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   **inhomogeneous**   **homogeneous or inhomogeneous**

pairwise   *multiple*   *pairwise*   *multiple*   *pairwise*   *multiple*

| Student t-Test | Dunnett or Williams t-Test | Welch t-Test | Holm`s Bonferro. Welch t-Test | Mann-Whitney U-Test | Holm`s Bonferro. U-Test or Jonckheere -Terpstra |