Statistical Analysis of Developmental Neurotoxicity (DNT) Data

Regulatory agencies recognize that data collected from motor activity, auditory startle, learning and memory, and behavioral testing present unique characteristics that require appropriate statistical methodology. Failure to address these distinctive issues may yield false statistical conclusions in terms of test article effects and lead to delays in submissions.

Litter Effect: It is generally accepted that the litter as a whole presents a single experimental unit and therefore must be accounted for in the statistical analysis (1,2,3,4,5). Studies have shown there is a much higher correlation between offspring from the same litter than between offspring from different litters. This correlation between littermates is commonly known as the 'litter effect'. Ignoring the litter effect, and treating littermate observations as independent, may increase false positives by artificially deflating the estimate of random variation (3). The proper way to address litter effects is to include a random term for litter in the statistical model.

Sex Effect: Because they are littermates, when a male and female from the same litter are tested, the sex effect should be evaluated in the statistical analysis (1,2,3,4,5). Analyzing males and females separately not only reduces statistical power and increases the number of p-values, it also fails to answer the fundamental question of whether or not there are sex differences in test article effects (2,3). The proper statistical approach is to include sex nested within litter as a random factor in the analysis model. If the analysis suggests sex differences in test article effects (treatment by sex interaction), the analysis proceeds separately for each sex. Otherwise, the pooling of sexes is justified statistically given that the treatment effects are similar across sexes (2,3).

Repeated Measures: Because DNT testing consists of repeated measurements (trials or time blocks) on the same animal, a repeated measures model should be used for statistical analysis (1,2,3,4,5). A repeated measures model utilizes correlated observations from the same animal in a single model and allows for the direct evaluation of habituation (time effect) and test article effect on habituation (treatment by time interaction) (2).

Regulatory Perspective: The 2007 OECD Guideline (1) specifically states that the statistical analysis of DNT data must employ methodology that uses the litter as the experimental analysis unit and the 2016 Guidance Document of the NAFTA Tech Working Group on Pesticides (2) specifically outlines the litter effects, sex effects and repeated measures modeling discussed here.

BioSTAT Perspective: BioSTAT has participated on expert panels and co-authored manuscripts (3) that have become the basis for regulatory guidance on the statistical analysis of DNT data. The unique characteristics of DNT testing require sophisticated statistical modeling that goes beyond the simple one-way ANOVA. Proper analysis of data from DNT studies aids in the submission process and avoids delays caused by reviewers arriving at different conclusions after employing the correct statistical analysis.

References:

1) OECD (2007), *Test No. 426: Developmental Neurotoxicity Study*, OECD Guidelines for the Testing of Chemicals, Section 4, OECD Publishing, Paris,

2) Moser VC, Bailey F, Bowers W, Raffaele K, Crofton K, Gilbert M (2016) *Developmental Neurotoxicity Study Guidance Document*, North American Free Trade Agreement (NAFTA) Technical Working Group on Pesticides (TWG)

3) Holson RR, Freshwater L, Maurissen JP, Moser VC, Phang W. (2008) *Statistical issues and techniques appropriate for developmental neurotoxicity testing: a report from the ILSI Research Foundation/Risk Science Institute expert working group on neurodevelopmental endpoints.* Neurotoxicol. Teratol. 30, 326-348.

4) Vidmar T, Freshwater L, Collins R, *Developmental and Reproductive Toxicology: A Practical Approach, Third Edition. Chapter "*Understanding Statistics in Developmental and Reproductive Toxicology". Edited by Ron Hood., CRC Press, Inc., December 2011.

5) Vidmar T, Freshwater L, Collins R, *A Comprehensive Guide to Toxicology in Preclinical Drug Development, First Edition. Chapter "*Biostatistics for Toxicologists". Edited by Ali S Faqi, Elsevier Inc., November 2012.