# Statistical Report

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| Project #: | E2190.03 |
| Project Title: | Two Year Chronic Toxicology Study of Bisphenol A (BPA) [CAS # 80-05-7] Administered by Gavage to Sprague-Dawley Rats (NCTR) from Gestational Day 6 Until Birth and Directly to F1 Pups from Postnatal Day 1 (PND 1); Continuous and Stop Dose (PND 21) Exposures |
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| Task: | Statistical Analysis of Neoplastic and Non-neoplastic Lesions (Terminal Sacrifice) - Addendum |
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# Statistical Analysis of Neoplastic and Non-neoplastic Lesions (Terminal Sacrifice) - Addendum

## 1. Objectives

### *1.1 Project Objectives*

The goal of this study is to study the long term toxicity of orally administered Bisphenol-A (BPA) over a broad dose range.

### *1.2 Analysis Objectives*

The objective of this analysis is to determine the effects on histopathology of a broad range of continuous and stop doses of BPA after two years. Statistical analyses of data from continuous doses of the reference estrogen $EE_2$ are also presented. This addendum presents analyses regarding pooled adenomas and carcinomas for the pituitary gland, as this pool was not included in the original statistical report.

## 2. Experimental Design

The study design consisted of first generation female and male rats ($F_0$) for up to 600 mating pairs randomized to treatment groups in 5 loads. The goal of the $F_0$ matings was to obtain 352 study litters, 50 per dose group for vehicle controls and five BPA dose groups, 2.5, 25, 250, 2500, and 25000 µg/kg bw/day, and 26 for each of two $EE_2$ dose groups, 0.05 and 0.5 µg/kg bw/day. Dams were dosed daily from gestation day (GD) 6 until parturition. Dosing was by gavage for $F_0$ dams and $F_1$ pups, the second study generation. Litters were culled to 10 pups on PND 1.There were two study dosing arms of $F_1$ animals, daily continuous dosing to termination, and daily dose stopped at post-natal day (PND) 21. There was a vehicle control group and five BPA groups (2.5, 25, 250, 2500, and 25000 µg/kg) for each study dosing arm, and $EE_2$ daily dose groups (0.05 and 0.50 µg/kg) for the continuous dosing arm only. From the $F_1$ litters, pups were allocated at weaning, PND 21, to the interim (1 year) and terminal (2 year) sacrifices for the core study. For vehicle and BPA terminal sacrifice groups, there were 50 pups each; for the interim sacrifice and the $EE_2$ terminal sacrifice groups, there were 20-26 pups each. Pups within litter and sex were assigned to different dosing arms and sacrifice times.

## 3. Statistical Methods

Statistical analyses were performed separately for the BPA study arms, stop dose and continuous dose, and for the $EE_2$ continuous dose.

For neoplasm incidence, the poly-3 age-adjusted test (Bailer & Portier, 1988) with variance correction (Bieler & Williams, 1993) and the NIEHS continuity-correction (Peddada & Kissling, 2006) was used to test for linear dose trend and to compare dosed groups to the vehicle control.

Pituitary gland adenoma and carcinoma pooled lesions with an incidence of four or more in any treatment group (or two or more for an $EE_2$ group) were included in each analysis. Pituitary gland adenoma incidence and carcinoma incident are repeated from the original statistical report for comparison purposes. The tests for the comparisons to vehicle are one-sided, while the trend tests are two-sided. No adjustments are made for multiplicity.

# 4. Results

Tables are included in Appendix A1. Generally, simple incidence refers to all animals for which the given tissue was microscopically examined, poly-k incidence refers to the number of animals with lesions as a proportion of the number of mortality-adjusted animals examined, terminal incidence refers to animals which survived to scheduled sacrifice, and time-to-first indicates the number of study days of the earliest observance of the lesion.

As presented in Table 5, there was a significantly (p=0.011) greater incidence of pituitary gland pars distalis adenoma or carcinoma for the high $EE_2$ group, for females. There was also a significant dose trend for the $EE_2$ groups, for females. These differences were not significant when considering pituitary gland pars distalis adenoma and carcinoma individually. There were no other statistically significant differences for pituitary gland adenomas or carcinomas, pooled or individually.

# 5. Conclusions

For females, there was a significantly (p=0.011) greater incidence of pituitary gland pars distalis adenoma or carcinoma for the high $EE_2$ group. This difference was not significant when considering pituitary gland pars distalis adenoma and carcinoma individually. There were no other statistically significant differences for pituitary gland adenomas or carcinomas, pooled or individually.

# References

Bailer, AJ and CJ Portier. "Effects of treatment-induced mortality and tumor-induced mortality on tests for carcinogenicity in small samples." Biometrics 44 (1988): 417-431.

Bieler, GS and RL Williams. "Ratio estimates, the delta method, and quantal response tests for increased carcinogenicity." Biometrics 49 (1993): 793-801.Brunner, E, S Domhof and F Langer. *Nonparametric Analysis of Longitudinal Data in Factorial Experiments*. John Wiley and Sons, 2002.

Peddada, SD and GE Kissling. "A survival-adjusted quantal-response test for analysis of tumor rates in animal carcinogenicity studies." Environmental Health Perspectives 114 (2006): 537-541.

# Appendices

## *A1 Statistical Tables*

| *Table 1. Neoplasms by Body System for Terminal Sacrifice Females Bisphenol-A Stop Dose Arm* | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | | | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***2.5*** | ***25*** | ***250*** | ***2500*** | ***25000*** |
| Endocrine System | | | | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 23/49 ( 46.9%) | 16/50 ( 32.0%) | 14/48 ( 29.2%) | 20/50 ( 40.0%) | 20/50 ( 40.0%) | 20/46 ( 43.5%) |
| | Poly-K Incidence | 23/38.5 ( 59.8%) | 16/36.3 ( 44.1%) | 14/33.5 ( 41.8%) | 20/39.4 ( 50.8%) | 20/38.5 ( 51.9%) | 20/35.5 ( 56.3%) |
| | Terminal Incidence | 7/11 ( 63.6%) | 5/12 ( 41.7%) | 8/13 ( 61.5%) | 6/13 ( 46.2%) | 9/17 ( 52.9%) | 6/13 ( 46.2%) |
| | Time-to-First | 502 | 397 | 442 | 448 | 520 | 445 |
| | Poly-K P-Value | 0.470 | 0.112N | 0.081N | 0.271N | 0.308N | 0.469N |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 23/49 ( 46.9%) | 16/50 ( 32.0%) | 14/48 ( 29.2%) | 21/50 ( 42.0%) | 20/50 ( 40.0%) | 21/46 ( 45.7%) |
| | Poly-K Incidence | 23/38.5 ( 59.8%) | 16/36.3 ( 44.1%) | 14/33.5 ( 41.8%) | 21/39.7 ( 52.9%) | 20/38.5 ( 51.9%) | 21/35.5 ( 59.1%) |
| | Terminal Incidence | 7/11 ( 63.6%) | 5/12 ( 41.7%) | 8/13 ( 61.5%) | 6/13 ( 46.2%) | 9/17 ( 52.9%) | 7/13 ( 53.8%) |
| | Time-to-First | 502 | 397 | 442 | 448 | 520 | 445 |
| | Poly-K P-Value | 0.373 | 0.112N | 0.081N | 0.340N | 0.308N | 0.574N |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.

| *Table 2. Neoplasms by Body System for Terminal Sacrifice Males Bisphenol-A Stop Dose Arm* | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | | | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***2.5*** | ***25*** | ***250*** | ***2500*** | ***25000*** |
| Endocrine System | | | | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 29/46 ( 63.0%) | 22/48 ( 45.8%) | 19/48 ( 39.6%) | 19/49 ( 38.8%) | 19/50 ( 38.0%) | 17/43 ( 39.5%) |
| | Poly-K Incidence | 29/39.9 ( 72.7%) | 22/38.4 ( 57.2%) | 19/37.7 ( 50.4%) | 19/34.3 ( 55.4%) | 19/40.1 ( 47.4%) | 17/30.8 ( 55.2%) |
| | Terminal Incidence | 13/17 ( 76.5%) | 8/16 ( 50.0%) | 9/16 ( 56.3%) | 9/13 ( 69.2%) | 5/15 ( 33.3%) | 5/9 ( 55.6%) |
| | Time-to-First | 552 | 529 | 477 | 558 | 551 | 465 |
| | Poly-K P-Value | 0.023N* | 0.098N | 0.026N* | 0.074N | 0.012N* | 0.082N |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 29/46 ( 63.0%) | 22/48 ( 45.8%) | 20/48 ( 41.7%) | 19/49 ( 38.8%) | 19/50 ( 38.0%) | 17/43 ( 39.5%) |
| | Poly-K Incidence | 29/39.9 ( 72.7%) | 22/38.4 ( 57.2%) | 20/38.0 ( 52.7%) | 19/34.3 ( 55.4%) | 19/40.1 ( 47.4%) | 17/30.8 ( 55.2%) |
| | Terminal Incidence | 13/17 ( 76.5%) | 8/16 ( 50.0%) | 9/16 ( 56.3%) | 9/13 ( 69.2%) | 5/15 ( 33.3%) | 5/9 ( 55.6%) |
| | Time-to-First | 552 | 529 | 477 | 558 | 551 | 465 |
| | Poly-K P-Value | 0.022N* | 0.098N | 0.042N* | 0.074N | 0.012N* | 0.082N |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.

| *Table 3. Neoplasms by Body System for Terminal Sacrifice Females Bisphenol-A Continuous Dose Arm* | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | | | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***2.5*** | ***25*** | ***250*** | ***2500*** | ***25000*** |
| Endocrine System | | | | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 21/50 ( 42.0%) | 22/48 ( 45.8%) | 12/46 ( 26.1%) | 20/49 ( 40.8%) | 19/49 ( 38.8%) | 21/46 ( 45.7%) |
| | Poly-K Incidence | 21/39.9 ( 52.7%) | 22/37.1 ( 59.2%) | 12/31.4 ( 38.2%) | 20/36.6 ( 54.7%) | 19/34.1 ( 55.8%) | 21/35.4 ( 59.3%) |
| | Terminal Incidence | 7/16 ( 43.8%) | 12/19 ( 63.2%) | 4/14 ( 28.6%) | 8/13 ( 61.5%) | 5/10 ( 50.0%) | 4/8 ( 50.0%) |
| | Time-to-First | 477 | 497 | 434 | 561 | 456 | 510 |
| | Poly-K P-Value | 0.313 | 0.356 | 0.153N | 0.523 | 0.487 | 0.356 |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 22/50 ( 44.0%) | 23/48 ( 47.9%) | 12/46 ( 26.1%) | 20/49 ( 40.8%) | 19/49 ( 38.8%) | 21/46 ( 45.7%) |
| | Poly-K Incidence | 22/40.3 ( 54.6%) | 23/37.7 ( 61.0%) | 12/31.4 ( 38.2%) | 20/36.6 ( 54.7%) | 19/34.1 ( 55.8%) | 21/35.4 ( 59.3%) |
| | Terminal Incidence | 7/16 ( 43.8%) | 12/19 ( 63.2%) | 4/14 ( 28.6%) | 8/13 ( 61.5%) | 5/10 ( 50.0%) | 4/8 ( 50.0%) |
| | Time-to-First | 477 | 497 | 434 | 561 | 456 | 510 |
| | Poly-K P-Value | 0.408 | 0.361 | 0.115N | 0.595 | 0.559 | 0.425 |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.

| *Table 4. Neoplasms by Body System for Terminal Sacrifice Males Bisphenol-A Continuous Dose Arm* | | | | | | | |
|---|---|---|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | | | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***2.5*** | ***25*** | ***250*** | ***2500*** | ***25000*** |
| Endocrine System | | | | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 21/48 ( 43.8%) | 25/48 ( 52.1%) | 23/48 ( 47.9%) | 21/50 ( 42.0%) | 21/50 ( 42.0%) | 17/45 ( 37.8%) |
| | Poly-K Incidence | 21/36.9 ( 56.9%) | 25/37.6 ( 66.6%) | 23/38.4 ( 59.8%) | 21/38.4 ( 54.7%) | 21/38.0 ( 55.2%) | 17/33.7 ( 50.5%) |
| | Terminal Incidence | 7/14 ( 50.0%) | 10/16 ( 62.5%) | 8/17 ( 47.1%) | 9/14 ( 64.3%) | 8/16 ( 50.0%) | 4/11 ( 36.4%) |
| | Time-to-First | 494 | 613 | 412 | 460 | 546 | 386 |
| | Poly-K P-Value | 0.147N | 0.254 | 0.490 | 0.515N | 0.535N | 0.374N |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 21/48 ( 43.8%) | 25/48 ( 52.1%) | 23/48 ( 47.9%) | 21/50 ( 42.0%) | 22/50 ( 44.0%) | 17/45 ( 37.8%) |
| | Poly-K Incidence | 21/36.9 ( 56.9%) | 25/37.6 ( 66.6%) | 23/38.4 ( 59.8%) | 21/38.4 ( 54.7%) | 22/38.4 ( 57.3%) | 17/33.7 ( 50.5%) |
| | Terminal Incidence | 7/14 ( 50.0%) | 10/16 ( 62.5%) | 8/17 ( 47.1%) | 9/14 ( 64.3%) | 8/16 ( 50.0%) | 4/11 ( 36.4%) |
| | Time-to-First | 494 | 613 | 412 | 460 | 546 | 386 |
| | Poly-K P-Value | 0.172N | 0.254 | 0.490 | 0.515N | 0.584 | 0.374N |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.

| *Table 5. Neoplasms by Body System for Terminal Sacrifice Females EE2 Continuous Dose Arm* | | | | |
|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***0.05*** | ***0.50*** |
| Endocrine System | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 21/50 ( 42.0%) | 10/26 ( 38.5%) | 17/26 ( 65.4%) |
| | Poly-K Incidence | 21/39.9 ( 52.7%) | 10/18.3 ( 54.6%) | 17/23.2 ( 73.3%) |
| | Terminal Incidence | 7/16 ( 43.8%) | 4/7 ( 57.1%) | 2/4 ( 50.0%) |
| | Time-to-First | 477 | 484 | 360 |
| | Poly-K P-Value | 0.055 | 0.561 | 0.068 |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 22/50 ( 44.0%) | 10/26 ( 38.5%) | 20/26 ( 76.9%) |
| | Poly-K Incidence | 22/40.3 ( 54.6%) | 10/18.3 ( 54.6%) | 20/24.2 ( 82.8%) |
| | Terminal Incidence | 7/16 ( 43.8%) | 4/7 ( 57.1%) | 2/4 ( 50.0%) |
| | Time-to-First | 477 | 484 | 360 |
| | Poly-K P-Value | 0.009 ** | 0.615N | 0.011 * |
| Pituitary Gland * Carcinoma,Pars Distalis | Simple Incidence | 1/50 ( 2.0%) | 0/26 ( 0.0%) | 3/26 ( 11.5%) |
| | Poly-K Incidence | 1/34.6 ( 2.9%) | 0/15.9 ( 0.0%) | 3/16.1 ( 18.7%) |
| | Terminal Incidence | 0/16 ( 0.0%) | 0/7 ( 0.0%) | 0/4 ( 0.0%) |
| | Time-to-First | 615 | ---- | 610 |
| | Poly-K P-Value | 0.053 | 0.652N | 0.084 |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.

| *Table 6. Neoplasms by Body System for Terminal Sacrifice Males EE2 Continuous Dose Arm* | | | | |
|---|---|---|---|---|
| ***System and Neoplasm*** | | ***Treatment (ug/kg)*** | | |
| ***Lesion*** | ***Statistic*** | ***Control*** | ***0.05*** | ***0.50*** |
| Endocrine System | | | | |
| Pituitary Gland * Adenoma,Pars Distalis | Simple Incidence | 21/48 ( 43.8%) | 12/26 ( 46.2%) | 6/26 ( 23.1%) |
| | Poly-K Incidence | 21/36.9 ( 56.9%) | 12/19.9 ( 60.2%) | 6/20.8 ( 28.9%) |
| | Terminal Incidence | 7/14 ( 50.0%) | 6/9 ( 66.7%) | 3/12 ( 25.0%) |
| | Time-to-First | 494 | 543 | 518 |
| | Poly-K P-Value | 0.030N* | 0.517 | 0.029N* |
| Pituitary Gland * Carcinoma or Adenoma,Pars Distalis | Simple Incidence | 21/48 ( 43.8%) | 12/26 ( 46.2%) | 6/26 ( 23.1%) |
| | Poly-K Incidence | 21/36.9 ( 56.9%) | 12/19.9 ( 60.2%) | 6/20.8 ( 28.9%) |
| | Terminal Incidence | 7/14 ( 50.0%) | 6/9 ( 66.7%) | 3/12 ( 25.0%) |
| | Time-to-First | 494 | 543 | 518 |
| | Poly-K P-Value | 0.030N* | 0.517 | 0.029N* |
| Pituitary Gland * Lymphoma Malignant | Simple Incidence | 2/48 ( 4.2%) | 2/26 ( 7.7%) | 1/26 ( 3.8%) |
| | Poly-K Incidence | 2/33.7 ( 5.9%) | 2/19.3 ( 10.4%) | 1/20.3 ( 4.9%) |
| | Terminal Incidence | 0/14 ( 0.0%) | 0/9 ( 0.0%) | 0/12 ( 0.0%) |
| | Time-to-First | 486 | 369 | 473 |
| | Poly-K P-Value | 0.588N | 0.482 | 0.676N |

P-values for dose trend are presented in the “Control” column.
N indicates a negative trend or negative treatment comparison to control with corresponding lower-tail p-values.