Food and Chemical Toxicology 132 (2019) 110728

Contents lists available at ScienceDirect

Food and Chemical Toxicology

journal homepage: www.elsevier.com/locate/foodchemtox

A two-year toxicology study of bisphenol A (BPA) in Sprague-Dawley rats: CLARITY-BPA core study results

L. Camacho[a], S.M. Lewis[a], M.M. Vanlandingham[a], G.R. Olson[b], K.J. Davis[b], R.E. Patton[b], N.C. Twaddle[a], D.R. Doerge[a], M.I. Churchwell[a], M.S. Bryant[a], F.M. McLellen[a], K.A. Woodling[a], R.P. Felton[a], M.P. Maisha[a], B.E. Juliar[a], G. Gamboa da Costa[a], K.B. Delclos[a,*]

[a] U.S. FDA, National Center for Toxicological Research, Jefferson, AR, 72079, USA
[b] Toxicologic Pathology Associates, Jefferson, AR, 72079, USA

ARTICLE INFO

Keywords:
Bisphenol A
Chronic toxicity
Sprague-Dawley
Safety assessment

ABSTRACT

We report the data from the guideline-compliant two-year toxicology study conducted as part of the Consortium Linking Academic and Regulatory Insights on Bisphenol A Toxicity (CLARITY-BPA). BPA (0, 2.5, 25, 250, 2,500, and 25,000 μg/kg body weight (bw)/day) was administered daily by gavage in 0.3% carboxymethylcellulose vehicle to NCTR Sprague-Dawley rats from gestation day 6 through the start of parturition and then directly to pups from the day after birth until postnatal day 21 (stop-dose arm) or continuously until termination at one or two years. The stop-dose arm was included to assess the potential for any BPA effects that were due to developmental exposure. No BPA-related effects were evident in the in-life and non-histopathology data. Neoplastic and nonneoplastic lesions diagnosed in both females and males were common age-associated lesions that were variable across control and BPA-treated groups. The lack of consistent responses within the continuous- and stop-dose arms within and across tissues brought into question the plausible relationship of most of these lesions to BPA treatment. There was a possible relationship between the increased incidences of lesions in the female reproductive tract and the male pituitary and exposure to the 25,000 μg BPA/kg bw/day dose level.

1. Introduction

Bisphenol A (BPA) has been widely used 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. Human exposure occurs predominantly through the migration of BPA from packaging material into food. Because of ubiquitous exposure, BPA has undergone extensive toxicological evaluations, but the conclusions derived from the aggregate results of the studies remain subjects of debate by certain groups. Current safety assessments by the preponderance of international regulatory agencies conclude that BPA at current exposure levels (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 at any life stage (Health Canada, 2012; EFSA, 2015; FDA, 2014). In contrast, others have concluded that the overall body of evidence indicates that BPA is likely to be a human health hazard. 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 (ANSES, 2013). The European Union has banned the use of BPA-containing polymers in bottles or cups intended for use by infants and young children based on the precautionary principle (EC, 2011), and 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 (OEHHA, 2015). The US FDA repealed its regulations establishing safe conditions of use for BPA in infant feeding bottles and spill-proof cups ("sippy cups") (77 FR 41899, July 17, 2012) and in epoxy resins as coatings in packaging for infant formula (78 FR 41840, July 12, 2013) because these uses have been abandoned. The US EPA established a reference dose of 50 μg BPA/kg bw/day based on the application of a 1,000-fold safety factor to a lowest-observed-adverse-effect level, which was a reduction in body weight at 50,000 μg/kg bw/day in a rat feeding study (EPA, 1988). EFSA established a tolerable daily intake (TDI) of 50 μg BPA/kg bw/day, which was later revised to a temporary TDI of 4 μg/kg bw/day after a reevaluation of the data (EFSA, 2006, 2015). Both the previous and updated EFSA TDIs are based on general toxicological effects in the liver and kidney and not on reproductive or endocrine-mediated effects. Following its

* Corresponding author.
E-mail address: barry.delclos@fda.hhs.gov (K.B. Delclos).

https://doi.org/10.1016/j.fct.2019.110728
Received 30 May 2019; Received in revised form 25 July 2019; Accepted 26 July 2019
Available online 28 July 2019
0278-6915/ Published by Elsevier Ltd.

comprehensive review of BPA toxicity and exposure literature in 2014, the FDA confirmed its position that the most appropriate no-observed-adverse-effect level from animal oral exposure studies was 50,000 μg/kg bw/day for systemic toxicity and that the margin of safety based on current exposures of all age groups was well over 1,000 (FDA, 2014).

The National Toxicology Program (NTP) previously assessed BPA in two-year dietary administration studies in both sexes of F344 rats and B6C3F1 mice, using dose levels ≥ 50,000 μg/kg bw/day (NTP, 1982), levels far above potential human dietary exposure. In addition, these studies started dosing at six weeks of age, and thus did not address any potential for toxicities resulting from developmental exposures. The NTP conclusions from these two-year studies were that there was no convincing evidence of BPA-induced carcinogenesis in rats or mice. Similarly, several other guideline-compliant studies only found clear adverse effects of BPA at doses several orders of magnitude above potential human exposures (e.g., Delclos et al., 2014; Stump et al., 2010; Tyl et al., 2002, 2008). Given the on-going public controversy on the potential toxicity of BPA to humans, the National Institute of Environmental Health Sciences (NIEHS) Division of Extramural Research and Training (DERT) and the NTP sponsored a research program termed the Consortium Linking Academic and Regulatory Insights on BPA Toxicity (CLARITY-BPA). CLARITY-BPA designed and conducted a rat study involving exposures to a broad range of BPA doses, included developmental exposure, and, in addition to evaluating endpoints typically used for regulatory decision making ("core study"), provided animals and biological samples to a group of NIEHS-funded university-based scientists to pursue hypothesis-driven studies in various organ systems ("grantee studies"). Most of these hypothesis-driven studies included methodology not typically used in guideline-compliant studies. The doses selected for the CLARITY-BPA study ranged from 2.5 μg BPA/kg bw/day, a dose reasonably close to human exposure levels and approximately 10-fold above the level that could potentially result from consumption of background BPA in the laboratory rodent diet, to 25,000 μg BPA/kg bw/day, a level at least 50,000-fold higher than the estimated 95th percentile human dietary exposures. Detailed descriptions of the general plan of the CLARITY-BPA project have been published (Birnbaum et al., 2012; Heindel et al., 2015; Schug et al., 2013), and all primary data generated in both the core and grantee studies are available to the public (NTP, 2018a, b, c). Here we report the conduct of and results from the core study. In addition to continuously dosed groups, the study design included BPA treatment groups that were dosed only until weaning and then were continued untreated until removal from the study at one or two years. Although the stop-dose exposure regimen is of dubious relevance to the existing continuous lifetime human exposure to BPA, this dosing arm was included because long-lasting effects following developmental exposure to BPA have been a focus of concern in the literature. An interim sacrifice at one year was included to allow for evaluation of histopathology and other endpoints that were less confounded by background lesions of aging than those encountered at the terminal sacrifice at two years. Since the interpretation of the study relied most heavily on the histopathology evaluation, those data are the focus of the data presentation and discussion.

2. Materials and methods

2.1. CLARITY-BPA core study

A comprehensive description of the materials and methods used in core study, which 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), is available in the NTP Research Report on the study (NTP, 2018b), and in an abbreviated form in Heindel et al. (2015). Essential elements of the study are recapitulated below and in Supplemental Table 1.

2.1.1. Test chemicals and vehicle

The sources and purities of chemicals used were as follows: BPA (CAS no. 80-05-7, TCI America, Portland, OR; product no. B0494, lot no. 6052012 [air-milled], > 99% purity); ethinyl estradiol (EE_2, CAS #57-63-6, Sigma-Aldrich Corporation, St. Louis, MO; product no. E4876, lot no. 071M1492V, > 99% purity); and the vehicle, carboxymethyl cellulose (CMC, Sigma-Aldrich, St. Louis, MO; product no. C5013, lot no. 041M0105V).

2.1.2. Animals and animal husbandry

The animal model used was the Sprague-Dawley rat maintained at NCTR (Sprague-Dawley/CD23/NctrBR). Animal care and use were in accordance with the Public Health Service Policy on Humane Care and Use of Animals (grants.nih.gov/grants/olaw/references/phspolicylabanimals.pdf). All animal use and procedures were approved in advance by the NCTR Animal Care and Use Committee and were conducted in an Association for Assessment and Accreditation of Laboratory Animal Care (AALAC)-accredited facility.

Animal husbandry was as described in the NTP Research Report (NTP, 2018b). A low phytoestrogen diet (5K96-verified casein diet 10 IF, round pellets, γ-irradiated, Test Diets, product no. 1810069, Purina Mills, Richmond, IN) and Millipore-filtered water in glass water bottles were available *ad libitum*. Polysulfone cage leachates, drinking water, and bedding extracts did not have BPA detectable above the analytical method blank. Bedding was hardwood chip (P.J. Murphy, Montville, NJ), but was replaced with Alpha-Dri® (Shepherd Specialty Papers, Watertown, TN), a softer virgin cellulose bedding, when recommended by Veterinary Services for animals with lower body lesions.

All diet lots were monitored for nutrient levels and for phytoestrogen and other contaminant levels. Diet lots and other study materials were monitored for BPA by liquid chromatography/tandem mass spectrometry, as described previously (Delclos et al., 2014).

Microbiological surveillance of the animal rooms, water, feed, and animals was conducted in accordance with NCTR's Sentinel Animal Program. All 46 sentinel animals evaluated periodically over the course of the study were determined to be free of pathogenic organisms.

2.1.3. Dose preparation and administration

The study included a vehicle, five BPA dose groups: 2.5, 25, 250, 2,500 and 25,000 μg/kg bw/day, and two dose groups of the reference estrogen EE_2: 0.05 and 0.5 μg/kg bw/day. Test chemicals were prepared in the vehicle, 0.3% aqueous CMC, and administered by gavage daily at a volume of 5 mL/kg bw using modified Hamilton Microlab ML511C programmable 115V pumps (Hamilton Co., Reno, NV; described in Lewis et al., 2010). Dosing vials and animals were marked so that the technicians dosing and caring for the animals were not blinded to the treatments. Dose formulations were certified to be within 10% of target as described (NTP, 2018b) and used within their established stability window.

2.1.4. Animal dose group assignment, breeding, dosing, and data collection

The overall study design is illustrated in Fig. 1 and details of mating, pup allocations, and in-life data collection for the core study are given in Supplemental Table 1.

Pregnant dams were dosed daily by gavage from gestation day (GD) 6 until start of parturition and their pups were directly dosed by gavage from postnatal day (PND) 1 (day of birth = PND 0). For animals younger than PND 5, the gavage needle did not enter the esophagus but rather was inserted to the opening of the esophagus (Butchbach et al., 2007).

2.1.5. Histopathology procedures

All gross lesions were processed for histological evaluation. For tissues specified for evaluation by the study pathologist (Supplemental Table 1), tissues from all dose groups were evaluated. Tissues were generally processed in accordance with NTP specifications (NTP, 2011,

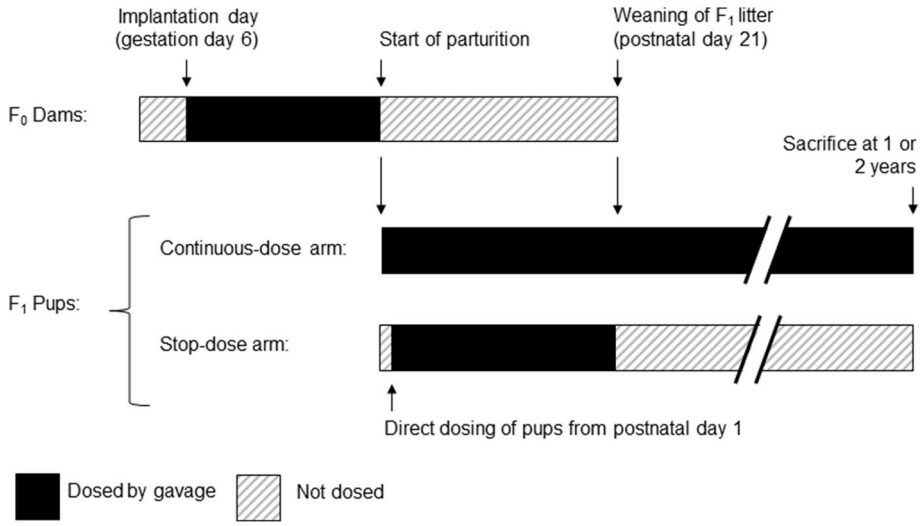

Fig. 1. CLARITY-BPA core study experimental design.

NTP, 2011), except that six step sections of each prostate were examined. All tissues, except testes and eyes, were fixed in 10% NBF and stained with hematoxylin and eosin for microscopic evaluation. Testes and eyes were fixed in modified Davidson's fixative and testes were stained with periodic acid-Schiff (PAS) stain. The International Harmonization of Nomenclature and Diagnostic Criteria (INHAND) guidelines (https://www.toxpath.org/inhand.asp) and the NTP's non-neoplastic lesion atlas (https://ntp.niehs.nih.gov/nnl/) 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) respectively, were used. Many of the nonneoplastic lesions were assigned severity scores on a four-level scale: 1, minimal; 2, mild; 3, moderate; 4, marked. Individual animal data records and pathology tables were evaluated by an independent quality assessment (QA) group, and QA pathologists evaluated selected histopathology slides, including 100% of reproductive organs, mammary gland, pituitary, and all neoplasms. The reviewed slides, along with the diagnoses made by the study pathologist and QA pathologists, were reviewed by a Pathology Working Group (PWG) consisting of the study pathologist and nine additional board-certified veterinary pathologists in a treatment group-blinded fashion. Final diagnoses for reviewed lesions represent a consensus of the PWG.

2.1.6. Statistical methods

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. Similarly, the two EE_2 dose groups were compared to the vehicle control. Tests were conducted at the 0.05 significance level and were two-sided, except where indicated to be one-sided. For non-histopathology data, comparisons to vehicle control were corrected for multiplicity by Dunnett's test or Holm's method, as appropriate. 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 neoplastic and nonneoplastic lesions, where trend tests were conducted also 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. Preweaning data analyses included nesting by litter.

For neoplasm and nonneoplasm 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 nonneoplasm incidence for terminal sacrifice animals, the Poly-3 method of Bailer and Portier (1988), as modified by Bieler and Williams (1993), and the NIEHS continuity-correction, discussed in Peddada and Kissling (2005), 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 nonneoplasm incidences, the trend test was two-sided, while the pairwise comparisons between treatment and vehicle control groups were one-sided and not adjusted for multiple comparisons.

Additional statistical tests were run to include information on lesion severity together with the incidence data. The Jonckheere-Terpstra test (Jonckheere, 1954; Terpstra, 1952) was run to test for monotonic dose trends, followed by Shirley's test (Shirley, 1977; Williams, 1986) for pairwise comparisons to controls. This combination of tests is referred to as JT/SW, and it presumes a monotonic dose response. A nonparametric relative treatment effects (RTE) method (Brunner et al., 2002), which does not presume a monotonic dose response, was also used.

2.1.6.1. Sensitivity analysis. Prior to weaning, most animals from the first cohort/load were housed in the same room as a separate cohort of CLARITY-BPA animals dosed with 250,000 μg BPA/kg bw/day (load 0, see Section 2.2, below). These animals were potentially exposed to low levels of BPA above the dietary background intake, which could lead to blood levels of BPA metabolites above the limit of detection and similar to those resulting from the 2.5 μg BPA/kg bw/day dose (Churchwell et al., 2014). Animals housed in rooms where the highest dose was 25,000 μg BPA/kg bw/day had no detectable BPA metabolites in their blood (Heindel et al., 2015). As a conservative approach to determine if this potential low-level exposure of the first cohort/load (representing ~20% of study animals) could impact the interpretation of study results, a post-hoc sensitivity analysis was conducted for each endpoint, in which 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 from statistical analysis. Any significant effects found in the sensitivity analysis that were not found in the analysis that includes all animals are listed in Supplemental Table 2.

2.2. Evaluation of a 90-day exposure to 250,000 μg BPA/kg bw/day on female reproductive tract

A separate group of animals (referred to as Load 0) was mated two weeks prior to the start of the core study described above. The animal model, husbandry, allocation, breeding, and dosing procedures were the same as used for the core study. Dams (ten per group) were dosed by daily gavage with vehicle or 250,000 μg BPA/kg bw/day from GD 6 until the start of parturition, and F_1 female pups (one per litter and siblings of the males assessed for sperm endpoints by Dere et al., 2018) were sacrificed on PND 90 without regard to stage of estrous. The reproductive tissues (ovary, uterus, and vagina) were removed for microscopic evaluation by a board-certified veterinary pathologist to compare results with those observed in the previous 90-day study (Delclos et al., 2014). The statistical methods applied were as reported previously (Delclos et al., 2014).

2.3. Evaluation of d6-BPA (2.5–250 μg/kg bw/day) dosimetry at PND 4 and PND 21

Deuterated (d6)-BPA (99.5 atom %) was obtained from CDN Isotopes (Pointe-Claire, Quebec). Four to five pups of each sex from the Load 0 were given a single dose of 2.5, 25, or 250 μg d6-BPA/kg bw by gavage on PND 4 or 21. Blood was collected 15 min after dosing, the approximate time of C_{max} (Doerge et al., 2010a), and serum was prepared for analysis. A previously described sensitive derivatization method using pyridine-3-sulfonyl chloride (Patterson et al., 2013) was used together with a validated high throughout liquid chromatography-electrospray ionization-tandem mass spectrometry (LC-ES/MS/MS) method to measure unconjugated d6-BPA from 100 μL of serum with (for total) or without (for unconjugated) pre-hydrolysis with β-glucuronidase/sulfatase (*Helix pomatia*, H1, 16 units/mg; Sigma Aldrich, St. Louis, MO).

3. Results

3.1. Exposure measurements with d6-BPA and background exposure to BPA through the diet

Dosing of pups with a single oral dose of 2.5 μg d6-BPA/kg bw resulted in mean peak levels of unconjugated d6-BPA at PND 4 and PND 21 of 0.24 and 0.03 nM, approximately 4.5% and 1.1% of the total d6-BPA, respectively (Table 1). As expected, the serum levels of total and unconjugated d6-BPA increased with increased doses in a near linear manner and the percentage of circulating unconjugated d6-BPA decreased with age (Table 1).

Ten of the 11 lots of diet used in the present study contained BPA above the analytical background levels, with an average (using 0 for the lot with no BPA detectable above the limit of blank) of 1.3 ± 0.9 (standard deviation, S.D.) ng/g diet (range 0–3.0), which was below the protocol-specified limit of 5 ng/g diet. Based on the measured food consumption in postweaning animals, this dietary intake of BPA resulted in a mean background BPA dose of approximately 0.05–0.06 μg/kg bw/day (2–2.5% of the lowest BPA dose tested), and maximum background BPA dose of approximately 0.12–0.15 μg BPA/kg bw/day (5–6% of the lowest BPA dose tested). Since younger animals consume higher quantities of food per unit of body weight, younger animals consumed higher background levels of dietary BPA. For example, on week 4, pups consumed 2- to 3-fold more BPA than the mean value calculated over the entire study (data not shown).

Table 1
Serum concentration of total and unconjugated d6-BPA at approximate time of maximum concentration after dosing with d6-BPA.

Age	d6-BPA dose (μg/kg bw)	n	Unconjugated d6-BPA (nM)	Total d6-BPA (nM)	% Unconjugated
PND 4	2.5	10	0.24 ± 0.31	5.4 ± 2.3	4.5 ± 3.9
	25	10	2.0 ± 1.2	51.0 ± 13.8	4.1 ± 2.4
	250	8	31 ± 34	405 ± 179	7.7 ± 6.9
PND 21	2.5	10	0.03 ± 0.05	2.3 ± 0.8	1.1 ± 1.7
	25	9	0.12 ± 0.13	21.7 ± 8.3	0.50 ± 0.30
	250	10	1.5 ± 1.5	325 ± 162	0.60 ± 0.90

Pups received a single oral gavage dose of d6-BPA at PND 4 or PND 21 and a blood sample was collected approximately 15 min later. Each dose group consisted of both male and female pups. The data are presented as the mean ± S.D.

Table 2
Microscopic evaluation of ovaries, uteri, and vagina from PND 90 females treated with vehicle or 250,000 μg BPA/kg bw/day.

Tissue	Morphology	Vehicle Control	BPA
Ovary	Anestrus[a]	0/10	2/9
	Corpus luteum, depletion	0/10	1/9
	Corpus luteum, hypertrophy	0/10	2/9
	Corpus luteum, cyst	0/10	1/9
	Follicle, cyst	0/10	1/9
	Interstitial cell, atrophy	0/10	2/9
Uterus	Anestrus[a]	0/10	0/9
	Endometrium, hyperplasia	0/10	2/9
Vagina	Anestrus[a]	0/10	0/9
	Hyperplasia, mucocyte	0/10	2/9

[a] A likely abnormal state of the estrous cycle, either arrested or prolonged, based on accompanying histopathologic changes in the ovary.

3.2. Evaluation of the effects of a 90-day exposure to 250,000 μg BPA/kg bw/day on female reproductive tract

The ovary, uterus, and vagina of females dosed with vehicle or 250,000 μg BPA/kg bw/day were examined microscopically at PND 90 (Table 2). There were no abnormal findings in the ten vehicle control animals, while two of nine animals dosed with 250,000 μg BPA/kg bw/day showed mild effects in all organs evaluated. These two animals had subtle mucification of vagina, endometrial folding (hyperplasia), and at least one set of hypertrophied corpora lutea, suggesting mildly aberrant estrous cyclicity culminating in prolonged diestrus of a short duration. Interstitial cell atrophy in the ovary of these two animals was also evident (Table 2).

3.3. Core study in-life data

The number of breeding pairs assigned to the study in each dose group, as well as the number of sperm-positive dams and the number of litters produced, are given in Supplemental Table 3. Mating success was not analyzed since dosing was not started until GD 6, the initiation of major organogenesis. Dam body weights during pregnancy, number of implantation sites in mated dams, litter size, sex ratio, litter weight by sex, or mean pup weight at birth by sex were not affected by BPA or EE_2 treatment (data not shown).

Survival was not affected by any treatment, except for a lower survival relative to vehicle control in the preweaning period in the 0.05 μg EE_2/kg bw/day group (Supplemental Table 4). There was a high moribund removal rate for both females and males between one and two years of age. Where possible, a primary cause of death or underlying cause of the moribund condition, along with any contributing causes, were assigned for animals removed early from the study (Appendix 32, Subappendix 6 in NTP, 2018c). For females, mammary gland fibroadenomas and pituitary adenomas/carcinomas were the primary causes of morbidity across all groups. These two lesions accounted for 56–90% of the identified causes for removal in

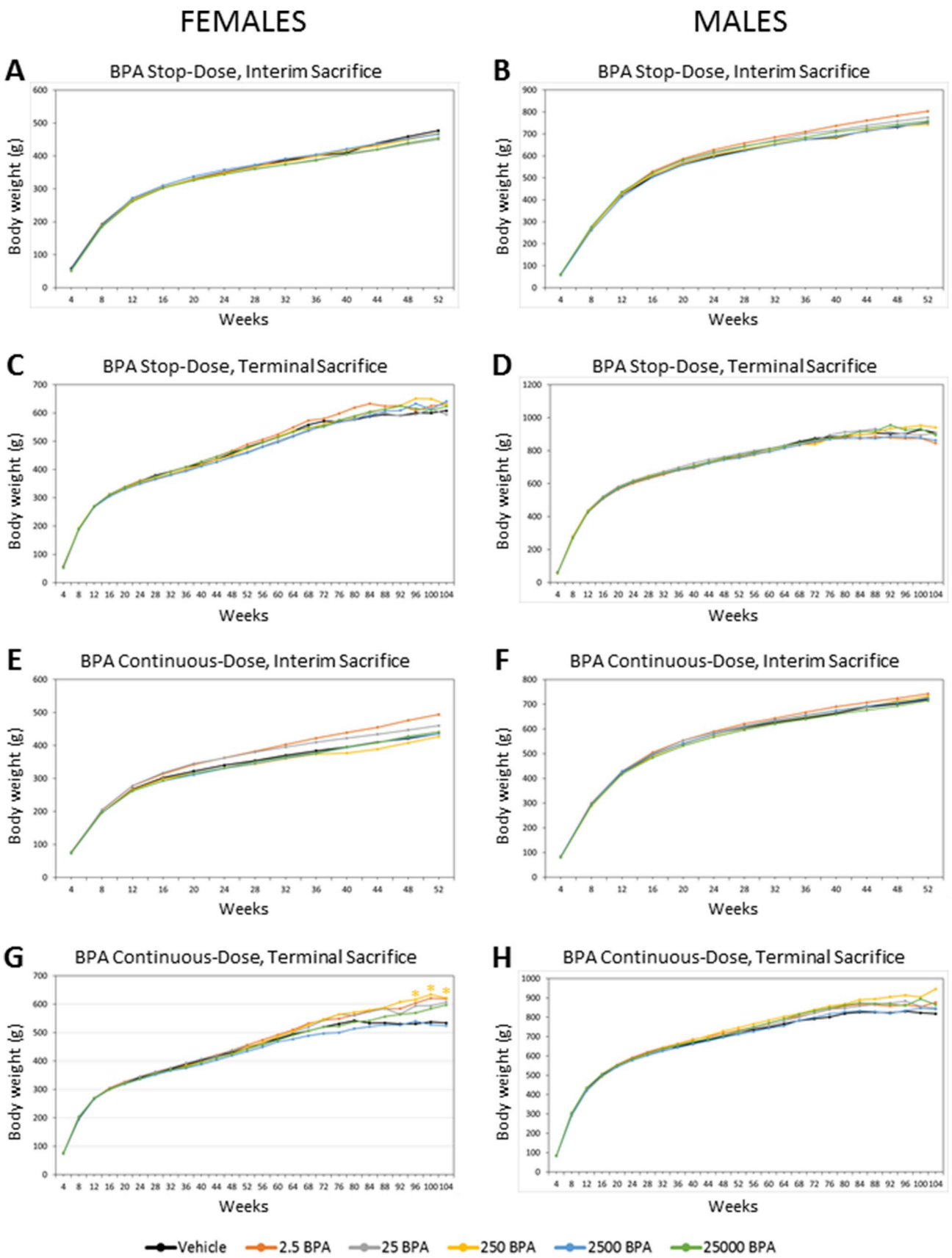

Fig. 2. Body weights for vehicle controls and (A) BPA stop-dose treatment groups, interim sacrifice females; (B) BPA stop-dose treatment groups, interim sacrifice males; (C) BPA stop-dose treatment groups, terminal sacrifice females; (D) BPA stop-dose treatment groups, terminal sacrifice males; (E) BPA continuous-dose treatment groups, interim sacrifice females; (F) BPA continuous-dose treatment groups, interim sacrifice males; (G) BPA continuous-dose treatment groups, terminal sacrifice females; (H) BPA continuous-dose treatment groups, terminal sacrifice males.

vehicle and BPA groups, with 71% (14 mammary fibroadenoma, 10 pituitary adenoma/carcinoma) in the continuous-dose vehicle controls and 90% (22 mammary fibroadenoma, 13 pituitary adenoma/carcinoma) in the stop-dose vehicle controls. For males, a broader range of primary causes for removal were identified, with pituitary adenomas/carcinomas, nephropathy, preputial gland carcinoma, and spleen malignant lymphoma among the primary causes of morbidity across all dose groups. These four lesions accounted for 37–66% of the identified causes for removal, with 49% (10 pituitary adenoma/carcinoma, 2 nephropathy, 3 preputial gland carcinomas, and 2 spleen malignant lymphomas) in the continuous-dose vehicle controls and 52% (6 pituitary adenoma/carcinoma, 9 nephropathy, 1 preputial gland carcinoma, and 1 spleen malignant lymphomas) in the stop-dose vehicle controls.

The post-weaning body weights of females and males in the vehicle control and BPA dose groups are shown in Fig. 2. Body weights of the animals in the EE_2 dose groups for interim and terminal sacrifice animals are shown in Supplemental Fig. 1. The sole difference in comparisons between treatment groups and vehicle controls was for terminal sacrifice females: mean body weights in the 250 μg BPA/kg bw/day dose group were statistically significantly higher by 16–18% than those of the vehicle control group for weeks 96–104 (Fig. 2G). While the mean body weights in the 2.5 μg BPA/kg bw/day females in weeks 36–52 of the interim sacrifice group were 10–13% higher than vehicle control means (Fig. 2E), these differences were not statistically significant, and a similar elevation of body weights over this period was not evident in the terminal sacrifice females (Fig. 2G).

There were no treatment effects on the time of vaginal opening. Effects on the estrous cycle were minimal in BPA-treated animals, with the sole statistically significant effect being a delay in the median time of onset in the stop-dose 2,500 μg BPA/kg bw/day dose group (57 weeks versus 42 weeks in vehicle controls, Supplemental Table 5). The 0.5 μg EE_2/kg bw/day dose altered the estrous cycle relative to vehicle control, with all but one animal in persistent estrus by that time.

3.4. Clinical chemistry, hematology, organ weights, and sperm parameter data obtained at the interim sacrifice

Full data sets for the hematology, clinical chemistry, and organ weight data collected are presented in the NTP Research Report on this study (NTP, 2018b). Endpoints that showed significant differences in any BPA dose group from the appropriate vehicle control are shown in Supplemental Figs. 2–4. Significant effects were generally confined to single dose groups in one study arm. Neither BPA nor EE_2 affected testicular spermatid head counts, caudal sperm counts, or caudal sperm motility and morphology in any dose group of the stop- or continuous-dose study arms (Supplemental Table 6).

3.5. Histopathology

The complete pathology report, with detailed individual animal and summary tables of all lesions evaluated in all study tissues in interim and terminal sacrifice animals, along with the associated statistical reports, are available online at the NTP website ((NTP), 2018b, c). All statistically indicated effects observed in the study are summarized for females in Table 3 and for males in Table 4. Selected lesions that exemplify the responses observed in the study are presented below. Since no statistically significant differences versus control were found regarding organ-specific neoplasms in males in any BPA treatment group, only nonneoplastic lesions are discussed for males.

3.5.1. Female mammary gland, neoplastic and nonneoplastic lesions

Table 5 summarizes the mammary gland neoplastic lesions observed in interim and terminal females. Fibroadenomas were observed in 4–25% of interim sacrifice females and 54–90% of terminal sacrifice animals, with no significant BPA or EE_2 treatment effects (Table 5). In the terminal sacrifice stop-dose females, 8% of the vehicle controls had mammary gland adenomas or adenocarcinomas, and the 2.5 μg BPA/kg bw/day stop-dose group had a statistically significant higher incidence of adenomas or adenocarcinomas (24% versus 8%, $p = 0.018$). In the terminal sacrifice continuous-dose females, 12% of the vehicle controls had adenomas or adenocarcinomas and incidences ranged from 9 to 20% in the BPA dose groups. There were no statistically significant increases relative to vehicle control in any continuous-dose BPA dose group. For the terminal sacrifice EE_2 treatment groups, there was a significant increase in adenocarcinomas in the 0.5 μg EE_2/kg bw/day dose group (38% versus 12%, $p < 0.001$; no adenomas diagnosed).

In the stop-dose BPA treatments, there were no statistically significant increased mammary gland nonneoplastic 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 6). 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 RTE test) for the 2.5 μg BPA/kg bw/day dose group in both the interim (14% versus 0%) and terminal (15% versus 4%) females. In the interim sacrifice animals, there was an increase in ductal dilatation (32% versus 9%) in the 25 μg BPA/kg bw/day dose group that was statistically significant by RTE test. There was a significant decrease (by RTE test) in ductal dilatation in the terminal sacrifice females in this dose group (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 6). In both interim and terminal animals, there were significant pairwise comparisons of the 0.5 μg EE_2/kg bw/day dose groups to vehicle control for ductal dilatation (85% versus 9%, interim; 81% versus 30%, terminal). In interim sacrifice animals, there was a significant pairwise comparison of the 0.5 μg EE_2/kg bw/day dose group to 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%).

3.5.2. Uterus, neoplastic and nonneoplastic lesions

Stromal polyps were the only neoplastic lesions noted in the uteri that showed statistically significant effects differences, with a significantly lower incidence in the interim stop-dose 25,000 μg BPA/kg bw/day females relative to the vehicle control (2% versus 14%, Supplemental Table 7) and a significant dose trend in the interim continuous-dose BPA females. There were no significant effects of EE_2 treatment.

Regarding nonneoplastic uterine lesions (Table 7), in the interim stop-dose 25,000 μg BPA/kg bw/day dose group there was a non-significant increase (27% versus 10%) in the incidence of apoptosis in luminal epithelial cells in the endometrium. In the interim continuous-dose BPA, there was a significant trend for this lesion, with the incidence in the 25,000 μg BPA/kg bw/day group significantly higher than the vehicle controls (38% versus 9%, all tests applied). The incidence of apoptosis in luminal epithelial cells was also increased relative to the vehicle control in the interim 0.5 μg EE_2/kg bw/day group (69% versus 9%, all tests applied).

Endometrial hyperplasia was significantly increased (RTE test) in the interim continuous-dose 2.5 and 250 μg BPA/kg bw/day dose groups (32% and 29%, respectively, versus 9%).

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%, JT/SW and/or RTE tests). Cystic endometrial hyperplasia was also

Table 3
Females, endpoints affected by any dose of BPA or EE_2 as indicated by statistical tests.

Endpoint	BPA, stop-dose (μg/kg bw/day)					BPA, continuous-dose (μg/kg bw/day)					EE_2 (μg/kg bw/day)	
	2.5	25	250	2,500	25,000	2.5	25	250	2,500	25,000	0.05	0.5
Prewean survival	na	na	na	na	na	–	–	–	–	–	–	↓ 0.005
Prewean body weight	na	na	na	na	na	–	–	–	–	–	–	↓ 0.006 (PNDs 4 & 7)
Postwean body weight (2y)	–	–	–	–	–	–	–	↑ 0.026 (wks 96–104)	–	–	–	↑ 0.002 (wks 4 & 8)
Abnormal estrous cycles at 16 weeks of age	–	–	–	–	–	–	–	–	–	–	–	↑ < 0.001
Early onset of aberrant estrous cycles	–	–	–	–	–	–	–	–	–	–	–	↑ < 0.001
Adrenal gland weight[a]	–	–	–	–	–	–	–	–	–	–	–	↑ < 0.001
Ovarian/parametrial fat pad weight[a]	–	–	–	–	–	–	–	–	–	–	–	↓ < 0.001
Heart weight[a]	–	–	–	–	–	–	–	–	–	–	–	↑ 0.015
Kidney weight[a]	–	–	–	–	–	–	–	–	–	–	–	↑ < 0.001
Liver weight[a]	–	–	–	–	–	–	–	–	–	–	–	↑ < 0.001
Ovary weight[a]	–	–	–	–	–	–	–	–	–	–	–	↓ 0.021
Pituitary gland weight[a]	–	–	–	–	–	–	–	–	–	–	–	↑ 0.002
Mean corpuscular hemoglobin concentration	–	–	–	–	–	–	↑ 0.007	–	–	–	–	–
Platelets	–	–	–	–	–	–	–	–	–	↓ 0.040	–	↓ 0.043
Eosinophils	–	–	–	–	–	–	–	↓ 0.015	–	–	–	–
% Eosinophils	–	–	–	–	–	–	–	–	–	–	–	↓ 0.024
Alkaline phosphatase	–	–	–	–	–	–	–	↑ 0.041	–	–	↑ 0.015	–
Thyroid-stimulating hormone	–	–	–	–	–	–	–	–	–	–	–	↑ 0.025
Mammary Gland, interim sacrifice												
Dilatation, duct	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Hyperplasia, lobular	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Dilatation, duct	–	–	–	–	–	–	#	–	–	–	–	–
Atypical focus	–	–	–	–	–	#	–	–	–	–	–	–
Mammary Gland, terminal sacrifice												
Dilatation, duct	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Dilatation, alveolus	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Atypical focus	–	–	–	–	–	#	–	–	–	–	–	
Adenocarcinoma	0.016	–	–	–	–	–	–	–	–	–	–	< 0.001
Uterus, interim sacrifice												
Apoptosis, endometrial luminal epithelium	–	–	–	–	–	–	–	–	0.022	–		< 0.001
Cystic endometrial hyperplasia	–	–	–	–	#	–	–	–	–	–		0.021
Squamous metaplasia	–	–	–	–	#	–	–	–	–	–		< 0.001
Dilatation, lumen	–	–	#	–	–	–	–	–	–	–		–
Endometrial hyperplasia	–	–	–	–	–	#	–	#	–	–		–
Uterus, terminal sacrifice												
Hyperplasia, endometrium	–	–	–	–	–	–	–	–	–	0.040		–
Atrophy	–	–	–	–	–	–	–	–	–	–		0.033
Cystic endometrial hyperplasia	–	–	–	#	#	–	–	–	–	–		–
Ovary, interim sacrifice												
Atrophy	–	–	–	–	–	–	–	–	–	–		< 0.001
Cyst, follicle	–	–	–	–	< 0.001	–	–	–	–	–		< 0.001
Depletion, corpora lutea	–	–	–	–	–	–	–	–	–	–		< 0.001
Hypertrophy, interstitial cell	–	–	–	–	–	–	–	–	#	–		< 0.001
Vagina, interim sacrifice												
Hyperplasia, epithelium	–	–	–	–	–	–	–	–	#	–		< 0.001
Mucification, epithelium	–	–	–	–	–	–	–	–	–	–		#
Vagina, terminal sacrifice												
Hyperplasia, epithelium	–	–	–	–	–	–	0.014	#	0.026	0.026	–	–
Degeneration, epithelium	–	–	–	–	–	#	–	–	#	–	–	–
Atrophy	–	–	–	–	–	–	–	–	–	–	–	#
Pituitary, interim sacrifice												
Angiectasis	–	–	–	–	–	–	–	–	–	–	–	#
Pars distalis, hyperplasia	–	–	–	–	–	–	–	–	–	–	–	#
Pituitary, terminal sacrifice												
Angiectasis	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Hemorrhage	–	–	–	–	–	–	–	–	–	–	–	< 0.001
Pars distalis, adenoma/carcinoma	–	–	–	–	–	–	–	–	–	–	–	0.011
Pars distalis, hyperplasia	#	#	–	–	–	–	–	–	–	–	–	–
Thyroid, interim sacrifice												
Hyperplasia, C-cell (1y)	#	–	–	–	–	–	–	–	–	–	–	–
Thyroid, terminal sacrifice												
Hyperplasia, follicular cells	–	–	–	–	–	#	–	–	–	0.034		–
Ultimobranchial cyst	–	–	0.038	0.017	–	–	–	–	–	–		–
Adrenal Gland, interim sacrifice												

(*continued on next page*)

Table 3 (*continued*)

Endpoint	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	2.5	25		250	2,500	25,000	2.5	25	250	2,500	25,000	0.05	0.5	
Cortex, cystic degeneration	–	–	#		–	–	–	–	–	–	–	–		–
Adrenal gland, terminal sacrifice														
Cortex, cystic degeneration	–	–	–		–	–	–	–	–	–	–	–		0.017
Cortex, hypertrophy	–	–	–		–	#	–	–	–	–	–	–		–
Cortex, angiectasis	–	–	–		–	–	–	–	–	–	–	–		#
Cortex, pigmentation	–	–	–		–	–	–	–	–	–	–	–		#
Medulla, hyperplasia	–	–	–		–	–	–	–	–	–	–	–		#
Kidney, interim sacrifice														
Cyst, renal tubule	–	–	–		–	–	0.004	–	–	–	–	0.034		–
Nephropathy	–	–	–		–	–	–	#	–	#	–	–		0.025
Mineralization	–	–	–		–	–	–	–	–	–	#	–		–
Kidney, terminal sacrifice														
Cyst, renal tubule	0.036	–	–		–	–	–	–	–	–	–	–		–
Nephropathy	–	–	–		–	#	0.033	–	–	–	–	#		#
Cyst, cortex	–	–	–		–	–	–	–	–	–	–	0.045		–
Liver, interim sacrifice														
Infiltration, mononuclear cells	0.012	–	–		–	0.048	–	–	–	–	–	–		–
Liver, terminal sacrifice														
Vacuolization, cytoplasmic	–	–	–		–	–	–	–	–	–	–	0.031		–
Degeneration, cystic	–	–	–		#	#	–	–	–	–	–	–		–
Angiectasis	–	–	–		–	–	–	–	–	–	#	–		–
Spleen, interim sacrifice														
Pigmentation	–	–	–		–	–	–	–	–	–	–	–		#
Spleen, terminal sacrifice														
Pigmentation	–	–	–		–	–	–	–	–	–	–	–		< 0.001
Heart, interim sacrifice														
Cardiomyopathy (1y)	–	–	–		–	–	–	–	–	–	–	–		0.015
Heart, terminal sacrifice														
Cardiomyopathy (2y)	#	–	#		#	#	–	–	–	–	–	–		–
Brain, terminal sacrifice														
Brain stem, compression	–	–	–		–	–	–	–	–	–	–	–		< 0.001
Brain stem, hemorrhage	–	–	–		–	–	–	–	–	–	–	–		0.009
Cerebrum, dilatation, ventricle	–	–	–		–	–	–	–	–	–	–	–		#
Pancreas, interim sacrifice														
Infiltration, lymphocyte	–	–	–		–	–	#	–	–	–	–	–		–
Pancreas, terminal sacrifice														
Hyperplasia, acinar cell	–	–	–		–	–	–	–	–	–	–	–		#
Thymus, interim sacrifice														
Atrophy	–	#	–		–	–	–	–	–	–	–	–		–
Thymus, terminal sacrifice														
Atrophy	–	#	–		#	–	–	–	–	–	–	–		–
Bone marrow, terminal sacrifice														
Hypocellularity	–	–	–		–	–	–	–	–	–	#	–		–
Hyperplasia, myeloid cell	–	–	–		–	–	–	–	–	–	–	–		#

na, not applicable; ↑, increased *versus* controls; ↓, decreased *versus* controls; –, no change *versus* controls.
p-Values are *versus* controls by primary statistical test. For histopathology endpoints, #, $p < 0.05$ *versus* controls by secondary, but not primary, statistical test.
[a] Results for organ weights adjusted for body weights are summarized in this table.

significantly increased in interim 0.5 μg EE_2/kg bw/day females (54% versus 22%, all tests applied). In the continuous dose BPA groups, the only significant effect on cystic endometrial hyperplasia was a decrease in terminal sacrifice females at 2.5 μg BPA/kg bw/day (60% versus 42%, RTE test).

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 (18% versus 0%, JT/SW and RTE tests) and an increased incidence of dilatation of the lumen in the 250 μg BPA/kg bw/day dose group (18% versus 5%, RTE test). There were no significant pairwise comparisons versus vehicle controls in these lesions for the continuous-dose BPA dose groups, except for significant trends for squamous metaplasia and dilatation of the lumen in the interim and terminal sacrifice, respectively.

In the interim 0.5 μg EE_2/kg bw/day females, there was a significant increase by all tests applied in squamous metaplasia (54% versus 4%).

3.5.3. Ovary, nonneoplastic lesions

Table 8 summarizes the nonneoplastic lesions observed in the ovaries in the interim and terminal sacrifices. In the stop-dose BPA-treated interim sacrifice females, there was a significant dose trend ($p < 0.001$) for follicular cysts in the ovary and the 25,000 μg BPA/kg bw/day dose group had a higher incidence than vehicle controls (82% versus 25%, CAFE test). The 2,500 μg BPA/kg bw/day dose group had an incidence of 55% ($p = 0.053$). There were no differences for this lesion between continuous-dose BPA groups and the vehicle controls. Ovarian follicular cysts were significantly higher in 0.5 μg EE_2/kg bw/day dose group relative to the vehicle control (100% versus 35%, all tests applied).

Females in the interim sacrifice continuous BPA dose arm showed significant dose trends for depletion of corpora lutea and interstitial cell hypertrophy. Interstitial cell hypertrophy was increased in the 2,500 and 25,000 μg BPA/kg bw/day dose groups relative to vehicle controls (40% and 38%, respectively, versus 17%), and the increase was significant by the RTE test in the former BPA dose level. All tests applied found significantly higher depletion of corpora lutea and interstitial cell hypertrophy (both 100% versus 17%) in interim 0.5 μg EE_2/kg bw/day females relative to vehicle controls.

Ovarian atrophy was also significantly higher in the interim (100%

Table 4
Males, endpoints affected by any dose of BPA or EE_2 as indicated by statistical tests.

Endpoint	BPA, stop-dose (μg/kg bw/day)					BPA, continuous-dose (μg/kg bw/day)					EE_2 (μg/kg bw/day)	
	2.5	25	250	2,500	25,000	2.5	25	250	2,500	25,000	0.05	0.5
Liver weight[a]	–	–	–	–	–	↓ 0.033	–	–	–	–	–	–
Hemoglobin concentration	–	–	–	–	–	–	–	–	–	↑ 0.042	↑ 0.023	–
% Eosinophils	–	–	–	–	–	–	–	↓ 0.024	–	–	–	–
Total protein	–	↓ 0.015	–	–	–	–	–	–	–	–	–	–
Total bile acids	–	↓ 0.011	–	–	–	–	–	–	–	–	–	–
Insulin	–	–	–	–	–	–	–	–	–	–	↓ 0.047	–
Triglycerides	–	–	–	–	–	–	–	–	–	–	–	↑ 0.032
Epididymis, interim sacrifice												
Exfoliated germ cells	0.034#	–	–	–	–	–	–	–	–	0.047	–	–
Lymphocyte infiltration	–	–	–	–	–	–	–	–	–	0.024	–	#
Epididymis, terminal sacrifice												
Lymphocyte infiltration	–	–	–	–	–	–	–	–	–	–	–	#
Polyarteritis	–	–	–	–	–	–	–	–	#	–	–	–
Testis, terminal sacrifice												
Polyarteritis	–	–	–	0.038	–	–	–	–	–	–	–	–
Dorsal/lateral prostate, interim sacrifice												
Lymphocyte infiltration	–	–	–	–	–	#	–	–	–	–	–	–
Inflammation, suppurative	–	–	–	–	–	#	–	#	#	#	–	–
Dorsal/lateral prostate, terminal sacrifice												
Suppurative inflammation	–	–	–	–	–	0.027	–	–	–	–	–	–
Coagulating gland, terminal sacrifice												
Atrophy	–	#	–	–	–	–	–	–	–	–	–	–
Pituitary, terminal sacrifice												
Hyperplasia, pars distalis	–	–	–	–	0.020	–	#	–	–	0.022	–	0.027
Pars distalis, cyst	–	–	0.047	–	–	–	–	–	–	–	–	–
Mammary Gland, terminal sacrifice												
Dilatation, alveolus	–	–	–	–	–	0.028	–	–	–	–	–	–
Thyroid, interim sacrifice												
Hyperplasia, C-cell	–	–	–	–	–	–	–	–	0.003	–	0.009	–
Hyperplasia, follicular cell	–	–	–	–	–	–	#	–	–	–	–	–
Parathyroid, terminal sacrifice												
Hyperplasia	–	–	–	–	–	–	0.004	–	#	–	–	–
Adrenal gland, interim sacrifice												
Vacuolization, cytoplasmic	–	–	–	–	–	#	–	–	–	–	–	–
Adrenal gland, terminal sacrifice												
Medulla, hyperplasia	–	–	–	0.030	–	–	–	–	–	–	–	–
Cortex, hypertrophy	–	–	–	–	–	–	–	–	–	–	0.005	–
Kidney, terminal sacrifice												
Hyperplasia, transitional epithelium	–	–	–	–	–	–	0.006	–	–	–	–	–
Cyst, renal tubule	–	–	–	–	–	–	–	0.024	0.033	–	0.002	–
Mineralization	–	–	–	–	–	–	–	#	–	–	–	–
Nephropathy	–	–	–	–	–	–	–	–	#	–	–	–
Liver, interim sacrifice												
Hepatodiaphragmatic nodule	–	–	–	–	–	–	–	–	0.038	–	–	–
Infiltration, mononuclear cells	–	–	–	–	–	#	–	0.029	0.023	#	–	–
Fatty change	–	–	–	–	–	–	#	–	–	–	–	#
Liver, terminal sacrifice												
Vacuolization, cytoplasmic	–	–	–	–	–	–	–	–	–	–	–	0.037
Angiectasis	–	#	–	–	–	0.016	–	–	–	–	–	–
Spleen, interim sacrifice												
Pigmentation	–	–	0.012	–	–	–	–	–	–	–	–	–
Hematopoietic cell proliferation	–	–	–	–	–	–	–	–	–	#	–	–
Spleen, terminal sacrifice												
Hyperplasia, lymphoid	–	–	0.039	#	–	–	–	–	–	–	–	–
Heart, interim sacrifice												
Cardiomyopathy	#	#	–	–	–	–	–	–	–	–	–	–
Heart, terminal sacrifice												
Metaplasia, osseus	–	–	–	–	–	–	–	–	–	–	–	#
Pancreas, terminal sacrifice												
Pigmentation	0.018	–	–	–	–	–	–	–	–	–	–	–
Polyarteritis	–	–	–	0.035	#	–	–	–	–	–	–	–
Bone marrow, interim sacrifice												
Hyperplasia, myeloid cells	–	#	–	–	–	–	–	–	–	–	–	–
Bone marrow, terminal sacrifice												
Hypocellularity	–	–	0.046	–	0.035	–	–	–	–	–	–	–

↑, increased *versus* controls; ↓, decreased *versus* controls; –, no change *versus* controls.
p-Values are *versus* controls by primary statistical test. For histopathology endpoints, #, $p < 0.05$ *versus* controls by secondary, but not primary, statistical test.
[a] Liver weight adjusted for body weight results summarized.

Table 5
Incidence of neoplastic lesions in the female mammary gland.

Lesion	BPA, stop-dose (μg/kg bw/day)					BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)		
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Adenoma or Adenocarcinoma														
Interim	0/20 (0%)	0/22 (0%)	0/20 (0%)	0/22 (0%)	0/20 (0%)	0/22 (0%)	0/23 (0%)	1/22 (4%)	1/22 (4%)	0/24 (0%)	0/20 (0%)	0/24 (0%)	2/26 (8%)	0/26 (0%)
Terminal	4/50 (8%)	12/50* (24%)	5/48 (10%)	9/49 (18%)	9/50 (18%)	6/46 (13%)	6/50 (12%)	7/48 (15%)	8/46 (17%)	6/49 (12%)	10/50 (20%)	4/46 (9%)	2/26 (8%)	10/26*** (38%)
Fibroadenoma														
Interim	4/20 (20%)	1/22 (4%)	1/20 (5%)	1/22 (4%)	1/20 (5%)	2/22 (9%)	2/23 (9%)	3/22 (13%)	3/22 (13%)	1/24 (4%)	2/20 (10%)	6/24 (25%)	2/26 (8%)	4/26 (15%)
Terminal	43/50 (86%)	45/50 (90%)	37/48 (77%)	42/49 (86%)	36/50 (72%)	34/46 (74%)	41/50 (82%)	40/48 (83%)	33/46 (72%)	39/49 (80%)	35/50 (70%)	38/46 (83%)	18/26 (69%)	14/26 (54%)

Number of animals affected/number of animals examined (%). *, $p < 0.05$; ***, $p < 0.001$ by Poly-3 test.

versus 44%, all tests applied) and terminal (100% versus 94%, JT/SW and RTE tests) 0.5 μg EE_2/kg bw/day dose groups relative to vehicle controls.

3.5.4. Vagina, nonneoplastic lesions

In the stop-dose arm, there were no statistically significant BPA effects on vaginal histopathology in the interim or terminal sacrifice females (Table 9). In the continuous-dose arm, there were significant increases in the incidence of epithelial hyperplasia in both the interim and terminal sacrifice BPA-treated animals. 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 (33% versus 13%, JT/SW and RTE tests), with the incidence in the 2,500 μg BPA/kg bw/day continuous-dose group also elevated (30%) without statistical significance. 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, with a similar response 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 significant increase in epithelial hyperplasia in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (77% versus 13%). There was also a significant increase in epithelial mucification in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control (69% versus 44%, JT/SW and RTE tests).

3.5.5. Female and male pituitary, neoplastic and nonneoplastic lesions

Adenomas or carcinomas of the pars distalis were observed at low incidence in interim females, and higher incidences, primarily of adenomas, were observed in terminal animals (Supplemental Table 8). There were no statistically significant effects in BPA continuous- or stop-dose interim or terminal sacrifice females. In the terminal EE_2 females, the incidence of combined adenomas and carcinomas of the pars distalis was significantly increased in the 0.5 μg/kg bw/day dose group (77% versus 44%).

There were no statistically significant differences in the incidence of nonneoplastic lesions in the pituitaries of continuous BPA dose arm interim or terminal sacrifice females (Table 10). In the interim stop-dose BPA arm, there was an increase in angiectasis in the 2.5 μg BPA/kg bw/day stop-dose group (9% versus 0%, RTE test). Angiectasis was also significantly increased in the 0.5 μg EE_2/kg bw/day dose group relative to vehicle control in the interim (23% versus 4%, JT/SW and RTE tests) and terminal (65% versus 20%, all statistical tests) sacrifices.

The incidence of hyperplasia in the pars distalis was increased in the terminal stop-dose 2.5 and 25 μg BPA/kg bw/day female groups (64% and 71%, respectively, versus 51%, RTE test). A significant increase in pars distalis hyperplasia was also observed in interim 0.5 μg EE_2/kg bw/day dose group relative to the vehicle control (96% versus 78%, JT/SW and RTE tests).

There were no significant treatment effects in interim sacrifice males in the stop- or continuous-dose BPA arms or in the EE_2 treatment groups (Table 10). In the terminal sacrifice, the incidence of pars distalis hyperplasia was significantly increased in stop-dose 25,000 μg BPA/kg bw/day males relative to vehicle controls (44% versus 26%, Poly-3 test). This lesion was also significantly increased relative to vehicle controls in terminal sacrifice males treated continuously with 25 μg BPA/kg bw/day (40% versus 23%, RTE test), 25,000 μg BPA/kg bw/day (42% versus 23%, all tests applied), and 0.5 μg EE_2/kg bw/day (50% versus 23%, all tests applied).

Table 6
Incidence and mean severity of nonneoplastic lesions in the female mammary gland.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Atypical focus														
Interim								^						
	1/20[a]	0/22	0/20	0/22	0/20	0/22	0/23	3/22	2/22	2/24	0/20	0/24	0/26	0/26 (0%)
	(5%)[b]	(0%)	(0%)	(0%)	(0%)	(0%)	(0%)	(14%)	(9%)	(8%)	(0%)	(0%)	(0%)	
	1.0/0.1[c]	–	–	–	–	–	–	1.7/0.2	2.0/0.2	1.0/0.1	–	–	–	–
Terminal								^						
	6/50	2/50	6/48	8/49	7/50	5/46	2/50	7/48	1/46	5/49	3/50	3/46	2/26	3/26 (12%)
	(12%)	(4%)	(12%)	(16%)	(14%)	(11%)	(4%)	(15%)	(2%)	(10%)	(6%)	(6%)	(8%)	
	2.0/0.2	2.5/0.1	1.8/0.2	1.8/0.2	2.6/0.4	1.6/0.2	2.0/0.1	1.9/0.3	2.0/0.0	2.0/0.2	1.7/0.1	1.7/0.1	1.5/0.1	1.3/0.2
Dilatation, duct														
Interim	*, #, ^			^		#, ^			^^					***, ###, ^^^
	N			N		N								
	4/20	2/22	1/20	1/22	1/20	1/22	2/23	2/22	7/22	1/24	2/20	2/24	3/26	22/26 (85%)
	(20%)	(9%)	(5%)	(4%)	(5%)	(4%)	(9%)	(9%)	(32%)	(4%)	(10%)	(8%)	(12%)	
	1.8/0.4	1.5/0.1	2.0/0.1	1.0/0.1	2.0/0.1	1.0/0.1	1.5/0.1	4.0/0.4	1.9/0.6	3.0/0.1	2.0/0.2	2.0/0.2	1.7/0.2	2.0/1.7
Terminal		##, ^^	#, ^	#, ^	##, ^^	#			^					###, ^^^
		N	N	N	N	N			N					
	16/50	5/50	9/48	9/49	7/50	11/46	15/50	16/48	7/46	9/49	9/50	14/46	6/26	21/26 (81%)
	(32%)	(10%)	(19%)	(18%)	(14%)	(24%)	(30%)	(33%)	(15%)	(18%)	(18%)	(30%)	(23%)	
	2.6/0.8	2.0/0.2	2.2/0.4	2.3/0.4	2.1/0.3	2.4/0.6	2.3/0.7	2.4/0.8	2.1/0.3	2.3/0.4	2.6/0.5	2.1/0.6	2.5/0.6	2.7/2.2
Hyperplasia, lobular														
Interim			*, ^		*, ^									***, ##, ^^
			N		N									
	15/20	12/22	8/20	12/22	7/20	12/22	10/23	14/22	13/22	15/24	13/20	12/24	13/26	23/26 (88%)
	(75%)	(54%)	(40%)	(54%)	(35%)	(54%)	(44%)	(64%)	(59%)	(62%)	(65%)	(50%)	(50%)	
	1.4/1.1	1.4/0.8	1.6/0.7	1.4/0.8	1.4/0.5	1.7/0.9	1.5/0.7	1.5/1.0	1.5/0.9	1.3/0.8	1.4/0.9	1.5/0.8	1.6/0.8	1.6/1.4
Terminal									^^		^			
									N		N			
	41/50	40/50	39/48	39/49	36/50	38/46	43/50	41/48	30/46	38/49	40/50	37/46	13/26	23/26 (88%)
	(82%)	(80%)	(81%)	(80%)	(72%)	(83%)	(86%)	(85%)	(65%)	(78%)	(80%)	(80%)	(50%)	
	3.2/2.6	3.3/2.6	2.8/2.3	3.1/2.4	3.3/2.4	3.1/2.5	3.3/2.8	3.3/2.8	3.1/2.0	3.2/2.5	3.0/2.4	3.1/2.5	3.0/2.8	2.8/2.5
Dilatation, alveolus[d]														
Terminal					^									***,###, ^^^
					N									
	8/50	4/50	4/48	8/49	3/50	7/46	9/50	14/48	5/46	7/49	7/50	11/46	5/26	22/26 (85%)
	(16%)	(8%)	(8%)	(16%)	(6%)	(15%)	(18%)	(29%)	(11%)	(14%)	(14%)	(24%)	(19%)	
	2.5/0.4	2.0/0.2	2.0/0.2	2.2/0.4	1.7/0.1	1.9/0.3	2.0/0.4	2.1/0.6	2.0/0.2	2.0/0.3	2.1/0.3	2.2/0.5	2.0/0.4	2.2/1.9

[a] Number of animals affected/number of animals examined.
[b] Lesion incidence (%).
[c] Mean severity score based on number of affected animals/mean severity score based on total number of animals examined. Lesion severities were graded as 0, none; 1, minimal; 2, mild; 3, moderate; 4, marked. Asterisks indicate statistical significance by the primary statistical test applied, CAFE for interim sacrifice animals and Poly-3 for terminal sacrifice animals (*, $p < 0.05$; ***, $p < 0.001$). # and ^ indicate statistical significance by the secondary statistical tests JT/SW and RTE, respectively. # or ^, $p < 0.05$; ## or ^^, $p < 0.01$; ### or ^^^, $p < 0.001$. Significance markers in the vehicle control ("0") columns indicate trends. "N" indicates decreased response relative to appropriate vehicle control group or negative trend.
[d] There were no diagnoses of "dilatation, alveolus" in interim sacrifice females.

3.5.6. Female and male heart, nonneoplastic lesions

There were no statistically significant increases in nonneoplastic lesions in the heart of interim stop-dose BPA females (Table 11). In terminal sacrifice, there were significant increases in cardiomyopathy in 2.5, 250, 2,500, and 25,000 μg/kg bw/day stop-dose BPA groups relative to the vehicle control (incidences of 74%, 74%, 70%, and 76%, respectively, versus 64%; JT/SW and/or RTE tests). The diagnostic criteria outlined in NTP's nonneoplastic lesion atlas (https://ntp.niehs.nih.gov/nnl/) were used for cardiomyopathy: "a temporally progressive lesion characterized at the early stage by focal to multifocal necrosis of individual or small numbers of cardiomyocytes and myofiber vacuolation. These changes are often accompanied by a mixed inflammatory cell infiltrate (i.e., mononuclear and polymorphonuclear cells) that progresses to mixed mononuclear inflammatory cells only (i.e., lymphocytes and macrophages)". There were no statistical increases in cardiomyopathy in female rats dosed continuously with BPA. In interim sacrifice females dosed with EE_2, all three statistical tests applied indicated 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%). The JT/SW and RTE tests also detected an increase in the terminal sacrifice females (85% versus 70%).

In males, there was a statistically significant increase (RTE test) in cardiomyopathy in the interim 2.5 and 25 μg BPA/kg bw/day stop-dose groups (100% and 95%, respectively, versus 85%; Table 11). There was a statistically significant trend for the incidence of this lesion in the terminal stop-dose BPA groups, but no significant pairwise comparisons were found between any BPA group and the vehicle control. There were no statistically significant differences in cardiomyopathy in the continuous-dose BPA or EE_2 interim or terminal sacrifice dose groups.

3.5.7. Epididymis, nonneoplastic lesions

The sole statistically significant effect in the stop-dose BPA males was an increase in exfoliated germ cells in the 2.5 μg BPA/kg bw/day dose group (15% versus 0%, RTE test; Supplemental Table 9). In the interim continuous-dose BPA males, exfoliated germ cells and lymphocyte infiltration had statistically significant trends and increases in the 25,000 μg BPA/kg bw/day dose group relative to the vehicle control group (27% versus 4% and 23% versus 0%, respectively, all statistical tests). There were no significant BPA treatment effects in the terminal sacrifice males in the continuous BPA dose groups. In the 0.5 μg EE_2/kg

Table 7
Incidence and mean severity of nonneoplastic lesions in the uterus.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Apoptosis, Luminal epithelium														
Interim							**, ##, ^^					*, #, ^^		***, ###,^^^
	2/20 (10%)	3/22 (14%)	2/20 (10%)	2/22 (9%)	1/20 (5%)	6/22 (27%)	2/23 (9%)	1/22 (4%)	4/21 (19%)	5/24 (21%)	5/20 (25%)	9/24 (38%)	6/25 (24%)	18/26 (69%)
	4.0/0.4	3.3/0.5	3.5/0.4	3.0/0.3	4.0/0.2	3.7/1.0	4.0/0.4	4.0/0.2	3.3/0.6	3.6/0.8	3.4/0.9	3.2/1.2	3.3/0.8	3.4/2.4
Hyperplasia, endometrium														
Interim								^		^				
	6/20 (30%)	9/22 (41%)	5/20 (25%)	7/22 (32%)	6/20 (30%)	9/22 (41%)	2/23 (9%)	7/22 (32%)	5/21 (24%)	7/24 (29%)	5/20 (25%)	2/24 (8%)	4/25 (16%)	0/26 (0%)
	2.0/0.6	2.1/0.9	1.8/0.5	1.9/0.6	2.0/0.6	2.2/0.9	2.0/0.2	1.7/0.6	2.2/0.5	1.9/0.5	2.2/0.6	2.0/0.2	2.0/0.3	–
Terminal													^	
	18/49 (37%)	14/49 (29%)	17/48 (35%)	14/49 (29%)	12/49 (24%)	10/46 (22%)	10/50 (20%)	15/48 (31%)	12/45 (27%)	15/49 (31%)	15/48 (31%)	12/46 (26%)	10/26 (38%)	2/26 (8%)
	1.7/0.6	1.4/0.4	1.7/0.6	1.6/0.5	1.8/0.5	2.0/0.4	1.8/0.4	1.8/0.6	2.0/0.5	1.9/0.6	1.3/0.4	2.3/0.6	1.9/0.7	1.5/0.1
Hyperplasia, cystic, endometrium														
Interim						#,^								*,#,^
	2/20 (10%)	4/22 (18%)	2/20 (10%)	2/22 (9%)	1/20 (5%)	7/22 (32%)	5/23 (22%)	1/22 (4%)	4/21 (19%)	3/24 (12%)	7/20 (35%)	4/24 (17%)	6/25 (24%)	14/26 (54%)
	3.0/0.3	2.5/0.5	2.0/0.2	2.0/0.2	4.0/0.2	2.0/0.6	2.4/0.5	1.0/0.1	2.0/0.4	2.0/0.3	2.1/0.8	2.5/0.4	1.5/0.4	2.1/1.2
Terminal	#, ^				#, ^	#		^ N						
	18/49 (37%)	23/49 (47%)	22/48 (46%)	25/49 (51%)	28/49 (57%)	24/46 (52%)	30/50 (60%)	20/48 (42%)	26/45 (58%)	23/49 (47%)	22/48 (46%)	26/46 (56%)	14/26 (54%)	14/26 (54%)
	2.3/0.9	2.1/1.0	2.3/1.0	2.2/1.1	2.5/1.4	2.3/1.2	2.2/1.3	2.2/0.9	2.5/1.4	2.2/1.0	2.4/1.1	2.4/1.4	2.5/1.4	2.5/1.4
Squamous metaplasia														
Interim						##, ^^	*, #, ^							***, ###,^^^
	0/20 (0%)	2/22 (9%)	1/20 (5%)	1/22 (4%)	0/20 (0%)	4/22 (18%)	1/23 (4%)	1/22 (4%)	4/21 (19%)	3/24 (12%)	3/20 (15%)	5/24 (21%)	2/25 (8%)	14/26 (54%)
	–	1.0/0.1	2.0/0.1	1.0/0.1	–	1.8/0.3	2.0/0.1	2.0/0.1	1.3/0.2	1.0/0.1	1.7/0.3	1.0/0.2	2.0/0.2	1.6/0.9
Terminal		^ N												
	5/49 (10%)	1/49 (2%)	2/48 (4%)	2/49 (4%)	4/49 (8%)	3/46 (6%)	2/50 (4%)	4/48 (8%)	4/45 (9%)	1/49 (2%)	4/48 (8%)	6/46 (13%)	2/26 (8%)	4/26 (15%)
	1.4/0.1	1.0/0.0	1.5/0.1	2.0/0.08	1.3/0.1	1.0/0.1	1.5/0.1	1.5/0.1	2.3/0.2	1.0/0.0	1.8/0.2	1.5/0.2	2.0/0.2	1.5/0.2
Dilatation, lumen														
Interim				^										
	1/20 (5%)	0/22 (0%)	1/20 (5%)	4/22 (18%)	1/20 (5%)	0/22 (0%)	0/23 (0%)	1/22 (4%)	2/21 (10%)	2/24 (8%)	1/20 (5%)	2/24 (8%)	1/25 (4%)	0/26 (0%)
	4.0/0.2	–	4.0/0.2	3.8/0.7	3.0/0.2	–	–	3.0/0.1	4.0/0.4	4.0/0.3	4.0/0.2	4.0/0.3	4.0/0.2	–
Terminal	*, #, ^ N						*,#, ^							
	3/49 (6%)	6/49 (12%)	2/48 (4%)	4/49 (8%)	2/49 (4%)	0/46 (0%)	2/50 (4%)	2/48 (4%)	3/45 (7%)	4/49 (8%)	5/48 (10%)	6/46 (13%)	2/26 (8%)	3/26 (12%)
	3.7/0.2	4.0/0.5	4.0/0.2	4.0/0.3	3.5/0.1	–	4.0/0.2	4.0/0.2	4.0/0.3	3.8/0.3	4.0/0.4	3.8/0.5	4.0/0.3	4.0/0.5

See legend to Table 6.

Table 8
Incidence and mean severity of nonneoplastic lesions in the ovary.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Atrophy														
Interim														***,###,^^^
	10/20	9/22	11/20	6/22	12/20	15/22	10/23	7/22	9/22	14/24	11/20	11/24	9/25	26/26 (100%)
	(50%)	(41%)	(55%)	(27%)	(60%)	(68%)	(44%)	(32%)	(41%)	(58%)	(55%)	(46%)	(36%)	
	2.2/1.1	2.8/1.1	1.6/0.9	2.5/0.7	2.1/1.3	2.5/1.7	2.9/1.3	3.1/1.0	3.6/1.5	2.1/1.3	3.3/1.8	3.6/1.7	3.3/1.2	4.0/4.0
Terminal														###, ^^^
	47/49	48/49	46/47	50/50	49/50	44/46	47/50	45/48	44/46	46/49	45/50	46/46	25/26	26/26 (100%)
	(96%)	(98%)	(98%)	(100%)	(98%)	(96%)	(94%)	(94%)	(96%)	(94%)	(90.0%)	(100%)	(96%)	
	2.6/2.5	2.4/2.3	2.5/2.4	2.5/2.5	2.7/2.6	2.7/2.6	2.7/2.5	2.5/2.4	2.6/2.5	2.6/2.4	2.7/2.6	2.7/2.7	2.8/2.7	3.9/3.9
Cyst, Follicle														
Interim	***					***								***
	5/20	6/22	4/20	7/22	11/20	18/22	8/23	4/22	10/22	5/24	10/20	11/24	9/25	26/26 (100%)
	(25%)	(27%)	(20%)	(32%)	(55%)	(82%)	(35%)	(18%)	(46%)	(21%)	(50%)	(46%)	(36%)	
	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Terminal	4/49	4/49	2/47	1/50	2/50	4/46	3/50	3/48	2/46	7/49	6/50	4/46	0/26	3/26 (12%)
	(8%)	(8%)	(4%)	(2%)	(4%)	(9%)	(6%)	(6%)	(4%)	(14%)	(12%)	(9%)	(0%)	
	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Depletion, Corpus Luteum														
Interim							*							***
	2/20	4/22	2/20	2/22	3/20	6/22	4/23	4/22	7/22	4/24	8/20	9/24	6/25	26/26 (100%)
	(10%)	(18%)	(10%)	(9%)	(15%)	(27%)	(17%)	(18%)	(32%)	(17%)	(40%)	(38%)	(24%)	
	–	–	–	–	–	–	–	–	–	–	–	–	–	–
Hypertrophy, Interstitial Cell														
Interim							*, #, ^				^			***, ###,^^^
	4/20	3/22	1/20	2/22	3/20	5/22	4/23	4/22	6/22	3/24	8/20	9/24	5/25	26/26 (100%)
	(20%)	(14%)	(5%)	(9%)	(15%)	(23%)	(17%)	(18%)	(27%)	(12%)	(40%)	(38%)	(20.0%)	
	2.8/0.6	2.3/0.3	2.0/0.1	2.0/0.2	2.0/0.3	2.2/0.5	2.0/0.4	2.3/0.4	2.7/0.7	3.3/0.4	2.3/0.9	2.0/0.8	2.2/0.4	2.5/2.5

See legend to Table 6.

Table 9
Incidence and mean severity of nonneoplastic lesions in the vagina.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Hyperplasia, epithelium														
Interim							**, ##, ^^					#, ^		***,###,^^^
	2/20	4/22	2/20	1/22	2/20	6/22	3/23	2/22	2/21	4/24	6/20	8/24	7/25	20/26 (77%)
	(10%)	(18%)	(10%)	(4%)	(10%)	(27%)	(13%)	(9%)	(10%)	(17%)	(30%)	(33%)	(28%)	
	3.0/0.3	3.0/0.6	3.0/0.3	4.0/0.2	2.5/0.3	2.8/0.8	2.7/0.4	2.5/0.2	2.5/0.2	3.0/0.5	2.8/0.9	2.9/1.0	2.4/0.7	2.6/2.0
Terminal							**, ##, ^^		*, ##, ^	#	*, #, ^	*, #, ^		
	6/49	10/50	3/47	7/49	7/49	7/46	4/49	5/48	12/45	10/49	11/50	12/46	5/26	2/26 (8%)
	(12%)	(20%)	(6%)	(14%)	(14%)	(15%)	(8%)	(10%)	(27%)	(20%)	(22%)	(26%)	(19%)	
	2.7/0.3	2.6/0.5	3.0/0.2	2.9/0.4	3.0/0.4	3.3/0.5	3.0/0.2	2.8/0.3	2.8/0.7	2.3/0.5	3.0/0.7	2.7/0.7	3.2/0.6	2.5/0.2
Mucification, epithelium														
Interim														#, ^
	8/20	11/22	9/20	11/22	8/20	10/22	10/23	12/22	7/21	9/24	7/20	8/24	15/25	18/26 (69%)
	(40%)	(50%)	(45%)	(50%)	(40%)	(45%)	(44%)	(54%)	(33%)	(38%)	(35%)	(33%)	(60%)	
	3.0/1.2	3.1/1.6	2.9/1.3	2.7/1.4	3.4/1.4	3.3/1.5	2.7/1.2	2.5/1.4	3.6/1.2	3.2/1.2	3.7/1.3	2.8/0.9	3.3/2.0	3.1/2.1
Terminal									*N	*N	**, ^ N			
	40/49	46/50	37/47	44/49	39/49	34/46	46/49	37/48	34/45	39/49	34/50	40/46	21/26	23/26 (88%)
	(82%)	(92%)	(79%)	(90%)	(80%)	(74%)	(94%)	(77%)	(76%)	(80%)	(68%)	(87%)	(81%)	
	3.4/2.7	3.3/3.0	3.3/2.6	3.1/2.7	3.4/2.7	3.3/2.4	3.2/3.0	3.4/2.6	3.3/2.5	3.4/2.7	3.3/2.2	3.2/2.8	3.4/2.7	3.1/2.8

See legend to Table 6.

bw/day dose group, lymphocyte infiltration was increased relative to the vehicle control group in the interim (12% versus 0%, JT/SW and RTE tests) and terminal (38% versus 20%, RTE test).

3.5.8. Dorsal/lateral and ventral prostate, nonneoplastic lesions

In the dorsal/lateral prostate, there were no significant increases in observed lesions in any stop-dose BPA group relative to the vehicle control group (Supplemental Table 10). In interim continuous-dose BPA males, there were statistically significant increases relative to vehicle controls of lymphocyte infiltration in the 2.5 μg BPA/kg bw/day dose group (46% versus 18%, RTE test). 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 continuous-dose males. The incidence of suppurative inflammation was increased (Poly-3 test) in the 2.5 μg BPA/kg bw/day dose group in

Table 10
Incidence and mean severity of nonneoplastic lesions in the female and male pituitary glands.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Females														
Angiectasis														
Interim		^												#, ^
	0/20	2/22	1/20	0/22	0/20	0/22	*1/23*	*0/22*	*1/22*	*0/24*	*0/20*	*0/24*	*2/25*	*6/26 (23%)*
	(0%)	(9%)	(5%)	(0%)	(0%)	(0%)	*(4%)*	*(0%)*	*(5%)*	*(0%)*	*(0%)*	*(0%)*	*(8%)*	
	-/0	2.5/0.2	2.0/0.1	-/0	-/0	-/0	2.0/0.1	-/0	2.0/0.1	-/0	-/0	-/0	3.0/0.2	2.5/0.6
Terminal														***,###,^^^
	12/49	11/50	8/48	12/50	14/50	11/46	10/50	8/48	4/46	9/49	9/49	9/46	5/26	17/26 (65%)
	(24%)	(22%)	(17%)	(24%)	(28%)	(24%)	(20%)	(17%)	(9%)	(18%)	(18%)	(20%)	(19%)	
	4.0/1.0	3.9/0.9	3.5/0.6	3.4/0.8	3.7/1.0	3.2/0.8	3.7/0.7	4.0/0.7	4.0/0.4	3.8/0.7	3.3/0.6	3.7/0.7	4.0/0.8	4.0/2.6
Hyperplasia, pars distalis														
Interim														##, ^^
	18/20	16/22	14/20	20/22	16/20	18/22	18/23	17/22	18/22	15/24	16/20	20/24	20/25	25/26 (96%)
	(90%)	(73%)	(70%)	(91%)	(80%)	(82%)	(78%)	(77%)	(82%)	(62%)	(80%)	(83%)	(80%)	
	1.4/1.3	1.5/1.1	1.9/1.4	1.6/1.4	1.8/1.5	1.6/1.3	1.8/1.4	1.9/1.5	1.9/1.5	1.7/1.1	1.7/1.4	2.0/1.6	1.7/1.3	2.4/2.3
Terminal		^	^											##, ^^ N
	25/49	32/50	34/48	26/50	28/50	21/46	27/50	22/48	32/46	26/49	29/49	23/46	16/26	6/26 (23%)
	(51%)	(64%)	(71%)	(52%)	(56%)	(46%)	(54%)	(46%)	(70%)	(53%)	(59%)	(50%)	(62%)	
	2.8/1.4	3.3/2.1	3.1/2.2	3.2/1.7	3.0/1.7	3.2/1.5	3.3/1.8	3.5/1.6	3.4/2.4	3.2/1.7	2.9/1.7	3.1/1.5	3.3/2.0	3.3/0.8
Males														
Hyperplasia, pars distalis														
Interim	8/20	9/20	3/20	4/19	4/20	7/22	4/22	6/22	2/20	4/24	2/20	4/22	7/2	2/26 (8%)
	(40%)	(45%)	(15%)	(21%)	(20%)	(32%)	(18%)	(27%)	(10%)	(17%)	(10%)	(18%)	(27%)	
	1.0/0.4	1.4/0.7	2.0/0.3	1.0/0.2	1.0/0.2	1.1/0.4	1.3/0.2	1.0/0.3	1.5/0.2	1.5/0.3	1.0/0.1	1.5/0.3	1.6/0.4	1.5/0.1
Terminal	*					*	**, #, ^		^			,#,^		*, #, ^
	12/46	16/48	18/48	15/49	19/50	19/43	11/48	9/48	19/48	15/50	17/50	19/45	10/26	13/26 (50%)
	(26%)	(33%)	(38%)	(31%)	(38%)	(44%)	(23%)	(19%)	(40%)	(30%)	(34%)	(42%)	(38%)	
	2.8/0.7	2.2/0.7	2.3/0.9	2.4/0.7	2.3/0.9	2.4/1.1	2.1/0.5	2.3/0.7	2.3/0.9	2.2/0.7	2.2/0.7	2.4/1.0	2.2/0.9	2.0/1.0

See legend to Table 6.

terminal sacrifice animals (96% versus 82%). There were no statistically significant differences for nonneoplastic lesions in interim or terminal EE_2 dose groups relative to the vehicle control group.

In the ventral prostate, lymphocyte cellular infiltration and suppurative inflammation were significantly decreased across continuous-dose BPA or EE_2 dose groups relative to the vehicle control group (Supplemental Table 11). The only significant increase relative to control was in epithelial hyperplasia in the terminal continuous-dose 250 μg BPA/kg bw/day dose group (37% versus 20%, RTE test).

4. Discussion

In the voluminous literature related to BPA toxicity, there were previously no 2-year guideline-compliant rodent toxicology studies that included exposure during any early developmental stage. The CLARITY-BPA project was designed to fill that gap and, in addition, attempt to

Table 11
Incidence and mean severity of cardiomyopathy in female and male hearts.

Lesion/Study Arm	BPA, stop-dose (μg/kg bw/day)						BPA, continuous-dose (μg/kg bw/day)						EE_2 (μg/kg bw/day)	
	0	2.5	25	250	2,500	25,000	0	2.5	25	250	2,500	25,000	0.05	0.5
Females														
Interim														*, ##, ^^
	6/20	8/22	7/20	7/22	6/20	7/22	7/23	10/22	9/22	8/24	9/20	7/24	8/26	17/26
	(30%)	(36%)	(35%)	(32%)	(30%)	(32%)	(30%)	(46%)	(41%)	(33%)	(45%)	(29%)	(30.8%)	(65%)
	1.0/0.3	1.0/0.4	1.1/0.4	1.3/0.4	1.0/0.3	1.3/0.4	1.1/0.4	1.2/0.6	1.2/0.5	1.0/0.3	1.1/0.5	1.1/0.3	1.0/0.3	1.2/0.8
Terminal	##, ^^	^		#, ^	#, ^	##, ^^			^ N					#, ^
	32/50	37/50	38/48	37/50	35/50	35/46	35/50	30/48	24/46	35/49	33/50	33/46	19/26	22/26
	(64%)	(74%)	(79%)	(74%)	(70%)	(76%)	(70%)	(62%)	(52%)	(71%)	(66%)	(72%)	(73%)	(85%)
	1.3/0.8	1.5/0.9	1.3/1.0	1.5/1.1	1.7/1.2	1.7/1.3	1.3/0.9	1.6/1.0	1.3/0.7	1.3/0.9	1.4/0.9	1.4/1.0	1.3/0.9	1.5/1.3
Males														
Interim		^	^^											
	17/20	20/20	19/20	13/19	18/20	19/22	16/22	16/22	16/20	18/24	15/20	20/22	24/26	23/26
	(85%)	(100%)	(95%)	(68%)	(90%)	(86%)	(73%)	(73%)	(80%)	(75%)	(75%)	(91%)	(92%)	(89%)
	1.4/1.2	1.7/1.7	2.0/1.9	1.2/0.8	1.3/1.2	1.6/1.4	1.4/1.0	1.4/1.0	1.3/1.1	1.6/1.2	1.1/0.8	1.4/1.2	1.5/1.3	1.6/1.4
Terminal	*													
	45/50	44/48	45/48	48/50	47/50	41/46	44/50	44/48	43/48	45/50	44/50	41/46	24/26	24/26
	(90%)	(92%)	(94%)	(96%)	(94%)	(89%)	(88%)	(92%)	(90%)	(90%)	(88%)	(89%)	(92%)	(92%)
	2.3/2.1	2.1/2.0	2.4/2.2	2.0/1.9	2.4/2.3	2.3/2.1	2.0/1.8	2.0/1.9	2.1/1.9	2.0/1.8	1.9/1.7	2.0/1.8	1.8/1.7	2.4/2.2

See legend to Table 6.

move toward resolving the controversy that has existed between some researchers and most worldwide regulatory agencies, including the FDA, regarding the safety of BPA in the general population at current exposure levels. To address the latter issue, a group of university-based scientists selected by the NIEHS participated in discussions on the study design and utilized siblings of the core study animals to evaluate various endpoints not usually included in guideline-compliant studies (Heindel et al., 2015; Schug et al., 2013). The core study report and all core and grantees studies data are publicly available (NTP, 2018a, 2018b, 2018c). In addition, some of the grantee studies' findings have been published (Arambula et al., 2016; 2017; 2018; Bansal and Zoeller, 2019; Cheong et al., 2018; Dere et al., 2018; Gear et al., 2017; Johnson et al., 2016; Li et al., 2018a, b; Patel et al., 2017; Prins et al., 2018a; Rebuli et al., 2015; Witchey et al., 2019). The core study report focused solely on the data generated in that study and its interpretation and did not discuss the results in terms of related literature, including directly relevant results reported in publications by CLARITY-BPA grantees. Additional data collected outside the core study on internal dosimetry after administration of d6-BPA and microscopic evaluation of reproductive tissues at 90 days in females treated continuously with 250,000 μg BPA/kg bw/day are also presented here.

Given the broad scope of potential toxicities that have been reported for BPA and the wide dose range over which they have been reported, the current study utilized a 10,000-fold BPA dose range, with the spacing between consecutive doses equivalent to the usual maximum dose range covered in chronic toxicity/carcinogenicity studies. Also, in contrast to the typical chronic toxicity study design, a stop-dose arm was included in which dosing of the animals was terminated at weaning and the animals continued on study untreated until termination at one or two years. This stop-dose exposure does not reflect the continuous lifetime exposure situation in humans, but was included due to the interest in potential delayed responses to developmental BPA exposures and the fact that many reported studies of BPA use dosing restricted to developmental time periods. The wide dose spacing sacrifices granularity that would be useful in defining the shape of the dose-response curve, if any, in the low dose region; however, it was considered important to cover a BPA dose range from near human exposures to a dose that provided a large margin of safety over current estimated dietary exposure levels. A 90-day study conducted under the same conditions used in the core chronic study resulted in clear adverse effects only at doses ≥100,000 μg BPA/kg bw/day, with most of the effects observed in females at the highest tested dose of 300,000 μg BPA/kg bw/day (Delclos et al., 2014). These results were consistent with what had been reported for detectable BPA estrogenic activity in the uterotrophic assay or other short-term assays for estrogenic activity (Ashby and Tinwell, 1998; Kanno et al., 2003; Kim et al., 2005; Yamasaki et al., 2002). While the 90-day study results would have led to a selection of 250,000 μg BPA/kg bw/day as a high dose in the chronic study, that dose was considered well beyond the range of regulatory concern and was not selected given that it would have had no impact on current safety assessments. Despite this, a limited assessment of the effects of 250,000 μg BPA/kg bw/day was conducted in female littermates of males utilized for the sperm study reported by Dere et al. (2018), since females were found to be more responsive to BPA in our previous 90-day study (Delclos et al., 2014). In that 90-day study, 100,000 μg BPA/kg bw/day had no-to-minimal effects on the female reproductive tract endpoints assessed (0–5% lesion incidence), while over 70% of the females in the 300,000 μg BPA/kg bw/day dose group had cystic ovarian follicles and depleted corpora lutea and endometrial hyperplasia (Delclos et al., 2014). Accordingly, in the present study, these ovarian and uterine lesions were observed in 11% and 22%, respectively, at 250,000 μg BPA/kg bw/day, while the vehicle controls had none (Table 2).

Direct measurements of d6-BPA at C_{max} were made on the low end of the BPA dose scale used in the core study to estimate internal dose without interference from incidental background BPA (Table 1). The mean peak serum levels of unconjugated d6-BPA in the 2.5 μg d6-BPA/kg bw group were 0.24 nM and 0.03 nM on PNDs 4 and 21, respectively. These data indicate that even the lowest BPA dose tested in the study only approached the estimated upper bound human exposure levels at PND 21, a time when the metabolic capacity of the rats had matured (Yang et al., 2015; Doerge et al., 2010a).

Our consistent finding of trace (low ppb) BPA in commercial rodent feed (Camacho et al., 2016; Delclos et al., 2014, and the present study) needs to be considered when planning to test BPA doses lower than those used in the present study to ensure a sufficient margin between the lowest BPA dose to be tested and the background dietary BPA exposure. While there has been concern about potential exposure of test animals to BPA from polycarbonate cages and drinking water (Howdeshell et al., 2003; Le et al., 2008), there has been less consideration given to exposure to BPA from the diet (Camacho et al., 2016; Delclos et al., 2014; Yoshida et al., 2004), for which most concern for potential confounding background exposures has focused on phyto- and myco-estrogens (Brown and Setchell, 2001; Thigpen et al., 2004). While tests of diet extracts for estrogenic activity using estrogen-sensitive cell lines have been reported and utilized to assess the estrogenic content of rodent diets (Soto et al., 1992; Welshons et al., 1990), these methods do not accurately evaluate the estrogen burden seen by the animal due to metabolism considerations and do not have the selectivity of mass spectrometry to detect BPA or other specific estrogenic agents. In addition, the limits of detection for BPA in the complex extract matrix, which are likely to vary across diet lots due to varying content of potential modifying factors, have not been established for these cell-based assays.

The use of gavage as a dosing method has been discussed by some as a potential concern in toxicology studies due to the potential for causing stress to the dosed animals that could impact their response to treatment or confound interpretation of results (Cao et al., 2013; Marty et al., 2018; Vandenberg et al., 2014). The degree and duration of stress induced by gavage relative to other potential animal room stressors is not well-defined in those presentations and appears to vary according to volume and vehicle, as well as the skills of the individuals performing the procedure (Arantes-Rodrigues et al., 2012; Balcombe et al., 2004; Brown et al., 2000; He et al., 2014; Turner et al., 2012). In addition, it is known that bolus dosing by gavage will result in different pharmacokinetic behavior than dietary or drinking water exposure, and this may impact the response (Kapetanovic et al., 2006, 2009; Martin-Jimenez et al., 2008; Marty et al., 2007). In the case of BPA, gavage dosing in adult rodents has been shown to result in faster time-to-peak concentrations compared to dietary administration, although the area-under-the-curves produced by the two dosing regimens are similar (Sieli et al., 2011). In the present study, gavage dosing was selected as the method of delivery because of the poor lactational transfer of BPA to pups (Doerge et al., 2010b). Efficient dosing of the large number of neonatal rats in the CLARITY-BPA core and ancillary studies with known and consistent concentrations of BPA necessitated gavage administration for oral dosing.

The oral bioavailability of EE_2 in the rat is also known to be low (Dusterberg et al., 1986; Fotherby, 1996; Hirai et al., 1981), and previous studies with dietary administration up to 50 ppb (approximately 5 μg/kg bw/day) resulted in undetectable serum levels in adult rats (NTP, 2010a, 2010b; Twaddle et al., 2003). We had previously reported the peak serum levels of EE_2 in rats at various ages using the same animal model and dosing regimen used in the current study (Churchwell et al., 2014). As with BPA, exposure to EE_2 was highest in neonates and decreased with age (for 0.5 μg EE_2/kg bw/day: 500 pM at PND 4, 10 pM at PND 21, and < 5 pM (LOD) at PND 80). Outside of studies conducted at NCTR (Delclos et al., 2014; Ferguson et al., 2011, 2012, 2014; He et al., 2012, and the present study), we are unaware of any rodent studies of EE_2 that have used a dosing regimen that includes direct oral gavage of neonates. In any case, gavage administration of BPA with corn or sesame oil vehicles has been used in studies in which

the authors reported effects at the lowest doses tested that in some cases were not seen at higher doses (e.g., Betancourt et al., 2010; Christiansen et al., 2014; Hass et al., 2016; Jenkins et al., 2009; Mandrup et al., 2016; Moral et al., 2008; Wang et al., 2014; Wei et al., 2011).

With respect to the question of potential gavage- or handling-induced stress and its impact on the study, Gear et al. (2017), reporting on a CLARITY-BPA study that utilized siblings of the core study animals, indicated statistically significant higher body weights in male stop-dose controls versus continuous-dose controls at PND 90 and 6 months, with mean weights 9.6% and 9.1%, respectively, higher in the stop-dose groups. Our reanalysis of the data from this study (NTP, 2018a) resulted in somewhat different values, with mean weights at PND 90 and 6 months that were 5.6% and 10.3%, respectively, higher in the stop-dose groups and with only the weights at 6 months significantly different from continuous-dose arm vehicle controls (*t*-test, $p = 0.03$). Gear et al. (2017) indicated that a significant body weight difference between the study arms was not observed in the females. The authors suggested that this observation was "consistent with previous studies showing that prolonged postnatal stress in males decreases weight gain over time, and that female SD rats are resistant to these effects of stress". However, the supporting references cited by Gear et al. (2017) did not use SD rats, used restraint only during pregnancy or in adulthood, used stressing methods (holding for 75 min per day in a plastic restraint device or electric shock pad) that were very different from the restraint used in the present study (manual holding for < 1 min per gavage dose), and did not always show body weight differences consistent with those reported by Gear et al. (2017). In our previous 90-day study, there were no significant body weight differences for either sex between unhandled naïve controls and the vehicle controls that were dosed daily by gavage, while naïve control females had a 7% higher heart weight adjusted for body weight ($p = 0.047$; Delclos et al., 2014). In the core study, we did not collect data other than body weights at PND 90 and 6 months, but we did collect data on several potentially stress-related endpoints (Everds et al., 2013) (Supplemental Tables 12 and 13). There were no significant differences between the dosing arms in these endpoints at any age tested.

Survival was not affected by any BPA dose and there were minimal effects on body weights. 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. Literature reports of BPA effects on body weights are mixed and it is difficult to discern any clear pattern of effects given the differences in dosing windows, doses, routes, and species/strains (reviewed in Thayer et al., 2012; Wassenaar et al., 2017). Several studies have utilized exposure to BPA during development (gestation, lactation, or a combination) and observed body weights later in life after dosing had stopped. However, Rubin et al. (2017) in a study in CD-1 mice with BPA administered by implanted osmotic pumps, reported that the addition of peripubertal exposure enhanced changes in body weight and composition in the lowest dose groups tested (2.5 and 25 μg BPA/kg bw/day) that started at relatively young ages (less than 15 weeks). In a well-powered study in which Wistar rat dams were dosed from GD 7 through PND 22, Hass et al. (2016) reported an increased body weight in females only in the lowest dose group (25 μg BPA/kg bw/day) that was evident after 9 months, but the difference was less than 10%.

There were few statistically significant effects of BPA treatment, in either the continuous- or stop-dose arms, on clinical pathology endpoints or organ weights in either sex, 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%, were not associated with organ lesions and, in the case of organ weights, were not significant when adjusted for body weight.

Based on the wide range of organ systems reported in the literature to be affected by BPA and the fact that one purpose of the CLARITY-BPA program was to compare the results from endpoints typically assessed in guideline-compliant studies to those assessed in investigational studies, a broad range of tissues and endpoints was evaluated. The issue of the impact of multiplicity of endpoints on the statistical analysis of both clinical trials and preclinical toxicology studies has long been a topic of discussion (Haseman, 1984; Kissling et al., 2015; FDA, 2017). The statistical analyses used in the core study were pre-specified in the study protocol and generally followed the procedures used in NTP studies (NTP, 2018d). While statistical analyses for neoplastic and nonneoplastic lesions were conducted based on incidence, non-standard statistical analyses that utilized severity scores assigned by the study pathologist were also utilized for nonneoplastic lesions. The Society for Toxicological Pathologists generally recommends against the use of statistical analyses that utilize severity scores due to the nonlinear scale, semi-quantitative nature, and potential dependence on pathologist (Schafer et al., 2018), although the use of the PWG review in the case of this study somewhat mitigates the last concern. In addition, the tests that incorporate incidence and severity scores do not utilize the survival correction applied in the Poly-3 test used for the two-year animals. Despite these concerns, we report the results of the statistical tests that incorporated severity scores as secondary tests to minimize false negatives.

In no case were corrections made for the multiple histopathology endpoints analyzed. For carcinogenicity studies, both NTP and the FDA's Center for Drug Evaluation and Research (CDER) have proposed and utilized modified decision criteria, that is, applying a more stringent p-value cut-off, in evaluating neoplastic lesions to reduce false positives (Haseman, 1983; Haseman, 1984, 1990; Lin and Rahman, 1998; Rahman and Lin, 2008; FDA, 2001). The modified decision criteria values differ for rare neoplasms, defined as neoplasms with a background incidence of < 1% ($p < 0.05$ for NTP, $p < 0.025$ for CDER), and common tumors ($p < 0.01$ for NTP, $p < 0.005$ for CDER). In the core study, all neoplastic and nonneoplastic lesions diagnosed and analyzed were common in this strain of rat, and no lesion in BPA-dosed groups would have met the more stringent decision criteria. In addition, the distribution of statistically significant effects, as defined at the $p < 0.05$ level, in pairwise comparisons to the vehicle control in a direction consistent with toxicity was nearly even across dose groups (Supplemental Table 14) and did not show a consistent pattern within study arms (Tables 3 and 4). In addition, there were similar numbers and a similar distribution across BPA dose groups, for statistically significant effects consistent with a beneficial or protective effect of BPA in comparison to the vehicle control (Supplemental Table 15**)**. In any case, the evaluation of histopathology results in a toxicology study for a safety assessment is not based on an indication of statistical significance alone, but is rather based on a consideration of biological significance and plausibility (EFSA, 2011; Haseman, 1984, 1990; Kissling et al., 2015). As stated by Kissling et al. (2015) regarding carcinogenicity evaluations, "its response(s) must not only be statistically significant, but coherent, and part of a pattern of responses." Just as a statistically significant result may not be biologically relevant, a biologically significant result may not be statistically significant. In the core study, none of the histopathology results were interpreted as clearly BPA treatment-related by the study pathologist, pathology review group, or the investigators involved in study conduct and reporting. The selection of endpoints presented and discussed was based solely on the low stringency statistical tests applied.

One set of results from the current study that merits more detailed consideration is the group of neoplastic and nonneoplastic lesions observed in the female mammary gland. Several reports in the literature identify the female mammary gland as a potential target organ of BPA (reviewed in Mandrup et al., 2016; Perrot-Applanat et al., 2018; Seachrist et al., 2016; Soto et al., 2013). Given the various exposures (implanted osmotic minipump or gavage) and timings of exposure (*in utero* only, *in utero* plus lactation, lactation only, or adults only) to BPA that would lead to substantially different internal dose exposures,

straight comparisons of administered doses among those studies or between any of those studies and the present study are not appropriate. Some of the studies reported effects at lower or intermediate BPA doses that were not seen at higher doses, although these studies would not have met suggested criteria for establishing a credible non-monotonic dose response (Beausoleil et al., 2016; Lagarde et al., 2015; Varret et al., 2018) based on the number of doses tested. For example, Mandrup et al. (2016) reported an increase of intraductal hyperplasia only at 250 μg BPA/kg bw/day at a later age (PND 400, but not PND 100), but not at 25, 5,000, or 25,000 μg BPA/kg bw/day after dosing of the dam during pregnancy and lactation by gavage. Furthermore, the increase was demonstrated by a post-hoc pooling of severity classes (compared > 30% area affected to < 30% area affected), rather than a standard pre-specified statistical analysis. Acevedo et al. (2013) reported pre-neoplastic lesions in females treated gestationally or gestationally and lactationally with doses from 0.25 to 250 μg BPA/kg bw/day administered from an implanted osmotic pump, although there were not statistically significant effects. Jenkins et al. (2011) reported a non-monotonic effect on mammary carcinogenesis using an adult-only drinking water exposure of MMTS-erbB2/neu transgenic mice, which have an increased susceptibility to mammary neoplasms. Tumor multiplicity, time to tumor, tumor volume, and lung metastases were all reported to be affected in an adverse manner at the lowest doses tested (estimated as approximately 0.5 and 5 μg BPA/kg bw/day), but not at the higher doses (approximately 50 and 500 μg BPA/kg bw/day; Jenkins et al., 2011). Cell proliferation and apoptosis were evaluated, but the responses did not correspond with the tumor data, and no mechanistic basis for the reported dose response was elucidated.

In our previous 90-day study, in which animals were dosed as in the present study, a single mammary adenocarcinoma was observed in a female in the 2.5 μg BPA/kg bw/day dose group and was considered an incidental lesion (Delclos et al., 2014). While relatively rare, spontaneous adenomas/adenocarcinomas have been reported in young Sprague-Dawley rats from various sources, including the NCTR Sprague-Dawley rat (NTP, 2010a; Boudreau et al., 2016; Faustino-Rocha et al., 2017; Kuzutani et al., 2012; Oishi et al., 1995). The background rate of female mammary gland adenoma/adenocarcinoma increases in older Sprague-Dawley rats. In our previous 2-year diet studies with the NCTR Sprague-Dawley rat on the same low phytoestrogen diet used in the present study, the background rate of mammary gland adenocarcinoma in 2-year-old vehicle control rats was 11–16% (NTP, 2008, 2010b). Although these studies were conducted approximately a decade before the present study and differed in design (e.g., used individually housed animals rather than pair-housed animals, dietary administration rather than gavage), they are the most relevant studies for comparison with the current study. In interim sacrifice females in the continuous-dose BPA study arm, single adenoma/adenocarcinomas were observed in the 2.5 (1/22, 4%) and 25 (1/22, 4%) μg BPA/kg bw/day dose groups, while no mammary gland adenomas/adenocarcinomas were observed in any interim stop-dose BPA females. The only other such neoplasms observed in interim sacrifice females were in the 0.05 μg EE_2/kg bw/day group (Table 5). At the terminal sacrifice of females in the continuous-dose BPA study arm, the vehicle control group incidence of adenoma/adenocarcinoma was 12%, with incidences ranging from 9 to 20% in the BPA groups, none of which were statistically significantly different from the vehicle controls.

In the terminal stop-dose study arm, there was a statistically significant increase in the incidence of mammary gland adenoma/adenocarcinoma (24% versus 8%, $p = 0.018$) in the 2.5 μg BPA/kg bw/day dose group. Incidences in the other BPA stop-dose groups varied from 10 to 18% and did not differ statistically from the vehicle control. There were no statistically significant nonneoplastic changes in BPA groups versus vehicle controls in the mammary gland of interim or terminal sacrifice female stop-dose animals.

In contrast to BPA treatments, the reference estrogen EE_2 had a clearly interpretable impact on the female mammary gland. The high dose (0.5 μg EE_2/kg bw/day) induced significant increases in the incidences of adenocarcinoma (38% versus 12% 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 (Table 5). In addition, the significant increases observed in the incidence of combined adenomas and carcinomas in the pituitary pars distalis at 0.5 μg EE_2/kg bw/day may be related to the increased mammary neoplasm incidence (Table 10). 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 (Blankenstein et al., 1984; Sarkar et al., 1982). 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 our previous 90-day study (Delclos et al., 2014). It is also interesting to note the increase in adenocarcinomas in the current study at an administered dose of 0.5 μg EE_2/kg bw/day, while such an increase was not observed in the earlier 2-year diet study of EE_2 conducted in this strain of rat with the high dietary concentration of 50 ppb EE_2, which resulted in an ingested dose of approximately 5 μg EE_2/kg bw/day (NTP, 2010b). As mentioned previously, even at this dietary dose in adults, serum levels of EE_2 were undetectable (NTP, 2010b; Twaddle et al., 2003), which would have led to far lower exposure of neonates through the milk than the direct dosing procedure used in this study (Churchwell et al., 2014). This difference in neonatal exposure to EE_2 likely explains many of the differences between the results observed in the present study and the results of the earlier 2-year dietary study.

Our interpretation of the overall evidence gathered in the study was that there is not a clear biologically plausible relationship between BPA exposure and female mammary gland adenoma/adenocarcinoma in this study, but rather that this was the result of chance fluctuation in incidences of a common rat neoplasm. The elevated incidence occurs in a single BPA dose group of the stop-dose arm and was not observed in the continuous-dose arm. In addition, there were no treatment-related nonneoplastic lesions in the mammary gland of interim or terminal sacrifice stop-dose animals. There is not a clear mechanism whereby mammary adenomas/adenocarcinoma would be induced only at the lowest dose of BPA in stop-dose animals.

Yoshizawa et al. (2009) suggested that a higher incidence of uterine tumors in treated rats at a lower dose of PCB 118 than was observed at the highest dose may have been due to the > 10% lower body weight in the high dose group. Mammary gland fibroadenomas, a neoplasm whose incidence had been shown to be sensitive to body weight changes, was also lower in the high dose group of the PCB study (Yoshizawa et al., 2009). In the present study, the body weights of continuous-dose animals were generally lower than stop-dose animals over the course of the study, while the development of spontaneous mammary gland fibroadenomas was not suppressed in the BPA continuous-dose females nor was the development of mammary gland adenocarcinomas in high dose EE_2 animals. In addition, evidence of a dose response has long been considered as an important component of evaluation of a chronic toxicity study (Haseman, 1984, 1990; FDA, 2001). While mechanisms for low dose responses and non-monotonic dose responses for hormonally active agents have been proposed based on interactions with various receptor types or other targets involved in hormone responses (Acconcia et al., 2015; Nomiri et al., 2019; Shafei et al., 2018; Thayer, 2010; Villar-Pazos et al., 2017), no clear supporting data for these mechanisms in a particular case have been developed. It has been argued that the understanding of mechanism should not be an issue for the use of such data in safety assessments (e.g., Zoeller and Vandenberg, 2015). However, a clear and plausible mechanism would provide confidence in drawing conclusions from the data and other expert groups have pointed out that an ultimate resolution of the debate on these issues will be dependent on an

understanding of mechanisms (Solecki et al., 2017). For BPA, this is particularly the case when extrapolating any effects observed in animals to the levels of exposure in humans, where interactions with the various proposed receptors are minimal and would occur in the presence of higher concentrations of more potent endogenous estrogen receptor ligands (Autrup et al., 2015; Borgert et al., 2013; Pande et al., 2018; Teeguarden et al., 2013).

There are a few endpoints in the core study that can be compared to published data on the same organs in the grantee studies conducted under CLARITY-BPA. Patel et al. (2017) reported on the effects of BPA and EE_2 on the ovary. Statistically significant effects of BPA on ovarian follicle populations were confined to effects at PND 21 and to the 2.5 and 250 μg BPA/kg bw/day dose groups, with decreases in primordial, primary, preantral, and total healthy follicles. There were no significant effects at the other ages examined (PND 1, PND 90, 6 months, and 1 year). The unusual dose response observed and the restricted timing of the effects were not understood. The high dose of EE_2 had more significant effects on ovarian follicle populations at various ages examined, but cystic follicles were not noted (Patel et al., 2017).

The ovary pathology evaluations in the core study did not enumerate follicle populations, but rather followed the recommendations of the Society for Toxicological Pathologists for first tier assessments (Regan et al., 2005). At the interim sacrifice, there were significant increases of cystic follicles, atrophy, depletion of corpora lutea, and interstitial cell hypertrophy in the high EE_2 dose group. The profound effects of the high dose of EE_2 on the ovary were reflected in the almost complete lack of cycling animals at the 16-week evaluation (Supplemental Table 5). Statistically significant effects of BPA in the ovary were limited to an increase of cystic follicles in the 25,000 μg BPA/kg bw/day stop-dose group and an elevation of interstitial cell hypertrophy in the 2,500 μg BPA/kg bw/day continuous dose group. The 25,000 μg BPA/kg bw/day dose effect on cystic follicles in the stop-dose arm appeared to be a strong effect (82% in the BPA group versus 25% in vehicle controls, $p < 0.001$), although not clearly understandable due to the lack of effect in the continuous-dose arm. Similarly, the lack of observation of cystic follicles in any dose group, including EE_2 groups, in the Patel et al. (2017) study cannot be readily explained given the observation of cystic follicles in the previous 90-day study (Delclos et al., 2014) and in the current study's interim sacrifice females.

The primary purpose of the study of Gear et al. (2017), which was discussed earlier regarding reported sex-dependent body weight differences, was the evaluation of cardiotoxicity. Gear et al. (2017) reported evaluation of the hearts of animals at PND 21, PND 90, and 6 months. The authors concluded that BPA-dependent cardiotoxicity occurred in females at PND 21 across multiple dose groups from 2.5 to 25,000 μg BPA/kg bw/day and occurrences of a more severe diffuse degeneration of the myocardium in one male (250 μg BPA/kg bw/day) and two females (one each in the 2.5 and 25 μg BPA/kg bw/day dose groups). The incidence of cardiomyopathy was diagnosed as near 100% in both control and treatment groups in older animals, but several incidences of diffuse degeneration of the myocardium were diagnosed in PND 90 animals of both sexes in multiple BPA and EE_2 dose groups. In comparison to the core study, which only evaluated animals at 1- and 2-years, as well as our previous 90-day study (Delclos et al., 2014), it seems clear that there was a fundamental difference in the histopathological evaluations conducted in Gear et al. (2017) and the NCTR studies. While Gear et al. (2017) found virtually 100% incidence of cardiomyopathy in PND 90 vehicle control animals of both sexes, our previous 90-day evaluation reported 0% incidence in females and 35% in male vehicle controls (Supplemental Table 16 and NTP, 2014). Even at 1-year in the core study, incidences of cardiomyopathy in female vehicle controls were approximately 30% and in males were 85% and 73% in stop-dose and continuous-dose controls, respectively. No incidences of diffuse degeneration were diagnosed in either sex at any dose group in the 90-day study (NTP, 2014) or core study. It is likely that differences in diagnoses of cardiomyopathy resulted from diagnostic criteria used in the NCTR studies that were not used in the Gear et al. (2017) study, that is, a background level of cardiomyopathy (threshold) was set by the NCTR study pathologist that was not diagnosed as a lesion, a common practice in toxicological pathology (Gibson-Corley et al., 2013; Schafer et al., 2018). Both the 90-day study and the core study underwent a PWG review by pathologists not involved in the study (see Materials and Methods). The fluctuations in incidence/severity in cardiomyopathy in the core study were considered within the confines of background variation, rather than treatment-related lesions.

In the males, there were few effects of BPA or EE_2 noted in the current study. The lack of effects of EE_2 on the male mammary gland were likely related to the advanced age of the animals at evaluation (NTP, 2018b; Latendresse et al., 2009). We found no biologically relevant effects of BPA on spermatogenesis or on prostate pathology, consistent with the results reported from the CLARITY-BPA grantee studies related to these endpoints that have been published thus far (Dere et al., 2018; Prins et al., 2018a). On the other hand, Prins et al. (2018a) did report an enhancement of prostatic intraepithelial neoplasia in the lateral prostate and dorsal lateral prostate ductal adenocarcinoma at doses as low as 2.5 μg BPA/kg bw/day in animals that had been implanted with subcutaneous pellets of testosterone and estradiol. Existing literature on the effects of BPA on spermatogenesis is mixed, with some studies reporting impacts and others not (reviewed in Dere et al., 2018; Hass et al., 2016).

While there was an increase in exfoliated germ cells and lymphocyte infiltration in epididymis in the 25,000 μg BPA/kg bw/day continuous-dose group, there were no associated testicular lesions, decreasing the biological plausibility that this effect was related to the BPA exposure. The only potentially BPA treatment-related lesion in males in the current study was a significant increase of hyperplasia in the pars distalis of the pituitary in the 25,000 μg BPA/kg bw/day stop- and continuous-dose groups, as well as the 0.5 μg EE_2/kg bw/day dose group at 2-years.

Evaluations by regulatory authorities of the body of toxicological data available for BPA have, with few exceptions (ANSES, 2013), concluded that the reported effects of BPA at the lower end of the dose range tested have not demonstrated a consistent interpretable pattern with biological plausibility. Our interpretation of the data generated in the present study is consistent with these assessments of the earlier published literature. Others have offered a different interpretation of the results of the core study, based primarily on accepting the validity of effects indicated by the various statistical tests applied and suggesting that the pattern, or lack thereof, is consistent with those of other studies found in the literature (Prins et al., 2018b; Hill et al., 2018). However, while there were statistically significant differences from the vehicle control, particularly with the low stringency tests applied with the histopathology endpoints, these effects did not show a coherent and plausible pattern consistent with BPA-induced lesions (Tables 3 and 4). In contrast, the reference estrogen, EE_2, demonstrated a consistent pattern of adverse effects in females. Based on a weight of evidence approach, we conclude that the core study data do not suggest a plausible hazard of BPA exposure in the lower end of the dose range tested.

Acknowledgments

We are grateful for the extraordinary efforts of the NCTR Animal Care, Diet Preparation, Information Technology, Microbiology, Pathology, Quality Assurance, and Veterinary Services staffs, as well as Ms. Kathy Carroll of the Office of Scientific Coordination in the planning, conduct, and reporting of this study. This study was funded by the National Toxicology Program under an Interagency Agreement between FDA and NIEHS (FDA IAG #224-17-0502 and NIEHS IAG #AES12013). This manuscript was reviewed in accordance with FDA procedures prior to submission. The opinions expressed in this paper do not necessarily

reflect those of the FDA or the NIEHS/NTP.

Transparency document

Transparency document related to this article can be found online at https://doi.org/10.1016/j.fct.2019.110728.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fct.2019.110728.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Acconcia, F., Pallottini, V., Marino, M., 2015. Molecular mechanisms of action of BPA. Dose Response 13 1559325815610582.

Acevedo, N., Davis, B., Schaeberle, C.M., Sonnenschein, C., Soto, A.M., 2013. Perinatally administered bisphenol a as a potential mammary gland carcinogen in rats. Environ. Health Perspect. 121, 1040–1046.

ANSES, 2013. Opinion of the French Agency for Food, Environmental and Occupational Health & Safety on the assessment of the risks associated with bisphenol A for human health, and on toxicological data and data on the use of bisphenols S, F, M, B, AP, AF and BADGE. https://www.anses.fr/en/system/files/CHIM2009sa0331Ra-0EN.PDF.

Arambula, S.E., Belcher, S.M., Planchart, A., Turner, S.D., Patisaul, H.B., 2016. Impact of low dose oral exposure to bisphenol a (BPA) on the neonatal rat hypothalamic and hippocampal transcriptome: a CLARITY-BPA consortium study. Endocrinology 157, 3856–3872.

Arambula, S.E., Fuchs, J., Cao, J., Patisaul, H.B., 2017. Effects of perinatal bisphenol A exposure on the volume of sexually-dimorphic nuclei of juvenile rats: a CLARITY-BPA consortium study. Neurotoxicology 63, 33–42.

Arambula, S.E., Jima, D., Patisaul, H.B., 2018. Prenatal bisphenol A (BPA) exposure alters the transcriptome of the neonate rat amygdala in a sex-specific manner: a CLARITY-BPA consortium study. Neurotoxicology 65, 207–220.

Arantes-Rodrigues, R., Henriques, A., Pinto-Leite, R., Faustino-Rocha, A., Pinho-Oliveira, J., Teixeira-Guedes, C., Fernanda Seixas, F., Gama, A., Colaço, B., Colaço, A., Oliveira, P.A., 2012. The effects of repeated oral gavage on the health of male CD-1 mice. Lab. Anim. (NY) 41, 129–134.

Ashby, J., Tinwell, H., 1998. Uterotrophic activity of bisphenol A in the immature rat. Environ. Health Perspect. 106, 719–720.

Autrup, H., Barile, F.A., Blaauboer, B.J., Degen, G.H., Dekant, W., Dietrich, D., Domingo, J.L., Gori, G.B., Greim, H., Hengstler, J.G., Kacew, S., Marquardt, H., Pelkonen, O., Savolainen, K., Vermeulen, N.P., 2015. Principles of pharmacology and toxicology also govern effects of chemicals on the endocrine system. Toxicol. Sci. 146, 11–15.

Bailer, A.J., Portier, C.J., 1988. Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples. Biometrics 44, 417–431.

Balcombe, J.P., Barnard, N.D., Sandusky, C., 2004. Laboratory routines cause animal stress. Contemp. Topics 43, 42–51.

Bansal, R., Zoeller, R.T., 2019. CLARITY-BPA: bisphenol A or propylthiouracil on thyroid function and effects in the developing male and female rat brain. Endocrinology 160, 1771–1785.

Beausoleil, C., Beronius, A., Bodin, L., Bokkers, B.G.H., Boon, P.E., Burger, M., Cao, Y., De Wit, L., Fischer, A., Hanberg, A., Leander, K., Litens-Karlsson, S., Rousselle, C., Slob, W., Varret, C., Wolterink, G., Zilliacus, J., 2016. Review of Non-monotonic Dose-Responses of Substances for Human Risk Assessment. pp. 290.

Betancourt, A.M., Eltoum, I.A., Desmond, R.A., Russo, J., Lamartiniere, C.A., 2010. In utero exposure to bisphenol A shifts the window of susceptibility for mammary carcinogenesis in the rat. Environ. Health Perspect. 118, 1614–1619.

Bieler, G.S., Williams, R.L., 1993. Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity. Biometrics 49, 793–801.

Birnbaum, L.S., Bucher, J.R., Collman, G.W., Zeldin, D.C., Johnson, A.F., Schug, T.T., Heindel, J.J., 2012. Consortium-based science: the NIEHS's multipronged, collaborative approach to assessing the health effects of bisphenol A. Environ. Health Perspect. 120, 1640–1644.

Blankenstein, M.A., Broerse, J.J., van Zweiten, M.J., van der Molen, H.J., 1984. Prolactin concentration in plasma and susceptibility to mammary tumors in female rats from different strains treated chronically with estradiol-17beta. Breast Canc. Res. Treat. 4, 137–141.

Borgert, C.J., Baker, S.P., Matthews, J.C., 2013. Potency matters: thresholds govern endocrine activity. Regul. Toxicol. Pharmacol. 67, 83–88.

Boudreau, M.D., Imam, M.S., Paredes, A.M., Bryant, M.S., Cunningham, C.K., Felton, R.P., Jones, M.Y., Davis, K.J., Olson, G.R., 2016. Differential effects of silver nanoparticles and silver ions on tissue accumulation, distribution, and toxicity in the Sprague Dawley rat following daily oral gavage administration for 13 weeks. Toxicol. Sci. 150, 131–160.

Brown, N.M., Setchell, K.D.R., 2001. Animal models impacted by phytoestrogens in commercial chow: implications for pathways induced by hormones. Lab. Investig. 81, 735–747.

Brown, A.P., Dinger, N., Levine, B.S., 2000. Stress produced by gavage administration in the rat. Contemp. Topics 39, 17–21.

Brunner, E., Domhof, S., Langer, F., 2002. Nonparametric Analysis of Longitudinal Data in Factorial Experiments.

Butchbach, M.E., Edwards, J.D., Schussler, K.R., Burghes, A.H., 2007. A novel method for oral delivery of drug compounds to the neonatal SMNDelta7 mouse model of spinal muscular atrophy. J. Neurosci. Methods 161, 285–290.

California Office of Environmental Health Hazard Assessment, O., 2015. Proposition 65: Bisphenol A Listed as Known to the State of California to Cause Reproductive Toxicity. http://oehha.ca.gov/prop65/CRNR_notices/list_changes/051115listBPA.html.

Camacho, L., Lewis, S.M., Vanlandingham, M.M., Juliar, B.E., Olson, G.R., Patton, R.E., Gamboa da Costa, G., Woodling, K., Sepehr, E., Bryant, M.S., Doerge, D.R., Basavarajappa, M.S., Felton, R.P., Delclos, K.B., 2016. Comparison of endpoints relevant to toxicity assessments in 3 generations of CD-1 mice fed irradiated natural and purified ingredient diets with varying soy protein and isoflavone contents. Food Chem. Toxicol. 94, 39–56.

Cao, J., Rebuli, M.E., Rogers, J., Todd, K.L., Leyrer, S.M., Ferguson, S.A., Patisaul, H.B., 2013. Prenatal bisphenol A exposure alters sex-specific estrogen receptor expression in the neonatal rat hypothalamus and amygdala. Toxicol. Sci. 133, 157–173.

Cheong, A., Johnson, S.A., Howald, E.C., Ellersieck, M.R., Camacho, L., Lewis, S.M., Vanlandingham, M., Ying, J., Ho, S.M., Rosenfeld, C.S., 2018. Gene expression and DNA methylation changes in the hypothalamus and hippocampus of adult rats developmentally exposed to bisphenol A or ethinyl estradiol: a CLARITY-BPA consortium study. Epigenetics 1–17.

Christiansen, S., Axelstad, M., Boberg, J., Vinggaard, A.M., Pedersen, G.A., Hass, U., 2014. Low-dose effects of bisphenol A on early sexual development in male and female rats. Reproduction 147, 477–487.

Churchwell, M.I., Camacho, L., Vanlandingham, M.M., Twaddle, N.C., Sepehr, E., Delclos, K.B., Fisher, J.W., Doerge, D.R., 2014. Comparison of life-stage-dependent internal dosimetry for bisphenol A, ethinyl estradiol, a reference estrogen, and endogenous estradiol to test an estrogenic mode of action in Sprague Dawley rats. Toxicol. Sci. 139, 4–20.

Creasy, D., Bube, A., de Rijk, E., Kandori, H., Kuwahara, M., Masson, R., Nolte, T., Reams, R., Regan, K., Rehm, S., Rogerson, P., Whitney, K., 2012. Proliferative and non-proliferative lesions of the rat and mouse male reproductive system. Toxicol. Pathol. 40, 40S–121S.

Delclos, K.B., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Latendresse, J.R., Olson, G.R., Davis, K.J., Patton, R.E., Gamboa da Costa, G., Woodling, K.A., Bryant, M.S., Chidambaram, M., Trbojevich, R., Juliar, B.E., Felton, R.P., Thorn, B.T., 2014. Toxicity evaluation of bisphenol A administered by gavage to Sprague Dawley rats from gestation day 6 through postnatal day 90. Toxicol. Sci. 139, 174–197.

Dere, E., Anderson, L.M., Huse, S.M., Spade, D.J., McDonnell-Clark, E., Madnick, S.J., Hall, S.J., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Boekelheide, K., 2018. Effects of continuous bisphenol A exposure from early gestation on 90day old rat testes function and sperm molecular profiles: a CLARITY-BPA consortium study. Toxicol. Appl. Pharmacol. 347, 1–9.

Dixon, D., Alison, R., Bach, U., Colman, K., Foley, G.L., Harleman, J.H., Haworth, R., Herbert, R., Heuser, A., Long, G., Mirsky, M., Regan, K., Van Esch, E., Westwood, F.R., Vidal, J., Yoshida, M., 2014. Nonproliferative and proliferative lesions of the rat and mouse female reproductive system. J. Toxicol. Pathol. 27, 1S–107S.

Doerge, D.R., Twaddle, N.C., Vanlandingham, M., Fisher, J.W., 2010a. Pharmacokinetics of bisphenol A in neonatal and adult Sprague-Dawley rats. Toxicol. Appl. Pharmacol. 247, 158–165.

Doerge, D.R., Vanlandingham, M., Twaddle, N.C., Delclos, K.B., 2010b. Lactational transfer of bisphenol A in Sprague-Dawley rats. Toxicol. Lett. 199, 372–376.

Dusterberg, B., Kuhne, G., Tauber, U., 1986. Half-lives in plasma and bioavailability of ethinylestradiol in laboratory animals. Arzneimittelforschung 36, 1187–1190.

EFSA, 2006. Opinion of the scientific panel on food additives, flavourings, processing aids and materials in contact with food on a request from the Commission related to 2,2-bis(4-hydroxyphenyl)propane (Bisphenol A). EFSA J. 428, 1–75.

EFSA, 2011. Statistical significance and biological relevance. EFSA J. 9.

EFSA, 2015. Scientific Opinion on the risks to public health related to the presence of bisphenol A (BPA) in foodstuffs: PART II - toxicological assessment and risk characterization. EFSA J. 13, 3978.

EPA, 1988. Integrated Risk Information System (IRIS) Chemical Assessment Summary: Bisphenol A; CASRN 80-05-7.

European Commission, 2011. Commission Directive 2011/8/EU of 28 January 2011 Amending Directive 2002/72/EC as Regards the Restricition of Use of Bisphenol A in Plastic Infant Feeding Bottles.

Everds, N.E., Snyder, P.W., Bailey, K.L., Bolon, B., Creasy, D.M., Foley, G.L., Rosol, T.J., Sellers, T., 2013. Interpreting stress responses during routine toxicity studies: a review of the biology, impact, and assessment. Toxicol. Pathol. 41, 560–614.

Faustino-Rocha, A.I., Gama, A., Oliveira, P.A., Alvarado, A., Ferreira, R., Ginja, M., 2017. A spontaneous high-grade undifferentiated mammary carcinoma in a seven-week-old female rat. Exp. Toxicol. Pathol. 69, 241–244.

FDA, 2001. Draft Guidance for Industry, Statistical Aspects of the Design, Analysis, and Interpretation of Chronic Rodent Carcinogenicity Studies of Pharmaceuticals.

FDA, 2014. Updated Safety Assessment of Bisphenol A (BPA) for Use in Food Contact Applications. 2014.

FDA, 2017. Multiple Endpoints in Clinical Trials, Guidance for Industry (Draft Guidance).

Ferguson, S.A., Law Jr., C.D., Abshire, J.S., 2011. Developmental treatment with bisphenol A or ethinyl estradiol causes few alterations on early preweaning measures.

Toxicol. Sci. 124, 149–160.
Ferguson, S.A., Law, C.D., Abshire, J.S., 2012. Developmental treatment with bisphenol A causes few alterations on measures of postweaning activity and learning. Neurotoxicol. Teratol. 34, 598–606.
Ferguson, S.A., Law, C.D., Kissling, G.E., 2014. Developmental treatment with ethinyl estradiol, but not bisphenol A, causes alterations in sexually dimorphic behaviors in male and female Sprague Dawley rats. Toxicol. Sci. 140, 374–392.
Fotherby, K., 1996. Bioavailability of orally administered sex steroids used in oral contraception and hormone replacement therapy. Contraception 54, 59–69.
Gear, R., Kendziorski, J.A., Belcher, S.M., 2017. Effects of bisphenol A on incidence and severity of cardiac lesions in the NCTR-Sprague-Dawley rat: a CLARITY-BPA study. Toxicol. Lett. 275, 123–135.
Gibson-Corley, K.N., Olivier, A.K., Meyerholz, D.K., 2013. Principles for valid histopathologic scoring in research. Vet. Pathol. 50, 1007–1015.
Haseman, J.K., 1983. A reexamination of false-positive rates for carcinogenesis studies. Fundam. Appl. Toxicol. 3, 334–339.
Haseman, J.K., 1984. Statistical issues in the design, analysis and interpretation of animal carcinogenicity studies. Environ. Health Perspect. 58, 385–392.
Haseman, J.K., 1990. Use of statistical decision rules for evaluating laboratory animal carcinogenicity studies. Fundam. Appl. Toxicol. 14.
Hass, U., Christiansen, S., Boberg, J., Rasmussen, M.G., Mandrup, K., Axelstad, M., 2016. Low-dose effect of developmental bisphenol A exposure on sperm count and behaviour in rats. Andrology 4, 594–607.
He, Z., Paule, M.G., Ferguson, S.A., 2012. Low oral doses of bisphenol A increase volume of the sexually dimorphic nucleus of the preoptic area in male, but not female, rats at postnatal day 21. Neurotoxicol. Teratol. 34, 331–337.
He, Y.D., Karbowski, C.M., Werner, J., Everds, N., Di Palma, C., Chen, Y., Higgins-Garn, M., Tran, S., Afshari, C.A., Hamadeh, H.K., 2014. Common handling procedures conducted in preclinical safety studies result in minimal hepatic gene expression changes in Sprague-Dawley rats. PLoS One 9, e88750.
Health Canada, 2012. Health Canada's updated assessment of bisphenol a (BPA) exposure from food sources. In: F.D. Bureau of Chemical Safety, Health Products and Food Branch, Health Canada.
Heindel, J.J., Newbold, R.R., Bucher, J.R., Camacho, L., Delclos, K.B., Lewis, S.M., Vanlandingham, M., Churchwell, M.I., Twaddle, N.C., McLellen, M., Chidambaram, M., Bryant, M., Woodling, K., Gamboa da Costa, G., Ferguson, S.A., Flaws, J., Howard, P.C., Walker, N.J., Zoeller, R.T., Fostel, J., Favaro, C., Schug, T.T., 2015. NIEHS/FDA CLARITY-BPA research program update. Reprod. Toxicol. 58, 33–44.
Hill, C.E., Myers, J.P., Vandenberg, L.N., 2018. Nonmonotonic dose–response curves occur in dose ranges that are relevant to regulatory decision-making. Dose-Response 16 155932581879828.
Hirai, S., Hussain, A., Haddadin, M., Smith, R.B., 1981. First-pass metabolism of ethinyl estradiol in dogs and rats. J. Pharm. Sci. 70, 403–406.
Howdeshell, K.L., Peterman, P.H., Judy, B.M., Taylor, J.A., Orazio, C.E., Ruhlen, R.L., Vom Saal, F.S., Welshons, W.V., 2003. Bisphenol A is released from used polycarbonate animal cages into water at room temperature. Environ. Health Perspect. 111, 1180–1187.
Jenkins, S., Raghuraman, N., Eltoum, I., Carpenter, M., Russo, J., Lamartiniere, C.A., 2009. Oral exposure to bisphenol a increases dimethylbenzanthracene-induced mammary cancer in rats. Environ. Health Perspect. 117, 910–915.
Jenkins, S., Wang, J., Eltoum, I., desmond, R.A., Lamartiniere, C.A., 2011. Chronic oral exposure to Bisphenol A results in a nonmonotonic dose response in mammary carcinogenesis and metastasis in MMTV-erbB2 mice. Environ. Health Perspect. 119, 1604–1609.
Johnson, S.A., Javurek, A.B., Painter, M.S., Ellersieck, M.R., Welsh Jr., T.H., Camacho, L., Lewis, S.M., Vanlandingham, M.M., Ferguson, S.A., Rosenfeld, C.S., 2016. Effects of developmental exposure to bisphenol A on spatial navigational learning and memory in rats: a CLARITY-BPA study. Horm. Behav. 80, 139–148.
Jonckheere, A.R., 1954. A distribution-free k-sample test against ordered alternatives. Biometrika 41, 133–145.
Kanno, J., Onyon, L., Peddada, S., Ashby, J., Jacob, E., Owens, W., 2003. The OECD program to validate the rat uterotrophic bioassay. Phase 2: dose-response studies. Environ. Health Perspect. 111, 1530–1549.
Kapetanovic, I.M., Krishnaraj, R., Martin-Jimenez, T., Yuan, L., van Breemen, R.B., Lyubimov, A., 2006. Effects of oral dosing paradigms (gavage versus diet) on pharmacokinetics and pharmacodynamics. Chem. Biol. Interact. 164, 68–75.
Kapetanovic, I.M., Bauer, K.S., Tessier, D.M., Lindeblad, M.O., Zakharov, A.D., Lubet, R., Lyubimov, A., 2009. Comparison of pharmacokinetic and pharmacodynamic profiles of aspirin following oral gavage and diet dosing in rats. Chem. Biol. Interact. 179, 233–239.
Kim, H.S., Kang, T.S., Kang, I.H., Kim, T.S., Moon, H.J., Kim, I.Y., Ki, H., Park, K.L., Lee, B.M., Yoo, S.D., Han, S.Y., 2005. Validation study of OECD rodent uterotrophic assay for the assessment of estrogenic activity in Sprague-Dawley immature female rats. J. Toxicol. Environ. Health 68, 2249–2262.
Kissling, G.E., Haseman, J.K., Zeiger, E., 2015. Proper interpretation of chronic toxicity studies and their statistics: a critique of "Which level of evidence does the US National Toxicology Program provide? Statistical considerations using the Technical Report 578 on Ginkgo biloba as an example. Toxicol. Lett. 237, 161–164.
Kuzutani, K., Shibanushi, T., Kangawa, Y., Kihara, T., 2012. Spontaneous mammary adenocarcinoma in a twelve-week-old female Sprague-Dawley rat. J. Toxicol. Pathol. 25, 221–224.
Lagarde, F., Beausoleil, C., Belcher, S.M., Belzunces, L.P., Emond, C., Guerbet, M., Rousselle, C., 2015. Non-monotonic dose-response relationships and endocrine disruptors: a qualitative method of assessment. Environ. Health 14, 13–28.
Latendresse, J.R., Bucci, T.J., Olson, G., Mellick, P., Weis, C.C., Thorn, B., Newbold, R.R., Delclos, K.B., 2009. Genistein and ethinyl estradiol dietary exposure in multigenerational and chronic studies induce similar proliferative lesions in mammary gland of male Sprague-Dawley rats. Reprod. Toxicol. 28, 342–353.
Le, H.H., Carlson, E.M., Chua, J.P., Belcher, S.M., 2008. Bisphenol A is released from polycarbonate drinking bottles and mimics the neurotoxic actions of estrogen in developing cerebellar neurons. Toxicol. Lett. 176, 149–156.
Lewis, S.M., Lee, F.W., Ali, A.A., Allaben, W.T., Weis, C.C., Leakey, J.E., 2010. Modifying a displacement pump for oral gavage dosing of solution and suspension preparations to adult and neonatal mice. Lab. Anim. 39, 149–154.
Li, J., Bach, A., Crawford, R.B., Phadnis-Moghe, A.S., Chen, W., D'Ingillo, S., Kovalova, N., Suarez-Martinez, J.E., Zhou, J., Kaplan, B.L.F., Kaminski, N.E., 2018a. CLARITY-BPA: effects of chronic Bisphenol A exposure on the immune system: Part 1 - quantification of the relative number and proportion of leukocyte populations in the spleen and thymus. Toxicology 396–397, 46–53.
Li, J., Bach, A., Crawford, R.B., Phadnis-Moghe, A.S., Chen, W., D'Ingillo, S., Kovalova, N., Suarez-Martinez, J.E., Zhou, J., Kaplan, B.L.F., Kaminski, N.E., 2018b. CLARITY-BPA: effects of chronic bisphenol A exposure on the immune system: Part 2 - characterization of lymphoproliferative and immune effector responses by splenic leukocytes. Toxicology 396–397, 54–67.
Lin, K.K., Rahman, M.A., 1998. Overall false positive rates in tests for linear trend in tumor incidence in animal carcinogenicity studies of new drugs. J. Biopharm. Stat. 8, 1–15 discussion 17-22.
Mandrup, K., Boberg, J., Isling, L.K., Christiansen, S., Hass, U., 2016. Low-dose effects of bisphenol A on mammary gland development in rats. Andrology 4, 673–683.
Martin-Jimenez, T., Lindeblad, M., Kapetanovic, I.M., Chen, Y., Lyubimov, A., 2008. Comparing pharmacokinetic and pharmacodynamic profiles in female rats orally exposed to lovastatin by gavage versus diet. Chem. Biol. Interact. 171, 142–151.
Marty, M.S., Domoradzki, J.Y., Hansen, S.C., Timchalk, C., Bartels, M.J., Mattsson, J.L., 2007. The effect of route, vehicle, and divided doses on the pharmacokinetics of chlorpyrifos and its metabolite trichloropyridinol in neonatal Sprague-Dawley rats. Toxicol. Sci. 100, 360–373.
Marty, M.S., Borgert, C., Coady, K., Green, R., Levine, S.L., Mihaich, E., Ortego, L., Wheeler, J.R., Yi, K.D., Zorrilla, L.M., 2018. Distinguishing between endocrine disruption and non-specific effects on endocrine systems. Regul. Toxicol. Pharmacol. 99, 142–158.
Moral, R., Wang, R., Russo, I.H., Lamartiniere, C.A., Pereira, J., Russo, J., 2008. Effect of prenatal exposure to the endocrine disruptor bisphenol A on mammary gland morphology and gene expression signature. J. Endocrinol. 196, 101–112.
Nomiri, S., Hoshyar, R., Ambrosino, C., Tyler, C.R., Mansouri, B., 2019. A mini review of bisphenol A (BPA) effects on cancer-related cellular signaling pathways. Environ. Sci. Pollut. Res. Int. 26, 8459–8467.
NTP, 1982. Carcinogenesis bioassay of bisphenol a (CAS No. 80-05-7) in F344 rats and B6C3F1 mice (feed study). Natl. Toxicol. Program Tech. Rep. Ser. 215, 1–116.
NTP, 2008b. Toxicology and carcinogenesis studies of genistein (Cas No. 446-72-0) in Sprague-Dawley rats (feed study). Natl. Toxicol. Program Tech. Rep. Ser. 1–240.
NTP, 2010a. Multigenerational reproductive toxicology study of ethinyl estradiol (CAS No. 57-63-6) in Sprague-Dawley rats. Natl. Toxicol. Program Tech. Rep. Ser. 1–312.
NTP, 2010b. Toxicology and carcinogenesis study of ethinyl estradiol (CAS No. 57-63-6) in Sprague-Dawley rats (feed study). Natl. Toxicol. Program Tech. Rep. Ser. 1–210.
NTP, 2011a. Specifications for the Conduct of Studies to Evaluate the Reproductive and Developmental Toxicity of Chemical, Biological, and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP). Available from. https://ntp.niehs.nih.gov/ntp/test_info/finalntp_reprospecsmay2011_508.pdf.
NTP, 2011b. Specifications for the Conduct of Studies to Evaluate the Toxic and Carcinogenic Potential of Chemical, Biological and Physical Agents in Laboratory Animals for the National Toxicology Program (NTP). Available from. https://ntp.niehs.nih.gov/ntp/test_info/finalntp_toxcarspecsjan2011.pdf.
NTP, 2014. Evaluation of the Toxicity of Bisphenol A (BPA) in Male and Female Sprague-Dawley Rats Exposed Orally from Gestation Day 6 through Postnatal Day 90: Study Tables. Available from. https://tools.niehs.nih.gov/cebs3/views/?action=main.dataReview&bin_id=3148.
NTP, 2018a. CLARITY-BPA. Chemical effects in biological systems (CEBS). In: NTP (Ed.), Research Triangle Park, NC (USA), . https://manticore.niehs.nih.gov/cebssearch/program/CLARITY-BPA.
NTP, 2018b. NTP Research Report on the CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats: Research Report 9. pp. 223. https://ntp.niehs.nih.gov/ntp/results/pubs/rr/reports/rr09_508.pdf.
NTP, 2018c. RR-9: CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats, Supplemental Appendices. National Toxicology Program, Research Triangle Park, NC. https://doi.org/10.22427/NTP-DATA-018-00015-0001-000-6.
NTP, 2018d. NTP Statistical Procedures, Expanded Overviews.
Oishi, Y., Yoshizawa, K., Suzuki, J., Makino, N., Hase, K., Yamauchi, K., Tsubura, A., 1995. Spontaneously occurring mammary adenocarcinoma in a 10-wk-old female rat. Toxicol. Pathol. 23, 696–700.
Pande, P., Fleck, S.C., Twaddle, N.C., Churchwell, M.I., Doerge, D.R., Teeguarden, J.G., 2018. Comparative estrogenicity of endogenous, environmental and dietary estrogens in pregnant women II: total estrogenicity calculations accounting for competitive protein and receptor binding and potency. Food Chem. Toxicol. 125, 341–353.
Patel, S., Brehm, E., Gao, L., Rattan, S., Ziv-Gal, A., Flaws, J.A., 2017. Bisphenol a exposure, ovarian follicle numbers, and female sex steroid hormone levels: results from a CLARITY-BPA study. Endocrinology 158, 1727–1738.
Patterson, T.A., Twaddle, N.C., Roegge, C.S., Callicott, R.J., Fisher, J.W., Doerge, D.R., 2013. Concurrent determination of bisphenol A pharmacokinetics in maternal and fetal rhesus monkeys. Toxicol. Appl. Pharmacol. 267, 41–48.
Peddada, S.D., Kissling, G.E., 2005. A survival-adjusted quantal-response test for analysis of tumor incidence rates in animal carcinogenicity studies. Environ. Health Perspect.

114, 537–541.
Perrot-Applanat, M., Kolf-Clauw, M., Michel, C., Beausoleil, C., 2018. Alteration of mammary gland development by bisphenol a and evidence of a mode of action mediated through endocrine disruption. Mol. Cell. Endocrinol. 475, 29–53.
Prins, G.S., Hu, W.Y., Xie, L., Shi, G.B., Hu, D.P., Birch, L., Bosland, M.C., 2018a. Evaluation of bisphenol a (BPA) exposures on prostate stem cell homeostasis and prostate cancer risk in the NCTR-Sprague-Dawley rat: an NIEHS/FDA CLARITY-BPA consortium study. Environ. Health Perspect. 126, 117001.
Prins, G.S., Patisaul, H.B., Belcher, S.M., Vandenberg, L.N., 2018b. CLARITY-BPA academic laboratory studies identify consistent low-dose Bisphenol A effects on multiple organ systems. Basic Clin. Pharmacol. Toxicol. https://doi.org/10.1111/bcpt.13125. Sep 12.
Rahman, M.A., Lin, K.K., 2008. A comparison of false positive rates of peto and poly-3 methods for long-term carcinogenicity data analysis using multiple comparison adjustment method suggested by Lin and Rahman. J. Biopharm. Stat. 18, 949–958.
Rebuli, M.E., Camacho, L., Adonay, M.E., Reif, D.M., Aylor, D.L., Patisaul, H.B., 2015. Impact of low-dose oral exposure to bisphenol a (BPA) on juvenile and adult rat exploratory and anxiety behavior: a CLARITY-BPA consortium study. Toxicol. Sci. 148, 341–354.
Regan, K.S., Cline, J.M., Creasy, D., Davis, B., Foley, G.L., Lanning, L., Latendresse, J.R., Makris, S., Morton, D., Rehm, S., Stebbins, K., 2005. STP position paper: ovarian follicular counting in the assessment of rodent reproductive toxicity. Toxicol. Pathol. 33, 409–412.
Rubin, B.S., Paranjpe, M., DaFonte, T., Schaeberle, C., Soto, A.M., Obin, M., Greenberg, A.S., 2017. Perinatal BPA exposure alters body weight and composition in a dose specific and sex specific manner: the addition of peripubertal exposure exacerbates adverse effects in female mice. Reprod. Toxicol. 68, 130–144.
Rudmann, D., Cardiff, R., Chouinard, L., Goodman, D., Kuttler, K., Marxfeld, H., Molinolo, A., Treumann, S., Yoshizawa, K., Inhand Mammary, Z.s.P., Clitoral Gland Organ Working, G., 2012. Proliferative and nonproliferative lesions of the rat and mouse mammary, Zymbal's, preputial, and clitoral glands. Toxicol. Pathol. 40, 7S–39S.
Sarkar, D.K., Gottschall, P.E., Meites, J., 1982. Damage to hypothalamic dopaminergic neurons is associated with development of prolactin-secreting pituitary tumors. Science 218, 684–686.
Schafer, K.A., Eighmy, J., Fikes, J.D., Halpern, W.G., Hukkanen, R.R., Long, G.G., Meseck, E.K., Patrick, D.J., Thibodeau, M.S., Wood, C.E., Francke, S., 2018. Use of severity grades to characterize histopathologic changes. Toxicol. Pathol. 46, 256–265.
Schug, T.T., Heindel, J.J., Camacho, L., Delclos, K.B., Howard, P., Johnson, A.F., Aungst, J., Keefe, D., Newbold, R., Walker, N.J., Thomas Zoeller, R., Bucher, J.R., 2013. A new approach to synergize academic and guideline-compliant research: the CLARITY-BPA research program. Reprod. Toxicol. 40, 35–40.
Seachrist, D.D., Bonk, K.W., Ho, S.M., Prins, G.S., Soto, A.M., Keri, R.A., 2016. A review of the carcinogenic potential of bisphenol A. Reprod. Toxicol. 59, 167–182.
Shafei, A., Ramzy, M.M., Hegazy, A.I., Husseny, A.K., El-Hadary, U.G., Taha, M.M., Mosa, A.A., 2018. The molecular mechanisms of action of the endocrine disrupting chemical bisphenol A in the development of cancer. Gene 647, 235–243.
Shirley, E., 1977. A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. Biometrics 33, 386–389.
Sieli, P.T., Jasarevic, E., Warzak, D.A., Mao, J., Ellersieck, M.R., Liao, C., Kannan, K., Collet, S.H., Toutain, P.L., Vom Saal, F.S., Rosenfeld, C.S., 2011. Comparison of serum bisphenol A concentrations in mice exposed to bisphenol A through the diet versus oral bolus exposure. Environ. Health Perspect. 119, 1260–1265.
Solecki, R., Kortenkamp, A., Bergman, A., Chahoud, I., Degen, G.H., Dietrich, D., Greim, H., Hakansson, H., Hass, U., Husoy, T., Jacobs, M., Jobling, S., Mantovani, A., Marx-Stoelting, P., Piersma, A., Ritz, V., Slama, R., Stahlmann, R., van den Berg, M., Zoeller, R.T., Boobis, A.R., 2017. Scientific principles for the identification of endocrine-disrupting chemicals: a consensus statement. Arch. Toxicol. 91, 1001–1006.
Soto, A.M., Lin, T.-M., Honorato, J., Silva, R.M., Sonnenschein, C., 1992. An "in culture" bioassay to assess the estrogenicity of xenobiotics (E-screen). In: Colborn, T., Clement, C. (Eds.), Chemically Induced Alterations in Sexual Development: the Wildlife/human Connection. Princeton Scientific Publishing, Princeton, NJ, pp. 295–309.
Soto, A.M., Brisken, C., Schaeberle, C., Sonnenschein, C., 2013. Does cancer start in the womb? altered mammary gland development and predisposition to breast cancer due to in utero exposure to endocrine disruptors. J. Mammary Gland Biol. Neoplasia 18, 199–208.
Stump, D.G., Beck, M.J., Radovsky, A., Garman, R.H., Freshwater, L.L., Sheets, L.P., Marty, M.S., Waechter Jr., J.M., Dimond, S.S., Van Miller, J.P., Shiotsuka, R.N., Beyer, D., Chappelle, A.H., Hentges, S.G., 2010. Developmental neurotoxicity study of dietary bisphenol A in Sprague-Dawley rats. Toxicol. Sci. 115, 167–182.
Teeguarden, J., Hanson-Drury, S., Fisher, J.W., Doerge, D.R., 2013. Are typical human serum BPA concentrations measurable and sufficient to be estrogenic in the general population? Food Chem. Toxicol. 62, 949–963.
Terpstra, T.J., 1952. The asymptotic normality and consistency of Kendall's test against trend when ties are present in one ranking. Indag. Math. 14, 327–333.
Thayer, K.A.a.B., S., 2010. Mechanisms of Action of Bisphenol A and Other Biochemical/Molecular Interactions. FAO/WHO Expert Meeting on Bisphenol A (BPA), Ottawa, Canada.
Thayer, K.A., Heindel, J.J., Bucher, J.R., Gallo, M.A., 2012. Role of environmental chemicals in diabetes and obesity: a National Toxicology Program workshop review. Environ. Health Perspect. 120, 779–789.
Thigpen, J.E., Setchell, K.D., Saunders, H.E., Haseman, J.K., Grant, M.G., Forsythe, D.B., 2004. Selecting the appropriate rodent diet for endocrine disruptor research and testing studies. ILAR J. 45, 401–416.
Turner, P.V., Vaughn, E., Sunohara-Neilson, J., Ovari, J., Leri, F., 2012. Oral gavage in rats: animal welfare evaluation. JAALAS 51, 25–30.
Twaddle, N.C., Churchwell, M.I., Newbold, R.R., Delclos, K.B., Doerge, D.R., 2003. Determination using liquid-chromatography-electrospray tandem mass spectroscopy of ethinylestradiol serum pharmacokinetics in adult Sprague-Dawley rats. J. Chromatogr. B Analyt. Technol. Biomed. Life Sci. 793, 309–315.
Tyl, R.W., Myers, C.B., Marr, M.C., Thomas, B.F., Keimowitz, A.R., Brine, D.R., Veselica, M.M., Fail, P.A., Chang, T.Y., Seely, J.C., Joiner, R.L., Butala, J.H., Dimond, S.S., Cagen, S.Z., Shiotsuka, R.N., Stropp, G.D., Waechter, J.M., 2002. Three-generation reproductive toxicity study of dietary Bisphenol A in CD Sprague-Dawley rats. Toxicol. Sci. 68, 121–146.
Tyl, R.W., Myers, C.B., Marr, M.C., Sloan, C.S., Castillo, N.P., Veselica, M.M., Seely, J.C., Dimond, S.S., Van Miller, J.P., Shiotsuka, R.N., Beyer, D., Hentges, S.G., Waechter Jr., J.M., 2008. Two-generation reproductive toxicity study of dietary bisphenol A in CD-1 (Swiss) mice. Toxicol. Sci. 104, 362–384.
Vandenberg, L.N., Welshons, W.V., Vom Saal, F.S., Toutain, P.L., Myers, J.P., 2014. Should oral gavage be abandoned in toxicity testing of endocrine disruptors? Environ. Health 13, 46.
Varret, C., Beronius, A., Bodin, L., Bokkers, B.G.H., Boon, P.E., Burger, M., De Wit-Bos, L., Fischer, A., Hanberg, A., Litens-Karlsson, S., Slob, W., Wolterink, G., Zilliacus, J., Beausoleil, C., Rousselle, C., 2018. Evaluating the evidence for non-monotonic dose-response relationships: a systematic literature review and (re-)analysis of in vivo toxicity data in the area of food safety. Toxicol. Appl. Pharmacol. 339, 10–23.
Villar-Pazos, S., Martinez-Pinna, J., Castellano-Munoz, M., Alonso-Magdalena, P., Marroqui, L., Quesada, I., Gustafsson, J.A., Nadal, A., 2017. Molecular mechanisms involved in the non-monotonic effect of bisphenol-a on ca2+ entry in mouse pancreatic beta-cells. Sci. Rep. 7, 11770.
Wang, J., Jenkins, S., Lamartiniere, C.A., 2014. Cell proliferation and apoptosis in rat mammary glands following combinational exposure to bisphenol A and genistein. BMC Canc. 14, 379.
Wassenaar, P.N.H., Trasande, L., Legler, J., 2017. Systematic review and meta-analysis of early-life exposure to bisphenol a and obesity-related outcomes in rodents. Environ. Health Perspect. 125, 106001.
Wei, J., Lin, Y., Li, Y., Ying, C., Chen, J., Song, L., Zhou, Z., Lv, Z., Xia, W., Chen, X., Xu, S., 2011. Perinatal exposure to bisphenol A at reference dose predisposes offspring to metabolic syndrome in adult rats on a high-fat diet. Endocrinology 152, 3049–3061.
Welshons, W.V., Rottinghaus, G.E., Nonneman, D.J., Dolan-Timpe, M., Ross, P.F., 1990. A sensitive bioassay for detection of dietary estrogens in animal feeds. J. Vet. Diagn. Investig. 2, 268–273.
Williams, D.A., 1986. A note on Shirley's nonparametric test for comparing several dose levels with a zero-dose control. Biometrics 42, 183–186.
Witchey, S.K., Fuchs, J., Patisaul, H.B., 2019. Perinatal bisphenol A (BPA) exposure alters brain oxytocin receptor (OTR) expression in a sex- and region-specific manner: a CLARITY-BPA consortium follow-up study. Neurotoxicology 74, 139–148.
Yamasaki, K., Sawaki, M., Noda, S., Imatanaka, N., Takatsuki, M., 2002. Subacute oral toxicity study of ethynylestradiol and bisphenol A, based on the draft protocol for the "Enhanced OECD Test Guideline no. 407". Arch. Toxicol. 76, 65–74.
Yang, X., Doerge, D.R., Teeguarden, J.G., Fisher, J.W., 2015. Development of a physiologically based pharmacokinetic model for assessment of human exposure to bisphenol A. Toxicol. Appl. Pharmacol. 289, 442–456.
Yoshida, M., Shimomoto, T., Katashima, S., Watanabe, G., Taya, K., Maekawa, A., 2004. Maternal exposure to low doses of bisphenol A has no effects on development of female reproductive tract and uterine carcinogenesis in Donryu rats. J. Reprod. Dev. 50, 349–360.
Yoshizawa, K., Brix, A.E., Sells, D.M., Jokinen, M.P., Wyde, M., Orzech, D.P., Kissling, G.E., Walker, N.J., Nyska, A., 2009. Reproductive lesions in female Harlan Sprague-Dawley rats following two-year oral treatment with dioxin and dioxin-like compounds. Toxicol. Pathol. 37, 921–937.
Zoeller, R.T., Vandenberg, L.N., 2015. Assessing dose-response relationships for endocrine disrupting chemicals (EDCs): a focus on non-monotonicity. Environ. Health 14, 42.