

CLARITY-BPA Core Study: A Perinatal and Chronic Extended-Dose-Range Study of Bisphenol A in Rats

Results and Preliminary Conclusions

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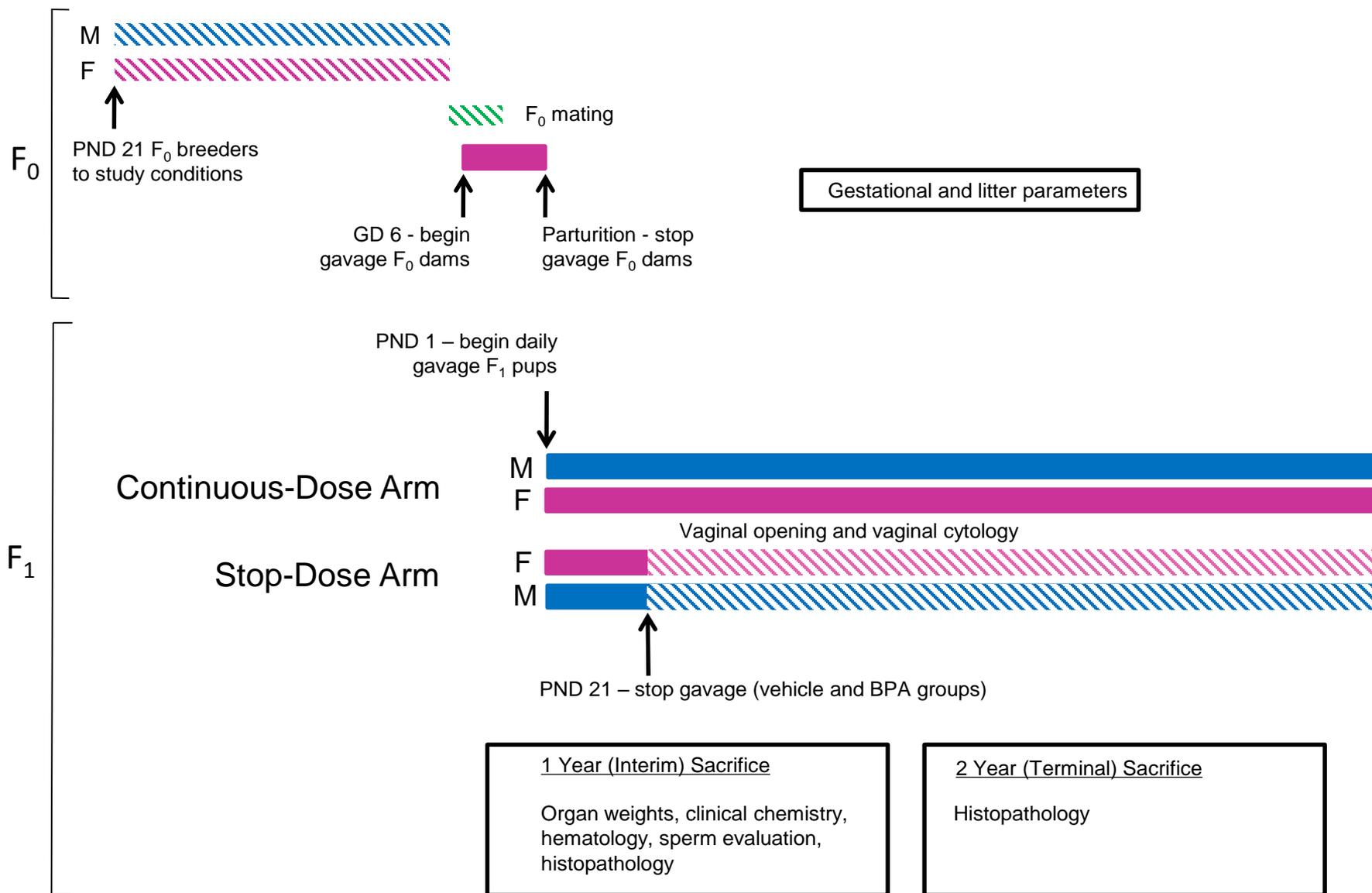
Food and Drug Administration (FDA)

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CLARITY-BPA Core 2-Year Toxicology Study





- Survival, reasons for early removals
- Gestation and litter parameters
- Body weights
- Vaginal opening, vaginal cytology, and time to onset of aberrant cycles
- Organ weight, clinical chemistry, hematology, and sperm parameter summaries
- Histopathology
 - Female neoplasms and nonneoplastic lesions
 - Male neoplasms and nonneoplastic lesions
- Summary



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- Survival was monitored/analyzed for:
 - The preweaning period
 - The period between weaning and the interim and terminal sacrifices
- BPA: No statistically significant effects in any phase of the study
- EE₂: Statistically significant effect in low dose preweaning only
- Moribund removals/early deaths increased in all study groups after 1 year of age



Preweaning and Interim Survival

Percent Survival

- No statistically significant effects on survival except preweaning females, low EE₂

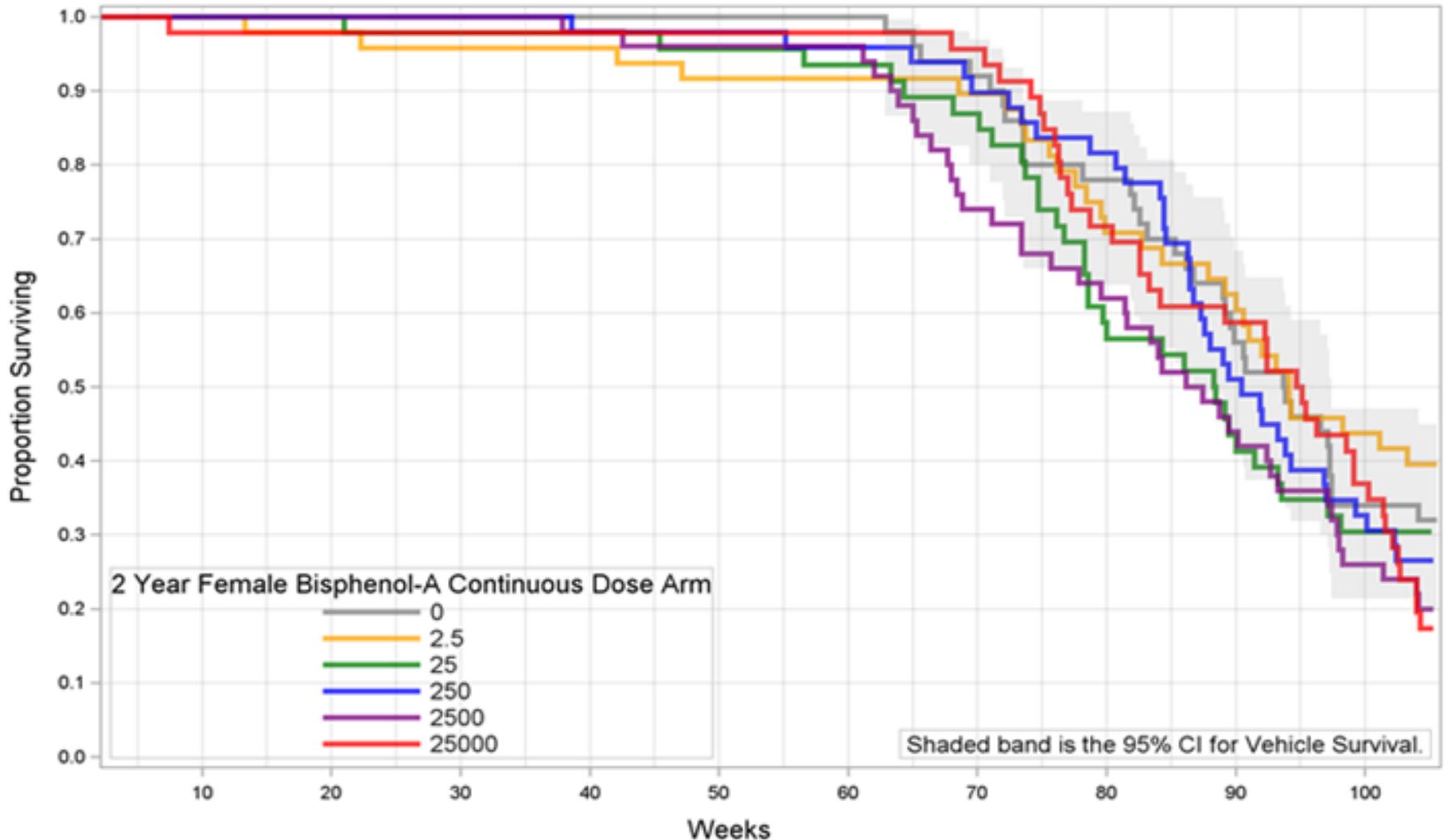
	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Preweaning (PND 1-21)								
Female	95%	91%	92%	91%	91%	92%	85%**	91%
Male	93%	91%	94%	91%	93%	95%	90%	95%
Interim Sacrifice, Continuous-Dose								
Female	91%	100%	95%	92%	100%	100%	92%	100%
Male	82%	100%	90%	100%	90%	95%	85%	88%
Interim Sacrifice, Stop-Dose								
Female	100%	100%	100%	100%	100%	91%		
Male	100%	100%	95%	100%	100%	100%		

** p<0.01



Example: Continuous-Dose Females

- Moribund removals (predominant) and early deaths increased after one year of age





Percent Survival

- No significant differences from controls for either study arm or sex

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Female	32%	40%	30%	27%	20%	17%	27%	15%
Male	30%	33%	35%	28%	32%	24%	35%	46%
Stop-Dose								
Female	22%	24%	27%	26%	34%	28%		
Male	34%	33%	33%	26%	30%	20%		

- The majority of animals removed between one and two years were removed as moribund



Reasons for Early Removals and Deaths

Most common causes of removal/death after 1 year of age

- Females
 - Mammary gland fibroadenoma
 - Pituitary adenoma/carcinoma
 - Mammary gland adenocarcinoma
- Males
 - Pituitary adenoma/carcinoma
 - Nephropathy
 - Preputial gland carcinoma
 - Spleen, malignant lymphoma



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Gestation and Litter Parameters

- No significant treatment effects on:
 - Implantation sites
 - Gestational body weight gain
 - Litter size
 - Litter sex ratio
 - Number of pups born dead
 - Litter weight
 - Preweaning survival in BPA groups (shown previously)



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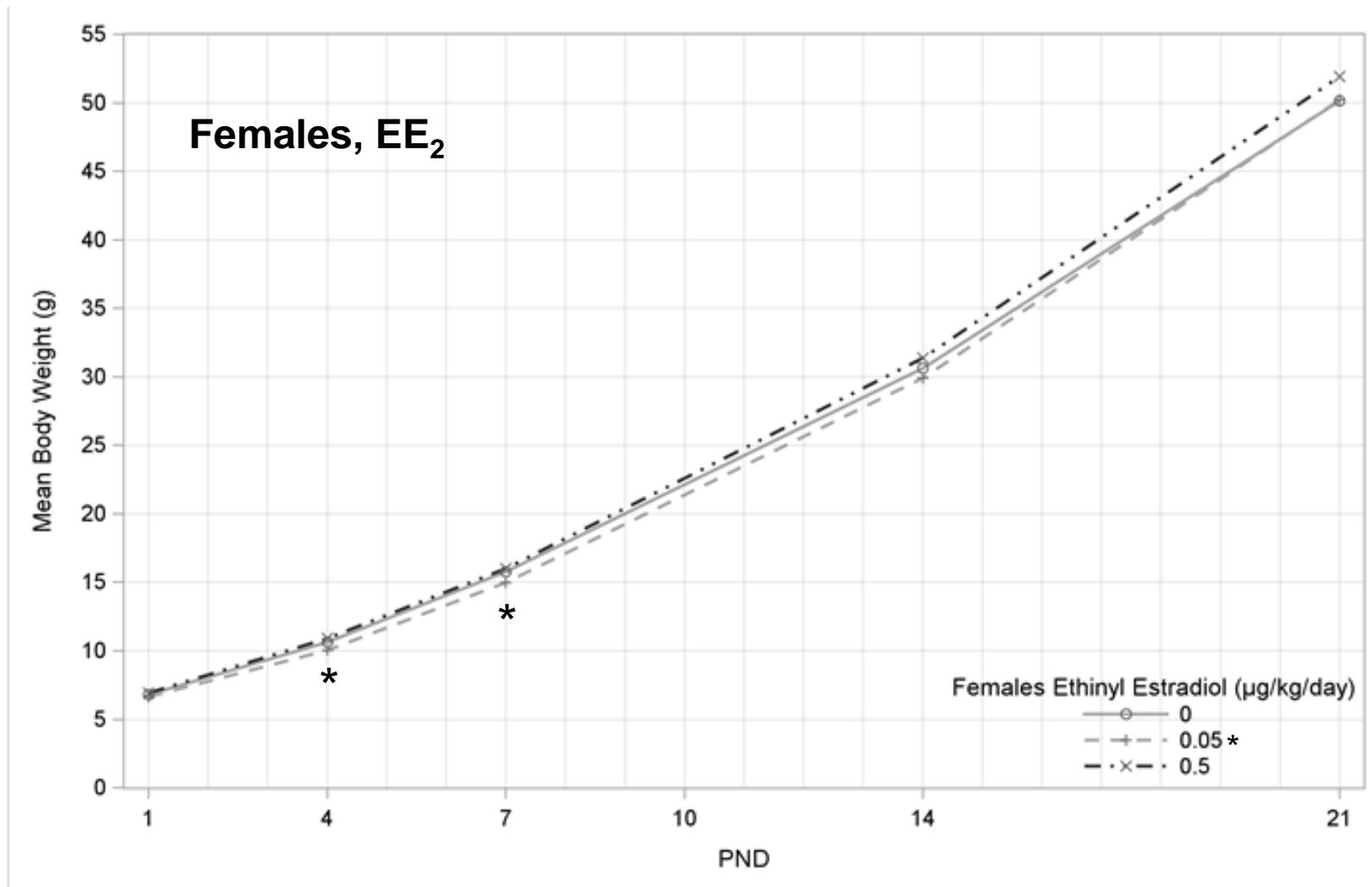


- Body weights were monitored at varied intervals across the study, as discussed in the earlier presentation
- Males: No statistically significant effects of BPA or EE₂
- Females: Statistically significant effects of BPA or EE₂ treatments were few and confined to restricted time periods in intermediate dose groups (shown in following slides)



Prewaning Body Weight

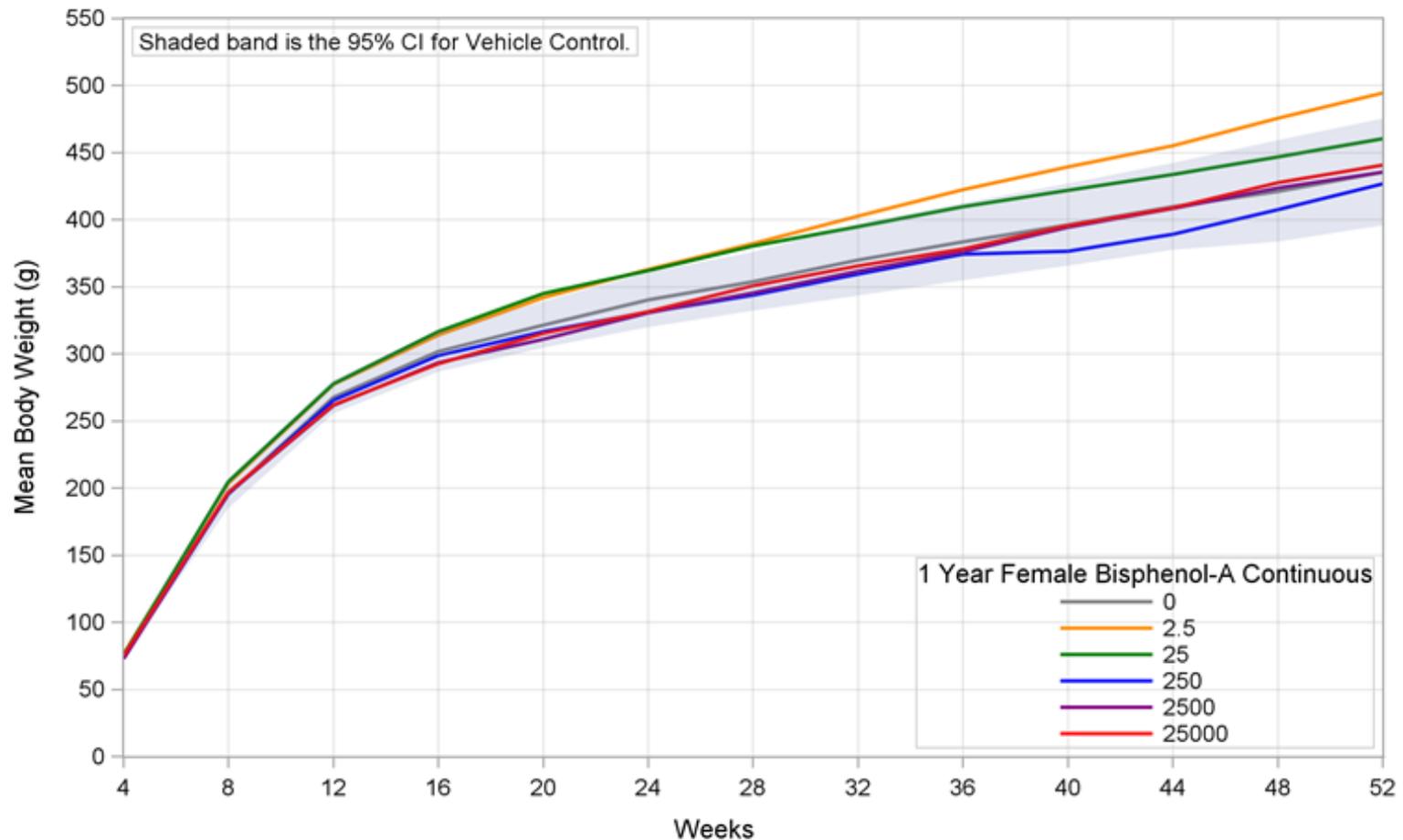
- Statistically significant effects (~5%) only in low dose EE₂ females; no differences from control for BPA groups of either sex or EE₂ males





Postweaning Body Weights – Interim

- No statistically significant BPA or EE₂ effects in either sex or study arm
- For continuous-dose females, body weights were 10 – 13% higher than controls from weeks 36 to 52 in the 2.5 µg BPA/kg bw/day group, although not statistically significant

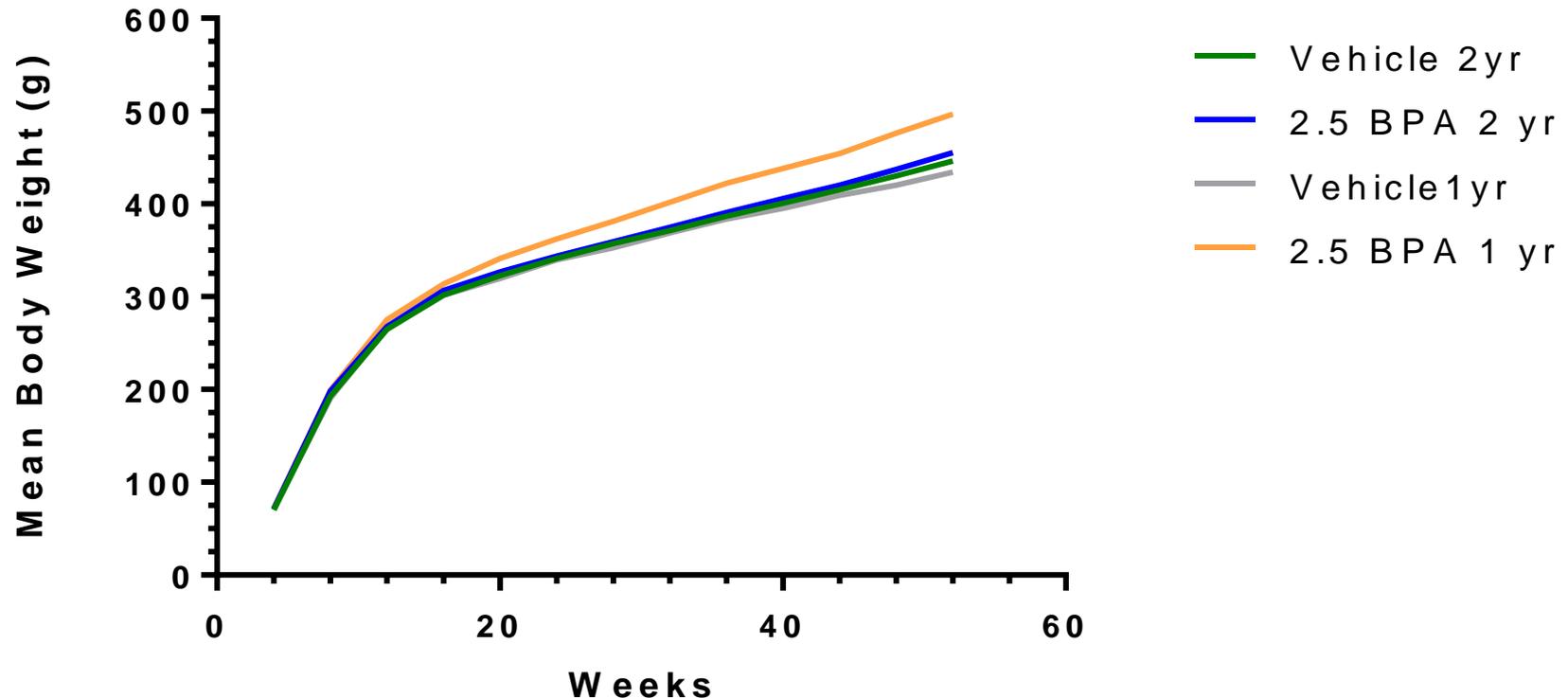




Continuous-Dose Females – Vehicle and 2.5 BPA

Postweaning body weights, interim and terminal to one year

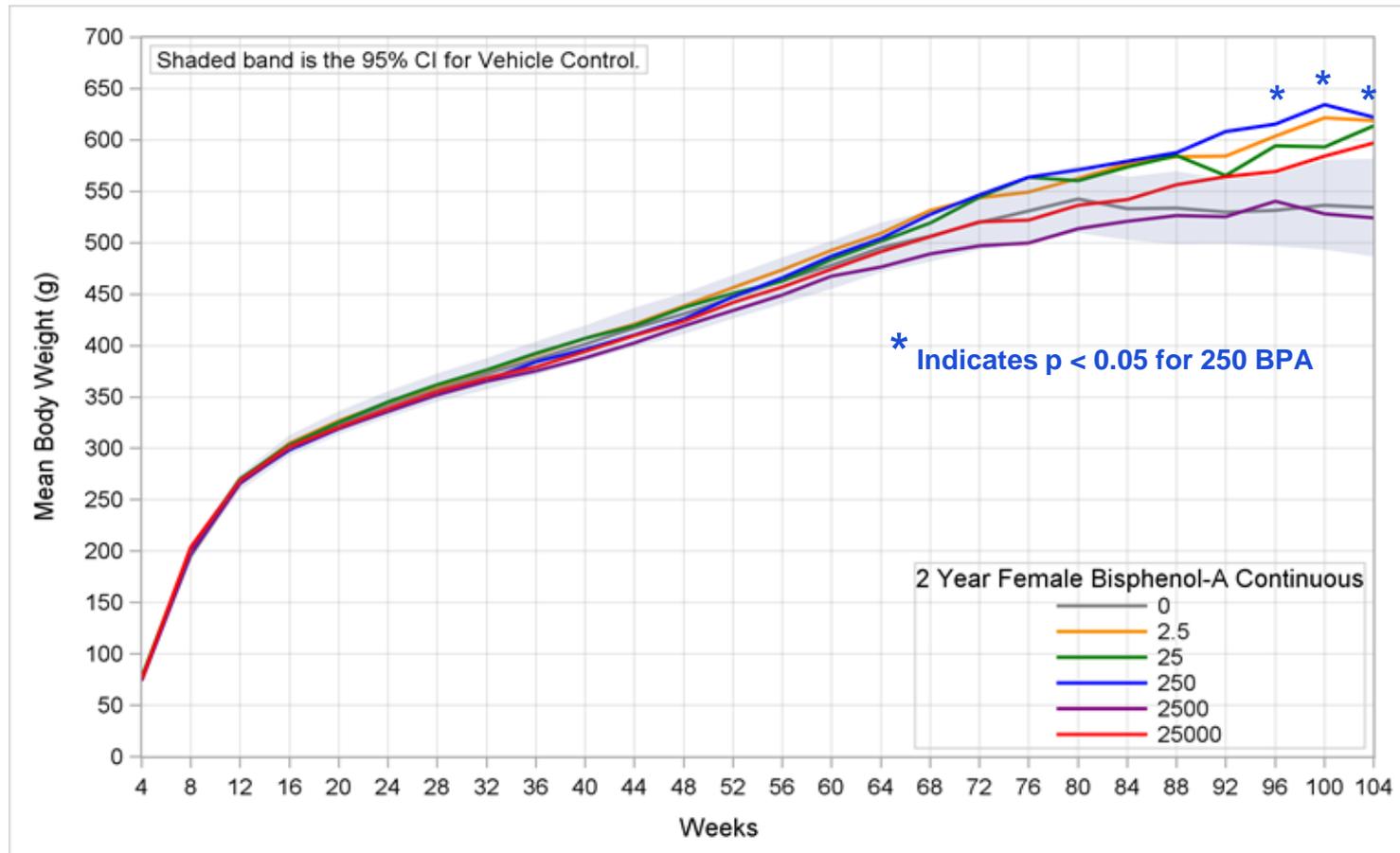
- The non-significant trend for increased body weight over weeks 36 to 52 in the interim 2.5 BPA group was not observed in the terminal 2.5 BPA group





Postweaning Body Weights – Terminal

- Males: No statistically significant BPA or EE₂ effects in either study arm
- Females: No statistically significant BPA or EE₂ effects, except for the 250 µg BPA/kg bw/day continuous-dose group (weeks 96 to 104)





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Vaginal Opening/Cytology Data

- 26 females per dose group monitored for vaginal opening
- At 16 weeks of age, collected 14 consecutive vaginal smears from the animals for which time of vaginal opening had been evaluated
- From these same animals, 5 consecutive days of vaginal smears were subsequently taken monthly
- Animals showing 3 consecutive days of estrus or 5 days without an estrus smear for 2 consecutive months were considered to have started aberrant cycles and were no longer monitored
- Neither BPA nor EE_2 affected the timing of vaginal opening at the doses tested
- As shown in subsequent slides, the high EE_2 treatment had clear effects on the estrous cycle, while adverse effects of BPA treatments were not evident



Age (days \pm SEM) and Body Weight (g \pm SEM)

- No statistically significant differences from vehicle control

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Age	35.9 \pm 1.1	35.2 \pm 0.7	36.5 \pm 0.8	37.8 \pm 1.4	34.1 \pm 0.5	35.4 \pm 2.8	35.5 \pm 2.8	34.8 \pm 2.8
Body Weight	120.5 \pm 4.6	117.1 \pm 5.0	128.6 \pm 4.6	131.1 \pm 5.8	109.6 \pm 2.6	121.0 \pm 4.3	123.0 \pm 4.8	117.1 \pm 11.8
Stop-Dose								
Age	41.1 \pm 1.8	42.1 \pm 2.5	40.0 \pm 1.5	39.6 \pm 1.2	42.4 \pm 1.2	38.0 \pm 1.3		
Body Weight ^a	-	-	-	-	-	-		

^a Body weight at vaginal opening was not available for stop-dose animals due to technical error.



Percent Abnormal Estrous Cycles

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Diestrus	15.4%	8.0%	15.4%	4.0%	15.4%	20.0%	23.1%	7.7%
Estrus	11.5%	12.0%	15.4%	16.0%	7.7%	16.0%	15.4%	96.2%***
Proestrus	0%	0%	0%	4.0%	3.8%	4.0%	0%	0%
Combined	26.9%	20.0%	30.8%	20.0%	26.9%	36.0%	38.5%	100%***
Stop-Dose								
Diestrus	19.2%	19.2%	7.7%	3.8%	15.4%	19.2%		
Estrus	19.2%	7.7%	11.5%	19.2%	7.7%	11.5%		
Proestrus	7.7%	3.8%	3.8%	0%	0%	0%		
Combined	38.5%	26.9%	19.2%	23.1%	23.1%	30.8%		

Abnormal definitions: > 2 days consecutive estrus
 > 4 days consecutive diestrus
 ≥ 2 days consecutive proestrus

*** p<0.001



Onset of Aberrant Estrous Cycling, Weeks

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Week	56.8	47.0	51.9	56.9	52.0	46.9	51.8	21.9
Lower 95% CI	42.0	36.9	42.1	46.9	46.9	41.7	37.0	21.7
Upper 95% CI	66.9	52.0	56.9	61.9	56.7	56.9	62.1	22.0
p-value	-	0.732	0.788	0.788	0.788	0.788	0.356	<0.001
Stop-Dose								
Week	41.9	51.7	46.8	51.9	56.9	52.1		
Lower 95% CI	41.3	36.9	41.9	41.9	51.7	41.9		
Upper 95% CI	51.7	57.0	56.9	56.9	66.6	61.9		
p-value	-	1.000	0.827	1.000	0.027	0.524		

Red font indicates significant differences



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Organ Weights, Clinical Chemistry, Hematology, Sperm

- Organ weight, clinical chemistry, hematology, and sperm parameter data were collected at the interim sacrifice
- High dose EE₂ affected multiple organ weights in females, while BPA had minimal effects
- Male organ weights were not affected by BPA or EE₂ treatments
- BPA and EE₂ had statistically significant effects on several clinical chemistry and hematology endpoints measured, but these differences were not judged to be adverse effects
- No effects of BPA or EE₂ on sperm parameters were observed



Female Organ Weights

Endpoint	Stop-Dose BPA	Continuous-Dose BPA	EE ₂
Adrenal glands	-	-	↑ (0.5)
Fat pad, ovarian/parametrial	-	-	↓ (0.5)
Fat pad, retroperitoneal	-	↑ (2.5)	-
Heart	-	-	↑ (0.5)
Kidney	-	-	↑ (0.5)
Liver	-	↑ (trend)	↑ (0.5)
Ovary	↓ (25,000)	-	↓ (0.5)
Pituitary gland	-	-	↑ (0.5)

- The retroperitoneal fat pad and ovary weights in the BPA-treated groups were not significantly different from control when adjusted for body weight



Male Organ Weights

- There were no significant effects of BPA or EE₂ on the male organs weighed in either study arm
- Prostate lobes were not dissected and weighed so as not to interfere with processing and sectioning for microscopic evaluation



Interim Sacrifice

Stop-Dose BPA	Continuous-Dose BPA	EE ₂
Red Blood Cells, ↑ trend	Hemoglobin (Hb), ↑ trend	Platelets, ↓ 0.5
% Basophils, ↑ trend	Mean Corpuscular Hb Concentration, ↑ 25	Eosinophils, ↓ 0.5
Albumin, ↑ trend	Platelets, ↓ trend, ↓ 25,000	Alkaline Phosphatase, ↑ 0.05
	Monocytes, ↑ trend	TSH, ↑ 0.5
	Eosinophils, ↓ 250	
	Alkaline Phosphatase, ↑ 250	



Interim Sacrifice

Stop-Dose BPA	Continuous-Dose BPA	EE ₂
% Neutrophils, ↓ trend	Hematocrit, ↑ trend	Hb, ↑ 0.05
Total Protein, ↓ 25	Hb, ↑ trend, ↑ 25,000	Triglycerides, ↑ 0.5
Total Bile Acids, ↓ trend, ↓ 25	Packed Cell Volume, ↑ trend	Insulin, ↓ 0.05
T4, ↓ trend	Mean Corpuscular Volume, ↑ trend	
	Mean Corpuscular Hb, ↑ trend	
	Platelets, ↓ trend	
	% Eosinophils, ↓ 250	
	Total Bile Acids, ↓ trend	
	Troponin T, ↑ trend	
	T4, ↑ trend	



Interim Sacrifice

- No significant BPA or EE₂ effects in either study arm
 - Testicular spermatid head counts
 - Cauda sperm
 - Counts
 - Motility
 - Morphology



Clarification Questions?



- Survival, reasons for early removals
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Narrative Report

- Pathology report, based on diagnoses of the Study Pathologist, which were reviewed and agreed to by the Pathology Working Group, indicated that many of the lesions identified were common lesions of aging in Sprague-Dawley rats with variable incidences across dose groups
- The lesions presented in the report and the following slides were selected based on the results obtained in subsequent statistical analyses



Statistical Analyses

- As discussed in the earlier presentation, CAFE and Poly-3 tests were considered the primary statistical tests
 - Consider incidence only for both neoplastic and nonneoplastic lesions, trend tests for increasing effect (positive and negative) with increasing dose, consistent with general NTP practice
 - Secondary tests that take into account severity scores (JT/SW and RTE) were also applied for nonneoplastic lesions; the RTE test was included because it does not presume a monotonic response; no survival corrections for these tests
 - Results of the primary tests are reported in subsequent slides, all results of secondary tests are found in the report and some are noted in the slides
- All tests designed to minimize false negatives (one-sided, no correction for multiple comparisons, $p < 0.05$ significance level), at the risk of increasing false positives
- Statistical significance \neq biological significance



Concurrent and Past NCTR Control Incidences

- Concurrent controls are primary
 - Continuous- and stop-dose vehicle control animals were treated differently and were not statistically compared
 - Variation between continuous- and stop-dose vehicles was considered in evaluating incidence range in unexposed animals
- In the Draft Report and slides where historical control data are noted, the results referenced are from NTP Technical Reports 545 (genistein) and 548 (ethinyl estradiol)
 - 2-Year dietary exposure studies conducted at NCTR in Sprague-Dawley rats using the same diet
 - Both studies had 2 unexposed control groups (F_1 and F_3 generations were followed to 2 years in these studies; no 1 year data)
 - These data are one aspect used in considering variability and biological significance



Mammary Gland, Fibroadenomas

- Mammary gland fibroadenomas are a high incidence background lesion in this rat strain
 - Major cause of early removals
 - Diagnosed in both interim and terminal sacrifice animals, with no treatment effect other than a slight decreasing trend in terminal sacrifice stop-dose BPA animals



Female Mammary Gland Neoplasms

Interim Sacrifice – Fibroadenomas

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	2/23 (9%)	3/22 (13%)	3/22 (13%)	1/24 (4%)	2/20 (10%)	6/24 (25%)	2/26 (8%)	4/26 (15%)
p-value	0.150 ^a	0.478	0.478	0.484N ^b	0.641	0.136	0.647N	0.395
Stop-Dose								
Incidence	4/20 (20%)	1/22 (4%)	1/20 (5%)	1/22 (4%)	1/20 (5%)	2/22 (9%)		
p-value	0.191N	0.144N	0.171N	0.144N	0.171N	0.286N		

^a In all histopathology tables, the p-value in the vehicle column is the result of the trend test over all BPA dose groups.

^b In all histopathology tables, an “N” indicates reduced incidence relative to vehicle control.



Female Mammary Gland Neoplasms

Terminal Sacrifice – Fibroadenomas

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	41/50 (82%)	40/48 (83%)	33/46 (72%)	39/49 (80%)	35/50 (70%)	38/46 (83%)	18/26 (82%)	14/26 (54%)
p-value	0.410N	0.366	0.567N	0.565N	0.366N	0.457	0.354N	0.056N
Stop-Dose								
Incidence	43/50 (86%)	45/50 (90%)	37/48 (77%)	42/49 (86%)	36/50 (72%)	34/46 (74%)		
p-value	0.021N*	0.319	0.489N	0.600	0.099N	0.257N		

NCTR historical control incidence: 132/210 (63%); range 59 – 69% in 4 control groups

* $p < 0.05$; N, negative trend



Female Mammary and Pituitary Glands

- Adenoma/adenocarcinomas were diagnosed in interim and terminal animals, with statistically indicated significance only in the terminal sacrifice animals
 - Low dose BPA (2.5 $\mu\text{g}/\text{kg}$ bw/day), stop-dose arm
 - High dose EE₂ (0.5 $\mu\text{g}/\text{kg}$ bw/day)
- Nonneoplastic lesions (ductal and alveolar dilatation, lobular hyperplasia), not related to neoplasms, were observed in high dose EE₂ animals in both interim and terminal sacrifices (not shown)
- A secondary statistical test (RTE) indicated an increase in atypical foci in continuous-dose, but not stop-dose, low dose BPA animals
- EE₂, but not BPA, increased pituitary adenoma/carcinoma in the pars distalis
 - In the rat, estrogen induced-increases in prolactin are correlated with mammary gland neoplasms



Female Mammary Gland Neoplasms

Interim Sacrifice – Adenoma/Adenocarcinoma

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	0/23 (0%)	1/22 (4%)	1/22 (4%)	0/24 (0%)	0/20 (0%)	0/24 (0%)	2/26 (8%)	0/26 (0%)
p-value	-	-	-	-	-	-	0.276	-
Stop-Dose								
Incidence	0/20 (0%)	0/22 (0%)	0/20 (0%)	0/22 (0%)	0/20 (0%)	0/22 (0%)		
p-value	-	-	-	-	-	-		



Female Mammary Gland Neoplasms

Terminal Sacrifice – Adenoma/Adenocarcinoma

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	6/50 (12%)	7/48 (15%)	8/46 (17%)	6/49 (12%)	10/50 (20%)	4/46 (9%)	2/26 (8%)	10/26 (38%)
p-value	0.513N	0.452	0.271	0.624	0.126	0.428N	0.667	<0.001
Stop-Dose								
Incidence	4/50 (8%)	12/50 (24%)	5/48 (10%)	9/49 (18%)	9/50 (18%)	6/46 (13%)		
p-value	0.483	0.018 ^a	0.482	0.140	0.149	0.360		

^a Also significant for adenocarcinoma alone (p = 0.016).

Red text indicates significant differences

NCTR historical control incidences: adenoma, 2/210 (1%); adenocarcinoma, 31/210 (14.8%); combined, 32/210 (15.2%); range for combined 12 – 17% in 4 control groups



Female Mammary Gland – Nonneoplastic

Interim and Terminal – Atypical Focus

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Interim, Continuous-Dose								
Incidence	0%	14%^ [^]	9%	8%	0%	0%	0%	0%
Severity	-	1.7	2.0	1.0	-	-	-	-
Interim, Stop-Dose								
Incidence	5%	0%	0%	0%	0%	0%		
Severity	1.0	-	-	-	-	-		
Terminal, Continuous-Dose								
Incidence	4%	15%^ [^]	2%	10%	6%	7%	8%	12%
Severity	2.0	2.0	2.0	2.0	1.7	1.7	1.5	1.3
Terminal, Stop-Dose								
Incidence	12%	4%	13%	16%	14%	11%		
Severity	2.0	2.5	1.8	1.8	2.6	1.6		

[^] Indicates significance by RTE; not significant by CAFE (interim), Poly-3 (terminal)

NCTR historical control incidence: 51/210 (24%); range 11 – 37% in 4 control groups



Female Pituitary (pars distalis) Neoplasms

Terminal Sacrifice – Adenoma/Carcinoma

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	22/50 (44%)	23/48 (48%)	12/46 (26%)	20/49 (41%)	19/49 (39%)	21/46 (46%)	10/26 (38%)	20/26 (77%)
p-value	0.408	0.361	0.115N	0.595	0.559	0.425	0.615N	0.011*
Stop-Dose								
Incidence	23/49 (47%)	16/50 (32%)	14/48 (29%)	21/50 (42%)	20/50 (40%)	21/46 (46%)		
p-value	0.373	0.112N	0.081N	0.340N	0.308N	0.574N		

This analysis was unintentionally omitted in the NTP Research Report.

*** p<0.05**

NCTR historical controls: pituitary, pars distalis, adenoma/carcinoma, 149/210 (71%); range 62 – 77% in 4 control groups



Factors for Questioning the Biological Significance of the Low Dose BPA Statistical Result

- Occurred only in a single dose group
- Not dose-responsive
- Occurred only in the stop-dose arm
- Incidences variable across all dose groups in continuous- and stop-dose arms
- Statistically significant incidence near high end of control incidences observed in previous NCTR studies; control incidences lower than observed in previous NCTR studies
- Reference high dose EE₂ shows a pattern consistent with estrogen-induced mammary neoplasms in Sprague-Dawley rats
 - Both mammary adenoma/adenocarcinoma and pituitary pars distalis neoplasms in terminal animals



Neoplasms and Nonneoplastic Lesions

- Uterine stromal polyps were the only other neoplasm in females in which there were statistically significant effects
 - A significant trend was indicated in interim sacrifice continuous-dose animals
 - A significant negative trend and reduction in the high BPA dose group was indicated in terminal sacrifice stop-dose animals
- A significant increase in apoptosis of the luminal epithelium was noted in the interim sacrifice females in the continuous high BPA and the high dose EE₂ groups



Interim Sacrifice – Stromal Polyps

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	1/23 (4%)	0/22 (0%)	1/21 (5%)	0/24 (0%)	3/20 (15%)	3/24 (12%)	1/25 (4%)	0/26 (0%)
p-value	0.037	0.511N	0.733	0.489N	0.252	0.321	-	-
Stop-Dose								
Incidence	0/20 (0%)	1/22 (5%)	0/20 (0%)	1/22 (5%)	0/20 (0%)	0/22 (0%)		
p-value	-	-	-	-	-	-		

p-value in vehicle column indicates statistically significant trend



Terminal Sacrifice – Stromal Polyps

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	5/50 (10%)	3/48 (6%)	7/45 (16%)	2/49 (4%)	4/48 (8%)	3/46 (7%)	3/26 (12%)	1/26 (4%)
p-value	0.333N	0.383N	0.231	0.247N	0.638N	0.424N	0.529	0.389N
Stop-Dose								
Incidence	7/49 (14%)	4/49 (8%)	5/48 (10%)	6/49 (12%)	4/49 (8%)	1/46 (2%)		
p-value	0.041N*	0.263N	0.384N	0.433N	0.207N	0.032N*		

p<0.05, N indicates lower incidence relative to control

Historical controls, 12/206 (6%); range 2 – 9%



Interim Sacrifice

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Apoptosis, Endometrium, Luminal Epithelium								
Continuous-Dose								
Incidence	2/23** (9%)	1/22 (4%)	4/21 (19%)	5/24 (21%)	5/20 (25%)	9/24* (38%)	6/25 (24%)	18/26*** (69%)
Mean Severity	4.0	4.0	3.2	3.6	3.4	3.2	3.3	3.4
Stop-Dose								
Incidence	2/20 (10%)	3/22 (14%)	2/20 (10%)	2/22 (9%)	1/20 (5%)	6/22 (27%)		
Mean Severity	4.0	3.3	3.5	3.0	4.0	3.7		

*****, p <0.05; ******, p<0.01; *******, p<0.001



Ovary and Vagina

- Treatment effects were indicated in the ovary in interim sacrifice animals and the vagina of both interim and terminal sacrifice animals
 - The incidence of cystic follicles was increased in stop-dose interim high dose BPA females and a dose trend was evident
 - High dose EE₂ (continuous-dose) also increased the incidence of cystic follicles
 - Epithelial hyperplasia in the vagina was increased in continuous-dose interim high dose BPA females and a dose trend was evident; the incidence was also increased in interim high dose EE₂ females
 - Epithelial hyperplasia in the vagina was increased in multiple BPA dose groups in continuous-dose terminal females



Interim Sacrifice

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Ovary, cyst, follicle								
Continuous-Dose								
Incidence	8/23 (35%)	4/22 (18%)	10/22 (46%)	5/24 (21%)	10/20 (50%)	11/24 (46%)	9/25 (36%)	26/26*** (100%)
Stop-Dose								
Incidence	5/20*** (25%)	6/22 (27%)	4/20 (20%)	7/22 (32%)	11/20 (55%)	18/22*** (82%)		

No severity scores were assigned for this lesion.

*** p<0.001



Interim and Terminal, Epithelial Hyperplasia

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Interim, Continuous-Dose								
Incidence	13%**	9%	10%	17%	30%	33%#	28%	77%***
Severity	2.7	2.5	2.5	3.0	2.8	2.9	2.4	2.6
Interim, Stop-Dose								
Incidence	10%	18%	10%	4%	10%	27%		
Severity	3.0	3.0	3.0	4.0	2.5	2.8		
Terminal, Continuous-Dose								
Incidence	8%**	10%	27%*	20%#	22%*	26%*	19%	8%
Severity	3.0	2.8	2.8	2.3	3.0	2.7	3.2	2.5
Terminal, Stop-Dose								
Incidence	12%	20%	6%	14%	14%	15%		
Severity	2.7	2.6	3.0	2.9	3.0	3.3		

***, p<0.05; **, p<0.01; ***, p<0.001, by Poly-3 or CAFE #, p<0.05, by RTE test**



Females – Summary of Statistically Significant Effects

Summary chart that follows includes all affected endpoints

- The high dose of EE₂ induced multiple effects, particularly in female reproductive organs, including increases in mammary gland and pituitary neoplasms
- Statistically significant effects of BPA were observed in multiple tissues in various dose groups, including the lowest dose, but a coherent pattern of effects was difficult to discern



Females – Summary of Statistically Significant Effects

Endpoint Grouping	Specific Endpoint	Stop BPA					Continuous BPA					EE ₂		
		2.5	25	250	2,500	25,000	2.5	25	250	2,500	25,000	0.05	0.5	
Survival and Body Weight	Preweaning pup survival, female													
	Preweaning pup body weight, female													
	Postweaning body weight, female, 2 years													
Vaginal Cytology	Abnormal estrous cycles at 16 weeks of age													
	Early onset of aberrant estrous cycles													
Organ Weights	Adrenal gland weight													
	Fat pad, ovarian/parametrial weight													
	Heart weight													
	Kidney weight													
	Liver weight													
	Ovary weight													
	Pituitary gland weight													
Clinical Chemistry/Hematology	Mean corpuscular hemoglobin concentration													
	Platelets													
	Eosinophils													
	% Eosinophils													
	Alkaline phosphatase													
	Thyroid-stimulating hormone (TSH)													
Mammary Gland, Histopathology	Mammary gland, dilatation, duct (1y)													
	Mammary gland, hyperplasia, lobular (1y)													
	Mammary gland, dilatation, duct (2y)													
	Mammary gland, dilatation, alveolus (2y)													
	Mammary gland, adenocarcinoma (2y)													
Uterus, Histopathology	Uterus, apoptosis (1y)													
	Uterus, hyperplasia, cystic, endometrium (1y)													
	Uterus, metaplasia, squamous (1y)													
	Uterus, hyperplasia, endometrium (2y)													
	Uterus, metaplasia, squamous (2y)													
Ovary, Histopathology	Uterus, atrophy (2y)													
	Ovary, atrophy (1y)													
	Ovary, cyst, follicle (1y)													
	Ovary, depletion, corpora lutea (1y)													
Vagina, Histopathology	Ovary, hypertrophy, interstitial cell (1y)													
	Vagina, hyperplasia, epithelium (1y)													
	Vagina, hyperplasia, epithelium (2y)													
Pituitary, Histopathology	Vagina, degeneration, epithelium (2y)													
	Pituitary, angiectasis (2y)													
	Pituitary, hemorrhage (2y)													
Kidney, Histopathology	Pituitary, adenoma/carcinoma													
	Kidney, mineralization (1y)													
	Kidney, cyst, renal tubule (1y)													
	Kidney, nephropathy (1y)													
	Kidney, nephropathy (2y)													
	Kidney, cyst, renal tubule (2y)													
Liver, Histopathology	Kidney, cyst, cortex (2y)													
	Liver, infiltration, mononuclear cells (1y)													
Thyroid Histopathology	Liver, vacuolization, cytoplasmic (2y)													
	Thyroid, hyperplasia, follicular cells (2y)													
Spleen, Adrenal, Heart, Brain Stem Histopathology	Thyroid, ultimobranchial cyst (2y)													
	Spleen pigmentation (2y)													
	Adrenal cortex, degeneration, cystic (2y)													
	Heart, cardiomyopathy (1y)													
	Brain stem, compression (2y)													
	Brain stem, hemorrhage (2y)													



p < 0.05



p < 0.01



p < 0.001

For histopathology endpoints, results of CAFE and Poly-3 tests are summarized



Additional results using secondary tests

- In most cases of nonneoplastic lesions with severity scores that were statistically significant by the CAFE or Poly-3 tests, the secondary tests (JT/SW and RTE) also showed significance
- In some cases, additional statistically significant results were indicated by the secondary tests with a distribution across dose groups and study arms that was not clearly interpretable
- In a few cases, multiple contiguous dose groups were indicated as significant, pituitary hyperplasia (pars distalis) and cardiomyopathy are shown as examples
 - Relatively high background incidences and minimal differences from control are common features



Female Pituitary – Nonneoplastic

Interim and Terminal – Hyperplasia (pars distalis)

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Interim, Continuous-Dose								
Incidence	78%	77%	82%	62%	80%	83%#	80%	96%##
Severity	1.8	1.9	2.1	1.7	1.7	2.0	1.6	2.4
Interim, Stop-Dose								
Incidence	90%	73%	70%	91%	80%	82%		
Severity	1.4	1.5	1.9	1.6	1.8	1.6		
Terminal, Continuous-Dose								
Incidence	54%	46%	70%	53%	59%	50%	62%	23%**N
Severity	3.3	3.5	3.4	3.2	2.9	3.1	3.3	3.3
Terminal, Stop-Dose								
Incidence	51%	64%#	71%#	52%	56%	46%		
Severity	2.8	3.3	3.1	3.2	3.0	3.2		

#, p<0.05; ##, p<0.01 by RTE test.

** , p<0.01 by Poly-3 test, negative direction



Female Heart – Nonneoplastic

Interim and Terminal – Cardiomyopathy

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Interim, Continuous-Dose								
Incidence	30%	46%	41%	33%	45%	29%	31%	65%
Severity	1.1	1.2	1.2	1.0	1.1	1.1	1.0	1.2
Interim, Stop-Dose								
Incidence	30%	36%	35%	32%	30%	32%		
Severity	1.0	1.0	1.1	1.3	1.0	1.3		
Terminal, Continuous-Dose								
Incidence	70%	62%	52%#N	71%	66%	72%	73%	85%#
Severity	1.3	1.6	1.3	1.3	1.4	1.4	1.3	1.5
Terminal, Stop-Dose								
Incidence	64%##	74%#	79%	74%#	70%#	76%##		
Severity	1.3	1.5	1.3	1.5	1.7	1.7		

#, p<0.05; ##, p<0.01 by RTE test

#, p<0.05 by RTE test; N, negative direction



Clarification Questions?



- Survival, reasons for early removals
- Gestation and litter parameters
- Body weights
- Vaginal opening, vaginal cytology, and time to onset of aberrant cycles
- Organ weight, clinical chemistry, hematology, and sperm parameter summaries
- **Histopathology**
 - Female neoplasms and nonneoplastic lesions
 - Male neoplasms and nonneoplastic lesions
- Conclusions



Interim and Terminal

- There were no statistically significant increases in any organ-specific neoplasm in interim or terminal sacrifice males in the continuous- or stop-dose arms for either BPA or EE₂
- There was a significant increasing trend ($p < 0.01$) in stop-dose BPA males for systemic malignant lymphoma, which was diagnosed in the liver, dorsal/lateral prostate, bone marrow, spleen, and kidney
 - Vehicle control incidence varied from 0 – 2% and the highest incidences, seen in the 25,000 μg BPA/kg bw/day dose group, ranged from 9 – 11%
- Two neoplasms, an adenoma in a 25,000 $\mu\text{g}/\text{kg}$ BPA continuous-dose animal and an adenocarcinoma in a 25 $\mu\text{g}/\text{kg}$ BPA stop-dose animal, were noted in the dorsal/lateral prostate lobes
- Adenomas in the ventral prostate were diagnosed across all groups, with no statistically significant differences from controls



Male Nonneoplastic Lesions

- Statistically significant differences in males were observed in the epididymis of interim sacrifice animals and in the pituitary, dorsal/lateral prostate, and mammary gland of terminal sacrifice animals
- In the ventral prostate, suppurative inflammation and fibrosis were decreased relative to controls (data not shown)
 - Such statistically significant decreases relative to control occurred in many endpoints across the study



Interim Sacrifice

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Exfoliated germ cells, Continuous-Dose								
Incidence	4%*	4%	5%	4%	0%	27%*	15%	8%
Severity	1.0	1.0	2.0	1.0	-	1.0	1.2	1.0
Exfoliated germ cells, Stop-Dose								
Incidence	0%	15%#	5%	10%	5%	4%		
Severity	-	1.7	2.0	1.0	2.0	1.0		
Lymphocyte infiltration, Continuous-Dose								
Incidence	0%*	4%	15%	8%	0%	23%*	4%	12%#
Severity	-	2.0	1.0	1.0	-	1.0	1.0	1.0
Lymphocyte infiltration, Stop-Dose								
Incidence	5%	5%	20%	5%	10%	9%		
Severity	1.0	1.0	1.0	1.0	1.0	1.0		

*****, p<0.05

#, p<0.05 by RTE test, but not CAFE

There were no treatment differences in any testicular lesions



Male Pituitary – Nonneoplastic

Terminal Sacrifice – Hyperplasia (pars distalis)

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	11/48** (23%)	9/48 (19%)	19/48 (40%)	15/50 (30%)	17/50 (34%)	19/45* (42%)	10/26 (38%)	13/26* (50%)
Severity	2.1	2.7	1.7	2.2	2.2	2.4	2.2	2.0
Stop-Dose								
Incidence	12/46* (26%)	16/48 (33%)	18/48 (38%)	15/49 (31%)	19/50 (38%)	19/43* (44%)		
Severity	2.8	2.2	2.3	2.4	2.3	2.7		

***, p<0.05; **, p<0.01**

NCTR historical control incidence 51/193 (26.2%), range 16-39% in 4 control groups



Dorsal/Lateral Prostate – Nonneoplastic

Interim and Terminal Sacrifice – Suppurative Inflammation

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Interim Sacrifice, Continuous-Dose								
Incidence	82%	91%#	90%	92%#	90%#	86%#	96%	96%
Severity	1.4	1.7	1.5	1.6	1.8	1.7	1.6	1.5
Interim Sacrifice, Stop-Dose								
Incidence	90%	95%	80%	89%	95%	82%		
Severity	1.7	1.4	1.6	1.6	1.5	1.8		
Terminal Sacrifice, Continuous-Dose								
Incidence	82%	96%*	98%	90%	86%	89%	100%	88%
Severity	2.0	2.0	2.0	1.8	2.0	1.8	1.8	2.0
Terminal Sacrifice, Stop-Dose								
Incidence	85%	96%	85%	84%	90%	84%		
Severity	2.2	2.0	1.9	2.1	2.0	2.1		

* p<0.05 by Poly-3 test ; # p<0.05 by RTE test

NCTR historical control incidence 132/198 (67%), range 64 – 69% in 4 control groups (single prostate sections per animal evaluated versus 6 in present study)



Male Mammary Gland – Nonneoplastic

Terminal Sacrifice – Dilatation, alveolus

	Vehicle	2.5 BPA	25 BPA	250 BPA	2500 BPA	25000 BPA	0.05 EE ₂	0.5 EE ₂
Continuous-Dose								
Incidence	8/50 (16%)	17/48* (35%)	11/48 (23%)	10/50 (20%)	11/50 (22%)	9/45 (20%)	4/25 (16%)	1/25 (4%)
Severity	2.4	2.5	2.7	2.2	2.4	2.2	2.2	2.0
Stop-Dose								
Incidence	15/49 (31%)	7/48 (15%)	6/47 (13%)	7/50 (14%)	6/49 (12%)	7/45 (16%)		
Severity	2.3	2.7	2.5	2.4	2.2	2.3		

* $p < 0.05$

- The above was the only difference from controls indicated by statistical tests in this study. This is not considered an adverse or pathologically meaningful lesion.
- In the previous 90-day BPA/EE₂ gavage study, ductal and alveolar hyperplasia were noted at 0.5 µg EE₂/kg bw/day. In the dietary EE₂ study in this rat model, induction of male mammary gland hyperplasia was observed at 2 ppb (~ 0.2 µg/kg bw/day) EE₂ in younger males (PND 140), but only at higher doses (10 and 50 ppb) in older males (2 years)



Males – Summary of Statistically Significant Effects

Summary chart that follows includes all affected endpoints

- In contrast to the females, effects of EE₂ in males were minimal
- As with females, statistically significant effects of BPA were observed in multiple tissues in various dose groups, but the magnitude of most effects was small and a coherent pattern of effects was generally difficult to discern
 - There was an increased incidence of hyperplasia in the pars distalis of the pituitary at the highest BPA dose in both the continuous- and stop-dose study arms
 - Effects in the epididymis were observed at the high dose of BPA without findings in the testes



Males – Summary of Statistically Significant Effects

Endpoint	BPA Stop					BPA Continuous					EE ₂	
	2.5	25	250	2,500	25,000	2.5	25	250	2,500	25,000	0.05	0.5
Liver weight						■						
Hemoglobin concentration										■	■	
% Eosinophils								■				
Total protein		■										
Total bile acids		■										
Epididymis, exfoliated germ cells (1y)										■		
Epididymis, infiltration cellular, lymphocyte (1y)										■		
Liver, hepatodiaphragmatic nodule (1y)									■			
Liver, infiltration, mononuclear cells (1y)								■	■		■	
Spleen, pigmentation (1y)			■									
Dorsal/lateral prostate, suppurative inflammation (2y)						■						
Mammary gland, dilatation, alveolus (2y)						■						
Kidney, hyperplasia, transitional epithelium (2y)							■					
Kidney, cyst, renal tubule (2y)				■				■	■		■	
Pituitary gland, hyperplasia, pars distalis (2y)					■					■		■
Pituitary, cyst, pars distalis (2y)			■									
Thyroid gland, hyperplasia, C-cell (2y)									■		■	
Parathyroid gland, hyperplasia (2y)							■					
Pancreas, pigmentation (2y)	■											
Pancreas, polyarteritis (2y)				■								
Adrenal medulla, hyperplasia (2y)				■								
Adrenal cortex, hypertrophy (2y)											■	
Testes, polyarteritis (2y)				■								
Bone marrow, hypocellularity (2y)			■		■							
Spleen, hyperplasia, lymphoid (2y)			■									
Liver, angiectasis (2y)						■						
Liver, vacuolization, cytoplasmic (2y)												■

 p<0.05
  p<0.01
  p<0.001

For histopathology endpoints, results of CAFE and Poly-3 tests are summarized.



Males – Summary of Statistically Significant Effects

Additional results using secondary tests

- In most cases of nonneoplastic lesions with severity scores that were statistically significant by the CAFE or Poly-3 tests, the secondary tests (JT/SW and RTE) also showed significance
- In some cases, additional statistically significant results were indicated by the secondary tests with a distribution across dose groups and study arms that was not clearly interpretable
- In a few cases, multiple contiguous dose groups were indicated as significant
 - An example of suppurative inflammation in the dorsal/lateral prostate was shown in an earlier slide, where there was a relatively high background incidence and minimal differences from control



Clarification Questions?



- Survival, reasons for early removals
- Gestation and litter parameters
- Body weights
- Vaginal opening, vaginal cytology, and time to onset of aberrant cycles
- Organ weight, clinical chemistry, hematology, and sperm parameter summaries
- Histopathology
 - Female neoplasms and nonneoplastic lesions
 - Male neoplasms and nonneoplastic lesions
- **Summary**



- BPA had effects that were distinguishable statistically from background, but with questionable biological relevance
- These effects were largely not dose-responsive, often occurred in only one BPA dose group, and there was not a clear pattern of consistent responses in the stop-dose and continuous-dose study arms
- The observed variations in endpoints, particularly below the highest BPA dose, were within the range of normal biological variation
- Some of the observed effects at the highest BPA dose (25,000 μg BPA/kg bw/day) may be treatment-related (pituitary hyperplasia and epididymal effects in males, effects in uterus and vagina in females)
- EE₂ produced clear adverse effects in females