**STATISTICAL ANALYSIS**

**PND4 and PND22 Plasma Data**

**Toxico Genomic Study**

**of**

**BDE-47 and DE71 in Wistar Han Rats**

**(C98090B and C20287C)**

**PREPARED FOR**

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| **Table 1: BDE47 Alion Study C98098B CHEM13419 Wistar Han Rats:  PND Plasma Data Pups and Dams** | | | | | |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Control** | **.1 mg/kg** | **15 mg/kg** | **50 mg/kg** | **Trenda** |
| **PND 4b,c,d,e,f** |  |  |  |  |  |
| **Male Pups** | 0.000 ± 0.000 [6] | 0.035 ± 0.008 [6]\*\* | 6.976 ± 1.166 [5]\*\* | 19.367 ± 2.300 [6]\*\* | <0.001+ |
| **Female Pups** | 0.003 ± 0.003 [6] | 0.047 ± 0.010 [6]\*\* | 7.243 ± 0.907 [6]\*\* | 21.317 ± 2.393 [6]\*\* | <0.001+ |
| **PND 22b,c,d,e** |  |  |  |  |  |
| **Dams** | 0.000 ± 0.000 [6] | 0.000 ± 0.000 [6] | 0.560 ± 0.051 [5]\*\* | 2.032 ± 0.426 [5]\*\* | <0.001+ |
| **Male Pups** | 0.009 ± 0.009 [6] | 0.044 ± 0.007 [6]\* | 3.850 ± 0.573 [6]\*\* | 15.053 ± 4.059 [6]\*\* | <0.001+ |
| **Female Pups** | 0.007 ± 0.007 [6] | 0.042 ± 0.008 [6]\* | 3.392 ± 0.345 [6]\*\* | 10.663 ± 1.247 [6]\*\* | <0.001+ |
| a: P-value and direction of Trend Test | | | | | |
| b: Each dose was compared to the control with Shirley test when a trend was present [P<.01 from Jonckheere’s trend test] | | | | | |
| or with Dunn test when no trend was present [\* = P<0.05, \*\* = P<0.01] | | | | | |
| c: Values below limit of detection[LOD] subsituted with 1/2 the LOD.   d: Values listed as ND set = 0 | | | | | |
| e: Mean ± standard error [number] | | | | | |
| f: One outlier, Male PND4, Animal 4046 Dose .1mg/kg  was removed from the analysis. | | | | | |
|  | | | | | |

| **Table 2: DE71 Alion Study C20287C Wistar Han Rats:  PND4 Plasma Data Pups and Dams** | | | | | |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Control** | **.1 mg/kg** | **15 mg/kg** | **50 mg/kg** | **Trenda** |
| **Male Pups** |  |  |  |  |  |
| **BDE47** | 0.0000 ± 0.0000 [6] | 0.0040 ± 0.0019 [6]\* | 2.9733 ± 0.5792 [6]\*\* | 8.8033 ± 0.9709 [6]\*\* | <0.001+ |
| **BDE99** | 0.0000 ± 0.0000 [6] | 0.0035 ± 0.0018 [6] | 3.2967 ± 0.5534 [6]\*\* | 11.0733 ± 1.5298 [6]\*\* | <0.001+ |
| **BDE153** | 0.0000 ± 0.0000 [6] | 0.0000 ± 0.0000 [6] | 1.3613 ± 0.2200 [6]\*\* | 4.6167 ± 0.7182 [6]\*\* | <0.001+ |
| **Female Pups** |  |  |  |  |  |
| **BDE47** | 0.0000 ± 0.0000 [6] | 0.0024 ± 0.0009 [5]\* | 2.6163 ± 0.8845 [7]\*\* | 9.6740 ± 1.2615 [5]\*\* | <0.001+ |
| **BDE99** | 0.0000 ± 0.0000 [6] | 0.0033 ± 0.0033 [5] | 2.7293 ± 0.8838 [7]\*\* | 12.9000 ± 2.1338 [5]\*\* | <0.001+ |
| **BDE153** | 0.0000 ± 0.0000 [6] | 0.0000 ± 0.0000 [5] | 1.1333 ± 0.3305 [7]\*\* | 5.6600 ± 0.7836 [5]\*\* | <0.001+ |
| a: P-value and direction of Trend Test | | | | | |
| b: Each dose was compared to the control with Shirley test when a trend was present [P<.01 from Jonckheere’s trend test] | | | | | |
| or with Dunn test when no trend was present [\* = P<0.05, \*\* = P<0.01] | | | | | |
| c: Values below limit of detection[LOD] subsituted with 1/2 the LOD.   d: Values listed as ND set = 0 | | | | | |
| e: Mean ± standard error [number] | | | | | |
|  | | | | | |

| **Table 3: DE71 Alion Study C20287C Wistar Han Rats:  PND22 Plasma Data Pups and Dams** | | | | | |
| --- | --- | --- | --- | --- | --- |
| **Parameter** | **Control** | **.1 mg/kg** | **15 mg/kg** | **50 mg/kg** | **Trenda** |
| **Dams** |  |  |  |  |  |
| **BDE47** | 0.0017 ± 0.0009 [6] | 0.0013 ± 0.0009 [6] | 0.1732 ± 0.0247 [6]\*\* | 0.5283 ± 0.0562 [6]\*\* | <0.001+ |
| **BDE99** | 0.0102 ± 0.0047 [6] | 0.0054 ± 0.0045 [6] | 0.1608 ± 0.0175 [6]\*\* | 0.4400 ± 0.0523 [6]\*\* | <0.001+ |
| **BDE153** | 0.0017 ± 0.0017 [6] | 0.0017 ± 0.0017 [6] | 0.0975 ± 0.0188 [6]\*\* | 0.2645 ± 0.0381 [6]\*\* | <0.001+ |
| **Male Pups** |  |  |  |  |  |
| **BDE47** | 0.0003 ± 0.0002 [6] | 0.0013 ± 0.0003 [6]\* | 1.1883 ± 0.1599 [6]\*\* | 3.5733 ± 0.3153 [6]\*\* | <0.001+ |
| **BDE99** | 0.0008 ± 0.0008 [6] | 0.0039 ± 0.0030 [6] | 0.6637 ± 0.0975 [6]\*\* | 2.4367 ± 0.2904 [6]\*\* | <0.001+ |
| **BDE153** | 0.0000 ± 0.0000 [6] | 0.0017 ± 0.0017 [6] | 0.6755 ± 0.0985 [6]\*\* | 2.8750 ± 0.2086 [6]\*\* | <0.001+ |
| **Female Pups** |  |  |  |  |  |
| **BDE47** | 0.0003 ± 0.0002 [6] | 0.0047 ± 0.0024 [6]\* | 0.9795 ± 0.0915 [6]\*\* | 2.2800 ± 0.1348 [6]\*\* | <0.001+ |
| **BDE99** | 0.0025 ± 0.0011 [6] | 0.0119 ± 0.0031 [6]\* | 0.5197 ± 0.0658 [6]\*\* | 1.2955 ± 0.1312 [6]\*\* | <0.001+ |
| **BDE153** | 0.0000 ± 0.0000 [6] | 0.0017 ± 0.0017 [6] | 0.5455 ± 0.0607 [6]\*\* | 1.7650 ± 0.0637 [6]\*\* | <0.001+ |
| a: P-value and direction of Trend Test | | | | | |
| b: Each dose was compared to the control with Shirley test when a trend was present [P<.01 from Jonckheere’s trend test] | | | | | |
| or with Dunn test when no trend was present [\* = P<0.05, \*\* = P<0.01] | | | | | |
| c: Values below limit of detection[LOD] subsituted with 1/2 the LOD.   d: Values listed as ND set = 0 | | | | | |
| e: Mean ± standard error [number] | | | | | |
|  | | | | | |

**Appendix A: Extreme Values**

The following was removed from the analysis as an outlier:

BDE47 Male Pup, PND4, Dose Group .1mg/kg, animal 4046 (value=21.7 ug/mL).

**Appendix B: Statistical Methods**

Analysis of Continuous Variables

Two approaches were employed to assess the significance of pairwise comparisons between dosed and control groups in the analysis of continuous variables. Organ and body weight data, which have approximately normal distributions, were analyzed using the parametric multiple comparison procedures of Williams (1971, 1972) and Dunnett (1955). Hematology, clinical chemistry, thyroid hormone and plasma data, which typically have skewed distributions, were analyzed using the nonparametric multiple comparison methods of Shirley (1977) and Dunn (1964). Jonckheere's test (Jonckheere, 1954) was used to assess the significance of dose-response trends and to determine whether a trend-sensitive test (Williams' or Shirley's test) was more appropriate for pairwise comparisons than a test that does not assume a monotonic dose-response (Dunnett's or Dunn's test). Trend-sensitive tests were used when Jonckheere's test was significant at p<0.01.

Values below the limit of detection (LOD) were substituted with ½ the LOD. Values listing as ND were assigned the value of 0.

Prior to analysis, extreme values identified by the outlier test of Dixon and Massey (1951) were examined by NTP personnel. Implausible values, extreme values from animals that were suspected of being sick due to causes other than treatment, and values that the laboratory indicated as being inadequate due to measurement problems were eliminated from the analysis. A list of these values is provided in the Appendix.

**Appendix C: References**

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