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Estimation of effective concentrations from *in vitro* dose-response data using the log-logistic model

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Dose-response studies are performed to investigate the toxicity or potency of a chemical. *In vitro* dose-response data usually exhibit a monotonic sigmoidal relationship, which can often be evaluated properly using a log-logistic model. Fixing the two parameters of the log-logistic model that relate to the lower or upper asymptote of the dose-response curve may lead to erroneous estimates. Thus, the four-parameter log-logistic model should be used even in the case that response data are normalized to a range between 0 and 1 before investigating the dose-response relationship.

This thesis focuses on the following two issues that are often arising when estimating effective concentrations by applying log-logistic model to dose-response data:

(1) Estimation of absolute low effective concentration (ALEC) and construction of an accurate confidence interval for it.

The lowest observed effect concentration used as a summary statistic for low toxic effects is determined by hypothesis testing procedures. The drawback of hypothesis testing procedures is strong dependence on the experimental design (e.g. concentration levels and sample size). As an alternative approach, the relative low effective concentration can be derived from dose-response models. It is hardly to estimate accurately, if the dose-response curve is not sufficiently supported by data points. In this context, the absolute low effective concentration (ALEC) derived from a dose-response model can be used as another characteristic value to reflect low toxic effects. To quantify the uncertainty in ALEC estimates, statistical methods for construction of accurate confidence intervals for the estimates are required.

(2) Averaging EC50 estimates from a series of experiments performed for the same *in vitro* endpoint and the same chemical.

When fitting log-logistic models, main interest lies in estimating EC50 value, i.e., the concentration of the chemical that produces half-maximal response. Often, more than one experiment is carried out to determine the EC50 value for a chemical, requiring averaging of EC50 estimates from a series of experiments. However, as the ranges of tested concentrations are chosen differently from experiment to experiment, some experiments show clear toxic effects of a chemical while others do not. Systematic analysis strategies for averaging EC50 estimates from multiple dose-response experiments are required.

Statistical methods that can resolve these issues are investigated in detail both in simulated and in real dose-response studies.

In this thesis, a formula to derive the ALEC based on the four-parameter log-logistic model is provided. Theoretically, confidence intervals for the model-based ALEC estimates can be constructed by application of the delta method, profile likelihood or bootstrap resampling

techniques, as well as their extended approaches. However, the bootstrap resampling techniques are not appropriate in practical situations with only a few replicate (e.g., typically triplicate) measurements performed per concentration level, as in these cases they lead to low probabilities of the confidence interval to capture the true ALEC value. If dose-response data exhibit an explicit relationship and do not vary greatly, the standard and the back-transformed version of the delta method are recommended to construct confidence intervals for the ALEC. If a lower (an upper) plateau of a decreasing (an increasing) dose-response curve is not supported by data points, but nevertheless the variability of the data is small, the back-transformed version of the delta method is suggested for construction of confidence intervals for the ALEC. If in this case extremely large confidence intervals occur, the profile likelihood based confidence interval is proposed as the best option. If highly variable data is present, again the profile likelihood method is recommended, although it is computationally a bit more elaborate.

Mixed-effects models can be utilized to estimate the average behavior of EC50 values over all experiments by considering the variabilities within and among experiments simultaneously. However, fitting nonlinear mixed-effects models is more complicated as compared to linear mixed-effects models, convergence problems are thus often encountered. An alternative strategy is the application of a meta-analysis approach, which combines EC50 estimates obtained from separate log-logistic model fittings. In this thesis, these two proposed strategies are compared in two settings of multiple dose-response experiments: complete and explicit dose-response relationships are observed (1) in all experiments; (2) merely in a subset of experiments. In the case that complete dose-response relationships are observed in all experiments, the meta-analysis strategy was found to be a simple and robust method to average EC50 estimates from multiple experiments, which is especially suited in situations with only a small number of experiments. In the case that multiple experiments exhibit both complete and incomplete dose-response relationships, all experimental data should be first screened using the DORES (dose-response screening) plot created for the purpose of visualization and comparison of multiple dose-response experimental results. As long as the number of experiments that provide information about complete dose-response relationships is more than three, those experiments that do not show complete dose-response relationships in the DORES plot can be excluded from the procedure of averaging EC50 estimates and the meta-analysis approach will still perform well. If only two experiments exhibit complete dose-response relationships, the mixed-effects modeling approach outperforms the meta-analysis approach.

The approaches discussed here can further be elaborated. The proposed methods for estimation of ALEC and associated confidence interval from log-logistic model fits can be extended and applied to other dose-response models, e.g., log-normal models or Weibull models. The analytical strategies for averaging EC50 estimates from multiple dose-response experiments can be applied to average also other effective concentrations of interest (e.g. EC10, IC10, ALEC).