Be more realistic: Predicting the effects of short-term exposure events on Zebrafish Early Life Stages using the GUTS-model

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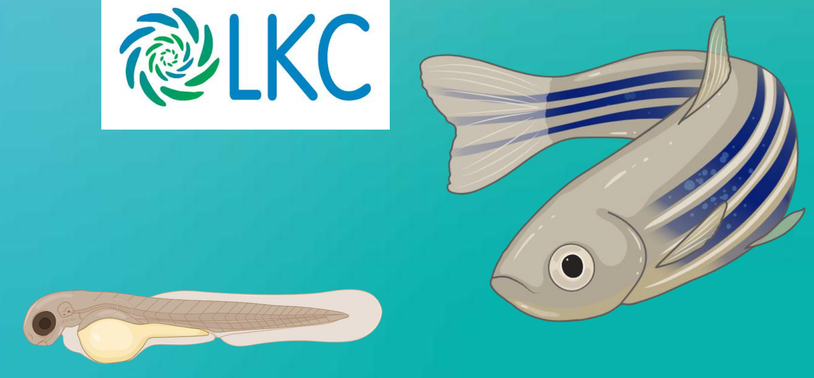

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Toxicokinetic-toxicodynamic (TKTD) models are increasingly used to analyse and predict effects of time-variable exposure on survival and sublethal endpoints. Here we present an example on the use of the GUTS model to describe effects of short-term exposure events on survival of fish larvae.

Motivation

A Fish Full Life Cycle Test was conducted to assess the impact of an insecticide to zebrafish (*Danio rerio*) under flow-through conditions to achieve constant exposure. The survival of larvae of the parental (P) and filial generation (F1) was identified as the most sensitive endpoint (Figure 1). Since predicted exposure patterns are highly dynamic and often characterized by short-term exposure events, GUTS modelling [1] was used as higher tier option for a more realistic risk assessment.

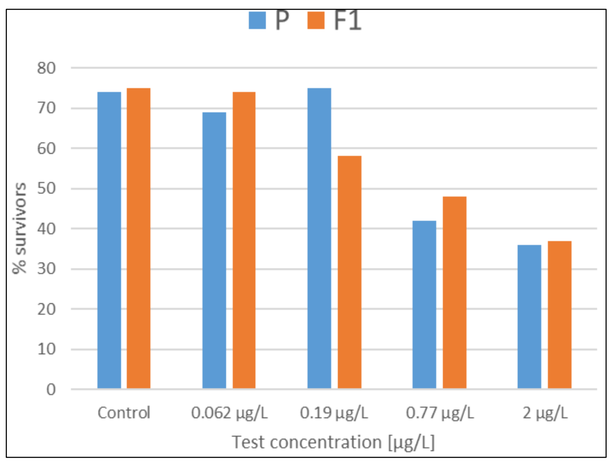

Figure 1: Survival of parental (P) and filial (F1) early life stage zebrafish in a Full Life Cycle Tests under flow through conditions.

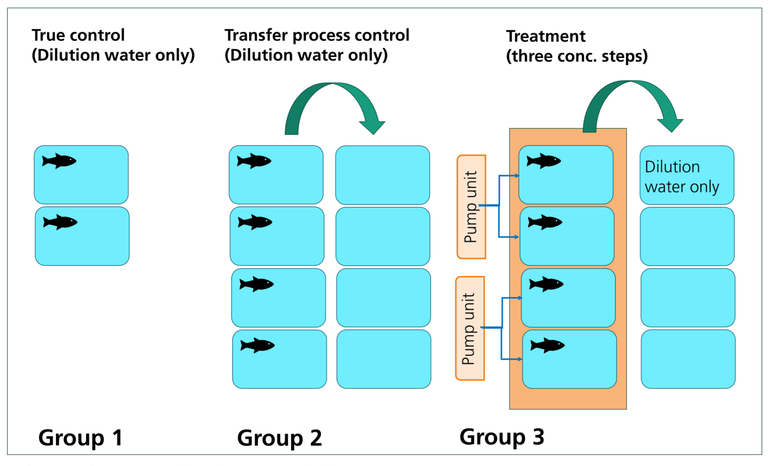

Figure 2: Early Life Stage Toxicity Test, pulsed exposure setup
For each pulse, the animals were transferred to the exposure vessels and transferred back to dilution water at the end (Group 3). To check the impact of the transfer process, a transfer process control (Group 2) was applied. The "True control" (Group 1) was used to evaluate the control performance in accordance with the defined validity criteria.

Model calibration

Since results for both generations in the full life cycle test did not differ significantly, the pooled data were used to calibrate the model by means of the openGUTS software [2]. Both, the stochastic death (SD) and the individual tolerance (IT) variants of the reduced GUTS model could be fitted well to this data set (as shown for the IT variant in Figure 3a).

Validation

For the model validation, a Zebrafish Early Life Stage Toxicity Test over 35 d (OECD 210) was designed to provide independent data on effects of short-term exposure as recommended by EFSA [3]. The exposure pattern was based on available FOCUS exposure estimations and practical considerations.

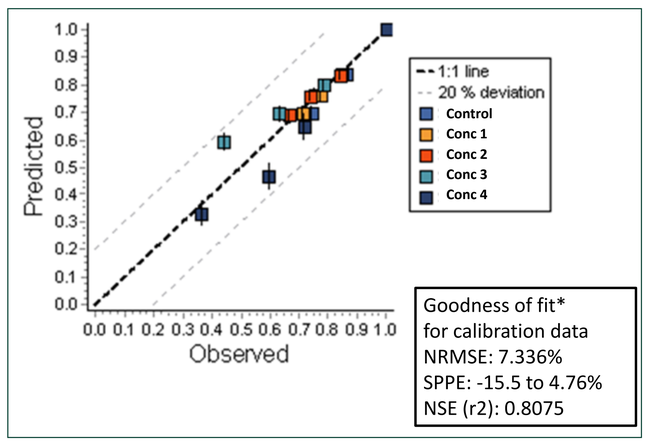

Figure 3a: **Calibration** of the reduced GUTS-IT model. Observed versus predicted survival plot and further goodness of fit parameters*

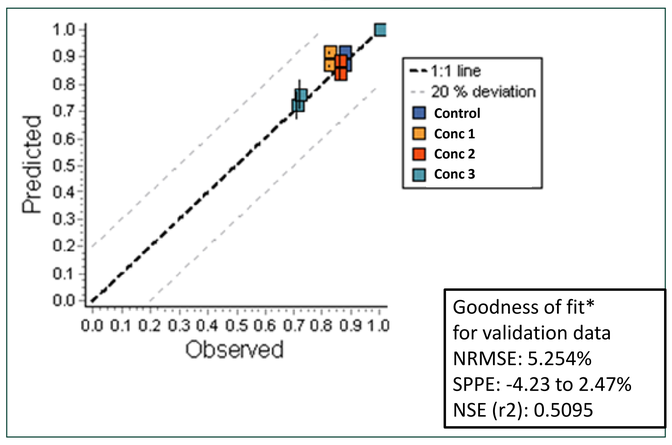

Figure 3b: **Validation** of the reduced GUTS-IT model. Observed versus predicted survival plot and further goodness of fit parameters*

*NRMSE = Normalised root-means-square error (should be < 50%). SPPE: Survival probability prediction error. NSE = Model efficiency.

Three exposure concentrations, each resulted from two pulse exposure events of 24 h on day 7 and 15 post fertilization were tested (Figure 2). A second, larger interval to test toxicologically independent peaks was not tested because independent peaks would require an interval exceeding the duration of the test. Animal welfare was a further strong argument to skip testing of a second interval.

The reduced GUTS model was able to predict reasonably well the experimentally observed survival over time in this pulse exposure validation test (Figure 3b and 4).

Conclusion

Since the model validation shows acceptable goodness of fit, we conclude that the model can be used as a Tier 2C approach to assess effects of the diverse dynamic exposure pattern predicted by FOCUS Step 3 or 4 modelling on the survival of zebrafish larvae.

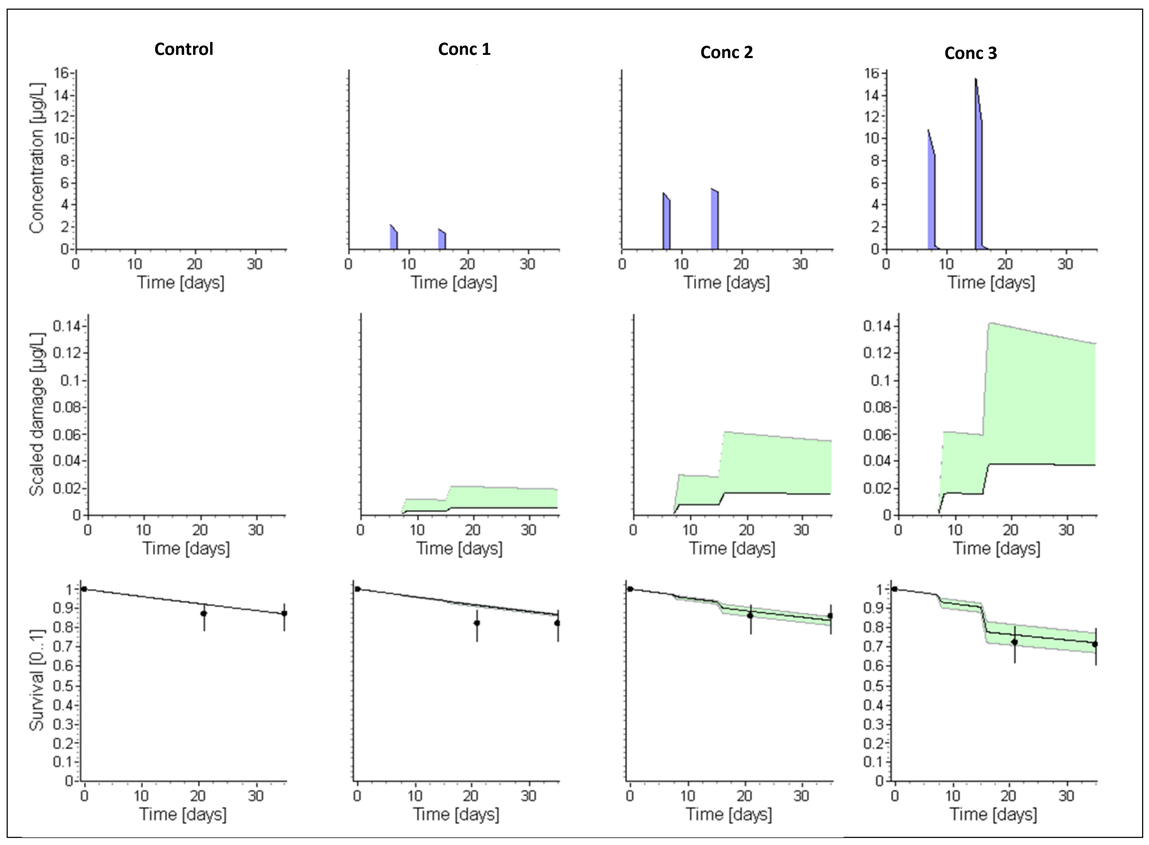

Figure 4: **Validation** using the GUTS-Red-IT variant. Symbols = observations with confidence intervals (Wilson scores). Lines = model prediction with 95 % confidence intervals (green area)

References

[1] Jager et al. (2011): General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology. DOI: 10.1021/es103092a

[2] Jager (2024): OpenGUTS Version 1.2. Available on: OpenGUTS software for analysing survival data over time. https://openguts.info/about.html

[3] EFSA PPR Panel (2018): Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms. doi: 10.2903/j.efsa.2018.5377