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TSCA 8(e) Substantial Risk
Bisphenol A
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This submission is made by:
The Society of the Plastics Industry (SPI), Inc.
SPI Bisphenol A Global Industry Group
1801 K Street, NW, Suite 600K
Washington, DC 20006-1301

SPI is submitting the final report for a study sponsored by the SPI Bisphenol A Global Industry Group entitled *Three-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD® (Sprague-Dawley) Rats*, conducted at Research Triangle Institute. SPI previously submitted interim results, on June 30th, 1999, pursuant to current guidance issued by the Environmental Protection Agency (EPA) indicating EPA's interpretation of section 8(e) of the Toxic Substances Control Act. The previous submission indicated the final draft report for the study was expected in October 2000; this submission is being made to provide the final study report. Neither SPI nor any of its members has determined that the interim results, or the results contained within the final report, indicate any potential risk of injury to human health or the environment.

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Please do not hesitate to contact me if you have any questions at (202) 974-5217.

Sincerely,

Lynne R. Harris
Director
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FINAL REPORT

VOLUME I OF IX

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Title:	Three-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD® (Sprague-Dawley) Rats
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TABLE OF CONTENTS

Page

VOLUME I OF IX

Title Page 1
Table of Contents 2
List of Figures 5
Abstract 6
Objectives 10
Materials and Methods 10
Test Material and Dietary Formulations 10
Animals and Husbandry 12
Text Table A. Study Schedule 13
Study Design 19
Text Table B. Organization and Target Dietary Concentrations and Intakes 19
Text Figure A. BPA Three-Generation Study Design 20
Parental F0, F1, and F2 Animals and Retained F3 Offspring 21
Offspring Animals (F1, F2, and F3) 25
Necropsy and Histology 26
Reproductive and Offspring Indices 29
Text Table C. Reproductive and Offspring Indices 30
Calculation of Standard Reproductive Toxicity Parameters 31
Text Table D. Formulas for Calculating Standard Reproductive Toxicity Study Parameters 31
Statistical Analyses 34
Text Figure B. Decision Trees for Statistical Analysis 36
Personnel 38
Storage of Records 39
Compliance 39
Results 40
Dosed Feed Formulations 40
F0 Prebreed Exposure Period 40
Gestation - F0 Females for F1 Litters 46
Lactation - F0 Females for F1 Litters 46
Necropsy – F1 Weanlings 51
F0 Parental Holding Period 52
Necropsy – F0 Parental Animals 53
F1 Prebreed Exposure Period 54

TABLE OF CONTENTS (continued)

Page

Gestation - F1 Females for F2 Litters 60
Lactation - F1 Females for F2 Litters 62
Necropsy – F2 Weanlings 65
F1 Parental Holding Period 66
Necropsy – F1 Parental Animals 68
F2 Prebreed Exposure Period 70
Gestation - F2 Females for F3 Litters 75
Lactation - F2 Females for F3 Litters 77
Necropsy - F3 Weanlings 80
F2 Parental Holding Period 81
Necropsy - F2 Parental Animals 82
Retained F3 Weanlings 84
F3 Postwean Exposure Period 85
Necropsy – F3 Adults 89
Discussion 92
Parental Systemic Parameters 94
Parental Reproductive Parameters 95
Offspring Parameters 96
Conclusions 103
References 105
Text Table E. Summary of F0, F1, F2, and F3 Adult Systemic Toxicity 109
Text Table F. Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters 116
Text Table G. Summary of F1, F2, and F3 Offspring Parameters 121
Text Table H. Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females 131
Text Table I. Bisphenol A Intake in mg/kg Body Weight/Day 141
Protocol Deviations 144
Good Laboratory Practices Compliance Statement 147
Quality Assurance Statement 148

TABLE OF CONTENTS (continued)

VOLUME II OF IX

Summary Data Tables 1 Through 62

VOLUME III OF IX

Summary Data Tables 63 Through 106

VOLUME IV OF IX

Analytical Chemistry Reports ..... Appendix I

Pathology Summary ..... Appendix II

VOLUME V OF IX

F0 Individual Tables

VOLUME VI of IX

F1 Individual Tables

VOLUME VII of IX

F2 Individual Tables

VOLUME VIII of IX

F3 Individual Tables

VOLUME IX of IX

Historical Control Data Set ..... Appendix III

Protocol and Five Amendments ..... Appendix IV

LIST OF FIGURES

Figure	Title	Page
1	F0 Male Body Weights During the Prebreed and Mating Periods	41
2	F0 Male Bisphenol A Intake During the Prebreed Period	43
3	F0 Female Body Weights During the Prebreed Period	44
4	F0 Female Bisphenol A Intake During the Prebreed Period	45
5	F0 Female Body Weights During Gestation	47
6	F0 Female Bisphenol A Intake During Gestation	48
7	F0 Female Body Weights During Lactation	48
8	F0 Female Bisphenol A Intake During Lactation	49
9	F1 Male Body Weights During the Prebreed and Mating Periods	57
10	F1 Male Bisphenol A Intake During the Prebreed Period	57
11	F1 Female Body Weights During the Prebreed Period	59
12	F1 Female Bisphenol A Intake During the Prebreed Period	59
13	F1 Female Body Weights During Gestation	61
14	F1 Female Bisphenol A Intake During Gestation	61
15	F1 Female Body Weights During Lactation	63
16	F1 Female Bisphenol A Intake During Lactation	64
17	F2 Male Body Weights During the Prebreed and Mating Periods	71
18	F2 Male Bisphenol A Intake During the Prebreed Period	72
19	F2 Female Body Weights During the Prebreed Period	73
20	F2 Female Bisphenol A Intake During the Prebreed Period	74
21	F2 Female Body Weights During Gestation	76
22	F2 Female Bisphenol A Intake During Gestation	76
23	F2 Female Body Weights During Lactation	77
24	F2 Female Bisphenol A Intake During Lactation	78
25	F3 Male Body Weights During the Post wean Period	86
26	F3 Male Bisphenol A Intake During the Post wean Period	87
27	F3 Female Body Weights During the Post wean Period	88
28	F3 Female Bisphenol A Intake During the Post wean Period	89

RTI Project No.: 65C-07036-000
RTI Protocol No.: RTI-637

Three-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD® (Sprague-Dawley) Rats

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ABSTRACT

Male and female Sprague-Dawley derived outbred albino rats [Crl:CD® (SD)BR] were administered Bisphenol A (BPA; CAS No. 80-05-7) in the feed at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm (resulting in BPA intakes of approximately 0.001, 0.02, 0.3, 5, 50, and 500 mg/kg/day, respectively), available *ad libitum*, 30 animals/sex/dose, for ten weeks. This study was conducted according to the OPPTS Testing Guidelines (870.3800; 1998) with additions (a third generation; seven treatment groups; examination for retained nipples in F1, F2, and F3 preweanling males; retention of F3 offspring, 30/sex/group, until adulthood with exposures continuing; measuring anogenital distance and acquisition of puberty in both sexes; epididymal sperm and testicular spermatids; estrous cyclicity; and full necropsy and histopathology). Body weights and feed consumption were recorded weekly, and clinical signs were recorded at least once daily. Vaginal cytology was evaluated for the last three weeks of the prebreed period. Animals were then randomly mated within treatment groups for a two-week mating period to produce the F1 generation, with treatment continued throughout breeding. All surviving F0 males were necropsied after the F1 delivery period, with histologic evaluation of reproductive and other organs and andrological assessments (reproductive organ weights, epididymal sperm number, motility and morphology, testicular homogenization-resistant spermatid head counts, daily sperm production, and efficiency of daily sperm production).

F1 litters were culled to ten pups on postnatal day (pnd) 4 and weaned on pnd 21. At weaning, up to three weanlings/sex/litter were randomly selected and necropsied with selected organ weights, and 30/sex/dose were randomly selected as F1 parents of the F2 generation. All surviving F0 females were then necropsied with organ weights, stage of estrus at necropsy,

enumeration of ovarian primordial follicles (high dose and control), and histopathology of reproductive and other selected organs. Selected F1 weanlings were administered BPA in the diet for a ten-week prebreed exposure period, with assessment of acquisition of vaginal patency (VP) in females and preputial separation (PPS) in males; vaginal cytology for estrous cyclicity was evaluated during the last three weeks. They were then mated for a two-week mating period as described above. At weaning of F2 litters, up to three weanlings/sex/litter were randomly selected and necropsied, and 30/sex/dose were randomly selected as F2 parents of the F3 generation. All surviving F1 males were necropsied after the delivery period, with histopathology and andrological assessments (as described above). After weaning of the F2 litters, all surviving parental F1 females were then necropsied with histopathology as described above. Selected F2 weanlings were administered BPA in the diet for a ten-week prebreed exposure period, with acquisition of VP (females) and PPS (males) and vaginal cytology for estrous cyclicity evaluated during the last three weeks. They were then mated as described above to generate F3 litters. At weaning of F3 litters, up to three weanlings/sex/litter were randomly selected and necropsied, and 30/sex/dose were randomly selected and retained until adulthood, with exposures continuing, with acquisition of VP, PPS, and estrous cyclity evaluated. At necropsy of these retained adult F3 offspring, they were evaluated as described above for F0, F1, and F2 parental animals. The results of this study were as follows:

7500 ppm (approximately 500 mg/kg/day). Adult systemic toxicity was present for both sexes in all generations (F0, F1, F2, and F3), expressed as consistent and persistent reduced body weights and weight gains, typically greater than 15%, occasional reductions in feed consumption as g/day, and occasional increases in feed consumption as g/kg/day. Food efficiency was occasionally reduced. For nonreproductive organs, absolute weights were consistently reduced in both sexes, while relative organ weights were commonly increased. Histopathologic findings on nonreproductive organs included increased incidence of renal tubular degeneration and chronic hepatic inflammation only in F0, F1, and F2 females (but not F3). There were no treatment- or dose-related direct effects on reproductive organ weights, histopathology, or function. Consistent with the reduced body weights and body weight gains in this group, paired ovarian weights were significantly reduced for F0, F1, and F2 females (absolute and relative weights) and for F3 females (absolute weights only). Paired ovarian follicle counts (ten females each at 0 and 7500 ppm) were significantly increased at 7500 ppm in

F0 females but were unaffected in F1, F2, and F3 females. There were no effects of treatment on any andrological parameters at any dose in any generation (except for significantly reduced epididymal sperm counts only in F1 males at 7500 ppm, and for significantly reduced daily sperm production per testis, but not efficiency of daily sperm production only in F3 males at 7500 ppm). The only reproductive effect was a significant decrease in total and live pups per litter (with no significant increase in prenatal postimplantation loss per litter) on pnd 0 and 4 (precull) for F1, F2, and F3 litters at 7500 ppm, associated with reduced uterine weight in parental F0, F1, and F2 females. There were no effects on mating, fertility, or gestational indices, on precoital interval, gestational length, offspring sex ratios, or on nipple and/or areola retention in preweanling F1, F2, and F3 male pups. The only offspring effects at 7500 ppm included statistically significant reductions in pup body weights per litter, beginning on pnd 7 for F1, F2, and F3 offspring, and statistically significant delayed acquisition of both vaginal patency and preputial separation for F1, F2, and F3 postweanling pups. Anogenital distance (measured in F2 and F3 offspring on pnd 0) was unaffected for F2 and F3 males and for F3 females. Only F2 female anogenital distance was significantly increased: by 0.03 mm at 0.015, 0.3, and 4.5 ppm, and by 0.04 mm at 750 ppm versus the control value. Although statistically significant, these variations were only 0.03 to 0.04 mm and were considered to be due to the extremely precise measurement technique used and were not considered to be of biological or toxicological significance. These changes (referring to preputial separation, vaginal patency, and anogenital distance) did not affect reproductive function in the animals exhibiting them. The effects observed on reproductive function or postnatal toxicity were present in a clearly toxic dose (adult systemic toxicity) and were not observed at doses below 7500 ppm.

750 ppm (approximately 50 mg/kg/day). Adult systemic toxicity was present for F0, F1, F2, and F3 generations, expressed only as reduced body weights for F0 females (but not males) during prebreed, gestation, and lactation; F1 females (but not males) during gestation and lactation (but not prebreed); F2 males (but not females) only during prebreed, but not for F3 males or females. Feed consumption in g/day or g/kg/day and food efficiency were not consistently or persistently affected. In males, absolute liver weight was significantly reduced in all generations, absolute pituitary weight was significantly reduced in the F1 generation, and absolute kidney and spleen weights were significantly reduced in the F2 generation. In females, absolute and relative nonreproductive organ weights were not affected in any generation. There

were no treatment-related gross or histopathologic findings. Reproductive organ weights, histopathology, and reproductive function were all unaffected in both males and females. There were no effects on reproductive, gestational, or offspring parameters, on pre- or postnatal survival, anogenital distance (F2 and F3 offspring, except for a significant increase of 0.04 mm for F2 female anogenital distance), or onset of puberty (except for a small delay in acquisition of preputial separation in F1 males, but not in F2 or F3 males).

75 ppm (approximately 5 mg/kg/day). There were no consistent or persistent treatment-related effects in F0, F1, F2, or F3 animals, nor in F1, F2, or F3 offspring.

4.5 - 0.015 ppm (approximately 0.3 - 0.001 mg/kg/day). There were no treatment-related effects in paired F0, F1, F2, or F3 animals, nor in F1, F2, or F3 offspring. Anogenital distance was significantly increased in F2 females at 0.015, 0.3, and 4.5 ppm. These findings were considered unrelated to treatment and of no biological or toxicological significance since there were no other effects on growth and development, including reproductive development. All of the values rounded to 1.0 mm and were not found in any other generation or in the males.

Overall Study. In conclusion, exposure to dietary BPA for three generations, one litter per generation, resulted in adult systemic toxicity only at 750 and 7500 ppm. Because of the lack of adverse effects below these doses, the adult systemic toxicity NOAEL (no observable adverse effect level) was established at 75 ppm (equivalent to approximately 5 mg/kg/day) in CD® (Sprague-Dawley) rats, under the conditions of this study. Similarly, because there were no treatment- or dose-related reproductive or postnatal toxicity effects below 7500 ppm, the reproductive and postnatal toxicity NOAELs were concluded to be 750 ppm (equivalent to approximately 50 mg/kg/day) in CD® (Sprague-Dawley) rats, under the conditions of this study. There were no effects of BPA dietary exposure on any adult or offspring parameter at 0.015, 0.3, 4.5, or 75 ppm (resulting in BPA intakes of approximately 0.001, 0.02, 0.3, or 5 mg/kg/day, respectively).

OBJECTIVES

This study was performed to evaluate the potential of BPA, administered in the feed to CD® rats, to produce alterations in male or female fertility, pregnancy and lactation, and growth and development of the offspring for three generations, one litter per generation.

MATERIALS AND METHODS

Test Material and Dietary Formulations

The test material was Bisphenol A (BPA; 4,4'-isopropylidene-2'diphenol; CAS No. 80-05-7) and was received from Acros Organics, NV, Fairlawn, NJ, through purchase from Fisher Scientific, Pittsburgh, PA, in one shipment. The Acros Organics catalog number was AC15824, and the Fisher Scientific catalog number was NC9472612. The test material was a white crystalline solid and was identified by the supplier as Lot No. B00701-38, with purity of 99.9% BPA, as provided on the Analytical Certificate of Analysis from the supplier, Acros Organics. The identity, purity, and stability of the test material were the initial responsibility of the Sponsor and were confirmed annually at RTI (RTI Project No. 64C-07158.000). The analytical characterization indicated the purity to be greater than 99% throughout the study. The shipment was received at RTI on January 26, 1998, in ten five-gallon polydrums and received the RTI Log Book No. 9028-06-01. The approximate net weight was 100 kg, and it was stored at room temperature under controlled conditions. Archive samples were taken from the bulk material upon arrival and stored frozen. At the completion of this study, any remaining bulk chemical was returned to the RTI repository under separate contract (RTI Project No. 64C-07158.000) with the Sponsor.

The basic diet was ground Purina Certified Rodent Chow® (No. 5002, PMI Feeds, Inc., St. Louis, MO). Dosed diet preparations were formulated as follows. All measurements of the test material were made by weight and were not corrected for purity of the active ingredient. Different amounts of BPA were weighed and dissolved in a fixed volume of acetone (CAS No. 67-64-1), as separate stock solutions, one for each dietary dose. As needed, the chemical was ground to a fine powder with a mortar and pestle. The volume of acetone was 1200 ml/premix for all doses (regardless of the final amount of feed made), except for the high dose mixes. The volume of acetone for the high dose mixes varied from approximately 1200-2600 ml (depending on the total amount of feed made, to keep the concentration below 300 mg BPA/ml acetone).

Each stock solution was then added to premix feed aliquots (fixed at 5 kg for all doses, except at 7 kg for the top dose) for each dietary dose level. Acetone was allowed to evaporate from each premix in a fume hood, the premix was sieved and/or blended as needed to assure homogeneity as it dried, and each premix feed aliquot was then added to an additional known amount of feed to formulate the total amount of feed needed and used to dose each group. Control diets were prepared by adding acetone alone to a premix feed aliquot and blending with additional diet after acetone evaporation, as described above (1200 ml/5 kg premix), but no test material added (see RTI SOP CSC-422 for mixing test materials in solvents for dosed feed formulations). All diets were mixed in a Lowe stainless steel, five cubic foot V-shell blender with neoprene rubber port gaskets (Lowe Industries, Inc., Crestwood, IL). Diets were prepared in a manner to ensure homogeneous distribution of the test material. Details of the procedures, including mixing times, were documented in the raw data. All premixes and diets were prepared at a frequency determined by the stability data (see below) and stored prior to use at approximately -20°C in appropriate containers (polyethylene tubs with fitted lids and polyethylene insert liners which did not contain BPA).

Homogeneity and stability of the test material in the animal diets, at (or above and below) the concentrations used in this study, were determined by RTI, using negative ion CI (chemical ionization) gas chromatography-mass spectrometry (GC-MS) analysis, as follows. The mass spectrometer (MS) was Hewlett Packard No. 5989A, the gas chromatograph (GC) was a Hewlett-Packard No. 5990 Series 2 with Hewlett-Packard Software ChemStation® Version B.07.00 (Unix) in use until September 19, 1999, and a Hewlett Packard HP 6890 Series GC system with a Hewlett Packard No. 5973 Mass Selective Detector, with Hewlett Packard Software Enhanced ChemStation® G1701B, Version A.01.00 (Windows NT), in use from September 19, 1999, until completion of the study. (There were typographical errors in the protocol in the designation of the initial HP GC number and in the designation of the ChemStation® software number and version. They are corrected in this report and are, therefore, "deviations from the protocol.") Prior to the performance of this study, aliquots of treated diets, encompassing the range of dietary concentrations to be used in this study, were used to assess the homogeneity (at 15 ppb and 10,000 ppm) and stability (15 ppb and 7500 ppm and 10,000 ppm) of the prepared diets. Homogeneity (one sample each, assayed in triplicate, from the left, right, and bottom of the V-shell blender) was established for the high and low

dietary concentrations. Stability analyses were also performed on dosed feed samples at 15 ppb and 10,000 and 7500 ppm at room temperature in open containers (to simulate cageside exposures) for 0, 4, 7, 9, and 14 days for the low dose and at 0 and 9 days for the high doses, and on dosed feed samples stored in sealed containers frozen (approximately -20°C) for 0, 7, 14, 33, and 55 days for 15 ppb; for 0, 9, 28, and 50 days for 7500 ppm; and for 0, 9, 29, and 48 days for 10,000 ppm. The cageside stability was at least nine days, and the frozen (approximately -20°C) stability was at least 50 days. The dosed feed for this study was formulated approximately monthly (to allow for formulation, analysis and, if necessary, reformulation and reanalysis), based on the storage stability of the frozen (-20°C) feed. Aliquots from all dosed feed levels for each formulation date were analyzed for BPA concentration (see Table 1 and Appendix I for details of methods and results). Feed formulations were stored frozen, and feed in the feed jars was changed at least every seven days. Standards for acceptable stability and dose level verification were: the mean of the analyzed samples for each dosed feed level and timepoint was within ±15% of nominal (for dose analyses) or of the time 0 mean value (for stability studies), and the % RSD (relative standard deviation) for the triplicate analyses did not exceed 10%. Standards for acceptable homogeneity were: the values within each sampling location were ±10% of nominal, the range of means was ≤15% of nominal, and the % RSD for triplicate assays from a single sampling location was ≤10%. Study animals were administered the control feed or dosed diets *ad libitum*, seven days per week, 24 hours per day, throughout the study.

Animals and Husbandry

Two hundred forty (240) virgin female rats and the same number of virgin male rats were ordered for the study in two shipments, 120/sex/shipment, for arrival eight days apart. Animals from the first shipment were designated as Cohort 1, and animals from the second shipment were designated as Cohort 2. Females were nulliparous and nonpregnant. There were 420 animals (210 males and 210 females) assigned to the study at the initiation of the treatment period (105/sex/cohort). Of the 120 animals/sex/shipment ordered, five/sex were for quality control, four/sex were used as sentinels for the monitoring of disease, six/sex were available to replace any animals inappropriate for use (e.g., outlying body weights, injury, clinical signs of illness), and 105/sex went on study.

For Cohort 1, 122 virgin female rats and 120 virgin male outbred albino CD® (Sprague-Dawley) rats (Crl:CD®[SD]BR; birth date August 17, 1998) were received from Charles River Breeding Laboratories, Raleigh, NC (males from Area R12, females from Area R04) on September 28, 1998, 42 days old (animals were ordered age critical with weights of males 151-175 g, females 126-150 g; weights provided by the vendor) upon arrival. For Cohort 2, 120 virgin females and 120 virgin males of the same rat strain were received from the same source (females from Area R04, males from Area R12) on October 5, 1998, 42 days old upon arrival (date of birth August 24, 1998), males 151-175 g and females 126-150 g (with weights provided by the vendor) upon arrival. (The actual dates of all major phases of the study are presented in Text Table A.) These animals were quarantined for approximately one week upon arrival, during which time they were weighed, examined by a veterinarian, and representative animals were subjected to fecal examination and serum viral antibody analysis. For serum viral antibody analysis, within one day after receipt, five rats/sex/cohort were arbitrarily chosen from the shipment of animals, sacrificed, and blood collected for assessment of viral antibody status. Heat-inactivated serum was sent to MA Bioservices, Inc. (Rockville, MD) for their Level 1 Rat Antibody Screen. The viral screen consisted of evaluation for the presence of antibodies against the following: Toolan H-1 virus (H-1), Sendai virus, Pneumonia virus of mice (PVM), Rat coronavirus/sialodacryoadenitis (RCV/SDA), Kilham rat virus (KRV), CAR Bacillus, and Mycoplasma pulmonis (*M. pul.*). Results of the physical examination, serology, and parasitology were negative for signs of infectious disease; the animals were considered to be in good health and suitable for use in this study.

Text Table A. Study Schedule

Event	Dates	
	Cohort 1	Cohort 2
Animals arrived at RTI	09/28/98	10/05/98
F0 prebreed (10 weeks)	10/06/98 - 12/15/98	10/14/98 - 12/23/98
F0 vaginal cytology (21 days)	11/25/98 - 12/15/98	12/03/09 - 12/23/98
F0 mating (2 weeks)	12/15/98 - 12/29/98	12/23/98 - 01/06/99
F0 gestation (~5 weeks)	12/16/98 - 01/21/99	12/24/98 - 01/29/99
F0 lactation (~5 weeks)	01/06/99 - 02/11/99	01/13/99 - 02/19/99

(continued)

Text Table A (continued)

Event	Dates	
	Cohort 1	Cohort 2
F0 male necropsy	01/25/99 - 01/27/99	02/01/99 - 02/03/99
F0 female necropsy	01/28/99 - 02/11/99	02/04/99 - 02/19/99
F1 weaning (~2 weeks)	01/28/99 - 02/11/99	02/04/99 - 02/19/99
F1 pnd 21 necropsy (~2 weeks)	01/28/99 - 02/11/99	02/04/99 - 02/19/99
F1 prebreed (10 weeks)	02/02/99 - 04/13/99	02/17/99 - 04/28/99
F1 vaginal patency (pnd 22 - ~pnd 40)	01/28/99 - 02/21/99	02/05/99 - 03/07/99
F1 preputial separation (pnd 35 - ~pnd 50)	02/10/99 - 03/03/99	02/18/99 - 03/17/99
F1 vaginal cytology (21 days)	03/24/99 - 04/13/99	04/08/99 - 04/28/99
F1 mating (2 weeks)	04/13/99 - 04/27/99	04/28/99 - 05/12/99
F1 gestation (~5 weeks)	04/14/99 - 05/16/99	04/29/99 - 06/02/99
F1 lactation (~5 weeks)	05/05/99 - 06/06/99	05/20/99 - 06/23/99
F1 male necropsy	05/24/99 - 05/27/99	06/07/99 - 06/10/99
F1 female necropsy	05/26/99 - 06/06/99	06/10/99 - 06/23/99
F2 weaning (~2 weeks)	05/26/99 - 06/06/99	06/10/99 - 06/23/99
F2 pnd 21 necropsy (~2 weeks)	05/26/99 - 06/06/99	06/10/99 - 06/23/99
F2 prebreed (10 weeks)	06/08/99 - 08/17/99	06/23/99 - 09/01/99
F2 vaginal patency (pnd 22 - ~pnd 40)	05/27/99 - 06/25/99	06/11/99 - 07/12/99
F2 preputial separation (pnd 35 - ~pnd 50)	06/09/99 - 07/05/99	06/24/99 - 07/22/99
F2 vaginal cytology (21 days)	07/28/99 - 08/17/99	08/12/99 - 09/01/99
F2 mating (2 weeks)	08/17/99 - 08/31/99	09/01/99 - 09/15/99
F2 gestation (~5 weeks)	08/18/99 - 09/22/99	09/02/99 - 09/29/99
F2 lactation (~5 weeks)	09/08/99 - 10/13/99	09/23/99 - 10/20/99
F2 male necropsy	09/27/99 - 09/30/99	10/11/99 - 10/14/99
F2 female necropsy	09/29/99 - 10/13/99	10/14/99 - 10/20/99
F3 weaning (~2 weeks)	09/29/99 - 10/13/99	10/14/99 - 10/20/99
F3 pnd 21 necropsy (~2 weeks)	09/29/99 - 10/13/99	10/14/99 - 10/20/99
F3 prebreed (10-12 weeks)	10/19/99 - 12/28/99	10/20/99 - 01/06/00
F3 vaginal patency (pnd 22 - ~pnd 40)	09/30/99 - 11/01/99	10/15/99 - 11/08/99
F3 preputial separation (pnd 35 - ~pnd 50)	10/13/99 - 11/11/99	10/28/99 - 11/18/99
F3 vaginal cytology (21 days)	12/08/99 - 12/28/99	12/09/99 - 12/29/99
F3 male necropsy (99 to 111 days old)	12/28/99 - 12/30/99	01/03/00 - 01/06/00
F3 female necropsy (113 to 124 days old)	01/10/00 - 01/13/00	01/17/00 - 01/20/00

At the end of the quarantine period, all remaining animals were weighed and ordered from lightest to heaviest by sex. Any animals with clinical signs and the lightest and/or heaviest animals were removed (by the Study Director) to obtain the 210/sex (105/sex/cohort) and be the F0 animals. These study animals were distributed into treatment groups by stratified randomization procedures as follows. The animals were distributed by sex and body weight into groups or strata with seven rats per sex per stratum (equal to the number of treatment groups). The rats within each stratum were then randomly assigned, one to each treatment group, using a random number table.

After the selection of quality control and study animals, four of the remaining (nonselected) rats/sex/cohort were used as sentinels to monitor the health status of the study animals. They were housed in the study room(s), singly housed in stainless steel wire mesh hanging cages, with feed and automatic water available *ad libitum*. They were examined once daily by cageside observation for morbidity or mortality at the same time as the clinical observations or morbidity/mortality checks for the study animals. The clinical condition of sentinel animals was recorded only if an animal was moribund or found dead. If a sentinel animal was terminated moribund, blood was collected at termination and serum samples frozen. During the F1, F2, and F3 weanling necropsy periods and at the termination of the retained F3 animals, a maximum of one surviving sentinel animal per sex per necropsy period was terminated, blood samples collected, and serum samples prepared. All sentinel serum samples were submitted to MA Bioservices (Rockville, MD) for serological evaluation; all samples were negative for the rat viral antibody screen.

F0 animals were identified prior to the start of exposures by uniquely numbered ear tags. Selected F1, F2, and F3 weanlings were identified by uniquely numbered eartags. Pups prior to weaning were not uniquely identified. The method of identification, uniquely numbered eartags, and the animal numbers were documented in the study records. The animal numbers for this study were established with even numbers for females and odd numbers for males.

All weanling and adult animals were euthanized by CO_2 asphyxiation. This includes animals not used in the study; F0, F1, F2, and F3 adults; and F1, F2, and F3 weanlings at scheduled sacrifice; those weanlings not selected for parents or necropsy; and/or any sacrificed moribund. All pups sacrificed moribund during the lactation period were euthanized by CO_2 asphyxiation and decapitation. F1 and F2 pups culled on pnd 4 were sacrificed by decapitation.

Animals received with the initial shipment, but not used in the study, were euthanized or removed from the study room prior to the start of the treatment period and used for methods development and training of the RTI staff. Records were kept documenting the fate of all animals received for the study.

The experiment was carried out under standard laboratory conditions. The animals were individually housed upon arrival, during the acclimation period, and upon the initiation of the treatment period in stainless steel wire mesh hanging cages (11.5"x9"x7" high), in stainless steel racks with pans, and all stainless steel piping was used for the automatic watering system for all animals during prebreed and holding periods, for the males after mating, and the females when their litters were weaned, until termination. Some of the singly-housed F0 animals at the start of the prebreed exposure period had difficulty adjusting to the automatic watering system, as evidenced by dehydration, as did selected F1, F2, and F3 postweanlings at the high dose. These animals were assisted by placing glass petri dishes with water in the cages and gently putting the animals against the automatic watering system nipples at the back of the hanging cages. The affected animals then rapidly adjusted to the automatic watering. (This initial period of adjustment did not affect the study design, conduct, or conclusions.) During the mating period, mating pairs (one male:one female) and sperm/plug positive females, from gestational day (gd) 0 until weaning of their litters on pnd 21, were housed in solid-bottom polypropylene cages (8"x19"x10.5" high) with stainless steel wire lids (Laboratory Products, Rochelle Park, NJ), with Sani-Chip® cage bedding (P. J. Murphy Forest Products, Inc., Montville, NJ) and glass water bottles with propylene screw-cap lids and stainless steel sipper tubes.

Study animals were housed two per cage (one male:one female from the same dose level) during the mating periods. Females were caged individually once they had been successfully mated (or at the end of the mating period). Females were housed with their litters throughout the lactation period. At all times, the animals were handled, cared for, and used in compliance with the NRC Guide (NRC, 1996).

All animals were housed in the RTI Animal Research Facility (ARF) following arrival at RTI and for the duration of the study. Temperature and relative humidity in the ARF animal rooms were continuously monitored, controlled, and recorded using an automatic system (Siebe/Barber-Colman Network 8000 System with Signal Software Versions 4.1 [until December 1999] and 4.4.1 [Y2K compliant upgrade completed in December 1999], Siebe

Environmental Controls [SEC]/Barber-Colman Company, Loves Park, IL). The target environmental ranges were 64-79°F (18-26°C) for temperature and 30-70% relative humidity, with a 12-hour light cycle per day (NRC, 1996). The specific animal rooms used were documented in the study records and are as follows:

F0 Animals

(1) Room 200: Cohort 1 F0 females from September 28, 1998 (date of arrival at RTI), Cohort 2 females from October 13, 1998 (moved from Room 202) -- December 15, 1998 (Cohort 1) and December 23, 1998

(2) Room 201: Cohort 1 F0 males from September 28, 1998 (date of arrival at RTI), Cohort 2 males from October 13, 1998 (moved from Room 203) -- December 15, 1998 (Cohort 1) and December 23, 1998 (Cohort 2) -- from December 16, 1998 to January 27, 1999 (Cohort 1 males through necropsy; January 25-27, 1999), from December 24, 1998 to February 3, 1999 (Cohort 2 males through necropsy, February 1-3, 1999)

(3) Room 202: F0 females October 5, 1998 (date of arrival at RTI) -- October 12, 1998 (quarantine); moved to Room 200 on October 13, 1998

(4) Room 203: Cohort 2 F0 males from October 5, 1998 (date of arrival at RTI) -- October 12, 1998 (quarantine); moved to Room 201 on October 13, 1998

(5) Room 301: Cohort 2 mating, gestation, and lactation (December 23, 1998 mating began - February 16, 1999 last day of weaning); Cohort 2 F1 weanlings transferred to Rooms 200 and 202

(6) Room 303: Cohort 1 mating, gestation, and lactation (December 15, 1998 mating began - February 2, 1999 last day of weaning); Cohort 1 F1 weanlings moved to Rooms 200 and 202

F1 Animals

(1) Room 200: F1 female weanlings (transferred from Rooms 301 and 303) on January 27, 1999 (Cohort 1) and February 4, 1999 (Cohort 2) -- April 13, 1999 (Cohort 1) and April 28, 1999 (Cohort 2); F1 females moved to Rooms 301 and 303 for mating

(2) Room 202: F1 male weanlings (transferred from Rooms 301 and 303) on January 27, 1999 (Cohort 1) and February 4, 1999 (Cohort 2) -- April 13, 1999 (Cohort 1) and April 28, 1999 (Cohort 2), F1 males moved to Rooms 301 and 303 for mating. F1 males returned to Room 202 after mating, starting on April 14, 1999 (Cohort 1) and April 29, 1999 (Cohort 2) until necropsy; last day of necropsy May 27, 1999 (Cohort 1) and June 10, 1999 (Cohort 2)

(3) Room 301: Cohort 1 F1 mating, gestation, and lactation from April 13, 1999 (start of mating) -- June 6, 1999 (last day of F2 weaning); F2 weanlings were transferred to Rooms 200 and 201

(4) Room 303: Cohort 2 F1 mating, gestation, and lactation from April 28, 1999 (start of mating) -- June 23, 1999 (last day of F2 weaning); F2 weanlings were transferred to Rooms 200 and 201

F2 Animals

(1) Room 200: F2 female weanlings for prebreed transferred from Room 301 on May 26, 1999 (Cohort 1) and from Room 303 on June 10, 1999 (Cohort 2) -- August 17, 1999 (Cohort 1) and September 1, 1999 (Cohort 2); moved to Rooms 301 and 303 for mating

(2) Room 201: F2 male weanlings for prebreed, transferred from Room 301 on May 26, 1999 (Cohort 1) and from Room 303 on June 10, 1999 (Cohort 2) -- August 17, 1999 (Cohort 1) and September 1, 1999 (Cohort 2); moved to Rooms 301 and 303 for mating. F2 males returned to Room 201 after mating starting on August 18, 1999 (Cohort 1) and September 2, 1999 (Cohort 2) until necropsy; last day of necropsy September 30, 1999 (Cohort 1) and October 14, 1999 (Cohort 2)

(3) Room 301: Cohort 1 F2 mating, gestation, and lactation from August 17, 1999 (start of mating) -- October 13, 1999 (last pnd 21); F3 weanlings moved to Rooms 200 and 202

(4) Room 303: Cohort 2 F2 mating, gestation, and lactation from September 1, 1999 (start of mating) -- October 20, 1999 (last pnd 21); F3 weanlings were moved to Rooms 200 and 202.

F3 Animals

(1) Room 200: F3 female weanlings from September 29, 1999 (first day of weaning) -- January 13, 2000 (last day of necropsy; Cohort 1) and from October 14, 1999, first day of weaning -- January 20, 2000, last day of necropsy (Cohort 2).

(2) Room 202: F3 male weanlings from September 29, 1999, first day of weaning -- December 30, 1999, last day of necropsy (Cohort 1) and from October 14, 1999, first day of weaning -- January 6, 2000, last day of necropsy (Cohort 2)

Except for a few, brief, minor excursions outside the target range in relative humidity, all animal rooms were in range throughout the study. There were no excursions outside the target range in temperature in any animal room throughout this study. The brief and minor excursions in relative humidity did not affect the design, conduct, or conclusions of this study (see Protocol Deviations, Section A, at the end of this volume, No. I). Purina Certified Ground Rodent Chow (No. 5002, PMI Feeds, Inc., St. Louis, MO; batch numbers documented in the study records) was available *ad libitum*. The analyses of each feed batch for nutrient levels and possible contaminants were performed by the supplier, examined by the Study Director, and maintained in the study records. For all feed batches, nutrient levels were at or above, and contaminant levels were below the certified levels, and suitable for use.

Water (tap water; City of Durham, Department of Water Resources, Durham, NC) was available *ad libitum* by an automatic watering system (Edstrom Industries, Inc., Waterford, WI) during the time the animals were in hanging cages, and by glass water bottles with polypropylene

screw-on caps with stainless steel sipper tubes during the time the females were in polypropylene solid-bottom cages. Contaminant levels of the Durham City water were measured monthly by the supplier (and reported annually), and tap water was analyzed at least annually (per EPA specifications) by Balazs Analytical Laboratory, Sunnyvale, CA. The analyses were examined by the Study Director and maintained in the study records. At all times, the tap water contaminants were below the maximum contaminant levels defined for potable (drinking) water.

Study Design

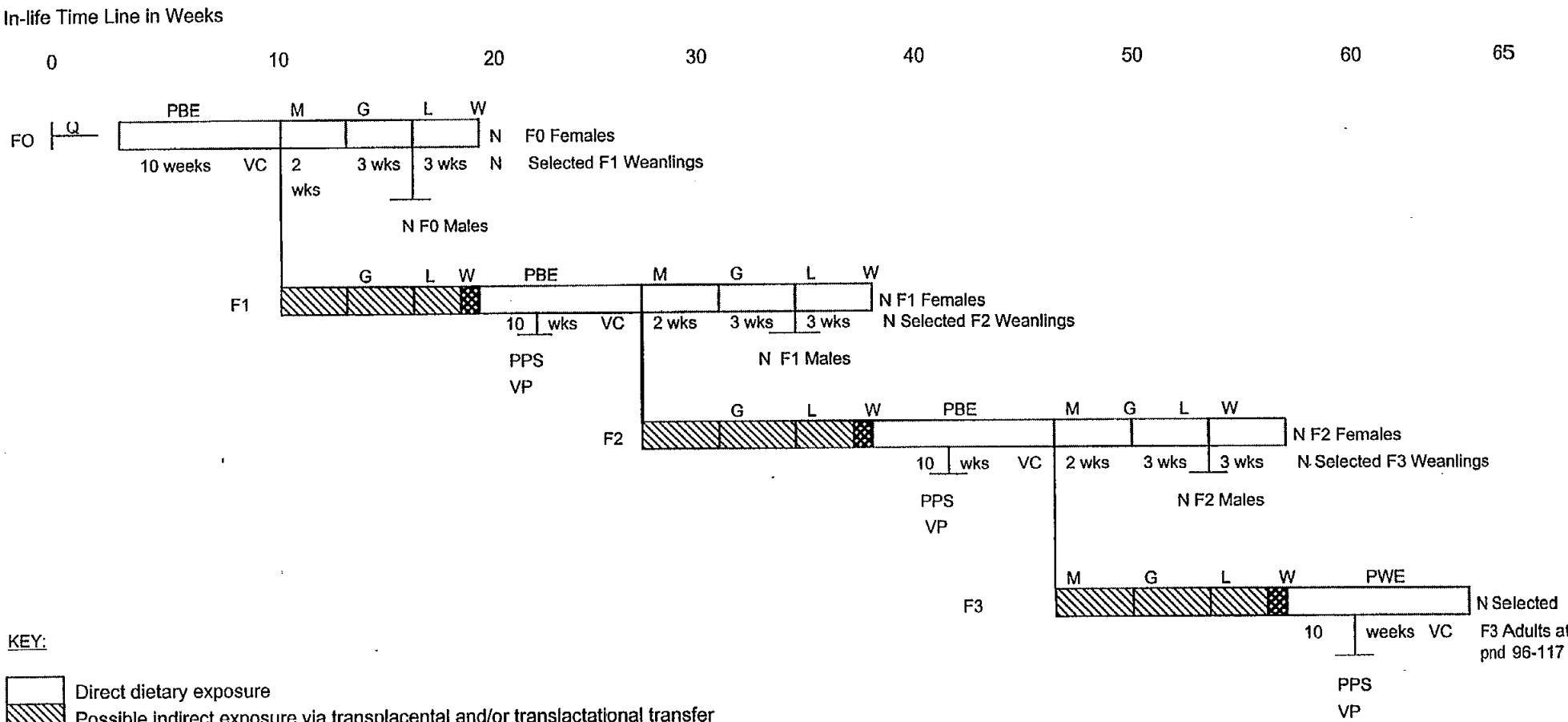
A graphic representation of the study design is presented in Text Figure A. The study began with 30 males/group and 30 females/group, to yield at least 20 pregnant females/group at or near term, and seven groups (see Text Table B). The target dietary doses were selected to provide BPA intakes to encompass the ranges of low BPA doses at which male offspring prostate weights were reported to be significantly increased, and testis weight and daily sperm production were reported to be significantly reduced, 2 and 20 µg/kg/day (in pregnant mice by oral administration; Nagel et al., 1997; vom Saal et al., 1998). The doses were also chosen to include the range at which other compounds (e.g., DES) administered in the drinking water to pregnant and lactating rats were reported to cause reductions in testis size and sperm production in adult male offspring, 0.01 - 1.0 ppm (Sharpe et al., 1995). In addition, to conform with published regulatory guidelines, the highest dose chosen was expected to produce a reduction in body weight or other indications of systemic toxicity. The target dietary concentrations were based on the targeted intakes for the female rats, at the request of the Sponsor.

Text Table B. Organization and Target Dietary Concentrations and Intakes

Group No.	No. Animals		Dosage Level of Test Material (ppm)	Target Female Rat Intake[a]		Target Male Rat Intake[b]	
	Male	Female		µg/kg/day	mg/kg/day	µg/kg/day	mg/kg/day
1	30	30	0.0	0.00	0.00	0.00	0.00
2	30	30	0.015	1.00	0.001	0.90	0.00090
3	30	30	0.3	20.0	0.02	18.0	0.018
4	30	30	4.5	300	0.30	270	0.27
5	30	30	75	5000	5	4500	4.5
6	30	30	750	50,000	50	45,000	45
7	30	30	7500	500,000	500	450,000	450

a Assumptions for females: 300 g (0.3 kg) body weight and 20 g/day feed consumption.
b Assumptions for males: 500 g (0.5 kg) body weight and 30 g/day feed consumption.

Text Figure A. BPA Three-Generation Study Design



KEY:

Direct dietary exposure

Possible indirect exposure via transplacental and/or translactational transfer

Possible indirect and direct exposure during last week of lactation (pups self-feeding and nursing)

Q =	Quarantine (1 week)	W =	Weaning (on pnd 21)
PBE =	Pre-breed exposure period (10 weeks)	N =	Necropsy
PWE =	Postwean exposure period (10 weeks for F3 offspring, until pnd 96-117)	M =	Mating (2 weeks)
G =	Gestation (3 weeks; 22 ± 1 days)	L =	Lactation (3 weeks)
PPS =	Preputial separation evaluated in offspring males (pnd 35-acquisition)		
VP =	Vaginal patency evaluated in offspring females (pnd 22-acquisition)		
VC =	Vaginal cytology (evaluated in females during last three weeks of prebreed/postwean period)		

Parental F0, F1, and F2 Animals and Retained F3 Offspring

Observations for mortality were made twice daily (a.m. and p.m.), and the general condition of all animals was checked daily. Clinical examinations were conducted and recorded at least once daily throughout the course of the study. This record included the time of onset, degree, and duration of symptoms. These cage-side observations included, but were not limited to, changes in skin and fur, eyes, mucous membranes, respiratory and circulatory system, autonomic and central nervous system, somatomotor activity, and behavior pattern.

The body weights of the male F0, F1, and F2 rats were determined and recorded initially and weekly through mating. The body weights of female F0, F1, and F2 rats were recorded in the same manner until confirmation of mating. During gestation, females were weighed on gd 0, 7, 14, and 20. F0, F1, and F2 dams producing litters were weighed on lactational days (pnd) 0, 4, 7, 14, and 21. The body weights of retained F3 offspring (both sexes) were also recorded initially and weekly until termination. Body weight gains were computed.

Feed consumption measurements were recorded for all F0, F1, and F2 parental animals every seven days, each time the feed was changed, throughout the prebreed treatment periods. Feed consumption measurements were also recorded for retained F3 offspring every seven days, each time the feed was changed, until termination on pnd 104±8 for males and 108±9 for females. Feed consumption collection periods corresponded with the collection of the animals' weekly body weight data and were employed to calculate their actual exposure to the test chemical as mg (or µg) of test article/kg of body weight/day. During pregnancy of F0, F1, and F2 females, feed consumption was recorded for gd 0-7, 7-14, and 14-20. Feed consumption was not measured during the period of cohabitation, since two adult animals (one male and one female) were in the same cage, but consumption was visually monitored and the feed changed as necessary. During lactation of F1, F2, and F3 litters, maternal feed consumption was measured for pnd 0-4, 4-7, 7-14, and 14-21, although maternal feed consumption after pnd 14 was confounded by the contribution from the pups, since pups are self-feeding by this time. Feed was changed at least weekly throughout the study.

Estrous cyclicity and normality were evaluated by vaginal smears daily for all F0, F1, and F2 females during the last three weeks of the prebreed exposure period and during the two-week mating period. Estrous cyclicity and normality (based on presence and duration of estrous

stages) were also evaluated by daily vaginal smears for retained F3 female offspring during the last three weeks of the postwean exposure period.

Animals of the F0 generation were approximately seven weeks of age at the commencement of treatment. They were administered dosed feed at their respective formulations for ten weeks prior to mating (i.e., until they were approximately 17 weeks of age). The animals were then mated on the basis of one male to one female in solid-bottom polypropylene cages with bedding and water bottle. Mating pairs were selected randomly within each dose group, for each cohort, for a period of up to 14 days, with no change in mating partners. Females were examined daily during the cohabitation period for the presence of a copulation plug and/or sperm in the vaginal tract and for estrous cyclicity. The observation of a retained (in the vaginal tract) copulation plug and/or vaginal sperm was considered evidence of successful mating. The day vaginal sperm/plug were observed was designated gd 0 (Hafez, 1970). Once vaginal sperm or plug had been observed, the male and female from that mating pair were individually housed, and the female was transferred to a clean, solid-bottom polypropylene cage with bedding and water bottle. Any female which did not show evidence of successful mating after 14 days of cohabitation was weighed weekly and feed consumption measured weekly until termination. If a female without a confirmed gd 0 was, in fact, pregnant and delivered a litter, her lactational information was collected as described below.

Beginning on the first gd 20 for each cohort, all females in that cohort were observed twice daily (a.m. and p.m.) for evidence of littering. Offspring were designated the F1 generation. The dams were allowed to rear their young to pnd 21. On pnd 21, each F1 litter was weaned. When each F1 litter had reached pnd 21, at least one male and one female pup, if possible, were randomly selected to produce the F2 generation. Each litter was represented at least once per sex, if possible, until 30/sex/treatment group (15/sex/group/cohort) was attained. These selected animals were then transferred to wire-bottom hanging cages with automatic watering system and exposed to dosed feed at the same concentration of BPA as their parents. In addition, three F1 weanlings per sex per litter, if possible, were randomly selected, necropsied, and examined for external and visceral alterations (with special attention on the prepubertal reproductive organs), with gross lesions retained in buffered neutral 10% formalin, and brain, spleen, liver, thymus (both sexes) and testes, epididymides and seminal vesicles (males) and ovaries, uterus with cervix, and vagina (females) weighed and retained in fixative (all organs but

the testes in buffered neutral 10% formalin, testes in Bouin's fixative for approximately 24 hours, then in 70% ethanol). Any remaining nonselected F1 weanlings were examined grossly, euthanized, and discarded. Nonretained organs and carcasses were also discarded.

Male and female F1 animals selected for mating were administered dosed feed at their respective formulations *ad libitum* seven days per week for at least ten weeks. During the F1 prebreed exposure period, each F1 female was observed, beginning on pnd 22 for vaginal patency. The number of females with the vagina patent (open) was recorded daily until all females had this response. The body weight of each female was recorded on the day of acquisition of vaginal patency. In addition, during this time period, each F1 male was observed for cleavage of the balanopreputial gland (preputial separation) beginning on pnd 35. The characteristic is present when the prepuce can be completely retracted to expose the glans penis. The number of males with this separation were recorded daily until all males had this response. The body weight of each male was recorded on the day of acquisition of preputial separation. The F1 animals were approximately 13 to 15 weeks of age at the initiation of the mating period. Estrous cyclicity was evaluated for the last three weeks of the prebreed exposure period and for the two-week mating period for F1 females (as described above for F0 females). The F1 animals were mated as described above for the F0 animals within cohorts, including, but not limited to, pairing one male:one female, and cohabitation for 14 days or until vaginal sperm or a vaginal copulation plug was observed, whichever came first (with no change in pairing). There were no brother-sister matings. The offspring from F1 parents were designated the F2 generation.

At weaning on pnd 21, at least one male and one female F2 pup per litter were randomly selected to produce the F3 generation. Each litter was represented at least once per sex, if possible, until 30/sex/treatment group (15/sex/group/cohort) were attained. These selected animals were then transferred to wire-bottom, hanging cages with automatic watering system and exposed to dosed feed at the same concentration of BPA as their parents. In addition, three F2 weanlings per sex per litter, if possible, were randomly selected, necropsied, and subjected to a gross (external and visceral) necropsy examination (with special attention on the prepubertal reproductive organs), with gross lesions retained in buffered neutral 10% formalin, and brain, spleen, liver, thymus (in both sexes) and testes, epididymides and seminal vesicles (males) and ovaries, uterus with cervix, and vagina (females) weighed and retained in fixative (all organs but testes in buffered neutral 10% formalin; testes in Bouin's fixative for approximately 24 hours,

then in 70% ethanol), as described above. Any remaining nonselected F2 weanlings were examined grossly, euthanized, and discarded.

Male and female F2 animals selected for mating were administered dosed feed at their respective formulations *ad libitum* seven days per week for at least ten weeks. During the F2 prebreed exposure period, each F2 female was observed, beginning on pnd 22, for vaginal patency. The number of females with the vagina patent was recorded daily until all females had this response. The body weight of each female was recorded on the day of acquisition of vaginal patency. In addition during this time period, each F2 male was observed for cleavage of the balanopreputial gland (preputial separation), beginning on pnd 35. The characteristic is present when the prepuce can be completely retracted to expose the glans penis. The number of males with this separation was recorded daily until all males had this response. The body weight of each male was recorded on the day of acquisition of preputial separation.

The F2 animals were approximately 13 to 15 weeks of age at the initiation of the mating period. Estrous cyclicity was evaluated for the last three weeks of the prebreed exposure period and for the two-week mating period for F2 females (as described above for F0 and F1 females). The F2 animals were mated as described above for the F0 and F1 animals, including, but not limited to, pairing one male:one female within groups within cohorts, and cohabitation for 14 days or until vaginal sperm or a vaginal copulation plug was observed, whichever came first (with no change in pairing). There were no brother-sister matings. The offspring from F2 parents was designated the F3 generation. At weaning on pnd 21, at least one male and one female F3 pup per litter was randomly selected, 30/sex/group (15/sex/group/cohort), to be held for approximately ten weeks, with exposure continuing until they were 104±8 days of age for males and 108±9 days for females (postwean exposure period). In addition, three F3 weanlings per sex per litter, if possible, were randomly selected and subjected to a gross (external and visceral) necropsy examination (with special attention on the prepubertal reproductive organs), with gross lesions retained in buffered neutral 10% formalin, and brain, spleen, liver, thymus (in both sexes) and testes, epididymides and seminal vesicles (males) and ovaries, uterus with cervix, and vagina (females) weighed and retained in fixative (all organs but testes in buffered neutral 10% formalin; testes in Bouin's fixative for approximately 24 hours, then in 70% ethanol), as described above. Any remaining nonselected F3 weanlings were examined grossly, euthanized, and discarded.

Male and female F3 animals selected for postwean exposure, 30/sex/group (15/sex/group/cohort), were transferred to wire-bottom cages and automatic watering system on pnd 21, and were administered dosed feed at their respective formulations *ad libitum* seven days per week for approximately ten weeks (until they were 104±8 days of age for males and 108±9 days of age for females). During this F3 postwean exposure period, each F3 female was observed beginning on pnd 22 for vaginal patency. The number of females with the vagina patent was recorded daily until all females had this response. The body weight of each female was recorded on the day of acquisition of vaginal patency. In addition during this time period, each F3 male was observed for cleavage of the balanopreputial gland (preputial separation) beginning on pnd 35. The characteristic is present when the prepuce can be completely retracted to expose the glans penis. The number of males with this separation was recorded daily until all males had this response. The body weight of each male was recorded on the day of acquisition of preputial separation.

Offspring Animals (F1, F2, and F3)

All pups were counted, sexed, and examined as soon as possible on the day of birth (designated pnd 0) to determine the number of viable and stillborn members of each litter. Thereafter, litters were evaluated for survival on pnd 4, 7, and 14 and at weaning (pnd 21).

On day 4 after birth, the size of each litter was adjusted by eliminating extra pups by random selection from litters with more than ten pups to yield ten pups with, as near as possible, five males and five females per litter. Natural litters with ten or fewer pups were not culled. All culled pups were sacrificed by decapitation and discarded. Survival indices were calculated on pnd 0, 4, 7, and 14 and at weaning on pnd 21.

All live pups were individually counted, sexed externally (by visual examination of anogenital distance; the anogenital distance was not recorded unless triggered; see below), weighed, and examined grossly at birth (pnd 0) at pnd 4, 7, and 14 and at weaning (pnd 21). The body weights and sexes were recorded on an individual basis, but the pups were not uniquely identified at this stage. The presence or absence of retained nipples on the ventrum were evaluated and documented in offspring males at approximately pnd 11-13. Since there were treatment-related effects in F1 and F2 sexual maturation (i.e., a delay in the time to acquisition of vaginal patency in F1 and F2 females and preputial separation in F1 and F2 males), anogenital distance was measured (using a dissecting microscope, ocular micrometer, and eye piece grid).

Therefore, the anogenital distance, sex, and body weight of each F2 and F3 pup were recorded on pnd 0.

All pups were examined for physical abnormalities at birth and throughout the preweaning and postwean period. All pups dying during lactation were necropsied, when possible, to investigate the cause of death.

At weaning (pnd 21), at least one female and one male (whenever possible) from each F1 and F2 litter were selected on a random basis to become parents of the next generation (F1 parents to produce F2 litters and F2 parents to produce F3 litters), for a total of 30/sex/dosage level. In addition, selection was made, as described above, for F3 weanlings, 30/sex/group, for the postwean exposure period. All pups were available for selection. The parentage of each weanling was ascertained to avoid brother-sister matings. In addition, three F1, F2, and F3 weanlings per sex per litter, if possible, were randomly selected and subjected to a gross necropsy (external and visceral) examination, with gross lesions retained in fixative, and brain, liver, spleen, thymus (of both sexes), and testes, epididymides and seminal vesicles (males), and ovaries, uterus with cervix and vagina (females) weighed and retained in fixative. Any remaining F1, F2, and F3 offspring were examined for gross external abnormalities, euthanized, and discarded.

Any pup which appeared moribund or which died during lactation was necropsied, when possible, to investigate the cause of death, and preserved in buffered neutral 10% formalin. Organs were not weighed for these animals.

All individual animal in-life data are presented in Volumes V, VI, VII, and VIII.

Necropsy and Histology

All F0, F1, and F2 parental animals in all groups (in all three generations) and retained adult F3 offspring were subjected to a complete gross necropsy, with selected organs weighed (see below). On the day of necropsy, prior to euthanasia, all F0, F1, and F2 parental females and retained F3 females were subjected to vaginal lavage, with slides stained with Toluidine Blue, and retained to determine (and record) the stage of estrus at termination. The uterus of each parental F0, F1, and F2 female was examined, and the number of nidation scars (implantation sites) was documented. The tissues listed below were retained in buffered neutral 10% formalin (except the testes, for which one per male was retained in Bouin's fixative and transferred to

70% ethanol after 24 hours, and one was retained frozen; see below). All fixed tissues were embedded in paraffin except the testes (one per male), which were embedded in glycol methacrylate (GMA). The gross necropsy included examination of the external surfaces; all orifices; carcass; the thoracic, abdominal, and pelvic cavities and their viscera; and cervical tissues and organs. Full histopathology of the organs listed below was performed for all control F0, F1, and F2 animals selected for mating, all control retained F3 adult offspring, and ten randomly selected parental F0, F1, and F2 animals and retained F3 adult offspring from all remaining dose groups. Additionally, reproductive organs of the animals in any dose groups suspected of reduced fertility (e.g., those that failed to mate, conceive, sire, or deliver healthy offspring, or for which estrous cyclicity or sperm number, motility, or morphology were affected) were subjected to histopathological evaluation; the identity of these animals was documented in the study records. Specific attention was focused on the examination of the parental reproductive organs as follows.

Male Reproductive Tract. In addition to identification of gross lesions, such as atrophy or tumors, testicular histopathological examination was conducted to identify treatment-related effects, such as retained spermatids, missing germ cell layers or types, multinucleated giant cells, or sloughing of spermatogenic cells into the lumen. Examination of the intact epididymis included the caput, corpus, and cauda by evaluation of a longitudinal section, and was conducted in order to identify such lesions as sperm granulomas, leukocytic infiltration (inflammation), aberrant cell types within the lumen, or the absence of clear cells in the cauda epididymal epithelium (Russell et al., 1990).

Sacrifice of the F0, F1, and F2 parental males occurred after the completion of the delivery period. Sacrifice of retained F3 male offspring occurred on pnd 105±6 (pnd 99-111). At the time of sacrifice of F0, F1, and F2 parental males and retained F3 adult male offspring, one testis from each male was frozen at approximately -20°C for subsequent enumeration of testicular homogenization-resistant spermatid heads. (The other testis per male was retained in Bouin's fixative and then 70% ethanol for histopathologic examination; see above.) In addition, one cauda epididymis was immediately removed, weighed, and seminal fluid from the cauda assessed for sperm number, motility, and morphology. (The other epididymis per male was retained in fixative for histopathologic examination; see below.) Sperm motility (motile and progressively motile) was assessed immediately after necropsy; number and morphology (at least 200 sperm per

male, if possible) were evaluated at a later date using appropriately retained sperm samples. Sperm motility and number were assessed using an HTM-IVOS Automated Sperm Analysis System (Version 10.8S; Hamilton-Thorne Research, Beverly, MA). Sperm morphology was evaluated manually. The percent abnormal sperm was based on counting 500 sperm per male, if possible, and identification of the number of sperm with blunt hook heads, "banana" heads, amorphous heads, pinheads, two heads and/or two tails, and heads only, divided by the number of abnormal plus the number of normal sperm (x100) (modified from the methodology of Seed et al., 1996).

Female Reproductive Tract. The postlactational ovary contained primordial (small), growing, and antral follicles, as well as the large corpora lutea of lactation. Ten ovarian sections were taken at least 100 µm apart from the inner third of each ovary. Examination included enumeration of the total number of primordial follicles from these 20 sections per female for comparison with control ovaries. Examination also confirmed the presence or absence of small, growing, and antral follicles and corpora lutea in comparison with control ovaries (Heindel et al., 1989).

Sacrifice of the F0, F1, and F2 maternal animals occurred after F1, F2, and F3 litters had been weaned. Sacrifice of retained F3 female adult offspring occurred on pnd 118±6 (pnd 113-124). The gross lesions retained in fixative from the F1, F2, and F3 weanling examinations and the weighed organs retained in fixative, from up to three/sex/litter, were subjected to histopathologic examination with emphasis on reproductive organs, if appropriate.

Histopathology. Histopathologic evaluation was conducted on tissues from parental animals and retained F3 adult offspring (from all animals in the control group and from ten/sex/group from the remaining groups, selected randomly), as specified below:

ovaries	testis (1)	vagina
epididymis w/ contents & fluids (1)	uterus with oviducts (2)	seminal vesicles (2)
cervix	prostate	pituitary
preputial glands w/ fluid (2)	adrenal glands (2)	spleen
liver	kidneys (2)	coagulating glands w/ fluids (2)

other tissues with gross lesions identified as being potentially treatment related

Any of the above organs or tissues showing gross alterations were evaluated microscopically in additional animals, in the dose groups for which ten/sex were initially examined. The histopathology report is presented in Volume IV, Appendix II.

A complete gross necropsy (with no organs weighed) and histopathologic examination, as described above, were conducted for any parental animals dying on test.

The fixed (buffered neutral 10% formalin) uteri from any females of the F0, F1, or F2 generations failing to produce a litter were stained with potassium ferricyanide for confirmation of pregnancy status. This staining procedure did not interfere with subsequent histopathologic evaluation.

The following organs were weighed from all surviving F0, F1, and F2 parental animals and retained F3 adult offspring at scheduled sacrifice:

ovaries (2)	uterus with oviducts and cervix	testes (2)
prostate	preputial glands w/ fluids (2)	brain
pituitary	liver	kidneys (2)
adrenal glands (2)	spleen	

epididymides w/ contents and fluids (2; total and caudal weight for either one or both)
seminal vesicles (2; with coagulating glands and their fluids)

The following organs were weighed from selected F1, F2, and F3 weanlings, up to three/sex/litter, subjected to sacrifice and necropsy:

brain	ovaries (2)	epididymides w/ contents & fluids (2)
spleen	uterus w/ cervix & vagina	seminal vesicles w/ contents & fluids (2)
thymus	testes (2)	liver

Organ weights were reported as absolute and relative to terminal body weight.

Reproductive and Offspring Indices

The indices for reproductive performance and gestational and postnatal parameters that were calculated for this study are presented in Text Table C. The indices include those for F0, F1, and F2 males and females to produce F1, F2, and F3 litters, respectively.

Text Table C. Reproductive and Offspring Indices

Maternal Indices for the F0, F1, and F2 Generations:

a. Survival indices

b. Mating index (%) = $\frac{\text{No. females sperm positive}}{\text{No. females paired}} \times 100$

c. Fertility index (%) = $\frac{\text{No. females pregnant}}{\text{No. females sperm positive}} \times 100$

d. Pregnancy index (%) = $\frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$

e. Postimplantation loss/litter (%) = $\frac{\text{No. implantation sites - no. live pups on pnd 0}}{\text{No. implantation sites}} \times 100$

Paternal Indices for the F0, F1, and F2 Generations:

a. Survival indices

b. Mating index (%) = $\frac{\text{No. males impregnating females}}{\text{No. males paired}} \times 100$

c. Fertility index (%) = $\frac{\text{No. males siring litter}}{\text{No. males impregnating females}} \times 100$

d. Pregnancy index (%) = $\frac{\text{No. pregnant females}}{\text{No. males impregnating females}} \times 100$

Litter Indices for the F1, F2, and F3 Generations:

a. Pup survival indices (also expressed as percentages)

b. Sex ratio (% males per litter).

(continued)

Text Table C (continued)

c. Indices (%):

Gestational index = $\frac{\text{No. females with live litters}}{\text{No. females pregnant}} \times 100$

Live birth index = $\frac{\text{No. live pups at birth}}{\text{Total no. pups born}} \times 100$

4-day survival index = $\frac{\text{No. pups surviving 4 days (precull)}}{\text{Total no. live pups at birth}} \times 100$

7-day survival index = $\frac{\text{No. pups surviving 7 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$

14-day survival index = $\frac{\text{No. pups surviving 14 days}}{\text{Total no. live pups at 7 days}} \times 100$

21-day survival index = $\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 14 days}} \times 100$

Lactation index = $\frac{\text{No. pups surviving 21 days}}{\text{Total no. live pups at 4 days (postcull)}} \times 100$

Calculation of Standard Reproductive Toxicity Parameters

The calculations used to summarize standard reproductive toxicity parameters for this study are presented in Text Table D.

Text Table D

Formulas for Calculating Standard Reproductive Toxicity Study Parameters[a]

The following endpoints are calculated for each animal and then the mean is calculated using the animal values.

1. Body Weight Change.

   body weight at end of measurement period - body weight at beginning of measurement period

2. Feed Consumption in grams per day.

   ((feed weight at beginning of measurement period) - (feed weight at end of measurement period)) / number of days in measurement period

(continued)

Text Table D (continued)

3. Feed Consumption in grams per day per kilogram body weight.

feed consumption in grams per day / average of all body weights taken during measurement period in kilograms

4. Percent Food Efficiency.

(body weight change for measurement period / ((feed weight at beginning of measurement period) - (feed weight at end of measurement period))) x 100

5. Test Material Intake in milligrams per day per kilogram body weight.

feed consumption in grams per day per kilogram body weight * (dose in ppm / 1000)

6. Relative Organ Weight.

(organ weight / sacrifice body weight) x 100

7. Daily Spermatid Production per Testis

number of spermatids in testis / number of days for spermatid cycle (4.61 for rats)

8. Efficiency of Daily Spermatid Production

((number of spermatids in testis / testis weight in grams) / number of days for spermatid cycle)

9. Percent Abnormal Sperm

100 x ((no. blunt hook + no. banana + no. amorphous + no. pinhead + no. two heads and/or two tails + no. head only) / (no. normal + no. blunt hook + no. banana + no. amorphous + no. pinhead + no. two heads and/or two tails + no. head only))

The following endpoints are calculated for each litter (dam) and then the mean is calculated using the litter (dam) values.

10. Percent postimplantation Loss per Dam and Arcsine Root Transformation:

100 x ((no. implantation sites - no. live pups) / no. of implantation sites)
arcsine (square root ((no. implantation sites - no. live pups) / no. of implantation sites))

11. Stillbirth Index per Dam and Arcsine Root Transformation:

100 x (no. dead pups delivered / total number of pups delivered)
arcsine (square root (no. dead pups delivered / total number of pups delivered))

12. Live Birth Index per Dam and Arcsine Root Transformation:

100 x (no. live pups delivered / total number of pups delivered)
arcsine (square root (no. live pups delivered / total number of pups delivered))

(continued)

Text Table D (continued)

13. 4-Day Survival Index per Dam and Arcsine Root Transformation:

100 x (no. pups alive on pnd 4 / no. of pups alive on pnd 0)
arcsine (square root (no. pups alive on pnd 4 / no. of pups alive on pnd 0))

14. 7-Day Survival Index per Dam and Arcsine Root Transformation:

100 x (no. pups alive on pnd 7 / no. of pups alive on pnd 4 after culling)
arcsine (square root (no. pups alive on pnd 7 / no. of pups alive on pnd 4 after culling))

15. 14-Day Survival Index per Dam and Arcsine Root Transformation:

100 x (no. pups alive on pnd 14 / no. of pups alive on pnd 7)
arcsine (square root (no. pups alive on pnd 14 / no. of pups alive on pnd 7))

16. 21-Day Survival Index per Dam and Arcsine Root Transformation:

100 x (no. pups alive on pnd 21 / no. of pups alive on pnd 14)
arcsine (square root (no. pups alive on pnd 21 / no. of pups alive on pnd 14))

17. Lactational Index per Dam and Arcsine Root Transformation:

100 x (no. pups alive on pnd 21 / no. of pups alive on pnd 4 after culling)
arcsine (square root (no. pups alive on pnd 21 / no. of pups alive on pnd 4 after culling))

18. Percent Males per Litter and Arcsine Root Transformation:
100 x (no. males in litter for given pnd / no. sexed in litter for given pnd)
arcsine (square root (no. males in litter for given pnd / no. sexed in litter for given pnd))

19. Average Fetal Body Weight per Litter:

sum of all individual pup weights in litter for given pnd / no. pups weighed in litter for given pnd

20. Average Male Fetal Body Weight per Litter:

sum of all individual male pup weights in litter for given pnd / no. male pups weighed in litter for given pnd

21. Average Female Fetal Body Weight per Litter:

sum of all individual female pup weights in litter for given pnd / no. female pups weighed in litter for given pnd

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[a] As required for FDA Good Laboratory Practices (subpart J, paragraph 58.185, no. 11), FIFRA Good Laboratory Practice Standards (subpart J, paragraph 160.185, no. 11) and TSCA Good Laboratory Practice Standards (subpart J, paragraph 792.185, no. 11).

Statistical Analyses

The unit of comparison was the male, female, pregnant female, or the litter, as appropriate. Data from the cohorts were combined for summarization and statistical analyses. Quantitative continuous data (e.g., parental and pup body weights, organ weights, feed consumption, anogenital distance, etc.) were compared among the six treatment groups and the one vehicle control group by the use of Bartlett's test for homogeneity of variances. If Bartlett's test indicated lack of homogeneity of variances (i.e., $p<0.001$), then nonparametric statistical tests were employed for the continuous variables (see below; Winer, 1962).

If Bartlett's test indicated homogeneous variances (i.e., $p>0.001$), then parametric statistical tests were employed for the continuous variables as follows. Appropriate General Linear Models (GLM) procedures (SAS Institute Inc., 1989a,b, 1990a,b,c, 1996, 1997) for the proposed Analyses of Variance (ANOVA) are available at Research Triangle Institute (RTI). Prior to GLM analysis, an arcsine-square root transformation was performed on all litter-derived percentage data (Snedecor and Cochran, 1967) to allow use of parametric methods. For these litter-derived percentage data (e.g., periodic pup survival indices), the ANOVA was weighted according to litter size. GLM analysis was used to determine the significance of the dose-response relationship (Test for Linear Trend) and to determine whether significant dosage effects had occurred for selected measures (ANOVA). When a significant ($p<0.05$) main effect for dosage occurred, Dunnett's Multiple Comparison Test (Dunnett, 1955; 1964) was used to compare each treatment group to the vehicle control group for that measure. A one-tailed test (i.e., Dunnett's Test) was used for all pairwise comparisons to the vehicle control group, except that a two-tailed test was used for parental and pup body weight and organ weight parameters, feed consumption, percent males per litter, and anogenital distance, if measured.

Nonparametric tests used for continuous data, which did not have homogeneous variances, included the Kruskal-Wallis Test (Siegel, 1956) to determine if significant differences were present among the groups, followed by the Mann-Whitney U test for pairwise comparisons to the vehicle control group, if the Kruskal-Wallis test was significant. Jonckheere's test for k independent samples (Jonckheere, 1954) was used to identify significant dose-response trends for nonparametric continuous data. Frequency data, such as reproductive indices (e.g., mating and fertility indices) was not transformed. All indices were analyzed by Chi-Square Test for Independence for differences among treatment groups (Snedecor and Cochran, 1967) and by the

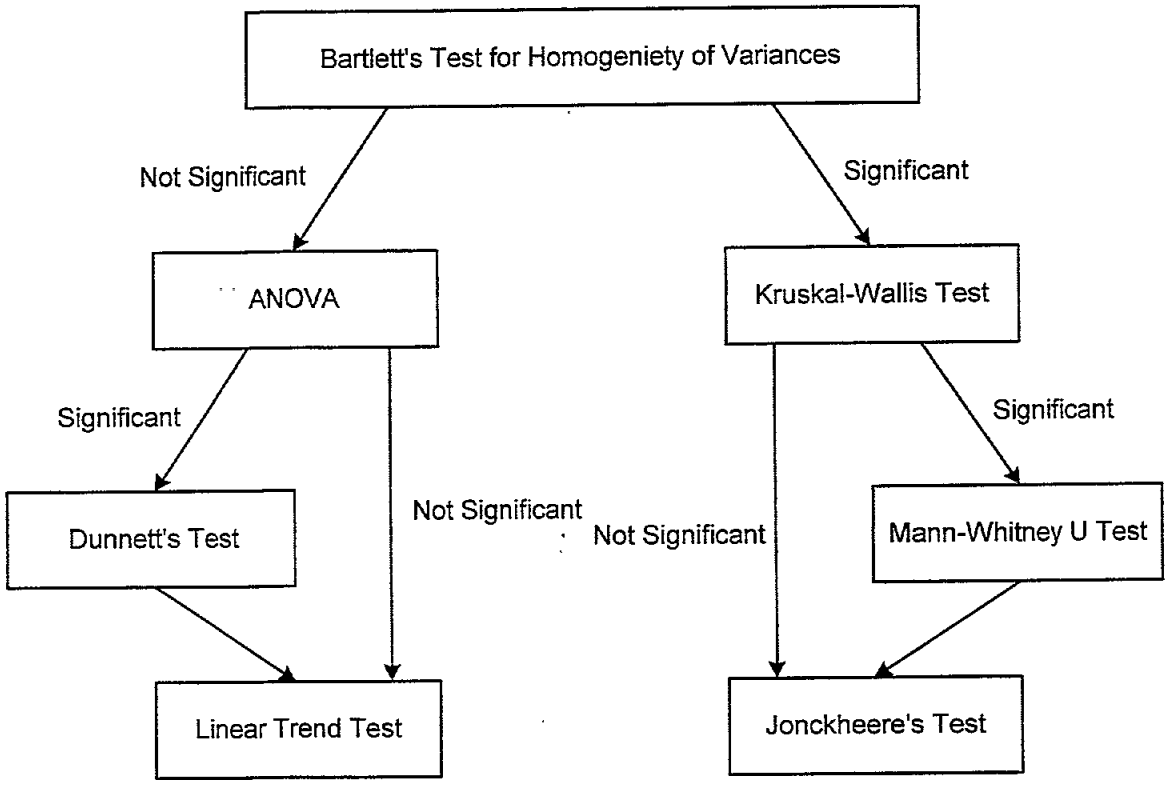
Cochran-Armitage Test for Linear Trend on Proportions (Cochran, 1954; Armitage, 1955; Agresti, 1990). When Chi-Square revealed significant ($p<0.05$) differences among groups, then a Fisher's Exact Probability Test, with appropriate adjustments for multiple comparisons, was used for pairwise comparisons between each treatment group and the control group. For acquisition of developmental landmarks (e.g., vaginal patency and preputial separation) and anogenital distance, each treatment percent or mean was compared to the control percent or mean by Mann-Whitney U test (Siegel, 1956). Acquisition of these developmental landmarks (e.g., vaginal patency and preputial separation), as well as anogenital distance, was also analyzed by Analysis of Covariance (ANCOVA) using body weight at acquisition or measurement as the covariate. For correlated data (e.g., body and organ weights at necropsy of weanlings, with more than one pup/sex/litter), SUDAAN® software (Shah et al., 1997) was used for analysis of overall significance, presence of trend, and pairwise comparisons to the control group values. For all statistical tests, the significance limit of 0.05 (one- or two-tailed) was used as the criterion for significance.

A test for statistical outliers (SAS, 1990b) was performed on parental body weights and feed consumption (in g/day) and parental and weanling offspring organ weights at necropsy. If examination of pertinent study data did not provide a plausible biologically sound reason for inclusion of the data flagged as "outlier," the data were excluded from summarization and analysis and were designated as outliers. If feed consumption data for a given animal for a given observational interval (e.g., study day [sd] 0-7, 7-14, 14-28, 28-35, etc., during the prebreed exposure period) were designated outliers or unrealistic, then summarized data encompassing this period (e.g., sd 0-70 for the prebreed exposure period) did not include this value.

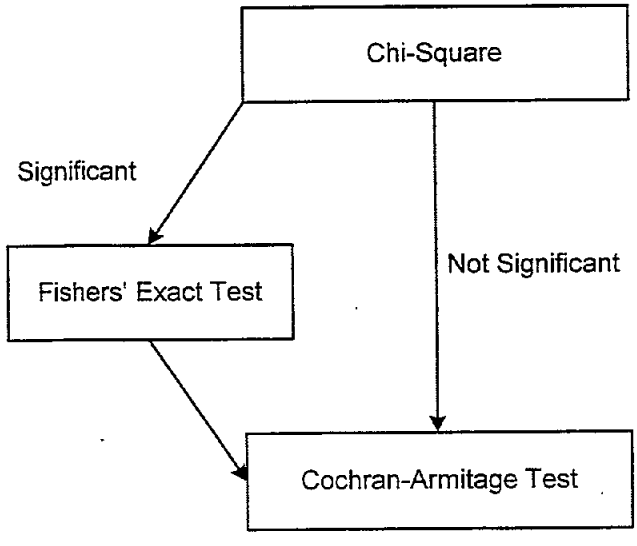
See Text Figure B for a graphical representation of the decision trees for statistical analysis.

Text Figure B. Decision Trees for Statistical Analysis

A. Continuous Data (i.e., body weights)

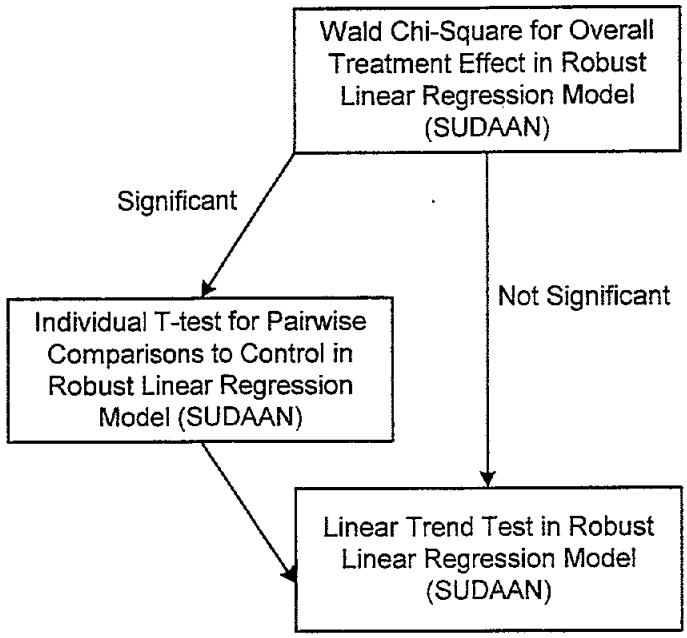


B. Binary Data (i.e., reproductive indices)

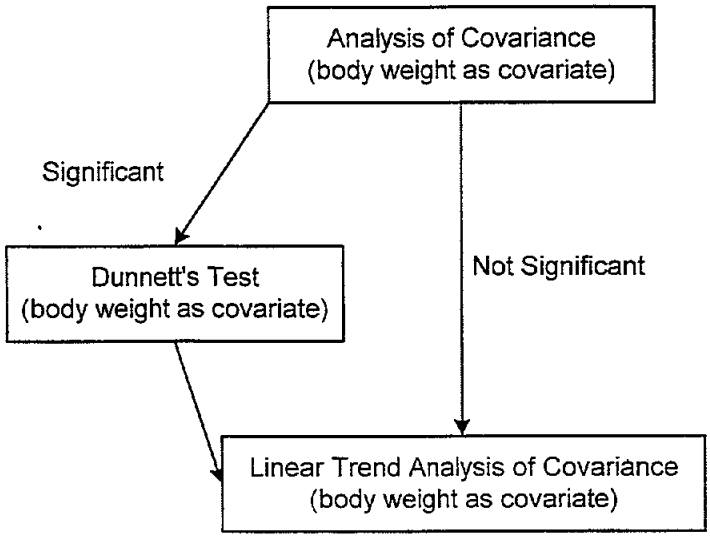


Text Figure B (continued)

C. Correlated Continuous Data (i.e., pup necropsy weights)



D. Continuous Data Weighted Analysis (i.e., vaginal opening)



Personnel

The evaluation of BPA for reproductive toxicity in CD® (Sprague-Dawley) rats was conducted at Research Triangle Institute (RTI), Research Triangle Park, NC, under contract to The Society of the Plastics Industry, Inc., Washington, DC. The lead Study Monitor was Dr. Ronald L. Joiner, GE Plastics. Co-study Monitors were Mr. Stephen S. Dimond, GE Plastics, and Mr. John H. Butala, Consultant to Aristech Chemical Corporation. Mr. Lynne R. Harris, The Society of the Plastics Industry, Inc., was the Sponsor's Representative. The RTI personnel indicated below contributed to the completion of this study.

Dr. R.W. Tyl served as Study Director. (Ms. C.B. Myers was Acting Study Director from July 26 - August 5, 1999, during the F2 prebreed period, when Dr. Tyl was out of the country.) Reproductive toxicology personnel included Ms. M.C. Marr (Laboratory Supervisor), Ms. C.B. Myers (Reproductive Toxicity Study Supervisor and Data Specialist), Ms. F.S. Gerling, Ms. V.I. Wilson, Mr. W.P. Ross (Biologists), Ms. L.B. Pelletier, Ms. B.J. McTaggart, Ms. M.V. Cheesborough, Ms. D.A. Wenzel, and Ms. A.M. D'Antonio (Study Team). Bulk chemical handling and dietary dosage formulations were provided by Mr. M.M. Veselica (Supervisor, RTI Materials Handling Facility), Mr. D.L. Hubbard, Mr. T.D. Burnette, and Mr. R.A. Price. Analyses of dosed feed formulations were performed by Ms. D.R. Brine (Analytical Chemistry Task Leader), Mr. W.O. Poteat, Mr. J.O. Farrar, and Mr. G.M. West. Dr. B.F. Thomas, Ms. A.R. Keimowitz, and Ms. S.S. Brown ran both of the GCMSs used in this study. Analyses of the test material were provided by the Sponsor and reanalyzed periodically by the RTI Analytical Chemistry staff (see Volume IV, Appendix I). Animal care was provided by Dr. D.B. Feldman, DVM, ACLAM, Veterinarian; Mr. F.N. Ali, Manager of RTI Animal Research Facility; and Animal Research Facility personnel. Andrological assessments were performed by Dr. P.A. Fail, Ms. C.S. Sloan, Mr. J.E. Larson, Ms. A. D'Antonio, Ms. C. Durkin, Ms. T. White, and Ms. K. Vick. Histologic preparations were made by Ms. T-Y. Chang (HT-ASCP), Mr. O. Lyght (HT-ASCP), Ms. D. Young (HT-ASCP), Ms. R. Sims (HT-ASCP), Ms. W. Batchelor (HT-ASCP), Ms. T. O'Neil (HT-ASCP), and Mr. D. Shepard (board eligible). Evaluation of histologic slides was by Dr. J.C. Seely, DVM, ACVP (at PATHCO, Inc., until February 21, 2000, and at Experimental Pathology Laboratories, Inc. from February 21, 2000, Research Triangle Park, NC). RTI Quality Assurance personnel were Ms. S.M. Taulbee, Manager (until October 1, 1998), Mr.

D.L. Brodish, Manager (from October 1, 1998), Ms. C.D. Keller, Ms. P.D. Hall, Mr. S.T. Sherrill, Mr. B.L. McCluney, Ms. D.B. Wells, Ms. M.L. Seagroves, Ms. M.E. Parker, and Ms. M.D. Phillips.

The final report was prepared by Dr. R.W. Tyl, with assistance from Ms. C.B. Myers for data compilation and statistical analyses, Ms. M.C. Marr for scheduling and coordinating activities across staff, maintenance of study records, tracking chain of custody documentation for study samples and archiving all study components, and Mr. Tim Wiley for data entry. The individual scientists' reports were prepared and signed by the author(s).

The protocol and five amendments detailing the design and conduct of the study are presented in Volume IX, Appendix IV. The protocol was signed by the Study Director on January 16, 1998.

Storage of Records

All original data sheets, records, biological specimens, blocks, and slides for the present study are stored in the RTI Archives, under the control of the RTI CLS Archivist, along with all biological samples collected during the course of the study which remain the responsibility of RTI. Work sheets and computer printouts, which were generated in the statistical analysis of data, are stored in the RTI Archives. Final disposition of original data sheets, records, biological specimens, blocks, and slides will be determined by the Sponsor and documented in the study records. Copies of this report are filed with the RTI Archives as well as with The Society of the Plastics Industry, Inc.

Compliance

All records, data, biological specimens, and reports will be maintained in storage for the time period specified by the appropriate testing guidelines or for as long as the quality of the preparation affords evaluation, whichever is less. The study was performed in compliance with the U.S. EPA Toxic Substances Control Act (TSCA) testing guidelines (U.S. EPA, 1997), U.S. EPA Office of Prevention, Pesticides and Toxic Substances (OPPTS) Health Effects Test Guidelines (U.S. EPA, 1998), and U.S. EPA TSCA Good Laboratory Practice Standards (U.S. EPA, 1989).

The toxicology laboratories at RTI are operated in compliance with Toxic Substances Control Act (TSCA) Good Laboratory Practice Standards (U.S. EPA, 1985; 1989), and the RTI

Animal Research Facility is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), International. This study was conducted in compliance with the TSCA GLP regulations and AAALAC accreditation standards.

RESULTS

Dosed Feed Formulations

Dosed feed formulations, encompassing the range of dosage concentrations employed in this study, were homogeneous and stable for at least 50 days under freezer conditions (-15 to -20°C), so formulations were used within the stability limits established and were stored frozen. All dietary formulations that were used were 85.0-115% of target dietary concentrations prior to use, except for two dose levels for the first formulation date (September 28-29, 1998), 4.5 ppm which assayed at 83.8%, and 75 ppm which assayed at 82.8% of nominal (see Protocol Deviations after the summary tables). These formulations were used only until they were reformulated and analyzed as acceptable. No BPA was detected in the vehicle control feed formulations, with an estimated detection limit of 0.0008 ppm (prior to September 19, 1999) and 0.0018 ppm (after September 19, 1999), due to a change in GC MS instruments on September 19, 1999 (see Materials and Methods), which resulted in a change in the estimated detection limit (Table 1, Volume II and Appendix I, Volume IV).

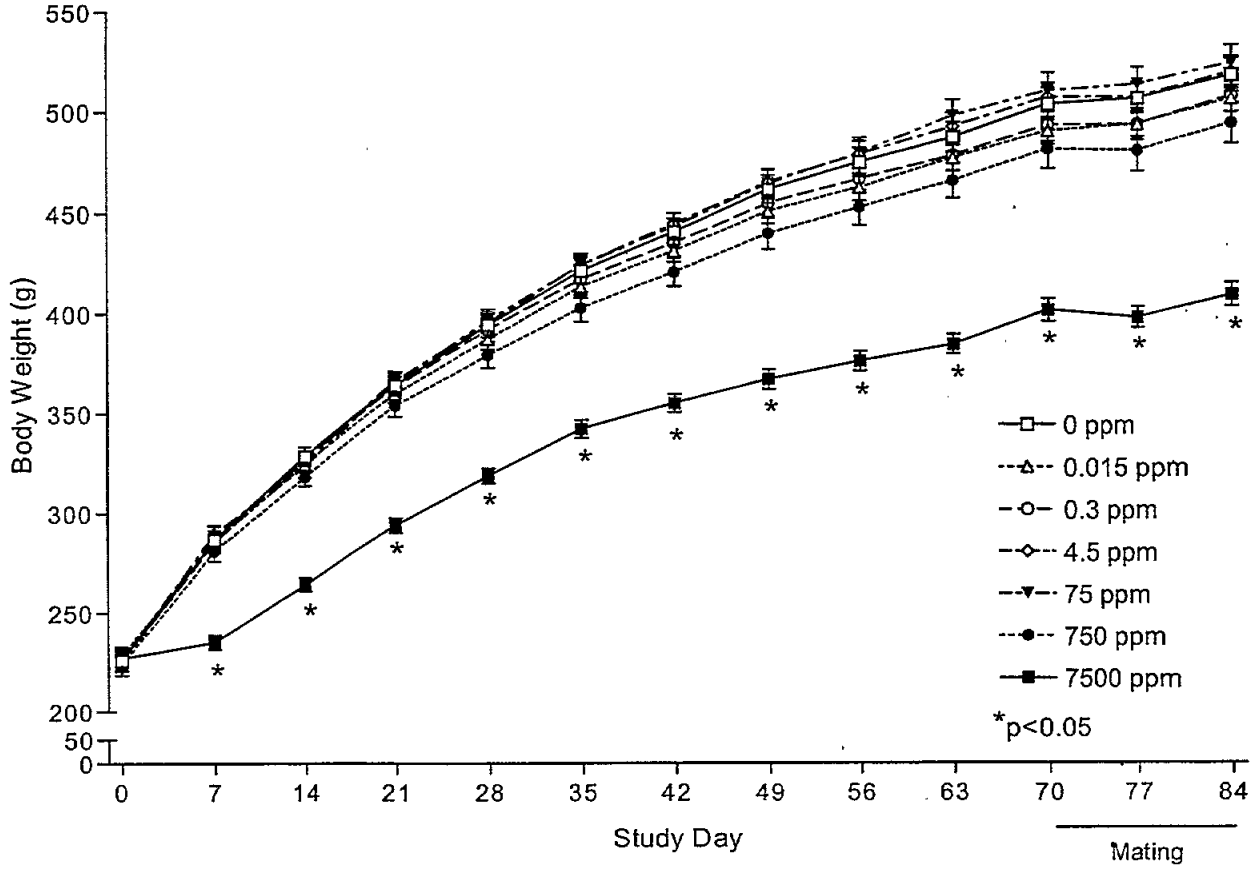
F0 Prebreed Exposure Period

Results are organized and reported chronologically. Figures are presented throughout the text to graphically illustrate body weights and test chemical intake; data in the figures are presented as mean ± standard error bars. All individual in-life and necropsy data are presented in Volumes V-VIII; all individual histopathology data are presented in Volume IV, Appendix II.

A summary of the fates of F0 males and females is presented in Table 2. One F0 male (No. 173) at 0.015 ppm was euthanized moribund on sd 57. All remaining F0 males in all groups survived to scheduled necropsy. There were therefore 30, 29, 30, 30, 30, 30, and 30 F0 males at scheduled sacrifice at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. All F0 females survived to scheduled necropsy.

F0 male body weights (Table 3) were statistically equivalent among treatment groups at the start of the study (sd 0). Body weights, recorded weekly during the ten-week prebreed dosing period, exhibited statistically significant reductions at 7500 ppm for all weeks evaluated, through the end of the prebreed dosing period (sd 70), and through the two-week mating period (to sd 84) (Table 3 and Figure 1). F0 male weekly body weight changes exhibited statistically significant reductions at 7500 ppm for weeks 1 (sd 0-7), 2 (sd 7-14), 3 (sd 14-20), 4 (sd 21-28), 6 (sd 35-42), 7 (sd 42-49), 8 (sd 49-56), and 9 (sd 56-63); for the entire prebreed period (sd 0-70); and at 750 ppm for week 5 (sd 28-35) (Table 3).

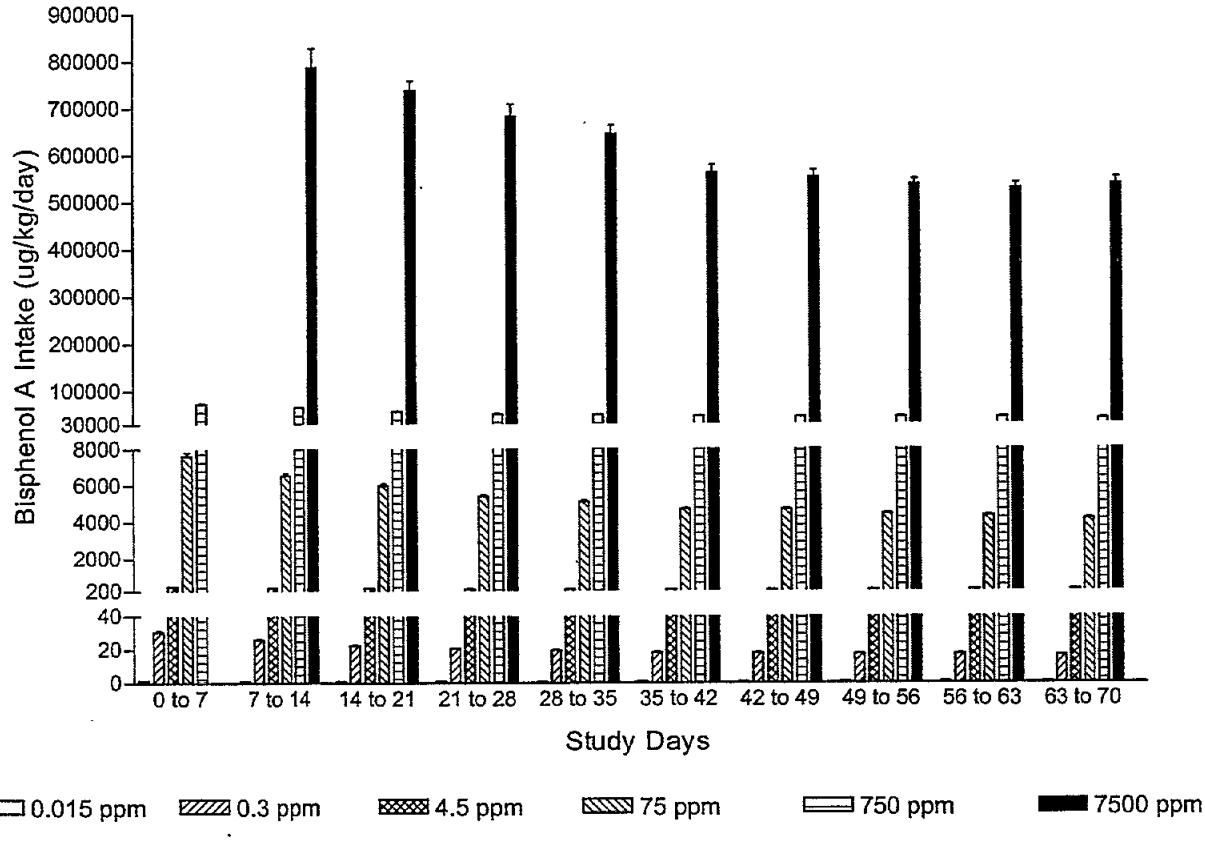
Figure 1. F_0 Male Body Weights During the Prebreed and Mating Periods



F0 male feed consumption, expressed as g/day, was equivalent across all groups for all intervals during the ten-week prebreed exposure period. It was clear that it took the F0 males approximately two weeks to adjust to the high-dose feed (feed was scratched out of the jars), so feed consumption values could not be determined during this period. When the data were expressed as g/kg/day, the values at 7500 ppm were significantly increased for weeks 2 through 10. Feed consumption for the entire prebreed exposure period (sd 0-70) could not be determined at 7500 ppm since there were no data at 7500 ppm for week 1 and only eight values at this dose for week 2. At 75 ppm for weeks 3, 4, and 10, food consumption, expressed as g/kg/day, was significantly increased. Percent food efficiency was significantly reduced at 7500 ppm for weeks 2, 3, 6, 7, and 8; this parameter was not available for the entire ten-week prebreed period (sd 0-70) due to feed consumption problems at 7500 ppm for weeks 1 and 2 (Table 4). BPA intake, expressed as µg/kg/day, exhibited the expected incremental increases across dose groups, averaging approximately 1 µg/kg/day at 0.015 ppm, 20 µg/kg/day at 0.3 ppm, 304 µg/kg/day at 4.5 ppm, 5092 µg/kg/day at 75 ppm, 49,628 µg/kg/day at 750 ppm, and not available at 7500 ppm for the entire period (sd 0-70), increasing approximately 20x from 0.015 to 0.3 ppm, 15x from 0.3 to 4.5 ppm, 17x from 4.5 to 75 ppm, 10x from 75 to 750 ppm, and unavailable for 750 to 7500 ppm. The BPA intake values also exhibited the expected decreases within each dose group over time. The test chemical intake was approximately 2, 31, 465, 7663, 73,370 (and not available) µg/kg/day during the first week (sd 0-7) of the prebreed period, and was approximately 1, 17, 247, 4130, 39,923, and 533,547 µg/kg/day for the last week (sd 63-70) of the prebreed period for the 0.015, 0.3, 4.5, 75, 750, and 7500 ppm groups, respectively. These decreases within groups are due to the feed consumption in g/day remaining relatively constant and the body weights increasing markedly over time. Therefore, the feed consumption in g/kg/day (the basis for the test chemical intake calculations) decreased over time within each group (Table 4 and Figure 2).

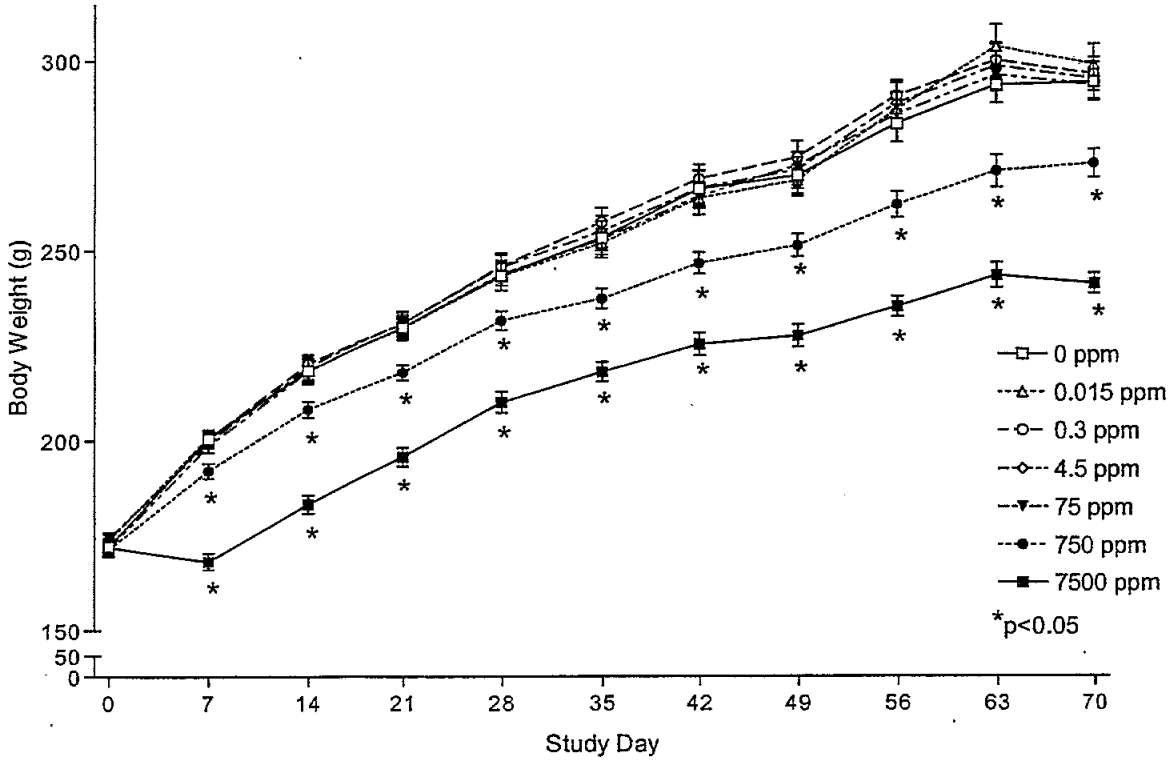
Clinical observations during the prebreed and mating periods for F0 males indicated no treatment-related clinical signs of toxicity. The recorded findings were those typically observed in rats housed in hanging metal cages and included sore(s) and alopecia (various locations); chromodacryorrhea; malocclusion and/or injury to teeth, mouth, nose, paws, and tail ("interactions" with the stainless steel hanging wire cages). One male died at 0.015 ppm, which was also considered incidental (euthanized moribund) (Table 5).

Figure 2. F_0 Male Bisphenol A Intake During the Prebreed Period



F0 female body weights (Table 6 and Figure 3) were equivalent across all groups at the start of the study (sd 0). Beginning immediately by the end of week 1 (sd 7) and continuing through the ten-week prebreed exposure period, the mean weights at 7500 and 750 ppm were significantly lower. Body weights at 0.015, 0.3, 4.5, and 75 ppm were unaffected. Weekly body weights of the three females remaining sperm-negative during week 1 of the two-week mating period were not statistically analyzed (Table 6). F0 female weekly body weight changes exhibited statistically significant reductions at 7500 ppm for weeks 1 (sd 0-7) and 8 (sd 49-56) and for the entire prebreed exposure period (sd 0-70). At 750 ppm, weekly weight change was significantly reduced for week 1 and the entire prebreed exposure period (sd 0-70) (Table 6).

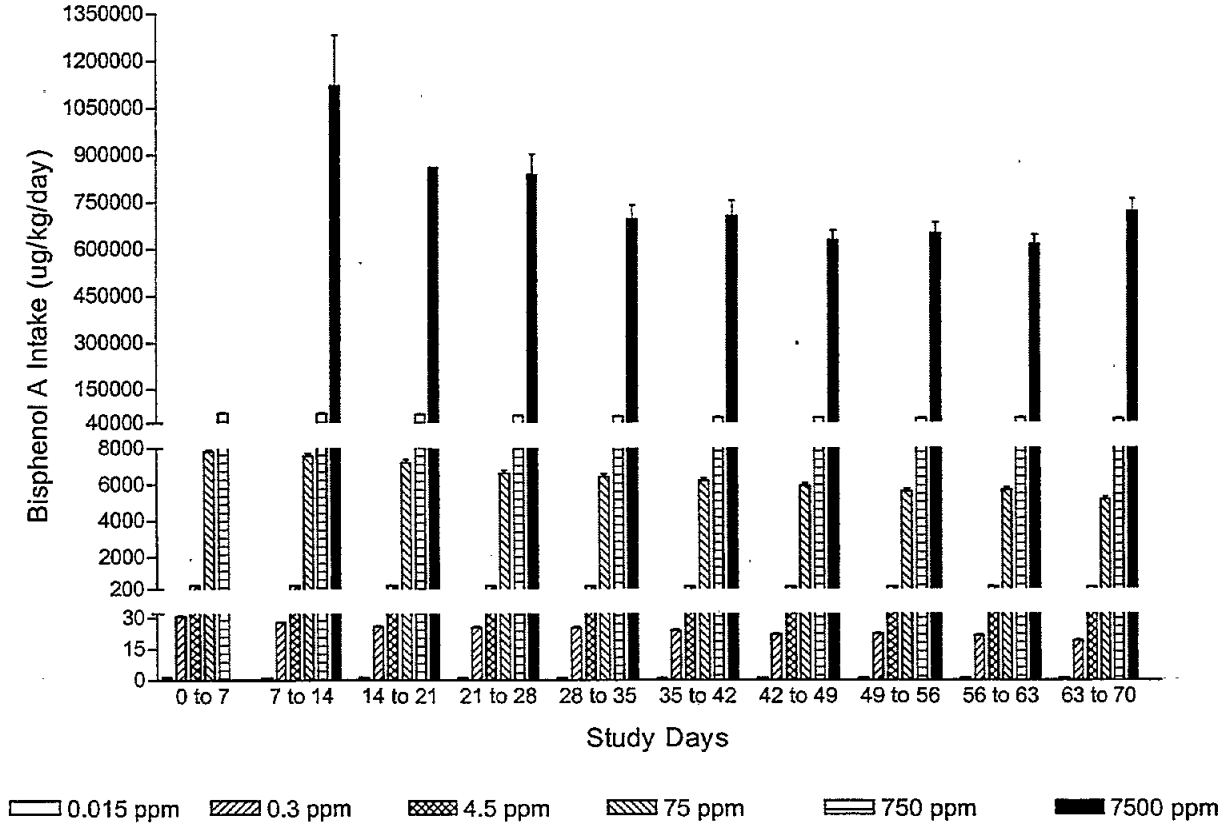
Figure 3. F_0 Female Body Weights During the Prebreed Period



F0 female feed consumption values (Table 7) during the ten-week prebreed dosing period, expressed as g/day and g/kg/day, exhibited sporadic effects. They were unaffected across all groups and all intervals except for significant reductions for week 1 (sd 0-7) at 4.5 and 750 ppm (with no data at 7500 ppm for week 1 and relatively few values for weeks 2 through 5); weeks 6 (sd 35-42), 7 (sd 42-49), and 8 (sd 49-56) and sd 0-70 at 750 ppm; and significantly increased feed consumption at 7500 ppm for week 2 (sd 7-14) and at 75 ppm for week 3 (sd 14-21), all as g/day. When the data were expressed as g/kg/day, the only significant effects were decreases at 4.5 and 750 ppm for week 1, and increases at 7500 ppm for weeks 2, 8, and 10 and at 75 ppm for weeks 2 and 3. Feed consumption as g/kg/day for the prebreed period (sd 0-70) was equivalent from 0 through 750 ppm (with no data available at 7500 ppm) (Table 7). Percent food efficiency was significantly reduced only at 750 ppm for week 1 and for the ten-week prebreed exposure period (sd 0-70). F0 female BPA intake (as µg/kg/day) exhibited comparable incremental increases across dosed-feed groups for the prebreed period and comparable decreases with increasing age (and weight) within dosed-feed groups, as did the F0 males. The

test chemical intake within groups was approximately 2, 31, 442, 7810, 72,251 µg/kg/day, and no data during the first week of the prebreed period for 7500 ppm, and was approximately 1, 19, 285, 5136, 51,236, and 713,421 µg/kg/day, during the last week of the prebreed period, at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Average intakes of test chemical during the ten-week prebreed were approximately 1, 24, 358, 6311, and 59,411 (and no data) µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 7 and Figure 4).

Figure 4. F_0 Female Bisphenol A Intake During the Prebreed Period



Clinical observations of F0 females during the prebreed dosing period are presented in Table 8. There were no treatment- or dose-related findings. Observations were essentially limited to occasional findings, such as alopecia (various locations), chromodacryorrhea, sore(s), and injury to teeth, tail, and/or mouth in one to five females in all groups.

Vaginal cytology was evaluated for F0 females daily during the last three weeks of the ten-week prebreed exposure period. The results are presented in Table 9. Of the 30 females evaluated in each group, 0, 1, 1, 0, 1, 0 and 0 at 0.0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, were not cycling (i.e., persistent diestrus). Of the females cycling, 5, 0, 2, 1, 5, 8, and 2 had abnormal cycles at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Cycle length was equivalent across all groups, with a mean duration of 4.58, 4.41, 4.48, 4.50, 4.57, 4.45, and 4.26 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. There were no statistically significant or toxicologically relevant differences among groups (Table 9).

Gestation - F0 Females for F1 Litters

Maternal gestational body weights (Table 10 and Figure 5) were significantly reduced at 750 and 7500 ppm for all timepoints evaluated (gd 0, 7, 14, and 20). Gestational weight change (gd 0-7, 7-14, 14-20, and 0-20) was significantly reduced only at 7500 ppm. There were no significant changes in feed consumption during any gestational interval for any group when expressed as g/day. However, maternal gestational feed consumption, expressed as g/kg/day, was significantly increased at 7500 ppm for gd 0-7, 14-20, and 0-20, and at 750 ppm for gd 14-20 and 0-20. Percent food efficiency was significantly reduced at 7500 ppm for the gestation period (gd 0-7, 2-14, 14-20, and 0-20) (Table 11). Maternal gestational (gd 0-20) intake of BPA was approximately 1, 19, 287, 4954, 51,587, and 648,545 µg/kg/day at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 11 and Figure 6).

Clinical observations of F0 dams during gestation are presented in Table 12 and included alopecia (various areas), chromodacryorrhea, red ears, and rust-colored facial fur across all groups, with no dose-related pattern of incidence (Table 12).

Lactation - F0 Females For F1 Litters

Maternal lactational body weights (Table 13 and Figure 7) were statistically significantly reduced at 7500 ppm for pnd 0, 4, 7, 14, and 21 and at 750 ppm for pnd 0, 4, 7, and 14. Lactational weight change was significantly reduced only at 7500 ppm during pnd 0-4, and significantly increased at 7500 ppm for pnd 14-21 and for the lactational period (pnd 0-21). Maternal lactational feed consumption, expressed as g/day, was significantly reduced only at 7500 ppm for pnd 4-7. When the data were expressed as g/kg/day, the values were statistically equivalent across all groups for all timepoints evaluated, except at 7500 ppm, at which feed

consumption in g/kg/day was significantly increased for pnd 0-4, 7-14, 14-21, and 0-21, and significantly decreased at 0.015 and 4.5 ppm for pnd 14-21. The feed consumption values for pnd 14-21 (and possibly pnd 7-14) were confounded by the pups self-feeding starting in the second week of postnatal life. Percent food efficiency at 7500 ppm was significantly decreased on pnd 0-4, significantly increased at 7500 ppm on pnd 14-21 and 0-21, and significantly increased at 750 ppm for pnd 14-21; it was unaffected for all other intervals and all other groups for the lactation period (pnd 0-21). Maternal lactational intake of BPA (pnd 0-21) was approximately 2, 50, 743, 12,924, 132,314, and 1,496,438 µg/kg/day at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, confounded by pups self-feeding, starting in the second week of postnatal life (Table 14 and Figure 8).

Figure 5. F_0 Female Body Weights During Gestation

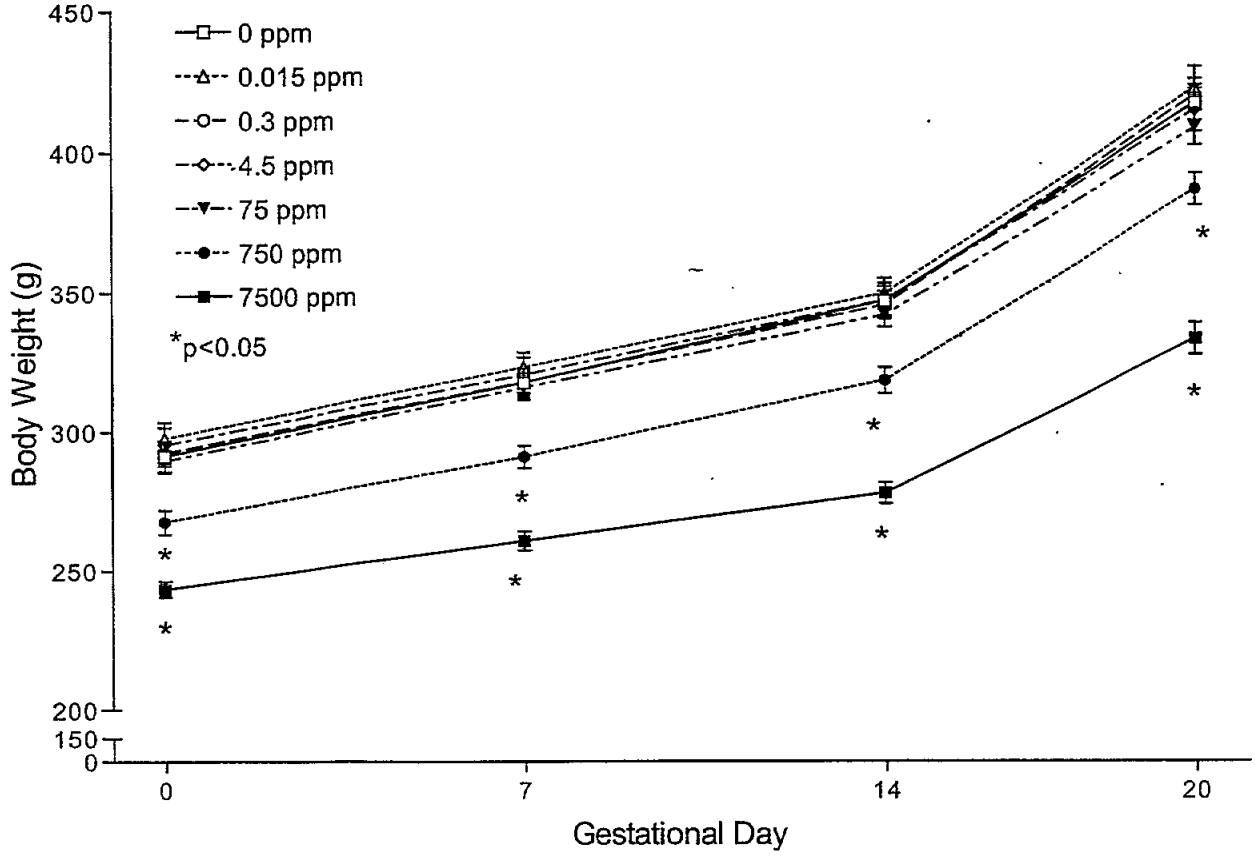


Figure 6. F_0 Female Bisphenol A Intake During Gestation

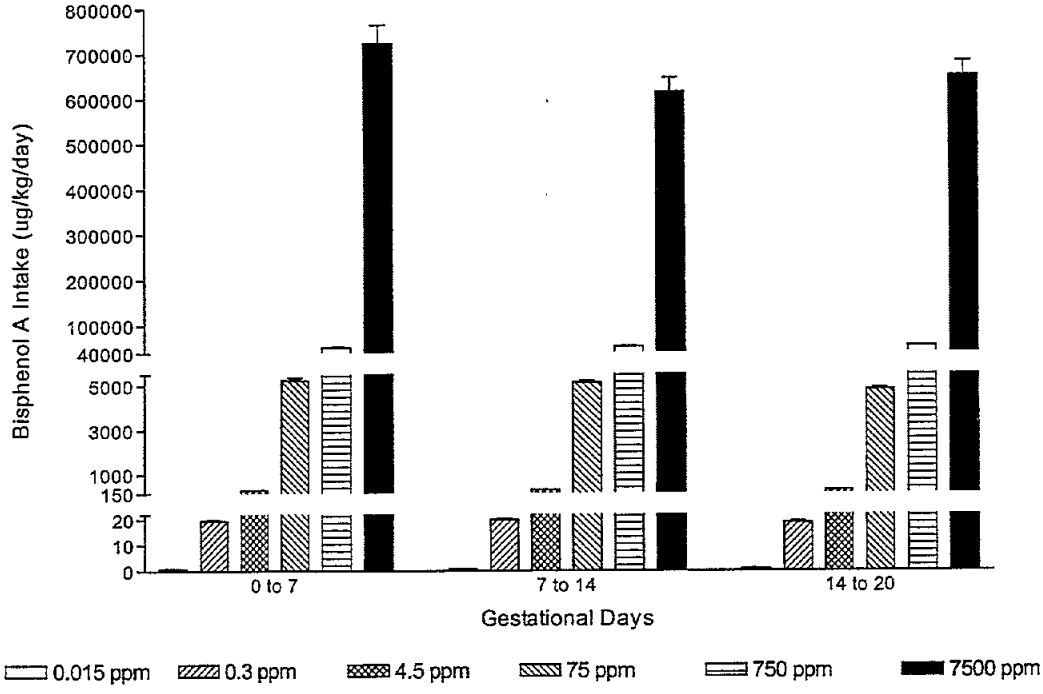


Figure 7. F_0 Female Body Weights During Lactation

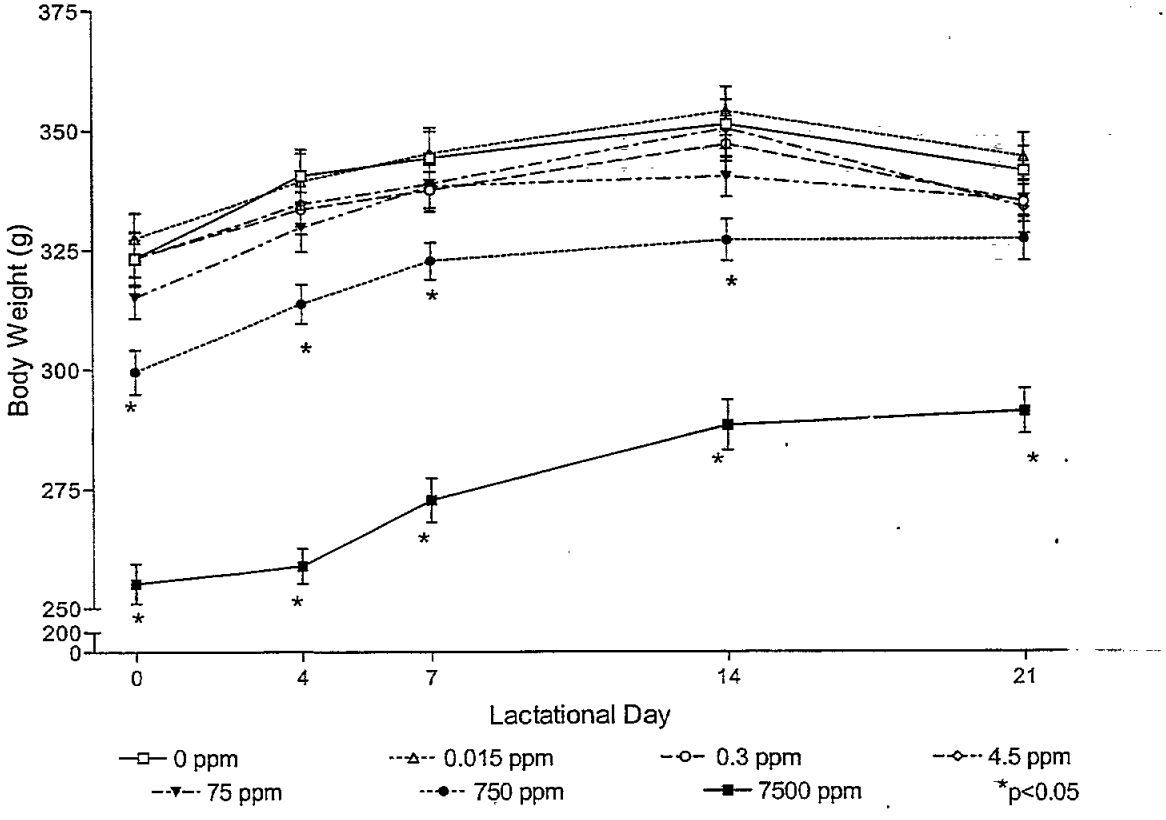
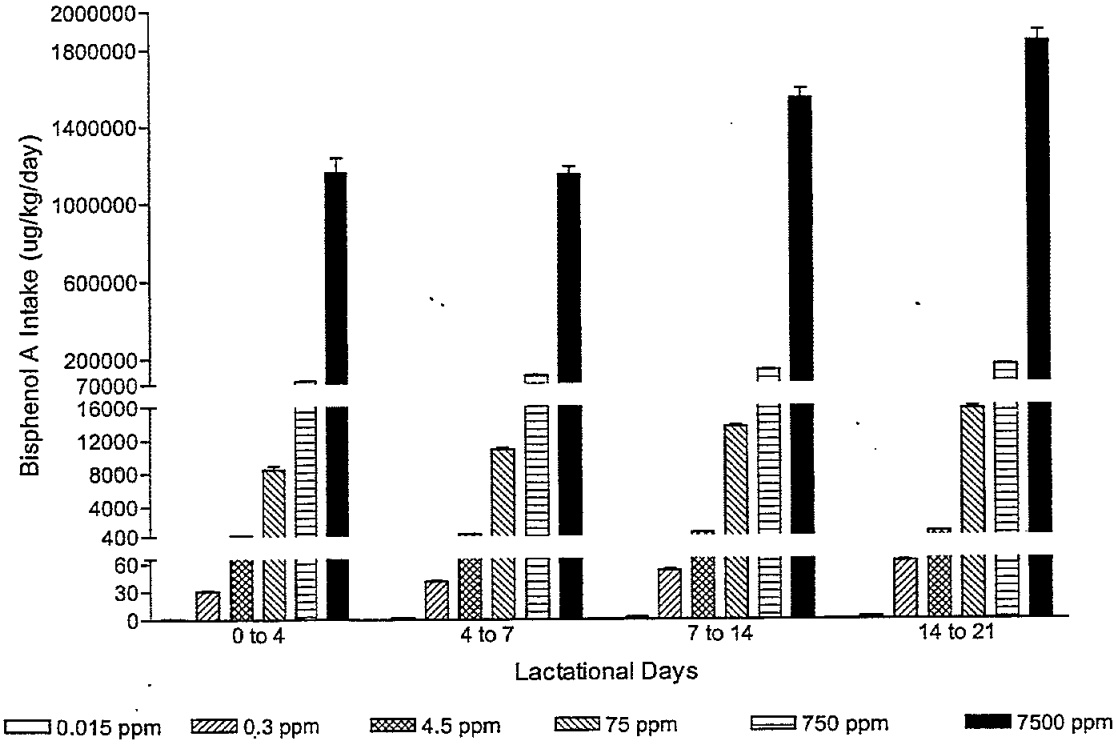


Figure 8. F_0 Female Bisphenol A Intake During Lactation



Clinical observations of F0 dams during lactation (Table 15) indicated no treatment- or dose-related findings. Observations included chromodacryorrhea, alopecia, red ears, piloerection, and rust-colored fur (Table 15).

Reproductive and lactational indices for F0 animals producing F1 litters are presented in Table 16. F0 male and female mating, fertility, and gestational and pregnancy indices were equivalent across all groups. Precoital interval was also equivalent across all groups. Gestational length in days was statistically equivalent across all groups, with mean values of 22.1, 22.1, 22.1, 22.1, 22.2, 22.0, and 22.1 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The number of implantation sites per dam was significantly reduced only at 7500 ppm, although postimplantation loss per litter and the number of dead pups per litter on pnd 0 were equivalent across all groups. The number of total and live pups per litter on pnd 0 were therefore also significantly reduced at 7500 ppm. The number of litters on pnd 0 were 26, 26, 27, 27, 26, 27, and 27 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Stillbirth index, live birth index, day 4, 7, 14, and 21 survival indices, and lactation index were all also statistically equivalent across all groups (Table 16).

The average number of pups per litter on pnd 0 and 4 were significantly reduced at 7500 ppm. After standardization of litter sizes on pnd 4, the number of pups per litter on pnd 7, 14, and 21 were statistically equivalent across all groups. Sex ratio (% males per litter) was equivalent across all groups at all time points. Mean pup body weights per litter were statistically equivalent across all groups (for all pups or separately by sex) for pnd 0. On pnd 4, pup body weight per litter for all pups was significantly reduced at 7500 ppm, but not when expressed separately by sex. Beginning on pnd 7 and for pnd 14 and 21, pup body weights per litter (for all pups and separately by sex) were significantly reduced at 7500 ppm. In addition, on pnd 21, pup body weights per litter (all pups) were significantly reduced at 0.015, 75, and 750 ppm, and for females only at 0.3 and 750 ppm. Male pup body weight per litter was significantly reduced only at 7500 ppm. The second week of lactation is the time of maximal milk production and the beginning of self-feeding in the pups, possibly as early as pnd 10 (especially when given access to powdered food), based on observations on CD® rats by the performing laboratory. The last week of the lactational period corresponds to the time the pups generally begin to self-feed continually. There were no retained nipples in F1 males on pnd 11-13; the number of areolae per male and the percent of males with one or more areolae exhibited no dose-related pattern, ranging from 0.0 (control) to 0.06 (75 ppm) and from 0.0 (control) to 3.0% (75 ppm), respectively (Table 17).

Clinical observations of F1 pups during the lactation period (Table 18) indicated no treatment- or dose-related changes in the number of pups found dead (and missing and presumed dead pups), failure to nurse (no milk band), thin fur, or chewed extremities (presumably by dam). These are all common findings during the lactation period. The total number of pups found dead (or euthanized moribund, or missing and presumed dead) from pnd 0 through pnd 4 were 18, 14, 6, 8, 9, 12, and 10, and from pnd 5-21 were 1, 1, 0, 1, 0, 1 and 6 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 18).

Gross necropsy of F1 pups that died during the lactation period and were available for examination (Table 19) included 14, 10, 5, 7, 8, 8, and 10 pups at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively; almost all deaths occurred during the first four days of life (see above). Many of the pnd 0 pups had patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of the pulmonary alveoli at birth) and no milk in stomach, although many pups died with closed ductus and air in lungs. These findings are

typical of pups dying at this age and are not considered unusual or treatment related. One pup each on pnd 0 at 750 ppm (a female with interventricular septal defect, double outlet right ventricle, situs inversus viscerum) and at 0.015 ppm (a male with interventricular septal defect, retrotracheal enlarged aorta, and pulmonary artery reduced in size) exhibited major visceral malformations, clearly not treatment-related (Table 19).

Necropsy – F1 Weanlings

Necropsy body and organ weights of F1 weanling (pnd 21) males and females, up to three per sex per litter, are presented in Table 20. A total of 69, 72, 76, 75, 72, 78, and 70 weanling males were necropsied at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F1 weanling male body weights were significantly reduced at 0.015, 75, and 7500 ppm. Absolute mean male liver, thymus, spleen, brain, testes (paired), and epididymides (paired) weights were significantly reduced at 7500 ppm. Absolute spleen weight was also significantly reduced at 750 ppm, and absolute thymus, paired testes, and paired epididymides weights were significantly reduced at 0.015 ppm. Absolute male seminal vesicles with coagulating gland weights were statistically equivalent across all groups. Relative male liver, thymus, epididymides, and seminal vesicles (with coagulating gland) weights were equivalent across all groups. Relative male spleen weights were significantly reduced at 750 and 7500 ppm, and relative brain weights were significantly increased at 0.015, 75, 750, and 7500 ppm. Relative paired testes weights were significantly increased at 7500 ppm (most likely due to reduced body weights, since absolute weights were lower at this dietary concentration) (Table 20).

Necropsy body and organ weights of F1 weanling females are also presented in Table 20. A total of 70, 70, 81, 77, 73, 75, and 77 weanling females were necropsied at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F1 weanling female body weight was significantly reduced at 7500 ppm. Absolute female liver, thymus, spleen, brain, paired ovary, and uterine weights were significantly reduced at 7500 ppm; absolute uterine weight was also reduced at 0.3 ppm. Relative female liver, thymus, and paired ovary weights were equivalent across all groups. Relative female spleen and uterine weights were significantly reduced at 7500 ppm. Relative brain weight was significantly increased at 0.015, 0.3, 75, 750, and 7500 ppm (Table 20).

Summary of gross findings for the F1 weanlings is presented in Table 21. There were no gross findings in F1 females. The only gross finding in F1 offspring was in a control group male that exhibited a reduction in the size of the right testis (Table 21).

F0 Parental Holding Period

F0 males were held after mating until the delivery of all F1 litters (sd 84-105). Male body weights were significantly reduced at 7500 ppm on sd 84, 91, 98, and 105. Body weight change was unaffected across all groups for sd 91-98 and 98-105; for sd 84-91, body weight change was significantly decreased at 7500 ppm (Table 22). F0 male feed consumption during this period was unaffected across groups when the data were expressed as g/day. When the data were expressed as g/kg/day, feed consumption was significantly increased at 7500 ppm for all three intervals (sd 84-91, 91-98, and 98-105). Percent food efficiency was significantly reduced only at 0.015 ppm and only for sd 84-91. BPA intake was approximately 0.8 (first week) - 0.8 (last week) µg/kg/day at 0.015 ppm, 16-15 µg/kg/day at 0.3 ppm, 233-223 µg/kg/day at 4.5 ppm, 3854-3856 µg/kg/day at 75 ppm, 39,079-37,559 µg/kg/day at 750 ppm, and 477,906-491,328 µg/kg/day at 7500 ppm (Table 23). Clinical observations in F0 males during this time (Table 24) indicated no treatment- or dose-related incidences or severities. Alopecia (various locations), chromodacryorrhea, red ears, and sore(s) (various locations) were observed across groups (Table 24).

F0 females that were not pregnant, did not deliver live litters, had litters that died during lactation, or had weaned the litters, were held through sd 112 until all dams were terminated at scheduled sacrifice. The summarized body weights and weight changes during this period are presented in Table 25; the data were not statistically analyzed due to small numbers of females per group, which also changed over time. Mean body weights appeared slightly reduced at 7500 ppm; mean weight changes were variable across groups (Table 25). Feed consumption in g/day and g/kg/day are also presented for these females in Table 26, with approximately equivalent values across all groups (slight increase at 7500 ppm as g/kg/day). Percent food efficiency was variable across groups within intervals and within groups across intervals with no consistent pattern (no statistical analyses were performed). BPA intake (first week to second week) was approximately 1-1, 18-18, 265-262, 4356-3833, 48,167-47,771, and 495,607-542,443 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Clinical observations for these females

are presented in Table 27. Dams across all groups exhibited alopecia (various locations) and/or red ears, with no treatment- or dose-related incidence or severity (Table 27).

Necropsy – F0 Parental Animals

The summary of F0 male terminal body weights, organ weights, and andrological assessments is presented in Table 28. Body weight at sacrifice was significantly reduced at 7500 ppm. There were no statistically significant effects of treatment on absolute brain, paired testes, paired epididymides, or preputial gland weights for any group. Absolute liver, paired kidneys, paired adrenal, spleen, pituitary, prostate, and seminal vesicles with coagulating gland weights were significantly reduced at 7500 ppm. Absolute liver weight was also significantly reduced at 750 ppm. Absolute weight of seminal vesicles with coagulating glands was significantly increased at 4.5 ppm. Relative organ weights (organ weight as a percentage of sacrifice body weight) were unaffected for adrenal glands, spleen, pituitary, prostate, preputial gland, and seminal vesicles with coagulating gland. Relative brain, paired kidney, testes, and epididymides weights were significantly increased at 7500 ppm (due to decreased body weights in this group); relative liver weight was significantly reduced at 750 ppm. There were no statistically significant differences among groups for any andrological parameters, including percent motile sperm, percent progressively motile sperm, epididymal sperm concentration (10^6/g), testicular spermatid head concentration (10^6/g), daily sperm production per testis, efficiency of daily sperm production, and percent abnormal sperm. The elevated mean value for percent abnormal sperm at 0 ppm was primarily due to one F0 male (No. 91) with low sperm motility, low sperm concentration, low daily sperm production, and 26.6% abnormal sperm; he sired a live litter (with F0 female No. 22) (Table 28 and individual animal data tables in Volume V).

Gross necropsy findings in F0 males included no apparent treatment- or dose-related findings in any males who were sacrificed on schedule. The one male that died on study at 0.015 ppm exhibited bilateral hydronephrosis, crusty ear, and necrotic tail tip; cause of death is unknown but not believed treatment related (Table 29). Similarly, there were no treatment- or dose-related histopathologic findings in any F0 male organs, including reproductive organs, or in organs with gross lesions. There was chronic inflammation of the liver in 2/11 males at 7500 ppm versus no males at 0 (out of 30), 0.015 (14), 0.3 (12), 75 (14), or 750 (12) and one (out of 13) at 4.5 ppm (Table 29, Volume II).

Summary of F0 female terminal body and organ weights is presented in Table 30. A total of 30 females per group were necropsied at scheduled termination. Mean sacrifice body weight was significantly reduced at 7500 ppm. Absolute liver, spleen, and brain weights were statistically equivalent across groups. Absolute pituitary weight was significantly increased at 0.3 ppm and significantly reduced at 7500 ppm. Absolute paired kidney, paired adrenal, pituitary, paired ovary, and uterine weights were significantly reduced at 7500 ppm. Absolute uterine weight was also significantly reduced at 0.015 ppm. Relative adrenal, spleen, and uterine weights were equivalent across all groups. Relative liver, paired kidney, and brain weights were significantly increased at 7500 ppm. Relative pituitary and paired ovary weights were significantly reduced at 7500 ppm. Paired ovarian follicle counts, examined in females at 0 and 7500 ppm, were significantly increased at 7500 ppm. Stage of estrus at necropsy exhibited significant decreases in the number (%) of females in proestrus at 4.5, 750, and 7500 ppm and significant increases in the number (%) of females in diestrus at 750 and 7500 ppm, relative to the control group values (Table 31). No treatment- or dose-related effects were seen at 750 ppm or below.

At scheduled sacrifice of all the F0 females, gross effects were limited to sore(s), alopecia (various locations), chromodacryorrhea, red ears, right kidney fused to liver (one female at 0.3 ppm), right side hydronephrosis (one female at 0.0 ppm), urinary caluli (one female at 750 ppm), fluid-filled uterus (one female at 7500 ppm), and retained fetus in left uterine horn (one female at 0.3 ppm) (Table 31). Histopathologic findings are presented in Table 31, Volume II. There was an apparent increase in renal tubular degeneration at 7500 ppm (4/13) versus no females with this finding at 0, 0.015, 0.3, 4.5, 75, or 750 ppm and a possible increase in chronic liver inflammation incidences as follows: 0 of 30, 0/12, 1/12, 0/12, 0/14, 1/12, and 3/13 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. No other findings were considered to be treatment or dose related.

F1 Prebreed Exposure Period

A summary of the fates of parental F1 males and females is presented in Table 32. One male (No. 1147) at 0.0 ppm (control) was found dead on sd 100, and one male (No. 1315) at 4.5 ppm was euthanized moribund on sd 72. There were therefore 29, 30, 30, 29, 30, 30, and 30 males at 0.0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. One female (No. 1238) at 0 ppm was found dead on pnd 0 (sd 92) while in the process of delivering. One female (No. 1204)

at 7500 ppm was euthanized moribund on sd 87 (during pregnancy). Also, one animal (No. 1214), initially identified as a female by external examination, was determined to be a male by histopathologic examination; no in-life or organ weight data are included for this animal. (Its gross and histopathologic data are presented with the F1 adult females; see footnote on Table 32 for further details.) Therefore, 29, 30, 30, 30, 30, 30, and 28 females were evaluated at scheduled sacrifice at 0.0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 32).

The assessment of acquisition of reproductive developmental landmarks is presented in Table 33. The mean day of acquisition of vaginal patency in F1 females ranged from 30.1 to 33.0 days, with the mean body weight at acquisition ranging from 92.32 to 106.04 g. The mean day of acquisition of preputial separation in F1 males ranged from 41.9 to 45.8 days, with the mean body weight at acquisition ranging from 225.06 to 194.02 g. When the age at acquisition was analyzed statistically by analysis of variance (ANOVA) and Dunnett's test (or Mann-Whitney U test) for pairwise comparisons, age at acquisition of vaginal patency was significantly delayed at 7500 ppm (33.0 days) relative to the control value (30.5 days), and the age at acquisition of preputial separation was significantly delayed at 750 ppm (43.6 days) and at 7500 ppm (45.8 days) relative to the control group value (41.9 days). F1 male and female body weights at acquisition exhibited a significant dose-related downward trend ($p<0.001$), with the mean weight at 7500 ppm, 92.32 g for females and 194.02 g for males, significantly reduced relative to the control value, 102.52 g for females and 215.70 g for males. When the age at acquisition was statistically analyzed by analysis of covariance (ANCOVA), with body weight as the covariate and with the Least Squares Means Test used for pairwise comparisons, the ages at acquisition of vaginal patency and preputial separation at 7500 ppm (and the age at acquisition of preputial separation at 750 ppm) were significantly delayed from the control group values (Table 33). The statistically significant delays in vaginal patency and preputial separation at 7500 ppm for F1 offspring required (U.S. EPA, 1998) the measurement of anogenital distance in F2 pups on pnd 0 (see Table 48). These changes (referring to preputial separation, vaginal patency, and anogenital distance) did not affect reproductive function in the animals exhibiting them.

F1 male weekly body weights during prebreed and mating periods are presented in Table 34 and Figure 9. The weekly body weights were statistically significantly reduced at 7500 ppm for all ten weeks (sd 0-70) of the prebreed treatment period, and were also statistically significantly reduced at 750 ppm on sd 35 through 70 of the ten-week (70 day) prebreed dosing

period. During the two-week mating period (sd 70-84), body weights were also significantly reduced at 750 and 7500 ppm (Table 34 and Figure 9). Weekly F1 male body weight changes were significantly reduced at 7500 ppm for weeks 1 (sd 0-7), 2 (sd 7-14), 3 (sd 14-21), 4 (sd 21-28), 5 (sd 28-35), 6 (sd 35-42), 7 (sd 42-49), 9 (sd 56-63), and 10 (sd 63-70) at 750 ppm for weeks 1, 3 and 4, and significantly increased for week 8 (sd 49-56) at 4.5 and 75 ppm. Weight changes for the entire 70-day prebreed period were statistically significantly decreased at 750 and 7500 ppm. There were no differences among groups for weight changes during the two-week mating period, sd 70-77 and sd 77-84 (Table 34). Feed consumption (Table 35), expressed as g/day, was significantly reduced at 7500 ppm for each of the ten weeks and for the entire prebreed period (sd 0-70), and at 750 ppm only for week 10. When feed consumption data were expressed as g/kg/day, feed consumption was significantly increased only at 7500 ppm for weeks 2 through 10 of the prebreed period and for the entire period (sd 0-70). Feed consumption as g/day and g/kg/day was statistically equivalent across all groups for the second week of the mating period (sd 77-84); when most males in all groups had already impregnated the females with which they were paired). Percent food efficiency was significantly reduced at 7500 ppm for weeks 3, 4, 5, 6, 7, 9, and 10, and was significantly reduced at 7500 ppm for the entire prebreed period (sd 0-70); it was unaffected for sd 77-84. BPA intake, µg/kg/day, exhibited the expected incremental increases across dose groups, averaging approximately 1, 25, 373, 6407, 62,305, and 748,860 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, over the ten-week prebreed exposure period. Test chemical intake also exhibited the expected decreases within each dosed group over time as the animals gained weight, with values from all six dosed groups dropping more than 50% over the ten-week period. The test chemical intake ranged from approximately 3, 49, 734, 12,229, 121,245, and 1,340,101 µg/kg/day for the first week, to approximately 1, 18, 269, 4566, 46,518, and 546,874 µg/kg/day for the last week of the ten-week prebreed period at 0.0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 35 and Figure 10).

Figure 9. F_1 Male Body Weights During the Prebreed and Mating Periods

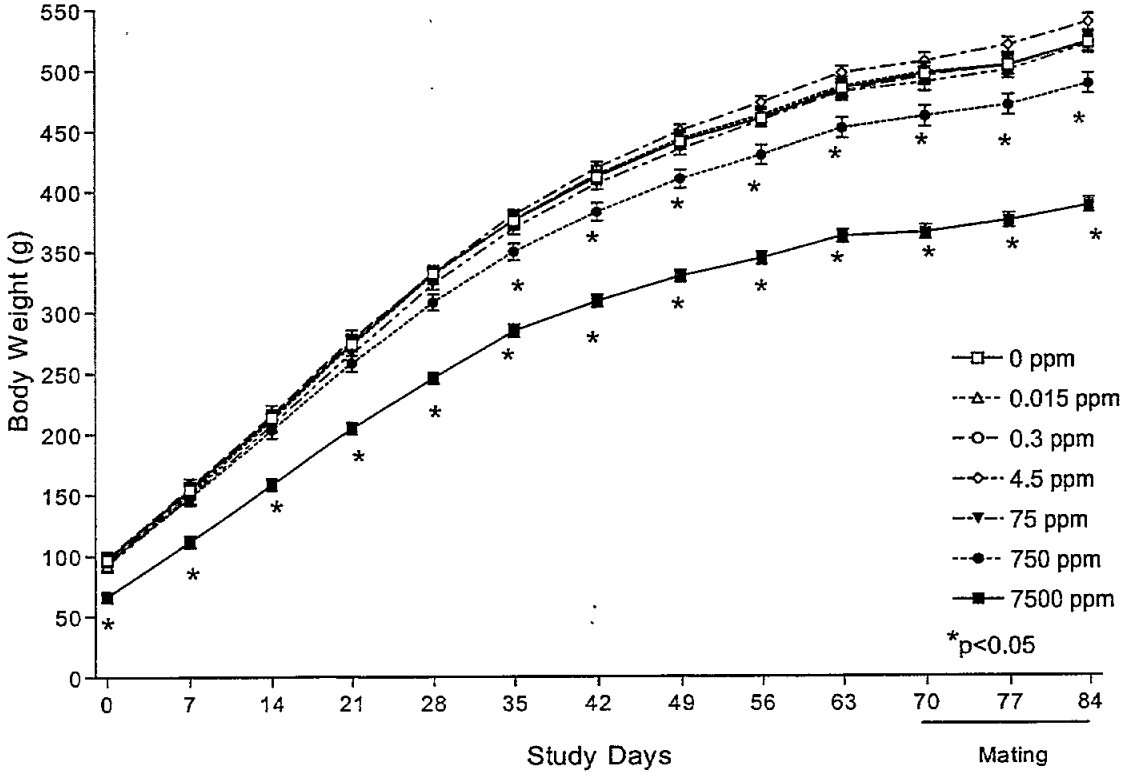
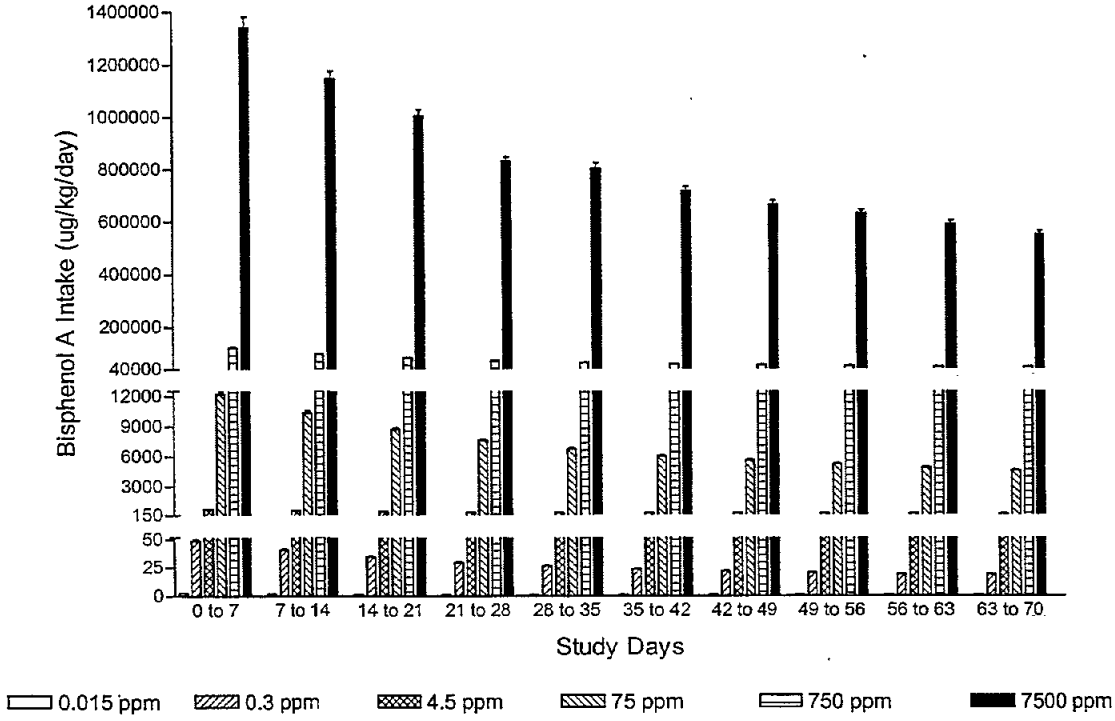


Figure 10. F_1 Male Bisphenol A Intake During the Prebreed Period



Clinical observations of F1 males during the prebreed and mating periods were limited to occasional reporting of alopecia (various locations), chromodacryorrhea, red or red and thickened ears, and sore(s) (various locations) in all groups, and injuries to the mouth, nose, toenails, tail, and teeth, with no treatment- or dose-related incidence or severity (Table 36).

F1 female body weights were significantly reduced during the ten-week prebreed dosing period only at 7500 ppm for all weekly measurements (Table 37 and Figure 11). Female weight changes were significantly reduced at 7500 ppm for weeks 1, 4, 5, 8, and 9, and for the entire period (sd 0-70). Body weight gain was also reduced at 750 ppm and only for week 1; it was significantly increased at 75 ppm only for week 6. Statistical analyses of body weights and weight changes during the mating period (for females not yet found sperm-positive) were not performed due to the decreasing numbers of females included, as more females became sperm-positive and were weighed and recorded based on gestational days, but there were no apparent differences among groups during this period (Table 37). F1 female feed consumption, expressed as g/day, was significantly reduced at 7500 ppm for weeks 1, 2, 3, 5, 6, 7, 8, 9, and 10, and for the entire prebreed period (sd 0-70). It was also significantly reduced at 750 ppm for weeks 2, 5, and 8 during the ten-week prebreed exposure period. When feed consumption data were expressed as g/kg/day, feed consumption was significantly increased at 7500 ppm for weeks 1, 2, 3, 4, 5, 7, and 10, and for the entire period (sd 0-70), and at 75 ppm for week 5. Percent food efficiency was statistically equivalent across all groups for all intervals during the ten-week prebreed exposure period, except for weeks 4 and 5 when the values at 7500 ppm were significantly reduced. BPA intake, calculated as µg/kg/day, exhibited the expected incremental increases across dosed groups, averaging approximately 1, 28, 407, 7004, 67,548, and 749,416 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, for the entire ten-week prebreed exposure period (sd 0-70). The intake values also exhibited the expected declines within groups over time as the F1 females gained weight over the ten-week period. Test chemical intake ranged from approximately 2, 48, 694, 11,817, 111,160, and 1,324,899 µg/kg/day during the first week, to approximately 1, 21, 298, 5249, 54,083, and 613, 983 µg/kg/day during the last week of the prebreed period at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 38 and Figure 12).

Figure 11. F_1 Female Body Weights During the Prebreed Period

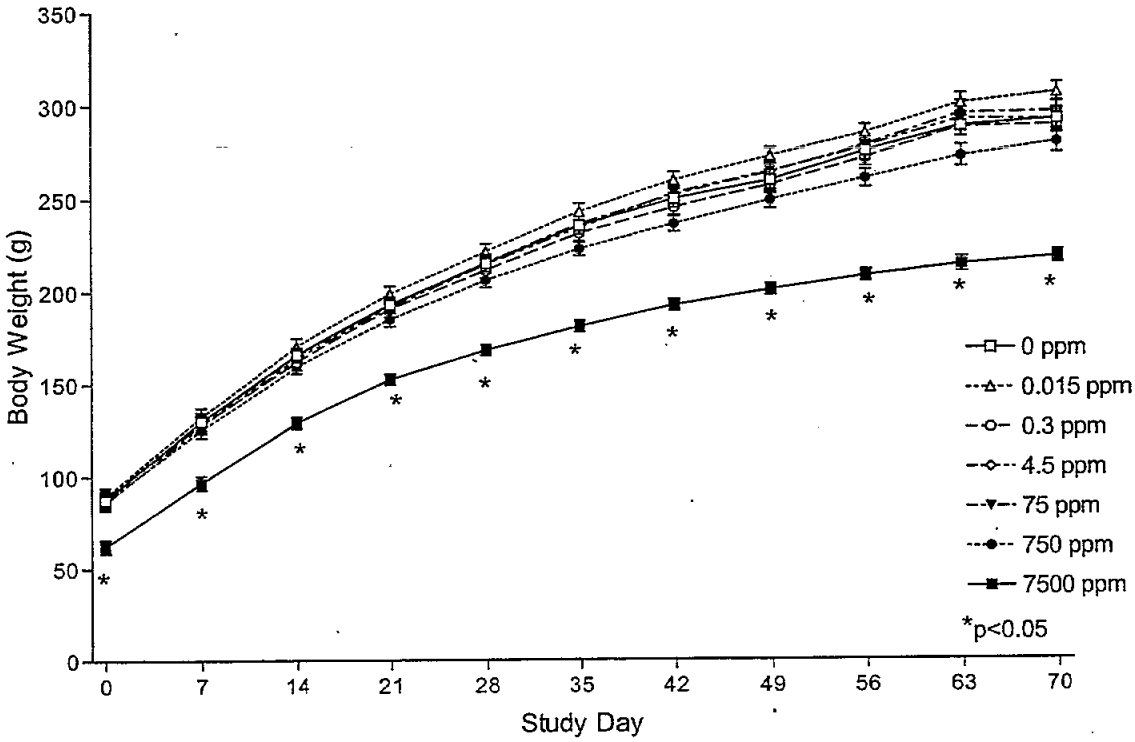
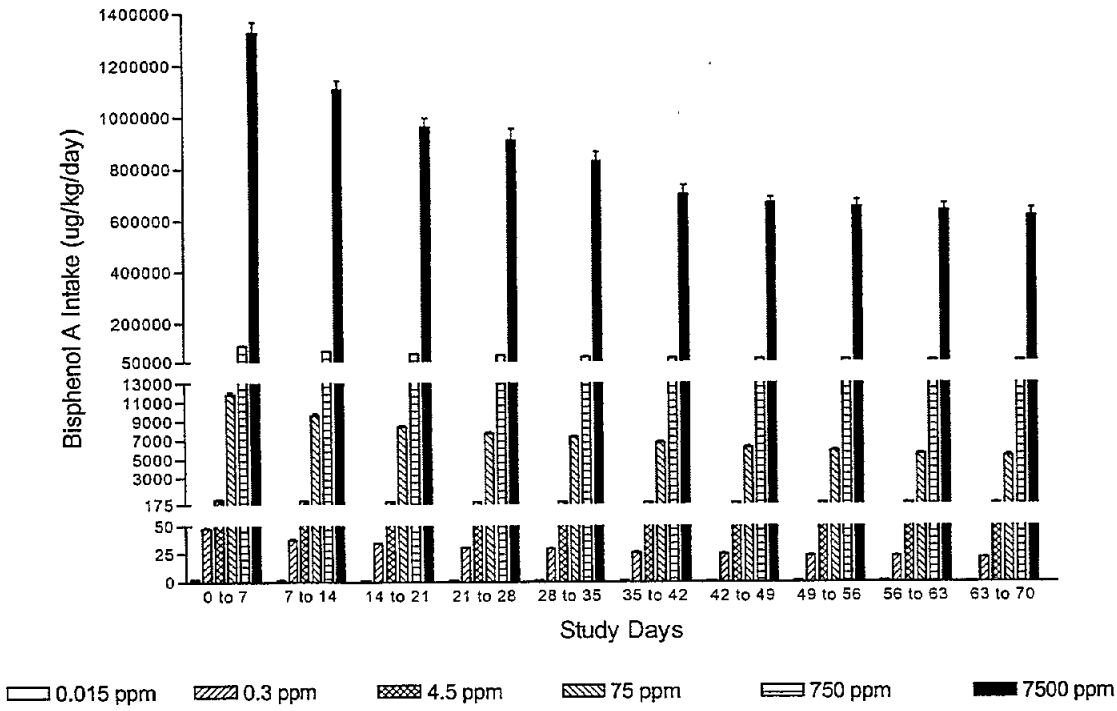


Figure 12. F_1 Female Bisphenol A Intake During the Prebreed Period



Clinical observations of F1 females during the prebreed exposure period included occasional findings of sores, alopecia, chromodacryorrhea, swollen and/or red ears, and teeth abscessed, broken, dark, maloccluded, and/or trimmed. There were no treatment- or dose-related findings (Table 39).

Results of the three-week vaginal cytology assessment are presented in Table 40. Thirty F1 females per group were assessed, with the exception of 7500 ppm where there were only 29. All females were cycling. The number of females with abnormal cycles were 4, 3, 0, 6, 7, 3, and 4 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Cycle length in days was equivalent across all groups, with means of 4.41, 4.47, 4.19, 4.70, 4.94, 4.40, and 4.54 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 40).

Gestation - F1 Females for F2 Litters

The number of females found sperm positive, and therefore included in gestational parameters, were 26, 28, 29, 27, 27, 26, and 27 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F1 maternal gestational body weights were significantly reduced at 7500 ppm for all timepoints, gd 0, 7, 14, and 20, and at 750 ppm for gd 7 and 14 (Table 41 and Figure 13). Maternal weight change was significantly reduced at 7500 ppm for gd 0-7, 7-14, 14-20, and 0-20; and at 750 ppm for gd 7-14 (Table 41). Maternal feed consumption during gestation, expressed as g/day, was significantly reduced at 7500 ppm for all timepoints and for the entire period (gd 0-20) and at 750 ppm for gd 7-14. When the data were expressed as g/kg/day, there were significant increases at 7500 ppm for gd 14-20 and 0-20 and at 750 ppm for gd 14-20. Percent food efficiency was significantly reduced at 7500 ppm for gd 0-7, 14-20 and 0-20, and was equivalent across all remaining groups for the gestational period (gd 0-20). Maternal intake of BPA exhibited the expected incremental increases across dose groups, averaging approximately 1, 20, 299, 5078, 50,536, and 573,440 µg/kg/day at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm for the entire gestational period, gd 0-20 (Table 42 and Figure 14).

Clinical observations of F1 females during gestation exhibited no treatment- or dose-related findings. Observations included occasional findings of alopecia, red ears, and sores across all groups (Table 43).

Figure 13. F_1 Female Body Weights During Gestation

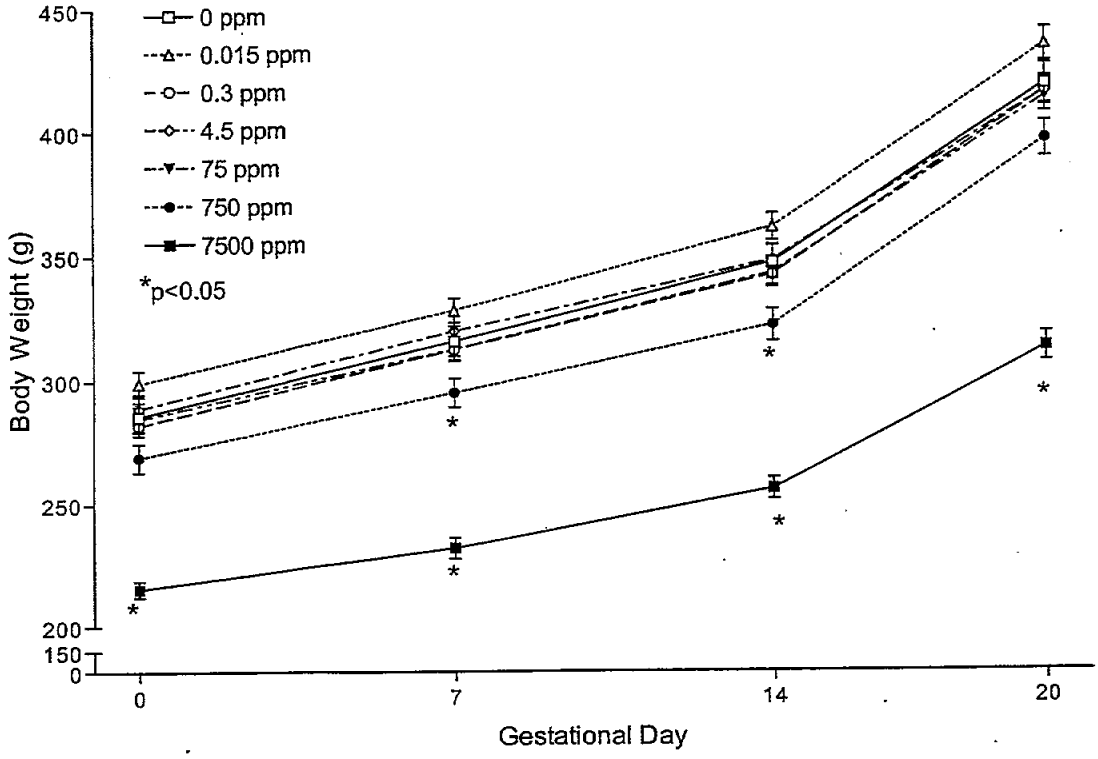
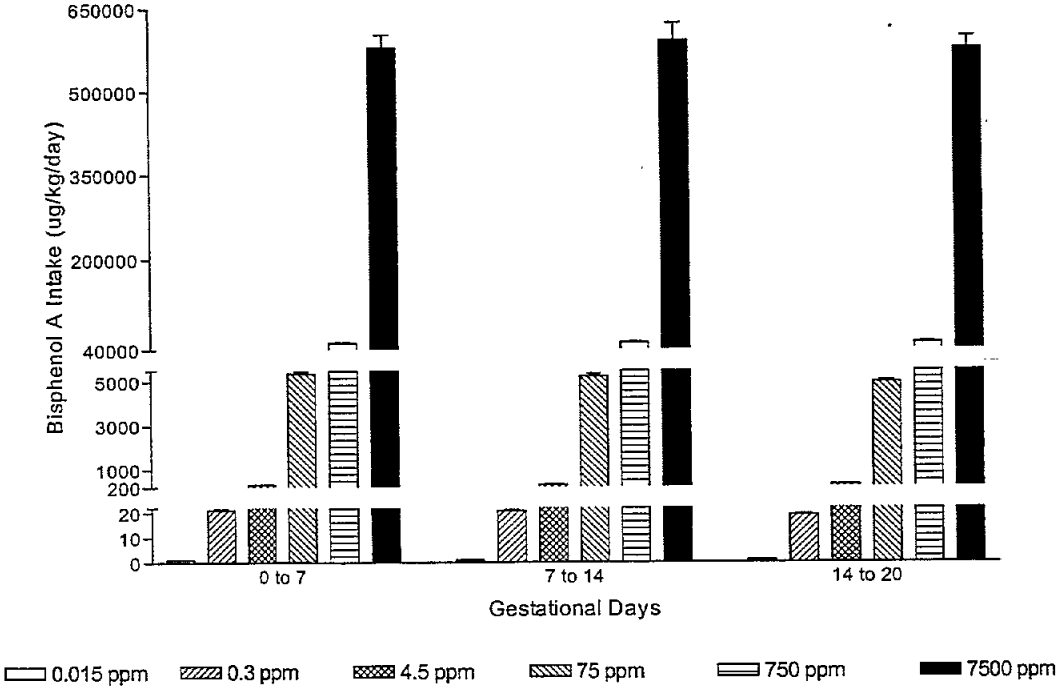


Figure 14. F_1 Female Bisphenol A Intake During Gestation



Lactation - F1 Females for F2 Litters

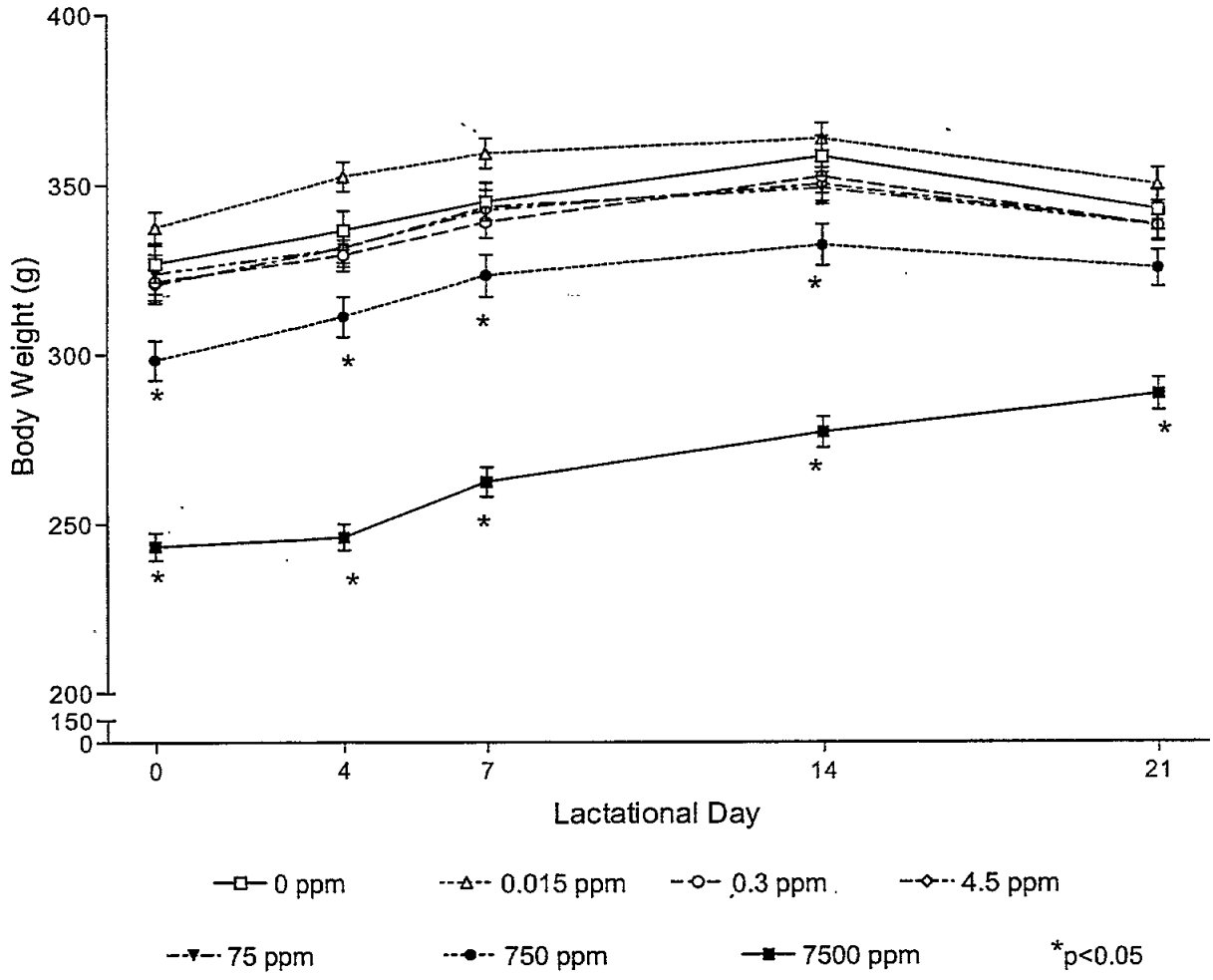
The number of females that delivered litters (with live pups) on pnd 0 were 27, 30, 30, 26, 27, 26, and 27 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. On pnd 21, the number of live litters were 27, 30, 30, 26, 27, 26 and 26 (one litter was lost at 7500 ppm) at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Maternal F1 body weights during lactation exhibited significant reductions at 7500 ppm on pnd 0, 4, 7, 14, and 21, and at 750 ppm on pnd 0, 4, 7, and 14 (but not 21) (Table 44 and Figure 15). Maternal lactational weight change (pnd 0-21) was significantly increased at 7500 ppm for pnd 4-7, 14-21, and 0-21; no other intervals at 7500 ppm or dams at other doses were affected (Table 44). Maternal F1 lactational feed consumption, expressed as g/day, was significantly reduced at 7500 for pnd 7-14, 14-21, and for pnd 0-21; at 0.3, 4.5, and 75 ppm for pnd 14-21; and at 0.3 ppm for pnd 0-21. Maternal F1 lactational feed consumption, expressed as g/kg/day, was significantly increased at 750 and 7500 ppm for pnd 0-4, 4-7, 7-14, 14-21, and pnd 0-21 (entire lactational period), and for pnd 0-4 at 0.015 ppm. Percent food efficiency was significantly increased at 7500 ppm for pnd 4-7 and 14-21 and for the entire lactation period (pnd 0-21). Maternal intake of BPA, expressed as µg/kg/day, exhibited the expected incremental increases across dosed-feed groups, with average intakes for the lactational period (pnd 0-21) of 2, 50, 763, 12,802, 139,741, and 1,510,054 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, confounded by the pups self-feeding, beginning during the second week of life (Table 45 and Figure 16).

Maternal F1 clinical observations during the lactation period indicated no treatment- or dose-related clinical signs. Observations included alopecia, chromodacryorrhea, red ears, and rust-colored fur across all groups (Table 46).

Reproductive and lactational indices for F1 parents and F2 litters (Table 47) exhibited no treatment-related changes in mating index, fertility index (either sex), gestational index, or pregnancy index. Precoital interval and mean gestational length in days were also equivalent across all groups. Mean gestational length was 21.8, 22.0, 21.9, 22.0, 21.9, 22.0, and 21.8 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The number of implantation sites per litter and, therefore, the total number of pups per litter and the number of live pups per litter on pnd 0 were significantly reduced at 7500 ppm. Percent postimplantation loss per litter, the number of dead pups per litter on pnd 0, stillbirth and live birth indices, and survival indices for

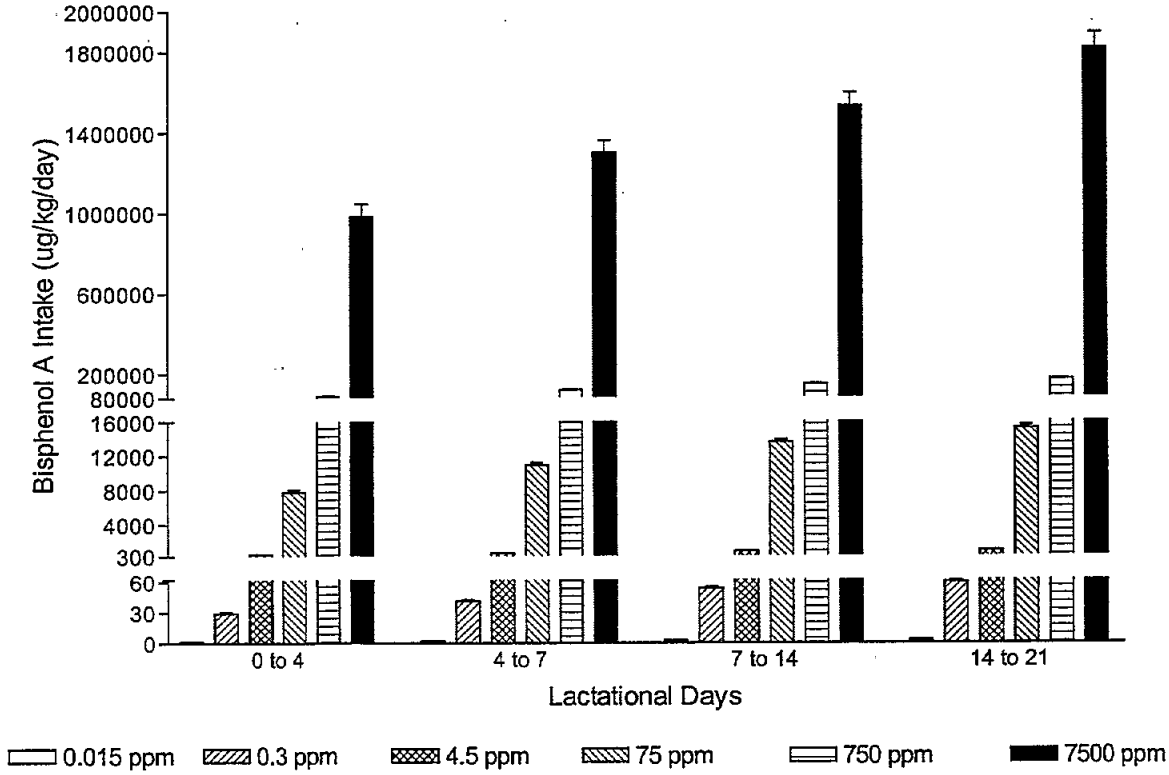
pnd 4, 7, 14, 21, and the lactational index (pnd 4 postcull to pnd 21) were equivalent across all groups (Table 47).

Figure 15. F_1 Female Body Weights During Lactation



F2 litter sex ratio (% male pups per litter) was equivalent across groups for all timepoints evaluated on pnd 0, 4, 7, 14 and 21, except for a significant increase (relatively more male pups) at 7500 ppm on pnd 7. Mean number of pups per litter was significantly reduced at 7500 ppm on pnd 0 and 4 (prior to standardization) and significantly increased at 750 ppm on pnd 7 (Table 48).

Figure 16. F_1 Female Bisphenol A Intake During Lactation



Since acquisition of both preputial separation and vaginal patency was significantly delayed in F1 offspring, anogenital distance was measured in F2 offspring at birth (pnd 0), as required by guidelines (U.S. EPA, 1998). Average male pup anogenital distance per litter was equivalent across all groups (1.98 mm at 0 ppm to 2.00 mm at 0.015 and 7500 ppm). Average female pup anogenital distance per litter was significantly increased at 0.015 ppm (0.98 mm), 0.3 ppm (0.98 mm), 4.5 ppm (0.98 mm), and at 750 ppm (0.99 mm) (but not at 7500 ppm, 0.96 mm), relative to the concurrent control value (0.95 mm); adjusted female anogenital distance (after ANCOVA) exhibited the same pattern. These changes (referring to preputial separation, vaginal patency, and anogenital distance) did not affect reproductive function in the animals exhibiting them (Table 48).

Pup body weights per litter (all pups or males and females separately) were equivalent across all groups for pnd 0 and 4. On pnd 7, 14, and 21, pup body weights per litter (all pups or separately by sex) were significantly reduced at 7500 ppm. On pnd 21, all pup body weights per

litter were also significantly reduced at 4.5, 75, and 750 ppm; male and female pup weights per litter, when evaluated separately by sex, were significantly reduced also at 75 ppm. As with F1 offspring during lactation, these effects on pup body weights were observed only when the pups were self feeding, beginning during the second week of life (based on previous observations in the performing laboratory) and, therefore, were directly exposed to the test material in the feed (possibly as early as pnd 10). Retained nipples in F2 preweanling pups were present only at 75 ppm, with 0.77% of male pups with one or more nipples at that dose. Areolae were present in one or more male pups in all groups, with the incidences equivalent across all groups; the percent of males with one or more areolae was 2.26, 2.10, 3.95, 1.57, 1.54, 2.40, and 2.26% at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 48).

Clinical observations of F2 pups during the lactation period (Table 49) indicated no treatment- or dose-related incidence of found dead, euthanized moribund, and missing and presumed dead pups. The total found dead, euthanized and moribund, or missing and presumed dead pups through pnd 4 were 14 (plus three pups euthanized since their dam died during delivery), 9, 28, 6, 10, 5, and 23, and from pnd 5 through pnd 21 were 2, 1, 1, 1, 6, 0, and 2 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Most observations occurred in all groups and indicated small, hypothermic (cold) pups, no milk in stomach (no milk band), and tail injuries (Table 49).

Gross necropsy of F2 pups that died during the lactation period and were available for examination (Table 50) included 13, 9, 17, 4, 12, 5, and 18 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Most deaths occurred in the first four days of life, many with patent (open) ductus arteriosus and no air in lungs, indicating primary atelectasis (defective expansion of pulmonary alveoli at birth), although many pups died with closed ductus and air in lungs. Many pups had autolyzed abdominal organs. All of these findings are typical for pnd 0-4 deaths and did not exhibit a treatment- or dose-related pattern of incidence or severity (Table 50).

Necropsy – F2 Weanlings

Necropsy body and organ weights of F2 weanling (pnd 21) males and females, up to three per sex per litter, are presented in Table 51. A total of 79, 86, 85, 74, 77, 74, and 70 male F2 pups were evaluated at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F2 weanling male terminal body weights were significantly reduced at 4.5, 75, and 7500 ppm, but not at 750

ppm. Absolute mean male liver, thymus, spleen, brain, and paired testes weights were significantly reduced at 7500 ppm. Absolute mean male liver weight was also significantly reduced at 4.5 and 75 ppm, as was absolute mean male thymus weight at 750 ppm. Absolute weights of male paired epididymides and seminal vesicles with coagulating glands, and relative male liver and seminal vesicles with coagulating gland weights were equivalent across all groups. F2 male relative thymus, brain, paired testes and epididymal weights were significantly increased at 7500 ppm, as well as relative brain weight at 0.3, 4.5, and 75 ppm. Relative paired testes weights were also significantly increased at 4.5 ppm. Relative paired epididymal weights were also significantly increased at 75 and 750 ppm. Relative thymus weight was significantly decreased at 750 ppm, and relative spleen weight was significantly decreased at 7500 ppm.

Necropsy body and organ weights of F2 weanling females are also presented in Table 51. A total of 77, 90, 79, 72, 80, 77, and 59 F2 weanling females were evaluated at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F2 weanling female body weights were significantly decreased at 75 and 7500 ppm (but not at 750 ppm). Absolute female liver, thymus, spleen, brain and paired ovary weights were significantly reduced at 7500 ppm, as were absolute female liver and spleen weights at 75 ppm. Absolute brain weight was increased at 750 ppm. Relative female paired ovary and uterus weights were equivalent across all groups. Relative female brain weight was significantly increased at 75, 750, and 7500 ppm. Relative thymus weight was significantly increased, and relative liver and spleen weights were significantly decreased at 7500 ppm (Table 51).

Summary of gross findings for the F2 weanlings is presented in Table 52. Almost all of the gross findings involved the kidneys. There was one male at 0.015 ppm with a pale liver and spleen. All of the renal lesions were hydronephrosis right, left, or bilateral (Table 52). Hydronephrosis (dilation of the renal pelvis) is a very common finding in CD® rats (from fetuses through adults; see historical control data, Volume IX, Appendix III).

F1 Parental Holding Period

F1 parental males were held from the end of mating until scheduled sacrifice after the delivery of the F2 litters (sd 84-105). F1 male body weights were significantly reduced at 750 and 7500 ppm for sd 84, 91, 98, and 105. Male weight change was equivalent across all groups for sd 84-91 and 91-98, and significantly reduced at 4.5, 75, 750, and 7500 ppm for sd 98-105

(Table 53). F1 male feed consumption, expressed as g/day, was significantly reduced at 7500 ppm for sd 91-98 and 98-105. F1 male feed consumption in g/kg/day was significantly increased at 7500 ppm for sd 84-91, 91-98, and 98-105. Percent food efficiency was statistically increased at 4.5 ppm for sd 91-98, significantly decreased at 4.5 ppm for sd 98-105, and significantly increased at 7500 ppm for sd 98-105. BPA intake in µg/kg/day ranged from approximately 0.9 (first week) to 0.8 (last week) µg/kg/day at 0.015 ppm, 17-16 µg/kg/day at 0.3 ppm, 252-236 µg/kg/day at 4.5 ppm, 4374-4090 µg/kg/day at 75 ppm, 44,281-41,773 µg/kg/day at 750 ppm, and 537,693-499,992 µg/kg/day at 7500 ppm (Table 54).

Clinical observations of the F1 males during this holding period involved alopecia (various locations), chromodacryorrhea, ears red or red and thickened, maloccluded teeth, foot and tail injuries, and sore(s) (various locations) across all groups, with no treatment- or dose-related pattern of incidence (Table 55).

F1 females that were not pregnant, did not deliver a live litter, lost the entire litter during lactation, or weaned the litter were also held until scheduled sacrifice through sd 112. Because of the small numbers per group, which changed over time, no statistical analyses were performed on body weight, weight gain or feed consumption parameters. Body weights appeared slightly reduced at 7500 ppm for sd 84, 91, 98, 105, and 112. F1 female body weight change during this period was variable (due to dam status and dam number) (Table 56).

F1 female feed consumption during this period in g/day and g/kg/day appeared approximately equivalent across all groups. Percent food efficiency was variable across all groups with zero to four dams per group. BPA intake ranged from approximately 1 µg/kg/day (first week) to no data (last week) at 0.015 ppm, 18 µg/kg/day-no data at 0.3 ppm, no data-264 µg/kg/day at 4.5 ppm, 3801-4657 µg/kg/day at 75 ppm, 50,183-48,875 µg/kg/day at 750 ppm, and 892,611-453,976 µg/kg/day at 7500 ppm (Table 57).

F1 female clinical observations during this period included only chromodacryorrhea, red ears, and piloerection in one dam/group (with no treatment- or dose-related pattern), and one dam at 7500 ppm with circling, disoriented behavior (cause unknown) (Table 58).

Necropsy – F1 Parental Animals

Summary of F1 male terminal body weights, organ weights, and andrological assessments is presented in Table 59. F1 male body weights at scheduled sacrifice were significantly reduced at 750 and 7500 ppm. Absolute liver, paired kidney, spleen, brain, pituitary, paired testes, paired epididymides, prostate, preputial gland, and seminal vesicles with coagulating gland weights were significantly reduced at 7500 ppm. Absolute liver and pituitary weights were also significantly reduced at 750 ppm, and absolute pituitary weight was significantly reduced at 0.015 ppm. Absolute paired adrenal weights were equivalent across all groups. Relative liver, prostate, and preputial gland weights were equivalent across all groups. Relative weights of paired kidneys, paired adrenals, spleen, brain, pituitary, paired testes, paired epididymides, and seminal vesicles with coagulating glands were significantly increased at 7500 ppm. Relative spleen weight was significantly reduced at 75 ppm, relative paired kidney weight was significantly reduced at 4.5 ppm, and relative brain weight was significantly increased at 750 ppm. Percent motile sperm and percent progressively motile sperm were statistically equivalent across all groups. Epididymal sperm concentration (10^6/g) was significantly reduced at 7500 ppm. Testicular homogenization-resistant spermatid head count (10^6/g), daily sperm production, efficiency of daily sperm production, and percent abnormal sperm were all statistically equivalent across all groups (Table 59). The mean values for percent abnormal sperm (with associated large variance terms) at 0.015 and 0.3 ppm were due to one male (no. 1173) at 0.015 ppm with 44% motility and 67.7% abnormal sperm, and one male (no. 1187) at 0.3 ppm with normal motility (77%) and 40.1% abnormal sperm (individual animal data table A-68, Volume VI). Both males sired live litters (male no. 1173 at 0.015 ppm with female no. 1034, and male no. 1187 at 0.3 ppm with female no. 1074; see individual animal data tables A-51 and A-52, Volume VI).

Gross findings for F1 males at scheduled sacrifice included alopecia (limbs), ears red and thickened, chromodacryorrhea, kidney with uni- (right) or bilateral hydronephrosis, adrenal glands reduced in size and/or soft, one male at 7500 ppm with a small left preputial gland (No. 1143), and one male at 0.015 ppm with an enlarged right testis (No. 1173). Both males with gross reproductive organ findings (Nos. 1143 and 1173) sired live litters (individual animal data tables in Volume VI). Of the two males who died on study, the one at 4.5 ppm (No. 1315) exhibited a fractured nose, hemorrhagic lungs, and blood in the cecum, and the control male (No.

1147) exhibited no findings at unscheduled necropsy. None of these findings were considered treatment or dose related (Table 60 and Table A-69 in Volume VI).

Microscopic findings in F1 males exhibited no treatment- or dose-related incidences or severities in the organs examined, including the reproductive organs and any organs identified from the gross findings (Table 60).

Summary of F1 female terminal body weights, organ weights, paired ovarian follicle counts (at high dose and control), and stage of estrus at necropsy is presented in Table 61. Body weights at scheduled sacrifice (29, 30, 30, 30, 30, 30, and 28 females at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm) were significantly reduced at 750 and 7500 ppm. Absolute female liver and spleen weights were statistically equivalent across all groups. Absolute paired kidney, paired adrenal, brain, pituitary, paired ovary, and uterine weights were significantly reduced at 7500 ppm. Absolute paired ovary weight was also significantly reduced at 750 ppm. Relative liver, paired kidney, paired adrenal gland, and uterine weights were equivalent across all groups. Relative pituitary and paired ovary weights were significantly reduced at 7500 ppm. Relative spleen and brain weights were significantly increased at 7500 ppm. Paired ovarian follicle counts at 0 and 7500 ppm were statistically equivalent, and distribution of stages of estrus at necropsy were equivalent across all groups (Table 61).

Gross findings for the two females that died on study included one female at 0.0 ppm with 11 retained dead fetuses in the uterus, and one female at 7500 ppm with right rear ankle swollen (Table 62). Gross findings for F1 females at scheduled sacrifice were unremarkable and included alopecia, chromodacryorrhea, or uni- (right) or bilateral red or red and thickened ears, unilateral (right side) or bilateral hydronephrosis, misshapen spleen (one), cyst on ovary (one), cyst on uterus (one), and one animal that appeared to be female externally at 7500 ppm (No. 1214), but had mid-abdominal testes with associated anchoring structures identified by the Study Director as anterior cranial ligaments and posterior gubernacula, with no male accessory sex organs or any female reproductive organs (Table 62).

F1 maternal microscopic findings are also presented in Table 62. Treatment-related findings included increased incidence of renal tubular degeneration (8/11 females) at 7500 ppm (versus none in any other group) and possibly of chronic hepatic inflammation at 7500 ppm (3/11) versus 3/30 in the control group. Animal No. 1214 (at 7500 ppm), externally identified as a female, exhibited mid-abdominal testes, as confirmed by histopathology. There were no

treatment- or dose-related incidences or severities of findings in other organs, including the reproductive system, or in other tissues with gross lesions (Table 62 in Volume II, and Table A-71 in Volume VI).

F2 Prebreed Exposure Period

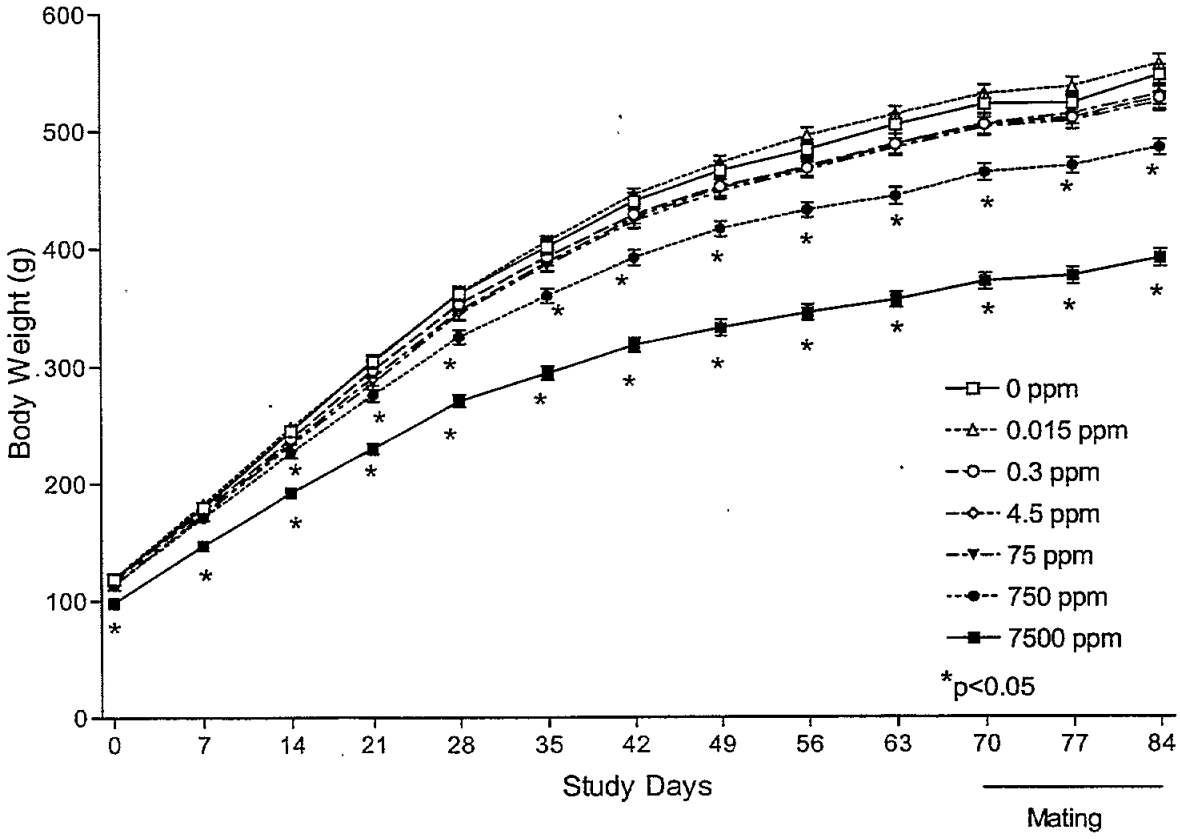
A summary of the fate of parental F2 males and females is presented in Table 63. Of the males (30/group) designated as F2 parents at weaning, one male (No. 2365) at 750 ppm was found dead on sd 63, and two males at 7500 ppm were found dead; one (No. 2265) on sd -8 and one (No. 2377) on sd -9. (The negative study days were during the holding period after weaning and prior to the start of the formal prebreed period.) Therefore, 30, 30, 30, 30, 30, 29, and 28 F2 males were sacrificed on schedule at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Of the 30 females/group, designated as F2 parents at weaning, two females were found dead at 7500 ppm; one (No. 2058) on sd -7 and one (No. 2354) on sd -8. Therefore, 30, 30, 30, 30, 30, 30, and 28 F2 females were sacrificed on schedule at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively.

The assessment of acquisition of reproductive developmental landmarks is presented in Table 64. The mean day of acquisition of vaginal patency in F2 females was significantly delayed at 75 ppm (by 1.1 days) and at 7500 ppm (by 3.5 days), but unaffected at 750 ppm. Mean body weights at acquisition were statistically equivalent across all groups. When the day of acquisition was analyzed by ANCOVA (with body weight at acquisition as the covariate), the adjusted mean day of acquisition was significantly delayed only at 7500 ppm (by 4.0 days).

The mean day of acquisition of preputial separation in F2 males was significantly delayed at 0.3 ppm (by 1.6 days), at 75 ppm (by 1.2 days), at 750 ppm (by 1.1 days), and at 7500 ppm (by 5.8 days) versus the control value. Mean body weights at acquisition were significantly reduced only at 7500 ppm. When the day of acquisition was analyzed by ANCOVA, the adjusted mean day of acquisition was significantly delayed only at 7500 ppm (by 7.4 days) (Table 64). The statistically significant delays in vaginal patency and preputial separation at 7500 ppm for F2 offspring required (U.S. EPA, 1998) the measurement of anogenital distance in F3 pups on pnd 0 (see Table 79). These changes (referring to preputial separation, vaginal patency, and anogenital distance) did not affect reproductive function in the animals exhibiting them.

F2 male weekly body weights during the prebreed and mating periods are presented in Table 65 and Figure 17. F2 male weekly body weights were significantly reduced at 7500 for all time points from sd 0 through 84, and at 750 ppm for all time points from sd 14 (not sd 0 or 7) through 84. Body weight change was significantly reduced at 7500 ppm from sd 0-7 (week 1) through 56-63 (week 9), for sd 0-70 (the entire prebreed period), and for sd 77-84 (the second week of mating), but not for sd 63-70 or 70-77. Body weight change was also significantly reduced at 750 ppm for sd 7-14, 14-21, 21-28, 35-42, 56-63, 0-70, and 77-84; at 75 ppm for sd 7-14, 14-21, and 77-84; and at 0.3 and 4.5 ppm only for sd 77-84 (Table 65).

Figure 17. F_2 Male Body Weights During the Prebreed and Mating Periods

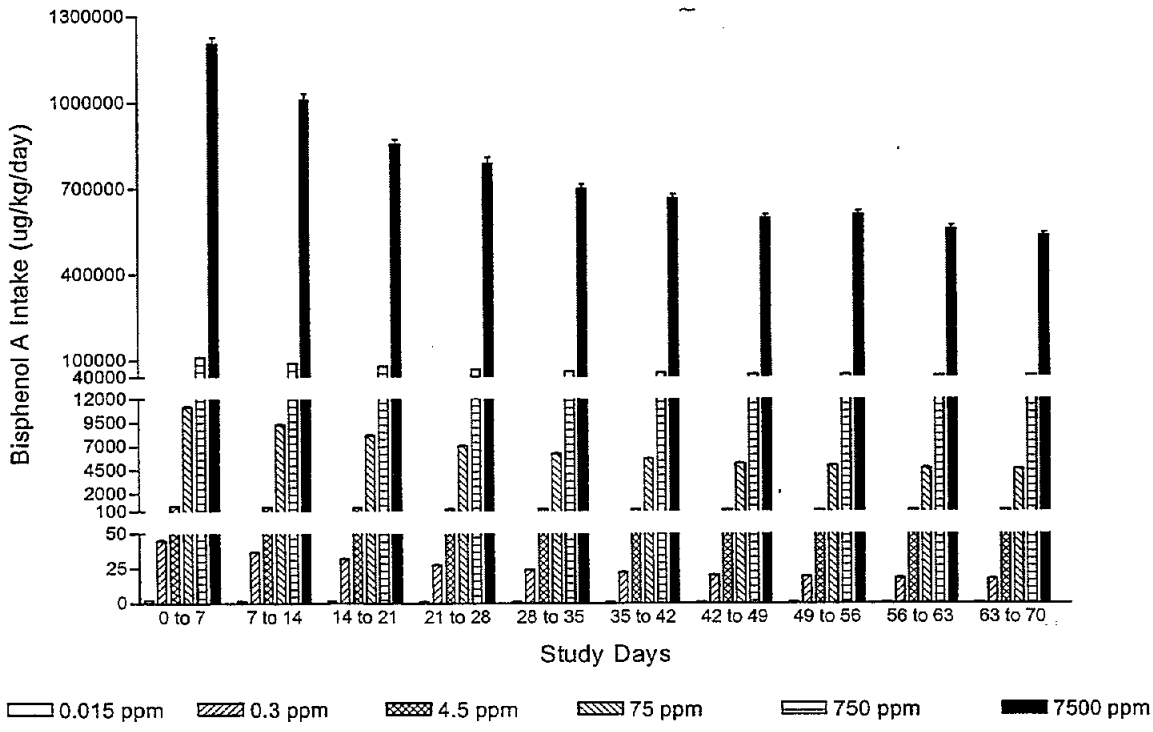


F2 male feed consumption, expressed as g/day, was significantly reduced at 7500 ppm for all intervals during the prebreed (sd 0-7 through sd 63-70) and for sd 0-70 (but not for sd 77-84). Feed consumption, as g/day, was also significantly reduced at 750 ppm for sd 7-14, 14-21, 21-28, 28-35, 35-42, 42-49, 49-56, 56-63, and 0-70. When the feed consumption data were

expressed as g/kg/day, it was significantly increased at 7500 ppm for all weekly prebreed intervals and for sd 0-70; at 750 ppm only for sd 49-56; and at 75 ppm for sd 21-28, 28-35, 35-42, 42-49, 49-56, 56-63, 63-70, and 0-70. It was also significantly increased at 4.5 ppm for sd 28-35 and 49-56. Percent feed efficiency was significantly reduced at 7500 ppm for all weekly intervals (except for week 10, sd 63-70), and for sd 0-70; at 750 ppm for sd 7-14, 14-21, 56-63, and 0-70; at 75 ppm only for sd 14-21 and 56-63; and at 4.5 ppm only for sd 14-21 (Table 66).

BPA intake, in µg/kg/day, exhibited the expected incremental increases across dose groups, averaging approximately 1, 24, 359, 6075, 59,402, and 696,952 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm over the entire prebreed period (sd 0-70). Test chemical intake also exhibited the expected decreases over time within each dose group, as the animals gained weight but did not exhibit major increases in feed consumption, with values for all six dosed groups dropping more than 50% over the ten-week period. The test chemical intake ranged from approximately 2, 45, 672, 11,163, 110,490, and 1,205,507 µg/kg/day for the first week (sd 0-7) down to approximately 1, 17, 261, 4542, 44,288, and 532,473 µg/kg/day for the last week (sd 63-70) of the ten-week prebreed period (Table 66 and Figure 18).

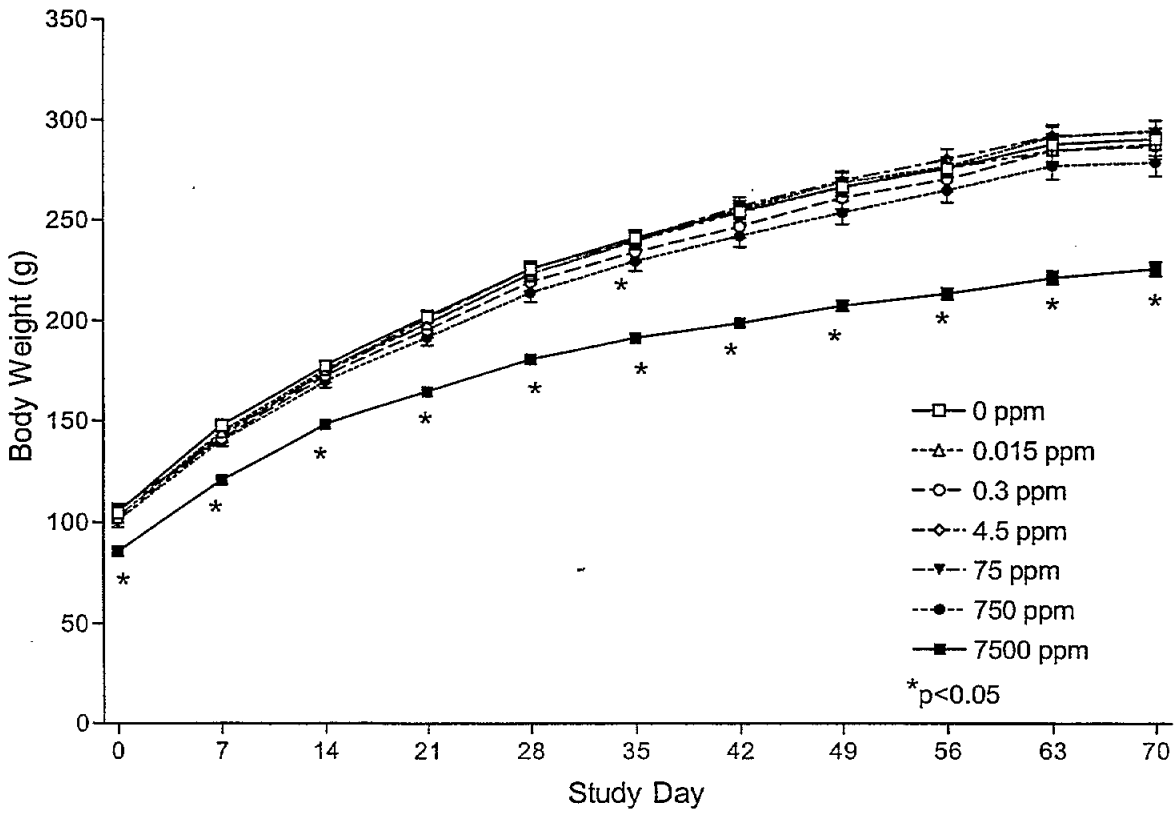
Figure 18. F_2 Male Bisphenol A Intake During the Prebreed Period



Clinical observations of F2 males during the prebreed and mating periods are presented in Table 67 and were limited to occasional findings of alopecia, chromodacryorrhea, dehydration (early in the prebreed period as the individually-housed males adjusted to automatic watering), red ears, sore(s), teeth broken, maloccluded or trimmed, and evidence of injury to the mouth and nose; none of these findings were treatment or dose related. Two males at 7500 ppm were found dead, and one male at 750 ppm was euthanized moribund during this period. (Table 67).

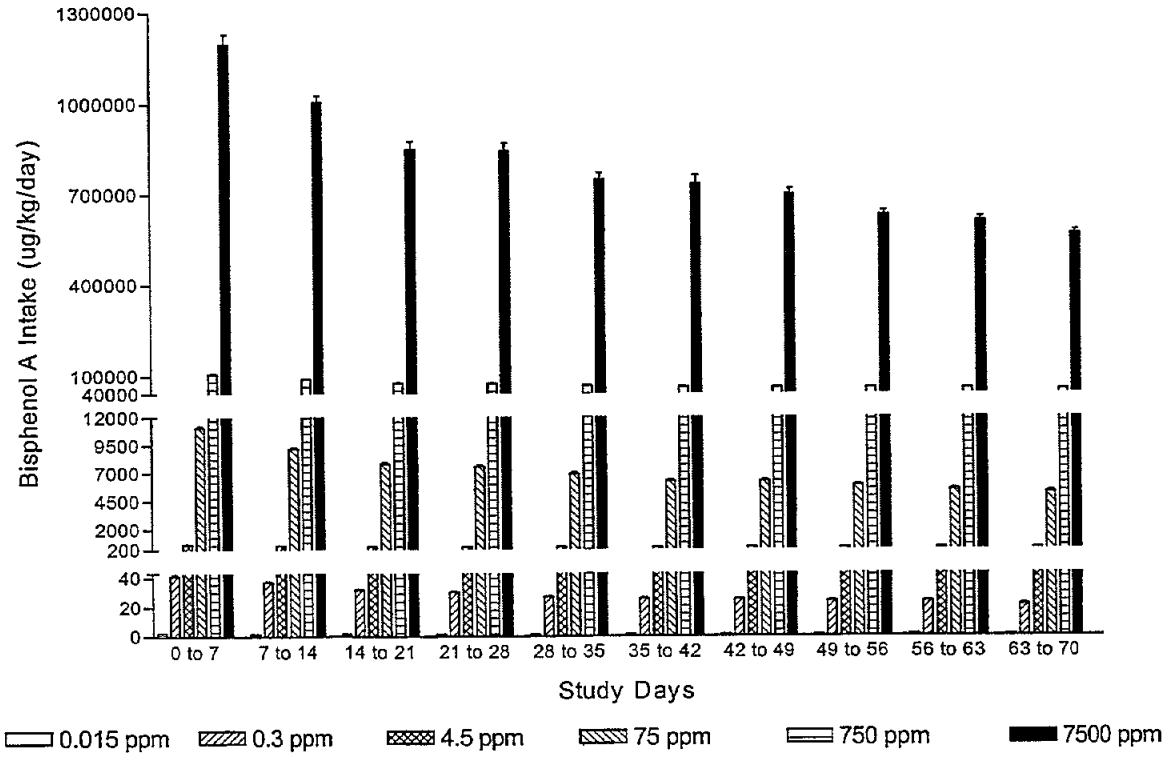
F2 female body weights were significantly reduced for all weekly measurements during the prebreed period (sd 0 through 70) at 7500 ppm. They were also significantly reduced at 750 ppm only for sd 35 (Table 68 and Figure 19). Body weight change was also significantly reduced at 7500 ppm for sd 0-7, 14-21, 21-28, 28-35, 35-42, and 0-70 (entire prebreed period). Body weight change was also significantly reduced for sd 0-7 at 0.3 ppm. Very few females remained sperm-negative during the two-week mating period (sd 70-77 and 77-84), but their data (not statistically analyzed) are also presented for completeness (Table 68).

Figure 19. F_2 Female Body Weights During the Prebreed Period



F2 female feed consumption in g/day was significantly reduced at 7500 ppm for all weekly intervals and for the entire prebreed period (sd 0-70). It was also significantly reduced at 750 ppm only for sd 28-35. When the feed consumption data were expressed as g/kg/day, the values at 7500 ppm were significantly increased for sd 0-7, 7-14, 21-28, 35-42, 42-49, 49-56, 63-70, and 0-70. Percent food efficiency was significantly reduced at 7500 ppm for sd 0-7, 14-21, 21-28, 35-42, and 0-70, and for sd 0-7 at 0.3 ppm. BPA intake, calculated as µg/kg/day, exhibited the expected incremental increases across dose groups, averaging approximately 1, 28, 410, 6808, 67,069, and 755,629 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, over the entire ten-week prebreed period (sd 0-70). The intake values also exhibited the expected declines within groups over time as the F2 females gained weight over the ten-week period. Test chemical intake ranged from approximately 2, 41, 687, 11,058, 106,479, and 1,196,984 µg/kg/day for the first week (sd 0-7) to 1, 21, 304, 5203, 51,394, and 564,708 µg/kg/day for the last week (sd 63-70) of the ten-week prebreed exposure period (Table 69 and Figure 20).

Figure 20. F_2 Female Bisphenol A Intake During the Prebreed Period



Clinical observations of F2 females during the prebreed exposure period (Table 70) included alopecia, chromodacryorrhea, dehydration, red ears, sore(s) (all common findings and with no treatment-related incidence), and damage to teeth, nose, and mouth (typical in rats housed in stainless steel wire mesh cages), and two females that died at 7500 ppm during this period.

Results of the three-week vaginal cytology assessment are presented in Table 71. All females, except for one each at 0.3, 4.5, and 750 ppm, were cycling. Of the females that were cycling, 5, 4, 4, 3, 1, 5, and 4 females exhibited abnormal cycles, and cycle length in days was equivalent across all groups, with means of 4.54, 4.61, 4.39, 4.47, 4.17, 4.56, and 4.56 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 71).

Gestation - F2 Females for F3 Litters

The number of F2 females found sperm-positive, and therefore included in gestational parameters, were 27, 28, 29, 26, 28, 27, and 27 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F2 maternal gestational weights were significantly reduced (approximately 22.6%) at 7500 ppm for the entire gestational period (gd 0, 7, 14, and 20) (Table 72 and Figure 21). Gestational body weight change was also significantly reduced at 7500 ppm for all gestational intervals: gd 0-7, 7-14, 14-20, and gd 0-20 (the entire period). Weight change was also significantly reduced at 750 ppm for gd 0-7, at 4.5 ppm for gd 0-20, and at 0.3 ppm for gd 14-20 and 0-20 (Table 72).

Maternal feed consumption during gestation is presented in Table 73. When the data were expressed as g/day, there were significant reductions at 7500 ppm for all intervals, gd 0-7, 7-14, 14-20, and 0-20; at 750 ppm for gd 0-7, 7-14, and 0-20; and at 0.3 ppm only for gd 0-7. When the data were expressed as g/kg/day, there were significant increases at 7500 ppm for gd 14-20 and 0-20, and significant decreases at 750 and 0.3 ppm only for gd 0-7. Percent food efficiency was significantly reduced only at 7500 ppm for gd 14-20 and 0-20. BPA intake in µg/kg/day for the gestational period (gd 0-20) was approximately 1, 19, 291, 4834, 48,815, and 547,557 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 73 and Figure 22).

Figure 21. F_2 Female Body Weights During Gestation

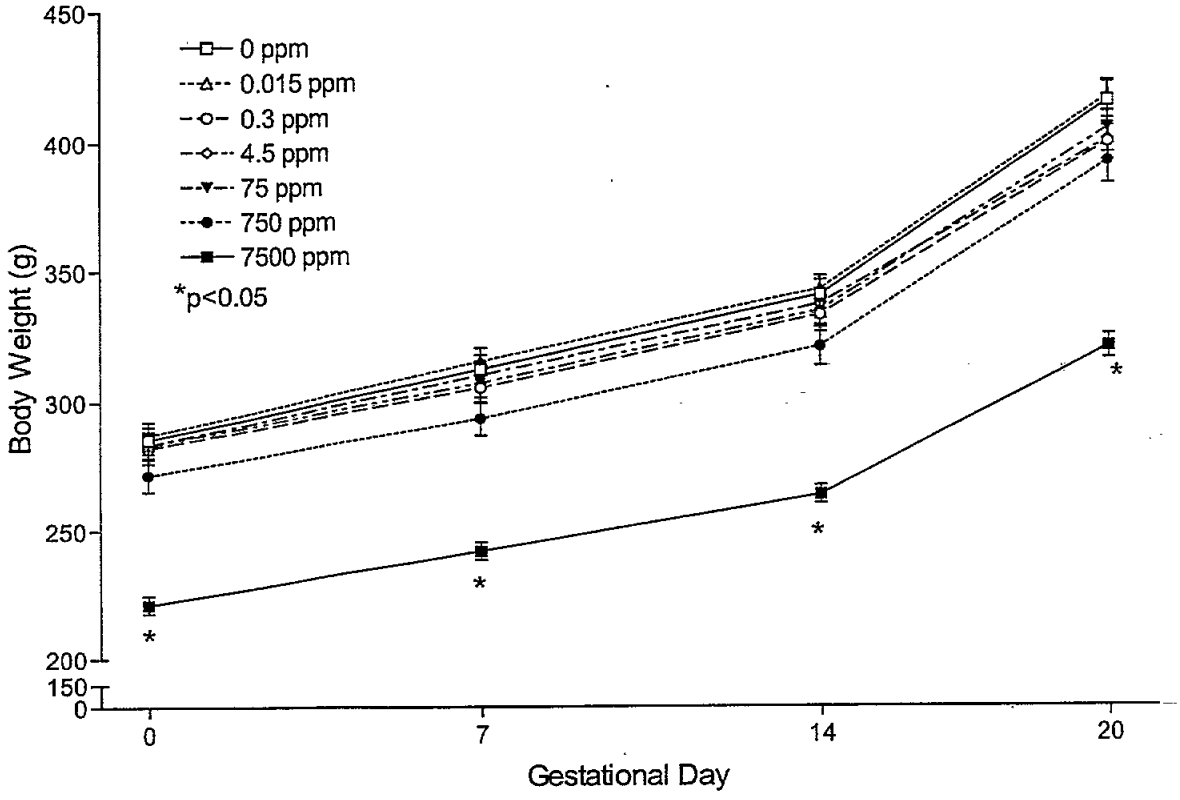
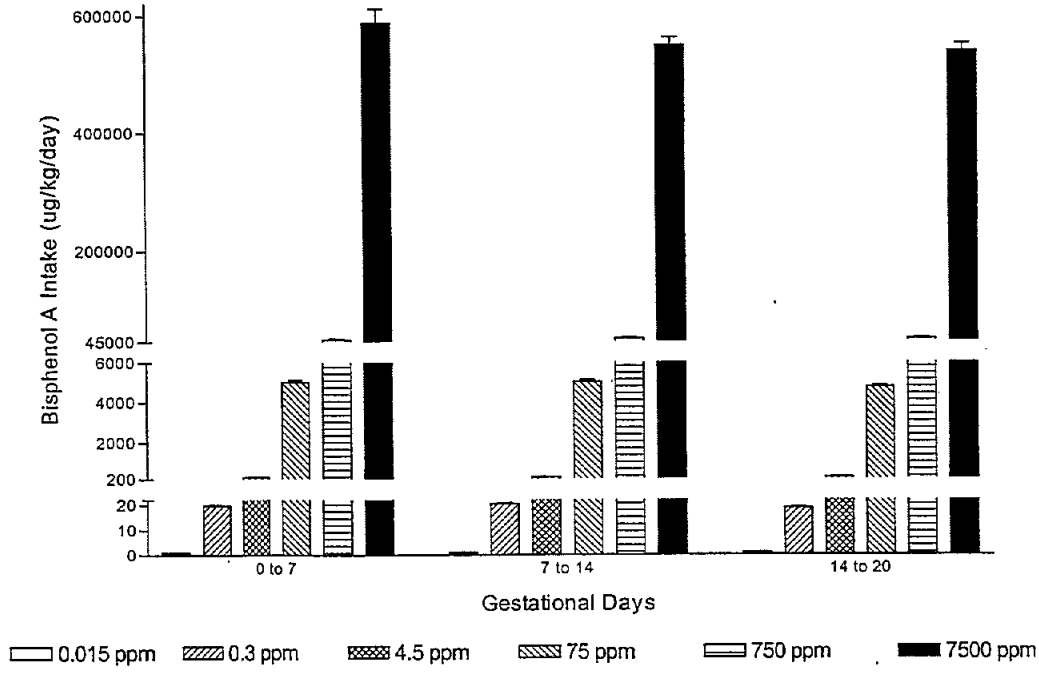


Figure 22. F_2 Female Bisphenol A Intake During Gestation

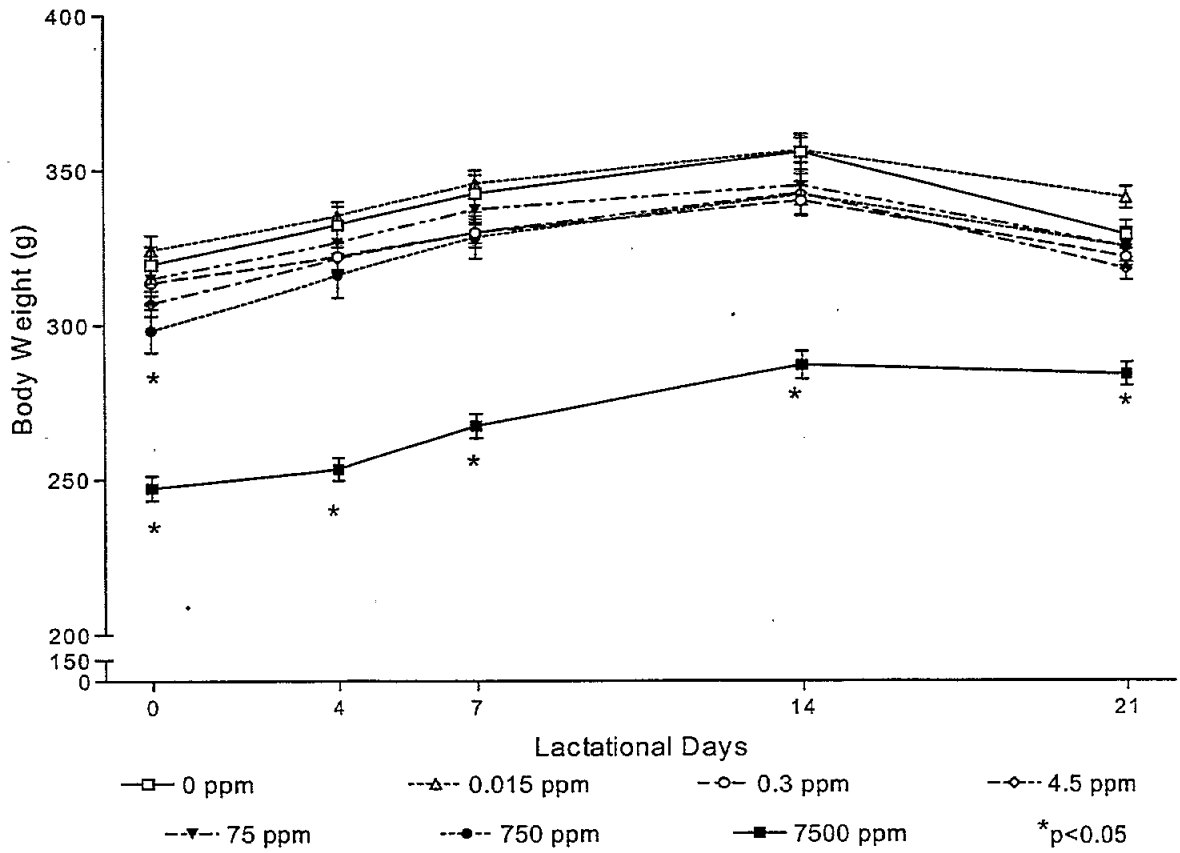


Clinical observations of F2 dams during gestation included alopecia, chromodacryorrhea, red ear(s), and trimmed teeth across all groups, with no treatment- or dose-related incidence or severity (Table 74).

Lactation - F2 Females for F3 Litters

The number of F2 females that delivered F3 litters (with live pups) on pnd 0 were 28, 29, 29, 25 (one additional female, No. 2178, was pregnant but did not deliver a live litter; only implant sites were present at necropsy), 28, 27, and 27, and on pnd 21 were 28, 29, 28, 25, 28, 26, and 27 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 75). Maternal F2 body weights during lactation were significantly reduced at 7500 ppm on pnd 0, 4, 7, 14, and 21, and at 750 ppm only on pnd 0 (Table 75 and Figure 23). Maternal body weight change was significantly increased at 7500 ppm for pnd 14-21 and 0-21, at 0.015 ppm only for pnd 14-21, and at 750 ppm only for pnd 0-21 (Table 75).

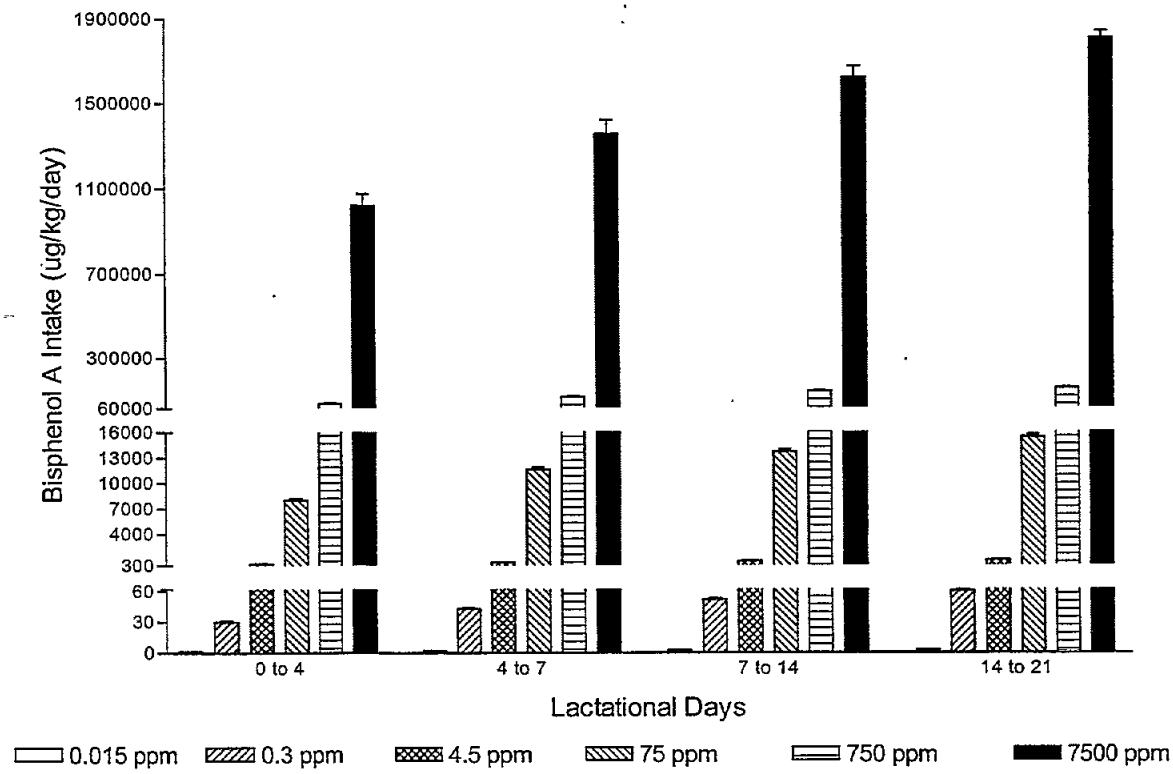
Figure 23. F_2 Female Body Weights During Lactation



Maternal F2 lactational feed consumption, expressed as g/day, was statistically equivalent across all groups for all lactational intervals. When the data were expressed as g/kg/day, values at 7500 ppm were significantly increased for all intervals, pnd 0-4, 4-7, 7-14, 14-21, and 0-21 (Table 76). Percent food efficiency was significantly increased at 7500 ppm for pnd 7-14, 14-21, and 0-21; at 0.015 ppm only for pnd 14-21; and at 750 ppm for pnd 0-21 (Table 76).

Maternal lactational BPA intakes, expressed as µg/kg/day, for the entire lactational period (pnd 0-21) were approximately 2, 50, 776, 13,060, 131,910, and 1,565,980 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively, confounded by pups self feeding, beginning during the second week of life (Table 76 and Figure 24).

Figure 24. F_2 Female Bisphenol A Intake During Lactation



Maternal F2 clinical observations during the lactation period were limited to alopecia, chromodacryorrhea, and red ear(s) across all groups, piloerection in three dams at 7500 ppm,

rust-colored fur in one dam at 0.3 ppm, and trimmed teeth in one dam each at 0.0, 0.015, and 0.3 ppm. None of these observations were treatment or dose related (Table 77).

Reproductive and lactational indices for F2 parents and F3 litters (Table 78) exhibited no treatment-related changes in the mating index, fertility index (either sex), gestational index (all pregnant dams delivered one or more live pups), or pregnancy index. Precoital and gestational length intervals were also equivalent across all groups. Mean gestational length was 22.0, 22.3, 22.0, 22.0, 22.1, 22.0, and 22.1 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 78). For the F3 litters, the number of implantation sites per litter was significantly reduced at 7500 ppm and at 0.3 ppm; percent postimplantation loss was statistically equivalent across all groups. The numbers of total and live pups per litter on pnd 0 were significantly reduced at 0.3 ppm (13.3 total, 13.2 live) and 7500 ppm (11.2, 10.9) versus the control values (14.9, 14.8). All of the survival indices were equivalent across all groups for all lactational intervals and for the entire lactational period, pnd 4 (postcull) to 21 (Table 78).

Information on the F3 litters is presented in Table 79. All pups from one litter (from female 2284) at 0.3 ppm were dead, missing, presumed dead, and/or euthanized moribund on or before pnd 3. All remaining live litters on pnd 0 survived to weaning on pnd 21. The average number of pups per litter was significantly reduced at 7500 ppm on pnd 0 and 4 prior to standardization of litters. For the rest of the lactational period, litter sizes were equivalent across all groups for all time points. Anogenital distance per litter by sex for F3 males and females on pnd 0 was equivalent across all groups when analyzed as distance alone, or when analyzed by ANCOVA with individual body weight as the covariate (anogenital distance values adjusted by ANCOVA analysis are also presented and were equivalent across all groups). These changes (referring to preputial separation, vaginal patency, and anogenital distance) were not associated with any alterations in reproductive structures (histopathology), andrology, or estrous cyclicity in the animals exhibiting them (F3 animals were not bred).

Mean pup body weight per litter, for all pups or for sexes separately, were equivalent across all groups on pnd 0 and 4. Starting on pnd 7 through 21, the body weight values for all pups and sexes separately per litter were significantly reduced only at 7500 ppm. Sex ratio (percent males per litter) was equivalent across all groups throughout lactation. There were no retained nipples in any F3 preweaning male; areolae were present in 0, 1.41, 0.75, 0, 2.92, 1.44, and 0% of the males at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 79).

Clinical observations of F3 pups during the lactation period (Table 80) indicated no treatment- or dose-related incidence of findings. The total found dead, euthanized, moribund, or missing and presumed dead pups through pnd 4 were 20, 14, 24, 10, 11, 13, and 11 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The total for pnd 5 through 21 were 2, 4, 3, 0, 4, 1, and 3 at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 80).

Gross necropsy of the F3 pups that died during the lactational period and were available for necropsy (Table 81) included 6, 9, 22, 7, 12, 6, and 10 pups at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Most deaths occurred in the first four days of life, many with patent ductus arteriosus and no air in lungs (primary atelectasis) and with autolyzed abdominal organs; hydronephrosis was also present; these are common findings in pups dying on pnd 0-4, with no treatment- or dose-related pattern (Table 81).

Necropsy - F3 Weanlings

Necropsy body and organ weights of F3 weanling (pnd 21) males and females, up to three per sex per litter, are presented in Table 82. A total of 76, 84, 81, 70, 83, 81, and 69 male F3 pups were necropsied at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively.

F3 male weanling body weights at necropsy were significantly reduced at 750 and 7500 ppm. Absolute liver, thymus, spleen, brain, and paired testes weights were significantly reduced at 7500 ppm; absolute liver, thymus, and spleen weights were also significantly reduced at 750 ppm. There were no effects in any group on absolute weights of paired epididymides or seminal vesicles with coagulating glands. Relative liver weight was significantly increased at 0.015 ppm and significantly decreased at 750 ppm. Relative thymus weight was significantly increased, and relative spleen weight was significantly reduced at 7500 ppm. Relative brain weight was significantly increased at 750 and 7500 ppm. Relative paired testes and epididymides weights were significantly increased at 7500 ppm, and relative epididymides weight was also significantly increased at 750 ppm. Relative seminal vesicles with coagulating gland weights were unaffected (Table 82).

A total of 84, 83, 80, 73, 83, 77, and 69 F3 female weanlings (pnd 21) were necropsied at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. F3 weanling female body weight was significantly reduced at 7500 ppm. Absolute liver, thymus, spleen, brain, paired ovary, and uterine weights were significantly reduced at 7500 ppm. Absolute liver and thymus weights

were also significantly reduced at 750 ppm. Relative liver weight was significantly increased at 0.015 ppm. Relative paired ovary and uterine weights were unaffected at any dose. Relative thymus and brain weights were significantly increased at 7500 ppm, and relative brain weight was also significantly increased at 750 ppm. Relative spleen weight was significantly reduced at 7500 ppm (Table 82).

Summary of F3 weanling necropsy findings indicated hydronephrosis at 0, 75, and 750 ppm and malocclusion of teeth at 4.5 ppm in females, and hydronephrosis at 0.3 and 4.5 ppm and situs inversus viscera (in one male at 75 ppm) in males. These findings were neither treatment- nor dose-related (Table 83).

F2 Parental Holding Period

F2 males were held from the end of mating until after the delivery of the F3 litters, sd 84-105. F2 male body weights were significantly reduced on sd 84, 91, 98, and 105 at both 750 and 7500 ppm. Body weight change was equivalent for all groups for sd 84-91 and 91-98, and significantly reduced at 7500 ppm for sd 98-105 (Table 84). F2 male feed consumption in g/day was significantly reduced at 7500 ppm for sd 84-91, 91-98, and 98-105, and at 750 ppm for sd 84-91 and 91-98. Feed consumption in g/kg/day was significantly increased at 7500 ppm for sd 84-91, 91-98, and 98-105, at 750 ppm for sd 84-91 and 98-105, and at 75 ppm for sd 84-91. Percent food efficiency was equivalent across all groups for sd 84-91 and 91-98 and significantly reduced at 7500 ppm for sd 98-105. BPA intake over the three-week period was approximately 1-1 µg/kg/day at 0.015 ppm, 16-15 µg/kg/day at 0.3 ppm, 243-229 µg/kg/day at 4.5 ppm, 4109-3852 µg/kg/day at 75 ppm, 40,975-39,427 µg/kg/day at 750 ppm, and 501,001-467,291 µg/kg/day at 7500 ppm (Table 85). Clinical observations of the F2 males during this period involved alopecia, chromodacryorrhea, red ear(s), sore(s), teeth maloccluded or trimmed, injury to nose and/or mouth in one to five males per group, and partial hindlimb paralysis (in one F2 male at 0.015 ppm) and kinked tail (in one F2 male at 750 ppm). None of the clinical findings were treatment or dose related (Table 86).

F2 females that were not pregnant, did not deliver a live litter, lost the entire litter during lactation, or weaned the litters on schedule on pnd 21 were also held until scheduled sacrifice for sd 84-112. Because of the small number per group, which changed over time, no statistical analyses were performed on female body weights, weight changes, or feed consumption

parameters during this period. Body weights and weight changes of 0 to 5 females per group are presented in Table 87. Feed consumption in g/day and g/kg/day, percent food efficiency, and BPA intake in μg/kg/day are presented for 0 to 5 females/group, for completeness, in Table 88. Clinical observations in the F2 females during this holding period are presented in Table 89 and consisted only of alopecia, chromodacryorrhea, and red ear(s) across almost all groups, with no treatment- or dose-related pattern.

Necropsy - F2 Parental Animals

Summary of F2 male terminal body weights, absolute and relative organ weights, and andrological assessments are presented in Table 90. F2 male body weights at scheduled sacrifice were significantly reduced at 750 and 7500 ppm. Absolute weights of liver, paired kidneys, spleen, brain, pituitary, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating glands were all significantly reduced at 7500 ppm. Absolute paired adrenal and preputial gland weights were unaffected. Absolute liver, paired kidney, spleen, and paired testes weights were also significantly reduced at 750 ppm. Absolute liver weight was also significantly reduced at 0.3 ppm, absolute spleen weight was also significantly reduced at 75 and 750 ppm, and absolute paired testes weights were also significantly reduced at 0.3 ppm. Relative paired kidney, paired adrenal, brain, pituitary, paired testes, and paired epididymides weights were all significantly increased at 7500 ppm. In addition, relative liver weight was decreased, and relative paired weights of kidneys, brain, epididymides, and seminal vesicles with coagulating glands were significantly increased at 750 ppm. Relative spleen, prostate, and preputial gland weights were unaffected across all groups.

Percent motile and progressively motile sperm were equivalent across all groups. There were also no effects of treatment on epididymal sperm concentration, testicular homogenization-resistant spermatid head counts, daily sperm production (DSP) per testis, or on efficiency of DSP. Percent abnormal sperm was statistically equivalent across all groups, with the mean values 2.19±0.38, 4.89±2.99, 4.10±2.30, 3.25±1.39, 3.47±1.12, 2.05±0.25, and 1.62±0.17% at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The mean values at 0.015-75 ppm were accompanied with high variance values; the elevated means in these groups were due to one male in each group with increased percentage of abnormal sperm, each of whom sired a live litter as follows: one male (no. 2287) at 0.015 ppm with no testicular spermatids, 3% motile

sperm, and 91.4% abnormal sperm (individual data table A-105, Volume VII; paired with female no. 2252 and sired a live litter; individual data table A-89, Volume VII); one male (no. 2019) at 0.3 ppm with reduced sperm motility and 70.7% abnormal sperm (paired with female no. 2114 and sired a live litter); one male (no. 2141) at 4.5 ppm with reduced sperm motility and 43.3% abnormal sperm (paired with female no. 2066 and sired a live litter); and one male (no. 2179) at 75 ppm with slightly reduced sperm motility and 34.1% abnormal sperm (paired with female no. 2088 and sired a live litter) (from individual animal data tables in Volume VII).

Gross findings for F2 males at scheduled sacrifice are presented in Table 91. They included alopecia; chromodacryorrhea; ear(s) red or thickened; hydronephrosis; reduced size of epididymis at 0.0, 0.015, and 0.3 ppm (one to two males each); reduced preputial gland (one male at 75 ppm); testis fluid filled (left; one at 0.015 ppm) and/or reduced in size at 0.0, 0.015, and 0.3 ppm (one to two males each); and malocclusion of teeth at 75 ppm (one) and 750 ppm (three) (Table 91).

Microscopic findings of F2 males at necropsy exhibited no treatment- or dose-related increases in incidence or severity in the organs examined, including the reproductive organs and any organs identified from the gross findings (Table 91).

Summary of F2 female terminal body weights, organ weights, paired ovarian primordial follicle counts (high dose and control), and stage of estrus at necropsy is presented in Table 92. Body weight at scheduled sacrifice (30 females per group for 0 through 4.5 ppm and 750 ppm, 29 females at 75 ppm, and 28 females at 7500 ppm) was significantly reduced at 7500 ppm. Absolute paired kidney, paired adrenal, pituitary, paired ovary, and uterine weights were significantly reduced at 7500 ppm. Absolute liver weight was significantly increased at 0.015 ppm. Absolute paired ovary weight was also significantly reduced at 0.015, 4.5, and 75 ppm. Absolute spleen and brain weights were unaffected across all groups.

Relative liver, paired kidney, spleen, and brain weights were significantly increased at 7500 ppm. Relative liver weight was also significantly increased at 0.015 ppm, relative spleen weight was significantly reduced at 75 ppm, and relative paired ovary weights were significantly reduced at 0.015, 4.5, 75, and 7500 ppm. Relative paired adrenal, pituitary, and uterine weights were unaffected. Paired ovarian primordial follicle counts (from ten randomly selected females each at 0 and 7500 ppm) were statistically equivalent. The stage of estrus at necropsy exhibited

a significant decrease in the number (and percent) of females in estrus and a significant increase in the number (and percent) of females in diestrus at 7500 ppm (Table 92).

Summary of F2 female gross and microscopic findings at necropsy are presented in Table 93. Gross findings included a number of females with alopecia (multiple areas), chromodacryorrhea, ear(s) red or thickened, hydronephrosis and renal cysts, ovarian cysts, bilateral undeveloped ovaries and uterus (one female at 4.5 ppm), and rust-colored fur (one female each at 0.3 and 4.5 ppm). None of the findings were treatment-related.

Microscopic findings included renal tubular degeneration only at 7500 ppm (7/13), with none at 0 through 750 ppm, and possibly increased incidence of chronic hepatic inflammation at 7500 ppm (5/13) versus the control incidence of 3/30 (and also zero to two out of 10-12 examined per group at 0.015 through 750 ppm). No other findings appeared related to treatment (Table 93).

Retained F3 Weanlings

At the weaning of F3 litters, 30/sex/group were randomly selected for retention through acquisition of vaginal patency for the F2 females and through acquisition of preputial separation for F2 males until adulthood. This retention period was designated the "postwean exposure period" and lasted until age 105±6 days for F3 males and age 118±6 days for F3 females. Exposure to the appropriate dosed feeds continued for both sexes until scheduled sacrifice with daily clinical observations and weekly body weights and feed consumption. In addition, at the time of acquisition of the reproductive landmarks, each male and female was weighed on the date of acquisition. Estrous cyclity was evaluated for the retained F3 females for the last three weeks of the postwean exposure period. At scheduled necropsy of the retained F2 males, organ weights, andrological assessments, and histopathology on scheduled organs were performed as for the F0, F1, and F2 parental males. At scheduled necropsy of the F3 females, organ weights, stage of estrus at necropsy, ovarian follicle counts, and histopathology on scheduled organs were performed as for the F0, F1, and F2 parental females.

F3 Postwean Exposure Period

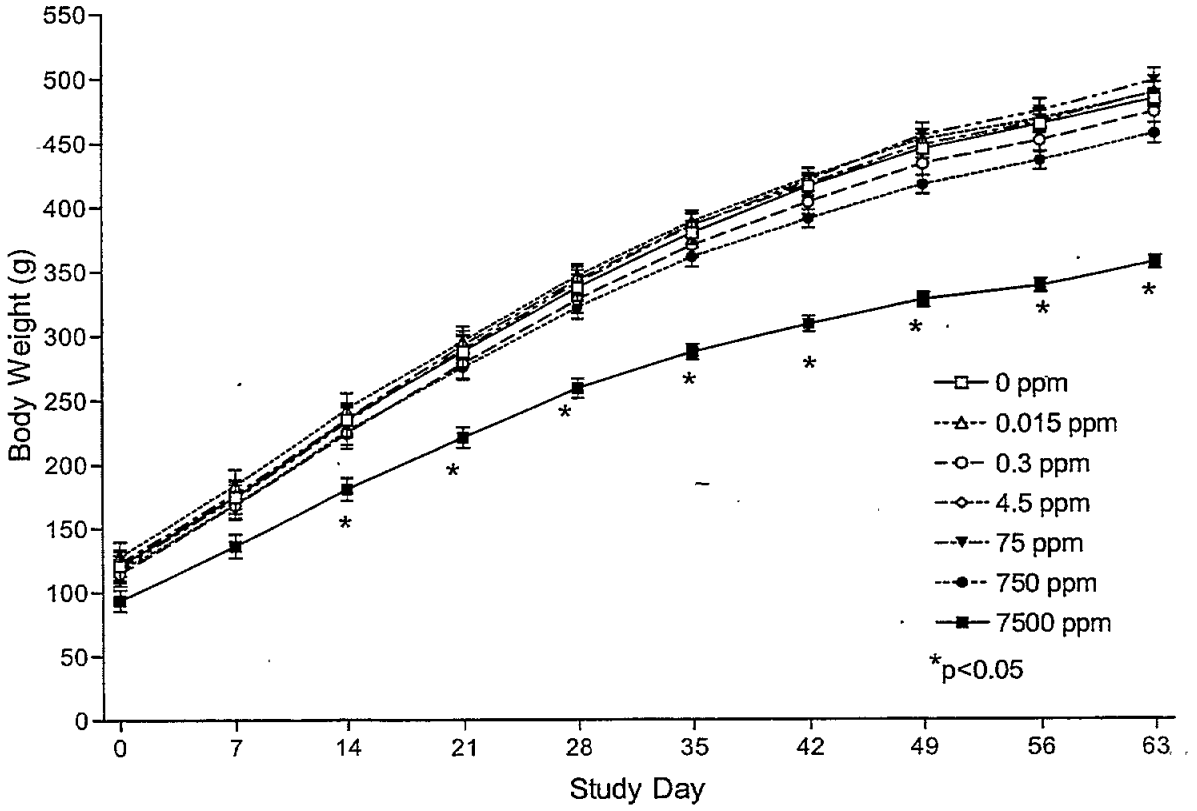
One F3 male at 0.3 ppm was euthanized moribund on sd -5. All remaining F3 males survived to scheduled sacrifice. There were therefore 30, 30, 29, 30, 30, 30, and 30 F3 males per group at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. All retained F3 females survived to scheduled sacrifice. There were therefore 30 F3 females per group (Table 94). The age at acquisition of vaginal patency for F3 females was significantly delayed at 7500 ppm (33.8 days), relative to the control age (31.3 days) when analyzed with Dunnett's test. Acquisition of vaginal patency at 7500 ppm was also delayed when analysis of covariance (ANCOVA), with the body weight at acquisition the covariate, and Least Squares Means Test were employed for statistical analysis. Average female body weight on the date of acquisition was equivalent across all groups (Table 95). The average age at acquisition of preputial separation for F3 males was also significantly delayed at 7500 ppm (45.2 days) relative to the control value (42.1 days) when analyzed by Dunnett's test and when analysis of covariance (ANCOVA) and Least Squares Means Test were employed for statistical analysis. Average male body weight on the date of acquisition was significantly reduced only at 7500 ppm (Table 95).

F3 male body weights during the postwean exposure period (Table 96 and Figure 25) were significantly reduced for sd 14, 21, 28, 35, 42, 49, 56, and 63 of the 63- to 70-day (nine- to ten-week) period at 7500 ppm. There were no effects on body weights in any other dose groups. F3 male body weight changes during this period were significantly reduced at 7500 ppm for weeks 1 (sd 0-7), 2 (sd 7-14), 3 (sd 14-21), 4 (sd 21-28), 5 (sd 28-35), 6 (sd 35-42), 7 (sd 42-49), 8 (sd 49-56), and for sd 0-63 (when all males were still present). Male weight change was unaffected in all other groups at all intervals (Table 96).

F3 male feed consumption (Table 97), as g/day, was significantly reduced at 7500 ppm for weeks 2 (sd 7-14), 3 (sd 14-21), 4 (sd 21-28), 5 (sd 28-35), 6 (sd 35-42), 7 (sd 42-49), 8 (sd 49-56), and sd 0-63. It was also significantly reduced at 750 ppm for week 5 (sd 28-35) and significantly increased at 75 ppm for week 8 (sd 49-56). When feed consumption data were expressed as g/kg/day, feed consumption was significantly increased at 7500 ppm for weeks 4, 5, 6, 7, 8, 9, and for the entire period (sd 0-63). It was also significantly increased at 0.3 and 75 ppm for weeks 8 and 9. Percent food efficiency was significantly reduced only at 7500 ppm and only for weeks 6, 7, 8, and gd 0-63. BPA intake, in µg/kg/day, exhibited the expected incremental increases across dose groups, averaging approximately 1, 24, 360, 6139, 59,288, and 698,676

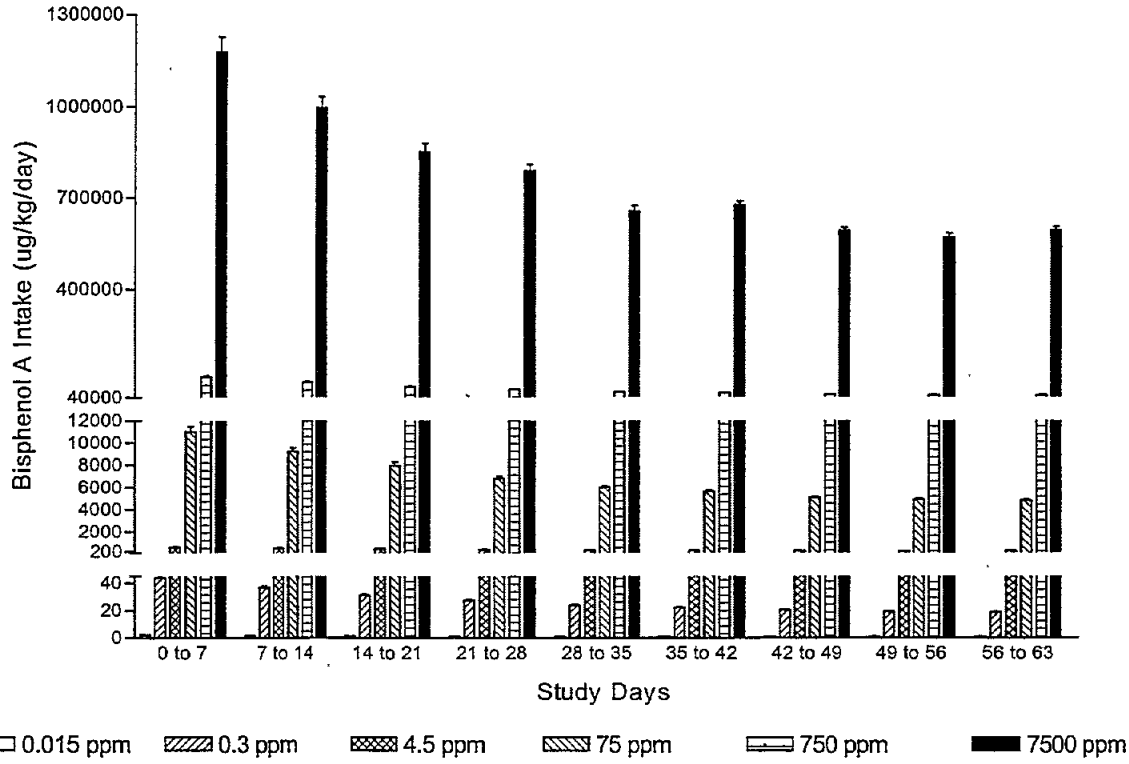
µg/kg/day for the entire period (sd 0-63) at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The chemical intake values also exhibited the expected decreases within each dosed group over time. The test chemical intake ranged from approximately 2 µg/kg/day for week 1 to 1 µg/kg/day for week 10 (sd 63-70) at 0.015 ppm, from 44 to 18 µg/kg/day at 0.3 ppm, from 633 to 268 µg/kg/day at 4.5 ppm, from 11,015 to 4609 µg/kg/day at 75 ppm, from 107,081 to 45,304 µg/kg/day at 750 ppm, and from 1,176,135-570,889 µg/kg/day at 7500 ppm (Table 97 and Figure 26).

Figure 25. F_3 Male Body Weights During the Post Wean Period



Clinical observations of the F3 males during the ten-week postwean exposure period (Table 98) included alopecia, chromodacryorrhea, dehydration, red ear(s), malocclusion (with teeth trimmed), and sore(s) in one to five males per group, and one male (No. 3095) euthanized moribund at 0.3 ppm (Table 98 and Volume VIII, Table A-114).

Figure 26. F_3 Male Bisphenol A Intake During the Post Wean Period



F3 female body weights were available for all females in all groups for sd 0 through sd 77 and for approximately 15/group for sd 77-84. F3 female mean body weight was significantly reduced at 7500 ppm on sd 7, 14, 21, 28, 35, 42, 49, 56, 63, 70, and 77, and not statistically analyzed for sd 84 due to the reduced numbers on sd 77-84 (Table 99 and Figure 27). F3 female body weight change was significantly reduced at 7500 ppm for week 5 (sd 28-35) and for sd 0-77 (Table 99). F3 female feed consumption, expressed as g/day, was significantly reduced at 7500 ppm for weeks 2 through 11 (sd 70-77) and for sd 0-77. It was also reduced at 75 ppm for weeks 10 (sd 63-70) and 11 (sd 70-77). F3 female feed consumption as g/kg/day was significantly increased at 7500 ppm for weeks 10 and 11 and at 75 ppm for week 10. Percent food efficiency was statistically equivalent across all groups for all intervals. BPA intake exhibited the expected incremental increases across groups, averaging approximately 1, 26, 386, 6871, 64,683, and 678,587 µg/kg/day at 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 100).

Test chemical intake also exhibited the expected decreases within each dosed group over time. The test chemical intake ranged from approximately 2 µg/kg/day for week 1 (sd 0-7) to 1 µg/kg/day for week 12 (sd 77-84) at 0.015 ppm, 43 to 22 µg/kg/day at 0.3 ppm, 664 to 316 µg/kg/day at 4.5 ppm, 11,675 to 6361 µg/kg/day at 75 ppm, 107,159 to 56,954 µg/kg/day at 750 ppm, and from 1,232,864 to 716,088 µg/kg/day at 7500 ppm (Table 100 and Figure 28).

F3 female clinical observations during the postwean holding period included alopecia, chromodacryorrhea, dehydration, red ears, sore(s), teeth maloccluded, missing, or trimmed, and injury to tail, teeth, and/or lips. None of the observations were treatment- or dose-related (Table 101).

Figure 27. F_3 Female Body Weights During the Post Wean Period

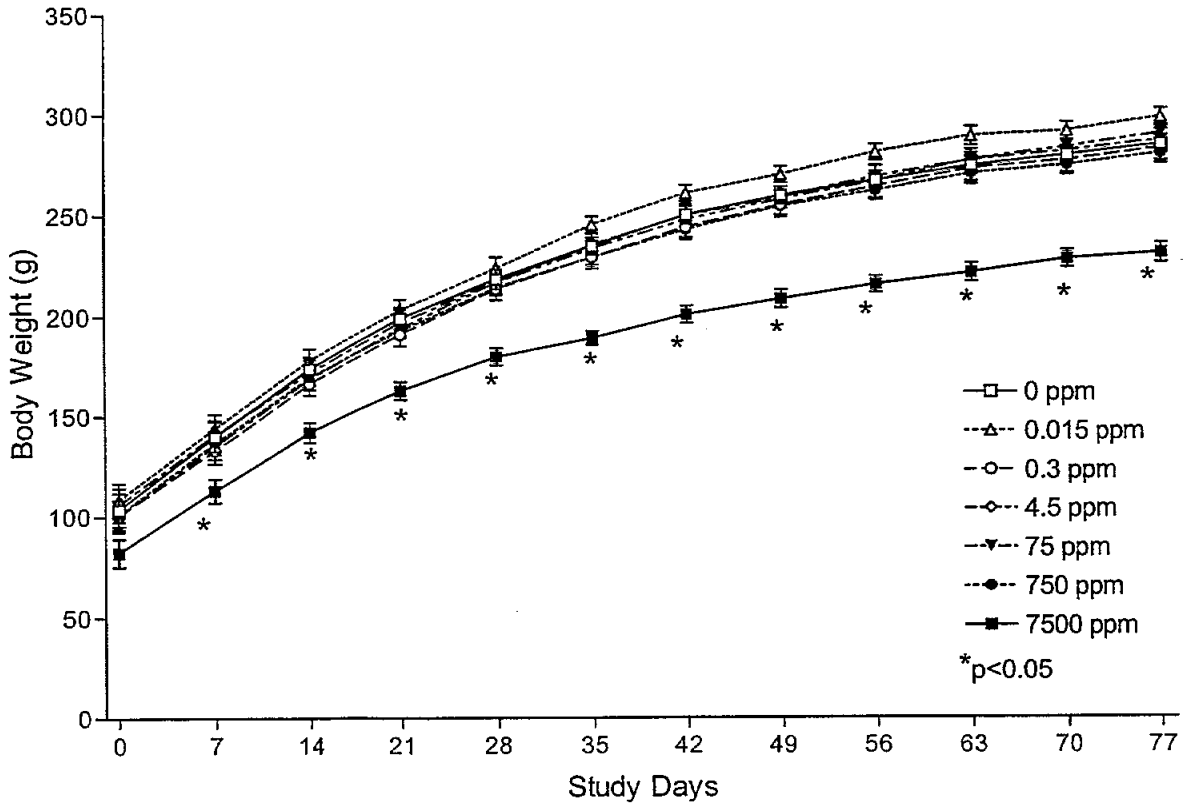
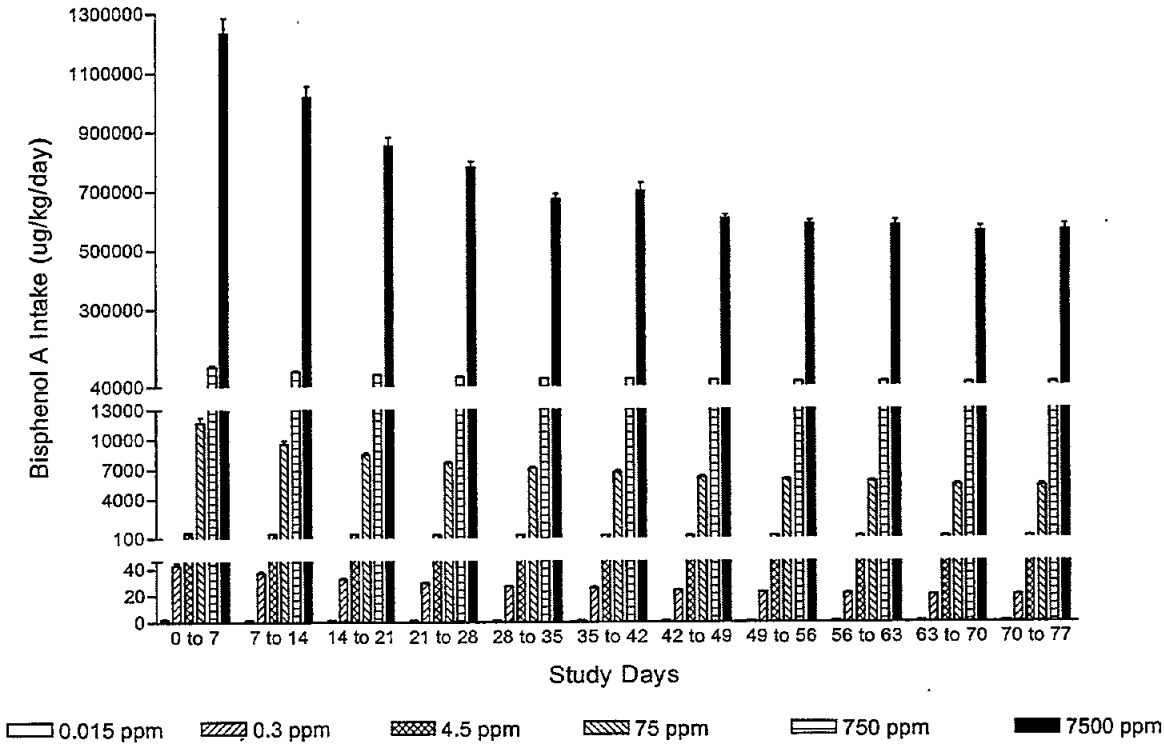


Figure 28. F_3 Female Bisphenol A Intake During the Post Wean Period



Estrous cyclity, evaluated for weeks 7 through 10 of the F3 female holding period, is presented in Table 102. Of the 30 per group assessed, all were cycling except for one each at 0.015, 4.5, and 75 ppm. Of the F3 females cycling, 5, 1, 4, 5, 6, 7, and 3 exhibited abnormal cycles at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. Cycle length was statistically and biologically equivalent across all groups, with the mean values 4.32, 4.34, 4.32, 4.39, 4.66, 4.59, and 4.31 days at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively (Table 102).

Necropsy – F3 Adults

Summary of F3 male terminal body weights, organ weights, and andrological assessments is presented in Table 103. F3 male body weight at scheduled sacrifice was significantly reduced at 7500 ppm. Absolute weights of liver, paired kidneys, spleen, brain, pituitary, paired testes, paired epididymides, prostate, and seminal vesicles with coagulating glands were all significantly reduced at 7500 ppm. Absolute liver weight was also significantly reduced at 750 ppm. Absolute paired testes weight was also significantly reduced at 0.015, 0.3,

and 750 ppm. Absolute paired epididymides weight was also significantly reduced at 750 ppm. Absolute paired adrenal and preputial gland weights were unaffected. Relative prostate, seminal vesicles with coagulating gland, and preputial gland weights were all statistically equivalent across all groups. Relative paired kidney, paired adrenal, brain, pituitary, paired testes, and paired epididymides weights were all significantly increased at 7500 ppm. Relative liver weight was significantly reduced at 750 ppm. Relative spleen weight was significantly reduced at 75 ppm (Table 103).

F3 adult male daily sperm production was significantly reduced at 7500 ppm. Percent motile sperm, percent progressively motile sperm, epididymal sperm concentration, testicular homogenization-resistant spermatid head concentration, efficiency of daily sperm production, and percent abnormal sperm were all statistically equivalent across all groups. The values for percent abnormal sperm were 1.75±0.16, 5.14±3.23, 1.93±0.20, 2.29±0.28, 8.33±4.20, 1.70±0.16, and 2.03±0.36% at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, respectively. The mean values (with associated large variance terms) at 0.015 and 75 ppm were due to one male (No. 3191) at 0.015 ppm with 0% motility and 98.7% abnormal sperm; and two males at 75 ppm, one (No. 3113) with low sperm motility and 88.4% abnormal sperm; and one (No. 3183) with 0% sperm motility and 97.1% abnormal sperm (individual animal data table A-120 in Volume VIII); F3 adult males were not mated (Table 103).

Summary of gross and microscopic findings for retained F3 males is presented in Table 104. Gross findings did not exhibit any treatment- or dose-related changes in incidence or severity. The gross observations included alopecia (various locations), chromodacryorrhea, sore(s), teeth maloccluded, uni- or bilateral hydronephrosis (in one to five animals at 0 through 750 ppm), enlarged testis (one each at 4.5 and 75 ppm), reduced testis (one each at 0.015 and 4.5 ppm), soft testis (one each at 0.3 and 750 ppm), small epididymides (in one to two males each at 0.015, 0.3, 4.5, 75, and 750 ppm), seminal vesicle reduced in size (one male at 75 ppm), and hydrocephaly in one male at 0.3 ppm (noted previously in-life as domed head) (Table 104).

F3 male microscopic evaluation from 30 males at 0 ppm and ten to 11 males per group in all other groups indicated that there were no treatment- or dose-related increases in the incidence or severity of any of the histopathologic findings in any organ examined, including the reproductive organs or in any organ with gross lesions (Table 104).

Summary and analysis of F3 female terminal body weights, organ weights, paired ovarian primordial follicle count (high dose and control), and stage of estrus at necropsy are provided in Table 105. F3 female terminal body weight was significantly reduced at 7500 ppm. Absolute liver, paired kidney, paired adrenal, spleen, pituitary, and paired ovary weights were significantly reduced only at 7500 ppm. Absolute brain and uterine weights were equivalent across all groups. Relative spleen and brain weights were significantly increased only at 7500 ppm. Relative liver, paired kidney, paired adrenal, pituitary, paired ovary, and uterine weights were equivalent across all groups (Table 105). Paired ovarian primodial follicle counts (ten females each at 0 and 7500 ppm) were statistically equivalent. The number (and percent) of females in each stage of estrus at necropsy were also equivalent across all groups (Table 105).

Summary of gross and microscopic findings for retained F3 females is presented in Table 106. The gross observations included alopecia (various locations), chromodacryorrhea, malocclusion of upper incisors in one to two females across all groups, red and/or thickened ears observed predominantly at 75 and 7500 ppm, uterus with various degrees of retained fluid in one female each at 0, 0.015, and 750 ppm and in two females each at 0.3 and 7500 ppm, and hydronephrosis in one female each at 0.015 and 75 ppm and two at 750 ppm (Table 106).

F3 female microscopic evaluation from 30 females at 0 ppm and seven to ten females per group in all other groups indicated no treatment- or dose-related increases in incidence or severity for any of the histopathologic findings, including those in the liver and kidneys. There appeared to be a reduction in the incidence of mineralization of the kidney corticomedullary junction at 7500 ppm (0/10) versus the incidence at 0 ppm (8/30) (Table 106).

DISCUSSION

Bisphenol A (BPA) is a high production volume chemical used principally as a monomer in the manufacture of numerous chemical products, including polycarbonate plastics and epoxy resins. Research published in the 1980s indicated that BPA was not a selective reproductive toxicant in mice, but did result in reproductive toxicity at very high dietary doses, which also resulted in substantial parental toxicity (Morrissey et al., 1989) and by intraperitoneal injection in rats (Hardin et al., 1981). Standard Segment II developmental toxicity studies in CD rats and CD-1 mice, administered BPA at high doses by gavage on gd 6-15, indicated that BPA was not a selective developmental toxicant (Morrissey et al., 1987).

Although it had been known for decades that BPA has weak estrogen-like activity *in vivo* by injection (Dodds and Lawson, 1936), there has been recent, renewed interest in its potential for estrogen-like activity. Krishnan et al. (1993) discovered that BPA leaching from polycarbonate flasks during autoclaving induced an estrogen-like response in yeast cultures. Gaido et al. (1997) confirmed its estrogen-like activity *in vitro,* calculating BPA as approximately 15,000-fold less potent than 17β-estradiol. Kuiper et al. (1997) demonstrated that BPA could interact with both the α- and β-estrogen receptors. Kuiper et al. (1998) also showed that the *in vitro* binding affinity of BPA was approximately 20,000-fold less potent than that of diethylstilbestrol (DES) for both ERα and ERβ. Milligan et al. (1998) showed BPA to be 10,000 times less potent in producing a uterotrophic effect than estradiol following subcutaneous injections into ovariectomized mice.

The *in vivo* estrogen-like activity of BPA was originally demonstrated in short-term uterotrophic assays in rodents using parenteral (injection) administration (e.g., Dodds and Lawson, 1936). Most of the more recent studies utilized oral administration (i.e., dosed feed, dosed water, or oral gavage) but at very high doses (e.g., Morrissey et al., 1987, 1989, who evaluated reproductive toxicity of BPA in mice using a Reproductive Assessment by Continuous Breeding [RACB] study design). Most recently, oral administration (presentation to the dam's buccal cavity) of BPA in corn oil, at much lower doses to pregnant CF-1 mice on gd 11-17, has been reported to affect adult male offspring reproductive organ parameters, such as increased prostate gland weight at 2 and 20 μg/kg/day and decreased daily sperm production per gram testis (efficiency of daily sperm production) at 20 μg/kg/day (Nagel et al., 1997; vom Saal et al., 1998). These reported low-dose effects of BPA were not replicated in more robust study designs

with the same and additional lower and higher dose levels and larger numbers of animals per group (Ashby et al., 1999; Cagen et al., 1999a).

In another recent study, female Wistar rats were exposed to 1 ppm BPA (corresponding to approximately 0.1-0.4 mg/kg/day) in their drinking water for eight to nine weeks (during prebreed, mating, gestation, and lactation), and their adult male offspring were reported to exhibit significantly reduced testes weights (Sharpe et al., 1995). This study could not be replicated by the original authors (Sharpe et al., 1998) nor by another study employing a larger number of dose groups, more animals per dose, and more reproductive parameters, but the same exposure route, timing, duration, and strain of rat (Cagen et al., 1999b). The validity and reproducibility of the purported low-dose effects of BPA is still questionable.

The finding of qualitatively and quantitatively different responses to different routes of BPA administration, with oral administration requiring much higher doses to produce an effect than parenteral subcutaneous administration (e.g., Twomey, 1999a,b), was resolved by work by Pottenger et al. (2000). They showed that there was a clear route dependency in the toxicokinetics and metabolism of ^{14}C-labeled BPA after a single oral, intraperitoneal (ip), or subcutaneous (sc) dose of either 10 or 100 mg/kg to Fischer 344 rats. The relative bioavailability of BPA and the plasma radioactivity was markedly lower after oral administration as compared to sc or ip administration, thus providing an explanation for the apparent route differences in estrogenic potency observed for BPA in rats.

The present study evaluated exposure of CD® (Sprague-Dawley) rats to Bisphenol A administered in the diet *ad libitum* at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm for three generations, one litter per generation. The selection of the very wide dose range for this study (see protocol, Volume IX, Appendix IV) was based on the need to evaluate the potential reproductive toxicity of BPA under standard EPA test guidelines, as well as an additional attempt to validate the potential for low-dose effects of BPA (see above). The interpretation of the results of this study, therefore, required not only the typical analysis of biological relevance of any effects in treated animals at the high dose in relationship to any effects observed at lower doses, but also careful consideration of potential estrogen-like effects resulting from receptor-mediated events that have been hypothesized by some investigators (vom Saal et al., 1998) as possible at lower doses. Additional assessments were added (beyond the guideline requirements) for this study, including a third generation, six dose groups from 0.015 to 7500 ppm (plus

control, 0 ppm), examination for retained nipples and areolae in F1, F2, and F3 preweanlings, and retention of F3 offspring (30/sex/group) until adulthood with exposure continuing, measuring anogenital distance and acquisition of puberty in both sexes, epididymal sperm and testicular spermatids, estrous cyclicity, and full necropsy and histopathology.

Parental Systemic Parameters

Adult systemic toxicity was evident for F0, F1, and F2 parental animals and F3 retained animals at 750 and 7500 ppm (as summarized in Text Table E). At 7500 ppm, there were consistent and persistent reductions in body weights and weight gains in both sexes and in F0, F1, F2, and F3 generations. Feed consumption in g/day and g/kg/day was variable and showed no clear treatment-related effects. There were no treatment- or dose-related clinical observations in either sex in any of the generations, except for dehydration at the start of the F0 prebreed in all groups as the animals adjusted to the automatic watering system, and at the start of the F1, F2, and F3 postwean (prebreed) exposure period at 7500 ppm, due to the small pups at this dose adjusting to the automatic watering system. Body weights during gestation and lactation were significantly reduced in F0, F1, and F3 females at 7500 ppm, and during gestation and lactation at 750 ppm for F0 and F2 females and for F1 females during lactation. There were no toxicologically significant, consistent, or persistent effects on these parameters at 75 ppm or below. At necropsy, F0, F1, and F2 parental and F3 retained adult absolute organ weights were almost uniformly reduced for liver, kidneys, adrenal glands, spleen, pituitary, and brain at 7500 ppm. Relative organ weights at 7500 ppm were typically significantly increased, with both effects most likely due to reduced terminal body weights at this dietary dose. Changes in absolute and relative organ weights did occur in other groups, but they were not consistent across generations and did not exhibit a dose-response pattern. There were no treatment- or dose-related gross or microscopic findings for the examined organs, for F0, F1, and F2 parental animals, and for F3 retained adults, except for renal tubular degeneration and chronic hepatic inflammation observed at a higher incidence in F0, F1, and F2 (but not F3) females at 7500 ppm. See Text Table E for a summary of adult parameters.

Parental Reproductive Parameters

A summary of parental reproductive parameters is presented in Text Table F. There were no effects of treatment in F0, F1, or F2 females on mating, fertility, pregnancy or gestational indices, dead pups per litter, or of percent postimplantation loss (prenatal mortality index). The number of implants, total pups, and live pups per litter at birth and on pnd 4 precull were significantly reduced at 7500 ppm for F1, F2, and F3 offspring.

The explanation for the reduced live litter size at birth at 7500 ppm for F1, F2, and F3 offspring is not known. It is not due to the male since there is no evidence of reproductive effects on the males at 7500 ppm (or any other dietary dose). It is not due to prenatal postimplantation loss of conceptuses, since postimplantation loss was unaffected at any dose for F0, F1, and F2 dams carrying F1, F2, and F3 litters. Preimplantation loss cannot be determined from this study design since by the time the parental females are sacrificed, the ovarian corpora lutea of pregnancy (which form after the eggs are ovulated) have involuted to corpora albicans, indistinguishable from corpora albicans from previous ovulation cycles. The possibilities, therefore, exist that there was preimplantation loss and/or fewer eggs ovulated. Although the absolute and relative paired ovarian weights were reduced in F0, F1, F2, and F3 (absolute only) females in the present study, there was no evidence of reduced ovarian primordial follicle counts at 7500 ppm in any generation.

There were no direct treatment-related effects on absolute or relative reproductive organ weights, except for significantly reduced absolute paired ovary weights in F0, F1, F2, and F3 females and relative paired ovary weights in F0, F1, and F2 (but not F3) females at 7500 ppm in the presence of significant systemic maternal toxicity. Estrous cycle length in days was equivalent across all groups for F0, F1, F2, and F3 females. Paired ovarian follicle counts were similar between the high dose and control F1, F2, and F3 females (and increased at 7500 ppm for F0 females). Precoital interval in days and gestational length in days were equivalent across all groups for all generations.

As summarized in Text Table F, there were no effects of treatment in F0, F1, or F2 males on mating or fertility indices, or treatment- or dose-related direct effects in F0, F1, F2 and retained F3 males on absolute or relative weights of the testes, epididymides, prostate, or seminal vesicles plus coagulating glands. The effects were consistently reduced absolute organ weights and increased relative organ weights, due to the reduced terminal body weights of the males at

750 and 7500 ppm. Also, there were no effects on epididymal sperm concentration (except for a significant reduction in epididymal sperm concentration in F1 males, but not F0, F2, or F3 males, at 7500 ppm), percent motile or progressively motile sperm, testicular homogenization-resistant spermatid head counts, daily sperm production (except for a significant reduction in daily sperm production at 7500 ppm for F3 males, but not for F0, F1, or F2 males), with no effect on efficiency of daily sperm production), or efficiency of daily sperm production. Percent abnormal sperm was also unaffected for all F0, F1, F2, and F3 males in all groups. The slightly higher (but not statistically significant) values for F0 males at 0 pppm, F1 males at 0.015 and 0.3 ppm, F2 males at 0.015, 0.3, 4.5, and 75 ppm, and for F3 males at 0.015 and 75 ppm were due to one or two males per group with few or no motile sperm and most or all abnormal sperm. In all cases for the F0, F1, and F2 males, the affected males sired live litters (F3 males were not bred). There were no treatment-related gross or microscopic findings in reproductive organs for F0, F1, F2, or F3 adult males or females.

Offspring Parameters

A summary of offspring parameters is presented in Text Table G. There were no differences among groups for F1, F2, or F3 stillbirth index, prenatal (postimplantation) loss per litter, sex ratio (% males) per litter at birth and throughout lactation, lactational survival index, or 4-, 7-, 14-, or 21-day survival indices. The number of total implants, total pups, and live pups was significantly reduced at 7500 ppm for F1, F2, and F3 litters, which persisted only until pnd 4 (precull). Pup body weights per litter were reduced at 7500 ppm for F1, F2, and F3 offspring for lactational time points pnd 7, 14, and 21. For F1 litters, pup body weights per litter were also significantly reduced on pnd 4 for all pups only, not for sexes separately. The reduced pup body weights occurred only during the mid and last portions of the lactational period when the pups were beginning to self-feed and, therefore, were being directly exposed to high daily doses of BPA in the diet by ingestion, possibly as early as pnd 10 (based on observations and evaluations of stomach contents by the performing laboratory, CD® rat pups begin to self-feed late in the second week of life). See below for a more detailed discussion of offspring BPA intakes.

Summarized absolute and relative organ weight data for F1, F2, and F3 weanling (pnd 21) pups are included in Text Table G. For F1, F2, and F3 males and females, the absolute organ weight changes were decreased at 7500 ppm (the dietary concentration at which the

terminal body weights were also decreased). There were reductions in absolute organ weights at lower doses, but they were not consistently affected in F1, F2, and F3 weanlings or reproducible in specific dose groups. Relative organ weights were increased (or unaffected) at 7500 ppm (again, due to reduced body weights at this dietary dose). The changes in absolute and relative F1, F2, and F3 male and female organ weights were not considered to be biologically significant and were most likely due to decreased body weights (Text Table G).

Because of changes observed in absolute and relative organ weights in F1, F2, and F3 weanlings (Text Table F) and adults (Text Tables E and F), a direct comparison of absolute and relative organ weights for F1, F2, and F3 weanlings (from Text Table G) and adults (from Text Tables E and F) is presented for those organs weighed in both weanlings and adults in Text Table H.

Many of the effects on absolute or relative organ weights in the F1, F2, and F3 weanlings were also present in the corresponding adults, including absolute and relative paired ovary weights significantly reduced in the weanling and adult F1, F2, and F3 females at 7500 ppm. Careful examination of the patterns of absolute and relative weights strongly suggests that test material-related effects may exist in the high-dose group but not other groups. The difference in effects on weanling versus adult animals is likely the result of the very high dietary intakes (greater than 750 [786-1205] mg/kg/day) of the test material being consumed by the weanling animals in the high-dose group (in their first postwean exposure week), which are approximately twice the daily doses in the adults at the same dose (less than 575 [534-570] mg/kg/day, during their last prebreed exposure week immediately prior to mating). Table H provides BPA intakes in mg/kg/day for F0, F1, F2, and F3 animals during all phases of the study, with values for weeks 1 and 10 of prebreed, pnd 4-7 and pnd 14-21 of lactation, and the first and last week of the post-mate holding period broken out. It is obvious that the first week of prebreed (when the pups are small and in a growth spurt) is the time of maximum exposure to BPA (as is probably also the last week of lactation, confounded by exposures directly from feed).

Maternal feed consumption in g/kg/day and, therefore, test chemical intake from dosed diets, is maximal for dams during the last week of lactation (pnd 14-21), confounded by the pups self-feeding. The feed consumption (in g/kg/day) and the test chemical intake in offspring from dosed diets are also maximal early in the prebreed/postwean period when the pups eat more relative to their body weight versus at the end of a long prebreed period (ten weeks), when the

body weights have approximately doubled and the feed consumption has changed very little or not at all (30 g/day for males, 20 g/day for females). The chemical intake in mg/kg/day from dosed diets therefore drops over 50% during this period, as is clear in Text Table I. Calculations by the performing laboratory on feed consumption in control dams and pups during lactation indicate that during the last week of lactation (pnd 14-21), 30% to 40% of the feed consumption (on a g/kg/day basis) designated as "maternal" is, in fact, from the pups. The exposure is therefore significantly underestimated for pups during the last week of lactation and significantly overestimated for the dams during this time period. This is supported by the work of Hanley and Watanabe (1985), who saw the same pattern in Fischer 344 rats during lactation by incorporating ^{141}Ce-radiolabeled microspheres into ground feed.

In the present study, age at acquisition of preputial separation was significantly delayed for F1, F2, and F3 males at 7500 ppm using ANCOVA with body weight at acquisition as the covariate (F1 male acquisition of preputial separation was also significantly delayed at 750 ppm; F2 and F3 males at this dietary dose were unaffected.) Age at acquisition of vaginal patency was also significantly delayed for F1, F2, and F3 females at 7500 ppm using ANCOVA with body weight at acquisition as the covariate. Analysis of the ages at acquisition alone (by nonparametric Kruskal-Wallis and Mann-Whitney U tests) did result in significant delays at lower doses (rarely and not consistently), but the values were not significant with ANCOVA. Therefore, at these lower doses, the effects were due to pups within groups within lower body weights. Body weight at acquisition was significantly reduced at 7500 ppm for F1 males and females and for F2 and F3 males only. Acquisition of developmental landmarks is dependent on both age and weight, *i.e.*, heavier animals acquire the landmark earlier, while lighter animals acquire the landmark later, but lighter animals do acquire the landmark (unless there is another cause for the delay) and in many cases acquire the landmark at a lighter weight than the heavier animals. In the present study, the lower body weight of the F1, F2, and F3 offspring at 7500 ppm presumably resulted in the observed delay in acquisition of vaginal patency and preputial separation. Since acquisition of both landmarks in both sexes of both generations was delayed, these results are not considered to be due to estrogen receptor-mediated events or other endocrine-related toxicity. The only endocrine-mediated mechanism currently known to result in delays in puberty in both sexes would be interference with steroidogenesis, thereby reducing

testosterone (and DHT) levels in males and estrogen levels in females, and there is no evidence that BPA interferes with steroidogenesis.

This interpretation is consistent with the recognition by the U.S. EPA (1996, p. 56295) that "body weight at puberty may provide a means to separate specific delays in puberty from those that are related to general delays in development." The delays in vaginal patency in females and in preputial separation in males at 7500 ppm in this study were relatively minor; 2.5 (F1, F3) to 3.5 (F2) days for females and 3.1 (F3), 3.9 (F1), and 5.8 (F2) days for males. The delay in acquisition in F1 males at 750 ppm was 1.7 days.

In addition, Kennedy and Mitra (1963) showed that body weight and food intake are factors in the initiation of puberty in the rat. Carney et al. (1998) fed SD pregnant dams at 70% or 50% of control feed intake levels from gd 7 through weaning of offspring. Feed restriction resulted in reduced body weights in dams and offspring and delayed vaginal patency and preputial separation (by one day at 70% and by six days at 50% restriction for both parameters).

In this study, ANCOVA was used to analyze acquisition of puberty (vaginal opening and preputial separation), with body weight at acquisition as the covariate, to attempt to ascertain if body weights were the critical parameter; i.e., with ANCOVA, if body weights were the "driver" (lighter animals acquired later), the statistical significance from ANOVA should not be present with ANCOVA. However, the ANCOVA analysis is intrinsically flawed, since the longer the delay, the older the animals and, therefore, the heavier the animals (all animals in all groups gained weight, and the first few weeks postweaning are the time of a major growth spurt in rats, and all animals did acquire puberty). In this study, the lighter animals acquired puberty at the later time (older age). Most of them were comparable in weight at the time of acquisition to the control (and low dose groups) animals who acquired puberty at an earlier time (younger age). There is much discussion among reproductive toxicologists as to the most appropriate body weight to use other than that at acquisition, with body weight on pnd 0 and pnd 21 (at weaning) the two main suggestions, but they also are problematic. At present, there is no better covariate than body weight at acquisition.

The significant effect on acquisition of reproductive landmarks in F1 and F2 offspring required measurement of anogenital distance in newborn F2 and F3 offspring, as specified in the guidelines (U.S. EPA, 1998). Therefore, anogenital distance was measured on pnd 0 for F2 and F3 pups and is summarized, along with pup body weights on pnd 0, in Text Table G by sex per

litter. Anogenital distances in newborn F2 and F3 males were statistically equivalent across all groups, with F2 and F3 male pup body weights per litter also statistically equivalent across all groups. Anogenital distance in the newborn F2 females was significantly longer at 0.015, 0.3, 4.5 (not 75), and 750 (not 7500) ppm, with mean values of 0.98, 0.98, 0.98, and 0.99 mm at 0.015, 0.3, 4.5, and 750 ppm, respectively, relative to the control group mean value of 0.95 mm. These effects represent an increase in anogenital distance of only 0.03, 0.03, 0.03, and 0.04 mm, equivalent to 3.16, 3.16, 3.16, and 4.21% increases at 0.015, 0.3, 4.5, and 750 ppm, respectively. F2 female pup body weights per litter on pnd 0 were statistically equivalent across all groups. F3 female pup anogenital distance and body weight on pnd 0 were equivalent across all groups. Anogenital distance is under androgenic control, specifically dihydrotestosterone (DHT) (Gray et al., 1998; Gray and Ostby, 1998), and does not appear to be affected by estrogens (Biegel et al., 1998a), and BPA has been shown not to be androgenic or anti-androgenic *in vivo* (Michna, 2000; Diel et al., 2000). Anogenital distance is affected by pup body weights (Ashby et al., 1997). For example, recent data (Gallavan, *et al.*, 1998) from 1501 control newborn CD® rats indicated that a one-gram increase in body weight leads to a 0.19-mm increase in anogenital distance. Carney et al. (1998) showed that anogenital distance was significantly reduced in newborn SD rats of dams fed 50% of control dietary feed levels, but anogenital distance:body weight ratios were essentially identical across groups.

In this study, all female anogenital distance values round to 1.00 mm (with historical control values in the performing laboratory from two recent studies of 0.73 ± 0.03 [SE] and 0.76 ± 0.02 mm in F2 female pups on pnd 0, with mean body weights of 6.08 ± 0.11 and 5.93 ± 0.11 g). These data suggest that changes in anogenital distance of 0.03 to 0.04 mm, as seen in the present study, are of no biological significance, based on the following:

- the magnitude of the differences is minimal;
- there were no correlating effects of any kind;
- there is no known biologically plausible mechanism for BPA to result in an increase in anogenital distance in females, since anogenital distance is under androgenic control, specifically dihydrotestosterone (DHT); and
- BPA is negative as either an androgen or antiandrogen *in vivo* (Michna, 2000; Diel et al., 2000)

These changes (referring to preputial separation, vaginal patency, and anogenital distance) did not affect reproductive function in the animals exhibiting them.

As noted earlier, BPA has been reported to have weak, estrogen-like activity in some screening assays. The present study evaluated a number of reproductive endocrine-sensitive endpoints, including preputial separation, vaginal patency, anogenital distance, estrous cyclicity, ovarian follicle counts, sperm and spermatid assessments, and reproductive organ weights and histopathology. A recent one-generation study with exposure of rats to 17β-estradiol (E2), at concentrations of 0.05, 2.5, 10, and 50 ppm in the diet (Biegel et al., 1998a,b), reported the following estrogenic responses (with the results from the present BPA study also provided):

Effect	E2 Dose	BPA	Comments
Estrous cycle changes (F0)	≥ 0.05 ppm	No	--
Total Infertility (F0)	≥ 10 ppm	No	--
Precocious (7 days) vaginal opening (F1)	≥ 0.05 ppm	No	F1, F2, and F3 VP delayed at 7500 ppm*
Delayed (8 days) preputial separation (F1)	≥ 2.5 ppm	Yes	F1, F2, and F3 at 7500 ppm*
Decreased F1 pup weights (Day 0)	≥ 0.05 ppm	No	--
Decreased F1 pup weights (Days 4-21)	2.5 ppm**	No	Reduced for pnd 7-21 for F1, F2, and F3 at 7500 ppm*
Reduced implantations/litter	2.5 ppm**	No	--
Reduced F1 litter size	2.5 ppm**	Yes	F1, F2, and F3 at birth at 7500 ppm*
Decreased testes/epididymal/accessory sex gland weights (F1)	2.5 ppm**	No	--
Decreased absolute and relative ovarian weight (F1)	2.5 ppm**	Yes	For F0, F1, F2, and F3 (absolute) and F0, F1, and F2 (relative) at 7500 ppm*
Reduced number of large antral follicles (F1)	2.5 ppm**	No	--
Vaginal mucosa thickening (F1)	2.5 ppm**	No	--

* Only in the presence of significant systemic maternal toxicity.

** At doses above 2.5 ppm E2, there was complete infertility (no F1 pups were born).

Assuming comparable kinetics for BPA and 17β-estradiol, and based on the kinetic information for 17-β-estradiol (*in vitro:* Gaido et al., 1997; *in vivo:* Milligan et al., 1998), the bioavailable function should be similar at comparable dietary concentrations, and BPA is generally reported to be approximately 15,000-fold less potent than 17β-estradiol from *in vitro* receptor binding/transcriptional activation screening assays (Gaido et al., 1997) and 10,000-fold less potent from *in vivo* subcutaneous injection in mice (Milligan et al., 1998). Therefore, if BPA were to have estrogen-like activity, it would be expected that doses of 750 ppm or greater

(50 mg/kg/day) should result in effects similar to those described above for 17β-estradiol at 0.05 ppm or greater. However, only two of the effects for dietary E2 exposure, reduced live litter size at birth and reduced paired ovary weights, were observed in the present study and only at 7500 ppm BPA in the presence of significant systemic maternal toxicity. The identification of these estrogen-relevant endpoints affected only at 7500 ppm supports the designation of BPA as possessing a very weak estrogen-like activity, but only at very high doses by oral administration and only in the presence of significant maternal systemic toxicity. No estrogen-like effects were observed at any doses below 7500 ppm in this study.

One last comment is that with all of the parameters evaluated, six BPA-exposed groups, 30/sex/group, three generations, and $p<0.05$ set as the criterion for statistical significance, by definition, one out of 20 values will be statistically significant by chance. Therefore, the most appropriate, scientific, and conservative approach is to identify only those statistically significant changes that are consistent generation-to-generation, exhibit a dose-response pattern of incidence and/or severity, and are biologically plausible, as treatment- and dose-related and biologically significant. The Study Director (author of this report) has endeavored to follow this approach.

CONCLUSIONS

Dietary exposure to BPA *ad libitum* for three generations, one litter per generation, at 0, 0.015, 0.3, 4.5, 75, 750, and 7500 ppm, resulted in:

- Decreased adult body weights and weight gains at 750 and 7500 ppm for F0, F1, and F2 parents and F3 retained adults
- Offspring toxicity (reduced body weights during lactation) beginning on pnd 7 at 7500 ppm for F1, F2, and F3 offspring (all pups and sexes separately)
- No consistent or dose-related effects on anogenital distance on pnd 0 for F2 and F3 offspring
- Delayed acquisition of vaginal patency and preputial separation for F1, F2, and F3 offspring at 7500 ppm; considered secondary to decreased body weights (delayed preputial separation also at 750 ppm for F1 males, but not F2 or F3 males)
- No treatment-related effects on reproductive performance or fertility parameters for any generation
- Significant reductions in implantations and total and live pups per litter at birth on pnd 0 (and on pnd 4 precull) at 7500 ppm for F1, F2, and F3 generations in the absence of any increased prenatal (postimplantation) loss
- Significant reductions in absolute and relative paired ovary weights at 7500 ppm in F1, F2, and F3 (absolute weights only) females, in the presence of profound maternal toxicity
- No treatment- or dose-related direct effects on testes weights, and no treatment- or dose-related testicular histopathology in any generation
- No treatment- or dose-related effects on epididymal sperm counts (reduced only in F1 males and only at 7500 ppm), motility, or morphology in any generation
- No treatment- or dose-related effects on testicular homogenization-resistant spermatid head counts, daily sperm production (reduced only in F3 males and only at 7500 ppm, with no effect on efficiency of daily sperm production in these F3 males), and no effects on efficiency of daily sperm production in any generation
- When compared to study findings for 17β-estradiol, only two estrogen-like effects (reduced ovarian weights and reduced litter size at birth) were observed in females only at 7500 ppm, a dose which was at or above the maximum tolerated dose (MTD) with substantial maternal toxicity

- No treatment- or dose-related effects on reproductive organ weights or histopathology for F0, F1, F2, or F3 male or female adults
- Histopathologic findings in females in F0, F1, and F2 (but not F3) generations, with increased incidences of renal tubular degeneration in the kidneys and chronic hepatic inflammation in the livers only at 7500 ppm
- No treatment- or dose-related increases in retained nipples and/or areolae in preweanling males at any dose for F1, F2, and F3 generations
- No treatment-related or consistent differences between high dose and control (ten females/group) paired ovarian primordial follicle counts; significantly increased at 7500 ppm for F0 females, unaffected for F1, F2, and F3 females

Exposure to dietary BPA for three generations, one litter per generation, resulted in adult systemic toxicity only at 750 and 7500 ppm. Because of the lack of adverse effects below these doses, the adult systemic toxicity NOAEL (no observable adverse effect level) was established at 75 ppm (equivalent to approximately 5 mg/kg/day) in CD® (Sprague-Dawley) rats, under the conditions of this study. Similarly, because there were no treatment- or dose-related reproductive or postnatal toxicity effects below 7500 ppm, the reproductive and postnatal toxicity NOAELs were concluded to be 750 ppm (equivalent to approximately 50 mg/kg/day) in CD® (Sprague-Dawley) rats, under the conditions of this study. There were no effects of BPA dietary exposure on any adult or offspring parameter at 0.015, 0.3, 4.5, or 75 ppm (equivalent to BPA intakes of approximately 0.001, 0.02, 0.3, or 5 mg/kg/day, respectively).

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Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 1 of 7)

Bisphenol A Groups:[a]		F0 1	F0 2	F0 3	F0 4	F0 5	F0 6	F0 7	F1 1	F1 2	F1 3	F1 4	F1 5	F1 6	F1 7
MALES															
Deaths		0	1	0	0	0	0	0	1	0	0	1	0	0	0
Prebreed Exposure															
Body Weights		--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
Weight Changes		--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
Clinical Observations		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption:	g/day	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	g/kg/day	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
Feed Efficiency		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Postmate Holding															
Body Weights		--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
Weight Changes[b]		--	--	--	--	--	--	--	--	--	--	↓	↓	↓	↓↓
Clinical Observations		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption:	g/day	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	g/kg/day	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
Feed Efficiency[b]		--	--	--	--	--	--	--	--	--	--	--	--	--	↓↓
Necropsy															
Body Weight		--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
Organ Weights:															
Liver	A	--	--	--	--	--	↓	↓↓	--	--	--	--	--	↓	↓↓
	R	--	--	--	--	--	↓	--	--	--	--	--	--	--	--
Kidneys	A	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	↑	--	--	--	↓	--	--	↑
Adrenal Glands	A	--	--	--	--	--	--	↓	--	--	--	--	--	--	--
	R	--	--	--	--	--	--	--	--	--	--	--	--	--	↑

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 2 of 7)

			F0							F1						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7	1	2	3	4	5	6	7
Spleen	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	--	--	--	--	--	↓	--	↑
Brain	A		--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↑	--	--	--	--	--	↑	↑↑
Pituitary	A		--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
	R		--	--	--	--	--	--	--	--	--	--	--	--	--	↑
Gross Findings			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Histopathology			--	--	--	--	--	--	↑[c]	--	--	--	--	--	--	--
FEMALES																
Deaths			0	0	0	0	0	0	0	1	0	0	0	0	0	1[d]
Prebreed Exposure																
Body Weights			--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Weight Changes			--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Clinical Observations			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption:	g/day		--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	g/kg/day		--	--	--	--	--	--	--	--	--	--	--	--	--	↑
Feed Efficiency			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gestation																
Body Weights			--	--	--	--	--	↓	↓↓	--	--	--	--	--	↓	↓↓
Weight Changes[b]			--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Clinical Observations			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption (gd 0-20):		g/day	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
		g/kg/day	--	--	--	--	--	↑	↑↑	--	--	--	--	--	--	↑
Feed Efficiency			--	--	--	--	--	--	↓	--	--	--	--	--	--	↓

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 3 of 7)

Bisphenol A Groups:[a]			F0							F1						
			1	2	3	4	5	6	7	1	2	3	4	5	6	7
Lactation																
Body Weights			--	--	--	--	--	↓	↓↓	--	--	--	--	--	↓	↓↓
Weight Changes			--	--	--	--	--	--	--	--	--	--	--	--	--	↑
Clinical Observations			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption (pnd 0-21):		g/day	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
		g/kg/day	--	--	--	--	--	--	↑	--	--	--	--	--	↑	↑↑
Feed Efficiency			--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
Necropsy																
Body Weight			--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
Organ Weights:																
Liver	A		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	R		--	--	--	--	--	--	↑	--	--	--	--	--	--	--
Kidneys	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↑	--	--	--	--	--	--	--
Adrenal Glands	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Spleen	A		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	R		--	--	--	--	--	--	--	--	--	--	--	--	--	↑
Brain	A		--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
Pituitary	A		--	--	↓	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Gross Findings			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Histopathology			--	--	--	--	--	--	↑[d]	--	--	--	--	--	--	↑[e]

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 4 of 7)

Bisphenol A Groups:[a]		F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7	F3 1	F3 2	F3 3	F3 4	F3 5	F3 6	F3 7
MALES															
Deaths		0	0	0	0	0	0	0	0	0	1	0	0	0	0
Prebreed Exposure															
Body Weights		--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Weight Changes		--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Clinical Observations		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption:	g/day	--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
	g/kg/day	--	--	--	--	↑	--	↑↑	--	--	--	--	--	--	↑
Feed Efficiency		--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Postmate Holding															
Body Weights		--	--	--	--	--	↓	↓↓			Not Applicable[f]				
Weight Changes[b]		--	--	--	--	--	--	↓							
Clinical Observations		--	--	--	--	--	--	--							
Feed Consumption:	g/day	--	--	--	--	--	↓	↓↓							
	g/kg/day	--	--	--	--	--	↑	↑↑							
Feed Efficiency[b]		--	--	--	--	--	--	↓							
Necropsy															
Body Weight		--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
Organ Weights:															
Liver	A	--	--	↓	--	--	↓	↓↓	--	--	--	--	--	↓	↓↓
	R	--	--	--	--	--	↓	--	--	--	--	--	--	↓	--
Kidneys	A	--	--	--	--	--	↓	↓↓	--	--	--	--	--	--	↓
	R	--	--	--	--	--	↑	↑↑	--	--	--	--	--	--	↑
Adrenal Glands	A	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	R	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 5 of 7)

Bisphenol A Groups:[a]			F2							F3						
			1	2	3	4	5	6	7	1	2	3	4	5	6	7
Spleen	A		--	--	--	--	↓	↓	↓↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	--	--	--	--	--	↓	--	--
Brain	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	↑	↑↑	--	--	--	--	--	--	↑
Pituitary	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
Gross Findings			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Histopathology			--	--	--	--	--	--	--	--	--	--	--	--	--	--
FEMALES																
Deaths			0	0	0	0	0	0	2	0	0	0	0	0	0	0
Prebreed Exposure																
Body Weights			--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Weight Changes			--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Clinical Observations			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Feed Consumption:	g/day		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	g/kg/day		--	--	--	--	--	--	↑	--	--	--	--	--	--	--
Feed Efficiency			--	--	--	--	--	--	↓	--	--	--	--	--	--	--
Gestation																
Body Weights			--	--	--	--	--	--	↓				Not applicable[f]			
Weight Changes			--	--	↓	↓	--	--	↓↓							
Clinical Observations			--	--	--	--	--	--	--							
Feed Consumption (gd 0-20):		g/day	--	--	--	--	--	↓	↓↓							
		g/kg/day	--	--	--	--	--	--	↑							
Feed Efficiency			--	--	--	--	--	--	↓							

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 6 of 7)

Bisphenol A Groups:[a]			F2							F3						
			1	2	3	4	5	6	7	1	2	3	4	5	6	7
Lactation																
Body Weights			--	--	--	--	--	--	↓			Not Applicable[f]				
Weight Changes			--	--	--	--	--	↑	↑↑							
Clinical Observations			--	--	--	--	--	--	--							
Feed Consumption (pnd 0-21):		g/day	--	--	--	--	--	--	--							
		g/kg/day	--	--	--	--	--	--	↑							
Feed Efficiency			--	--	--	--	--	↑	↑↑							
Necropsy																
Body Weight			--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
Organ Weights:																
Liver	A		--	↑	--	--	--	--	--	--	--	--	--	--	--	↓
	R		--	↑	--	--	--	--	↑↑	--	--	--	--	--	--	--
Kidneys	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	↑	--	--	--	--	--	--	--
Adrenal Glands	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Spleen	A		--	--	--	--	--	--	--	--	--	--	--	--	--	↓
	R		--	--	--	--	↓	--	↑	--	--	--	--	--	--	↑
Brain	A		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	R		--	--	--	--	--	--	↑↑	--	--	--	--	--	--	↑
Pituitary	A		--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	R		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gross Findings			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Histopathology			--	--	--	--	--	--	↑[g]	--	--	--	--	--	--	--

(continued)

Text Table E

Summary of F0, F1, F2, and F3 Adult Systemic Toxicity (page 7 of 7)

[a] Bisphenol A groups: 1 = 0.0 ppm, 2 = 0.015 ppm, 3 = 0.3 ppm, 4 = 4.5 ppm, 5 = 75 ppm, 6 = 750 ppm, 7 = 7500 ppm

[b] The significant reductions were observed only for the third week (sd 98-105).

[c] Chronic inflammation of the liver was observed in 2 of 11 F0 males at 7500 ppm versus 0/30, 0/14, 0/12, 1/13, 0/14, and 0/12 at 0, 0.015, 0.3, 4.5, 75, and 750 ppm, respectively

[d] One animal (No. 1214) was designated a female at birth and throughout in-life. At necropsy, it contained mid-abdominal testes. Therefore, none of No. 1214's in-life data were included in the summary or individual tables (see footnote to Table 32 for details). One female (No. 1204) was euthanized moribund as indicated.

[e] Increased incidences of renal tubular degeneration (8/11) and chronic hepatic inflammation (3/11) in F1 females at 7500 ppm versus 0 incidences for all other groups for renal tubular degeneration, and 3/30, 0/10, 0/10, 3/10, 1/10, and 0/10 for chronic hepatic inflammation at 0, 0.015, 0.3, 4.5, 75, and 7500 ppm, respectively.

[f] F3 offspring were not mated but necropsied after the prebreed (i.e., postwean) exposure period.

[g] Increased incidences of renal tubular degeneration (7/13) and chronic hepatic inflammation (5/13) in F2 females at 7500 ppm, with no incidence of renal tubular degeneration at 0 through 750 ppm, and incidences of chronic hepatic inflammation of 3/30, 1/11, 0/10, 2/12, 2/11, and 2/12 at 0, 0.015, 0.3, 4.5, 75, and 7500 ppm, respectively.

KEY:

↑ or ↓ = For those parameters analyzed statistically, one arrow means statistically significant increase (↑) or decrease (↓) in one group only, or in two or more groups if the effects were approximately comparable (equivalent). For those parameters not analyzed statistically, one arrow means an increase (↑) or decrease (↓) in incidence (clinical observations, histopathology) relative to the control group incidence.

↑↑ or ↓↓ = For those parameters analyzed statistically, two arrows mean statistically significant increase (↑↑) or decrease (↓↓) if there were effects in two or more groups, and the effects in the designated groups were obviously greater (e.g., $p<0.01$ or $p<0.001$ versus $p<0.05$), or if the "p" values are the same but the values for the two or more affected groups are clearly different. For those parameters not analyzed statistically, two arrows mean that the incidence in the designated group is clearly higher relative to the incidence in other affected groups.

-- = No statistically significant difference, or no apparent difference for clinical observations or histopathology

A = Absolute organ weight

R = Organ weight relative to terminal body weight

Text Table F

Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters (page 1 of 5)

	F0							F1						
Bisphenol A Groups:[a]	1	2	3	4	5	6	7	1	2	3	4	5	6	7
FEMALES														
Vaginal cyclicity	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Estrous cycle length, days	4.58	4.41	4.48	4.50	4.57	4.45	4.26	4.41	4.47	4.19	4.70	4.94	4.40	4.54
Indices: Mating	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Fertility	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Pregnancy	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Gestational	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Precoital interval, days	2.3	2.4	2.3	2.9	2.4	2.3	3.4	3.0	3.2	2.7	3.1	3.1	2.8	2.7
Gestational length, days	22.1	22.1	22.1	22.1	22.2	22.0	22.1	21.8	22.0	21.9	22.0	21.9	22.0	21.8
No. implant sites/litter	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
% postimplantation loss/litter (prenatal mortality index)	--	--	--	--	--	--	--	--	--	--	--	--	--	--
No. total pups/litter, pnd 0	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
No. live pups/litter, pnd 0	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
No. dead pups/litter, pnd 0	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Necropsy														
Weights: paired ovaries A	--	--	--	--	--	--	↓	--	--	--	--	--	↓	↓↓
R	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
uterus A	--	↓	--	--	--	--	↓↓	--	--	--	--	--	--	↓
R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Paired ovarian follicle counts[b]	--						↑	--						--
Stage of estrus at necropsy[c]	--	--	--	--	--	↑	↑↑	--	--	--	--	--	--	--
Reproductive system: gross findings	--	--	--	--	--	--	--	--	--	--	--	--	--	--
histopathology	--	--	--	--	--	--	--	--	--	--	--	--	--	--

(continued)

Text Table F

Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters (page 2 of 5)

			F0							F1						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7	1	2	3	4	5	6	7
MALES																
Indices:	Mating		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Fertility		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Necropsy																
Weights:	Testes	A	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
	Epididymides	A	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
	Prostate	A	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Preputial gland	A	--	--	--	--	--	--	--	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Seminal vesicles w/	A	--	--	--	↑	--	--	↓	--	--	--	--	--	--	↓
	coagulating glands	R	--	--	--	--	--	--	--	--	--	--	--	--	--	↑
Reproductive system:	gross findings		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	histopathology		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Andrological Assessments																
Epididymal sperm concentration			--	--	--	--	--	--	--	--	--	--	--	--	--	↓
% motile sperm			--	--	--	--	--	--	--	--	--	--	--	--	--	--
% progressively motile sperm			--	--	--	--	--	--	--	--	--	--	--	--	--	--
TSHC			--	--	--	--	--	--	--	--	--	--	--	--	--	--
DSP			--	--	--	--	--	--	--	--	--	--	--	--	--	--
Efficiency of DSP			--	--	--	--	--	--	--	--	--	--	--	--	--	--
% abnormal sperm			3.29[d]	1.72	2.01	2.03	5.16	2.35	1.70	1.98	4.13[d]	3.44[d]	1.98	2.41	1.82	1.91

(continued)

Text Table F

Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters (page 3 of 5)

	F2							F3						
Bisphenol A Groups:[a]	1	2	3	4	5	6	7	1	2	3	4	5	6	7
FEMALES														
Vaginal cyclicity	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Estrous cycle length, days	4.54	4.61	4.39	4.47	4.17	4.56	4.56	4.32	4.34	4.32	4.39	4.66	4.59	4.31
Indices: Mating	--	--	--	--	--	--	--			Not Applicable				
Fertility	--	--	--	--	--	--	--			(F3 females not bred)				
Pregnancy	--	--	--	--	--	--	--							
Gestational	--	--	--	--	--	--	--							
Precoital interval, days	3.1	2.0	2.8	2.7	2.9	2.7	3.1			Not Done				
Gestational length, days	22.0	22.3	22.0	22.0	22.1	22.0	22.1							
No. implant sites/litter	--	--	↓	--	--	--	↓↓							
% postimplantation loss/litter (prenatal mortality index)	--	--	--	--	--	--	--							
No. total pups/litter, pnd 0	--	--	↓	--	--	--	↓↓							
No. live pups/litter, pnd 0	--	--	↓	--	--	--	↓↓							
No. dead pups/litter, pnd 0	--	--	--	--	--	--	--							
Necropsy														
Weights: paired ovaries A	--	↓	--	↓	↓	--	↓↓	--	--	--	--	--	--	↓
R	--	↓	--	↓	↓	--	↓↓	--	--	--	--	--	--	--
uterus A	--	--	--	--	--	--	↓	--	--	--	--	--	--	--
R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Paired ovarian follicle counts[b]	--						--	--						--
Stage of estrus at necropsy[c]	--	--	--	--	--	--	↑	--	--	--	--	--	--	--
Reproductive system: gross findings	--	--	--	--	--	--	--	--	--	--	--	--	--	--
histopathology	--	--	--	--	--	--	--	--	--	--	--	--	--	--

(continued)

Text Table F

Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters (page 4 of 5)

			F2							F3						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7	1	2	3	4	5	6	7
MALES																
Indices:	Mating		--	--	--	--	--	--	--	Not Applicable						
	Fertility		--	--	--	--	--	--	--	(F3 males not bred)						
Necropsy																
Weights:	Testes	A	--	--	↓	--	--	↓	↓↓	--	↓	↓	--	--	↓	↓↓
		R	--	--	--	--	--	--	↑	--	--	--	--	--	--	↑
	Epididymides	A	--	--	--	--	--	↓	↓	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↑	↑↑	--	--	--	--	--	--	↑
	Prostate	A	--	--	--	--	--	--	↑	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Preputial gland	A	--	--	--	--	--	--	--	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--	--	--	--	--	--	--	--
	Seminal vesicles w/	A	--	--	--	--	--	--	↓	--	--	--	--	--	--	↓
	coagulating glands	R	--	--	--	--	--	↑	--	--	--	--	--	--	--	--
Reproductive system:	gross findings		--	--	--	--	--	--	--	--	--	--	--	--	--	--
	histopathology		--	--	--	--	--	--	--	--	--	--	--	--	--	--
Andrological Assessments																
Epididymal sperm concentration			--	--	--	--	--	--	--	--	--	--	--	--	--	--
% motile sperm			--	--	--	--	--	--	--	--	--	--	--	--	--	--
% progressively motile sperm			--	--	--	--	--	--	--	--	--	--	--	--	--	--
TSHC			--	--	--	--	--	--	--	--	--	--	--	--	--	--
DSP			--	--	--	--	--	--	--	--	--	--	--	--	--	↓
Efficiency of DSP			--	--	--	--	--	--	--	--	--	--	--	--	--	--
% abnormal sperm			2.19	4.89[d]	4.10[d]	3.25[d]	3.47[d]	2.05	1.62	1.75	5.14[d]	1.93	2.29	8.33[d]	1.70	2.03

(continued)

Text Table F

Summary of F0, F1, and F2 Parental and Retained F3 Male and Female Reproductive Parameters (page 5 of 5)

[a] Bisphenol A groups: 1 = 0.0 ppm, 2 = 0.015 ppm, 3 = 0.3 ppm, 4 = 4.5 ppm, 5 = 75 ppm, 6 = 750 ppm, 7 = 7500 ppm

[b] Both ovaries from ten females each in high dose and control groups were evaluated.

[c] Stage of estrus at necropsy was considered affected if there were significant decreases of the % in proestrus/estrus and increases of the % in diestrus.

[d] Increase in mean % abnormal sperm (with increased variance term) due to one to two males per designated group with low sperm motility and high % abnormal sperm. All of the identified F0, F1, and F2 males sired live litters (F3 males were not bred).

KEY:

↑ or ↓ = For those parameters analyzed statistically, one arrow means statistically significant increase (↑) or decrease (↓) in one group only, or in two or more groups if the effects were approximately comparable (equivalent). For those parameters not analyzed statistically, one arrow means an increase (↑) or decrease (↓) in incidence (clinical observations, histopathology) relative to the control group incidence.

↑↑ or ↓↓ = For those parameters analyzed statistically, two arrows mean statistically significant increase (↑↑) or decrease (↓↓) if there were effects in two or more groups, and the effects in the designated groups were obviously greater (e.g., $p<0.01$ or $p<0.001$ versus $p<0.05$), or if the "p" values are the same but the values for the two or more affected groups are clearly different. For those parameters not analyzed statistically, two arrows mean that the incidence in the designated group is clearly higher relative to the incidence in other affected groups.

-- = No statistically significant difference, or no apparent difference for clinical observations or histopathology

A = Absolute organ weight

R = Organ weight relative to terminal body weight

DSP = Daily sperm production

* = $p<0.05$ versus control value

TSHC = Testicular homogenization-resistant spermatid head counts

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 1 of 10)

		F1						
Bisphenol A Groups:[a]		1	2	3	4	5	6	7
LACTATION								
Stillbirth index		--	--	--	--	--	--	--
Live birth index		--	--	--	--	--	--	--
No. live pups/litter, pnd 0		--	--	--	--	--	--	↓
Survival indices:	4-day	--	--	--	--	--	--	--
	7-day	--	--	--	--	--	--	--
	14-day	--	--	--	--	--	--	--
	21-day	--	--	--	--	--	--	--
Lactational index (pnd 4 [postcull] - 21)		--	--	--	--	--	--	--
Sex ratio (% males/litter)		--	--	--	--	--	--	--
Pup body weights/litter:	pnd 0	--	--	--	--	--	--	--
	pnd 4	--	--	--	--	--	--	↓[b]
	pnd 7	--	--	--	--	--	--	↓
	pnd 14	--	--	--	--	--	--	↓
	pnd 21	--	--	--	--	--	--	↓
Anogenital distance/litter, pnd 0 (mm):	male[c]			NOT				
	female[c]			DONE				
Body weight/litter, pnd 0 (g):	male	6.53	6.36	6.69	6.60	6.62	6.49	6.37
	female	6.19	6.10	6.29	6.17	6.27	6.18	6.00
% males with one or more nipples		0.00	0.00	0.00	0.00	0.00	0.00	0.00
No. nipples per male		0	0	0	0	0	0	0
% males with one or more areolae		0.0	0.8	0.0	0.8	3.0	2.3	0.8
No. areolae per male		0.00	0.02	0.00	0.01	0.06	0.04	0.01

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 2 of 10)

Bisphenol A Groups:[a]			F1						
			1	2	3	4	5	6	7
WEANLING NECROPSY									
Males									
Terminal body weight			--	↓	--	--	↓	--	↓↓
Weights:	Liver	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	--
	Spleen	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↓	↓↓
	Brain	A	--	--	--	--	--	--	↓↓
		R	--	↑	--	--	↑	↑	↑↑
	Paired testes	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	--
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Gross findings			--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 3 of 10)

Bisphenol A Groups:[a]		F1 1	F1 2	F1 3	F1 4	F1 5	F1 6	F1 7
Females (cont.)								
Spleen	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	↓
Brain	A	--	--	--	--	--	--	↓
	R	--	↑	↑	--	↑	↑	↑↑
Paired ovaries	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	--
Uterus	A	--	--	↓	--	--	--	↓↓
	R	--	--	--	--	--	--	↓
Gross findings		--	--	--	--	--	--	--
POSTWEAN[b]								
Females								
Age at VP (days)		30.5	30.7	30.2	30.6	30.1	31.0	33.0***
Adjusted age at VP (days)		30.3	30.1	30.4	30.1	30.3	30.9	33.9***[d]
Weight at acquisition (g)		102.5	105.5	99.0	106.0	99.7	102.3	92.3**
Males								
Age at PPS (days)		41.9	43.2	43.1	42.2	42.8	43.6**	45.8***
Adjusted age at PPS (days)		41.9	42.8	42.7	42.0	42.7	43.6**[d]	46.8***[d]
Weight at acquisition (g)		215.7	221.3	225.1	220.6	216.7	214.2	194.0***

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 4 of 10)

Bisphenol A Groups:[a]		F2 1	F2 2	F2 3	F2 4	F2 5	F2 6	F2 7
LACTATION								
Stillbirth index		--	--	--	--	--	--	--
Live birth index		--	--	--	--	--	--	--
No. live pups/litter, pnd 0		--	--	--	--	--	--	↓
Survival indices:	4-day	--	--	--	--	--	--	--
	7-day	--	--	--	--	--	--	--
	14-day	--	--	--	--	--	--	--
	21-day	--	--	--	--	--	--	--
Lactational index (pnd 4 [postcull] - 21)		--	--	--	--	--	--	--
Sex ratio (% males/litter)		--	--	--	--	--	--	--
Pup body weights/litter:	pnd 0	--	--	--	--	--	--	--
	pnd 4	--	--	--	--	--	--	--
	pnd 7	--	--	--	--	--	--	↓
	pnd 14	--	--	--	--	--	--	↓
	pnd 21	--	--	--	↓[b]	↓	↓[b]	↓↓
Anogenital distance/litter, pnd 0 (mm):	male	1.98	2.00	1.98	1.97	1.95	1.96	2.00
	female	0.95	0.98*	0.98**	0.98*	0.97	0.99**	0.96
Body weight/litter, pnd 0 (g):	male	6.28	6.51	6.28	6.45	6.40	6.42	6.38
	female	5.97	6.10	5.94	6.13	6.01	6.00	5.99
% males with one or more nipples		0.00	0.00	0.00	0.00	0.77	0.00	0.00
No. nipples per male		0.0	0.0	0.0	0.00	0.01	0.00	0.00
% males with one or more areolae		2.26	2.10	3.95	1.57	1.54	2.40	2.26
No. areolae per male		0.05	0.06	0.09	0.04	0.02	0.05	0.05

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 5 of 10)

Bisphenol A Groups:[a]			F2						
			1	2	3	4	5	6	7
WEANLING NECROPSY									
Males									
Terminal body weight			--	--	--	↓	↓	--	↓↓
Weights:	Liver	A	--	--	--	↓	↓	--	↓↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↓	↑
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↓
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	↑	↑	--	↑↑
	Paired testes	A	--	--	--	--	--	--	↓
		R	--	--	--	↑	--	--	↑↑
	Paired epididymides	A	--	--	--	--	--	--	--
		R	--	--	--	--	↑	↑	↑↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Gross findings			--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	↓	--	↓↓
Weights:	Liver	A	--	--	--	--	↓	--	↓↓
		R	--	--	--	--	--	--	↓
	Thymus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 6 of 10)

		F2						
Bisphenol A Groups:[a]		1	2	3	4	5	6	7
Females (cont.)								
Spleen	A	--	--	--	--	↓	--	↓↓
	R	--	--	--	--	--	--	↓
Brain	A	--	--	--	--	--	↑	↓
	R	--	--	--	--	↑	↑	↑↑
Paired ovaries	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	--
Uterus	A	--	--	--	--	--	--	--
	R	--	--	--	--	--	--	--
Gross findings		--	--	--	--	--	--	--
POSTWEAN								
Females								
Age at VP (days)		31.0	31.2	31.2	33.5	32.1*	31.9	34.5***
Adjusted age at VP (days)		31.3	31.2	31.7	31.8	31.9	32.2	35.3***[d]
Weight at acquisition (g)		105.0	106.5	104.2	114.6	107.5	105.1	102.5
Males								
Age at PPS (days)		42.1	43.5	43.7**	42.9	43.3**	43.2*	47.9***
Adjusted age at PPS (days)		41.9	41.6	43.2	42.9	43.1	43.8	49.3***[d]
Weight at acquisition (g)		219.7	228.7	223.3	217.3	219.8	211.4	200.13**

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 7 of 10)

		F3						
Bisphenol A Groups:[a]		1	2	3	4	5	6	7
LACTATION								
Stillbirth index		--	--	--	--	--	--	--
Live birth index		--	--	--	--	--	--	--
No. live pups/litter, pnd 0		--	--	↓	--	--	--	↓
Survival indices:	4-day	--	--	--	--	--	--	--
	7-day	--	--	--	--	--	--	--
	14-day	--	--	--	--	--	--	--
	21-day	--	--	--	--	--	--	--
Lactational index (pnd 4 [postcull] - 21)		--	--	--	--	--	--	--
Sex ratio (% males/litter)		--	--	--	--	--	--	--
Pup body weights/litter:	pnd 0	--	--	--	--	--	--	--
	pnd 4	--	--	--	--	--	--	--
	pnd 7	--	--	--	--	--	--	↓
	pnd 14	--	--	--	--	--	--	↓
	pnd 21	--	--	--	--	--	--	↓
Anogenital distance/litter, pnd 0 (mm):	male	1.97	1.97	1.97	2.00	2.01	2.00	1.96
	female	0.92	0.96	0.91	0.93	0.95	0.95	0.94
Body weight/litter, pnd 0 (g):	male	6.36	6.71	6.51	6.66	6.40	6.49	6.49
	female	5.98	6.34	6.14	6.31	5.99	6.10	6.14
% males with one or more nipples		0.00	0.00	0.00	0.00	0.00	0.00	0.00
No. nipples per male		0.00	0.00	0.00	0.00	0.00	0.00	0.00
% males with one or more areolae		0.0	1.4	0.8	0.0	2.9	1.4	0.0
No. areolae per male		0.00	0.03	0.01	0.00	0.06	0.03	0.00

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 8 of 10)

Bisphenol A Groups:[a]			F3 1	F3 2	F3 3	F3 4	F3 5	F3 6	F3 7
WEANLING NECROPSY									
Males									
Terminal body weight			--	--	--	--	--	↓	↓↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	↑	--	--	--	↓	--
	Thymus	A	--	--	--	--	--	↓	↓
		R	--	--	--	--	--	--	↑
	Spleen	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	--	↓
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	↑↑
	Paired testes	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	↑	↑↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Gross findings			--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	↑	--	--	--	--	--
	Thymus	A	--	--	--	--	--	↓	↓
		R	--	--	--	--	--	--	↑

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 9 of 10)

Bisphenol A Groups:[a]		F3 1	F3 2	F3 3	F3 4	F3 5	F3 6	F3 7
Females (cont.)								
Spleen	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	↓
Brain	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	↑	↑↑
Paired ovaries	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	--
Uterus	A	--	--	--	--	--	--	↓
	R	--	--	--	--	--	--	--
Gross findings		--	--	--	--	--	--	--
POSTWEAN								
Females								
Age at VP (days)		31.3	31.1	31.1	31.1	31.6	30.9	33.8***
Adjusted age at VP (days)		31.1	30.8	31.3	30.9	31.4	31.1	34.3***d
Weight at acquisition (g)		105.6	106.3	101.9	105.6	105.2	102.4	99.0
Males								
Age at PPS (days)		42.1	42.2	43.1	41.9	42.8	43.1	45.2***
Adjusted age at PPS (days)		42.0	42.0	43.1	41.9	42.6	43.0	46.0***d
Weight at acquisition (g)		209.3	214.4	208.3	208.4	214.3	210.5	186.8***

(continued)

Text Table G

Summary of F1, F2, and F3 Offspring Parameters (page 10 of 10)

[a] Bisphenol A groups: 1 = 0.0 ppm, 2 = 0.015 ppm, 3 = 0.3 ppm, 4 = 4.5 ppm, 5 = 75 ppm, 6 = 750 ppm, 7 = 7500 ppm

[b] F1 pup body weight per litter was significantly reduced for sexes combined ($p<0.05$) but not for sexes separately.

[c] Anogenital distance of pups on pnd 0 was triggered in F2 and F3 offspring by significant delays in postwean reproductive maturation in F1 offspring.

[d] Adjusted value (age at acquisition) is statistically significantly different from control group value by analysis of covariance (ANCOVA), with body weight at acquisition the covariant.

KEY:

↑ or ↓ = For those parameters analyzed statistically, one arrow means statistically significant increase (↑) or decrease (↓) in one group only, or in two or more groups if the effects were approximately comparable (equivalent). For those parameters not analyzed statistically, one arrow means an increase (↑) or decrease (↓) in incidence (clinical observations, histopathology) relative to the control group incidence.

↑↑ or ↓↓ = For those parameters analyzed statistically, two arrows mean statistically significant increase (↑↑) or decrease (↓↓) if there were effects in two or more groups, and the effects in the designated groups were obviously greater (e.g., $p<0.01$ or $p<0.001$ versus $p<0.05$), or if the "p" values are the same but the values for the two or more affected groups are clearly different. For those parameters not analyzed statistically, two arrows mean that the incidence in the designated group is clearly higher relative to the incidence in other affected groups.

-- = No statistically significant difference, or no apparent difference for clinical observations or histopathology

f = female pup weights only (not males or all pups)

* = $p<0.05$, ** = $p<0.01$, *** = $p<0.001$ versus control group value

PPS = Preputial separation in males

VP = Vaginal patency in females

A = Absolute organ weight

R = Organ weight relative to terminal body weight

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 1 of 10)

			F1						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
WEANLINGS[b]									
Males									
Terminal body weight			--	↓	--	--	↓	--	↓↓
Weights:	Liver	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	--
	Spleen	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↓	↓↓
	Brain	A	--	--	--	--	--	--	↓↓
		R	--	↑	--	--	↑	↑	↑↑
	Paired testes	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	↓	--	--	--	--	↓↓
		R	--	--	--	--	--	--	--
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↓

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 2 of 10)

Bisphenol A Groups:[a]			F1 1	F1 2	F1 3	F1 4	F1 5	F1 6	F1 7
Females (cont.)									
	Brain	A	--	--	--	--	--	--	↓
		R	--	↑	↑	--	↑	↑	↑↑
	Paired ovaries	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Uterus	A	--	--	↓	--	--	--	↓↓
		R	--	--	--	--	--	--	↓
ADULTS[c]									
Males									
Terminal body weight			--	--	--	--	--	↓	↓↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	--	--
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	↓	--	↑
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	↑↑
	Paired testes	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Seminal vesicles w/ coagulating glands	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 3 of 10)

			F1						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
Females									
Terminal body weight			--	--	--	--	--	↓	↓↓
Weights:	Liver	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	↑
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired ovaries	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	--	↓
	Uterus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 4 of 10)

			F2						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
WEANLINGS[b]									
Males									
Terminal body weight			--	--	--	↓	↓	--	↓↓
Weights:	Liver	A	--	--	--	↓	↓	--	↓↓
		R	--	--	--	--	--	--	--
	Thymus	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↓	↑
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↓
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	↑	↑	--	↑↑
	Paired testes	A	--	--	--	--	--	--	↓
		R	--	--	--	↑	--	--	↑↑
	Paired epididymides	A	--	--	--	--	--	--	--
		R	--	--	--	--	↑	↑	↑↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	↓	--	↓↓
Weights:	Liver	A	--	--	--	--	↓	--	↓↓
		R	--	--	--	--	--	--	↓
	Thymus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Spleen	A	--	--	--	--	--	--	↓↓
		R	--	--	--	--	--	--	↓

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 5 of 10)

Bisphenol A Groups:[a]			F2						
			1	2	3	4	5	6	7
Females (cont.)									
	Brain	A	--	--	--	--	--	↑	↓
		R	--	--	--	--	↑	↑	↑↑
	Paired ovaries	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Uterus	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
ADULTS[c]									
Males									
Terminal body weight			--	--	--	--	--	↓	↓↓
Weights:	Liver	A	--	--	↓	--	--	↓	↓↓
		R	--	--	--	--	--	↓	--
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	↓	↓	↓↓
		R	--	--	--	--	--	--	--
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	↑↑
	Paired testes	A	--	--	↓	--	--	↓	↓↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↑	↑↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	--

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 6 of 10)

			F2						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	↑	--	--	--	--	--
		R	--	↑	--	--	--	--	↑↑
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	--	--	--
		R	--	--	--	--	↓	--	↑
	Brain	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	↑
	Paired ovaries	A	--	↓	--	↓	↓	--	↓↓
		R	--	↓	--	↓	↓	--	↓↓
	Uterus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 7 of 10)

			F3						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
WEANLINGS[b]									
Males									
Terminal body weight			--	--	--	--	--	↓	↓↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	↑	--	--	--	↓	--
	Thymus	A	--	--	--	--	--	↓	↓
		R	--	--	--	--	--	--	↑
	Spleen	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	--	↓
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	↑↑
	Paired testes	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	↑	↑↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	↑	--	--	--	--	--
	Thymus	A	--	--	--	--	--	↓	↓
		R	--	--	--	--	--	--	↑
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↓

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 8 of 10)

Bisphenol A Groups:[a]			F3						
			1	2	3	4	5	6	7
Females (cont.)									
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	↑	↑↑
	Paired ovaries	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Uterus	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
ADULTS[c]									
Males									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	↓	--
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	↓	--	--
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired testes	A	--	↓	↓	--	--	↓	↓↓
		R	--	--	--	--	--	--	↑
	Paired epididymides	A	--	--	--	--	--	↓	↓↓
		R	--	--	--	--	--	--	↑
	Seminal vesicles w/ coagulating gland	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 9 of 10)

			F3						
Bisphenol A Groups:[a]			1	2	3	4	5	6	7
Females									
Terminal body weight			--	--	--	--	--	--	↓
Weights:	Liver	A	--	--	--	--	--	--	↓
		R	--	--	--	↑	--	--	↑
	Thymus	A	NA						
		R							
	Spleen	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Brain	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	↑
	Paired ovaries	A	--	--	--	--	--	--	↓
		R	--	--	--	--	--	--	--
	Uterus	A	--	--	--	--	--	--	--
		R	--	--	--	--	--	--	--

(continued)

Text Table H

Comparison of Weanling and Adult Organ Weights for F1, F2, and F3 Males and Females (page 10 of 10)

[a] Bisphenol A groups: 1 = 0.0 ppm, 2 = 0.015 ppm, 3 = 0.3 ppm, 4 = 4.5 ppm, 5 = 75 ppm, 6 = 750 ppm, 7 = 7500 ppm

[b] Taken from Text Table F

[c] Taken from Text Tables D and E

KEY:

↑ or ↓ = For those parameters analyzed statistically, one arrow means statistically significant increase (↑) or decrease (↓) in one group only, or in two or more groups if the effects were approximately comparable (equivalent). For those parameters not analyzed statistically, one arrow means an increase (↑) or decrease (↓) in incidence (clinical observations, histopathology) relative to the control group incidence.

↑↑ or ↓↓ = For those parameters analyzed statistically, two arrows mean statistically significant increase (↑↑) or decrease (↓↓) if there were effects in two or more groups, and the effects in the designated groups were obviously greater (e.g., $p<0.01$ or $p<0.001$ versus $p<0.05$), or if the "p" values are the same but the values for the two or more affected groups are clearly different. For those parameters not analyzed statistically, two arrows mean that the incidence in the designated group is clearly higher relative to the incidence in other affected groups.

-- = No statistically significant difference, or no apparent difference for clinical observations or histopathology

A = Absolute organ weight

R = Organ weight relative to terminal body weight

NA = Not applicable

Text Table I

Bisphenol A Intake in mg/kg Body Weight/Day (page 1 of 3)

Parameter		BPA Concentration (ppm)					
		0.015	0.3	4.5	75	750	7500
PREBREED							
Males							
F0	week 1	0.0015	0.031	0.465	7.663	73.370	786.03[a]
	week 10	0.0008	0.016	0.247	4.130	39.923	533.55
	sd 0-70	0.0010	0.020	0.304	5.092	49.628	--[b]
F1	week 1	0.0026	0.049	0.734	12.229	121.245	1340.10
	week 10	0.0009	0.019	0.269	4.566	46.518	546.87
	sd 0-70	0.0013	0.025	0.373	6.407	62.305	748.86
F2	week 1	0.0022	0.045	0.672	11.163	110.490	1205.51
	week 10	0.0008	0.018	0.261	4.542	44.288	532.47
	sd 0-70	0.0012	0.024	0.359	6.075	59.402	696.95
F3	week 1[c]	0.0022	0.044	0.633	11.015	107.081	1176.14
	week 10[d]	0.0009	0.018	0.268	4.609	45.304	570.89
	sd 0-63[d]	0.0012	0.024	0.360	6.139	59.288	698.68
Females							
F0	week 1	0.0015	0.031	0.442	7.810	72.251	1119.04[a]
	week 10	0.0010	0.019	0.285	5.136	51.236	713.42
	sd 0-70	0.0012	0.024	0.358	6.311	59.411	--[b]
F1	week 1	0.0023	0.048	0.694	11.817	111.160	1324.90
	week 10	0.0010	0.021	0.298	5.249	54.083	613.98
	sd 0-70	0.0013	0.028	0.407	7.004	67.548	749.42

(continued)

Text Table I

Bisphenol A Intake in mg/kg Body Weight/Day (page 2 of 3)

Parameter		BPA Concentration (ppm)					
		0.015	0.3	4.5	75	750	7500
Females (continued)							
F2	week 1	0.0022	0.041	0.687	11.058	106.479	1196.98
	week 10	0.0010	0.021	0.304	5.203	51.394	564.71
	sd 0-70	0.0014	0.028	0.410	6.808	67.069	755.63
F3	week 1[c]	0.0022	0.043	0.664	11.675	107.160	1232.86
	week 11 (sd 70-77)	0.0010	0.020	0.296	5.306	50.623	558.34
	sd 0-77	0.0013	0.026	0.386	6.871	64.683	678.59
GESTATION							
F0	gd 0-20	0.0009	0.019	0.287	4.954	51.587	648.54
F1	gd 0-20	0.0010	0.020	0.299	5.078	50.536	573.44
F2	gd 0-20	0.0010	0.019	0.291	4.833	48.815	547.56
LACTATION							
F0	pnd 4-7	0.0020	0.041	0.631	10.816	109.845	1140.57
	pnd 14-21	0.0029	0.062	0.890	15.595	160.144	1822.82
	pnd 0-21	0.0024	0.050	0.743	12.924	132.314	1496.44
F1	pnd 4-7	0.0021	0.041	0.640	10.905	116.263	1293.55
	pnd 14-21	0.0030	0.060	0.897	15.168	167.236	1807.78
	pnd 0-21	0.0025	0.050	0.763	12.802	139.741	1510.05
F2	pnd 4-7	0.0022	0.043	0.673	11.573	113.452	1352.95
	pnd 14-21	0.0029	0.060	0.906	15.362	152.305	1798.51
	pnd 0-21	0.0025	0.050	0.776	13.060	131.910	1565.98

(continued)

Text Table I

Bisphenol A Intake in mg/kg Body Weight/Day (page 3 of 3)

Parameter		BPA Concentration (ppm)					
		0.015	0.3	4.5	75	750	7500
HOLDING PERIOD							
Males							
F0	week 1 (sd 84-91)	0.0008	0.016	0.233	3.854	39.079	477.91
	week 3 (sd 98-105)	0.0008	0.015	0.223	3.855	37.559	491.33
F1	week 1 (sd 84-91)	0.0009	0.017	0.252	4.373	44.281	537.69
	week 3 (sd 98-105)	0.0008	0.016	0.236	4.090	41.773	499.99
F2	week 1 (sd 84-91)	0.0008	0.016	0.243	4.109	40.975	501.00
	week 3 (sd 98-105)	0.0007	0.015	0.229	3.852	39.427	467.29
Females[e]							
F0	week 1 (sd 98-105)	0.0009	0.018	0.265	4.356	48.167	495.61
	week 2 (sd 105-112)	0.0009	0.018	0.262	3.833	47.711	542.42
F1	week 1 (sd 84-91)	0.0009	0.018	--	3.801	50.183	892.61
	week 4 (sd 105-112)	--	--	0.264	4.657	48.875	453.98
F2	week 1 (sd 84-91)	0.0009	--	0.249	4.129	40.514	--
	week 4 (sd 105-112)	0.0009	0.019	0.269	4.545	50.087	434.50

[a] There were no chemical intake values available for F0 males or females at 7500 ppm during prebreed week 1 since there were no feed consumption values. All animals removed significant amounts of feed from the feed jars, precluding accurate feed weights. The palatability problems were resolved, so week 2 intake values were used in place of week 1 values.

[b] Since there were no chemical intake values available for week 1 for F0 males or females at 7500 ppm (see footnote a), there were no values available for the entire prebreed period (sd 0-70).

[c] The F3 holding period is comparable to the F0, F1, and F2 prebreed period, with the same number of animals/sex/group, and of similar duration, but the F3 animals were not bred.

[d] For the F3 males during their holding period (see footnote c), 50% of the males in each group were necropsied between sd 63-70 and 50% between sd 70-77. Therefore, the intake values are presented for week 10 (sd 63-70), but the intake values for the entire period are presented for sd 0-63 when all males were present.

[e] The values for F0, F1, and F2 females during the postmate holding period were based on very few females per dose group, approximately one to five. These values are presented for completeness.

PROTOCOL DEVIATIONS

A. Temperature and Relative Humidity Excursions:

The protocol mandated temperature range was 64-79°F and relative humidity was 30-70% for the animal rooms (NRC, 1996). Excursions outside these ranges for the RTI Animal Research Facility animal rooms used in this study were as follows:

F0 Animals

1. Room 200: F0 females (October 6 - December 23, 1998)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			11/08/98	1 hour	29.8%
			11/30/98	2 hours	up to 73.8%

2. Room 201: F0 males (September 28, 1998 - February 3, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
	No Excursions		11/08/98	1 hour	28.6%
			11/30/98	2 hours	up to 78.4%
			12/12/98	1 hour	77.9%

3. Room 202: F0 females (October 5 - October 13, 1998)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

4. Room 203: F0 males (October 5 - October 13, 1998)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

5. Room 301: F0/F1 Cohort 2 (December 23, 1998 - February 16, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			01/05/99	1 hour	28.7%

6. Room 303: F0/F1 Cohort 1 (December 15, 1998 - February 2, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

F1 Animals

1. Room 200: F1 females (January 27 - April 28, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

2. Room 202: F1 males (January 27 - June 10, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

3. Room 301: F1/F2 Cohort 1 (April 13 - June 6, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

4. Room 303: F1/F2 Cohort 2 (April 28 - June 23, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

F2 Animals

1. Room 200: F2 females (May 26 - September 1, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			07/30/99	1 hour	70.1%

2. Room 201: F2 males (May 26 - October 14, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			07/30/99	1 hour	76.4%

3. Room 301: F2/F3 Cohort 1 (August 17 - October 13, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

4. Room 303: F2/F3 Cohort 2 (September 1 - October 20, 1999)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			10/01/99	1 hour	73.0%

F3 Animals

1. Room 200: F3 females (September 29, 1999 - January 20, 2000)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

2. Room 202: F3 males (September 29, 1999 - January 6, 2000)

Temperature			Relative Humidity		
Date	Duration	°F	Date	Duration	% RH
No Excursions			No Excursions		

The NRC *Guide for the Care and Use of Laboratory Animals* (Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council, revised 1996) recommends a temperature range of 64–79°F and relative humidity range of 30-70% for rats. There were no temperature excursions outside the protocol-mandated range in any animal room at any time. There were eight excursions for relative humidity, six for one hour, and two for up to two hours in duration. These relative humidity excursions did exceed the NRC recommended range, but they were few, brief, and minor. These few, brief, and minor excursions in relative humidity did not affect the design, conduct, or conclusions of this study in the Study Director's professional judgment.

B. Dosed Feed Formulations Outside the Protocol-Mandated Range

For the first dosed feed formulation date in this study (September 28-29, 1998), all of the analytical concentrations were within ±15% of the target concentrations except 4.5 ppm (83.8% of target) and 75 ppm (82.8% of target). Because there was not enough time to remix and reanalyze these formulations prior to use, they were fed to the animals from October 6, 1998 (Cohort 1) to October 9, 1998, until the new dosed feeds could be formulated and analyzed. As indicated in Table 1, these new dosed feeds were within ±15% of the target concentrations and were presented to the animals on October 9, 1998 (Cohort 1). Cohort 2 animals began exposure to dosed diets on October 14, 1998, with all dosed feed groups within mandated range. In the Study Director's professional opinion, this brief exposure of Cohort 1 test animals in Groups 4 (4.5 ppm) and 5 (75 ppm), to diets slightly lower than within ±15% of target concentrations for approximately the first four days of the F0 prebreed, had no effect on the design, conduct, or conclusions of the study.

Rochelle W. Tyl, Ph.D., DABT
Study Director

Date

GOOD LABORATORY PRACTICES COMPLIANCE STATEMENT

This study was performed in compliance with the Good Laboratory Practices (GLP) Standards promulgated by the U.S. Environmental Protection Agency Toxic Substances Control Act Good Laboratory Practice Standards, Final Rule, 40CFR Part 792 (*Federal Register* **54** [158], 34034-34050, August 17, 1989).

Prepared by:

Rochelle W. Tyl, Ph.D., DABT
Study Director/Research Director
Center for Life Sciences and Toxicology
Research Triangle Institute

10/5/00
Date

RTI

Quality Assurance Statement

Study Title: Three-Generation Reproductive Toxicity Evaluation of Bisphenol A Administered in the Feed to CD®(Sprague-Dawley) Rats

Sponsor: The Society of the Plastics Industry, Inc.

Study Code: Rt98-BPA

Protocol Number: RTI-637

This study was audited by the Chemistry and Life Sciences Quality Assurance Unit and the results of the inspections and audits were reported to the study director and management as identified below. To the best of our knowledge, the reported results accurately describe the study methods and procedures used, and the reported results accurately reflect the raw data.

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Protocol Review	12/22/97	12/22/97
Dose Formulation	09/28/98	09/28/98
Quarantine	10/09/98	10/09/98
Dosing	10/13/98	10/13/98
Dosing	10/14/98	10/16/98
Dose Analysis	10/20/98	10/20/98
Dose Analysis	10/26/98	10/26/98
Dose Analysis	12/08/98	12/10/98
Dose Analysis	12/08/98	12/10/98
Mating	12/15/98	12/15/98
Vaginal Cytology	12/15/98	12/15/98
Lactation	01/22/99	01/22/99
Necropsy	01/25/99	01/25/99
Necropsy	02/08/99	02/08/99

continued

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Vaginal Patency	02/9/99	02/09/99
Preputial Separation	02/15/99	02/15/99
Weaning	02/16/99	02/16/99
Dose Formulation	03/23/99	03/23/99
Vaginal Cytology, Clinical Observations	03/26/99	03/26/99
Gestation/Mating	04/22/99	04/23/99
Dose Formulation	05/18/99	05/18/99
Dose Analysis	05/19/99	05/21/99
Necropsy	05/24/99	05/25/99
Lactation/Weaning and Vaginal Patency	06/1/99	06/2/99
Prebreed Exposure, Preputial Separation and Clinical Signs	07/07/99	07/07/99
Dose Formulation	07/13/99	07/13/99
Vaginal Cytology	08/12/99	08/12/99
Mating/Sperm Check	09/02/99	09/02/99
Mating	09/20/99	09/21/99
Necropsy	09/27/99	09/27/99
Dose Formulation	10/06/99	10/07/99
Necropsy	10/14/99	10/15/99
Lactation/Weaning and Vaginal Patency	10/18/99	10/18/99

continued

Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Preputial Separation	11/08/99	11/08/99
Vaginal Smear	12/15/99	12/15/99
Necropsy	12/28/99	12/28/99
Dose Analysis Audit	2/26; 3/1; 5/11/99	05/11/99
Dose Analysis Audit	3/1; 5/11/99	05/11/99
Dose Analysis Audit	3/8-10; 5/11/99	05/11/99
Dose Analysis Audit	3/10; 5/11/99	05/11/99
Dose Analysis Audit	3/3-5; 5/12/99	05/12/99
Dose Analysis Audit	3/3-5; 5/12/99	05/12/99
Dose Analysis Audit	3/11-12; 5/13/99	05/13/99
Dose Analysis Audit	5/14,17,19; 7/13/99	07/13/99
Data Audit	7/2, 8, 9,15-16,19-20, 22-23, 27-28/99	07/28/99
Dose Analysis Audit	7/19-22; 8/27/99	08/27/99
Data Audit	8/02; 9/1-3,6-10,13,15/99	09/15/99
Dose Analysis Audit	11/10,12,22/99	11/22/99
Dose Analysis Audit	11/17-18/99; 1/20/00	01/21/00
Dose Analysis Audit	11/18-19, 22/99	02/09/00
Dose Analysis Audit	11/15-17/99; 1/21/00	01/21/00
Dose Analysis Audit	12/20/99; 2/2/00	02/09/00
Dose Analysis Audit	12/10/99	02/09/00
Dose Analysis Audit	2/29; 3/1-2/00	03/02/00

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Inspections and Audits	Inspection and Audit Date(s)	Date Inspection/Audit Report Sent to Study Director and Management
Dose Analysis Audit	12/3,6-9; 2/25,28/00	03/02/00
Dose Analysis Audit	12/16-17/99; 3/1-2/00	03/06/00
Dose Analysis Audit	12/17,20/99; 3/6/00	03/06/00
Data Audit	3/9-10,13-14,16-17,20-24,27-31; 4/3-6/00	04/06/00
Data Audit	4/7,10-14,17/00	04/17/00
Data Audit	4/20,24-28/00	04/28/00
Data Audit	5/1-5/00	05/05/00
Data Audit	5/5,9-12,16-18/00	05/18/00
Data Audit	6/1-23/00	06/23/00
Data Audit	6/23-29/00	06/29/00
Data Audit	7/1-14/00	07/18/00
Dose Analysis/Dose Formulation Data and Report Audit	6/22-23,26-30; 7/10-11, 19-21,24-26/00	07/26/00
Report and Data Audit	7/19,21,24-28,31; 8/2-5,7-11, 14-17/00	08/17/00
Report and Data Audit	8/28-30; 9/11,15,18,21,25-26/00	09/27/00

Approval:

David L. Brodish
Quality Assurance Manager

10/5/00
Date