0.18	4.82 ± 0.21	5.18 ± 0.19	4.77 ± 0.21	5.12 ± 0.13	5.14 ± 0.22	4.96 ± 0.18	5.09 ± 0.13
Thymus								
Absolute, mg	150 ± 11	138 ± 9	125 ± 12	158 ± 10	140 ± 10	150 ± 11	124 ± 11	154 ± 10
Ratio to Brain, g/g	65 ± 5	60 ± 4	54 ± 5	71 ± 4	62 ± 5	67 ± 5	54 ± 5	67 ± 4
Ratio to bw, mg/g	0.22 ± 0.02	0.20 ± 0.02	0.18 ± 0.02	0.22 ± 0.01	0.20 ± 0.02	0.22 ± 0.02	0.17 ± 0.01	0.22 ± 0.01
Thyroid								
Absolute, mg	43.5 ± 2.0	42.7 ± 2.4	44.0 ± 2.5	42.2 ± 1.6	40.9 ± 1.9	44.5 ± 1.8	43.6 ± 2.0	43.7 ± 1.5
Ratio to Brain,mg/g	18.8 ± 0.8	18.5 ± 1.0	19.1 ± 1.0	18.8 ± 0.7	17.8 ± 0.7	19.9 ± 0.8	19.0 ± 0.9	18.9 ± 0.6
Ratio to bw, mg/g	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0

[a]BPA and EE_2 doses are μg/kg bw/day. The indicated organs were collected from animals at the interim (one-year) necropsy and weights recorded. BPA and EE_2 groups were analyzed separately. Paired organs are presented and were analyzed as combined weights. ANOVA was performed for absolute organ weights to determine the effect of treatment on organ weight. Separate ANOCOVA were performed to determine the effect of treatment on organ weight adjusted for brain weight or receiving weight. Comparisons of dosed groups versus vehicle control were performed using Dunnett's method to adjust for multiple comparisons. Tests of trends, increasing treatment effect with increasing dose, were also performed for vehicle and BPA groups. Tests were conducted as two-sided at the 0.05 significance level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$). There were no additional statistically significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 μg BPA/kg bw/day (see Statistical Methods). The complete statistical report is found in Supplemental Appendix XXX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]For adrenal, n = 21 for the 0.05 EE_2 dose group.

[d]For kidneys, n = 17 for vehicle, 25 BPA, and 2,500 BPA dose groups; n = 21 for 0.05 EE_2 dose group.

Table 49. Male Organ Weights, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (20)	25 BPA (19)	250 BPA (19)	2500 BPA (20)	25000 BPA (22)
Body Weight at Necropsy						
g	735 ± 26	787 ± 23	760 ± 22	733 ± 21	738 ± 21	743 ± 17
Adrenal						
Absolute, mg	63.4 ± 2.0	65.7 ± 2.1	67.9 ± 3.3	68.9 ± 2.7	65.4 ± 3.5	68.3 ± 3.3
Ratio to Brain, mg/g	27.3 ± 0.8	28.2 ± 0.9	29.3 ± 1.5	30.0 ± 1.3	28.7 ± 1.4	29.6 ± 1.3
Ratio to bw, mg/g	0.09 ± 0	0.08 ± 0	0.09 ± 0	0.10 ± 0	0.09 ± 0	0.09 ± 0
Brain						
Absolute, g	2.32 ± 0.02	2.33 ± 0.02	2.32 ± 0.03	2.30 ± 0.03	2.28 ± 0.02	2.30 ± 0.03
Ratio to bw, mg/g	3.24 ± 0.12	3.00 ± 0.08	3.10 ± 0.09	3.17 ± 0.07	3.13 ± 0.09	3.13 ± 0.08
Epididymides						
Absolute, g	1.31 ± 0.03	1.28 ± 0.03	1.30 ± 0.03	1.32 ± 0.03	1.26 ± 0.04	1.37 ± 0.02
Ratio to Brain, g/g	0.56 ± 0.01	0.55 ± 0.02	0.56 ± 0.01	0.57 ± 0.02	0.56 ± 0.02	0.60 ± 0.01
Ratio to bw, mg/g	1.83 ± 0.08	1.65 ± 0.06	1.73 ± 0.05	1.83 ± 0.07	1.73 ± 0.07	1.87 ± 0.05
Epididymal Fat Pad						
Absolute, g	13.9 ± 0.7	16.0 ± 0.9	14.9 ± 1.0	13.5 ± 0.6	14.8 ± 1.0[c]	14.6 ± 0.7
Ratio to Brain, g/g	5.99 ± 0.31	6.85 ± 0.38	6.38 ± 0.44	5.82 ± 0.26	6.52 ± 0.41	6.38 ± 0.33
Ratio to bw, mg/g	18.8 ± 0.5	20.3 ± 0.9	19.2 ± 0.9	18.3 ± 0.7	19.8 ± 0.9	19.6 ± 0.7
Retroperitoneal Fat Pad						
Absolute, g	25.0 ± 2.3	27.8 ± 2.0	25.6 ± 2.3	23.0 ± 1.7	23.4 ± 2.7	25.0 ± 2.1
Ratio to Brain, g/g	10.8 ± 1.0	11.9 ± 0.8	11.0 ± 1.0	10.0 ± 0.7	10.3 ± 1.2	10.9 ± 0.9
Ratio to bw, mg/g	33.1 ± 2.1	34.7 ± 1.6	33.0 ± 2.3	31.3 ± 2.0	31.0 ± 2.9	33.0 ± 2.2
Heart						
Absolute, g	2.27 ± 0.07	2.45 ± 0.09	2.40 ± 0.08	2.25 ± 0.08	2.22 ± 0.07	2.35 ± 0.10
Ratio to Brain, g/g	0.98 ± 0.03	1.05 ± 0.04	1.03 ± 0.04	0.97 ± 0.03	0.98 ± 0.03	1.02 ± 0.05
Ratio to bw, mg/g	3.12 ± 0.08	3.13 ± 0.09	3.18 ± 0.11	3.09 ± 0.10	3.04 ± 0.10	3.20 ± 0.16
Kidney						
Absolute, g	4.38 ± 0.15	4.43 ± 0.15	4.54 ± 0.11	4.32 ± 0.11	4.17 ± 0.15	4.33 ± 0.10[d]
Ratio to Brain, g/g	1.89 ± 0.07	1.90 ± 0.06	1.95 ± 0.05	1.88 ± 0.05	1.83 ± 0.06	1.89 ± 0.04
Ratio to bw, mg/g	5.98 ± 0.09	5.62 ± 0.09	6.01 ± 0.13	5.94 ± 0.15	5.64 ± 0.08	5.78 ± 0.09
Liver						
Absolute, g	23.6 ± 0.9	24.4 ± 0.7	25.1 ± 1.0	23.2 ± 0.8	24.8 ± 1.0	25.1 ± 0.9
Ratio to Brain, g/g	10.2 ± 0.4	10.5 ± 0.3	10.8 ± 0.4	10.1 ± 0.3	10.9 ± 0.4	10.9 ± 0.4
Ratio to bw, mg/g	32.1 ± 0.4[*]	31.1 ± 0.5	33.1 ± 1.0	31.8 ± 0.8	33.6 ± 0.7	33.9 ± 1.2
Pituitary						
Absolute, mg	15.1 ± 0.6	16.5 ± 0.9	15.3 ± 0.3	15.6 ± 0.6	14.5 ± 0.4	15.1 ± 0.5
Ratio to Brain, mg/g	6.5 ± 0.2	7.1 ± 0.4	6.6 ± 0.1	6.8 ± 0.2	6.4 ± 0.2	6.6 ± 0.2
Ratio to bw, mg/g	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0	0.02 ± 0

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (20)	25 BPA (19)	250 BPA (19)	2500 BPA (20)	25000 BPA (22)
Seminal Vesicles						
Absolute, g	1.22 ± 0.05	1.23 ± 0.06	1.27 ± 0.09	1.12 ± 0.05	1.12 ± 0.07	1.17 ± 0.04
Ratio to Brain, g/g	0.53 ± 0.02	0.53 ± 0.02	0.54 ± 0.04	0.48 ± 0.02	0.49 ± 0.03	0.51 ± 0.02
Ratio to bw, mg/g	1.69 ± 0.08	1.58 ± 0.07	1.70 ± 0.13	1.54 ± 0.07	1.52 ± 0.09	1.59 ± 0.07
Spleen						
Absolute, g	1.00 ± 0.03	1.04 ± 0.04	1.02 ± 0.03	1.03 ± 0.05	1.07 ± 0.06	1.06 ± 0.06
Ratio to Brain, g/g	0.43 ± 0.01	0.45 ± 0.02	0.44 ± 0.01	0.45 ± 0.02	0.47 ± 0.03	0.46 ± 0.02
Ratio to bw, mg/g	1.38 ± 0.05	1.33 ± 0.06	1.34 ± 0.04	1.42 ± 0.07	1.46 ± 0.08	1.44 ± 0.10
Testes						
Absolute, g	3.64 ± 0.07	3.59 ± 0.08	3.63 ± 0.09	3.49 ± 0.09	3.55 ± 0.15	3.71 ± 0.06
Ratio to Brain, g/g	1.57 ± 0.03	1.54 ± 0.04	1.57 ± 0.05	1.52 ± 0.04	1.56 ± 0.07	1.62 ± 0.03
Ratio to bw, mg/g	5.05 ± 0.17	4.62 ± 0.13	4.82 ± 0.13	4.82 ± 0.18	4.84 ± 0.18	5.04 ± 0.14
Thymus						
Absolute, mg	137 ± 9	123 ± 5	154 ± 10	130 ± 8	134 ± 9	145 ± 8
Ratio to Brain, mg/g	59 ± 4	53 ± 2	66 ± 4	57 ± 3	59 ± 4	63 ± 4
Ratio to bw, mg/g	0.19 ± 0.01	0.16 ± 0.01	0.20 ± 0.01	0.18 ± 0.01	0.18 ± 0.01	0.20 ± 0.1
Thyroid						
Absolute, mg	43.1 ± 1.9	43.4 ± 2.5	44.9 ± 1.8	41.2 ± 1.9	42.2 ± 2.4	45.4 ± 2.7
Ratio to Brain, mg/g	18.6 ± 0.8	18.6 ± 1.1	19.4 ± 0.8	18.0 ± 0.9	18.6 ± 1.0	19.7 ± 1.1
Ratio to bw, mg/g	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0	0.06 ± 0

[a]BPA doses are µg/kg bw/day. The indicated organs were collected from animals at the interim (one-year) necropsy and weights recorded. Paired organs are presented and were analyzed as combined weights. ANOVA was performed for absolute organ weights to determine the effect of treatment on organ weight. Separate ANOCOVA were performed to determine the effect of treatment on organ weight adjusted for brain weight or receiving weight. Comparisons of dosed groups versus vehicle control were performed using Dunnett's method to adjust for multiple comparisons. Tests of trends, increasing treatment effect with increasing dose, were also performed for vehicle and BPA groups. Tests were conducted as two-sided at the 0.05 significance level. Statistically significant effects are indicated by asterisks (*, $p < 0.05$); asterisks in the vehicle column indicate a significant trend. There were no additional statistically significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods). The complete statistical report is found in Supplemental Appendix XXX.

[b]Numbers in parentheses are the number of animals examined. Any deviations from this number due to exclusions are indicated by footnotes.

[c]For epididymal fat pad, n = 19 for 2,500 BPA dose group.

[d]For kidney, n = 20 for 25,000 BPA dose group.

Table 50. Sperm Analysis, Vehicle, BPA, and EE_2 Continuous-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (18)[b]	2.5 BPA (22)	25 BPA (18)	250 BPA (24)	2500 BPA (18)	25000 BPA (21)	0.05 EE_2 (22)	0.5 EE_2 (23)
Testicular spermatid heads, 10^6/g	83.4 ± 9.5	76.8 ± 7.7	88.5 ± 6.1	81.5 ± 6.8	85.1 ± 7.1	76.6 ± 6.0	70.0 ± 4.7	73.7 ± 4.5
Cauda sperm counts, 10^6/g	991 ± 78	999 ± 88	1076 ± 68	998 ± 69	1062 ± 51	1027 ± 81	892 ± 72	857 ± 53
Cauda sperm, % Motility	65.9 ± 4.6	64.0 ± 5.1	72.4 ± 3.1	66.7 ± 4.5	69.9 ± 3.4	69.4 ± 4.2	67.2 ± 4.8	70.8 ± 2.7
Cauda sperm, head, abnormal counts per animal	0.00 ± 0	0.05 ± 0.05	0.06 ± 0.06	0.08 ± 0.06	0.11 ± 0.08	0.00 ± 0	0.23 ± 0.10	0.04 ± 0.04
Cauda sperm, tail, abnormal counts per animal	0.17 ± 0.10	0.36 ± 0.13	0.17 ± 0.10	0.08 ± 0.06	0.11 ± 0.08	0.14 ± 0.08	0.23 ± 0.10	0.13 ± 0.08
Cauda sperm, head and tail combined, abnormal counts per animal	0.17 ± 0.10	0.41 ± 0.14	0.22 ± 0.11	0.17 ± 0.08	0.22 ± 0.11	0.14 ± 0.08	0.45 ± 0.14	0.17 ± 0.09

[a]BPA and EE_2 doses are µg/kg bw/day. Values presented are means ± S.E.M. BPA and EE_2 groups were analyzed separately. Testicular spermatid head counts, cauda sperm counts, and percent sperm motility were analyzed using an ANOVA model. Analysis of sperm morphology data was performed using a generalized linear model with a Poisson distribution and a log link function. Pairwise comparisons to the vehicle control group were adjusted for multiple comparisons using Dunnett's method. Tests of trends, increasing treatment effect with increasing dose, were also performed for vehicle and BPA groups only. All tests were conducted as two-sided at the 0.05 significance level. There were no significant treatment effects. There were also no significant treatment effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods). The full statistical report is found in Supplemental Appendix XXXI.
[b]Numbers in parentheses are numbers of animals examined.

Table 51. Sperm Analysis, Vehicle and BPA Stop-Dose, Interim Sacrifice (Mean ± S.E.M.)[a]

Endpoint, Units	Vehicle (20)[b]	2.5 BPA (20)	25 BPA (19)	250 BPA (19)	2500 BPA (20)	25000 BPA (22)
Testicular spermatid heads, 10^6/g	76.5 ± 9.4	77.7 ± 4.8	72.2 ± 5.3	72.5 ± 6.3	73.6 ± 9.0	75.8 ± 4.7
Cauda sperm counts, 10^6/g	1,059 ± 87	1,186 ± 83	1,017 ± 66	1,111 ± 66	1,020 ± 89	1,016 ± 81
Cauda sperm % Motility	74.8 ± 4.3	75.8 ± 2.3	72.4 ± 3.4	70.2 ± 4.2	67.9 ± 5.6	77.7 ± 2.2
Cauda sperm, head, abnormal counts per animal	0.00 ± 0	0.00 ± 0	0.11 ± 0.07	0.05 ± 0.05	0.10 ± 0.07	0.00 ± 0
Cauda sperm, tail, abnormal counts per animal	0.15 ± 0.09	0.15 ± 0.09	0.21 ± 0.11	0.11 ± 0.07	0.20 ± 0.10	0.00 ± 0
Cauda sperm, head and tail combined, abnormal counts per animal	0.15 ± 0.09	0.015 ± 0.09	0.32 ± 0.13	0.16 ± 0.09	0.30 ± 0.12	0.00 ± 0

[a]BPA doses are µg/kg bw/day. Values presented are means ± S.E.M. Testicular spermatid head counts, cauda sperm counts, and percent sperm motility were analyzed using an ANOVA model. Analysis of sperm morphology data was performed using a generalized linear model with a Poisson distribution and a log link function. Pairwise comparisons to the vehicle control group were adjusted for multiple comparisons using Dunnett's method. Tests of trends, increasing treatment effect with increasing dose, were also performed. All tests were conducted as two-sided at the 0.05 significance level. There were no significant treatment effects. Also, there were no additional statistically significant effects in the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods). The full statistical report is found in Supplemental Appendix XXXI.

[b]Numbers in parentheses are numbers of animals examined.

Table 52. Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a, b]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenocarcinoma	Interim[c]	Incidence	0/23	1/22 (4%)	1/22 (4%)	0/24	0/20	0/24
	Terminal	Incidence	4/50 (8%)	6/48 (12%)	6/46 (13%)	5/49 (10%)	9/50 (18%)	3/46 (6%)
		Poly-3 Incidence	4/34.4 (12%)	6/32.9 (18%)	6/30.4 (20%)	5/34.6 (14%)	9/31.3 (29%)	3/31.2 (10%)
		Terminal Incidence	3/16 (19%)	6/19 (32%)	1/14 (7%)	0/13 (0%)	3/10 (30%)	0/8 (0%)
		Time-to-First	673	719 (T)	434	377	561	685
		Poly-3 *p*-value	0.412	0.336	0.286	0.504	0.071	0.555N
		Multiple Incidence[d]	2/50 (4%)	3/48 (6%)	3/46 (7%)	2/49 (4%)	3/50 (6%)	1/46 (2%)
Adenoma or Adenocarcinoma	Interim[c]	Incidence	0/23	1/22 (4%)	1/22 (4%)	0/24	0/20	0/24
	Terminal	Incidence	6/50 (12%)	7/48 (15%)	8/46 (17%)	6/49 (12%)	10/50 (20%)	4/46 (9%)
		Poly-3 Incidence	6/34.9 (17%)	7/32.9 (21%)	8/30.4 (26%)	6/34.8 (17%)	10/31.3 (32%)	4/31.8 (13%)
		Terminal Incidence	4/16 (25%)	7/19 (37%)	3/14 (21%)	0/13 (0%)	4/10 (40%)	0/8 (0%)
		Time-to-First	564	719 (T)	434	377	561	542
		Poly-3 *p*-value	0.513N	0.452	0.271	0.624	0.126	0.428N
		Multiple indicence[d]	2/50 (4%)	3/48 (6%)	3/46 (7%)	3/49 (6%)	3/50 (6%)	1/46 (2%)
Fibroadenoma	Interim	Incidence	2/23 (9%)	3/22 (13%)	3/22 (13%)	1/24 (4%)	2/20 (10%)	6/24 (25%)
		Terminal Incidence	2/21 (10%)	3/22 (14%)	2/21 (10%)	0/22 (0%)	2/20 (10%)	6/24 (25%)
		Time-to-First	361 (T)	356 (T)	311	256	362 (T)	362 (T)
		CAFE *p*-value	0.150	0.478	0.478	0.484N	0.641	0.136
		Multiple Incidence[d]	0/23 (0%)	0/22 (0%)	1/22 (5%)	0/24 (0%)	0/20 (0%)	1/24 (4%)
	Terminal	Incidence	41/50 (82%)	40/48 (83%)	33/46 (72%)	39/49 (80%)	35/50 (70%)	38/46 (83%)
		Poly-3 Incidence	41/47.0 (87%)	40/43.7 (92%)	33/38.4 (86%)	39/45.3 (86%)	35/42.3 (83%)	38/42.2 (90%)
		Terminal Incidence	13/16 (81%)	17/19 (90%)	12/14 (86%)	10/13 (77%)	7/10 (70%)	7/8 (88%)
		Time-to-First	431	321	434	261	419	467
		Poly-3 *p*-value	0.410N	0.366	0.567N	0.565N	0.366N	0.457
		Multiple Incidence[d]	21/50 (42%)	32/48 (67%)	20/46 (43%)	32/49 (65%)	21/50 (42%)	31/46 (67%)

[a]BPA doses are µg/kg bw/day. Statistical analyses were conducted for any lesion that was diagnosed in two animals in any dose group in the interim sacrifice groups or in the EE_2 terminal sacrifice groups or four animals in the control and BPA groups in the terminal sacrifice. A complete tabulation of all neoplastic lesions is found in Supplemental Appendix XXXII and complete results of the statistical analyses are found in Supplemental Appendices XXXIII (interim sacrifice) and XXXIV (terminal sacrifice). Details of the statistical methods are found in Materials and Methods and in Supplemental Appendices XXXIII (interim sacrifice) and XXXIV (terminal sacrifice). Data are presented as follows: Incidence, lesions observed/number of animals examined microscopically with percent animals affected in parentheses; Poly-3 Incidence, Poly-3 adjusted neoplasm incidence after adjustment for intercurrent mortality in terminal sacrifice animals; Terminal Incidence, lesions observed/number of animals reaching terminal sacrifice that were microscopically examined. Time-to-First, age of animal in which lesion was first observed, T in parentheses indicates that the first observation occurred at terminal sacrifice. For interim sacrifice data: CAFE *p*-value, *p*-value for Cochran-Armitage trend test in vehicle column, Fisher's exact test versus vehicle control in dose columns. For terminal sacrifice data, the Poly-3 *p*-value for the trend test is given in the vehicle column and values for pairwise comparisons to vehicle control are shown in dose columns. Significant trends are shown in the

vehicle column, while significant pairwise comparisons to the vehicle control are shown in BPA and EE_2 dose group columns. All *p*-values are one-sided and not corrected for multiple comparisons. "N" next to a *p*-value indicates a result that is a negative trend or negative relative to control. Significant effects are indicated with asterisks. *, $p < 0.05$; **, $p < 0.01$; ***, $p < 0.001$.

[b]There were no statistically significant effects in the female mammary gland in the continuous BPA dose groups in the interim or terminal sacrifice animals.

[c]Statistical analysis was not conducted since no group had 2 or more lesions diagnosed. There were no multiple adenocarcinomas in interim sacrifice animals.

[d]Proportion of animals examined that had multiple neoplasms. These animals with multiple neoplasms are included in the incidence row and were not analyzed separately. The numbers of mammary neoplasms found in each female are tabulated in Supplemental Appendix XXXII, Subappendix VII.

Table 53. Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Adenocarcinoma[c]	Interim	Incidence[d]	0/23 (0%)	2/26 (8%)	0/26 (0%)
		Terminal Incidence	0/21 (0%)	1/24 (4%)	0/26 (0%)
		Time-to-First	–	257	–
		CAFE *p*-value	0.639N	0.276	–
	Terminal	Incidence	4/50 (8%)	2/26 (8%)	10/26 (38%)
		Poly-3 Incidence	4/34.4 (12%)	2/16.8 (12%)	10/17.6 (57%)
		Terminal Incidence	3/16 (19%)	0/7 (0%)	3/4 (75%)
		Time-to-First	673	490	488
		Poly-3 *p*-value	<0.001***	0.667	<0.001***
		Multiple Incidence[e]	2/50 (4%)	0/26	4/26 (15%)
Fibroadenoma	Interim	Incidence[d]	2/23 (9%)	2/26 (8%)	4/26 (15%)
		Terminal Incidence	2/21 (10%)	2/24 (8%)	4/26 (15%)
		Time-to-First	361 (T)	361 (T)	360 (T)
		CAFE *p*-value	0.297	0.647N	0.395
	Terminal	Incidence	41/50 (82%)	18/26 (69%)	14/26 (54%)
		Poly-3 Incidence	41/47.0 (87%)	18/22.3 (81%)	14/20.2 (69%)
		Terminal Incidence	13/16 (81%)	5/7 (71%)	2/4 (50%)
		Time-to-First	431	460	360
		Poly-3 *p*-value	0.041N*	0.354N	0.056N
		Multiple Incidence[e]	21/50 (42%)	11/26 (42%)	4/26 (15%)

[a]EE_2 doses are μg/kg bw/day. See legend to Table 52 for description of data presentation. Adenocarcinoma incidence was significantly increased in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control and there was a significant dose trend.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 52.
[c]No mammary gland adenomas were diagnosed in interim or terminal sacrifice EE_2 females, so no tabulation of adenoma or adenocarcinoma is included.
[d]There were no animals with multiple mammary gland neoplasms in the interim sacrifice females.
[e]Proportion of animals examined that had multiple neoplasms. These animals with multiple neoplasms are included in the incidence row and were not analyzed separately. The numbers of mammary neoplasms found in each female are tabulated in Supplemental Appendix XXXII, Subappendix VII.

Table 54. Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenocarcinoma[b]	Terminal	Incidence	3/50 (6%)	11/50 (22%)	5/48 (10%)	7/49 (14%)	9/50 (18%)	5/46 (11%)
		Poly-3 Incidence	3/32.3 (9%)	11/33.3 (33%)	5/32.1 (16%)	7/35.4 (20%)	9/36.6 (25%)	5/32.0 (16%)
		Terminal Incidence	1/11 (9%)	2/12 (17 %)	2/13 (15%)	0/13 (0%)	5/17 (29%)	0/13 (0%)
		Time-to-First	681	573	458	450	488	615
		Poly-3 *p*-value	0.453	0.016*	0.348	0.189	0.083	0.346
		Multiple Incidence[d]	1/50 (2%)	1/50 (2%)	1/48 (2%)	2/49 (4%)	1/50 (2%)	2/46 (4%)
Adenoma or Adenocarcinoma[b]	Terminal	Incidence	4/50 (8%)	12/50 (24%)	5/48 (10%)	9/49 (18%)	9/50 (18%)	6/46 (13%)
		Poly-3 Incidence	4/33.0 (12%)	12/33.3 (36%)	5/32.1 (16%)	9/35.9 (25%)	9/36.6 (25%)	6/32.8 (18%)
		Terminal Incidence	1/11 (9%)	3/12 (25%)	2/13 (15%)	1/13 (8%)	5/17 (29%)	0/13 (0%)
		Time-to-First	514	573	458	450	488	463
		Poly-3 *p*-value	0.483	0.018*	0.482	0.140	0.149	0.360
		Multiple Incidence[d]	1/50 (2%)	1/50 (2%)	1/48 (2%)	2/49 (4%)	1/50 (2%)	2/46 (4%)
Fibroadenoma	Interim	Incidence[c]	4/20 (20%)	1/22 (4%)	1/20 (5%)	1/22 (4%)	1/20 (5%)	2/22 (9%)
		Terminal Incidence	4/20 (20%)	1/22 (4%)	1/20 (5%)	1/22 (4%)	1/20 (5%)	2/20 (10%)
		Time-to-First	363 (T)	365 (T)	365 (T)	363 (T)	364 (T)	363 (T)
		CAFE *p*-value	0.184N	0.144N	0.171N	0.144N	0.171N	0.286N
	Terminal	Incidence	43/50 (86%)	45/50 (90%)	37/48 (77%)	42/49 (86%)	36/50 (72%)	34/46 (74%)
		Poly-3 Incidence	43/47.7 (90%)	45/47.5 (95%)	37/42.2 (88%)	42/46.2 (91%)	36/45.5 (79%)	34/40.6 (84%)
		Terminal Incidence	8/11(73%)	11/12 (92%)	11/13 (85%)	11/13 (85%)	12/17 (71%)	12/13 (92%)
		Time-to-First	385	400	339	448	390	383
		Poly-3 *p*-value	0.021N*	0.319	0.489N	0.600	0.099N	0.257N
		Multiple Incidence[d]	29/50 (58%)	32/50 (64%)	28/48 (58%)	31/49 (63%)	29/50 (58%)	28/46 (61%)

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. There was a significant increase in adenocarcinoma, and combined adenoma or adenocarcinoma, in the 2.5 BPA µg/kg bw/day dose group relative to vehicle controls.

[b]No adenomas or adenocarcinomas were diagnosed in the mammary glands of interim sacrifice stop-dose females.

[c]There were no interim sacrifice females with multiple fibroadenomas.

[d]Proportion of animals examined that had multiple neoplasms. These animals with multiple neoplasms are included in the incidence row and were not analyzed separately. The numbers of mammary neoplasms found in each female are tabulated in Supplemental Appendix XXXII, Subappendix VII.

Table 55. Non-Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Atypical focus	Interim	Incidence	0/23 (0%)	3/22^ (14%)	2/22 (9%)	2/24 (8%)	0/20 (0%)	0/24 (0%)
		Severity Profile	–	1|2|0|0 (1.7)	0|2|0|0 (2.0)	2|0|0|0 (1.0)	–	–
	Terminal	Incidence	2/50 (4%)	7/48^ (15%)	1/46 (2%)	5/49 (10%)	3/50 (6%)	3/46 (6%)
		Severity Profile	0|2|0|0 (2.0)	3|2|2|0 (1.9)	0|1|0|0 (2.0)	0|5|0|0 (2.0)	1|2|0|0 (1.7)	1|2|0|0 (1.7)
		Poly-3 Incidence	2/34.4 (6%)	7/33.6 (21%)	1/28.2 (4%)	5/33.3 (15%)	3/29.7 (10%)	3/31.7 (10%)
Dilatation, duct	Interim	Incidence	2/23 (9%)	2/22 (9%)	7/22^^ (32%)	1/24 (4%)	2/20 (10%)	2/24 (8%)
		Severity Profile	1|1|0|0 (1.5)	0|0|0|2 (4.0)	2|4|1|0 1.9)	0|0|1|0 (3.0)	1|0|1|0 (2.0)	1|0|1|0 (2.0)
	Terminal	Incidence	15/50 (30%)	16/48 (33%)	7/46^ N (15%)	9/49 (18%)	9/50 (18%)	14/46 (30%)
		Severity Profile	0|10|5|0 (2.3)	0|12|2|2 (2.4)	0|6|1|0 (2.1)	0|6|3|0 (2.3)	0|4|5|0 (2.6)	2|9|3|0 (2.1)
		Poly-3 Incidence	15/38.1 (39%)	16/39.4 (41%)	7/31.9 (22%)	9/35.7 (25%)	9/33.1 (27%)	14/36.4 (38%)
Hyperplasia, lobular	Interim	Incidence	10/23 (44%)	14/22 (64%)	13/22 (59%)	15/24 (62%)	13/20 (65%)	12/24 (50%)
		Severity Profile	5|5|0|0 (1.5)	7|7|0|0 (1.5)	7|6|0|0 (1.5)	11|3|1|0 (1.3)	10|2|0|1 (1.4)	9|1|1|1 (1.5)
	Terminal	Incidence	43/50 (86%)	41/48 (85%)	30/46^^ N (65%)	38/49 (78%)	40/50^ N (80%)	37/46 (80%)
		Severity Profile	1|8|13|21 (3.3)	1|7|11|22 (3.3)	1|7|10|12 (3.1)	0|7|17|14 (3.2)	1|14|11|14 (3.0)	4|9|5|19 (3.1)
		Poly-3 Incidence	43/45.4* N (95%)	41/42.5 (96%)	30/36.7* N (82%)	38/43.7 (87%)	40/43.2 (93%)	37/43.4 (85%)
Dilatation, alveolus[b]	Terminal	Incidence	9/50 (18%)	14/48 (29%)	5/46 (11%)	7/49 (14%)	7/50 (14%)	11/46 (24%)
		Severity Profile	1|7|1|0 (2.0)	1|11|2|0 (2.1)	0|5|0|0 (2.0)	0|7|0|0 (2.0)	0|6|1|0 (2.1)	0|9|2|0 (2.2)
		Poly-3 Incidence	9/36.0 (25%)	14/38.0 (37%)	5/30.9 (16%)	7/35.2 (20%)	7/32.3 (22%)	11/34.6 (32%)

[a]BPA doses are µg/kg bw/day. Statistical analyses were conducted for any lesion that was diagnosed in two animals in any dose group in the interim sacrifice groups or in the EE_2 terminal sacrifice groups or four animals in the control and BPA groups in the terminal sacrifice. A complete tabulation of all non-neoplastic lesions is found in Supplemental Appendix XXXII and complete results of the statistical analyses are found in Supplemental Appendices XXXIII (interim sacrifice) and XXXIV (terminal sacrifice). Details of the statistical methods are found in Materials and Methods and in Supplemental Appendices XXXIII and XXXIV. Selected non-neoplastic lesions are tabulated in this report. Data are presented as follows: Incidence, lesions observed/number of animals examined microscopically, with percent animals affected in parentheses; Severity Profile, number of animals diagnosed with minimal/ mild/ moderate/ marked lesions, with the average severity in affected animals given in parentheses, based upon severity scores of 1, minimal; 2, mild; 3, moderate; and 4, marked.; Poly-3 Incidence, Poly-3 adjusted lesion incidence after adjustment for intercurrent mortality in the terminal sacrifice animals. Lesions in interim sacrifice animals were analyzed by the CAFE test (Cochran-Armitage trend test and Fisher's exact test to compare the incidence in each dose group to the vehicle control) and by

the Jonckheere-Terpstra (JT) trend test/Shirley-Williams (SW) pairwise comparison test to incorporate severity scores. Because the JT/SW test enforces an assumption of a monotonic response, a relative treatment effect (RTE) analysis that also incorporates severity scores, but does not enforce monotonicity, was also conducted. Significant JT/SW results that violated the monotonicity requirement are not shown in the tables, but are reported in Supplemental Appendices XXXIII and XXXIV. Lesions in terminal sacrifice animals were analyzed by the Poly-3 test to adjust for intercurrent mortality, as well as by the JT/SW and RTE tests. All pairwise tests were one-sided and not corrected for multiple comparisons. Significant trends are shown in the vehicle column, while significant pairwise comparisons to the vehicle control are shown in BPA or EE_2 dose group columns. The CAFE or Poly-3 tests for interim and terminal sacrifice animals, respectively, were considered as the primary statistical tests and positive significant results for those tests are shown with asterisks. Pound and caret signs indicate results from the JT/SW and RTE tests, respectively. *, #, ^, $p < 0.05$; **, ##, ^^, $p < 0.01$; ***, ###, ^^^, $p < 0.001$. "N" superscript next to significance markers indicates a result that is a negative trend or negative relative to control.
[b]No alveolar dilatation was diagnosed in interim sacrifice females.

Table 56. Non-Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Atypical focus[c]	Terminal	Incidence	2/50 (4%)	2/26 (8%)	3/26 (12%)
		Severity Profile	0|2|0|0 (2.0)	1|1|0|0 (1.5)	2|1|0|0 (1.3)
		Poly-3 Incidence	2/34.4 (6%)	2/16.1 (12%)	3/16.2 (19%)
Dilatation, duct	Interim	Incidence	2/23$^{***, ###, ^^^}$ (9%)	3/26 (12%)	22/26$^{***, ###, ^^^}$ (85%)
		Severity Profile	1|1|0|0 (1.5)	1|2|0|0 (1.7)	6|12|2|2 (2.0)
	Terminal	Incidence	15/50$^{###, ^^^}$ (30%)	6/26 (23%)	21/26$^{###, ^^^}$ (81%)
		Severity Profile	0|10|5|0 (2.3)	0|3|3|0 (2.5)	0|10|7|4 (2.7)
		Poly-3 Incidence	15/38.1*** (39%)	6/19.0 (32%)	21/24.2*** (87%)
Hyperplasia, lobular	Interim	Incidence	10/23$^{***, ##, ^^}$ (44%)	13/26 (50%)	23/26$^{***, ##, ^^}$ (88%)
		Severity Profile	5|5|0|0 (1.5)	6|6|1|0 (1.6)	11|11|1|0 (1.6)
	Terminal	Incidence	43/50 (86%)	24/26 (92%)	23/26 (88%)
		Severity Profile	1|8|13|21 (3.3)	1|8|4|11 (3.0)	1|10|4|8 (2.8)
		Poly-3 Incidence	43/45.4 (95%)	24/24.5 (98%)	23/25.1 (92%)
Dilatation, alveolus[d]	Terminal	Incidence	9/50$^{###, ^^^}$ (18%)	5/26 (19%)	22/26$^{###, ^^^}$ (85%)
		Severity Profile	1|7|1|0 (2.0)	0|5|0|0 (2.0)	0|18|4|0 (2.2)
		Poly-3 Incidence	9/36.0*** (25%)	5/18.8 (27%)	22/24.1*** (91%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 or description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 55.
[c]There were no atypical foci diagnosed in the interim vehicle, 0.05, or 0.5 µg EE_2/kg bw/day groups (0/23, 0/26, and 0/26, respectively), so there is no tabulation of data for the interim sacrifice animals for this lesion.
[d]No alveolar dilatation was diagnosed in interim sacrifice females.

Table 57. Non-Neoplastic Lesions in the Mammary Gland of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Atypical focus[b]	Terminal	Incidence	6/50 (12%)	2/50 (4%)	6/48 (12%)	8/49 (16%)	7/50 (14%)	5/46 (11%)
		Severity Profile	1|4|1|0 (2.0)	0|1|1|0 (2.5)	1|5|0|0 (1.8)	3|4|1|0 (1.8)	0|5|0|2 (2.6)	2|3|0|0 (1.6)
		Poly-3 Incidence	6/33.0 (18%)	2/32.0 (6%)	6/32.4 (18%)	8/36.2 (22%)	7/35.8 (20%)	5/32.6 (15%)
Dilatation, duct	Interim	Incidence	4/20$^{*, \#, \wedge N}$ (20%)	2/22 (9%)	1/20 (5%)	1/22$^{\wedge N}$ (4%)	1/20 (5%)	1/22$^{\#, \wedge N}$ (4%)
		Severity Profile	2|1|1|0 (1.8)	1|1|0|0 (1.5)	0|1|0|0 (2.0)	1|0|0|0 (1.0)	0|1|0|0 (2.0)	1|0|0|0 (1.0)
	Terminal	Incidence	16/50 (32%)	5/50$^{\#\#, \wedge\wedge N}$ (10%)	9/48$^{\#, \wedge N}$ (19%)	9/49$^{\#, \wedge N}$ (18%)	7/50$^{\#\#, \wedge\wedge N}$ (14%)	11/46$^{\# N}$ (24%)
		Severity Profile	0|9|4|3 (2.6)	0|5|0|0 (2.0)	2|4|2|1 (2.2)	0|6|3|0 (2.3)	0|6|1|0 (2.1)	0|7|4|0 (2.4)
		Poly-3 Incidence	16/37.0 (43%)	5/34.5$^{** N}$ (14%)	9/33.7 (27%)	9/35.3 (26%)	7/36.4$^{* N}$ (19%)	11/36.2 (30%)
Hyperplasia, lobular	Interim	Incidence	15/20 (75%)	12/22 (54%)	8/20$^{*, \wedge N}$ (40%)	12/22 (54%)	7/20$^{*, \wedge N}$ (35%)	12/22 (54%)
		Severity Profile	10|4|1|0 (1.4)	8|3|1|0 (1.4)	5|1|2|0 (1.6)	8|3|1|0 (1.4)	4|3|0|0 (1.4)	6|4|2|0 (1.7)
	Terminal	Incidence	41/50 (82%)	40/50 (80%)	39/48 (81%)	39/49 (80%)	36/50 (72%)	38/46 (83%)
		Severity Profile	1|8|15|17 (3.2)	0|8|12|20 (3.3)	3|14|11|11 (2.8)	2|9|13|15 (3.1)	3|5|7|21 (3.3)	2|10|9|17 (3.1)
		Poly-3 Incidence	41/45.5 (90%)	40/46.1 (87%)	39/42.7 (91%)	39/45.0 (87%)	36/45.4 (79%)	38/41.6 (91%)
Dilatation, alveolus[c]	Terminal	Incidence	8/50 (16%)	4/50 (8%)	4/48 (8%)	8/49 (16%)	3/50$^{\wedge N}$ (6%)	7/46 (15%)
		Severity Profile	0|4|4|0 (2.5)	0|4|0|0 (2.0)	0|2|2|0 (2.5)	0|6|2|0 (2.3)	1|2|0|0 (1.7)	1|6|0|0 (1.9)
		Poly-3 Incidence	8/34.8 (23%)	4/33.7 (12%)	4/30.5 (13%)	8/34.9 (23%)	3/35.7 (8%)	7/34.9 (20%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]There was a 5% incidence (1/20) of atypical focus in the mammary gland of female stop-dose vehicle control interim sacrifice animals and all BPA dose interim sacrifice dose groups had a 0% incidence (0/22, 0/20, 0/22, 0/20, 0/22 for 2.5, 25, 250, 2,500, and 25,000 µg BPA/kg bw/day, respectively).

[c] No alveolar dilatation was diagnosed in interim sacrifice females.

Table 58. Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Stromal polyps	Interim	Incidence	1/23 (4%)	0/22 (0%)	1/21 (5%)	0/24 (0%)	3/20 (15%)	3/24 (12%)
		Terminal Incidence	1/21 (5%)	0/22 (0%)	1/21 (5%)	0/22 (0%)	3/20 (15%)	3/24 (12%)
		Time-to-First	362 (T)	–	360 (T)	–	362 (T)	362 (T)
		CAFE *p*-value	0.037*	0.511N	0.733	0.489N	0.252	0.321
	Terminal	Incidence	5/50 (10%)	3/48 (6%)	7/45 (16%)	2/49 (4%)	4/48 (8%)	3/46 (6%)
		Poly-3 Incidence	5/36.4 (14%)	3/34.6 (9%)	7/29.5 (24%)	2/33.5 (6%)	4/29.2 (14%)	3/32.2 (9%)
		Terminal Incidence	1/16 (6%)	0/19 (0%)	4/14 (29%)	0/13 (0%)	0/10 (0%)	0/8 (0%)
		Time-to-First	506	321	441	542	673	569
		Poly-3 *p*-value	0.333N	0.383N	0.231	0.247N	0.638N	0.424N

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. There were no statistically significant effects in the terminal sacrifice animals. There was a significant trend across BPA dose levels in the interim sacrifice animals.

Table 59. Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Stromal polyps	Terminal	Incidence	5/50 (10%)	3/26 (12%)	1/26 (4%)
		Poly-3 Incidence	5/36.4 (14%)	3/17.4 (17%)	1/15.7 (6%)
		Terminal Incidence	1/16 (6%)	0/7 (0%)	0/4 (0%)
		Time-to-First	506	485	527
		Poly-3 *p*-value	0.383N	0.529	0.389N

[a]EE_2 doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. There were no statistically significant effects in the terminal sacrifice animals. Interim sacrifice animals are not tabulated as no group had two or more lesions.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 58.

Table 60. Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Stromal polyps	Terminal	Incidence	7/49 (14%)	4/49 (8%)	5/48 (10%)	6/49 (12%)	4/49 (8%)	1/46 (2%)
		Poly-3 Incidence	7/33.0 (21%)	4/32.3 (12%)	5/32.5 (15%)	6/35.9 (17%)	4/35.8 (11%)	1/31.0 (3%)
		Terminal Incidence	2/11 (18.2%)	1/12 (8%)	1/13 (8%)	1/13 (8%)	1/17 (6%)	0/13 (0%)
		Time-to-First	467	446	461	497	539	706
		Poly-3 *p*-value	0.041$^{*\ N}$	0.263^{N}	0.384^{N}	0.433^{N}	0.207^{N}	0.032$^{*\ N}$

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. Interim sacrifice animals are not tabulated as no group had two or more lesions.

Table 61. Non-Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Apoptosis	Interim	Incidence	2/23[**, ##, ^^] (9%)	1/22 (4%)	4/21 (19%)	5/24 (21%)	5/20 (25%)	9/24[*, #, ^^] (38%)
		Severity Profile	0/0/0/2 (4.0)	0/0/0/1 (4.0)	0/1/1/2 (3.2)	0/1/0/4 (3.6)	0/0/3/2 (3.4)	0/1/5/3 (3.2)
Hyperplasia, cystic, endometrium	Interim	Incidence	5/23 (22%)	1/22 (4%)	4/21 (19%)	3/24 (12%)	7/20 (35%)	4/24 (17%)
		Severity Profile	1|2|1|1 (2.4)	1|0|0|0 (1.0)	1|2|1|0 (2.0)	1|1|1|0 (2.0)	0|6|1|0 (2.1)	0|3|0|1 (2.5)
	Terminal	Incidence	30/50 (60%)	20/48[^ N] (42%)	26/45 (58%)	23/49 (47%)	22/48 (46%)	26/46 (56%)
		Severity Profile	4|18|5|3 (2.2)	2|13|5|0 (2.2)	0|16|7|3 (2.5)	4|12|6|1 (2.2)	4|8|8|2 (2.4)	1|14|10|1 (2.4)
		Poly-3 incidence	30/41.9 (72%)	20/40.1[* N] (50%)	26/36.9 (70%)	23/40.6 (57%)	22/35.8 (61%)	26/39.2 (66%)
Hyperplasia, endometrium	Interim	Incidence	2/23 (9%)	7/22[^] (32%)	5/21 (24%)	7/24[^] (29%)	5/20 (25%)	2/24 (8%)
		Severity Profile	0|2|0|0 (2.0)	3|3|1|0 (1.7)	0|4|1|0 (2.2)	2|4|1|0 (1.9)	1|2|2|0 (2.2)	0|2|0|0 (2.0)
	Terminal	Incidence	10/50 (20%)	15/48 (31%)	12/45 (27%)	15/49 (31%)	15/48 (31%)	12/46 (26%)
		Severity Profile	2|8|0|0 (1.8)	5|8|2|0 (1.8)	2|8|2|0 (2.0)	6|7|0|2 (1.9)	10|5|0|0 (1.3)	2|7|1|2 (2.2)
		Poly-3 incidence	10/39.1 (26%)	15/36.4 (41%)	12/31.9 (38%)	15/37.1 (40%)	15/35.9 (42%)	12/36.2 (33%)
Metaplasia, squamous	Interim	Incidence	1/23[*, #, ^] (4%)	1/22 (4%)	4/21 (19%)	3/24 (12%)	3/20 (15%)	5/24[b] (21%)
		Severity Profile	0|1|0|0 (2.0)	0|1|0|0 (2.0)	3|1|0|0 (1.2)	3|0|0|0 (1.0)	2|0|1|0 (1.7)	5|0|0|0 (1.0)
	Terminal	Incidence	2/50 (4%)	4/48 (8%)	4/45 (9%)	1/49 (2%)	4/48 (8%)	6/46 (13%)
		Severity Profile	1|1|0|0 (1.5)	2|2|0|0 (1.5)	1|2|0|1 (2.2)	1|0|0|0 (1.0)	1|3|0|0 (1.8)	4|1|1|0 (1.5)
		Poly-3 incidence	2/35.7 (6%)	4/35 (11%)	4/29.5 (14%)	1/32.8 (3%)	4/31.1 (13%)	6/33.9 (18%)
Dilatation, lumen	Interim	Incidence	0/23 (0%)	1/22 (4%)	2/21 (10%)	2/24 (8%)	1/20 (5%)	2/24 (8%)
		Severity Profile	-	0|0|1|0 (3.0)	0|0|0|2 (4.0)	0|0|0|2 (4.0)	0|0|0|1 (4.0)	0|0|0|2 (4.0)
	Terminal	Incidence	2/50[#, ^] (4%)	2/48 (4%)	3/45 (7%)	4/49 (8%)	5/48 (10%)	6/46 (13%)
		Severity Profile	0|0|0|2 (4.0)	0|0|0|2 (4.0)	0|0|0|3 (4.0)	0|0|1|3 (3.8)	0|0|0|5 4.0)	0|0|1|5 (3.8)
		Poly-3 incidence	2/35.6[*] (6%)	2/33.5 (6%)	3/28.5 (10%)	4/34.5 (12%)	5/30.1 (17%)	6/33.0[c] (18%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant (p = 0.048) difference for the CAFE test for the pairwise comparison of the 25,000 µg BPA/kg bw/day group to the vehicle control (squamous metaplasia, 5/20 (25%) versus 0/15 (0%)).

[c]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test (p = 0.035) for the pairwise comparison of the 25,000 µg BPA/kg bw/day group to the vehicle control group (lumen dilatation, Poly-3 incidences, 5/26.8 (19%) versus 0/24 (0%)).

Table 62. Non-Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	0.05 EE_2	0.5 EE_2
Apoptosis	Interim	Incidence	2/23***, ###, ^^^	6/25	18/26***, ###, ^^^
			(9%)	(24%)	(69%)
		Severity Profile	0|0|0|2 (4.0)	0|1|2|3 (3.3)	0|2|6|10 (3.4)
Hyperplasia, cystic, endometrium	Interim	Incidence	5/23*, ##, ^	6/25	14/26*, #, ^
			(22%)	(24%)	(54%)
		Severity Profile	1|2|1|1 (2.4)	3|3|0|0 (1.5)	4|6|2|2 (2.1)
	Terminal	Incidence	30/50	14/26	14/26
			(60%)	(54%)	(54%)
		Severity Profile	4|18|5|3 (2.2)	1|7|4|2 (2.5)	1|8|2|3 (2.5)
		Poly-3 Incidence	30/41.9	14/21.8	14/20.2
			(72%)	(64%)	(69%)
Hyperplasia, endometrium	Interim	Incidence	2/23	4/25	0/26
			(9%)	(16%)	(0%)
		Severity Profile	0|2|0|0 (2.0)	0|4|0|0 (2.0)	–
	Terminal	Incidence	10/50	10/26^	2/26
			(20%)	(38%)	(8%)
		Severity Profile	2|8|0|0 (1.8)	3|6|0|1 (1.9)	1|1|0|0 (1.5)
		Poly-3 Incidence	10/39.1	10/19.4*	2/16.2
			(26%)	(52%)	(12%)
Metaplasia, squamous	Interim	Incidence	1/23***, ###, ^^^	2/25	14/26***, ###, ^^^
			(4%)	(8%)	(54%)
		Severity Profile	0|1|0|0 (2.0)	1|0|1|0 (2.0)	8|4|2|0 (1.6)
	Terminal	Incidence	2/50#	2/26	4/26
			(4%)	(8%)	(15%)
		Severity Profile	1|1|0|0 (1.5)	0|2|0|0 (2.0)	3|0|1|0 (1.5)
		Poly-3 Incidence	2/35.7*	2/16.1	4/16.9
			(6%)	(12%)	(24%)
Dilatation, lumen	Interim	Incidence	0/23	1/25	0/26
			(0%)	(4%)	(0%)
		Severity Profile	–	0/0/0/1 (4.0)	–
	Terminal	Incidence	2/50	2/26	3/26
			(4%)	(8%)	(12%)
		Severity Profile	0|0|0|2 (4.0)	0|0|0|2 (4.0)	0|0|0|3 (4.0)
		Poly-3 Incidence[b]	2/35.6	2/16.9	3/16.0[c]
			(6%)	(12%)	(19%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 61.

[c]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant EE_2 dose trend ($p = 0.008$) and a significant pairwise comparison ($p = 0.013$) for the 0.5 µg EE_2/kg bw/day dose group versus the vehicle control group (lumen dilatation, Poly-3 incidences 3/10.5 (29%) versus 0/24 (0%)).

Table 63. Non-Neoplastic Lesions in the Uterus of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Apoptosis	Interim	Incidence	2/20 (10%)	3/22 (14%)	2/20 (10%)	2/22 (9%)	1/20 (5%)	6/22 (27%)
		Severity Profile	0|0|0|2 (4.0)	0|1|0|2 (3.3)	0|0|1|1 (3.5)	0|0|2|0 (3.0)	0|0|0|1 (4.0)	0|1|0|5 (3.7)
Hyperplasia, cystic, endometrium	Interim	Incidence	2/20 (10%)	4/22 (18%)	2/20 (10%)	2/22 (9%)	1/20 (5%)	7/22[#, ^] (32%)
		Severity Profile	0|1|0|1 (3.0)	0|2|2|0 (2.5)	0|2|0|0 (2.0)	0|2|0|0 (2.0)	0|0|0|1 (4.0)	2|4|0|1 (2.0)
	Terminal	Incidence	18/49[#, ^] (37%)	23/49 (47%)	22/48 (46%)	25/49 (51%)	28/49[#, ^] (57%)	24/46[#] (52%)
		Severity Profile	3|8|5|2 (2.3)	1|18|4|0 (2.1)	1|15|5|1 (2.3)	3|15|6|1 (2.2)	0|18|6|4 (2.5)	4|11|6|3 (2.3)
		Poly-3 Incidence	18/38.3 (47%)	23/38.1 (60%)	22/37.1 (59%)	25/41.6 (60%)	28/42.5[b] (66%)	24/38.6 (62%)
Hyperplasia, endometrium	Interim	Incidence	6/20 (30%)	9/22 (41%)	5/20 (25%)	7/22 (32%)	6/20 (30%)	9/22 (41%)
		Severity Profile	1|4|1|0 (2.0)	1|6|2|0 (2.1)	2|2|1|0 (1.8)	2|4|1|0 (1.9)	0|6|0|0 (2.0)	0|7|2|0 (2.2)
	Terminal	Incidence	18/49 (37%)	14/49 (29%)	17/48 (35%)	14/49 (29%)	12/49 (24%)	10/46 (22%)
		Severity Profile	6|12|0|0 (1.7)	8|6|0|0 (1.4)	7|9|0|1 (1.7)	6|7|1|0 (1.6)	2|10|0|0 (1.8)	1|8|1|0 (2.0)
		Poly-3 Incidence	18/38.8[* N] (46%)	14/37.4 (38%)	17/37.0 (46%)	14/39.2 (36%)	12/38.5 (31%)	10/33.6 (30%)
Metaplasia, squamous	Interim	Incidence	0/20 (0%)	2/22 (9%)	1/20 (5%)	1/22 (4%)	0/20 (0%)	4/22[##, ^^] (18%)
		Severity Profile	–	2|0|0|0 (1.0)	0|1|0|0 (2.0)	1|0|0|0 (1.0)	–	1|3|0|0 (1.8)
	Terminal	Incidence	5/49 (10%)	1/49[^ N] (2%)	2/48 (4%)	2/49 (4%)	4/49 (8%)	3/46 (6%)
		Severity Profile	3|2|0|0 (1.4)	1|0|0|0 (1.0)	1|1|0|0 (1.5)	0|2|0|0 (2.0)	3|1|0|0 (1.2)	3|0|0|0 (1.0)
		Poly-3 Incidence	5/33.1 (15%)	1/31.7 (3%)	2/30.8 (6%)	2/34.8 (6%)	4/36.2 (11%)	3/32.6 (9%)
Dilatation, lumen	Interim	Incidence	1/20 (5%)	0/22 (0%)	1/20 (5%)	4/22[^] (18%)	1/20 (5%)	0/22 (0%)
		Severity Profile	0|0|0|1 (4.0)	–	0|0|0|1 (4.0)	0|0|1|3 (3.8)	0|0|1|0 (3.0)	–
	Terminal	Incidence	3/49[#, ^ N] (6%)	6/49 (12%)	2/48 (4%)	4/49 (8%)	2/49 (4%)	0/46 (0%)
		Severity Profile	0|0|1|2 (3.7)	0|0|0|6 (4.0)	0|0|0|2 (4.0)	0|0|0|4 (4.0)	0|0|1|1 (3.5)	–
		Poly-3 Incidence	3/32.5[* N] (9%)	6/33.0 (18%)	2/31.3 (6%)	4/33.8 (12%)	2/35.2 (6%)	0/30.9 (0%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test (p = 0.038) for the comparison of 2,500 µg BPA/kg bw/day group to vehicle control (endometrial cystic hyperplasia, Poly-3 incidences, 23/43.3 (53%) versus 12/28.2 (43%)).

Table 64. Non-Neoplastic Lesions in the Ovary of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Atrophy	Interim	Incidence	10/23 (44%)	7/22 (32%)	9/22 (41%)	14/24 (58%)	11/20 (55%)	11/24 (46%)
		Severity Profile	1|4|0|5 (2.9)	0|3|0|4 (3.1)	0|2|0|7 (3.6)	6|4|0|4 (2.1)	2|1|0|8 (3.3)	0|2|0|9 (3.6)
	Terminal	Incidence	47/50 (94%)	45/48 (94%)	44/46 (96%)	46/49 (94%)	45/50 (90.0%)	46/46 (100%)
		Severity Profile	0|24|15|8 (2.7)	1|27|9|8 (2.5)	1|25|8|10 (2.6)	0|27|11|8 (2.6)	0|26|7|12 (2.7)	0|23|12|11 (2.7)
		Poly-3 Incidence	47/48.7 (96%)	45/45.1 (100%)	44/44.2 (100%)	46/47.0 (98%)	45/45.8 (98%)	46/46.0 (100%)
Cyst, follicle	Interim	Incidence	8/23 (35%)	4/22 (18%)	10/22 (46%)	5/24 (21%)	10/20 (50%)	11/24 (46%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	3/50 (6%)	3/48 (6%)	2/46 (4%)	7/49 (14%)	6/50 (12%)	4/46 (9%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 Incidence	3/35.7 (8%)	3/33.4 (9%)	2/28.9 (7%)	7/34.5 (20%)	6/30.8 (20%)	4/32.3 (12%)
Depletion, Corpus luteum	Interim	Incidence	4/23[*] (17%)	4/22 (18%)	7/22 (32%)	4/24 (17%)	8/20 (40%)	9/24 (38%)
		Severity Profile	–[b]	–	–	–	–	–
Hypertrophy, Interstitial cell	Interim	Incidence	4/23[*, #, ^] (17%)	4/22 (18%)	6/22 (27%)	3/24 (12%)	8/20[^] (40%)	9/24 (38%)
		Severity Profile	0|4|0|0 (2.0)	0|3|1|0 (2.2)	0|2|4|0 (2.7)	0|0|2|1 (3.3)	0|6|2|0 (2.2)	1|7|1|0 (2.0)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Severity scores were not assigned for this lesion.

Table 65. Non-Neoplastic Lesions in the Ovary of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Atrophy	Interim	Incidence	10/23***, ###, ^^^ (44%)	9/25 (36%)	26/26***, ###, ^^^ (100%)
		Severity Profile	1|4|0|5 (2.9)	0|3|0|6 (3.3)	0|0|0|26 (4.0)
	Terminal	Incidence	47/50###, ^^^ (94%)	25/26 (96%)	26/26###, ^^^ (100%)
		Severity Profile	0|24|15|8 (2.7)	0|12|7|6 (2.8)	0|1|1|24 (3.9)
		Poly-3 Incidence	47/48.7 (96%)	25/26.0 (96%)	26/26.0 (100%)
Cyst, follicle	Interim	Incidence	8/23*** (35%)	9/25 (36%)	26/26*** (100%)
		Severity Profile	–[c]	–	–
	Terminal	Incidence	3/50 (6%)	0/26 (0%)	3/26 (12%)
		Severity Profile	–[c]	–	–
		Poly-3 Incidence	3/35.7 (8%)	0/15.9 (0%)	3/16.1 (19%)
Depletion, Corpus luteum	Interim	Incidence	4/23*** (17%)	6/25 (24%)	26/26*** (100%)
		Severity Profile	–[c]	–	–
Hypertrophy, Interstitial cell	Interim	Incidence	4/23***, ###, ^^^ (17%)	5/25 (20.0%)	26/26***, ###, ^^^ (100%)
		Severity Profile	0|4|0|0 (2.0)	0|4|1|0 (2.2)	1|13|10|2 (2.5)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 64.
[c]Severity scores were not assigned for this lesion.

Table 66. Non-Neoplastic Lesions in the Ovary of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Atrophy	Interim	Incidence	10/20 (50%)	9/22 (41%)	11/20 (55%)	6/22 (27%)	12/20 (60%)	15/22 (68%)
		Severity Profile	2|6|0|2 (2.2)	1|4|0|4 (2.8)	8|1|0|2 (1.6)	1|3|0|2 (2.5)	5|4|0|3 (2.1)	4|5|0|6 (2.5)
	Terminal	Incidence	47/49 (96%)	48/49 (98%)	46/47 (98%)	50/50 (100%)	49/50 (98%)	44/46 (96%)
		Severity Profile	1|23|16|7 (2.6)	0|34|11|3 (2.4)	1|28|11|6 (2.5)	1|33|7|9 (2.5)	0|25|16|8 (2.7)	0|23|12|9 (2.7)
		Poly-3 Incidence	47/47.5 (99%)	48/48.1 (100%)	46/46.1 (100%)	50/50.0 (100%)	49/49.0 (100%)	44/44.8 (98%)
Cyst, follicle	Interim	Incidence	5/20*** (25%)	6/22 (27%)	4/20 (20%)	7/22 (32%)	11/20 (55%)	18/22*** (82%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	4/49 (8%)	4/49 (8%)	2/47 (4%)	1/50 (2%)	2/50 (4%)	4/46 (9%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 Incidence	4/32.0 12%)	4/33.5 (12%)	2/30.2 (7%)	1/34.6 (3%)	2/35.7 (6%)	4/32.4 (12%)
Depletion, Corpus luteum	Interim	Incidence	2/20 (10%)	4/22 (18%)	2/20 (10%)	2/22 (9%)	3/20 (15%)	6/22 (27%)
		Severity Profile	–[b]	–	–	–	–	–
Hypertrophy, Interstitial cell	Interim	Incidence	4/20 (20%)	3/22 (14%)	1/20 (5%)	2/22 (9%)	3/20 (15%)	5/22 (23%)
		Severity Profile	0|1|3|0 (2.8)	0|2|1|0 (2.3)	0|1|0|0 (2.0)	0|2|0|0 (2.0)	0|3|0|0 (2.0)	0|4|1|0 (2.2)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Severity scores were not assigned for this lesion.

Table 67. Non-Neoplastic Lesions in the Vagina of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, epithelium	Interim	Incidence	3/23$^{**, \#\#, \wedge\wedge}$ (13%)	2/22 (9%)	2/21 (10%)	4/24 (17%)	6/20 (30%)	8/24$^{\#, \wedge}$ (33%)
		Severity Profile	0|1|2|0 (2.7)	0|1|1|0 (2.5)	0|1|1|0 (2.5)	0|0|4|0 (3.0)	0|1|5|0 (2.8)	0|1|7|0 (2.9)
	Terminal	Incidence	4/49$^{\#\#, \wedge\wedge}$ (8%)	5/48 (10%)	12/45$^{\#\#, \wedge}$ (27%)	10/49$^{\#}$ (20%)	11/50$^{\#, \wedge}$ (22%)	12/46$^{\#, \wedge}$ (26%)
		Severity Profile	0|1|2|1 ((3.0)	0|1|4|0 (2.8)	1|3|6|2 (2.8)	0|7|3|0 (2.3)	0|3|5|3 (3.0)	0|6|4|2 (2.7)
		Poly-3 Incidence	4/35.2** (11%)	5/35.3 (14%)	12/33.7* (36%)	10/36.5 (27%)	11/33.4* (33%)	12/36.8* (33%)
Mucification, epithelium	Interim	Incidence	10/23 (44%)	12/22 (54%)	7/21 (33%)	9/24 (38%)	7/20 (35%)	8/24 (33%)
		Severity Profile	0|6|1|3 (2.7)	0|7|4|1 (2.5)	0|0|3|4 (3.6)	0|1|5|3 (3.2)	0|0|2|5 (3.7)	1|3|1|3 (2.8)
	Terminal	Incidence	46/49 (94%)	37/48 (77%)	34/45 (76%)	39/49 (80%)	34/50$^{\wedge\ N}$ (68%)	40/46 (87%)
		Severity Profile	0|13|12|21 (3.2)	0|7|8|22 (3.4)	0|7|11|16 (3.3)	0|4|14|21 (3.4)	0|8|9|17 (3.3)	1|10|8|21 (3.2)
		Poly-3 Incidence	46/47.2 (97%)	37/42.0 (88%)	34/39.3$^{*\ N}$ (87%)	39/45.2$^{*\ N}$ (86%)	34/42.8$^{**\ N}$ (79%)	40/43.6 (92%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 68. Non-Neoplastic Lesions in the Vagina of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia, epithelium	Interim	Incidence	3/23***, ###, ^^^	7/25	20/26***, ###, ^^^
			(13%)	(28%)	(77%)
		Severity Profile	0|1|2|0 (2.7)	0|4|3|0 (2.4)	0|9|11|0 (2.6)
	Terminal	Incidence	4/49	5/26	2/26
			(8%)	(19%)	(8%)
		Severity Profile	0|1|2|1 (3.0)	0|1|2|2 (3.2)	0|1|1|0 (2.5)
		Poly-3 Incidence	4/35.2	5/18.6	2/15.8
			(11%)	(27%)	(13%)
Mucification, epithelium	Interim	Incidence	10/23*, #, ^	15/25	18/26#, ^
			(44%)	(60%)	(69%)
		Severity Profile	0|6|1|3 (2.7)	0|5|1|9 (3.3)	1|4|6|7 (3.1)
	Terminal	Incidence	46/49	21/26	23/26
			(94%)	(81%)	(88%)
		Severity Profile	0|13|12|21 (3.2)	0|4|5|12 (3.4)	0|6|8|9 (3.1)
		Poly-3 Incidence	46/47.2	21/24.0	23/24.8
			(97%)	(87%)	(93%)

[a]EE_2 doses are μg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 67.

Table 69. Non-Neoplastic Lesions in the Vagina of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a, b]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, epithelium	Interim	Incidence	2/20 (10%)	4/22 (18%)	2/20 (10%)	1/22 (4%)	2/20 (10%)	6/22 (27%)
		Severity Profile	0|0|2|0 (3.0)	0|0|4|0 (3.0)	0|0|2|0 (3.0)	0|0|0|1 (4.0)	0|1|1|0 (2.5)	0|1|5|0 (2.8)
	Terminal	Incidence	6/49 (12%)	10/50 (20%)	3/47 (6%)	7/49 (14%)	7/49 (14%)	7/46 (15%)
		Severity Profile	0|3|2|1 (2.7)	0|5|4|1 (2.6)	0|1|1|1 (3.0)	0|2|4|1 (2.9)	0|1|5|1 (3.0)	0|0|5|2 (3.3)
		Poly-3 Incidence	6/33.7 (18%)	10/35.4 (28%)	3/31.8 (9%)	7/35.9 (20%)	7/37.6 (19%)	7/34.6 (20%)
Mucification, epithelium	Interim	Incidence	8/20 (40%)	11/22 (50%)	9/20 (45%)	11/22 (50%)	8/20 (40%)	10/22 (45%)
		Severity Profile	1|2|1|4 (3.0)	0|3|4|4 (3.1)	0|3|4|2 (2.9)	0|6|2|3 (2.7)	0|1|3|4 (3.4)	0|2|3|5 (3.3)
	Terminal	Incidence	40/49 (82%)	46/50 (92%)	37/47 (79%)	44/49 (90%)	39/49 (80%)	34/46 (74%)
		Severity Profile	0|7|12|21 (3.4)	1|7|15|23 (3.3)	0|9|9|19 (3.3)	2|12|12|18 (3.0)	0|8|9|22 (3.4)	1|5|11|17 (3.3)
		Poly-3 Incidence	40/45.8 (87%)	46/48.1 (96%)	37/42.4 (87%)	44/47.9 (92%)	39/44.6 (88%)	34/39.7 (86%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was also a significant Poly-3 dose trend ($p = 0.029$) for polymorphonuclear cellular infiltration (not shown in Table). The p-value for trend with all animals included was 0.136.

Table 70. Neoplastic Lesions in the Pituitary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenoma, pars distalis	Interim[b]	Incidence	0/23	0/22	1/22	1/24	0/20	0/24
Adenoma, pars distalis	Terminal	Incidence	21/50 (42%)	22/48 (46%)	12/46 (26%)	20/49 (41%)	19/49 (39%)	21/46 (46%)
		Poly-3 Incidence	21/39.9 (53%)	22/37.1 (59%)	12/31.4 (38%)	20/36.6 (55%)	19/34.1 (56%)	21/35.4 (59%)
		Terminal Incidence	7/16 (44%)	12/19 (63%)	4/14 (29%)	8/13 (62%)	5/10 (50%)	4/8 (50%)
		Time-to-First	477	497	434	561	456	510
		Poly-3 *p*-value	0.313	0.356	0.153N	0.523	0.487	0.356
Adenoma or carcinoma, pars distalis	Terminal	Incidence	22/50 (44%)	23/48 (48%)	12/46 (26%)	20/49 (41%)	19/49 (39%)	21/46 (46%)
		Poly-3 Incidence	22/40.3 (55%)	23/37.7 (61%)	12/31.4 (38%)	20/36.6 (55%)	19/34.1 (56%)	21/35.4 (59%)
		Terminal Incidence	7/16 (44%)	12/19 (63%)	4/14 (29%)	8/13 (62%)	5/10 (50%)	4/8 (50.0%)
		Time-to-First	477	497	434	561	456	510
		Poly-3 *p*-value	0.408	0.361	0.115N	0.595	0.559	0.425

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. There were no significant increases relative to vehicle control.
[b]No statistical analysis of lesions in interim animals was conducted since no group had two or more lesions.

Table 71. Neoplastic Lesions in the Pituitary Gland of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	0.05 EE_2	0.5 EE_2
Adenoma, pars distalis	Interim[b]	Incidence	0/23	1/25	1/26
Adenoma, pars distalis	Terminal	Incidence	21/50 (42%)	10/26 (38%)	17/26 (65%)
		Poly-3 Incidence	21/39.9 (53%)	10/18.3 (55%)	17/23.2 (73%)
		Terminal Incidence	7/16 (44%)	4/7 (57%)	2/4 (50%)
		Time-to-First	477	484	360
		Poly-3 *p*-value	0.055	0.561	0.068
Carcinoma, pars distalis	Terminal	Incidence	1/50 (2%)	0/26 (0%)	3/26 (12%)
		Poly-3 Incidence	1/34.6 (3%)	0/15.9 (0%)	3/16.1 (19%)
		Terminal Incidence	0/16 (0%)	0/7 (0%)	0/4 (0%)
		Time-to-First	615	–	610
		Poly-3 *p*-value	0.053	0.652N	0.084
Adenoma or carcinoma, pars distalis	Terminal	Incidence	22/50 (44%)	10/26 (38%)	20/26 (77%)
		Poly-3 Incidence	22/40.3 (55%)	10/18.3 (55%)	20/24.2 (83%)
		Terminal Incidence	7/16 (44%)	4/7 (57%)	2/4 (50%)
		Time-to-First	477	484	360
		Poly-3 *p*-value	0.009**	0.615N	0.011*

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation.
[b]No statistical analysis of lesions in interim animals was conducted since no group had two or more lesions.

Table 72. Neoplastic Lesions in the Pituitary Gland of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenoma or carcinoma, pars distalis	Interim	Incidence	0/20 (0%)	0/22 (0%)	2/20 (10%)[b]	0/22 (0%)	0/20 (0%)	0/22 (0%)
		Terminal Incidence	0/20 (0%)	0/22 (0%)	2/20 (10%)	0/22 (0%)	0/20 (0%)	0/22 (0%)
		Time-to-First	–	–	364 (T)	–	–	–
		CAFE *p*-value	0.408N	–	0.244	–	–	–
Adenoma, pars distalis	Terminal	Incidence	23/49 (47%)	16/50 (32%)	14/48 (29%)	20/50 (40%)	20/50 (40%)	20/46 (43%)
		Poly-3 Incidence	23/38.5 (60%)	16/36.3 (44%)	14/33.5 (42%)	20/39.4 (51%)	20/38.5 (52%)	20/35.5 (56%)
		Terminal Incidence	7/11 (64%)	5/12 (42%)	8/13 (62%)	6/13 (46%)	9/17 (53%)	6/13 (46%)
		Time-to-First	502	397	442	448	520	445
		Poly-3 *p*-value	0.470	0.112N	0.081N	0.271N	0.308N	0.469N
Adenoma or carcinoma, pars distalis	Terminal	Incidence	23/49 (47%)	16/50 (32%)	14/48 (29%)	21/50 (42%)	20/50 (40%)	21/46 (46%)
		Poly-3 Incidence	23/38.5 (60%)	16/36.3 (44%)	14/33.5 (42%)	21/39.7 (53%)	20/38.5 (52%)	21/35.5 (59%)
		Terminal Incidence	7/11 (64%)	5/12 (42%)	8/13 (62%)	6/13 (46%)	9/17 (53%)	7/13 (54%)
		Time-to-First	502	397	442	448	520	445
		Poly-3 *p*-value	0.373	0.112N	0.081N	0.340N	0.308N	0.574N

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation. There were no significant increases relative to vehicle control.
[b]One adenoma and one carcinoma.

Table 73. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, pars distalis	Interim	Incidence	18/23 (78%)	17/22 (77%)	18/22 (82%)	15/24 (62%)	16/20 (80%)	20/24 (83%)
		Severity Profile	8|6|3|1 (1.8)	7|6|3|1 (1.9)	7|4|5|2 (2.1)	8|4|2|1 (1.7)	8|6|1|1 (1.7)	7|8|4|1 (2.0)
	Terminal	Incidence	27/50 (54%)	22/48 (46%)	32/46 (70%)	26/49 (53%)	29/49 (59%)	23/46 (50%)
		Severity Profile	0|3|12|12 (3.3)	1|2|5|14 (3.5)	0|4|12|16 (3.4)	1|5|7|13 (3.2)	3|10|4|12 (2.9)	1|6|6|10 (3.1)
		Poly-3 Incidence	27/43.6 (62%)	22/40.2 (55%)	32/40.6[b] (79%)	26/44.2 (59%)	29/42.7 (68%)	23/39.7 (58%)
Angiectasis	Interim[c]	Incidence	1/23 (4%)	0/22 (0%)	1/22 (5%)	0/24 (0%)	0/20 (0%)	0/24 (0%)
		Severity Profile	0/1/0/0 (2.0)	–	0/1/0/0 (2.0)	–	–	–
	Terminal	Incidence	10/50 (20%)	8/48 (17%)	4/46 (9%)	9/49 (18%)	9/49 (18%)	9/46 (20%)
		Severity Profile	0|1|1|8 (3.7)	0|0|0|8 (4.0)	0|0|0|4 (4.0)	0|0|2|7 (3.8)	0|2|2|5 (3.3)	0|1|1|7 (3.7)
		Poly-3 Incidence	10/37.5 (27%)	8/35.4 (23%)	4/29.1 (14%)	9/34.8 (26%)	9/31.3 (29%)	9/32.2 (28%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test ($p = 0.027$) for the pairwise comparison of the 25 µg BPA/kg bw/day group to the vehicle control (hyperplasia of the pars distalis, Poly-3 incidences 30/37.0 (81%) versus 18/30.8 (58%)).

[c]This lesion was not statistically analyzed and is not included in Supplemental Appendix XXXIII since no dose group had two or more lesions. The data are found in Supplemental Appendix XXXII.

Table 74. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia, pars distalis	Interim	Incidence	18/23$^{\#\#, \wedge\wedge}$ (78%)	20/25 (80%)	25/26$^{\#\#, \wedge\wedge}$ (96%)
		Severity Profile	8|6|3|1 (1.8)	10|7|3|0 (1.6)	4|11|6|4 (2.4)
	Terminal	Incidence	27/50$^{\#, \wedge\wedge N}$ (54%)	16/26 (62%)	6/26$^{\#\#, \wedge\wedge N}$ (23%)
		Severity Profile	0|3|12|12 (3.3)	0|3|5|8 (3.3)	0|1|2|3 (3.3)
		Poly-3 Incidence	27/43.6 (62%)	16/23.6 (68%)	6/16.9 (36%)
Angiectasis	Interim	Incidence	1/23$^{*, \#, \wedge}$ (4%)	2/25 (8%)	6/26$^{\#, \wedge}$ (23%)
		Severity Profile	0|1|0|0 (2.0)	0|1|0|1 (3.0)	0|4|1|1 (2.5)
	Terminal	Incidence	10/50$^{\#\#\#, \wedge\wedge\wedge}$ (20%)	5/26 (19%)	17/26$^{\#\#\#, \wedge\wedge\wedge}$ (65%)
		Severity Profile	0|1|1|8 (3.7)	0|0|0|5 (4.0)	0|0|0|17 (4.0)
		Poly-3 Incidence	10/37.5*** (27%)	5/17.1 (29%)	17/22.0*** (77%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 73.

Table 75. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, pars distalis	Interim	Incidence	18/20 (90%)	16/22 (73%)	14/20 (70%)	20/22 (91%)	16/20 (80%)	18/22 (82%)
		Severity Profile	10|8|0|0 (1.4)	10|4|2|0 (1.5)	5|5|4|0 (1.9)	10|9|1|0 (1.6)	6|7|3|0 (1.8)	9|8|1|0 (1.6)
	Terminal	Incidence	25/49 (51%)	32/50^ (64%)	34/48^ (71%)	26/50 (52%)	28/50 (56%)	21/46 (46%)
		Severity Profile	3|7|7|8 (2.8)	0|5|11|16 (3.3)	2|6|14|12 (3.1)	0|6|9|11 (3.2)	3|7|5|13 (3.0)	1|3|7|10 (3.2)
		Poly-3 Incidence	25/42.2 (59%)	32/43.5 (74%)	34/45.0 (76%)	26/43.3 (60%)	28/45.3 (62%)	21/39.1 (54%)
Angiectasis	Interim	Incidence	0/20 (0%)	2/22^ (9%)	1/20 (5%)	0/22 (0%)	0/20 (0%)	0/22 (0%)
		Severity Profile	–	0|1|1|0 (2.5)	0|1|0|0 (2.0)	–	–	–
	Terminal	Incidence	12/49 (24%)	11/50 (22%)	8/48 (17%)	12/50 (24%)	14/50 (28%)	11/46 (24%)
		Severity Profile	0|0|0|12 (4.0)	0|0|1|10 (3.9)	0|1|2|5 (3.5)	0|3|1|8 (3.4)	0|1|2|11 (3.7)	0|2|5|4 (3.2)
		Poly-3 Incidence	12/35.3 (34%)	11/34.5 (32%)	8/32.7 (24%)	12/38.4 (31%)	14/37.6 (37%)	11/33.9 (32%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 76. Non-Neoplastic Lesions in the Heart of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Cardiomyopathy	Interim	Incidence	7/23 (30%)	10/22 (46%)	9/22 (41%)	8/24 (33%)	9/20 (45%)	7/24 (29%)
		Severity Profile	6|1|0|0 (1.1)	8|2|0|0 (1.2)	7|2|0|0 (1.2)	8|0|0|0 (1.0)	8|1|0|0 (1.1)	6|1|0|0 (1.1)
	Terminal	Incidence	35/50 (70%)	30/48 (62%)	24/46^ N (52%)	35/49 (71%)	33/50 (66%)	33/46 (72%)
		Severity Profile	24|10|1|0 (1.3)	18|7|4|1 (1.6)	18|5|1|0 (1.3)	25|9|1|0 (1.3)	24|7|1|1 (1.4)	23|9|0|1 (1.4)
		Poly-3 Incidence	35/43.5 (81%)	30/39.0 (77%)	24/34.6 (69%)	35/42.3 (83%)	33/38.4 (86%)	33/39.4 (84%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 77. Non-Neoplastic Lesions in the Heart of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Cardiomyopathy	Interim	Incidence	7/23[**, ##, ^^]	8/26	17/26[*, ##, ^^]
			(30%)	(30.8%)	(65%)
		Severity Profile	6|1|0|0 (1.1)	8|0|0|0 (1.0)	13|4|0|0 (1.2)
	Terminal	Incidence	35/50[^]	19/26	22/26[#, ^]
			(70%)	(73%)	(85%)
		Severity Profile	24|10|1|0 (1.3)	14|5|0|0 (1.3)	12|9|1|0 (1.5)
		Poly-3 Incidence	35/43.5	19/22.6	22/24.0
			(81%)	(84%)	(92%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 76.

Table 78. Non-Neoplastic Lesions in the Heart of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Cardiomyopathy	Interim	Incidence	6/20 (30%)	8/22 (36%)	7/20 (35%)	7/22 (32%)	6/20 (30%)	7/22 (32%)
		Severity Profile	6|0|0|0 (1.0)	8|0|0|0 (1.0)	6|1|0|0 (1.1)	5|2|0|0 (1.3)	6|0|0|0 (1.0)	5|2|0|0 (1.3)
	Terminal	Incidence	32/50[##, ^^] (64%)	37/50[^] (74%)	38/48 (79%)	37/50[#, ^] (74%)	35/50[#, ^] (70%)	35/46[##, ^^] (76%)
		Severity Profile	26|3|3|0 (1.3)	22|13|2|0 (1.5)	29|7|2|0 (1.3)	21|14|2|0 (1.5)	17|13|5|0 (1.7)	16|14|5|0 (1.7)
		Poly-3 Incidence	32/42.9 (74%)	37/42.9 (86%)	38/43.1 (88%)	37/44.0 (84%)	35/44.0 (80%)	35/39.7[b] (88%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test (p = 0.049) for the comparison of the 25,000 µg BPA/kg bw/day dose group to the vehicle controls (Poly-3 incidences 27/30.8 (88%) versus 22/31.6 (70%)).

Table 79. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Nephropathy	Interim	Incidence	6/23 (26%)	7/22 (32%)	11/22^ (50%)	8/24 (33%)	11/20^ (55%)	7/24 (29%)
		Severity Profile	4|1|0|1(1.7)	5|1|1|0 (1.4)	6|2|0|3 (2.0)	6|1|0|1 (1.5)	9|0|2|0 (1.4)	3|2|1|1 (2.0)
	Terminal	Incidence	19/50 (38%)	28/48^^ (58%)	21/46 (46%)	21/49 (43%)	21/50 (42%)	25/46#, ^ (54%)
		Severity Profile	18|0|1|0 (1.1)	16|9|0|3 (1.6)	17|2|1|1 (1.3)	12|5|2|2 (1.7)	12|4|3|2 (1.8)	18|3|1|3 (1.6)
		Poly-3 Incidence	19/39.9 (48%)	28/40.6* (69%)	21/34.4 (61%)	21/38.5 (54%)	21/35.5 (59%)	25/38.2 (65%)
Cyst, renal tubule	Interim	Incidence	0/23 (0%)	7/22** (32%)	3/22 (14%)	3/24 (12%)	3/20 (15%)	1/24 (4%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	9/50 (18%)	8/48 (17%)	12/46 (26%)	15/49 (31%)	12/50 (24%)	6/46 (13%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 Incidence	9/36.2 (25%)	8/34.3 (23%)	12/32.1 (37%)	15/36.1[c] (42%)	12/33.9 (35%)	6/33.4 (18%)
Mineralization	Interim	Incidence	11/23*, ##, ^^ (48%)	5/22 (23%)	11/22 (50%)	12/24 (50%)	11/20 (55%)	16/24#, ^ (67%)
		Severity Profile	11|0|0|0 (1.0)	3|1|1|0 (1.6)	7|2|1|1 (1.6)	11|1|0|0 (1.1)	8|3|0|0 (1.3)	10|3|3|0 (1.6)
	Terminal	Incidence	30/50 (60%)	23/48 (48%)	25/46 (54%)	25/49 (51%)	24/50 (48%)	26/46 (57%)
		Severity Profile	21|8|1|0 (1.3)	20|3|0|0 (1.1)	18|7|0|0 (1.3)	18|7|0|0 (1.3)	17|5|2|0 (1.4)	16|7|2|1 (1.5)
		Poly-3 Incidence	30/42.9 (70%)	23/37.8 (61%)	25/40.3 (62%)	25/40.6 (62%)	24/39.3 (61%)	26/38.6 (67%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]Severity scores were not assigned for this lesion.

[c]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test (p = 0.033) for the pairwise comparison of the 250 µg BPA/kg bw/day dose group to the vehicle control group (renal tubule cyst, Poly-3 incidences 15/32.9 (46%) versus 5/24.8 (20%)).

Table 80. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Nephropathy	Interim	Incidence	6/23[*, #, ^] (26%)	13/26 (50%)	15/26[*, #, ^] (58%)
		Severity Profile	4|1|0|1 (1.7)	8|5|0|0 (1.4)	10|4|1|0 (1.4)
	Terminal	Incidence	19/50[##, ^^] (38%)	14/26[#] (54%)	15/26[##, ^^] (58%)
		Severity Profile	18|0|1|0 (1.1)	10|3|1|0 (1.4)	6|5|3|1 (1.9)
		Poly-3 Incidence	19/39.9[*] (48%)	14/21.0 (67%)	15/21.6 (70%)
Cyst, renal tubule	Interim	Incidence	0/23 (0%)	5/26[*] (19%)	4/26 (15%)
		Severity Profile	–[c]	–	–
	Terminal	Incidence	9/50 (18%)	5/26 (19%)	6/26 (23%)
		Severity Profile	–[c]	–	–
		Poly-3 Incidence	9/36.2 (25%)	5/18.3 (27%)	6/17.6 (34%)
Mineralization	Interim	Incidence	11/23 (48%)	17/26[^] (65%)	14/26 (54%)
		Severity Profile	11|0|0|0 (1.0)	10|6|1|0 (1.5)	7|5|1|1 (1.7)
	Terminal	Incidence	30/50 (60%)	10/26 (38%)	17/26 (65%)
		Severity Profile	21|8|1|0 (1.3)	4|5|1|0 (1.7)	10|7|0|0 (1.4)
		Poly-3 Incidence	30/42.9 (70%)	10/20.7 (48%)	17/22.0 (77%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 79.
[c]Severity scores were not assigned for this lesion.

Table 81. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Nephropathy	Interim	Incidence	10/20 (50%)	10/22 (46%)	10/20 (50%)	11/22 (50%)	12/20 (60%)	13/22 (59%)
		Severity Profile	7|2|1|0 (1.4)	7|2|1|0 (1.4)	9|1|0|0 (1.1)	7|3|1|0 (1.5)	7|5|0|0 (1.4)	10|1|1|1 (1.5)
	Terminal	Incidence	28/49[##, ^^] (57%)	25/50 (50%)	25/47 (53%)	29/49 (59%)	33/50 (66%)	30/46[^] (65%)
		Severity Profile	17|8|1|2 (1.6)	16|4|2|3 (1.7)	17|3|5|0 (1.5)	22|5|1|1 (1.3)	15|11|3|4 (1.9)	10|11|6|3 (2.1)
		Poly-3 Incidence	28/39.2 (71%)	25/41.3 (60%)	25/38.4 (65%)	29/40.9 (71%)	33/43.3 (76%)	30/39.9 (75%)
Cyst, renal tubule	Interim	Incidence	4/20 (20%)	3/22 (14%)	4/20 (20%)	4/22 (18%)	6/20 (30%)	5/22 (23%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	7/49 (14%)	15/50 (30%)	11/47 (23%)	8/49 (16%)	16/50 (32%)	11/46 (24%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 Incidence	7/33.6 (21%)	15/34.9[*] (43%)	11/34.3 (32%)	8/36.1 (22%)	16/39.6 (40%)	11/34.4 (32%)
Mineralization	Interim	Incidence	13/20 (65%)	11/22 (50%)	11/20 (55%)	14/22 (64%)	13/20 (65%)	11/22 (50%)
		Severity Profile	8|4|0|1 (1.5)	9|2|0|0 (1.2)	6|4|0|1 (1.6)	8|6|0|0 (1.4)	6|6|1|0 (1.6)	8|3|0|0 (1.3)
	Terminal	Incidence	28/49 (57%)	22/50 (44%)	28/47 (60%)	26/49 (53%)	23/50 (46%)	23/46 (50%)
		Severity Profile	20|7|0|1 (1.4)	14|7|1|0 (1.4)	18|9|0|1 (1.4)	17|8|1|0 (1.4)	11|9|3|0 (1.7)	13|9|1|0 (1.5)
		Poly-3 Incidence	28/40.7 (69%)	22/39.1 (56%)	28/38.9 (72%)	26/42.4 (61%)	23/42.4 (54%)	23/39.1 (59%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Severity scores were not assigned for this lesion.

Table 82. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, Mononuclear cells	Interim	Incidence	4/23 (17%)	6/22 (27%)	4/22 (18%)	5/24 (21%)	5/20 (25%)	4/24 (17%)
		Severity Profile	4|0|0|0 (1.0)	5|1|0|0 (1.2)	4|0|0|0 (1.0)	5|0|0|0 (1.0)	5|0|0|0 (1.0)	4|0|0|0 (1.0)
	Terminal	Incidence	37/50$^{\#\#, \wedge\wedge N}$ (74%)	28/48 (58%)	35/46 (76%)	29/49$^{\#, \wedge N}$ (59%)	26/50$^{\#\#, \wedge\wedge N}$ (52%)	24/46$^{\#, \wedge N}$ (52%)
		Severity Profile	27|9|1|0 (1.3)	20|8|0|0 (1.3)	29|6|0|0 (1.2)	24|5|0|0 (1.2)	21|5|0|0 (1.2)	19|4|1|0 (1.2)
		Poly-3 Incidence	37/44.3$^{** N}$ (83%)	28/38.1 (74%)	35/38.7 (90%)	29/41.9 (69%)	26/38.7$^{* N}$ (67%)	24/37.9$^{* N}$ (63%)
Cystic degeneration	Terminal	Incidence	4/50 (8%)	3/48 (6%)	6/46 (13%)	3/49 (6%)	5/50 (10%)	1/46 (2%)
		Severity Profile	4|0|0|0 (1.0)	3|0|0|0 (1.0)	6|0|0|0 (1.0)	3|0|0|0 (1.0)	4|1|0|0 (1.2)	1|0|0|0 (1.0)
		Poly-3 Incidence	4/34.9 (12%)	3/33.3 (9%)	6/29.8 (20%)	3/33.2 (9%)	5/29.6 (17%)	1/31.2 (3%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 83. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Infiltration, Mononuclear cells	Interim	Incidence	4/23 (17%)	7/26 (27%)	2/26 (8%)
		Severity Profile	4|0|0|0 (1.0)	7|0|0|0 (1.0)	2|0|0|0 (1.0)
	Terminal	Incidence	37/50##, ^^^ N (74%)	17/26 (65%)	10/26##, ^^^ N (38%)
		Severity Profile	27|9|1|0 (1.3)	15|2|0|0 (1.1)	8|2|0|0 (1.2)
		Poly-3 Incidence	37/44.3** N (83%)	17/21.6 (79%)	10/18.7** N (54%)
Cystic degeneration	Terminal	Incidence	4/50 (8%)	4/26 (15%)	2/26 (8%)
		Severity Profile	4|0|0|0 (1.0)	4|0|0|0 (1.0)	1|1|0|0 (1.5)
		Poly-3 Incidence	4/34.9 (12%)	4/17.3 (23%)	2/15.2 (13%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 82.

Table 84. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a, b]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, Mononuclear cells	Interim	Incidence	2/20 (10%)	10/22[*, ^^] (46%)	7/20 (35%)	7/22 (32%)	2/20 (10%)	8/22[*, #, ^] (36%)
		Severity Profile	1|1|0|0 (1.5)	10|0|0|0 (1.0)	7|0|0|0 (1.0)	7|0|0|0 (1.0)	2|0|0|0 (1.0)	8|0|0|0 (1.0)
	Terminal	Incidence	29/49 (59%)	28/50 (56%)	33/48 (69%)	25/50 (50%)	31/50 (62%)	31/46 (67%)
		Severity Profile	22|6|1|0 (1.3)	18|10|0|0 (1.4)	26|7|0|0 (1.2)	16|9|0|0 (1.4)	25|5|1|0 (1.2)	23|8|0|0 (1.3)
		Poly-3 Incidence	29/39.7 (73%)	28/38.9 (72%)	33/41.5 (80%)	25/39.8 (63%)	31/41.0 (76%)	31/37.2 (83%)
Cystic degeneration	Terminal	Incidence	2/49[##, ^^] (4%)	1/50 (2%)	6/48 (12%)	5/50 (10%)	8/50[#, ^] (16%)	7/46[#, ^] (15%)
		Severity Profile	2|0|0|0 (1.0)	1|0|0|0 (1.0)	6|0|0|0 (1.0)	5|0|0|0 (1.0)	6|2|0|0 (1.2)	7|0|0|0 (1.0)
		Poly-3 Incidence	2/32.0[**] (6%)	1/31.7 (3%)	6/32.0 (19%)	5/35.3 (14%)	8/37.5 (21%)	7/32.3 (22%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a statistically significant Poly-3 dose trend ($p = 0.036$; in the analysis that included all animals, $p = 0.222$) for mixed cell foci in terminal sacrifice animals (not shown in Table).

Table 85. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Female Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, C-cell	Interim	Incidence	14/23 (61%)	11/22 (50%)	15/21 (71%)	12/24 (50%)	8/20 (40%)	16/24 (67%)
		Severity Profile	8|6|0|0 (1.4)	9|2|0|0 (1.2)	12|3|0|0 (1.2)	9|3|0|0 (1.2)	4|3|1|0 (1.6)	12|3|1|0 (1.3)
	Terminal	Incidence	22/50 (44%)	17/48 (35%)	22/46 (48%)	18/49 (37%)	20/50 (40%)	17/46 (37%)
		Severity Profile	11|9|2|0 (1.6)	12|3|2|0 (1.4)	13|7|2|0 (1.5)	11|7|0|0 (1.4)	8|10|2|0 (1.7)	7|8|2|0 (1.7)
		Poly-3 Incidence	22/40.7 (54%)	17/37.3 (46%)	22/36.0 (61%)	18/38.4 (47%)	20/35.9 (56%)	17/36.6 (46%)
Hyperplasia, Follicular cell[b]	Terminal	Incidence	1/50 (2%)	6/48^ (12%)	4/46 (9%)	3/49 (6%)	1/50 (2%)	4/46 (9%)
		Severity Profile	0|1|0|0 (2.0)	0|2|4|0 (2.7)	0|2|1|1 (2.8)	0|2|1|0 (2.3)	0|0|0|1 (4.0)	0|2|2|0 (2.5)
		Poly-3 Incidence	1/34.2 (3%)	6/34.1 (18%)	4/30.1 (13%)	3/33.3 (9%)	1/29.1 (3%)	4/32.9 (12%)
Ultimobranchial cyst	Interim	Incidence	7/23 (30%)	7/22 (32%)	5/21 (24%)	8/24 (33%)	6/20 (30%)	11/24 (46%)
		Severity Profile	–[c]	–	–	–	–	–
	Terminal	Incidence	8/50 (16%)	2/48 (4%)	6/46 (13%)	3/49 (6%)	4/50 (8%)	7/46 (15%)
		Severity Profile	–[c]	–	–	–	–	–
		Poly-3 Incidence	8/36.4 (22%)	2/33.5 (6%)	6/30.2 (20%)	3/33.3 (9%)	4/30.2 (13%)	7/32.8 (21%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Data for follicular cell hyperplasia are not tabulated for interim sacrifice animals since no dose groups had two or more diagnoses of this lesion.
[c]No severity scores were assigned for ultimobranchial cysts.

Table 86. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Female Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia, C-cell	Interim	Incidence	14/23 (61%)	11/26 (42%)	13/26 (50%)
		Severity Profile	8|6|0|0 (1.4)	7|4|0|0 (1.4)	11|1|1|0 (1.2)
	Terminal	Incidence	22/50 (44%)	7/26 (27%)	9/25 (36%)
		Severity Profile	11|9|2|0 (1.6)	2|5|0|0 (1.7)	6|2|0|1 (1.6)
		Poly-3 Incidence	22/40.7 (54%)	7/18.7 (37%)	9/18.1 (50%)
Hyperplasia, Follicular cell[c]	Terminal	Incidence	1/50 (2%)	4/26 ^^ (15%)	0/25 (0%)
		Severity Profile	0|1|0|0 (2.0)	0|1|3|0 (2.8)	–
		Poly-3 Incidence	1/34.2 (3%)	4/17.9* (22%)	0/14.7 (0%)
Ultimobranchial cyst	Interim	Incidence	7/23 (30%)	7/26 (27%)	11/26 (42%)
		Severity Profile	–[d]	–	–
	Terminal	Incidence	8/50 (16%)	5/26 (19%)	8/25 (32%)
		Severity Profile	–[d]	–	–
		Poly-3 Incidence	8/36.4* (22%)	5/17.5 (29%)	8/17.4 (46%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 85.
[c]Data for follicular cell hyperplasia are not tabulated for interim sacrifice animals since no dose groups had two or more diagnoses of this lesion.
[d]No severity scores were assigned for ultimobranchial cysts.

Table 87. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Female Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, C-cell	Interim	Incidence	10/20 (50%)	16/22^ (73%)	11/20 (55%)	12/22 (54%)	13/20 (65%)	9/22 (41%)
		Severity Profile	10|0|0|0 (1.0)	11|4|1|0 (1.4)	8|3|0|0 (1.3)	8|4|0|0 (1.3)	11|2|0|0 (1.2)	8|1|0|0 (1.1)
	Terminal	Incidence	26/48 (54%)	29/49 (59%)	17/45 (38%)	23/48 (48%)	28/50 (56%)	24/46 (52%)
		Severity Profile	8|15|2|1 (1.8)	15|11|1|2 (1.7)	11|5|0|1 (1.5)	15|7|1|0 (1.4)	10|12|4|2 (1.9)	11|11|2|0 (1.6)
		Poly-3 Incidence	26/40.5 (64%)	29/42.0 (69%)	17/34.9 (49%)	23/39.8 (58%)	28/42.6 (66%)	24/38.9 (62%)
Hyperplasia, Follicular cell[b]	Terminal	Incidence	4/48 (8%)	4/49 (8%)	7/45 (16%)	6/48 (12%)	5/50 (10%)	4/46 (9%)
		Severity Profile	0|2|2|0 (2.5)	1|3|0|0 (1.8)	0|5|2|0 (2.3)	0|4|2|0 (2.3)	0|3|2|0 (2.4)	0|3|1|0 (2.2)
		Poly-3 Incidence	4/32.8 (12%)	4/32.0 (12%)	7/31.6 (22%)	6/35.4 (17%)	5/35.9 (14%)	4/33.2 (12%)
Ultimobranchial cyst	Interim	Incidence	4/20 (20%)	6/22 (27%)	7/20 (35%)	4/22 (18%)	6/20 (30%)	6/22 (27%)
		Severity Profile	–[c]	–	–	–	–	–
	Terminal	Incidence	2/48 (4%)	7/49 (14%)	2/45 (4%)	9/48 (19%)	11/50 (22%)	3/46 (6%)
		Severity Profile	–[c]	–	–	–	–	–
		Poly-3 Incidence	2/31.4 (6%)	7/33.4 (21%)	2/29.5 (7%)	9/36.1* (25%)	11/38.6* (28%)	3/32.1 (9%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Data for follicular cell hyperplasia are not tabulated for interim sacrifice animals since no dose groups had two or more diagnoses of this lesion.
[c]No severity scores were assigned for ultimobranchial cysts.

Table 88. Non-Neoplastic Lesions in the Epididymis of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Exfoliated germ cells	Interim	Incidence	1/22[*, #, ^] (4%)	1/22 (4%)	1/20 (5%)	1/24 (4%)	0/20 (0%)	6/22[*, ##, ^^] (27%)
		Severity Profile	1|0|0|0 (1.0)	1|0|0|0 (1.0)	0|1|0|0 (2.0)	1|0|0|0 (1.0)	-	6|0|0|0 (1.0)
	Terminal	Incidence	10/49 (20%)	8/48 (17%)	10/48 (21%)	12/50 (24%)	13/50 (26%)	6/46 (13%)
		Severity Profile	5|3|1|1 (1.8)	5|1|2|0 (1.6)	1|9|0|0 (1.9)	4|5|2|1 (2.0)	4|7|2|0 (1.8)	4|0|2|0 (1.7)
		Poly-3 Incidence	10/36.5 (27%)	8/35.5 (23%)	10/35.4 (28%)	12/37.4 (32%)	13/38.0 (34%)	6/30.1 (20%)
Infiltration, cellular, lymphocyte	Interim	Incidence	0/22[*, #, ^] (0%)	1/22 (4%)	3/20 (15%)	2/24 (8%)	0/20 (0%)	5/22[*, ##, ^^] (23%)
		Severity Profile	-	0|1|0|0 (2.0)	3|0|0|0 (1.0)	2|0|0|0 (1.0)	-	5|0|0|0 (1.0)
	Terminal	Incidence	10/49 (20%)	12/48 (25%)	13/48 (27%)	15/50 (30%)	14/50 (28%)	15/46 (33%)
		Severity Profile	8|2|0|0 (1.2)	12|0|0|0 (1.0)	11|2|0|0 (1.2)	13|2|0|0 (1.1)	12|2|0|0 (1.1)	13|2|0|0 (1.1)
		Poly-3 Incidence	10/35.5 (28%)	12/36.5 (33%)	13/34.4 (38%)	15/36.2 (41%)	14/36.0 (39%)	15/34.1 (44%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 89. Non-Neoplastic Lesions in the Epididymis of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic[b]	Vehicle	0.05 EE_2	0.5 EE_2
Exfoliated germ cells	Interim	Incidence	1/22 (4%)	4/26 (15%)	2/26 (8%)
		Severity Profile	1|0|0|0 (1.0)	3|1|0|0 (1.2)	2|0|0|0 (1.0)
	Terminal	Incidence	10/49 (20%)	6/26 (23%)	4/26 (15%)
		Severity Profile	5|3|1|1 (1.8)	4|1|1|0 (1.5)	3|1|0|0 (1.2)
		Poly-3 Incidence	10/36.5 (27%)	6/18.3 (33%)	4/20.4 (20%)
Infiltration, cellular, lymphocyte	Interim	Incidence	0/22[#, ^] (0%)	1/26 (4%)	3/26[#, ^] (12%)
		Severity Profile	–-	1|0|0|0 (1.0)	3|0|0|0 (1.0)
	Terminal	Incidence	10/49[^] (20%)	5/26 (19%)	10/26[^] (38%)
		Severity Profile	8|2|0|0 (1.2)	4|1|0|0 (1.2)	8|2|0|0 (1.2)
		Poly-3 Incidence	10/35.5 (28%)	5/18.3 (27%)	10/20.4 (49%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 88.

Table 90. Non-Neoplastic Lesions in the Epididymis of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Exfoliated germ cells	Interim	Incidence	0/20 (0%)	3/20^ (15%)	1/20 (5%)	2/19 (10%)	1/20 (5%)	1/22 (4%)
		Severity Profile	–	1|2|0|0 (1.7)	0|1|0|0 (2.0)	2|0|0|0 (1.0)	0|1|0|0 (2.0)	1|0|0|0 (1.0)
	Terminal	Incidence	12/49 (24%)	15/48 (31%)	11/48 (23%)	13/50 (26%)	17/50 (34%)	9/46 (20%)
		Severity Profile	3|6|3|0 (2.0)	5|7|3|0 (1.9)	3|7|1|0 (1.8)	3|9|1|0 (1.8)	5|5|6|1 (2.2)	4|4|1|0 (1.7)
		Poly-3 Incidence	12/39.0 (31%)	15/38.3 (39%)	11/37.9 (29 %)	13/36.1 (36%)	17/39.8 [b] (43%)	9/29.5 (30%)
Infiltration, cellular, lymphocyte	Interim	Incidence	1/20 (5%)	1/20 (5%)	4/20 (20%)	1/19 (5%)	2/20 (10%)	2/22 (9%)
		Severity Profile	1|0|0|0 (1.0)	1|0|0|0 (1.0)	4|0|0|0 (1.0)	1|0|0|0 (1.0)	2|0|0|0 (1.0)	2|0|0|0 (1.0)
	Terminal	Incidence	14/49 (29%)	16/48 (33%)	16/48 (33%)	14/50 (28%)	12/50 (24%)	13/46 (28%)
		Severity Profile	9|5|0|0 (1.4)	13|3|0|0 (1.2)	14|2|0|0 (1.1)	11|3|0|0 (1.2)	10|2|0|0 (1.2)	12|1|0|0 (1.1)
		Poly-3 Incidence	14/40.2 (35%)	16/36.8 (44%)	16/38.3 (42%)	14/35.3 (40%)	12/39.9 (30%)	13/29.6 (44%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a statistically significant (p = 0.046) difference in the pairwise comparison between the 2,500 µg BPA/kg bw/day dose group and the vehicle control (exfoliated germ cells, Poly-3 incidences 16/32.4 (49%) versus 7/27.3 (26%)).

Table 91. Non-Neoplastic Lesions in the Dorsal/Lateral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, cellular, lymphocyte	Interim	Incidence	4/22 (18%)	10/22^ [b] (46%)	5/20 (25%)	6/24 (25%)	5/20 (25%)	7/22 (32%)
		Severity Profile	4|0|0|0 (1.0)	9|1|0|0 (1.1)	5|0|0|0 (1.0)	5|1|0|0 (1.2)	5|0|0|0 (1.0)	5|2|0|0 (1.3)
	Terminal	Incidence	33/50 (66%)	26/48 (54%)	27/48 (56%)	27/50 (54%)	27/50 (54%)	20/46 (44%)
		Severity Profile	19|11|2|1 (1.5)	18|7|1|0 (1.3)	21|5|1|0 (1.3)	16|6|3|2 (1.7)	18|8|0|1 (1.4)	10|7|1|2 (1.8)
		Poly-3 Incidence	33/40.4* N (82%)	26/38.2 (68%)	27/38.7 (70%)	27/39.6 (68%)	27/38.7 (70%)	20/33.0 (61%)
Suppurative inflammation	Interim	Incidence	18/22 (82%)	20/22^ (91%)	18/20 (90%)	22/24^ (92%)	18/20#, ^ (90%)	19/22# (86%)
		Severity Profile	11|7|0|0 (1.4)	6|14|0|0 (1.7)	9|9|0|0 (1.5)	9|12|1|0 (1.6)	6|10|2|0 (1.8)	7|11|1|0 (1.7)
	Terminal	Incidence	41/50 (82%)	46/48 (96%)	47/48 (98%)	45/50 (90%)	43/50 (86%)	41/46 (89%)
		Severity Profile	8|26|4|3 (2.0)	10|26|9|1 (2.0)	9|30|8|0 (2.0)	17|24|2|2 (1.8)	8|28|6|1 (2.0)	12|20|6|3 (2.0)
		Poly-3 Incidence	41/45.6 (90%)	46/46.2* (100%)	47/48.0 (98%)	45/47.7 (94%)	43/46.5 (92%)	41/43.3 (95%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), the lymphocyte cellular infiltration in the 2.5 BPA dose group was also significant in the CAFE analysis (lymphocyte cellular infiltration, 9/16 (56%), in treated group versus 3/17 (18%), in controls, $p = 0.025$).

Table 92. Non-Neoplastic Lesions in the Dorsal/Lateral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Infiltration, cellular, lymphocyte	Interim	Incidence	4/22 (18%)	9/26 (35%)	4/26 (15%)
		Severity Profile	4|0|0|0 (1.0)	9|0|0|0 (1.0)	4|0|0|0 (1.0)
	Terminal	Incidence	33/50 (66%)	17/26 (65%)	13/25 (52%)
		Severity Profile	19|11|2|1 (1.5)	10|6|1|0 (1.5)	6|6|0|1 (1.7)
		Poly-3 Incidence	33/40.4 (82%)	17/21.3 (80 %)	13/20.1 (65%)
Suppurative inflammation	Interim	Incidence	18/22 (82%)	25/26 (96%)	25/26 (96%)
		Severity Profile	11|7|0|0 (1.4)	13|11|0|1 (1.6)	12|13|0|0 (1.5)
	Terminal	Incidence	41/50 (82%)	26/26 (100%)	22/25 (88%)
		Severity Profile	8|26|4|3 (2.0)	8|16|1|1 (1.8)	6|12|3|1 (2.0)
		Poly-3 Incidence	41/45.6 (90%)	26/26.0 (100%)	22/23.1 (95%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 91.

Table 93. Non-Neoplastic Lesions in the Dorsal/Lateral Prostate of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, cellular, lymphocyte	Interim	Incidence	9/20 (45%)	5/20 (25%)	4/20 (20%)	8/18 (44%)	8/20 (40%)	6/22 (27%)
		Severity Profile	9|0|0|0 (1.0)	4|1|0|0 (1.2)	3|1|0|0 (1.2)	8|0|0|0 (1.0)	5|3|0|0 (1.4)	6|0|0|0 (1.0)
	Terminal	Incidence	31/46 (67%)	30/48 (62%)	28/48 (58%)	27/50 (54%)	35/49 (71%)	22/45[#, ^ N] (49%)
		Severity Profile	17|11|1|2 (1.6)	20|6|1|3 (1.6)	15|9|2|2 (1.7)	16|8|1|2 (1.6)	20|14|1|0 (1.5)	14|7|0|1 (1.5)
		Poly-3 Incidence	31/41.6 (75%)	30/41.1 (73%)	28/40.0 (70%)	27/38.9 (69%)	35/42.4 (83%)	22/33.2 (66%)
Suppurative inflammation	Interim	Incidence	18/20 (90%)	19/20 (95%)	16/20 (80%)	16/18 (89%)	19/20 (95%)	18/22 (82%)
		Severity Profile	5|13|0|0 (1.7)	12|7|0|0 (1.4)	6|10|0|0 (1.6)	7|9|0|0 (1.6)	9|10|0|0 (1.5)	5|12|1|0 (1.8)
	Terminal	Incidence	39/46 (85%)	46/48 (96%)	41/48 (85%)	42/50 (84%)	44/49 (90%)	38/45 (84%)
		Severity Profile	7|22|7|3 (2.2)	14|21|8|3 (2.0)	14|20|5|2 (1.9)	10|20|9|3 (2.1)	12|21|11|0 (2.0)	6|25|6|1 (2.1)
		Poly-3 Incidence	39/43.3 (90%)	46/46.7 (98%)	41/45.4 (90%)	42/45.2 (93%)	44/46.3 (95%)	38/41.2 (92%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 94. Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenoma	Terminal	Incidence	6/50	7/48	2/48	4/49	2/49	6/46
			(12%)	(15%)	(4%)	(8%)	(4%)	(13%)
		Poly-3 Incidence	6/34.4	7/34.4	2/32.3	4/32.9	2/33.1	6/29.4
			(17%)	(20%)	(6%)	(12%)	(6%)	(20%)
		Terminal Incidence	3/15	4/16	2/17	2/14	2/16	3/11
			(20%)	(25%)	(12%)	(14%)	(12%)	(27%)
		Time-to-First	603	683	726 (T)	713	726 (T)	613
		Poly-3 *p*-value	0.287N	0.499	0.148N	0.394N	0.139N	0.506
		Multiple Incidence[d]	0/50	2/48	0/48	0/49	0/49	3/46
			(0%)	(4%)	(0%)	(0%)	(0%)	(7%)

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation.

Table 95. Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.50 EE_2
Adenoma	Terminal	Incidence	6/50	2/26	2/26
			(12%)	(8%)	(8%)
		Poly-3 Incidence	6/34.4	2/17.6	2/20.7
			(17%)	(11%)	(10%)
		Terminal Incidence	3/15	2/9	0/12
			(20%)	(22%)	(0%)
		Time-to-First	603	725 (T)	462
		Poly-3 *p*-value	0.287N	0.433N	0.344N
		Multiple Incidence[d]	0/50	1/26	0/26
			(0%)	(4%)	(0%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 52 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 94.

Table 96. Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Adenoma	Terminal	Incidence	4/48 (8%)	4/47 (8%)	4/47 (8%)	2/50 (4%)	4/49 (8%)	6/45 (13%)
		Poly-3 Incidence	4/35.7 (11%)	4/33.0 (12%)	4/34.1 (12%)	2/30.8 (6%)	4/36.5 (11%)	6/28.7 (21%)
		Terminal Incidence	2/17 (12%)	2/16 (12%)	4/16 (25%)	2/13 (15%)	1/15 (7%)	1/9 (11%)
		Time-to-First	675	679	724 (T)	724 (T)	593	530
		Poly-3 *p*-value	0.273	0.601	0.619	0.407N	0.635N	0.233
		Multiple Incidence[d]	1/48 (2%)	2/47 (4%)	1/47 (2%)	1/50 (2%)	2/49 (4%)	2/45 (4%)

[a]BPA doses are µg/kg bw/day. See legend to Table 52 for description of data presentation.

Table 97. Non-Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, cellular, lymphocyte	Interim	Incidence	8/22 (36%)	10/22 (45%)	10/20 (50%)	8/24 (33%)	9/20 (45%)	8/22 (36%)
		Severity Profile	8|0|0|0	8|2|0|0	9|1|0|0	7|1|0|0	8|1|0|0	7|1|0|0
	Terminal	Incidence	25/50 (50%)	14/48$^{\#\#, \wedge\wedge\ N}$ (29%)	15/48$^{\#\#, \wedge\wedge\ N}$ (31%)	15/49$^{\#\#, \wedge\ N}$ (31%)	20/49$^{\#\ N}$ (41%)	15/46$^{\#, \wedge\ N}$ (33%)
		Severity Profile	12|5|5|3	9|4|1|0	14|1|0|0	9|4|0|2	15|3|1|1	9|3|1|2
		Poly-3 Incidence	25/40.8 (61%)	14/38.2$^{*\ N}$ (37%)	15/39.1$^{*\ N}$ (38%)	15/39.4$^{*\ N}$ (38%)	20/39.0 (51%)	15/33.3 (45%)
Suppurative inflammation	Interim	Incidence	10/22$^{**, \#\#, \wedge\wedge\ N}$ (45%)	3/22$^{*, \#, \wedge\wedge\ N}$ (14%)	4/20$^{\#, \wedge\ N}$ (20%)	5/24$^{\#, \wedge\ N}$ (21%)	3/20$^{*, \#, \wedge\wedge\ N}$ (15%)	1/22$^{**, \#\#\#, \wedge\wedge\wedge\ N}$ (5%)
		Severity Profile	9|1|0|0	3|0|0|0	2|2|0|0	4|1|0|0	3|0|0|0	1|0|0|0
	Terminal	Incidence	16/50 (32%)	5/48$^{\#\#, \wedge\wedge\ N}$ (10%)	5/48$^{\#\#, \wedge\wedge\ N}$ (10%)	6/49$^{\#\#, \wedge\wedge\ N}$ (12%)	5/49$^{\#\#, \wedge\wedge\ N}$ (10%)	11/46$^{\#\#\ N}$ (24%)
		Severity Profile	7|2|2|5	3|2|0|0	3|2|0|0	3|1|0|2	1|2|1|1	5|2|1|3
		Poly-3 Incidence	16/38.1 (42%)	5/34.6$^{**\ N}$ (14%)	5/34.6$^{**\ N}$ (14%)	6/35.3$^{*\ N}$ (17%)	5/34.9$^{**\ N}$ (14%)	11/31.9 (34%)
Hyperplasia, epithelium	Interim	Incidence	0/22	0/22	0/20	1/24	0/20	0/22
		Severity Profile	–	–	–	0/1/0/0	–	–
	Terminal	Incidence	10/50 (20%)	12/48 (25%)	10/48 (21%)	18/49$^{\wedge}$ (37%)	12/49 (24%)	8/46 (17%)
		Severity Profile	1|6|3|0	2|6|3|1	2|4|4|0	1|13|3|1	3|6|3|0	2|2|2|2
		Poly-3 Incidence	10/34.0 (29%)	12/36.4 (33%)	10/35.0 (28%)	18/36.3 (50%)	12/34.7 (35%)	8/30.5 (26%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 98. Non-Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Infiltration, cellular, lymphocyte	Interim	Incidence	8/22 (36%)	13/26 (50%)	13/26 (50%)
		Severity Profile	8|0|0|0	11|2|0|0	12|1|0|0
	Terminal	Incidence	25/50 (50%)	6/26$^{\wedge\wedge\ N}$ (23%)	14/26 (54%)
		Severity Profile	12|5|5|3	5|0|1|0	6|7|0|1
		Poly-3 Incidence	25/40.8 (61%)	6/19.2$^{*\ N}$ (31%)	14/22.3 (63%)
Suppurative inflammation	Interim	Incidence	10/22$^{*,\ \#,\ \wedge\ N}$ (45%)	4/26$^{*,\ \#,\ \wedge\ N}$ (15%)	5/26$^{\#,\ \wedge\ N}$ (19%)
		Severity Profile	9|1|0|0	3|1|0|0	5|0|0|0
	Terminal	Incidence	16/50 (32%)	4/26$^{\wedge\ N}$ (15%)	8/26 (31%)
		Severity Profile	7|2|2|5	3|0|0|1	5|2|0|1
		Poly-3 Incidence	16/38.1 (42%)	4/18.7 (21%)	8/22.2 (36%)
Hyperplasia, epithelium	Interim	Incidence	0/22 (0%)	0/26 (0%)	0/26 (0%)
		Severity Profile	–	–	–
	Terminal	Incidence	10/50 (20%)	4/26 (15%)	7/26 (27%)
		Severity Profile	1|6|3|0	0|1|2|1	1|4|2|0
		Poly-3 Incidence	10/34.0 (29%)	4/17.6 (23%)	7/19.9 (35%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 97.

Table 99. Non-Neoplastic Lesions in the Ventral Prostate of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Infiltration, cellular, lymphocyte	Interim	Incidence	9/20 (45%)	8/20 (40%)	9/20 (45%)	9/18 (50%)	8/20 (40%)	9/22 (41%)
		Severity Profile	8|1|0|0	7|1|0|0	8|1|0|0	8|1|0|0	7|1|0|0	9|0|0|0
	Terminal	Incidence	19/48 (40%)	17/47 (36%)	15/47 (32%)	16/50 (32%)	20/49 (41%)	18/45 (40%)
		Severity Profile	9|7|2|1	11|3|0|3	10|1|0|4	8|5|2|1	14|5|1|0	11|3|2|2
		Poly-3 Incidence	19/39.2 (48%)	17/37.7 (45%)	15/38.4 (39%)	16/34.5 (46%)	20/40.9 (49%)	18/33.2 (54%)
Suppurative inflammation	Interim	Incidence	3/20 (15%)	2/20 (10%)	2/20 (10%)	6/18 (33%)	4/20 (20%)	2/22 (9%)
		Severity Profile	2|1|0|0	2|0|0|0	2|0|0|0	6|0|0|0	4|0|0|0	2|0|0|0
	Terminal	Incidence	10/48 (21%)	9/47 (19%)	9/47 (19%)	8/50 (16%)	10/49 (20%)	9/45 (20%)
		Severity Profile	5|2|1|2	5|1|1|2	3|2|0|4	4|1|1|2	5|4|0|1	4|2|2|1
		Poly-3 Incidence	10/36.8 (27%)	9/35.2 (26%)	9/37.3 (24%)	8/33.7 (24%)	10/38.0 (26%)	9/30.1 (30%)
Hyperplasia, epithelium	Interim	Incidence	1/20 (5%)	1/20 (5%)	0/20 (0%)	2/18 (11%)	0/20 (0%)	0/22 (0%)
		Severity Profile	0|1|0|0	0|1|0|0	–	2|0|0|0	–	–
	Terminal	Incidence	17/48 (35%)	7/47[## N] (15%)	17/47 (36%)	16/50 (32%)	14/49 (29%)	13/45 (29%)
		Severity Profile	0|12|3|2	2|3|1|1	4|8|3|2	4|9|1|2	1|13|0|0	1|10|0|2
		Poly-3 Incidence	17/38.9 (44%)	7/32.9[* N] (21%)	17/36.0 (47%)	16/35.7 (45%)	14/37.4 (37%)	13/30.2 (43%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 100. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, pars distalis	Interim	Incidence	4/22 (18%)	6/22 (27%)	2/20 (10%)	4/24 (17%)	2/20 (10%)	4/22 (18%)
		Severity Profile	3|1|0|0 (1.2)	6|0|0|0 (1.0)	1|1|0|0 (1.5)	2|2|0|0 (1.5)	2|0|0|0 (1.0)	2|2|0|0 (1.5)
	Terminal	Incidence	11/48[#, ^] (23%)	9/48 (19%)	19/48[^] (40%)	15/50 (30%)	17/50 (34%)	19/45[#, ^] (42%)
		Severity Profile	3|4|4|0 (2.1)	0|4|4|1 (2.7)	5|8|2|4 (2.3)	4|6|3|2 (2.2)	4|7|5|1 (2.2)	3|9|3|4 (2.4)
		Poly-3 Incidence	11/36.2[**] (30%)	9/36.3 (25%)	19/37.8 (50%)	15/37.8 (40%)	17/38.5 (44%)	19/34.2[*] (56%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 101. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a, b]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[c]	0.05 EE_2	0.5 EE_2
Hyperplasia, pars distalis	Interim	Incidence	4/22 (18%)	7/26 (27%)	2/26 (8%)
		Severity Profile	3|1|0|0 (1.2)	4|2|1|0 (1.6)	1|1|0|0 (1.5)
	Terminal	Incidence	11/48[#, ^] (23%)	10/26 (38%)	13/26[#, ^] (50%)
		Severity Profile	3|4|4|0 (2.1)	2|4|4|0 (2.2)	3|7|3|0 (2.0)
		Poly-3 Incidence	11/36.2[*] (30%)	10/21.2 (47%)	13/22.6[*] (58%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant Poly-3 test ($p = 0.042$) for the pairwise comparison between the 0.05 µg EE_2/kg bw/day dose group and the vehicle control group for angiectasis in the pituitary gland (Poly-3 incidences 5/14.2 (35%) versus 2/24.2 (8%); data not shown in Table). In the analysis that included all animals, the Poly-3 incidences were 7/19.1 (37%) in the EE_2 group versus 6/33.5 (18%) in the vehicle control group.

[c]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 100

Table 102. Non-Neoplastic Lesions in the Pituitary of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a, b, c]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, pars distalis	Interim	Incidence	8/20 (40%)	9/20 (45%)	3/20 (15%)	4/19 (21%)	4/20 (20%)	7/22 (32%)
		Severity Profile	8|0|0|0 (1.0)	7|1|0|1 (1.4)	0|3|0|0 (2.0)	4|0|0|0 (1.0)	4|0|0|0 (1.0)	6|1|0|0 (1.1)
	Terminal	Incidence	12/46 (26%)	16/48 (33%)	18/48 (38%)	15/49 (31%)	19/50 (38%)	19/43 (44%)
		Severity Profile	0|6|3|3 (2.8)	5|4|6|1 (2.2)	4|8|2|4 (2.3)	3|6|3|3 (2.4)	3|9|5|2 (2.3)	4|8|3|4 (2.4)
		Poly-3 Incidence	12/37.2* (32%)	16/38.2 (42%)	18/40.8 (44%)	15/37.3 (40%)	19/38.9 (49%)	19/32.7* (58%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

[b]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant dose trend ($p = 0.048$) for pars distalis, cyst (not shown in Table). The p-value for the trend in the analysis including all animals was 0.056.

[c]Also in the sensitivity analysis mentioned in footnote b, there was a statistically significant pairwise comparison ($p = 0.041$, Poly-3 test) between the 25,000 µg BPA/kg bw/day dose group and the vehicle control for hypertrophy of the pars distalis (Poly-3 incidences 4/21.7 (18%) versus 0/24.3). In the analysis that included all animals, the Poly-3 incidences were 4/28.5 (14%) versus 1/35.1 (3%), $p = 0.118$. This lesion is not shown in the Table.

Table 103. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, C-cell	Interim	Incidence	10/22 (46%)	13/22 (59%)	9/20 (45%)	12/24 (50%)	7/18 (39%)	10/21 (48%)
		Severity Profile	3|4|3|0 (2.0)	7|4|2|0 (1.6)	6|2|1|0 (1.4)	5|4|3|0 (1.8)	2|4|0|1 (2.0)	6|3|1|0 (1.5)
	Terminal	Incidence	9/46 (20%)	13/40 (32%)	15/47 (32%)	15/44 (34%)	20/44^^ (46%)	11/44 (25%)
		Severity Profile	5|2|2|0 (1.7)	5|7|1|0 (1.7)	8|4|2|1 (1.7)	8|6|1|0 (1.5)	10|8|2|0 (1.6)	3|7|1|0 (1.8)
		Poly-3 Incidence	9/34.9* (26%)	13/32.8 (40%)	15/35.5 (42%)	15/34.1 (44%)	20/33.8** (59%)	11/30.5 (36%)
Hyperplasia, follicular cell	Interim	Incidence	1/22 (4%)	1/22 (4%)	2/20 (10%)	2/24 (8%)	2/18 (11%)	1/21 (5%)
		Severity profile	0|1|0|0 (2.0)	0|0|1|0 (3.0)	1|0|1|0 (2.0)	0|1|1|0 (2.5)	0|0|2|0 (3.0)	0|0|1|0 (3.0)
	Terminal	Incidence	3/46 (6%)	2/40 (5%)	9/47^ (19%)	6/44 (14%)	3/44 (7%)	3/44 (7%)
		Severity Profile	0|2|1|0 (2.3)	0|1|1|0 (2.5)	0|3|5|1 (2.8)	0|4|2|0 (2.3)	0|1|1|1 (3.0)	0|1|2|0 (2.7)
		Poly-3 Incidence	3/32.5 (9%)	2/31.2 (6%)	9/35.1 (26%)	6/32.0 (19%)	3/31.5 (10%)	3/28.6 (10%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 104. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia, C-cell	Interim	Incidence	10/22 (46%)	7/25 (28%)	9/24 (38%)
		Severity Profile	3|4|3|0 (2.0)	4|2|1|0 (1.6)	4|5|0|0 (1.6)
	Terminal	Incidence	9/46 (20%)	12/25^^ (48%)	7/25 (28%)
		Severity Profile	5|2|2|0 (1.7)	4|8|0|0 (1.7)	5|1|1|0 (1.4)
		Poly-3 Incidence	9/34.9 (26%)	12/20.3** (59%)	7/20.2 (35%)
Hyperplasia, follicular cell	Interim	Incidence	1/22 (4%)	4/25 (16%)	2/24 (8%)
		Severity Profile	0|1|0|0 (2.0)	0|0|4|0 (3.0)	0|0|2|0 (3.0)
	Terminal	Incidence	3/46 (6%)	2/25 (8%)	3/25 (12%)
		Severity Profile	0|2|1|0 (2.3)	0|1|0|1 (3.0)	0|1|2|0 (2.7)
		Poly-3 Incidence	3/32.5 (9%)	2/17.7 (11%)	3/19.9 (15%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 103.

Table 105. Non-Neoplastic Lesions in the Thyroid of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, C-cell	Interim	Incidence	13/20 (65%)	9/20 (45%)	10/20 (50%)	11/19 (58%)	10/20 (50%)	12/22 (54%)
		Severity Profile	5|7|1|0 (1.7)	1|4|4|0 (2.3)	6|2|2|0 (1.6)	3|7|1|0 (1.8)	7|2|1|0 (1.4)	5|5|2|0 (1.8)
	Terminal	Incidence	12/43 (28%)	18/45 (40%)	11/44 (25%)	19/45 (42%)	18/48 (38%)	13/42 (31%)
		Severity Profile	4|7|1|0 (1.8)	10|6|2|0 (1.6)	4|5|1|1 (1.9)	11|6|2|0 (1.4)	10|7|1|0 (1.5)	5|6|1|1 (1.8)
		Poly-3 Incidence	12/36.4 (33%)	18/35.2 (51%)	11/33.8 (33%)	19/35.2 (54%)	18/39.3 (46%)	13/31.0 (42%)
Hyperplasia, follicular cell	Interim	Incidence	1/20 (5%)	1/20 (5%)	4/20 (20%)	1/19 (5%)	0/20 (0%)	3/22 (14%)
		Severity Profile	0|0|1|0 (3.0)	0|0|1|0 (3.0)	0|1|3|0 (2.8)	0|0|1|0 (3.0)	-	0|0|3|0 (3.0)
	Terminal	Incidence	6/43 (14%)	6/45 (13%)	9/44 (20%)	10/45 (22%)	6/48 (12%)	7/42 (17%)
		Severity Profile	0|3|3|0 (2.5)	0|2|4|0 (2.7)	0|2|6|1 (2.9)	0|3|7|0 (2.7)	0|3|3|0 (2.5)	0|3|4|0 (2.6)
		Poly-3 Incidence	6/34.7 (17%)	6/34.0 (18%)	9/35.5 (25%)	10/32.3 (31%)	6/36.6 (16%)	7/28.9 (24%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 106. Non-Neoplastic Lesions in the Parathyroid Gland of Interim and Terminal Sacrifice Male Rats: Continuous BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia	Interim	Incidence	7/22[*, #, ^ N] (32%)	5/21 (24%)	7/19 (37%)	5/24 (21%)	2/19 (10%)	2/21[^ N] (10%)
		Severity Profile	3|4|0|0 (1.6)	2|3|0|0 (1.6)	3|3|1|0 (1.7)	1|4|0|0 (1.8)	0|2|0|0 (2.0)	1|1|0|0 (1.5)
	Terminal	Incidence	11/49 (22%)	11/46 (24%)	23/47[^^^] (49%)	18/50[^] (36%)	18/50 (36%)	12/46 (26%)
		Severity Profile	3|7|0|1 (1.9)	7|3|0|1 (1.5)	1|13|6|3 (2.5)	2|9|3|4 (2.5)	9|5|2|2 (1.8)	4|5|3|0 (1.9)
		Poly-3 Incidence	11/36.7 (30%)	11/33.9 (32%)	23/37.5[**] (61%)	18/38.4 (47%)	18/39.0 (46%)	12/32.3 (37%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 107. Non-Neoplastic Lesions in the Parathyroid Gland of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia	Interim	Incidence	7/22 (32%)	6/26 (23%)	4/26 (15%)
		Severity Profile	3|4|0|0 (1.6)	0|6|0|0 (2.0)	1|2|1|0 (2.0)
	Terminal	Incidence	11/49[#, ^] (22%)	7/25 (28%)	11/25[#, ^] (44%)
		Severity Profile	3|7|0|1 (1.9)	2|3|1|1 (2.1)	3|5|1|2 (1.8)
		Poly-3 Incidence	11/36.7[c] (30%)	7/18.2 (38%)	11/20.9 (53%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 106.
[c]In the sensitivity analysis that excluded all animals that overlapped with animals treated with 250,000 µg BPA/kg bw/day (see Statistical Methods), there was a significant EE_2 dose trend ($p = 0.046$). The p-value for the trend in the analysis that included all animals was 0.051.

Table 108. Non-Neoplastic Lesions in the Parathyroid Gland of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia	Interim	Incidence	4/20 (20%)	9/20 (45%)	3/19 (16%)	7/19 (37%)	4/19 (21%)	8/22 (36%)
		Severity Profile	1|2|1|0 (2.0)	7|1|1|0 (1.7)	1|2|0|0 (1.7)	5|2|0|0 (1.3)	4|0|0|0 (1.0)	1|6|1|0 (2.0)
	Terminal	Incidence	22/49 (45%)	17/46 (37%)	27/46 (59%)	23/49 (47%)	30/50 (60%)	23/43 (54%)
		Severity Profile	1|8|7|6 (2.8)	2|10|0|5 (2.5)	4|15|5|3 (2.3)	8|8|5|2 (2.0)	3|12|6|9 (2.7)	7|7|5|4 (2.3)
		Poly-3 Incidence	22/41.9* (52%)	17/36.6 (46%)	27/39.4 (69%)	23/38.0 (60%)	30/44.1 (68%)	23/34.1 (67%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 109. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, transitional epithelium	Terminal	Incidence	3/50 (6%)	4/48 (8%)	12/48^^ (25%)	7/50 (14%)	5/50 (10%)	4/45 (9%)
		Severity Profile	1|1|1|0 (2.0)	1|3|0|0 (1.8)	5|6|1|0 (1.7)	5|1|0|1 (1.6)	2|2|1|0 (1.8)	1|1|1|1 (2.5)
		Poly-3 Incidence	3/34.6 (9%)	4/34.5 (12%)	12/34.1** (35%)	7/34.8 (20%)	5/34.1 (15%)	4/28.9 (14%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 110. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Hyperplasia, transitional epithelium	Terminal	Incidence	3/50 (6%)	2/26 (8%)	1/26 (4%)
		Severity Profile	1|1|1|0 (2.0)	1|1|0|0 (1.5)	0|0|1|0 (3.0)
		Poly-3 Incidence	3/34.6 (9%)	2/17.8 (11%)	1/19.7 (5%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 109.

Table 111. Non-Neoplastic Lesions in the Kidney of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Hyperplasia, transitional epithelium	Terminal	Incidence	12/50 (24%)	9/48 (19%)	12/48 (25%)	12/50 (24%)	20/50 (40%)	10/45 (22%)
		Severity Profile	3|8|0|1 (1.9)	4|2|3|0 (1.9)	5|5|1|1 (1.8)	4|7|0|1 (1.8)	8|11|1|0 (1.6)	3|5|2|0 (1.9)
		Poly-3 Incidence	12/40.0* (30%)	9/36.7 (24%)	12/37.2 (32%)	12/34.9 (34%)	20/40.6 (49%)	10/29.4 (34%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.

Table 112. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Male Rats: Continuous-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Fatty change	Interim	Incidence	0/22 (0%)	0/22 (0%)	2/20^ (10%)	0/24 (0%)	0/19 (0%)	0/22 (0%)
		Severity Profile	–	–	0|2|0|0 (2.0)	–	–	–
	Terminal	Incidence	4/50 (8%)	8/47 (17%)	5/48 (10%)	5/50 (10%)	4/50 (8%)	2/45 (4%)
		Severity Profile	0|1|1|2 (3.3)	1|3|1|3 (2.8)	2|1|1|1 (2.2)	0|4|0|1 (2.4)	0|3|1|0 (2.2)	0|1|0|1 (3.0)
		Poly-3 incidence	4/35.1 (11%)	8/36.5 (22%)	5/33.2 (15%)	5/35.4 (14%)	4/34.4 (12%)	2/29.1 (7%)
Hepatodiaphragmatic Nodule	Interim	Incidence	0/22 (0%)	2/22 (9%)	2/20 (10%)	3/24 (12%)	4/19* (21%)	1/22 (4%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	6/50 (12.0%)	2/47 (4%)	4/48 (8%)	3/50 (6%)	7/50 (14%)	5/45 (11%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 incidence	6/34.6 (17.3%)	2/34.2 (6%)	4/32.8 (12%)	3/34.6 (9%)	7/35.4 (20%)	5/30.9 (16%)
Infiltration, cellular, mononuclear cells	Interim	Incidence	5/22 (23%)	11/22^ (50%)	9/20 (45%)	13/24*, #, ^ (54%)	11/19*, #, ^ (58%)	8/22# (36%)
		Severity Profile	5|0|0|0 (1.0)	11|0|0|0 (1.0)	9|0|0|0 (1.0)	12|1|0|0 (1.1)	11|0|0|0 (1.0)	8|0|0|0 (1.0)
	Terminal	Incidence	35/50 (70%)	29/47 (62%)	37/48 (77%)	36/50 (72%)	34/50 (68%)	28/45 (62%)
		Severity Profile	21|14|0|0 (1.4)	16|13|0|0 (1.4)	22|15|0|0 (1.4)	26|10|0|0 (1.3)	21|13|0|0 (1.4)	19|9|0|0 (1.3)
		Poly-3 incidence	35/42.3 (83%)	29/40.1 (72%)	37/42.6 (87%)	36/42.3 (85%)	34/40.5 (84%)	28/35.4 (79%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Severity scores were not assigned for this lesion.

Table 113. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Male Rats: Continuous-Dose EE_2[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle[b]	0.05 EE_2	0.5 EE_2
Fatty change	Interim	Incidence	0/22[*, #, ^] (0%)	1/26 (4%)	4/26[#, ^] (15%)
		Severity Profile	–	0|0|1|0 (3.0)	0|1|3|0 (2.8)
	Terminal	Incidence	4/50 (8.0%)	0/26 (0.0%)	1/25 (4.0%)
		Severity Profile	0|1|1|2 (3.2)	–	0|0|0|1 (4.0)
		Poly-3 Incidence	4/35.1 (11%)	0/17.6 (0%)	1/19.9 (5%)
Hepatodiaphragmatic Nodule	Interim	Incidence	0/22 (0%)	4/26 (15%)	4/26 (15%)
		Severity Profile	–[c]	–	–
	Terminal	Incidence	6/50 (12%)	3/26 (12%)	1/25 (4%)
		Severity Profile	–[c]	–	–
		Poly-3 Incidence	6/34.6 (17%)	3/17.8 (17%)	1/19.5 (5%)
Infiltration, cellular, mononuclear cells	Interim	Incidence	5/22 (23%)	13/26 (50%)	5/26 (19%)
		Severity Profile	5|0|0|0 (1.0)	13|0|0|0 (1.0)	5|0|0|0 (1.0)
	Terminal	Incidence	35/50 (70%)	22/26 (85%)	20/25 (80%)
		Severity Profile	21|14|0|0 (1.4)	16|6|0|0 (1.3)	12|8|0|0 (1.4)
		Poly-3 Incidence	35/42.3 (83%)	22/23.5[*] (94%)	20/23.1 (87%)

[a]EE_2 doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]The continuous vehicle control group data are the same as those shown for the continuous-dose BPA data in Table 112.
[c]Severity scores were not assigned for this lesion.

Table 114. Non-Neoplastic Lesions in the Liver of Interim and Terminal Sacrifice Male Rats: Stop-Dose BPA[a]

Lesion	Study Phase (Interim/Terminal)	Statistic	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA
Fatty change	Interim	Incidence	0/20 (0%)	1/20 (5%)	1/20 (5%)	0/19 (0%)	2/20 (10%)	1/22 (4%)
		Severity Profile	–	0|1|0|0 (2.0)	0|0|1|0 (3.0)	–	0|0|2|0 (3.0)	0|0|1|0 (3.0)
	Terminal	Incidence	5/50 (10%)	2/48 (4%)	7/48 (15%)	6/50 (12%)	1/50 (2%)	1/46 (2%)
		Severity Profile	1|3|1|0 (2.0)	0|0|0|2 (4.0)	2|2|1|2 (2.4)	2|1|1|2 (2.5)	0|0|0|1 (4.0)	0|0|0|1 (4.0)
		Poly-3 incidence	5/38.9 (13%)	2/34.4 (6%)	7/36.2 (19%)	6/33.4 (18%)	1/36.2 (3%)	1/28.1 (4%)
Hepatodiaphragmatic Nodule	Interim	Incidence	1/20 (5%)	2/20 (10%)	3/20 (15%)	3/19 (16%)	2/20 (10%)	1/22 (4%)
		Severity Profile	–[b]	–	–	–	–	–
	Terminal	Incidence	3/50 (6%)	3/48 (6%)	1/48 (2%)	5/50 (10%)	4/50 (8%)	5/46 (11%)
		Severity Profile	–[b]	–	–	–	–	–
		Poly-3 incidence	3/37.6 (8%)	3/34.3 (9%)	1/35.2 (3%)	5/33.1 (15%)	4/37.7 (11%)	5/28.8 (17%)
Infiltration, cellular, mononuclear cells	Interim	Incidence	11/20 (55%)	13/20 (65%)	9/20 (45%)	13/19 (68%)	11/20 (55%)	15/22 (68%)
		Severity Profile	11|0|0|0 (1.0)	13|0|0|0 (1.0)	9|0|0|0 (1.0)	13|0|0|0 (1.0)	11|0|0|0 (1.0)	15|0|0|0 (1.0)
	Terminal	Incidence	34/50 (68%)	40/48 (83%)	37/48 (77%)	33/50 (66%)	33/50 (66%)	29/46 (63%)
		Severity Profile	19|15|0|0 (1.4)	27|13|0|0 (1.3)	19|18|0|0 (1.5)	26|7|0|0 (1.2)	23|10|0|0 (1.3)	15|13|1|0 (1.5)
		Poly-3 incidence	34/43.3 (79%)	40/44.0 (91%)	37/43.7 (85%)	33/41.6 (79%)	33/42.3 (78%)	29/37.0 (78%)

[a]BPA doses are µg/kg bw/day. See legend to Table 55 for description of data presentation.
[b]Severity scores were not assigned for this lesion

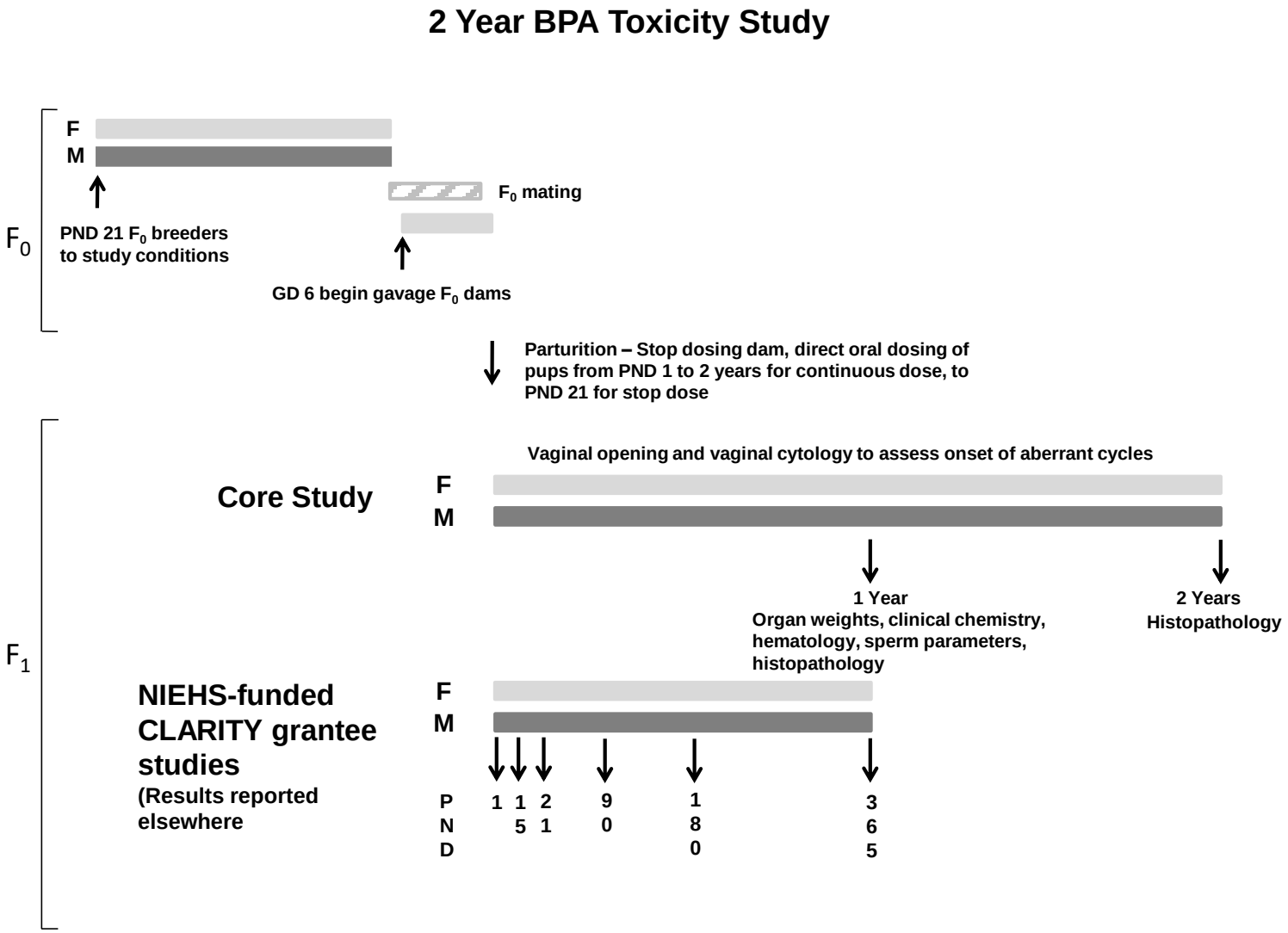

Figure 1. Scheme for Chronic BPA Toxicity Study Design

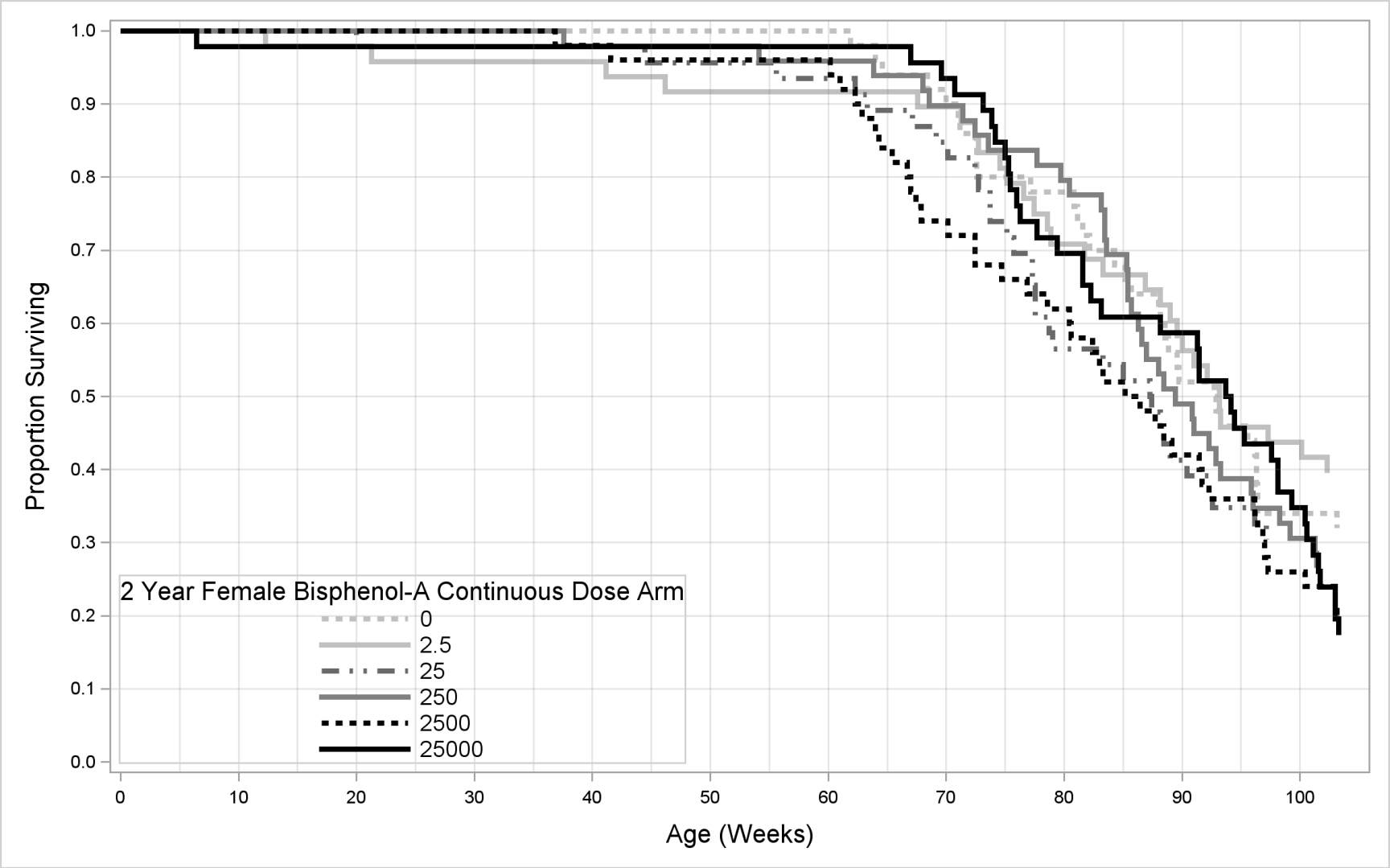

Figure 2. Kaplan-Meier Survival Curve for Terminal Sacrifice Female BPA Continuous-Dose Arm (Weeks 4–104)

See Table 18 for data analysis results.

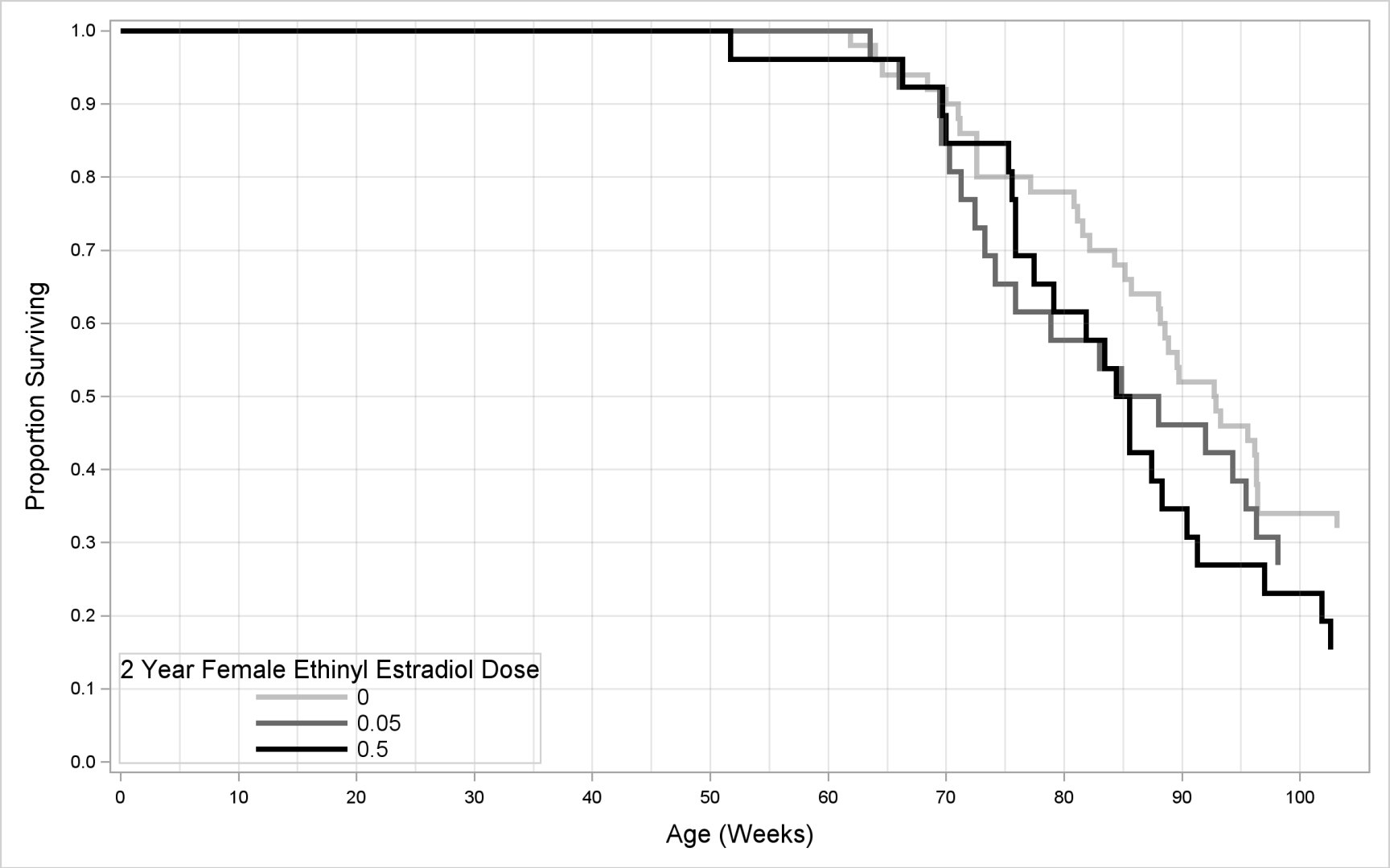

Figure 3. Kaplan-Meier Survival Curve for Terminal Sacrifice Female EE2 Continuous-Dose Arm (Weeks 4–104)

See Table 18 for data analysis results.

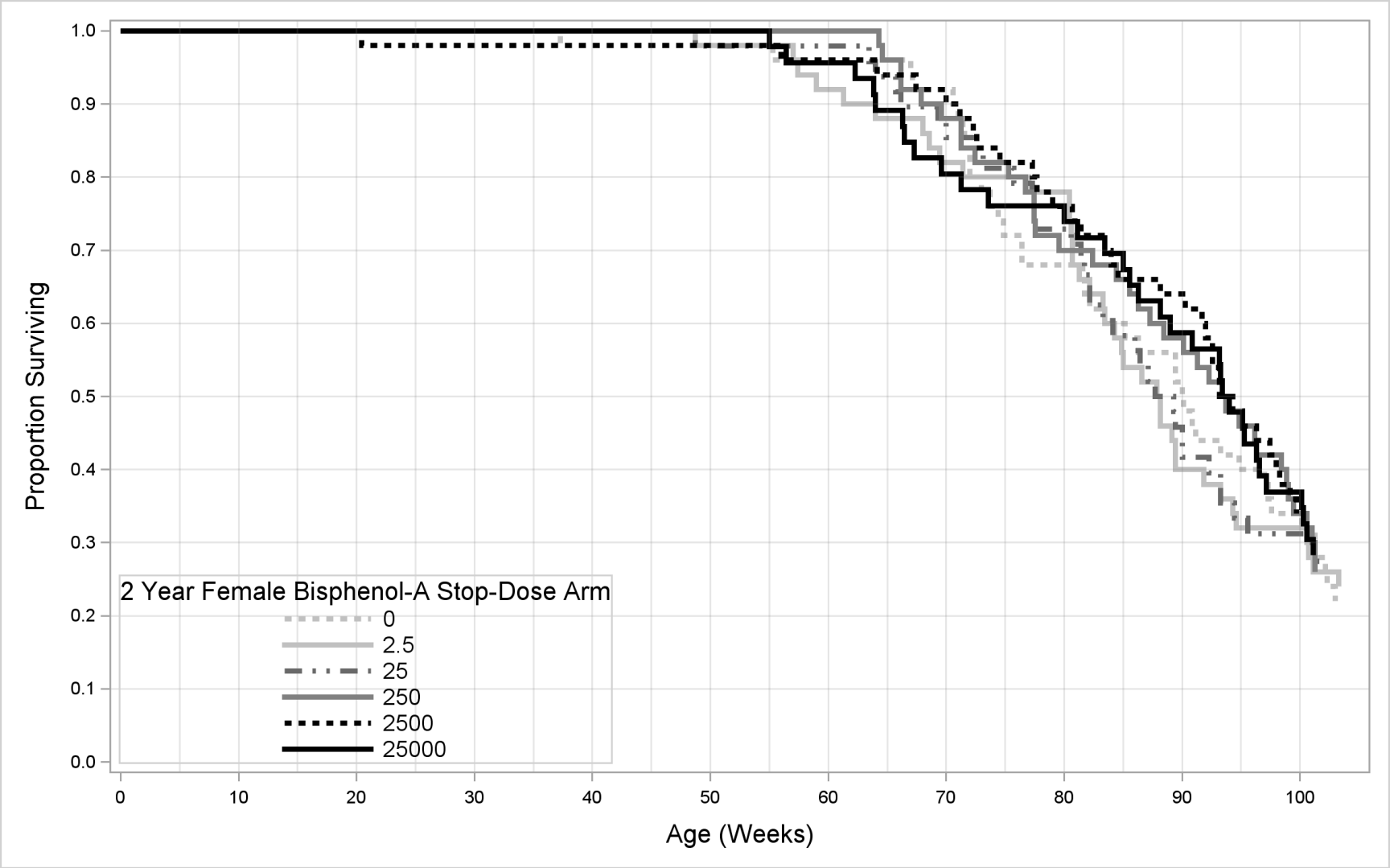

Figure 4. Kaplan-Meier Survival Curve for Terminal Sacrifice Female BPA Stop-Dose Arm (Week 4–104)

See Table 19 for data analysis results.

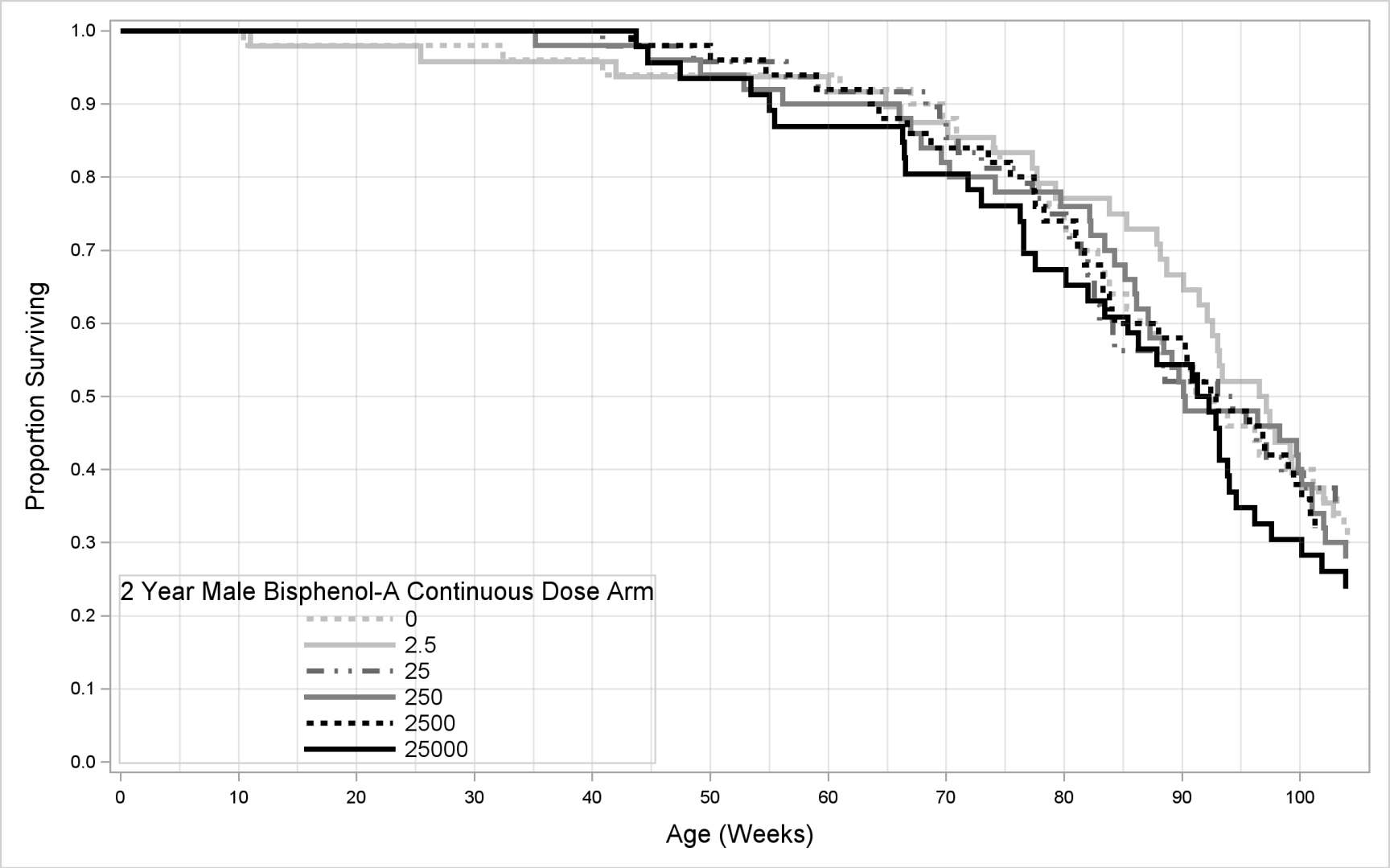

Figure 5. Kaplan-Meier Survival Curve for Terminal Sacrifice Male BPA Continuous-Dose Arm (Weeks 4–104)

See Table 20 for data analysis results.

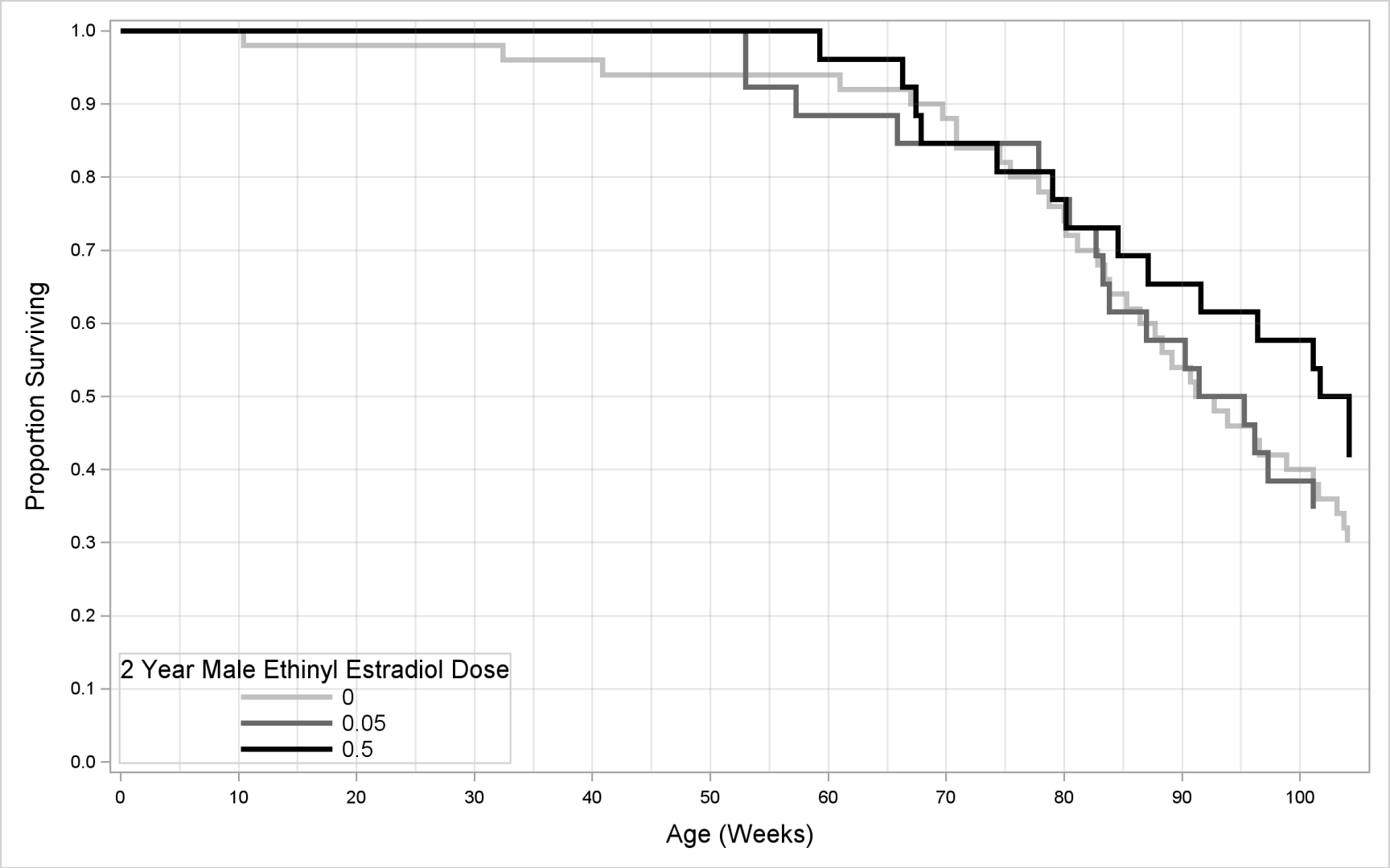

Figure 6. Kaplan-Meier Survival Curve for Terminal Sacrifice Male EE_2 Continuous-Dose Arm (Weeks 4–104)

See Table 20 for data analysis results.

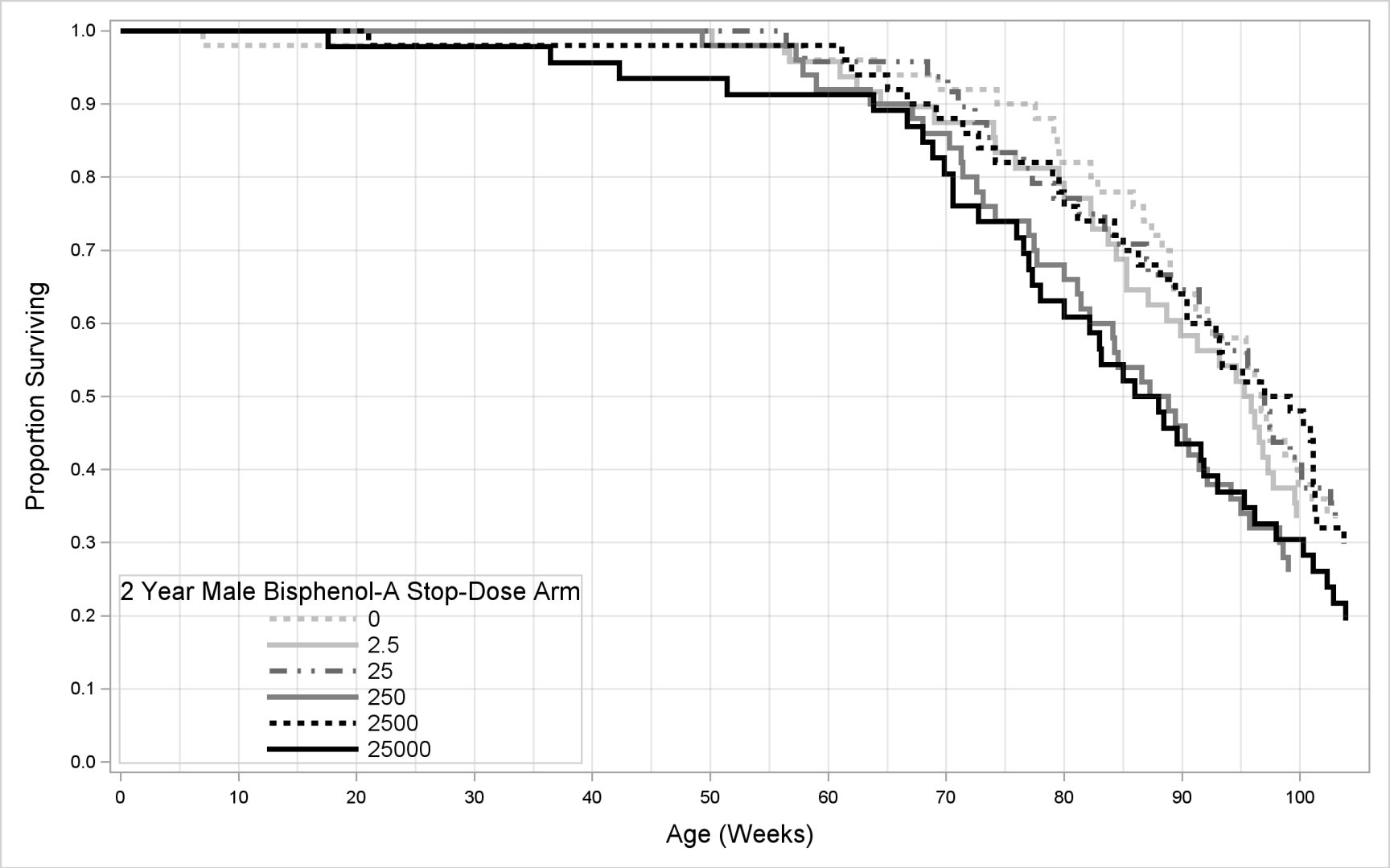

Figure 7. Kaplan-Meier Survival Curve for Terminal Sacrifice Male BPA Stop-Dose Arm (Week 4–104)

See Table 21 for data analysis results.

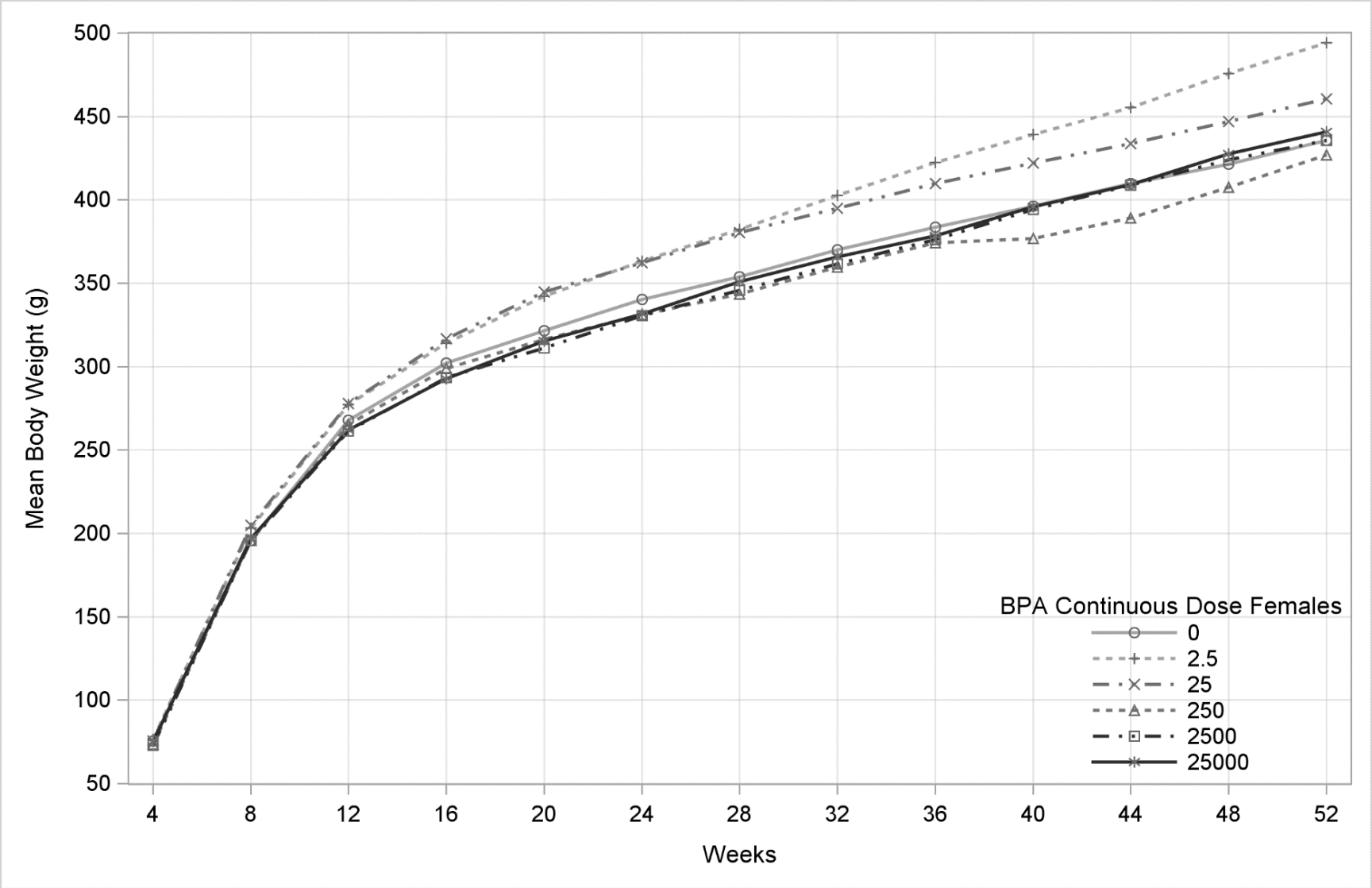

Figure 8. Body Weight for Interim Sacrifice Female BPA Continuous-Dose Arm

Data tabulated in Table 24.

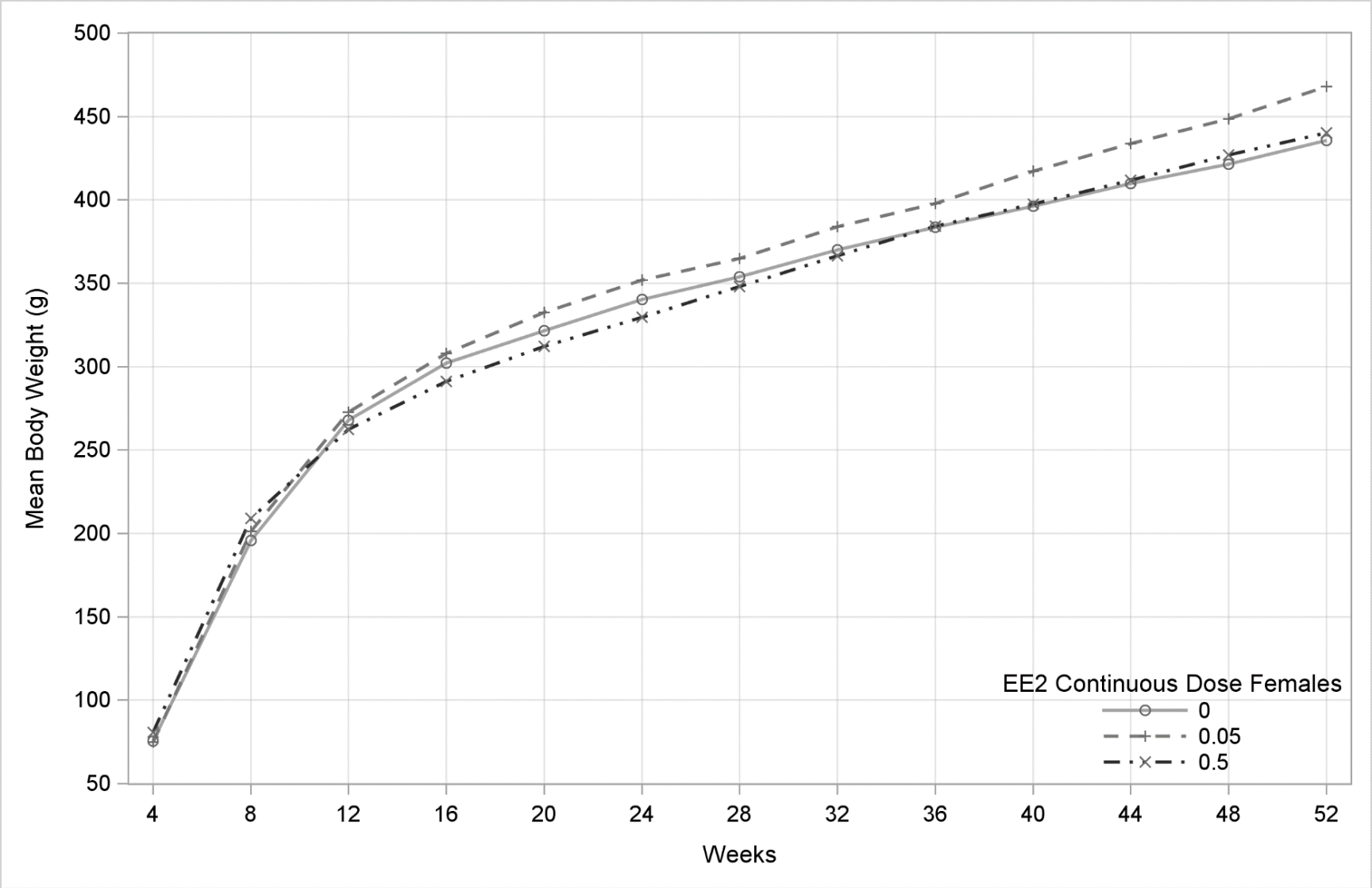

Figure 9. Body Weight for Interim Sacrifice Female EE_2 Continuous-Dose Arm

Data tabulated in Table 24.

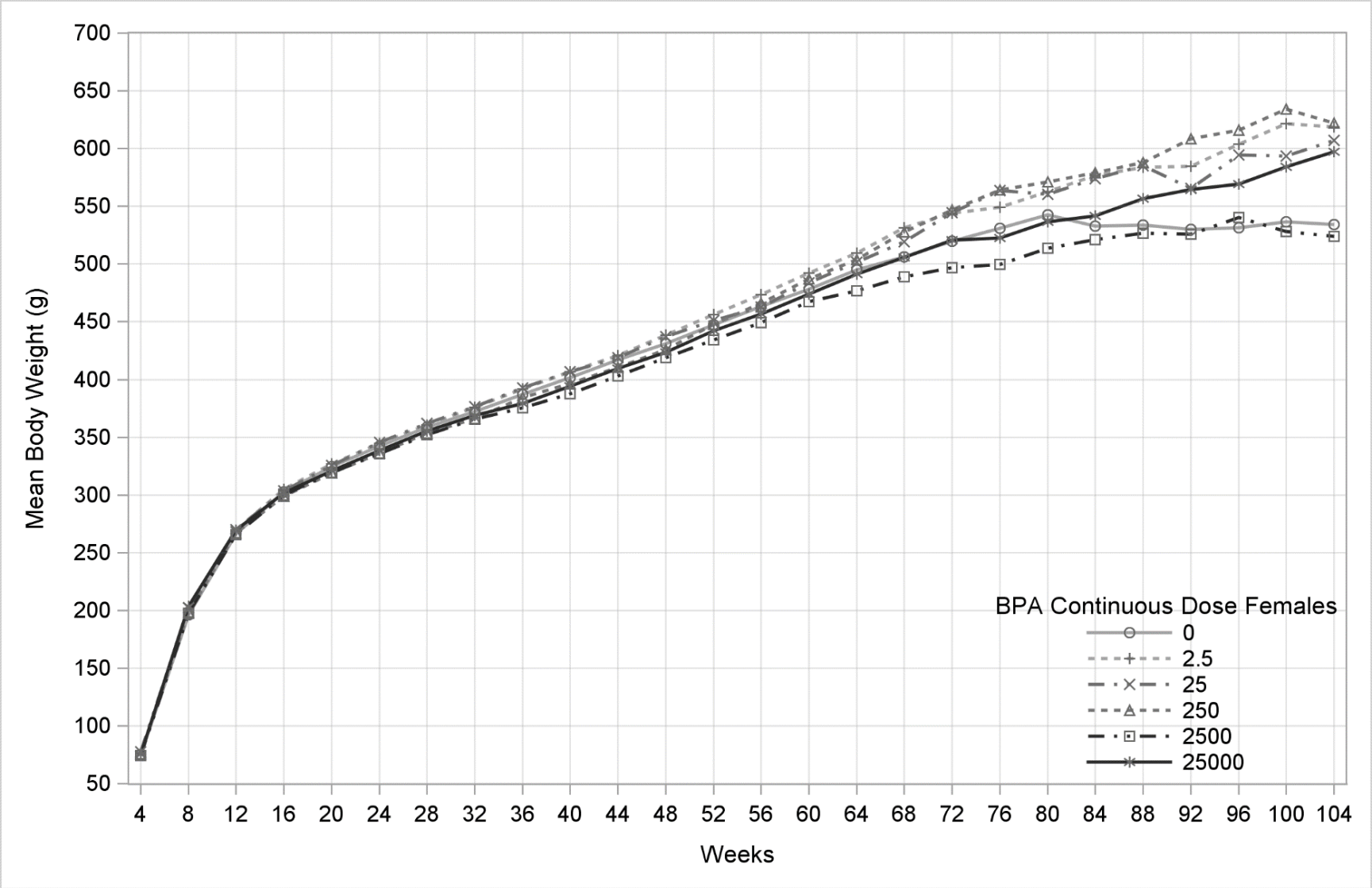

Figure 10. Body Weight for Terminal Sacrifice Female BPA Continuous-Dose Arm

Data tabulated in Table 25.

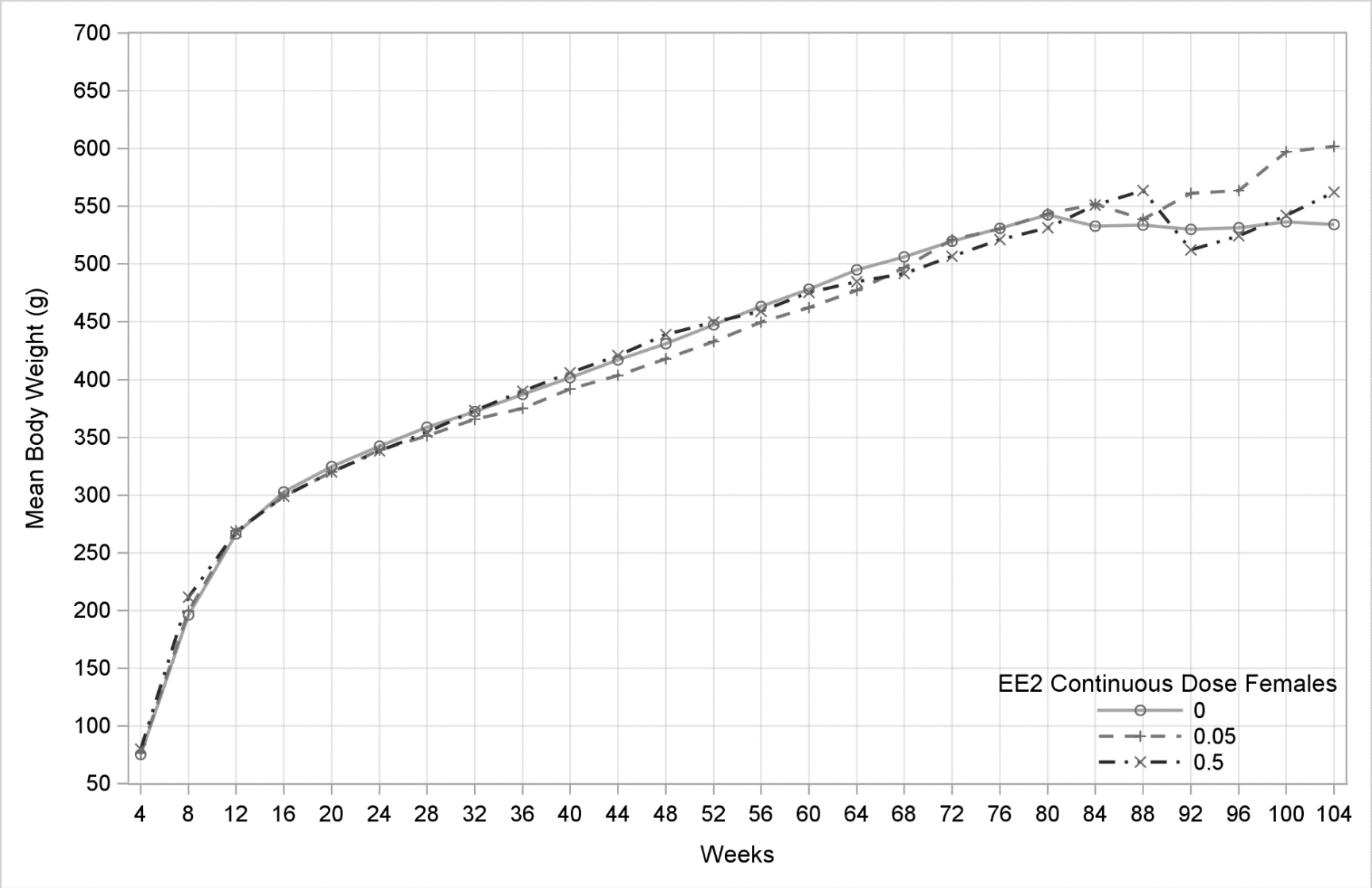

Figure 11. Body Weight for Terminal Sacrifice Female EE_2 Continuous-Dose Arm

Data tabulated in Table 25.

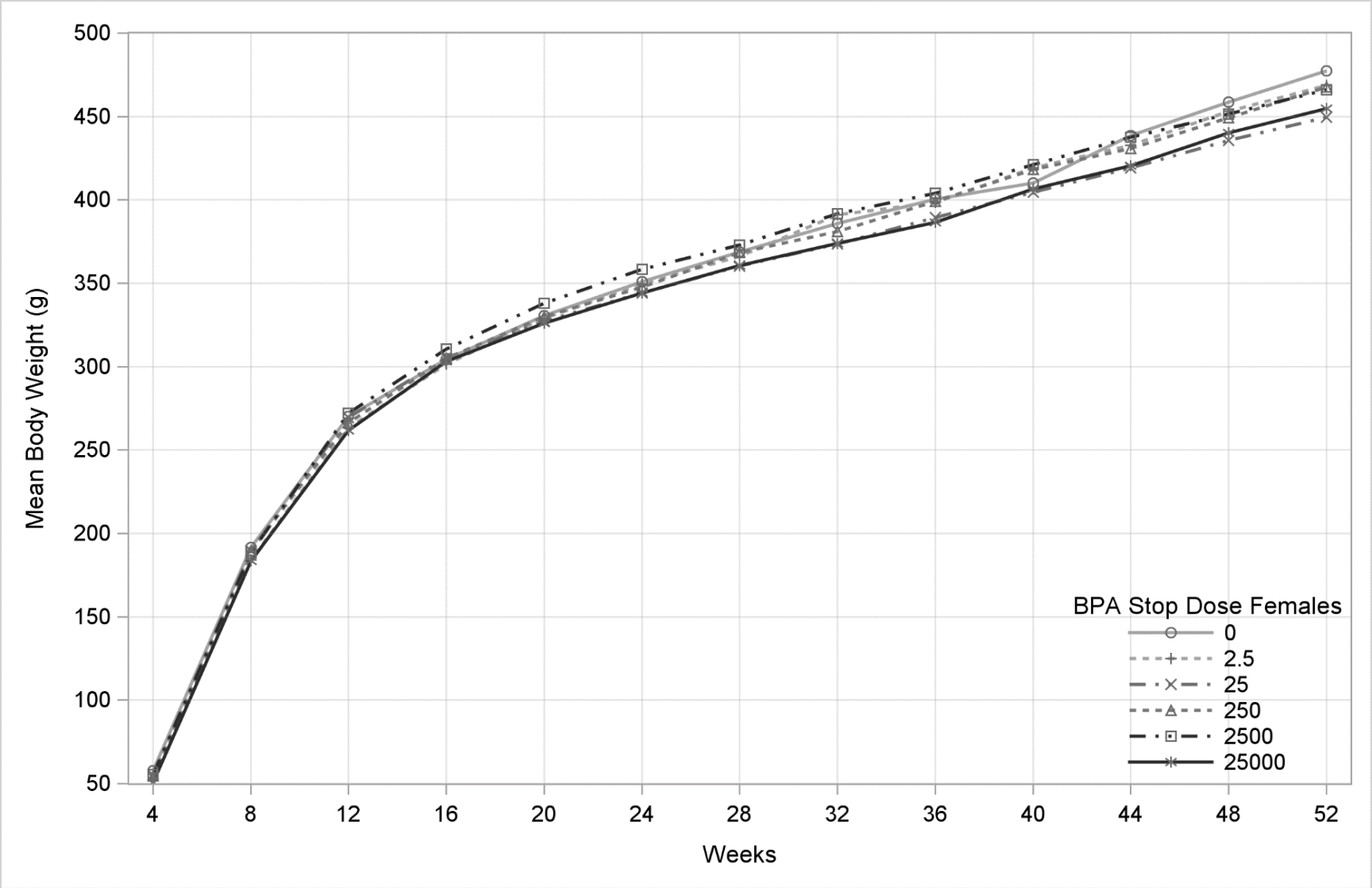

Figure 12. Body Weight for Interim Sacrifice Female BPA Stop-Dose Arm

Data tabulated in Table 26.

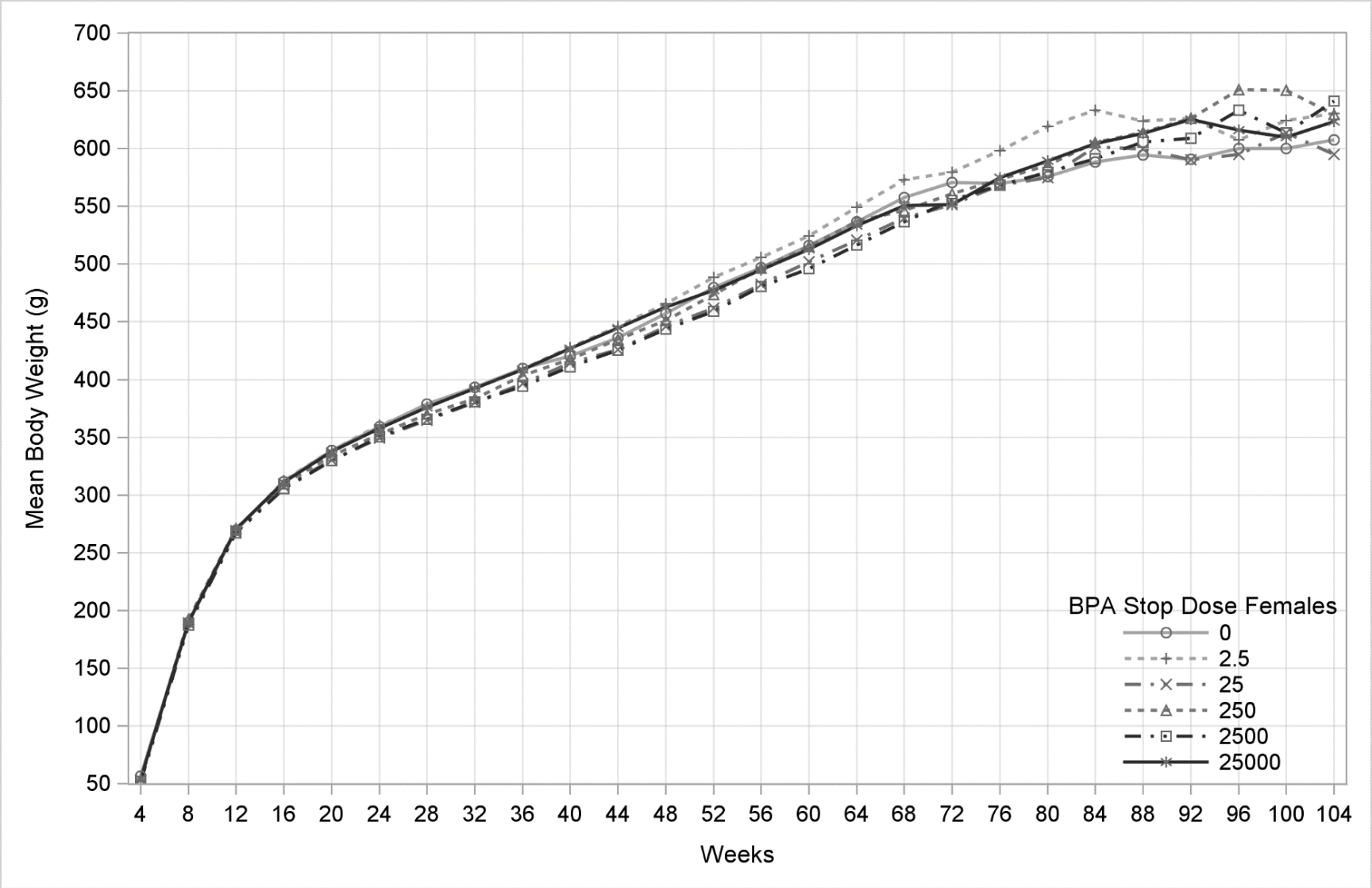

Figure 13. Body Weight for Terminal Sacrifice Female BPA Stop-Dose Arm

Data tabulated in Table 27.

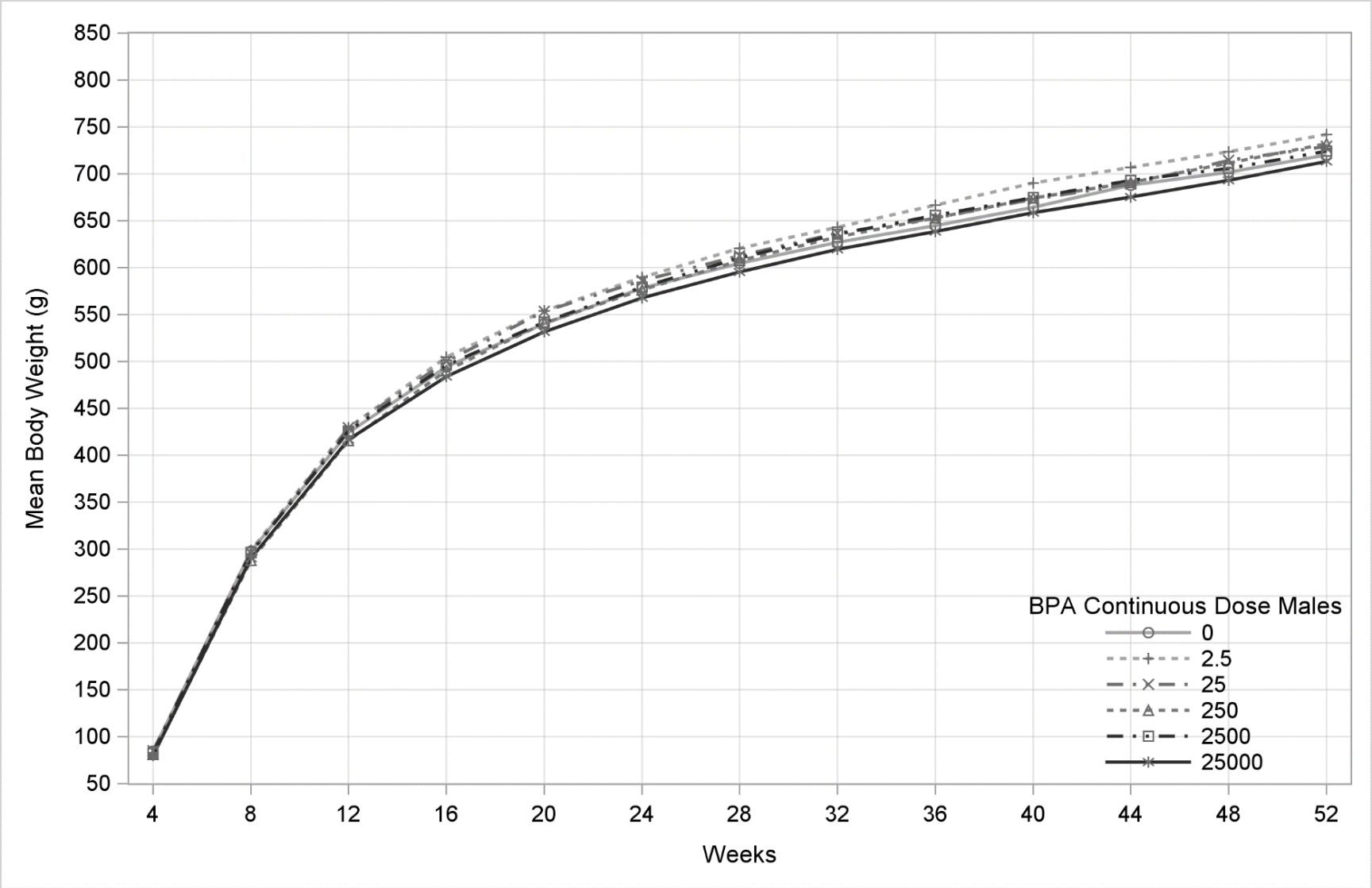

Figure 14. Body Weight for Interim Sacrifice Male BPA Continuous-Dose Arm

Data tabulated in Table 28.

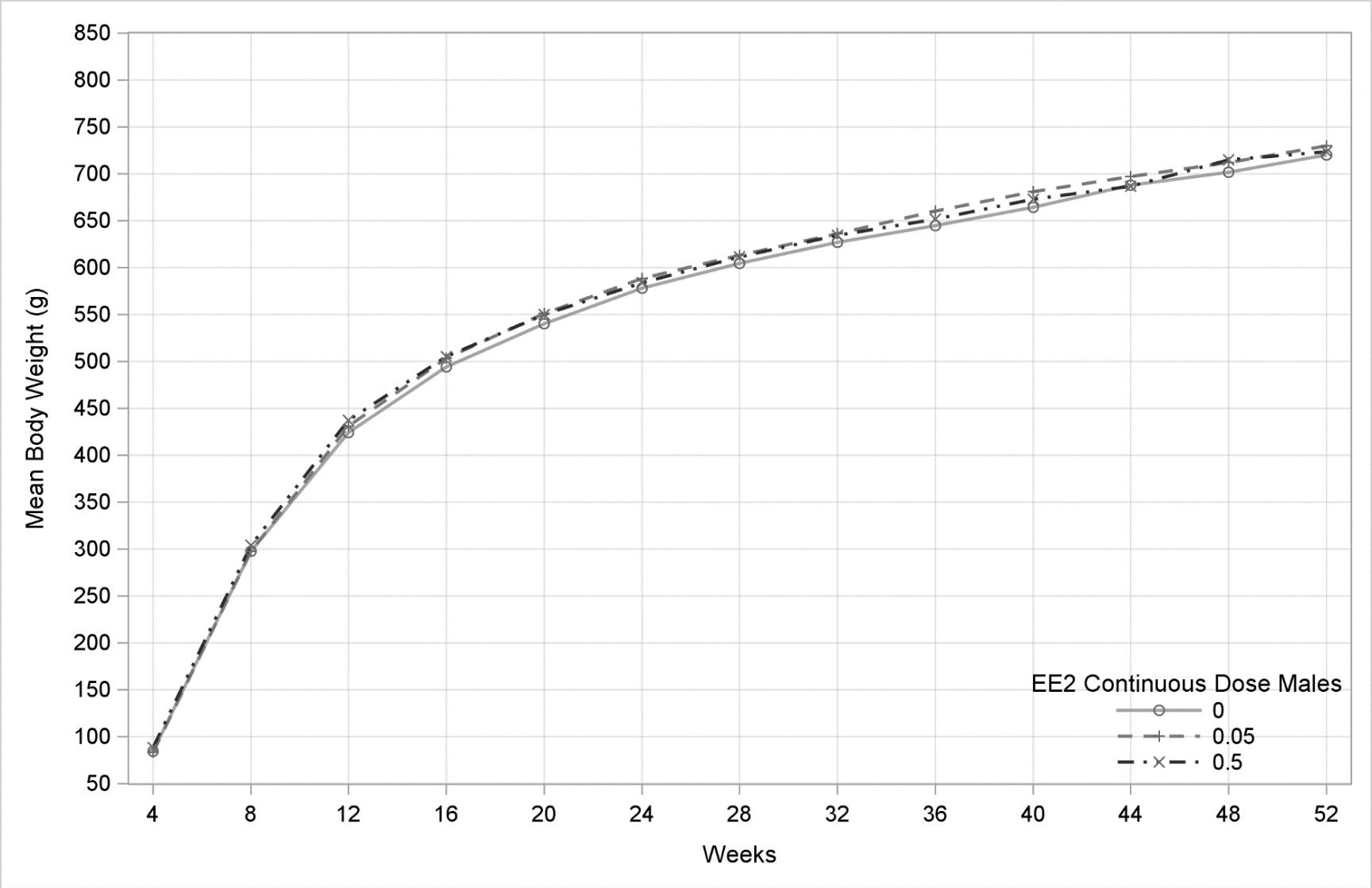

Figure 15. Body Weight for Interim Sacrifice Male EE_2 Continuous-Dose Arm

Data tabulated in Table 28.

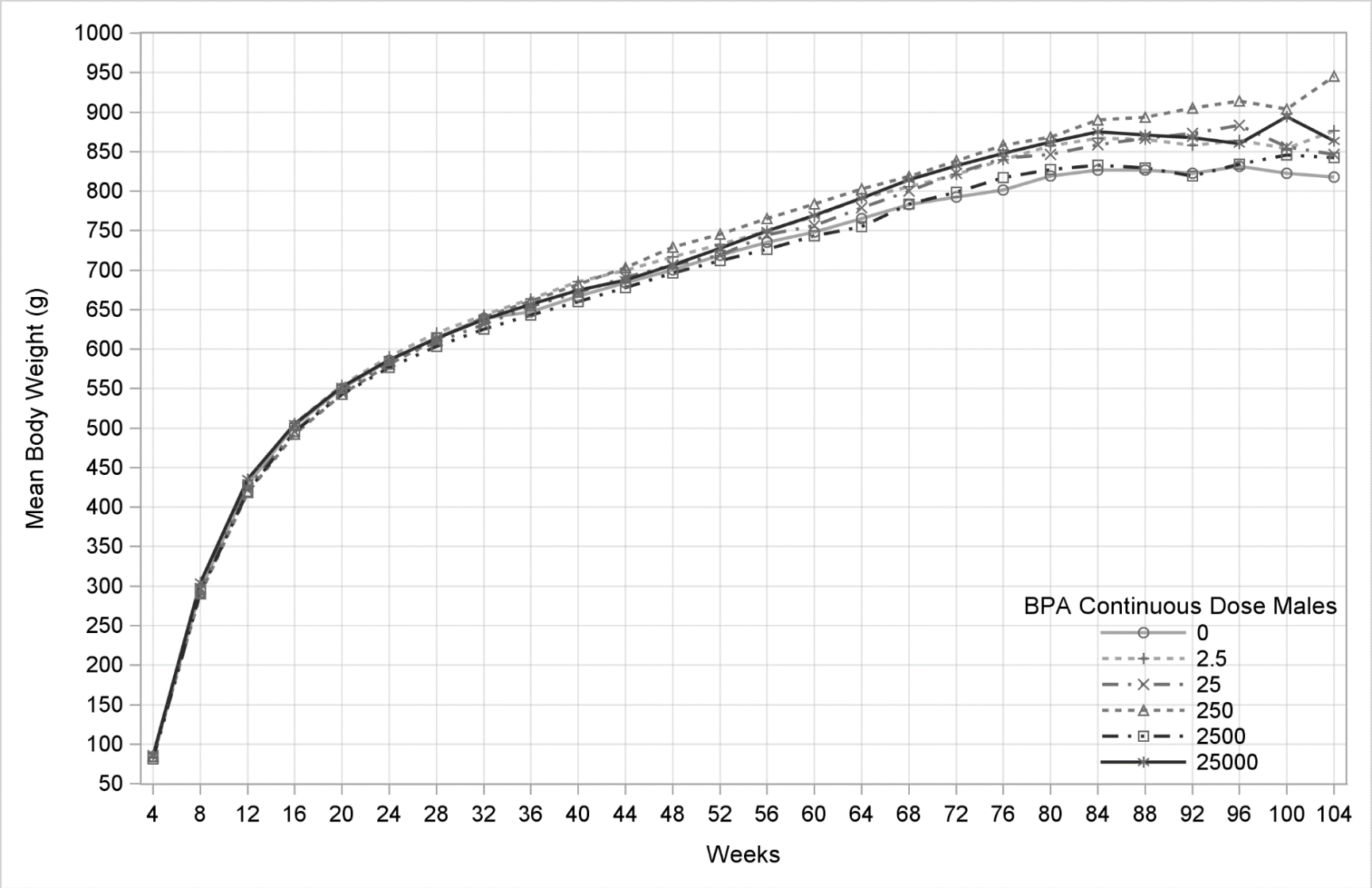

Figure 16. Body Weight for Terminal Sacrifice Male BPA Continuous-Dose Arm

Data tabulated in Table 29.

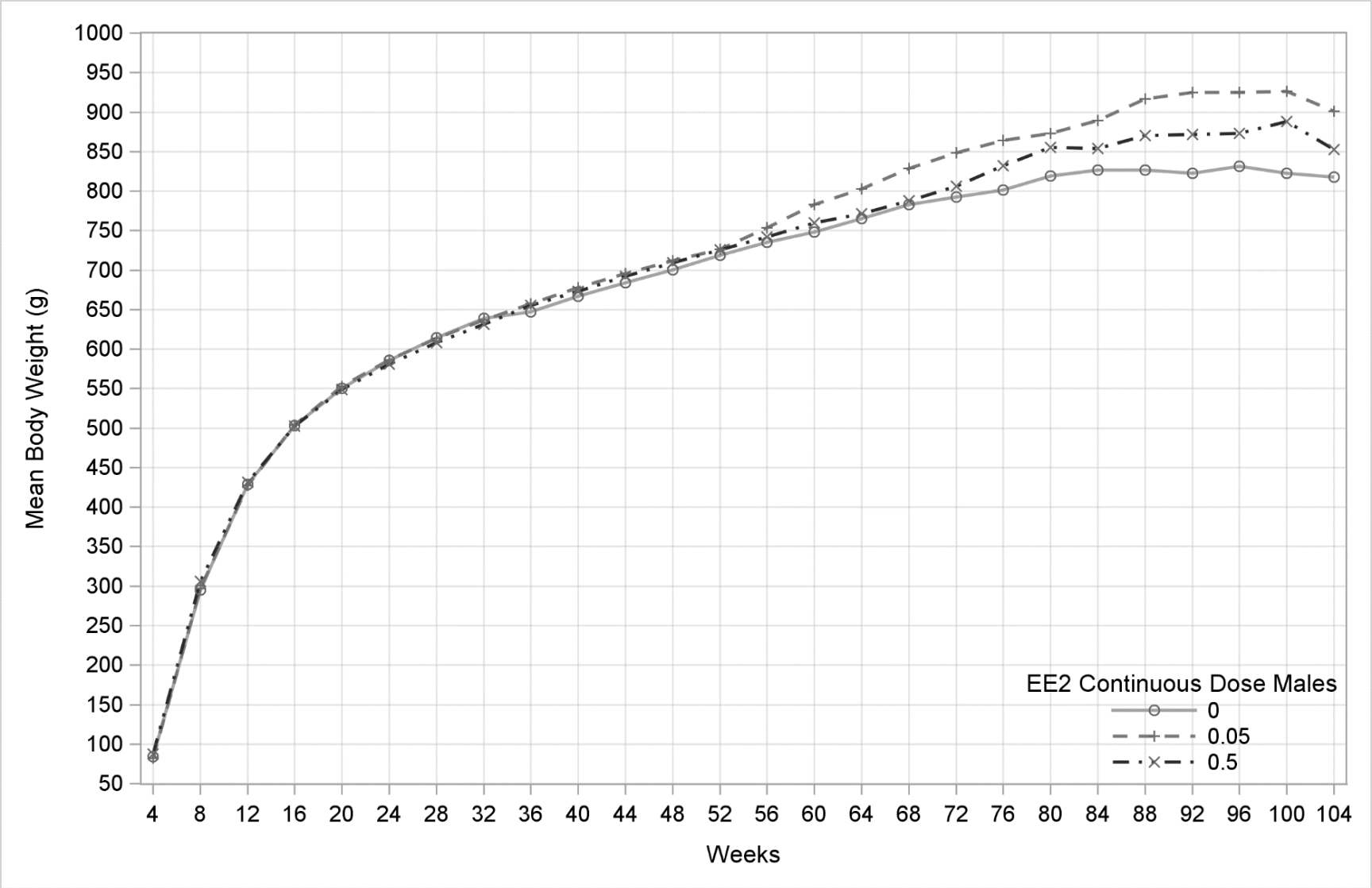

Figure 17. Body Weight for Terminal Sacrifice Male EE_2 Continuous-Dose Arm

Data tabulated in Table 29.

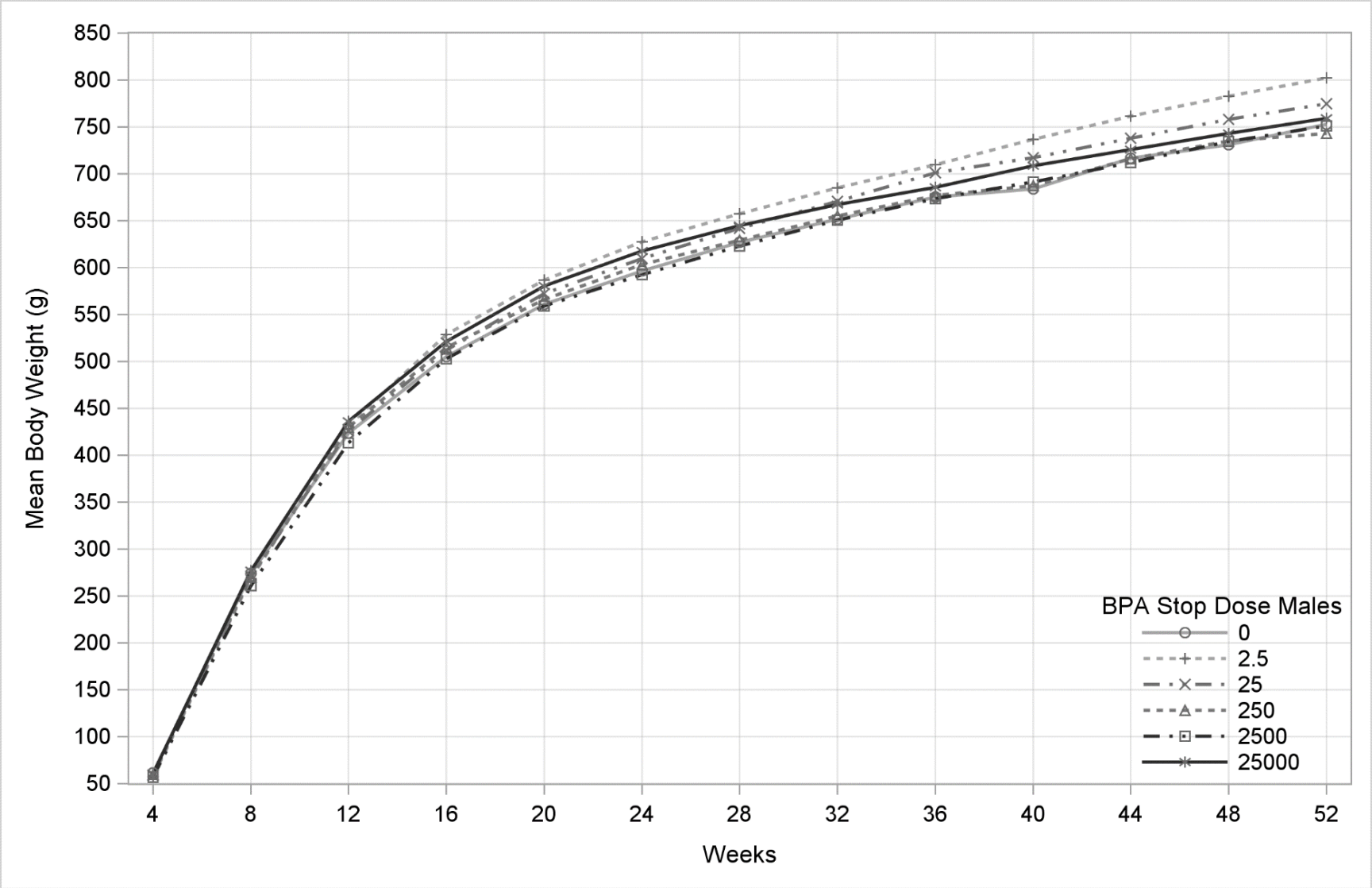

Figure 18. Body Weight for Interim Sacrifice Male BPA Stop-Dose Arm

Data tabulated in Table 30.

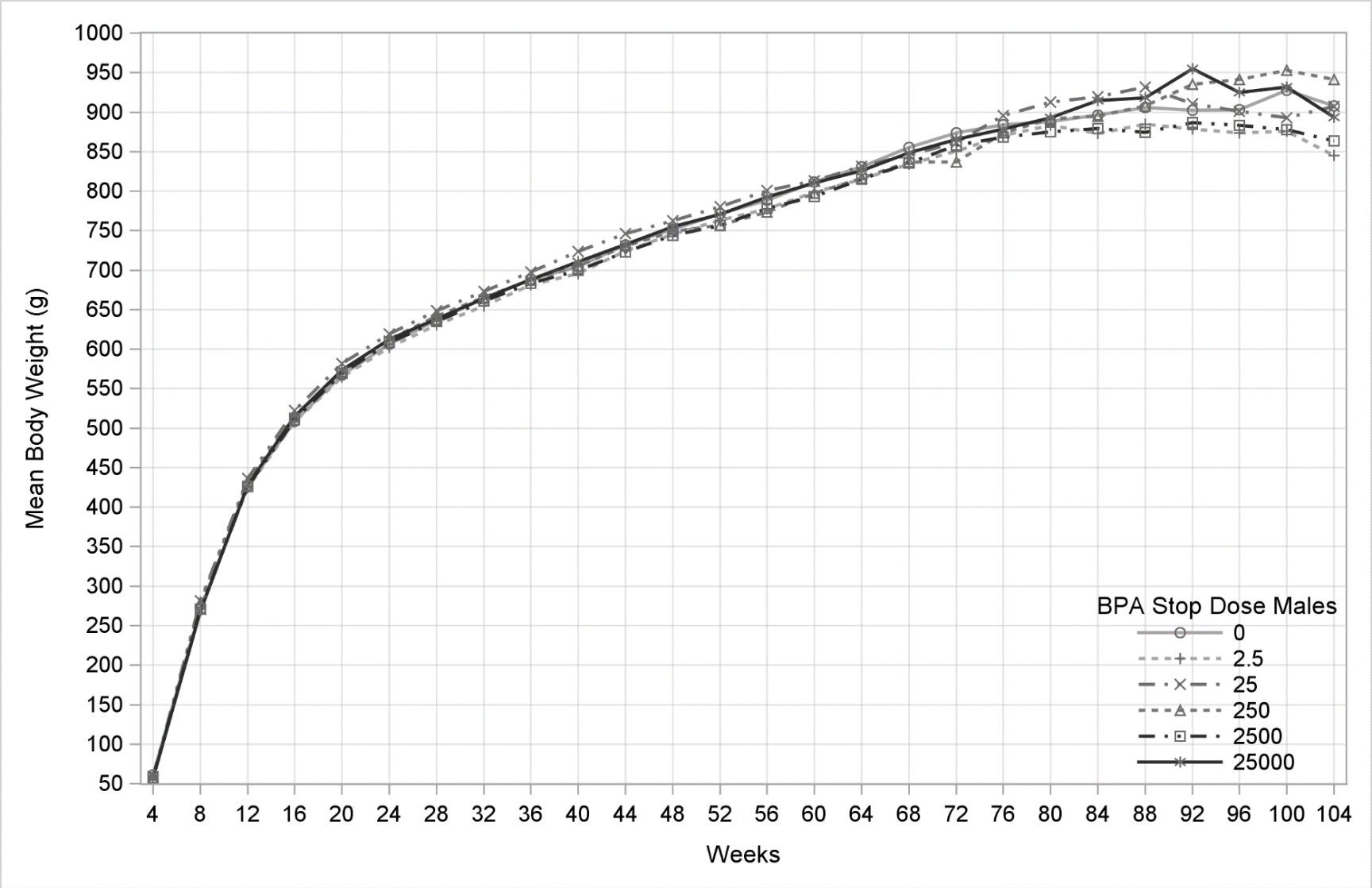

Figure 19. Body Weight for Terminal Sacrifice Male BPA Stop-Dose Arm

Data tabulated in Table 31.

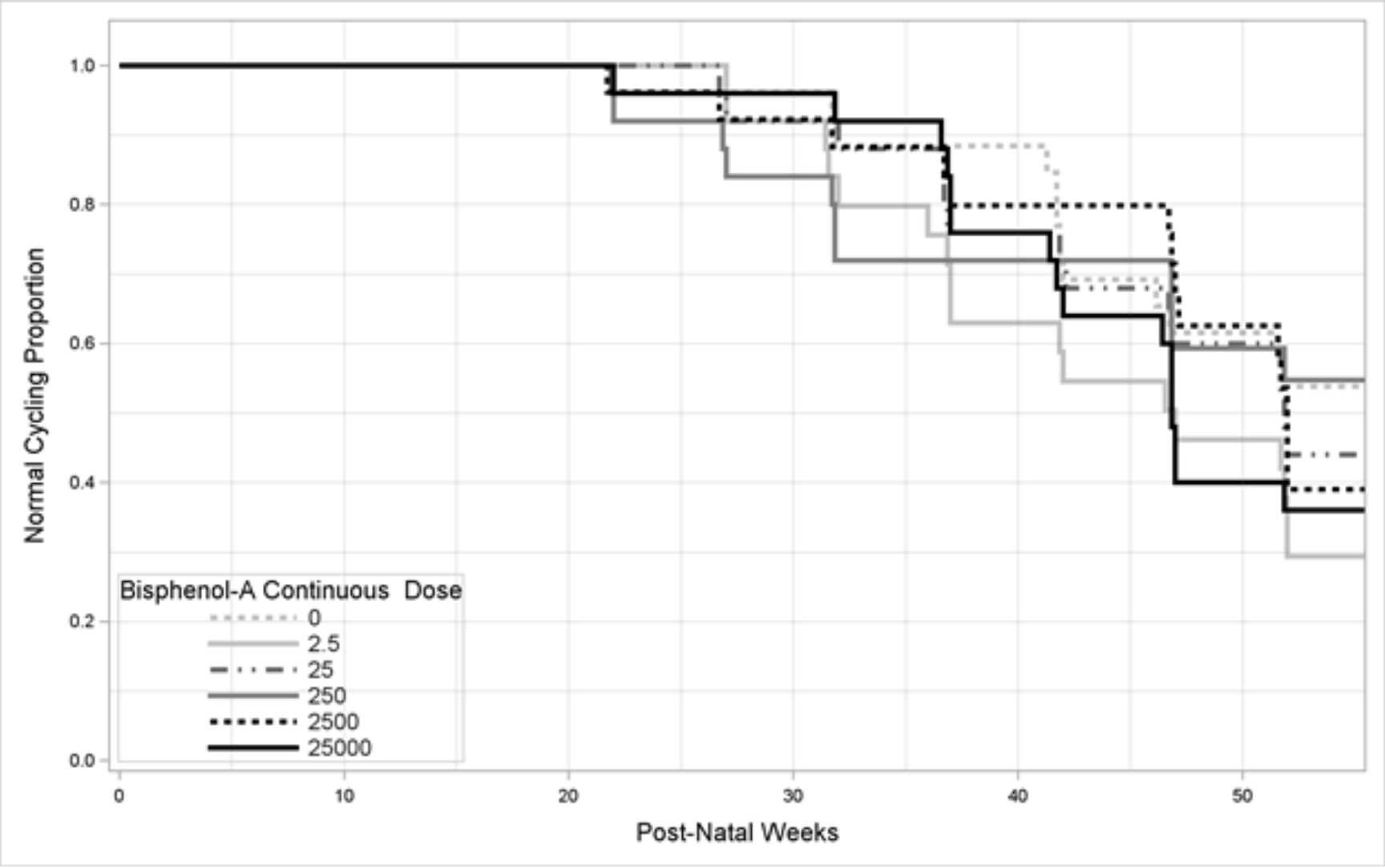

Figure 20. Kaplan-Meier Time to Aberrant Cycling Curve for BPA Continuous-Dose Arm

See Table 36 for data analysis results.

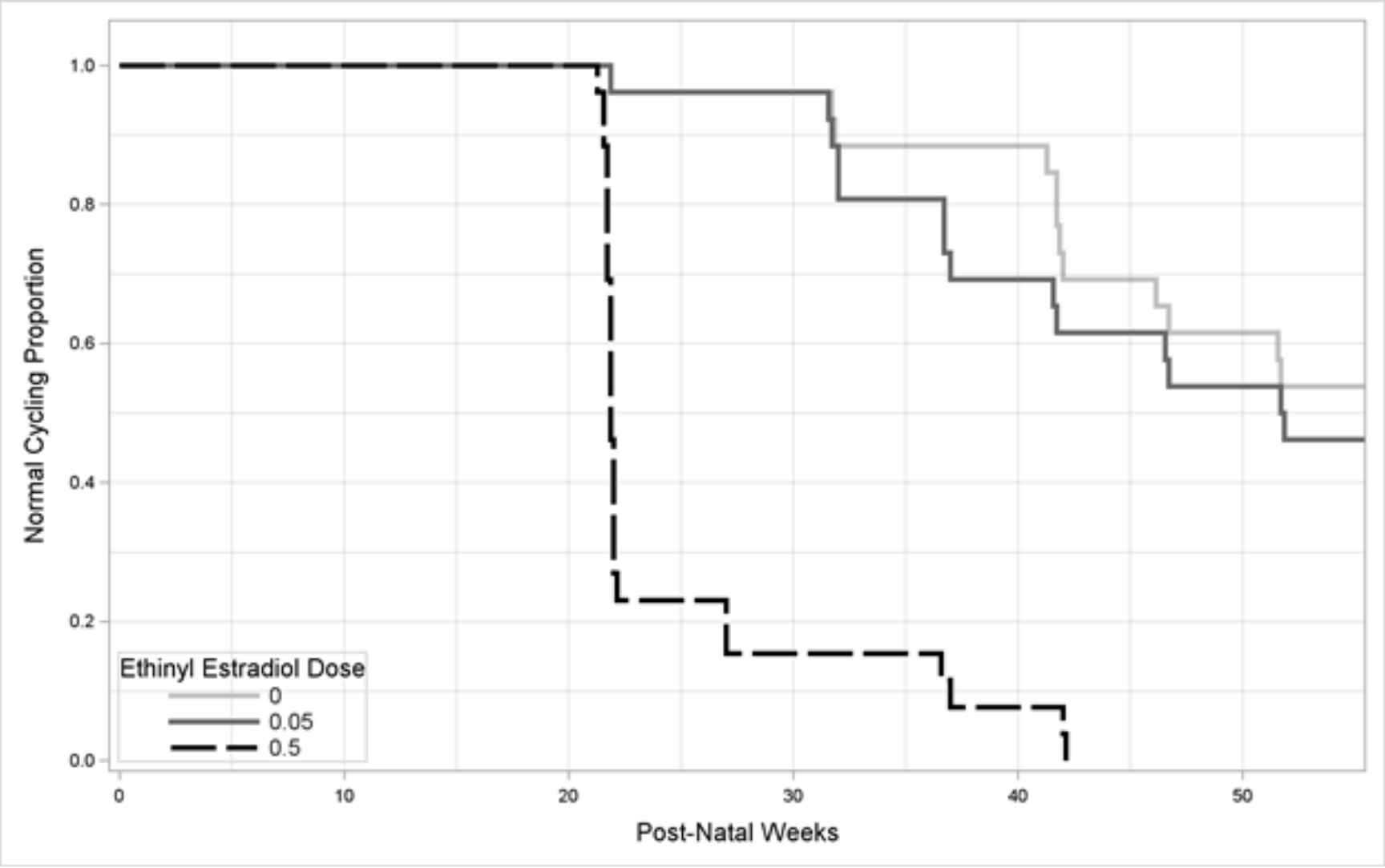

Figure 21. Kaplan-Meier Time to Aberrant Cycling Curve for EE_2 Continuous-Dose Arm

See Table 36 for data analysis results.

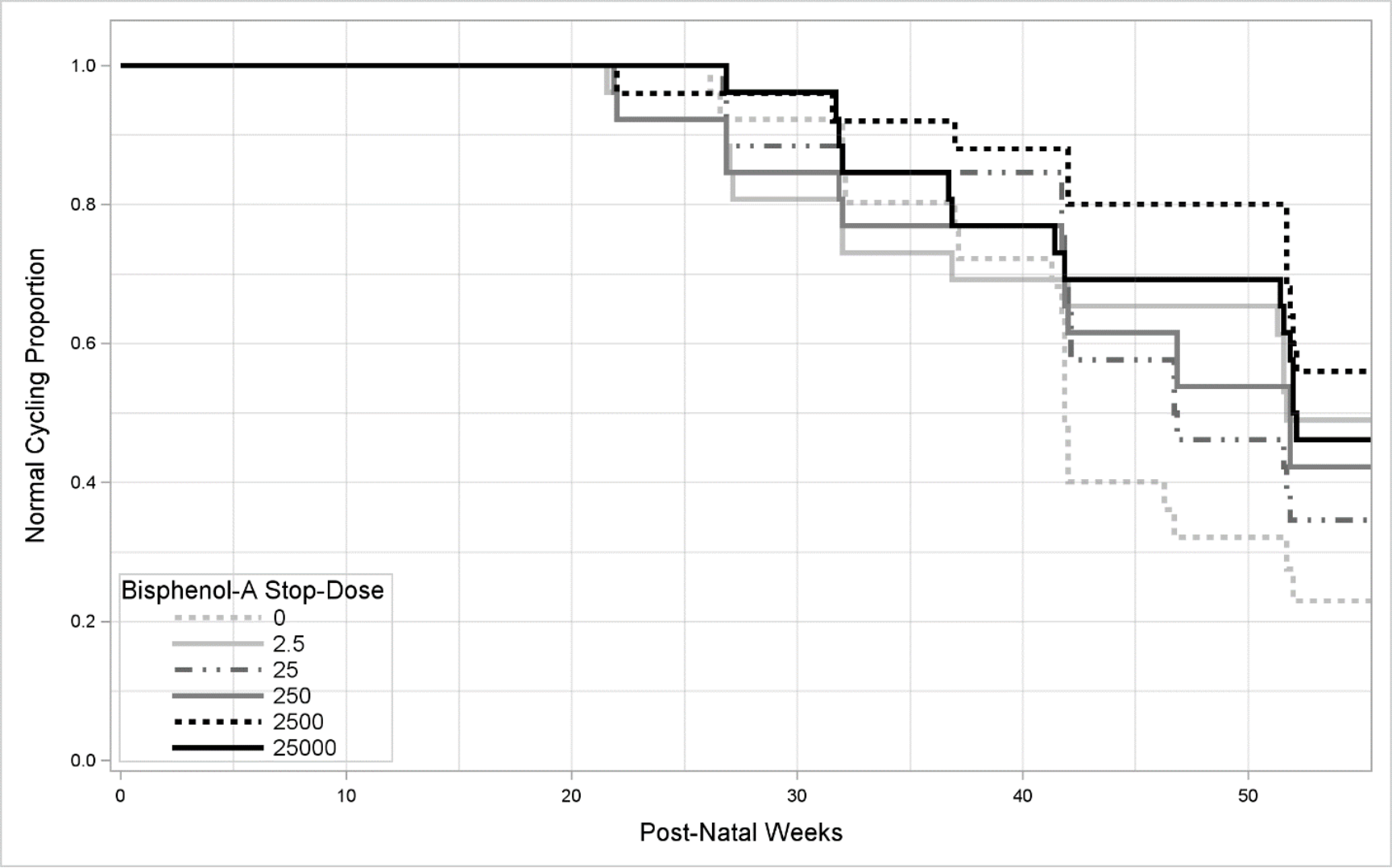

Figure 22. Kaplan-Meier Time to Aberrant Cycling Curve for BPA Stop-Dose Arm

See Table 37 for data analysis results.

Appendix A List of Supplemental Appendices

Supplemental appendices are available at https://doi.org/10.22427/NTP-DATA-018-00015-0001-000-6.

Supplemental Appendix	Title and Description
I	**Protocol and Amendments*** Approved NCTR study protocol (E0219001), which includes description of study sponsor, testing facility and responsible personnel, study objectives, background of the scientific problem being addressed, and a detailed description of the study design. Follow-up amendments to the approved study protocol and rationale for the changes, as well as a copy of the study protocol with all amendment changes incorporated, are also included.
II	**Protocol Deviations*** Documentation generated by Priority One (Animal Care contractor), Toxicologic Pathology Associates (Pathology services contractor), or the Study Director to report noted deviations from the procedures defined in the approved study protocol or follow-up amendments. It includes description of deviations, the corrective measurements taken to prevent re-occurrence, and the assessment by the Study Director on the impact of the deviation on study integrity. A summary table with all deviations is also included.
III	**Notes to Study File*** Documentation on various events related to the study conduct and not covered in the study protocol deviations documentation.
IV	**Study Startup Memo and Study Definition E0219002 (F_0 and F_1 Preweaning)*** Documentation generated by the Computer Support Group to describe the programming of the in-life tracking system used to support the study and its validation, animal allocation and breeding schemes, treatment group definitions, rack configurations used for animals of the F_0 generation and F_1 animals prior to weaning on postnatal day 21. Definitions of disposition and reasons for removal terms are also included.
V	**Study Startup Memo and Study Definition E0219003 (F_1 Postweaning)*** Documentation generated by the Computer Support Group to describe the programming of the in-life tracking system used to support the study and its validation, animal allocation scheme, treatment group definitions, and rack configurations used for F_1 animals after weaning on postnatal day 21.
VI	**Breeder Weight Ranking, Treatment Randomization, and Pairing Schedule*** Documentation to define the treatment randomization and weight ranking of the F_0 breeders, the breeding pair randomization, and to report the breeder pairings conducted in the study.
VII	**Chemistry Support Report** Report by the Chemistry Support Group on the analyses of the study test articles, dose preparations, BPA levels in study materials, and phyto/mycoestrogens in diet. The standard operating procedures followed to perform these analyses are also included.
VIII	**Microbiology Support Report** Report by the Surveillance/Diagnostic Program Support Group on the microbiological findings on sentinel animals, animal rooms, and animal husbandry supplies.
IX	**Diet Preparation Services Report** Report by the Diet Preparation Support Group describing the inventory and storage conditions of the study diet and test articles, dose preparations, and the standard operating procedures followed.
X	**Animal Rooms Temperature and Humidity Reports*** Report of the temperature and relative humidity recorded in the rooms used to house the animals over the course of the study.

Supplemental Appendix	Title and Description
XI	**Dosing Pump Volume Delivery Accuracy Determinations*** Documentation on the accuracy tests performed of the volumes dispensed by the Hamilton Microlab 500 Series Diluter/Dispenser instruments used in the oral dosing of the study animals over the course of the study.
XII	**5K96 Diet Nutrient and Contaminant Analyses from Diet Manufacturer** Certificates of analysis from the diet manufacturer of the nutrient and contaminants on each lot of study diet.
XIII	**Summary Statistics for Food Consumption** **a. Interim Sacrifice** **b. Terminal Sacrifice** Report by the Statistical Support Group on the analysis of feed consumption data collected after weaning from F_1 animals assigned to the interim (1-year) or terminal (2-year) sacrifice study arms. The analyses reported were limited to means and standard errors.
XIV	**Estimate of BPA Background Ingestion from Diet** Documentation on the calculations conducted to estimate the BPA background exposure of animals due to ingestion of study diet.
XV	**Animals Transferred to Cellulose (Alpha-Dri) Bedding*** Documentation on the animals transferred from hardwood bedding to Alpha-Dri cellulose bedding over the course of the study. These transfers were recommended by Veterinary Services due to foot lesions or ventral masses that could be further irritated by the hardwood chips.
XVI	**Rationale for Sensitivity Analyses in Statistical Reports*** Background on the rationale to conduct a sensitivity statistical analysis that excluded any animal that was co-housed in the same room as animals treated with 250,000 µg BPA/kg body weight/day, and list of animals excluded in the sensitivity analysis.
XVII	**Gestational Body Weight Statistical Analysis** Report by the Statistical Support Group on the analysis of gestational body weight data collected from F_0 dams. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XVIII	**Implantation Site Statistical Analysis** Report by the Statistical Support Group on the analysis of uterine implantation data collected from F_0 dams. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XIX	**Litter Parameters Statistical Analysis** Report by the Statistical Support Group on the analysis of litter data collected, including litter counts, sex proportions, and body weights. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XX	**Preweaning Animal Survival Analysis** Report by the Statistical Support Group on the analysis of survival data collected from F_1 animals prior to weaning. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXI	**Interim Sacrifice Survival Analysis** Report by the Statistical Support Group on the analysis of survival data collected after weaning from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.

Supplemental Appendix	Title and Description
XXII	**Terminal Sacrifice Survival Analysis** Report by the Statistical Support Group on the analysis of survival data collected after weaning from F_1 animals assigned to the terminal (2-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXIII	**Preweaning Body Weight Statistical Analysis** Report by the Statistical Support Group on the analysis of body weight data collected from F_1 animals prior to weaning. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXIV	**Postweaning Body Weight Statistical Analysis, Interim Sacrifice Animals** Report by the Statistical Support Group on the analysis of the body weight data collected after weaning from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXV	**Postweaning Body Weight Statistical Analysis, Terminal Sacrifice Animals** Report by the Statistical Support Group on the analysis of body weight data collected after weaning from F_1 animals assigned to the terminal (2-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXVI	**Vaginal Opening Time and Body Weight Statistical Analysis** Report by the Statistical Support Group on the analysis of vaginal opening and body weight data collected from a subset of F_1 animals assigned to the terminal (2-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXVII	**Estrous Cycle Statistical Analysis** Report by the Statistical Support Group on the analysis of vaginal cytology data collected from a subset of F_1 animals assigned to the terminal (2-year) sacrifice study arm to determine estrous cyclicity. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXVIII	**Time to Onset of Aberrant Cycling Statistical Analysis** Report by the Statistical Support Group on the analysis of vaginal cytology data collected from a subset of F_1 animals assigned to the terminal (2-year) sacrifice study arm to determine onset of abnormal estrous cycling. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXIX	**Hematology and Clinical Chemistry Statistical Analysis, Interim Sacrifice Animals** Report by the Statistical Support Group on the analysis of hematology and clinical chemistry data collected from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXX	**Organ Weight Statistical Analysis, Interim Sacrifice Animals** Report by the Statistical Support Group on the analysis of organ weight data collected from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.

Supplemental Appendix	Title and Description
XXXI	**Sperm Parameter Statistical Analysis, Interim Sacrifice Animals** Report by the Statistical Support Group on the analysis of sperm parameter data collected from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXXII	**Pathology Report, Interim and Terminal Sacrifice Animals** Report by Toxicologic Pathology Associates (Pathology services contractor) on the gross and microscopic lesions found in tissues collected from F_1 animals assigned to the interim (1-year) and terminal (2-year) sacrifice study arms. The incidence rates of neoplasms and non-neoplasms by anatomic site and by individual animal, severity scores for some non-neoplasms, the cause of death, and the mammary gland fibroadenoma/adenoma/ adenocarcinoma counts are also included.
XXXIII	**Neoplastic and Non-neoplastic Lesions Statistical Analysis, Interim Sacrifice Animals** Report by the Statistical Support Group on the analysis of neoplastic and non-neoplastic lesions data collected from F_1 animals assigned to the interim (1-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.
XXXIV	**Neoplastic and Non-neoplastic Lesions Statistical Analysis, Terminal Sacrifice Animals** Report by the Statistical Support Group on the analysis of neoplastic and non-neoplastic lesions data collected from F_1 animals assigned to the terminal (2-year) sacrifice study arm. The report includes a description of statistical methods used, tables of results of analyses conducted on the full set of study animals, and a summary of the results of the sensitivity analyses.

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ISSN 2473-4756