THE IMPORTANCE OF CONFIDENCE LIMITS FOR ECOTOXICOLOGICAL ENDPOINTS

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CAMBRIDGE ENVIRONMENTAL ASSESSMENTS

Background

In Europe, Tier I ecotoxicity tests using standard species are a regulatory risk assessment requirement for all chemicals prior to commercial use. The most common statistical endpoint used in acute and chronic ecotoxicological studies are Effect Concentration (EC_x) values. These describe the concentration at which the x percent of the organisms are affected for any given parameter, e.g. mortality, i.e. an EC_{50} would be where 50% of all tested organisms had died.

In recent years, there has been a requirement for the provision of both EC_{10} and EC_{20} values in addition to the traditional EC_{50} values. Therefore, older study data is routinely re-analysed to generate these values to be submitted to the regulatory authorities for current environmental risk assessments.

These re-analyses should also include an assessment of the reliability of each endpoint. This is usually carried out by investigating the corresponding confidence limits. Although confidence limits are a key indicator of the reliability of the data, they can sometimes be overlooked, or their importance misunderstood.

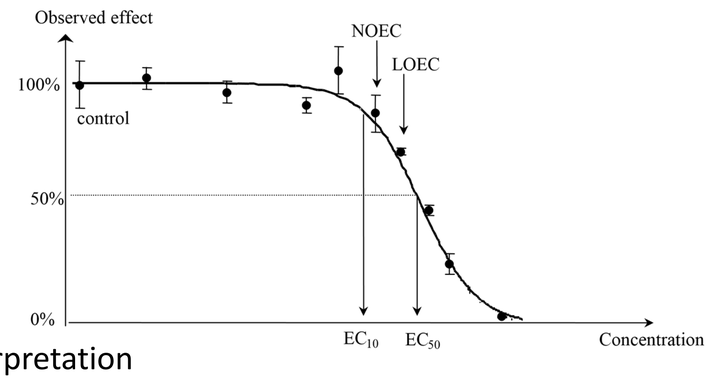
Calculation and assessment of reliable EC_x values

Available guidance

OECD 54 - Current Approaches in the statistical analysis of ecotoxicity data: A guidance to application (2006)

SUMMARY
- Provides an overview of the statistical criteria
- Includes aquatic, sediment and terrestrial tests
- Followed by regulators

LIMITATIONS
- Many of the tests in this guideline contradict the recommendations in specific OECD test guidelines
- Creates confusion on what statistical model to choose
- Provides information on how to calculate EC_x, confidence limits (cl), LOEC, NOEC values but no guidance for data interpretation



OECD 210 - Fish, Early-Life Stage Toxicity Tests (2013)

SUMMARY
- Annex 6 provides criteria to be used for the interpretation of EC_x estimates from statistical software
- Reliability is assessed using the calculated 95% confidence limits and the concentrations used within the test
  - The confidence limits should not span more than two test concentrations
  - The confidence limits should not contain zero
  - The EC_x value should be no more than 25% below the lowest test concentration or above the highest test concentration

We recommend using both guidance's (OECD 54 and 210) in conjunction with each other to calculate and interpret the result. Additionally, specific test guidelines should be consulted for any specific statistical requirements.

Species specific guidance

OECD 201: Freshwater Alga and Cyanobacteria, Growth Inhibition Test (2006)
- Required parameters: Average specific growth rate and yield (biomass).
- Objective: obtain a quantitative concentration-response relationship by regression analysis
- Data requirements: regression analysis should be performed using individual replicate responses, not treatment group means
- Statistical approach: Non-linear regression procedures are preferred techniques that better handle unavoidable data irregularities and deviations from smooth distributions

OECD 211: *Daphnia magna* Reproduction Test (2012)
- Required parameters: Survival and reproduction
- Data requirements: endpoint should be based on introduced (not surviving) parents to avoid data bias

Statistical Approaches

SOFTWARE
- Various statistical software programmes that can calculate statistical endpoint values
- It is important to use a package that can be validated to comply with GLP regulatory requirements

MODEL SELECTION
- Many models that can be used to run statistical data (i.e. Linear, non-linear, Weibul, etc.)
- Any test guidance recommendations should be followed when selecting the model
- The data should drive the model selection
  - It is not recommended to run all the models and just pick the best EC_x and cl values
  - Some approaches are designed to force a dose response onto data

REFERENCES

1. OECD Series on Testing Assessment No. 54: Current Approaches in the Statistical Analysis of Ecotoxicity Data: A Guidance to Application, 2006.
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3. OECD Guidelines for Testing of Chemicals, No. 201: "Alga, Growth Inhibition Test", adopted March 23, 2006.
4. OECD Guidelines for Testing of Chemicals, No. 211: "*Daphnia magna* Reproduction Test", adopted October 02, 2012.
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