Statistical evaluation of regulatory honeybee trials - a pragmatic approach

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Abstract

The sequential risk assessment scheme for honeybees incorporates different levels of testing based on relevant guidelines and guidance documents (OECD 213, 214, EPPO 170 and OECD GD 75/Oomen & de Ruijter test). Within the regulatory process statistical analysis of data derived from these honeybee trials is required. As a consequence, the ‘AG Bienenschutz’ (a German national expert group on honeybee and plant protection) developed a recommendation for statistical analysis of data from regulatory honeybee trials.

Statistical parameters are well established in standard laboratory trials (OECD 213/214). Depending on the data base, dose response relations (LDx) can be calculated via e.g. probit analysis > moving averages > binomial distribution. A NOED (No Observed Effect Dose) can be determined by e.g. Fisher Exact Test (corrected according to Holm’s/Bonferroni)

During higher tier bee testing comprehensive data on mortality, foraging activity or generic brood assessment can be evaluated at several levels.

Pre-treatment level: the parameters of the different treatment groups (colonies) before the treatment are determined in order to ensure an equal distribution among the groups (e.g. by analysis of variance, Tukey test or multiple t-test, two-sided).

Post-treatment level: effects of the treatment can be analysed via comparison to the control group or to the situation before the treatment.

A day-wise comparison of the treatment group(s) against the control as well as a comparison over distinct post-treatment intervals can be conducted (pairwise/multiple comparison, one-sided). The following flow chart gives guidance on these analyses of data derived from semi-field and field studies.

It should be generally acknowledged that statistical evaluation of semi-field and field trials is more challenging and sometimes limited. In view of these limits, the interpretation should primarily consider biological relevance of honeybee data. Therefore, a combination of statistical analyses and expert judgement is required.

In future, the current status will be complemented by statistically analysing brood parameters (e.g. brood termination rate) or combined data of field trials. Also, new approaches like evaluating the time dependency of data or the use of conceptual models (e.g. models excluding irrelevant variance components) will be considered.
Sequential Scheme for Statistical Analysis

**Testing Normal Distribution [N]**
- **Shapiro-Wilks:** \( n \geq 3 \), (combined data)
- **R/S Test:** \( n < 4 \), (single treatments)

(normal distribution can be achieved after data transformation (e.g. log-transf.)
\( n \) = number of replicates (sample size)

**N-distributed**

**not N-distributed**

**Testing Homogeneity of Variance**
- **Levene-Test:** independent on \( k \) and \( N \)
- **Bartletts Test:** N-distributed
  \( k \) = number of treatment groups

**Final Analysis**

**homogeneous**
- **pairwise:** Student t-Test
- **multiple:** Holm’s Bonferroni

**inhomogeneous**
- **pairwise:** Dunnett or Williams t-Test
- **multiple:** Welch t-Test

**homogeneous or inhomogeneous**
- **pairwise:** Holm’s Bonferroni, U-Test or Jonckheere-Terpstra
- **multiple:** Welch t-Test, Mann-Whitney U-Test