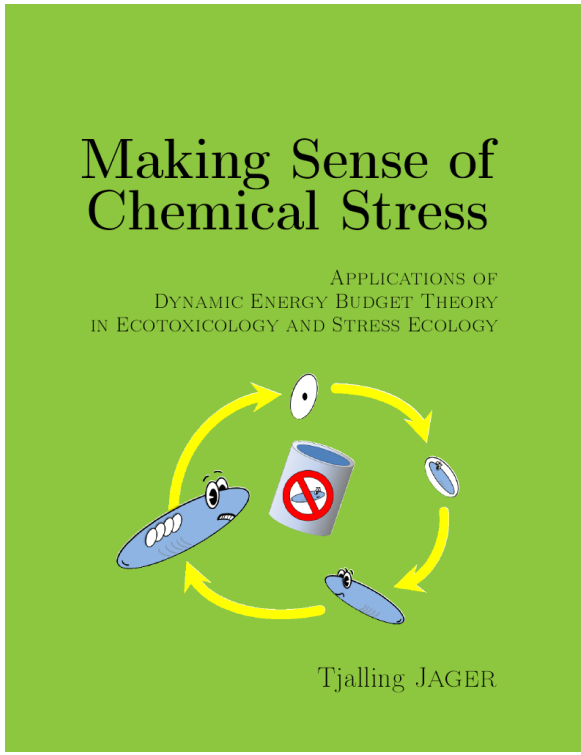
TKTD modelling
- Theoretical aspects –

Sandrine CHARLES



Inspired from Jager (2015)
Making sense of chemical stress: application of dynamic energy budget theory in ecotoxicology and stress ecology.

Leanpub: https://leanpub.com/debtox_book, Version 1.2.

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TC: GUTS modelling

What means TKTD?

- **TK stands for Toxico-Kinetics**
  - Provides a prediction of the chemical concentration inside an organism from the external chemical concentration to which the organism is exposed;
  - Models absorption, distribution, metabolism (biotransformation) and elimination of toxicants inside the organism (ADME processes);
  - Includes physiologically-based processes (PBPK).

- **TD stands for Toxico-Dynamics**
  - Translates the internal chemical concentration to an effect on life-history traits over time (e.g., survival, growth, reproduction,…);
  - Accounts for energy allocation and physiological compensation;
  - Accounts for damage inside the organism.

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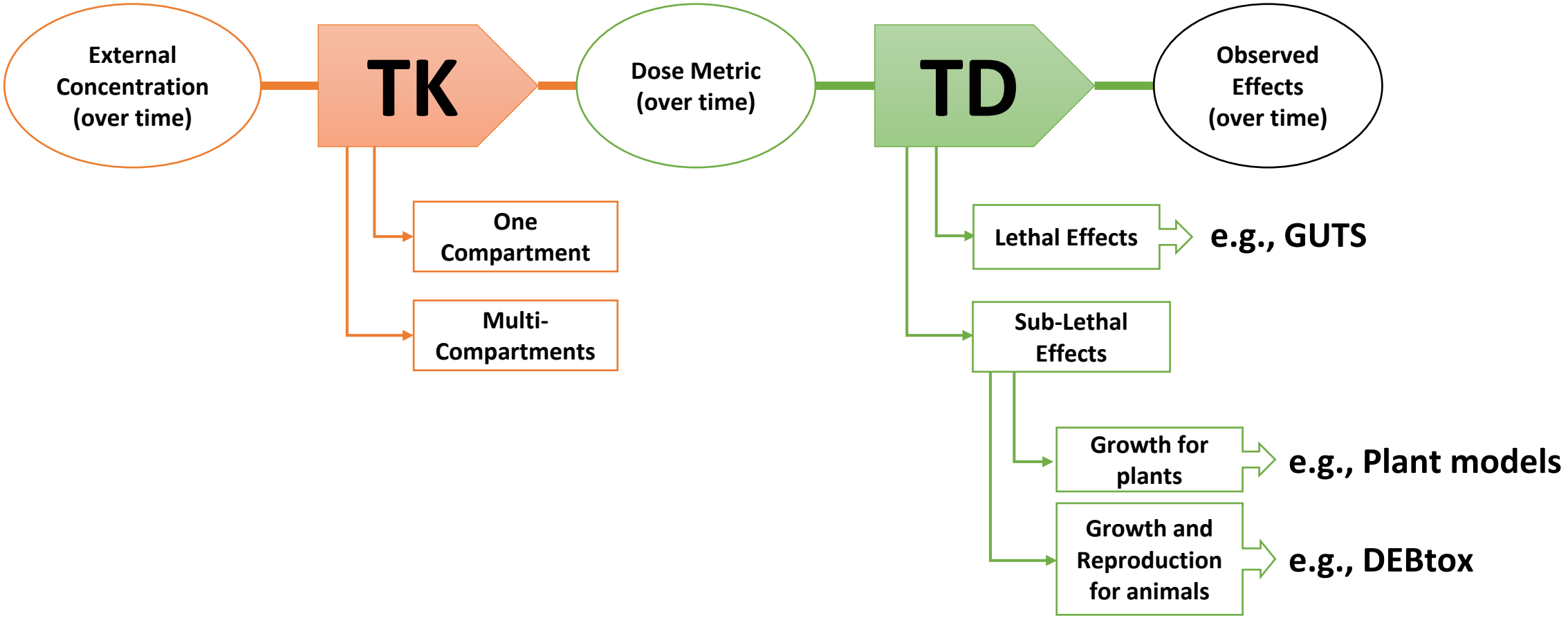
TKTD: the good compromise

Translate (time-varying) external concentrations to time patterns of effects.



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Lethal endpoints

The **G**eneral **U**nified
Threshold model of **S**urvival
GUTS

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What is GUTS?

ENVIRONMENTAL Science & Technology

CRITICAL REVIEW

2011, 45, 2529–2540

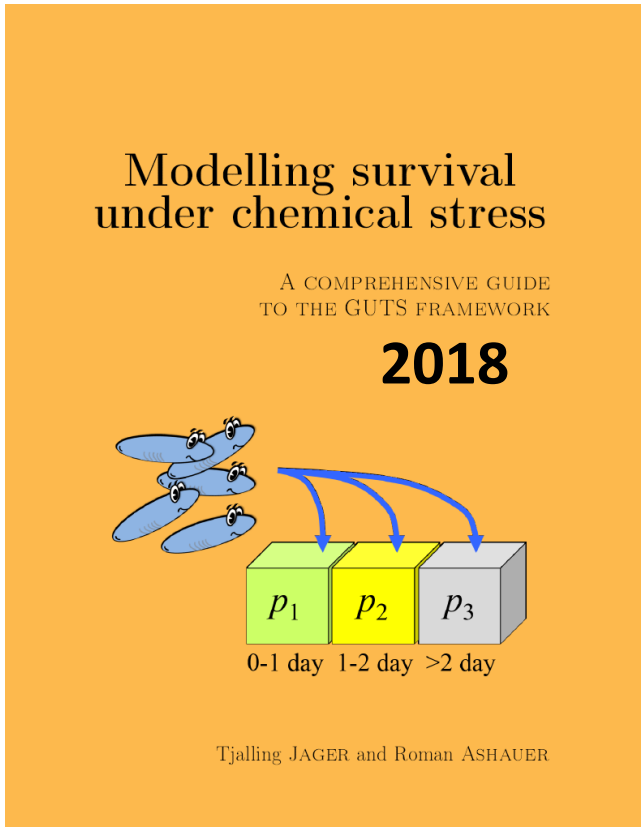
pubs.acs.org/est

General Unified Threshold Model of Survival - a Toxicokinetic-Toxicodynamic Framework for Ecotoxicology

Tjalling Jager,[†] Carlo Albert,[‡] Thomas G. Preuss,[§] and Roman Ashauer[‡,*]

→ A theoretical framework about stressors effects on survival over time, based on hypotheses related to:

- Stressor quantification (choice of a dose metric);
- Compensatory processes;
- Nature of the death process.



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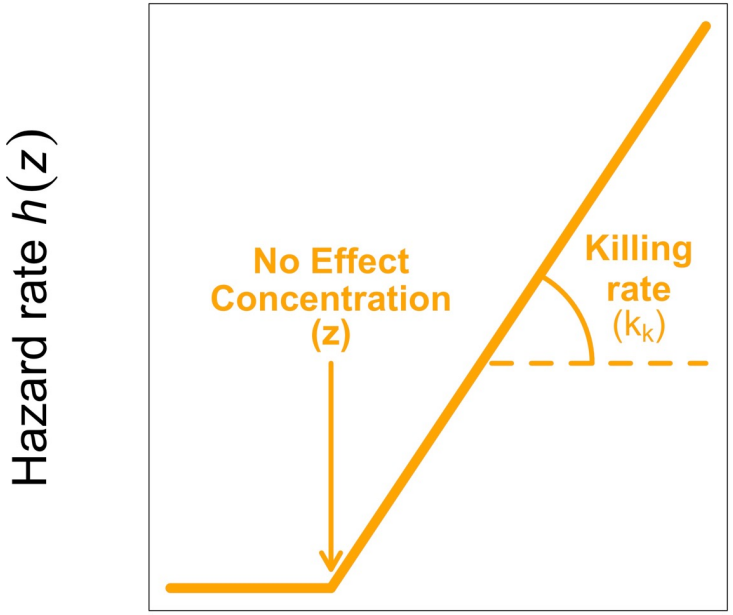
GUTS flavours depend on the death mechanism

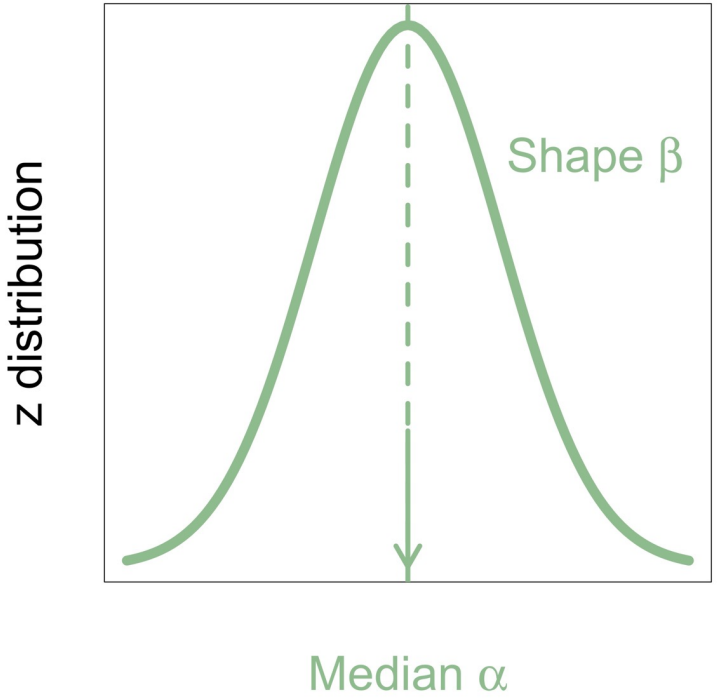
Stochastic death (SD)

- All individuals are identical
- Internal concentration increases probability to die



Individual tolerance (IT)

- Death is immediate if internal concentration > threshold
- Individuals differ in their threshold value



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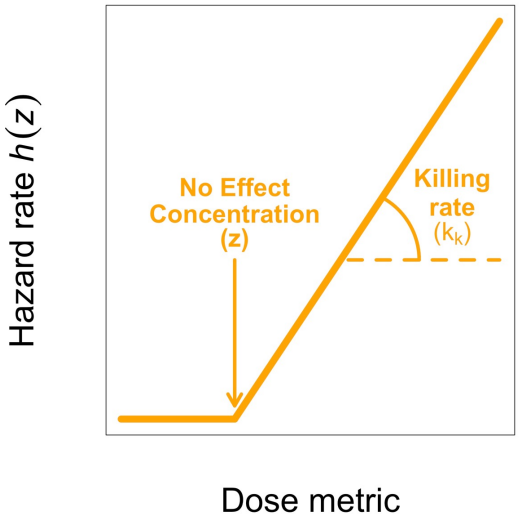
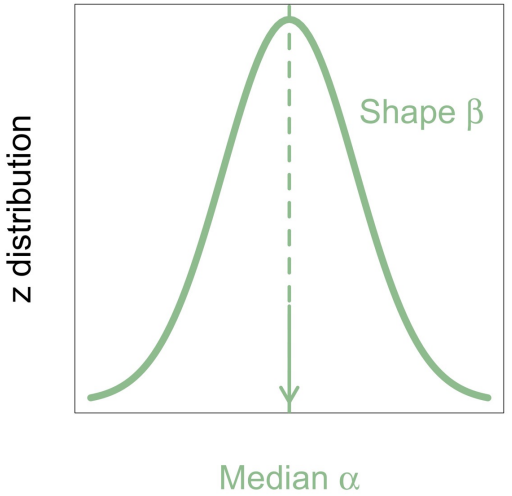
GUTS SD / IT unification





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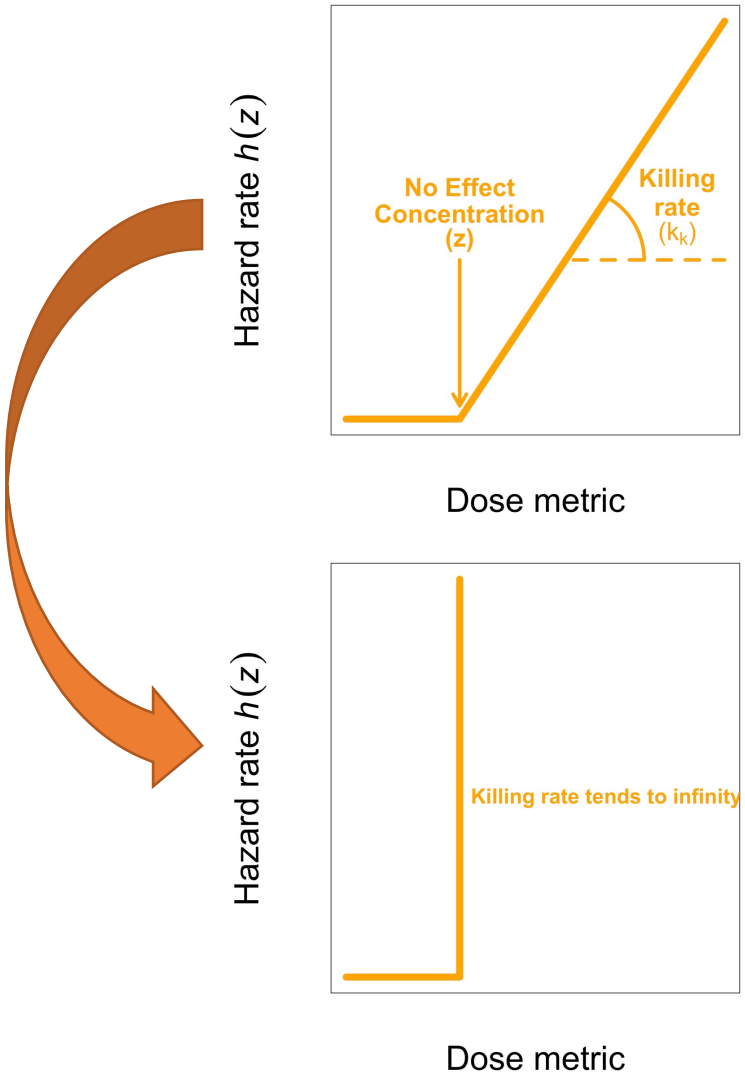
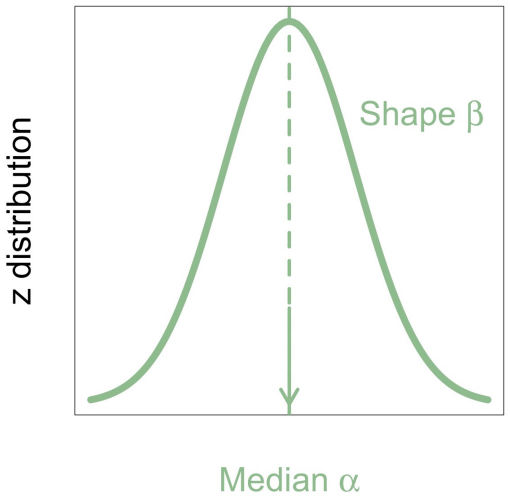
GUTS SD / IT unification





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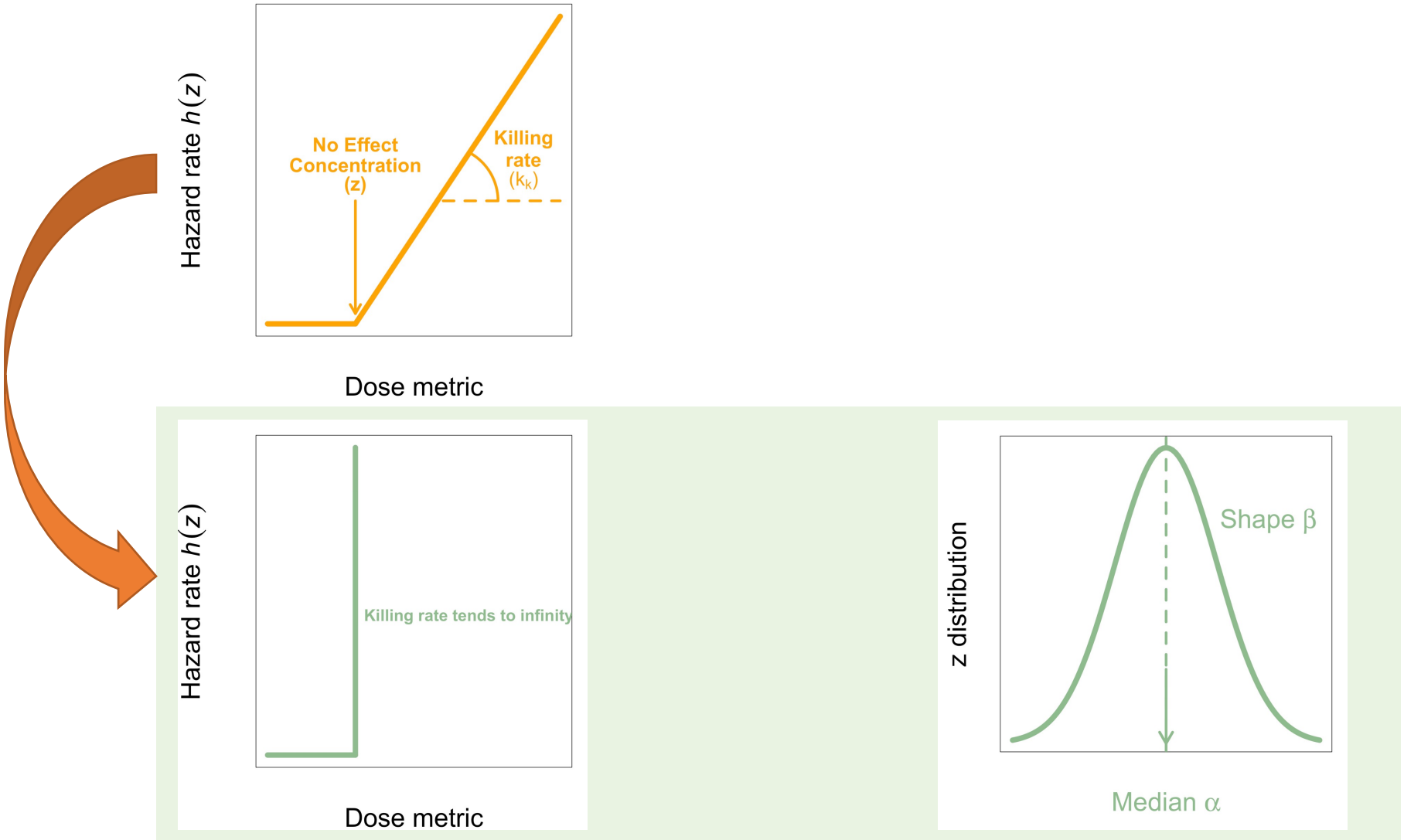
GUTS SD / IT unification



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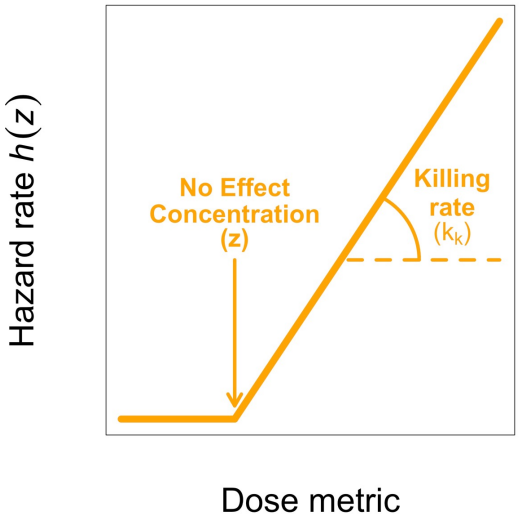
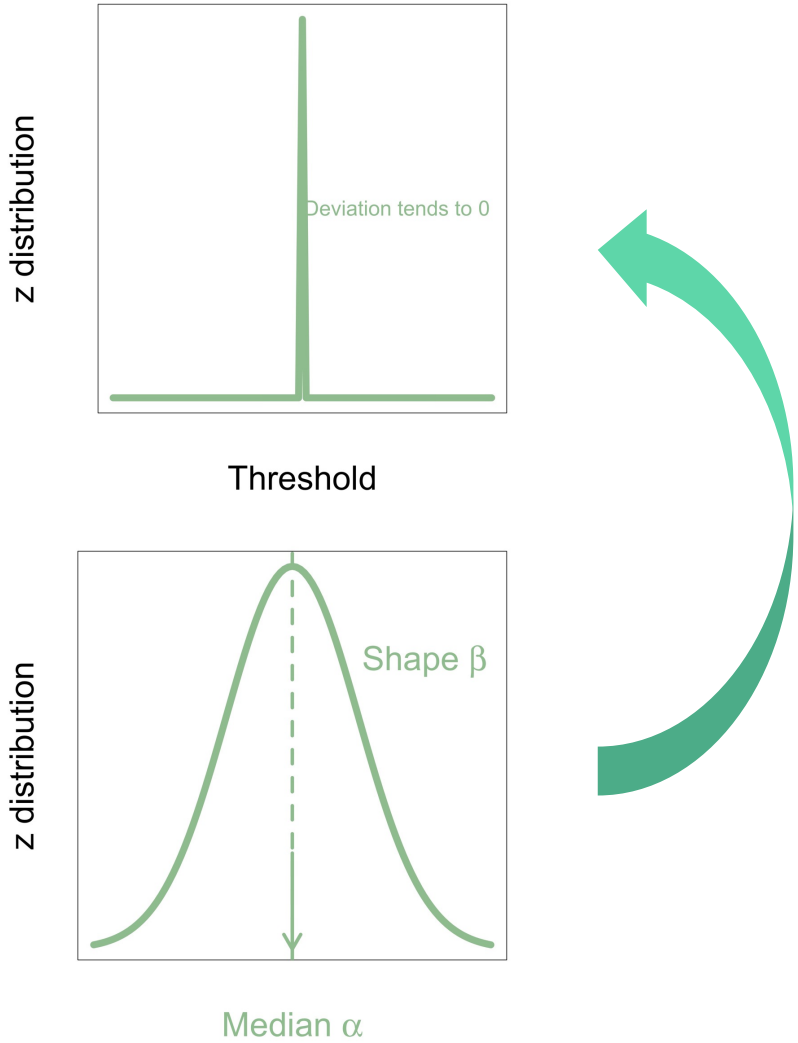
GUTS SD / IT unification





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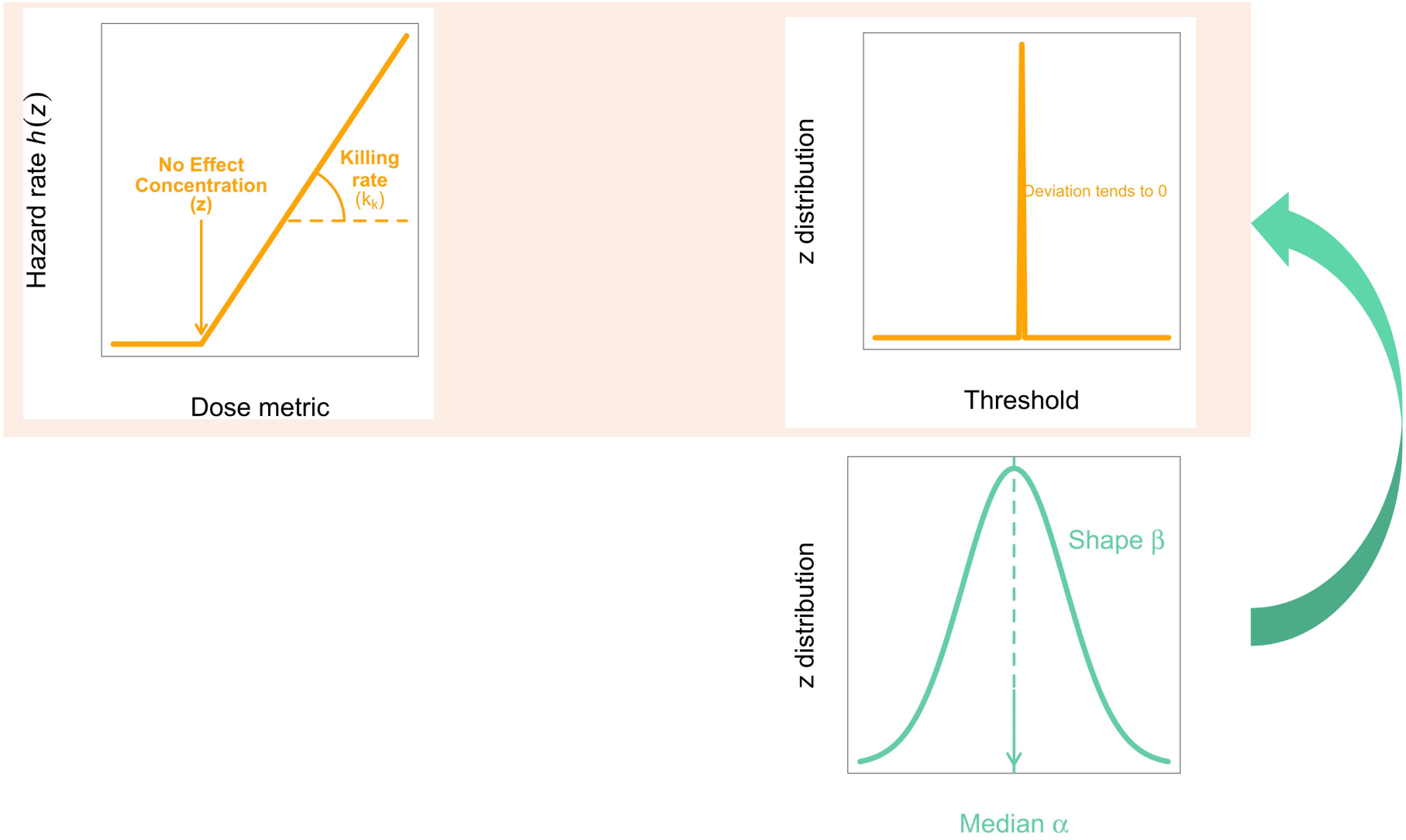
GUTS SD / IT unification



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GUTS flavours also depends on the dose metric

Dose metric

Effect model

Scaled internal concentration (**REDUCED**)	$C_i^*(t)$
Internal concentration (**IC**)	$C_i(t)$
Scaled damage (**D**)	$D^*(t)$

Stochastic death	**SD**
Individual tolerance	**IT**

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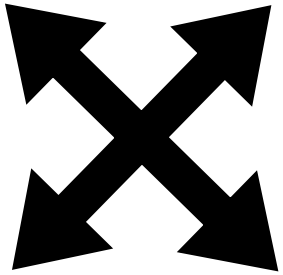
GUTS flavours also depends on the dose metric

REDUCED	IC	D
$C_i^*(t)$	$C_i(t)$	$D^*(t)$
$\frac{dC_i^*}{dt} = k_d\left(C_w - C_i^*\right)$	$\frac{dC_i}{dt} = k_u C_w - k_e C_i$	$\frac{dC_i}{dt} = k_u C_w - k_e C_i$ $\frac{dD^*}{dt} = k_r\left(C_i - D^*\right)$



$S_{SD}(t,\Theta)$

$S_{IT}(t,\Theta)$

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GUTS flavours

- GUTS-RED-SD
- GUTS-RED-IT

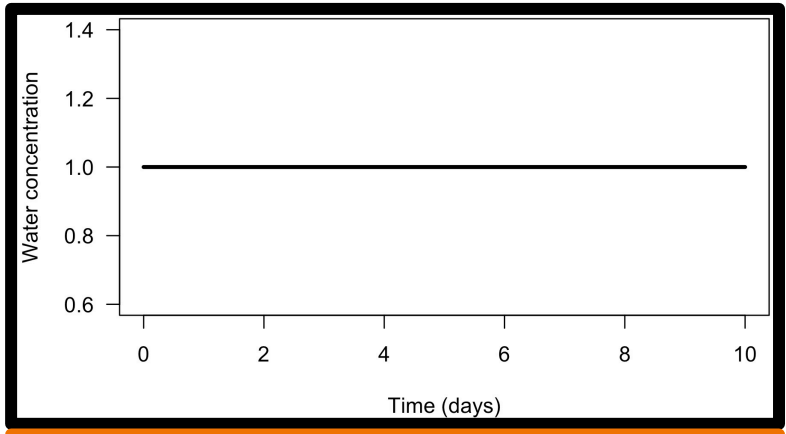
- GUTS-IC-SD
- GUTS-IC-IT

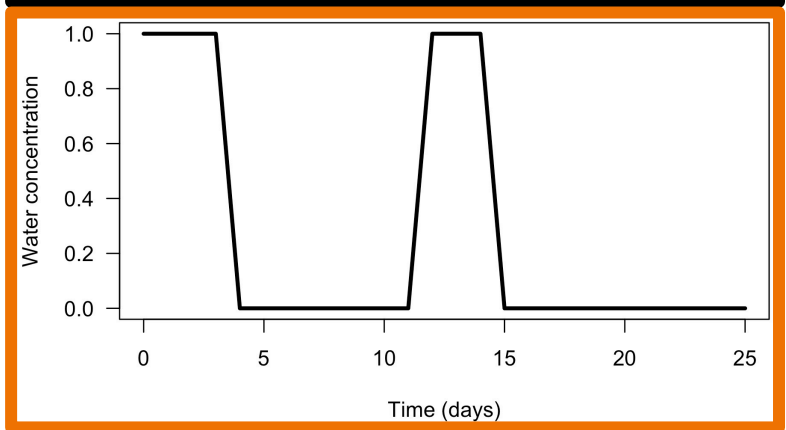
- GUTS-D-SD
- GUTS-D-IT

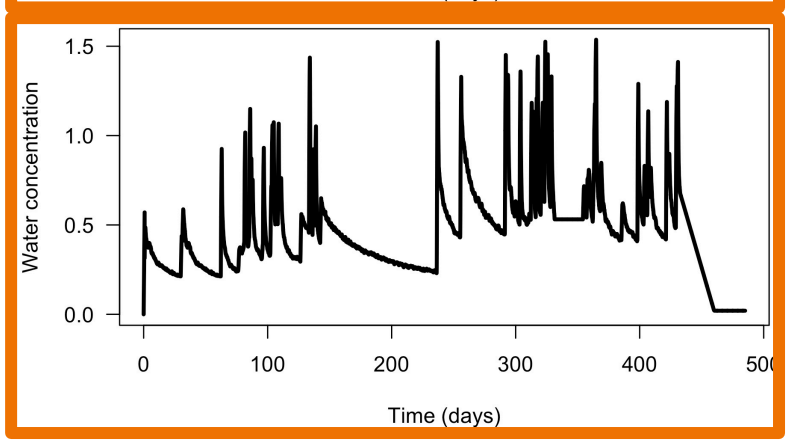
Six flavours

Constant exposure concentration

Variable exposure concentration







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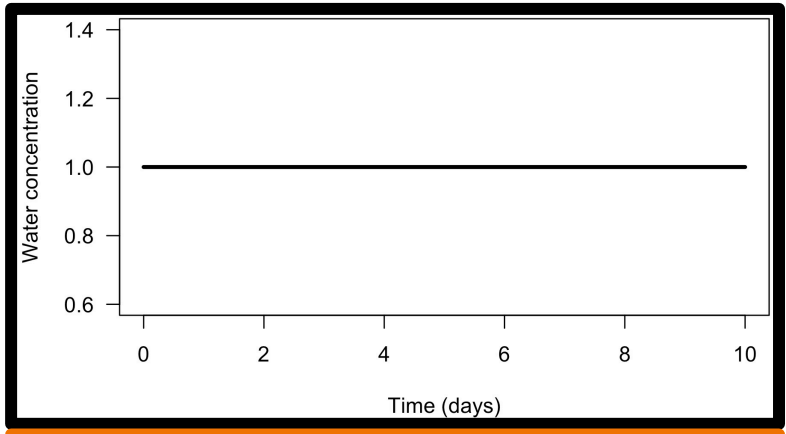
Training

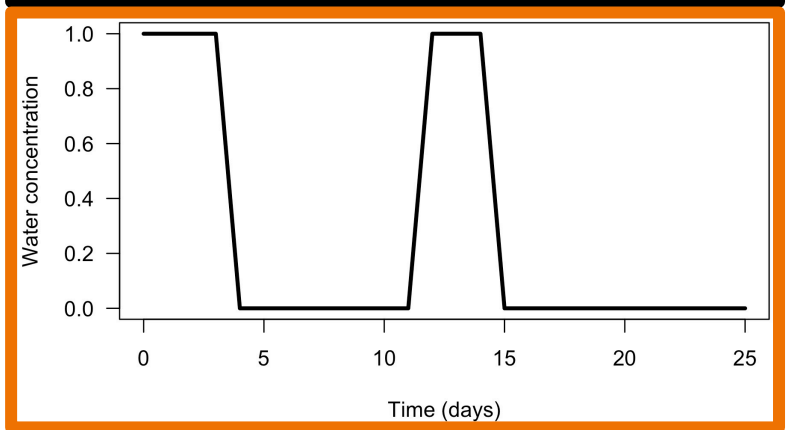
- GUTS-RED-SD
- GUTS-RED-IT

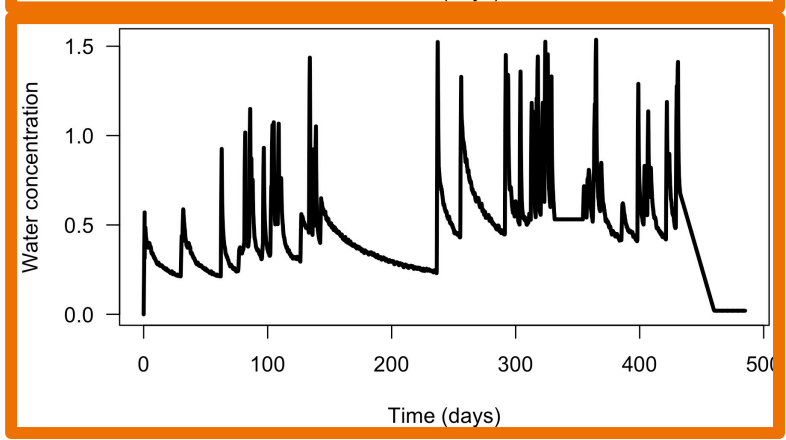
Constant exposure concentration

GUTS-RED

Variable exposure concentration







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TKTD models for Environmental Risk Assessment

Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms

efsa
European Food Safety Authority

EFSA Journal

https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5125#

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TKTD models for Environmental Risk Assessment

Scientific Opinion on the st… t of Toxicokinetic/Tox… D) effect model… isk assessment of … for aquatic organisms

APPROVED 27-06-2018

https://efsa.onlinelibrary.wiley.com/doi/full/10.2903/j.efsa.2018.5125#

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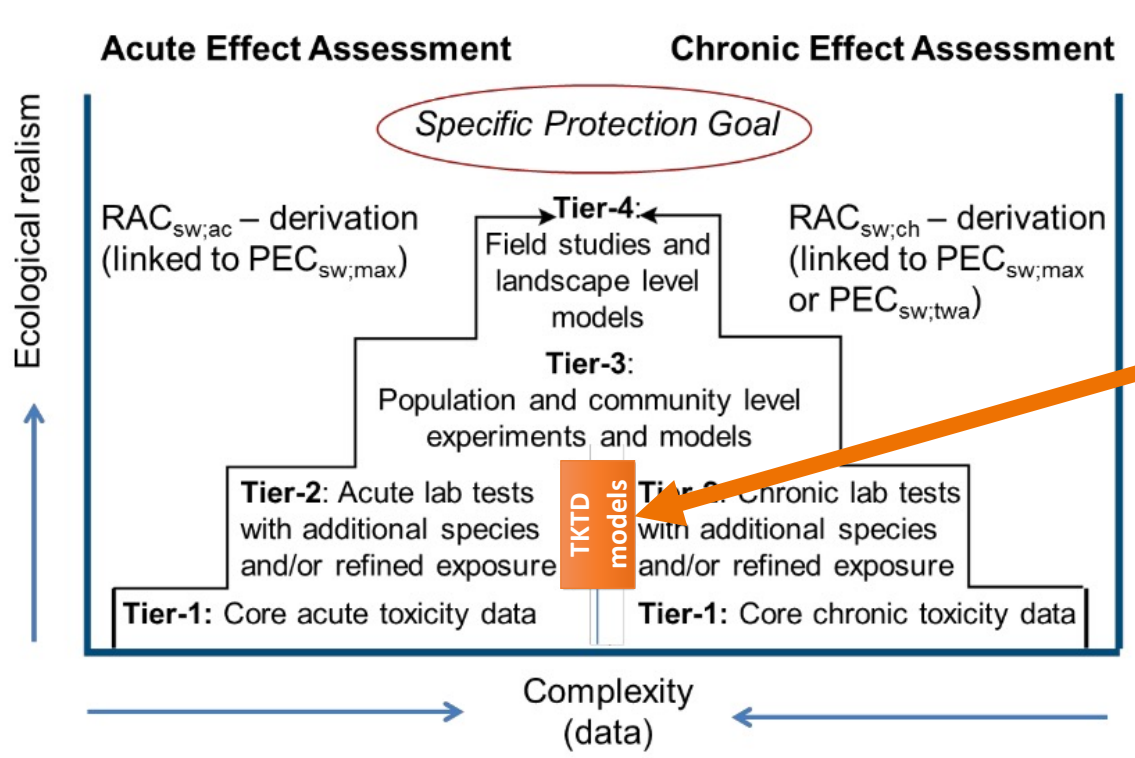
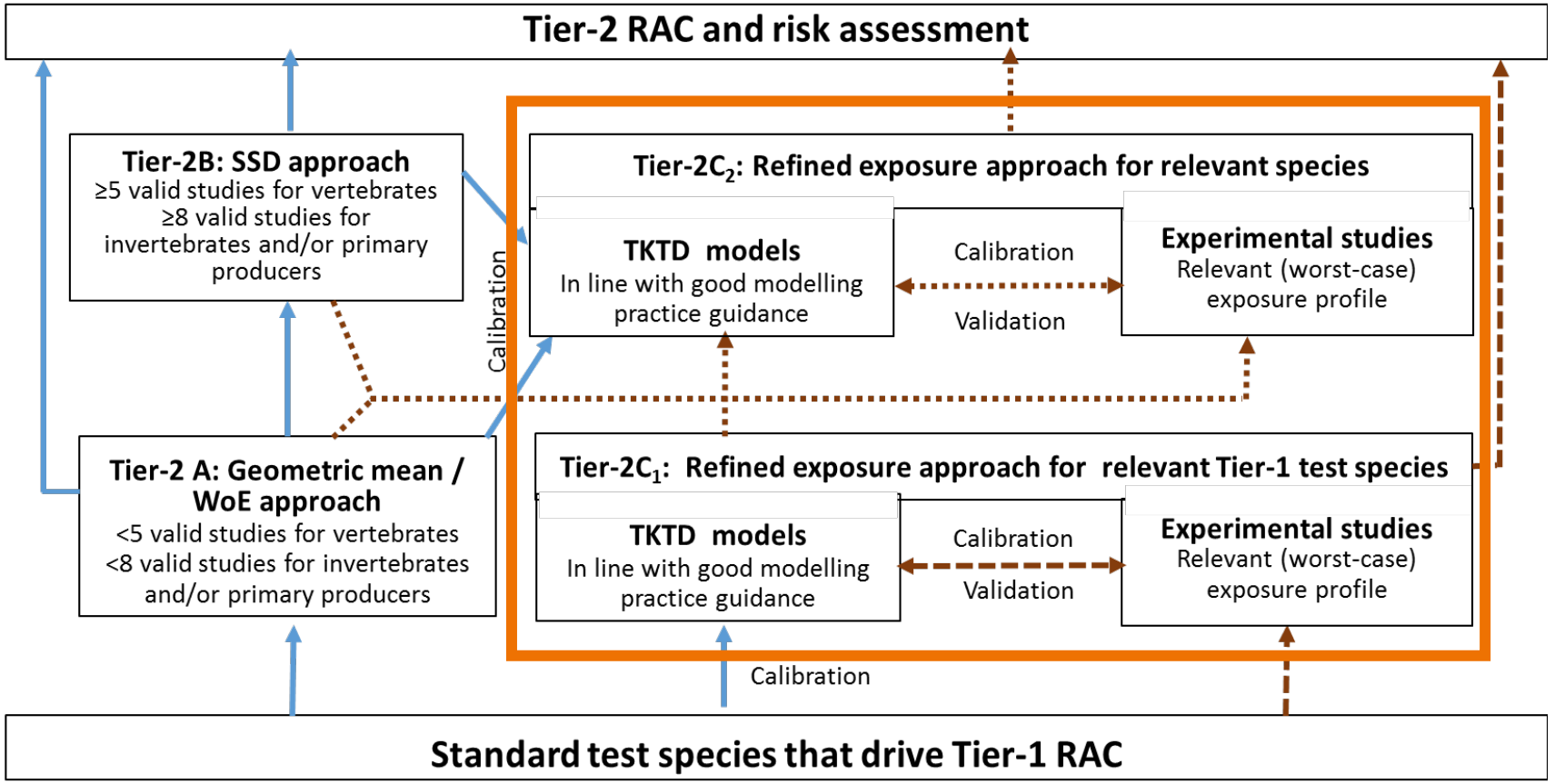
2013 – Aquatic Guidance Document



efsa

European Food Safety Authority

TKTD models in Tier-2C



Solid blue lines → **Standardised exposure**:
Experimental studies with standard and/or additional test species and exposure conditions in line with Tier-1 tests (worst-case approach)

Broken lines - - - → **Refined exposure (Tier-2C$_1$):**
····· → **Refined exposure (Tier-2C$_2$):**
Tests with standard and/or additional species and refined exposure conditions informed by predicted field exposure profiles

TC: GUTS modelling

TKTD models in Tier-2C

- TKTD modelling may be used to address (the threshold for) individual-level effects occurring from **time-variable exposure regimes** on aquatic vertebrates and invertebrates (Tier-2C), **even if TKTD models could also be used from data collected at Tier-1**;
- The **GUTS** framework is appropriate as it is for lethal effects in the acute and the chronic risk assessment scheme;
- The **DEBtox** framework is promising and seen as very relevant for sub-lethal effects in the chronic risk assessment scheme;
- **Plant models** need further standardisation, documentation, calibration and validation, except for *Lemna minor* which is ready-to-use.

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1 Calibration

Fit a TKTD model on toxicity test data and get parameter estimates associated with their uncertainty

2 Validation

Simulate an effect over time under a time-variable exposure profile and compare with observed data from a refined toxicity test

→ Three validation criteria are recommended by EFSA

3 Prediction

Make simulations under realistic scenarios to assess risk on how far is the exposure profile from causing a pre-defined effect.

Using GUTS models → Concept of the x% Lethal Profile (LPx) = Multiplication Factor leading to an additional x% reduction in the final survival rate.

efsa European Food Safety Authority

workflow for ERA

Step 1: calibration (inference)

- Tier-1, Tier-2A and/or Tier-2B toxicity data sets as well as dedicated refined exposure tests with the selected species of concern can be used.
- Data sets are expected to span from treatment levels with no effects up to large effects, ideally including full effects (e.g. 0% survival).
- At least 5 time points are expected.

- **Example for GUTS**:

  Survival data of chronic laboratory toxicity test with 10 organisms of *Gammarus pulex* freshwater invertebrates exposed to 8 tested concentrations of one fungicide (propiconazole, expressed in nmol.mL−1) with 2 replicates from day 0 to day 4. Concentration is constant over time.

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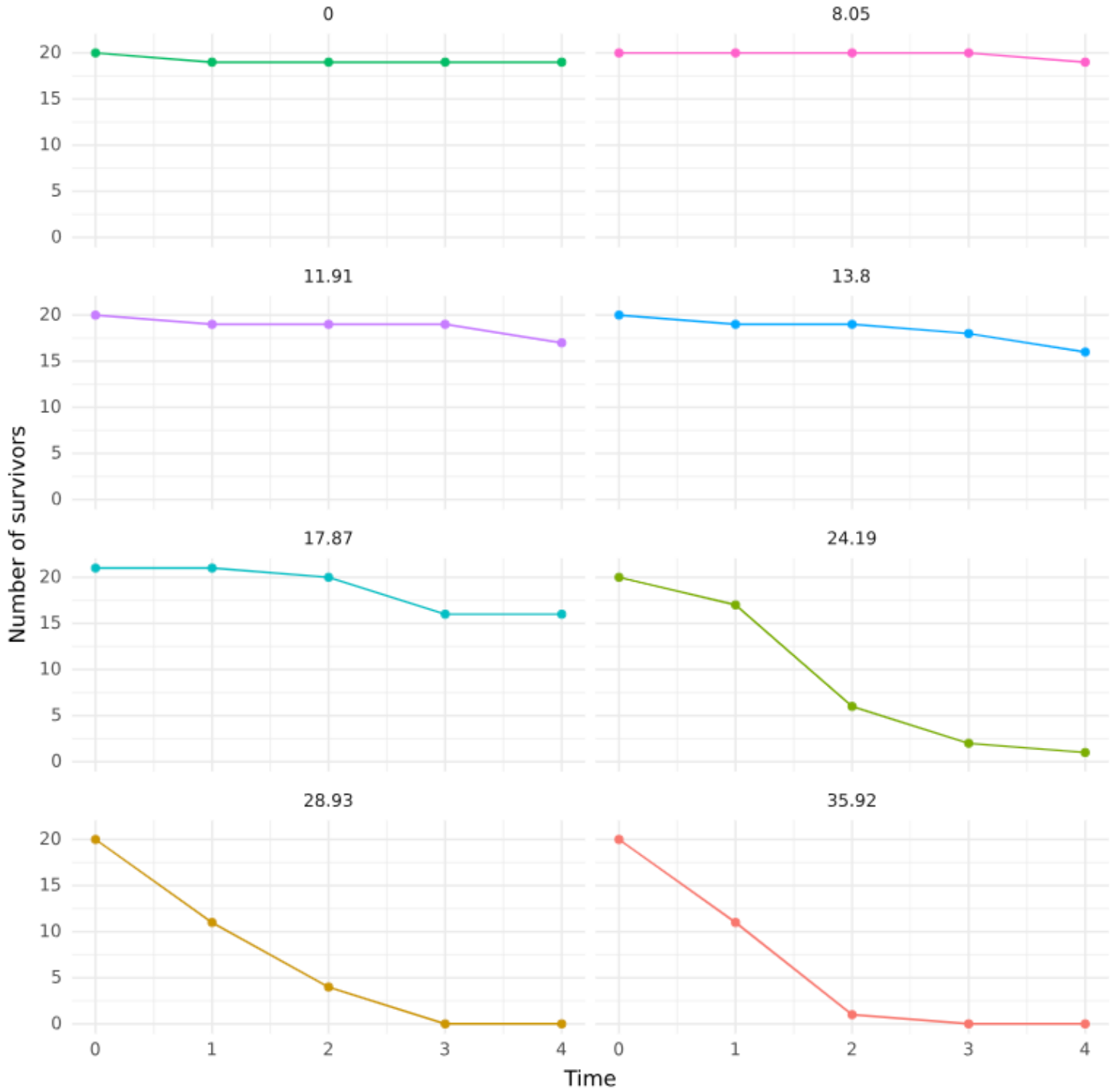


Example of calibration data:

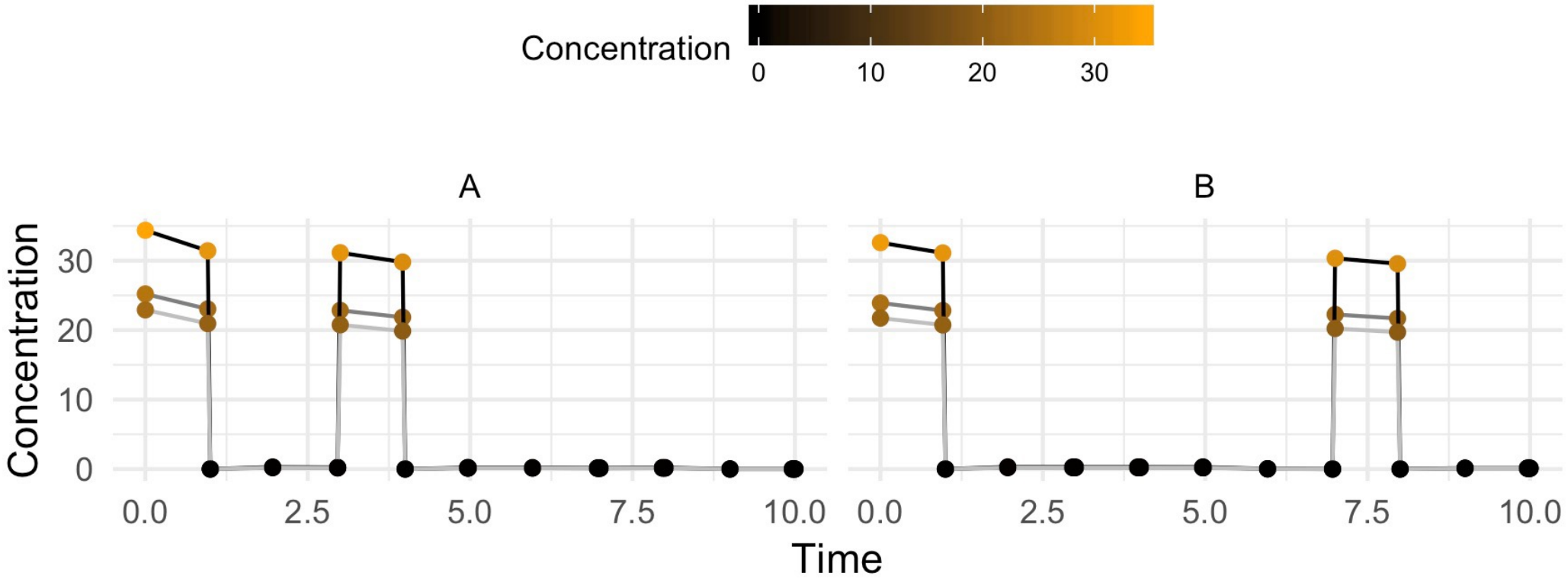
Gammarus pulex

Propiconazole

Nyman, A.-M., Schirmer, K., Ashauer, R., (2012) Toxicokinetic-toxicodynamic modelling of survival of *Gammarus pulex* in multiple pulse exposures to propiconazole: model assumptions, calibration data requirements and predictive power. *Ecotoxicology*, (21), 1828-1840.

Step 2: validation

- Validation experiments should include at least 2 different profiles with at least 2 pulses each;



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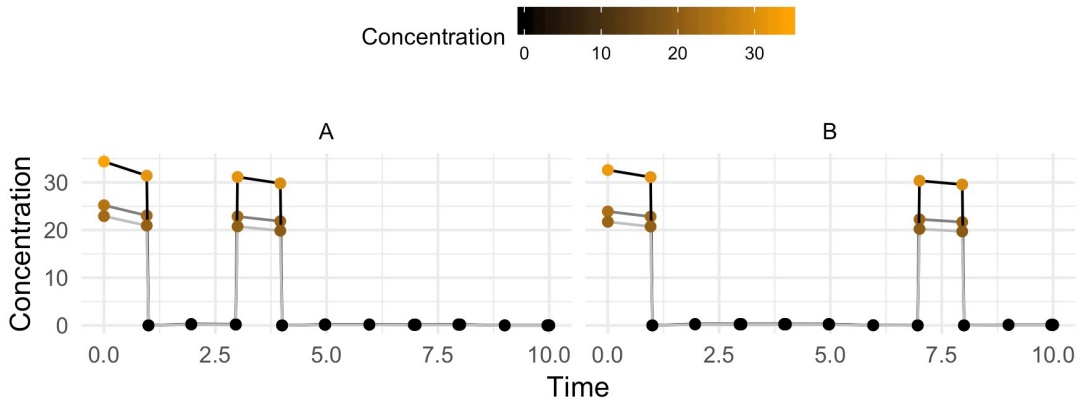
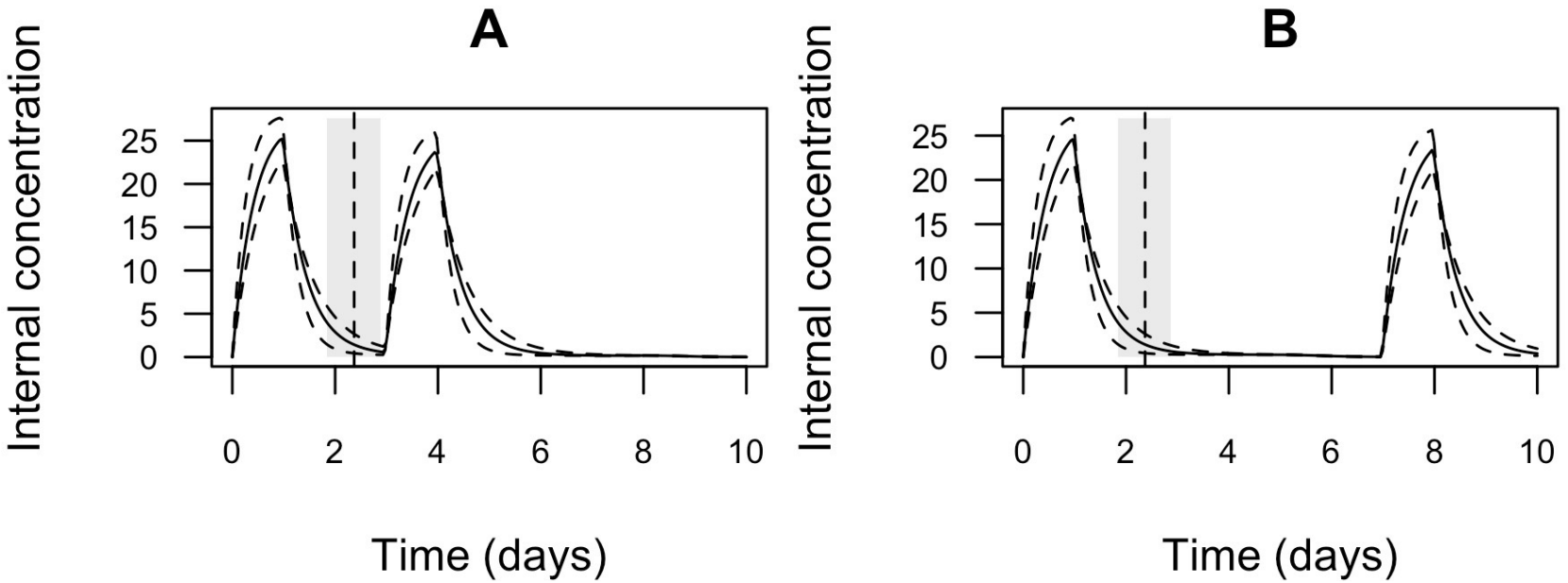
Step 2: validation



- Validation experiments should include at least 2 different profiles with at least 2 pulses each;

- The individual depuration and repair time (DRT_{95}) should be calculated and considered for the timing of the pulses.

$$DRT_{95} = -\frac{\ln(0.05)}{k_d}$$

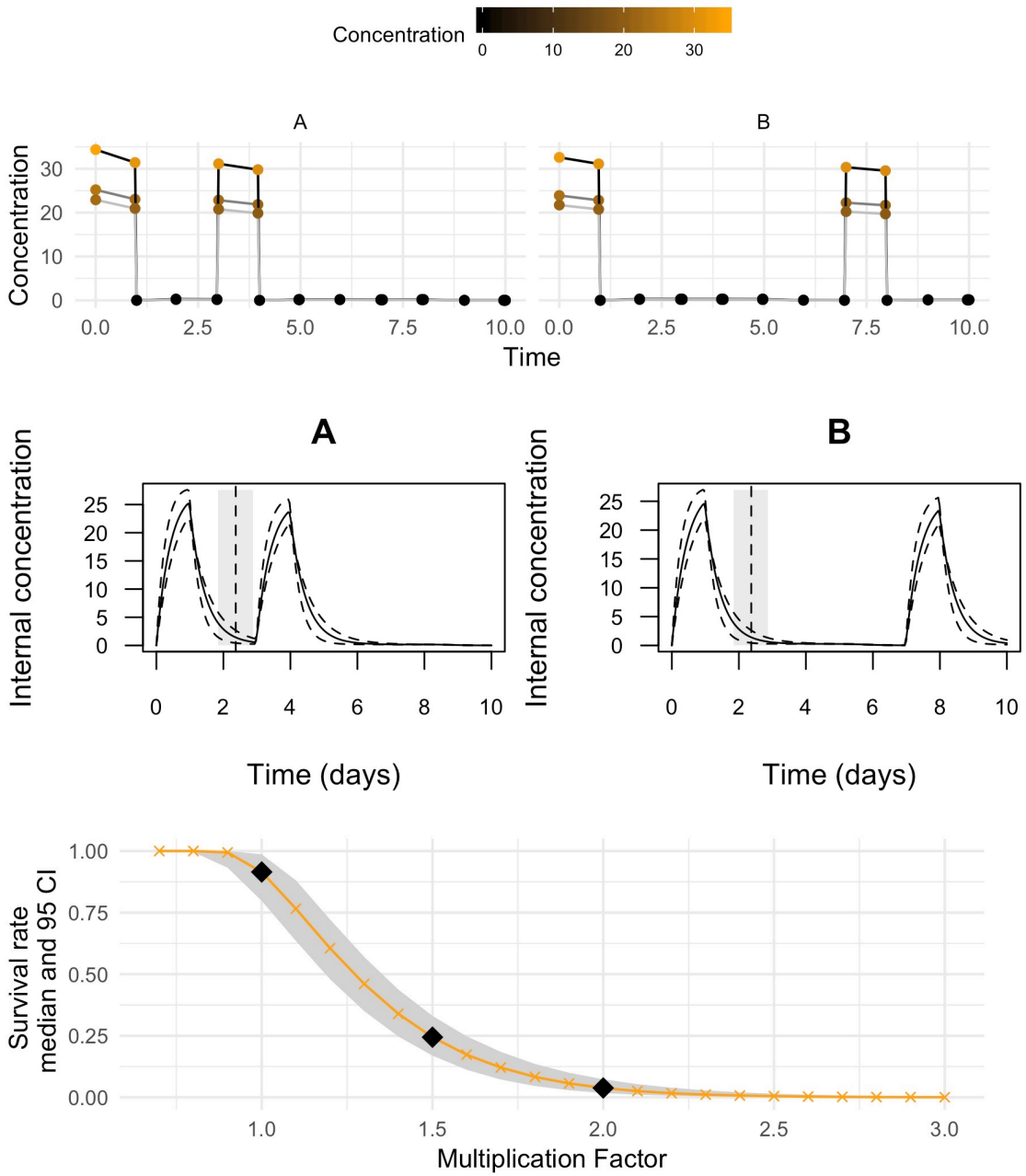


Contact

Step 2: validation

- Validation experiments should include at least 2 different profiles with at least 2 pulses each;
- The individual depuration and repair time (DRT_{95}) should be calculated and considered for the timing of the pulses.
- For each pulse at least 3 concentrations should be tested leading to low, medium, and strong effects;

→Calibrated TKTD models can be used with simulations to optimize the design of validation experiments



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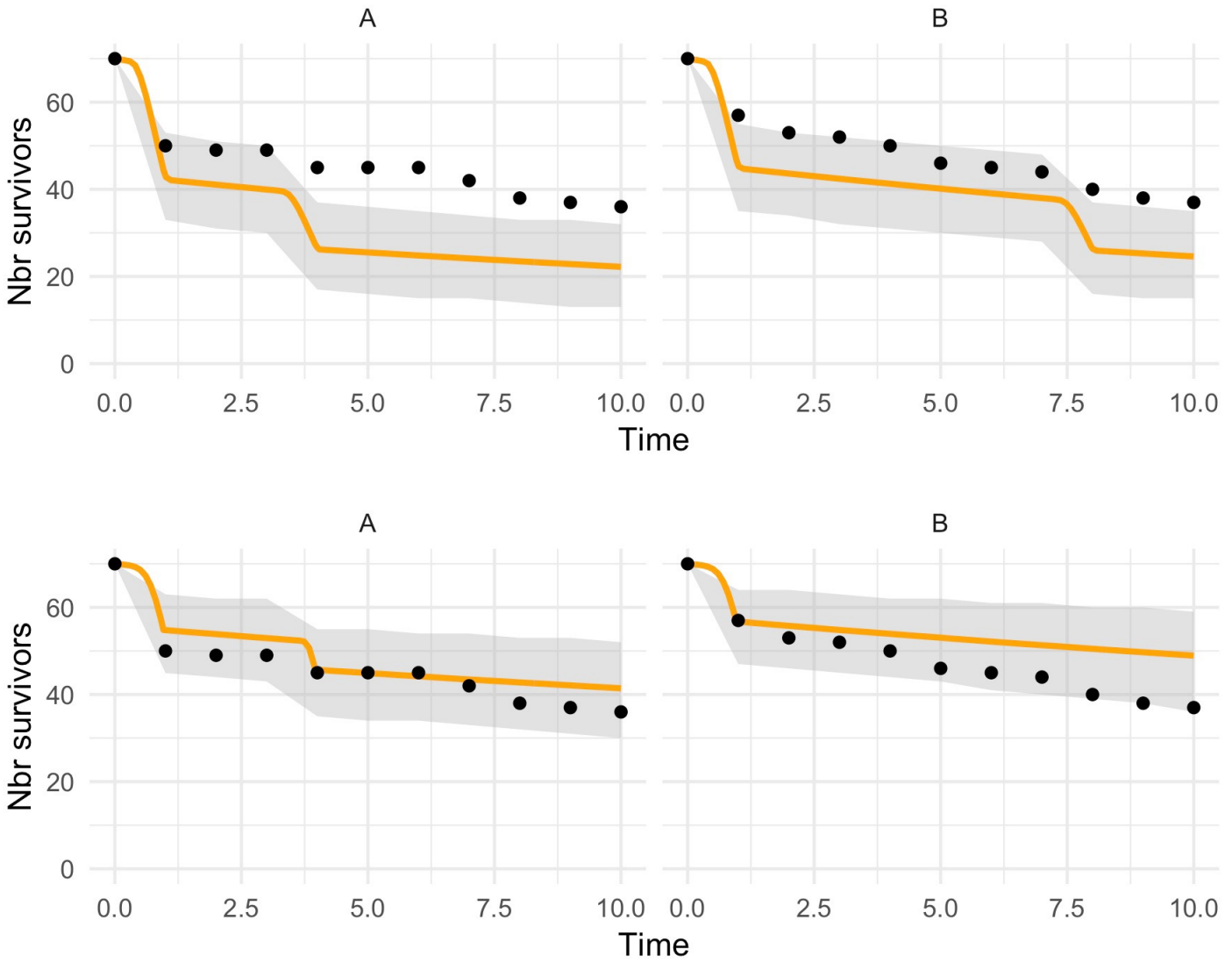
Step 2: validation output (GUTS models)

GUTS-RED-SD

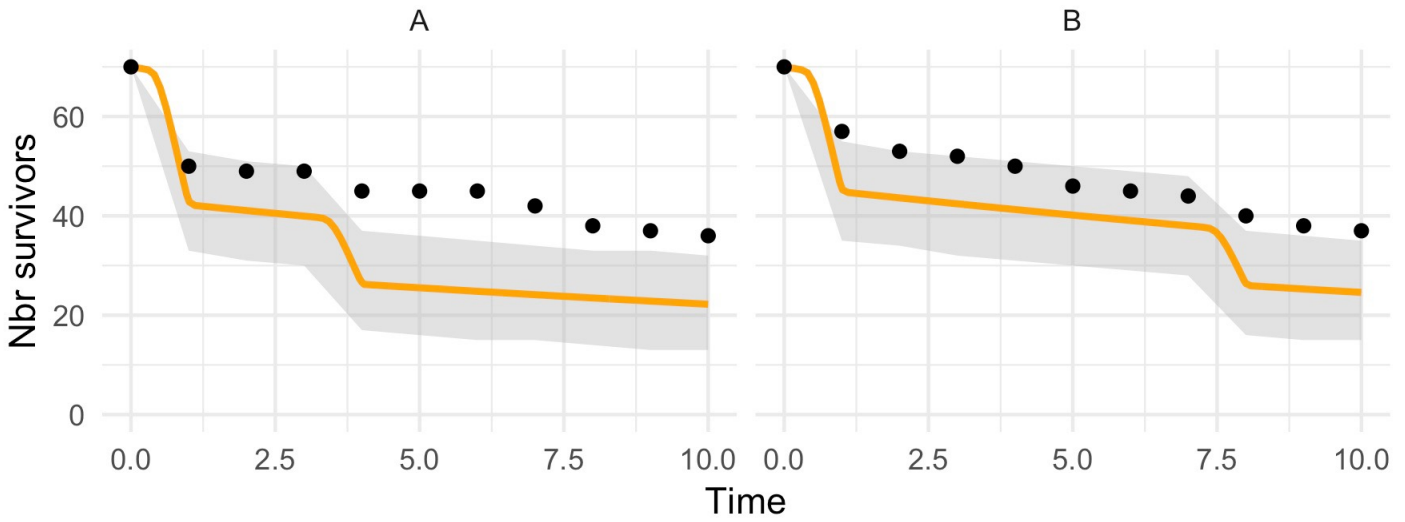
The expected number of survivors along the exposure profile

GUTS-RED-IT

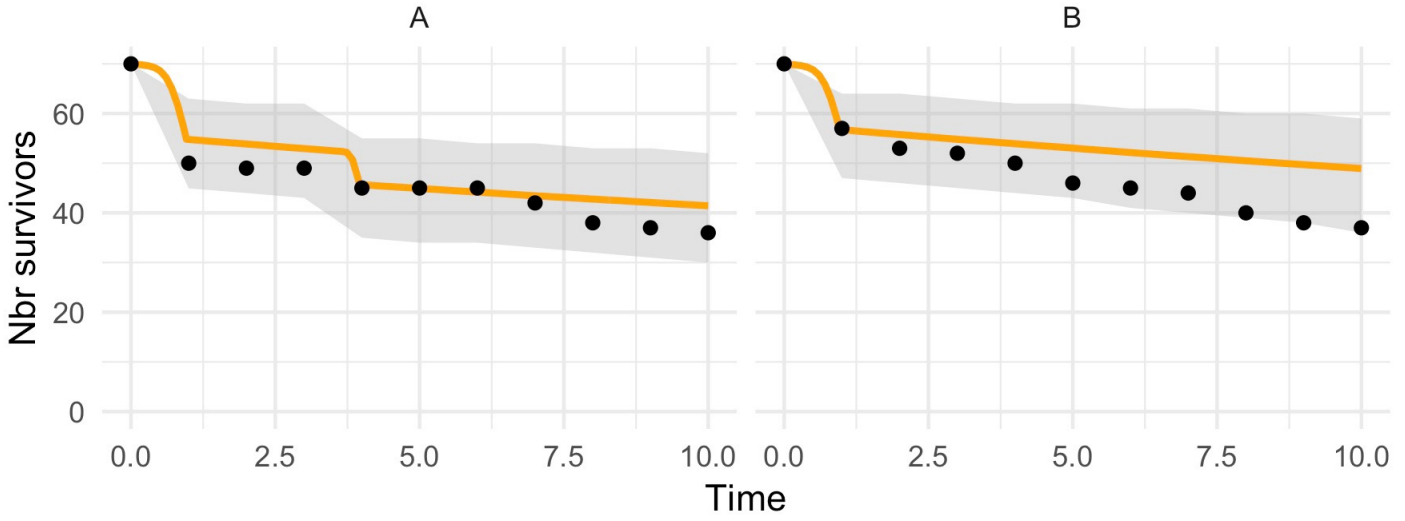


Step 2: 1st validation criterion → visual check

GUTS-RED-SD



GUTS-RED-IT



Step 2: quantitative validation criteria

1. **Posterior Predictive Check** (PPC)
   - Plot of the predicted numbers of survivors associated to their uncertainty limits versus the observed numbers of survivors;
   - Count of how frequently the uncertainty limits intersect with the 1:1 line

   → PPC resulting in less than 50% of the observations within the uncertainty limits indicates poor model performance for validation.

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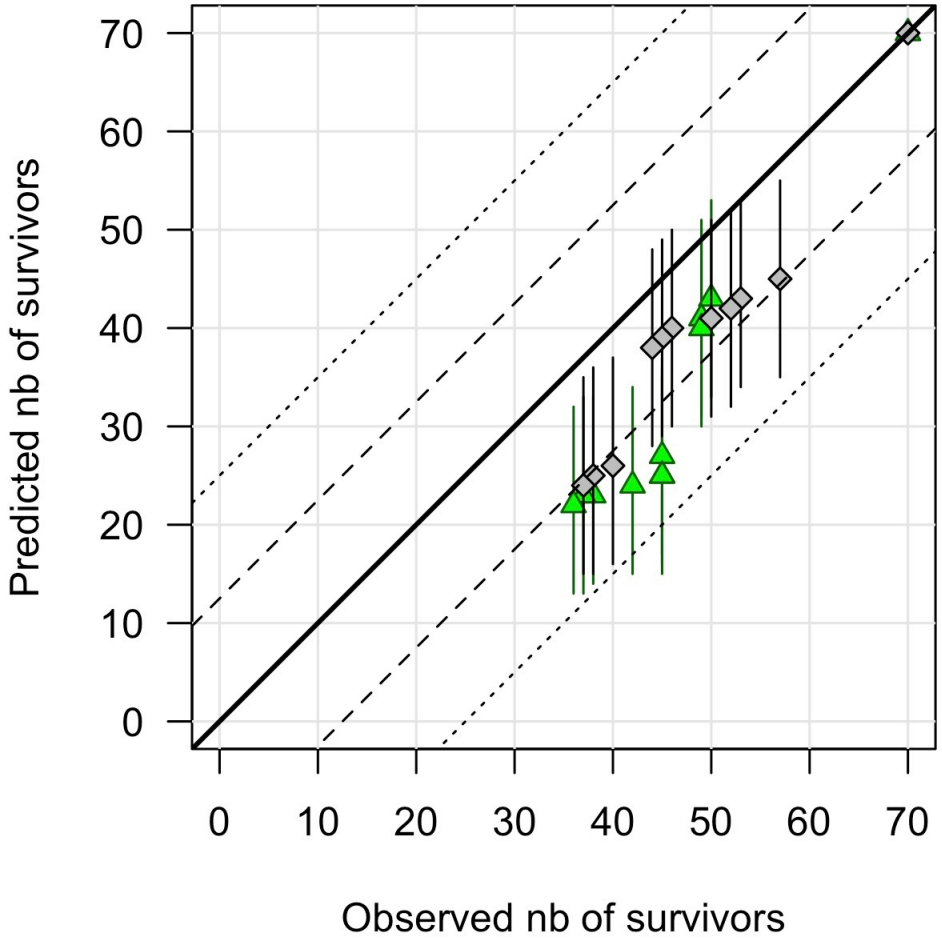
Step 2: quantitative validation criteria

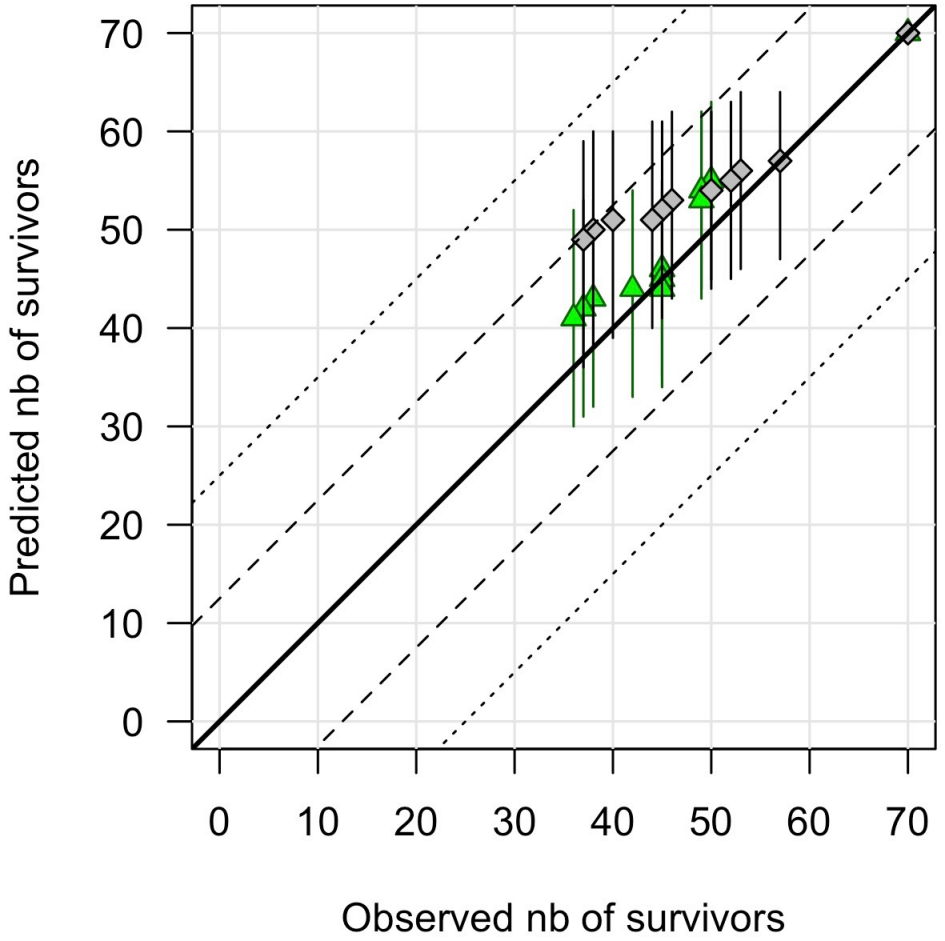
1. **Posterior Predictive Check** (PPC)



GUTS-RED-SD (left)
%PPC = 50%

GUTS-RED-IT (right)
%PPC = 100%

Green triangles: profile ‘A’
Grey diamonds: profile ‘B’



Step 2: quantitative validation criteria

1. **Posterior Predictive Check** (PPC)
2. **Normalized Root-Mean Square Error** (NRMSE)

$$NRMSE = \frac{1}{\overline{Y}} \sqrt{\frac{1}{n} \sum_{i=1}^{n} \left(y_{obs,i} - y_{pred,i} \right)^2}$$

→ It is expected that the NRMSE should not exceed the upper limit of 0.5 (50%).

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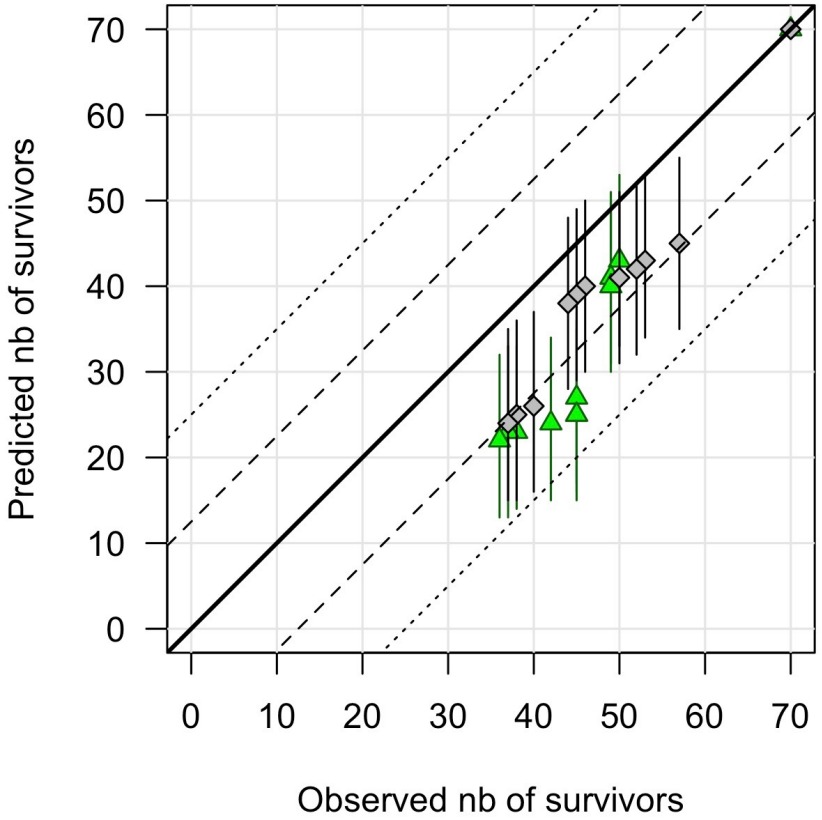
Step 2: quantitative validation criteria

1. **Posterior Predictive Check** (PPC)
2. **Normalized Root-Mean Square Error** (NRMSE)



GUTS-RED-SD (left)

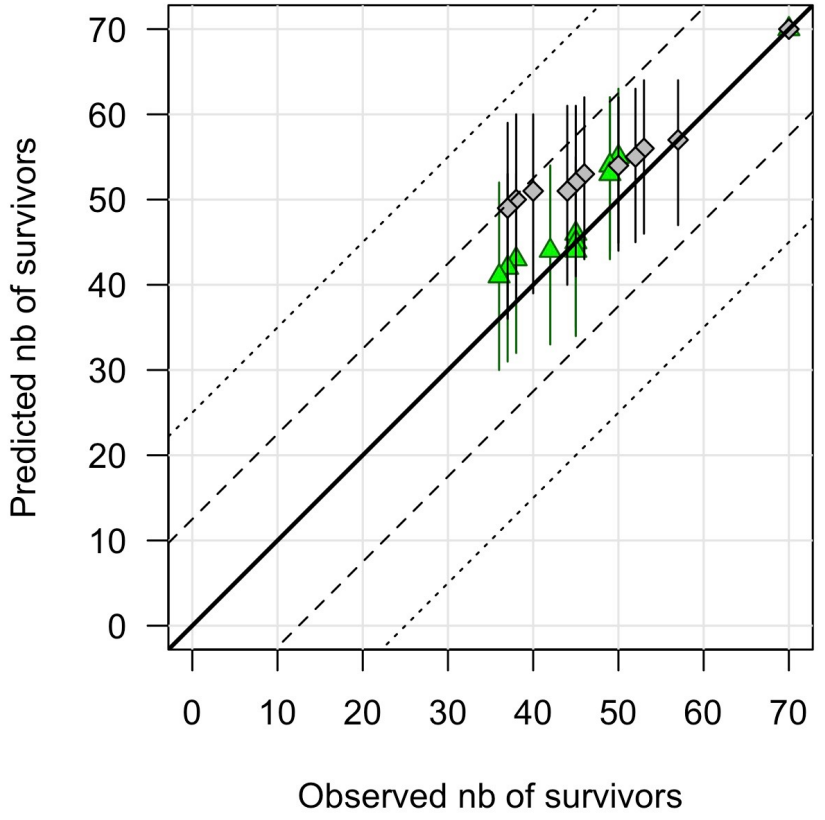
NRMSE-A = 31%

NRMSE-B = 20%

GUTS-RED-IT (right)

NRMSE-A = 8%

NRMSE-B = 15%



Step 2: quantitative validation criteria

1. **Posterior Predictive Check** (PPC)
2. **Normalized Root-Mean Square Error** (NRMSE)
3. **Survival Probability Prediction Error**

   Comparison survival probabilities between the beginning and the end of the exposure profile:

$$SPPE = \frac{y_{obs,t.end} - y_{pred,t.end}}{y_{init}} \times 100$$

→ SPPE is negative (resp. positive) for an underestimation (resp. overestimation) of effects. A 0% value means an exact prediction.

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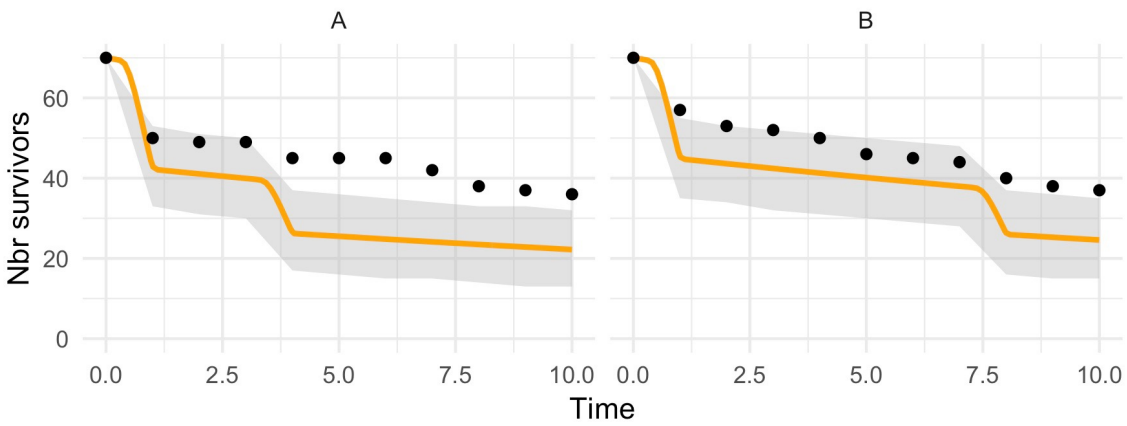
Step 2: quantitative validation criteria

1. **Posterior Predictive Check** (PPC)
2. **Normalized Root-Mean Square Error** (NRMSE)
3. **Survival Probability Prediction Error**

GUTS-RED-SD

SPPE-A = 20%

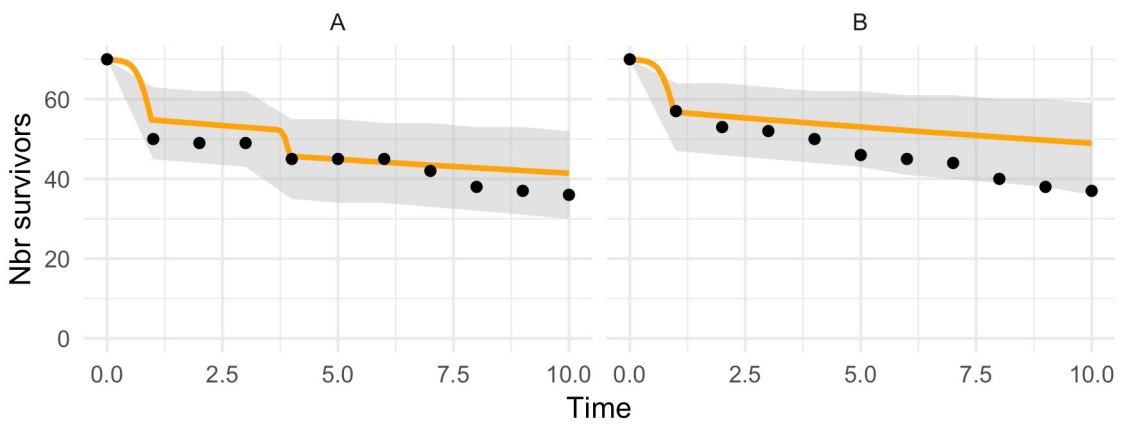
SPPE-B = 18%



GUTS-RED-IT

SPPE-A = -7%

SPPE-B = -17%

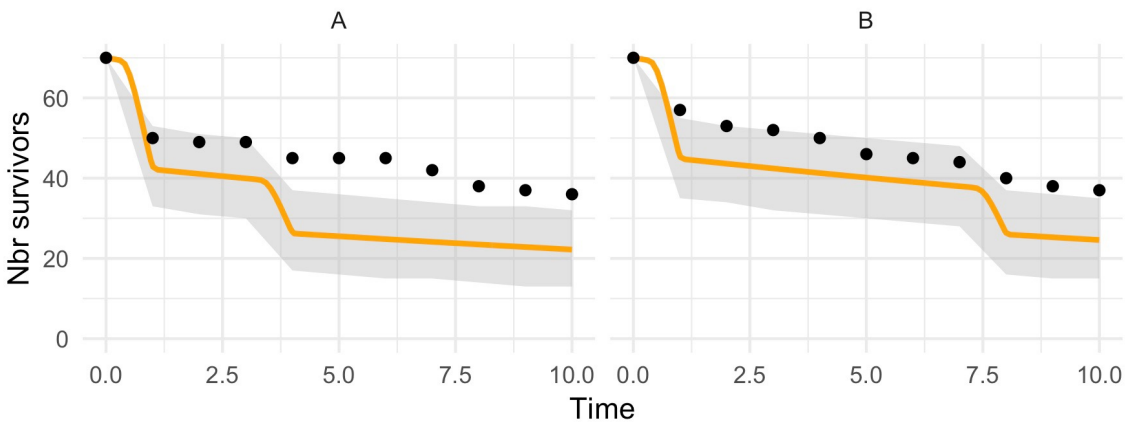


Step 2: validation performance

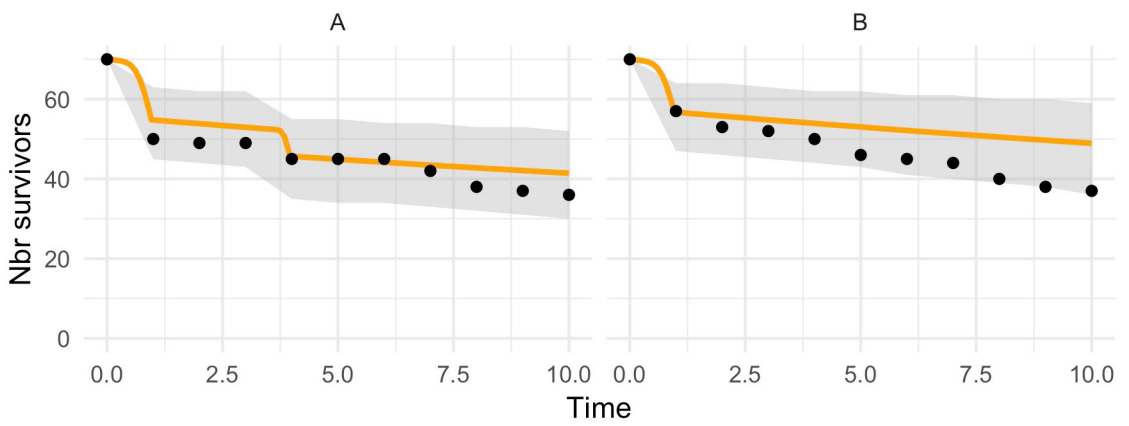
0. **Visual check ⟹ OK**
1. **%PPC ⟹ OK**
2. **NRMSE ⟹ OK**
3. **SPPE ⟹ OK**

⟹ GUTS-RED-SD could be finally preferred as more conservative

GUTS-RED-SD

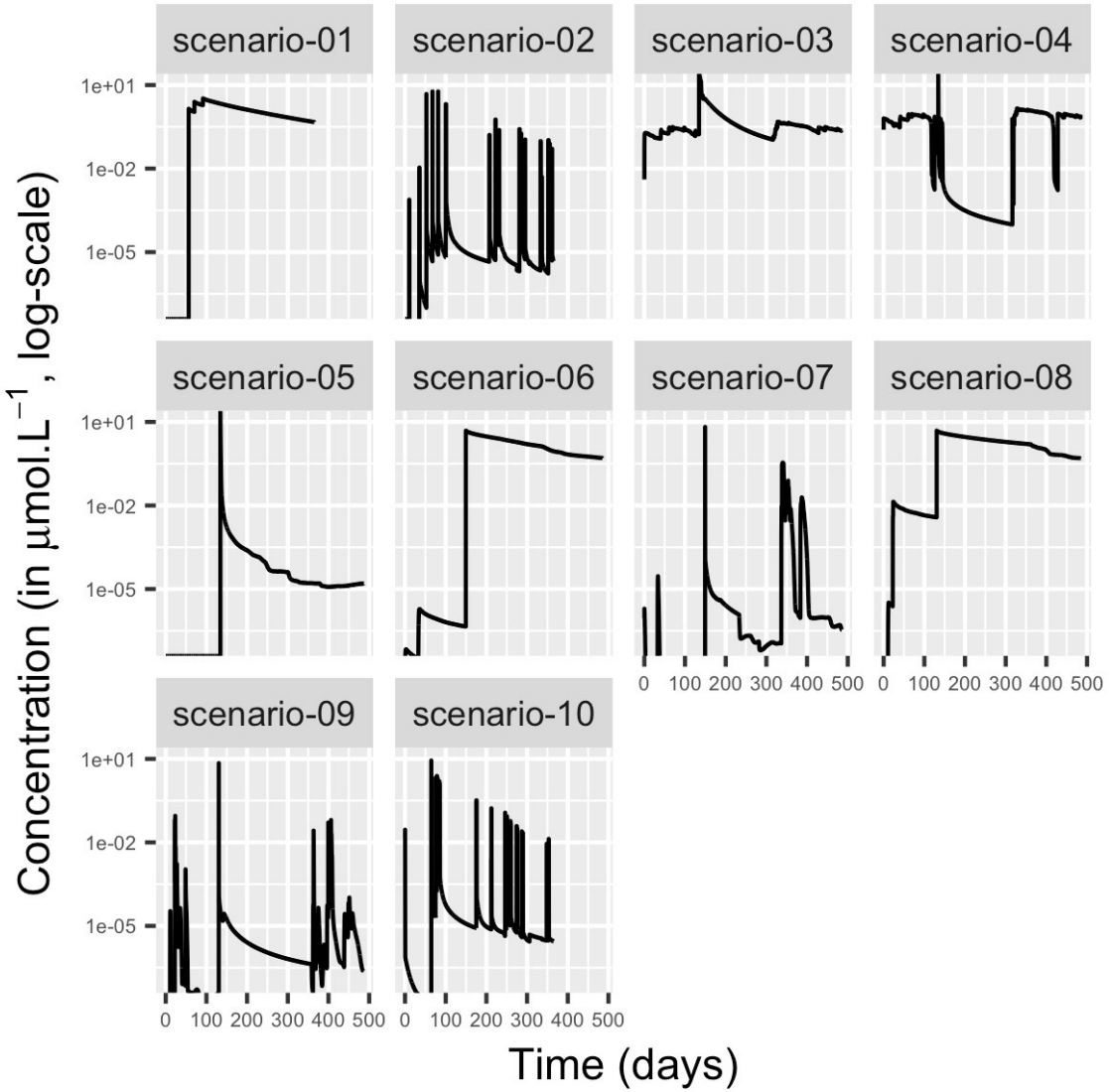


GUTS-RED-IT



Step 3: prediction under realistic profiles

Very often, no effects of realistic time-variable exposure scenarios on survival rate are predicted by GUTS models.



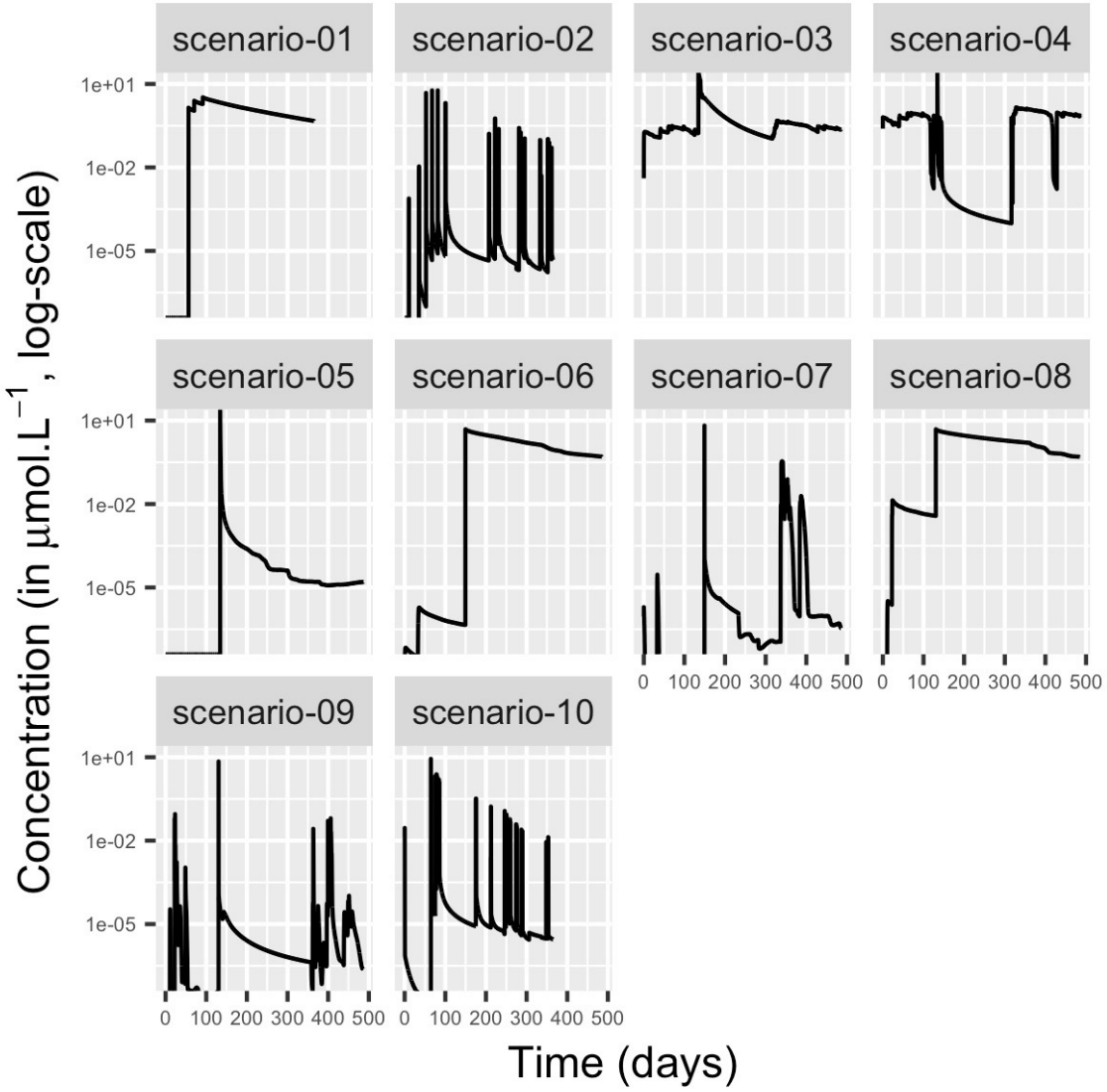
Contact :

Step 3: prediction under realistic profiles

Very often, no effects of relevant time-variable exposure scenarios on survival rate are predicted by GUTS models.

→ Is it the end of the assessment?

New question: How 'far' is the exposure profile from causing a pre-defined effect?



Step 3: the LPx concept

LPx = x% Lethal Profile (EFSA SO, 2018)

→This is the **Multiplication Factor** (or MF) leading to an additional x% reduction in the final survival rate (ie at the end of the exposure profile).

Original idea: "Margins of Safety" (Ashauer et al., 2013)

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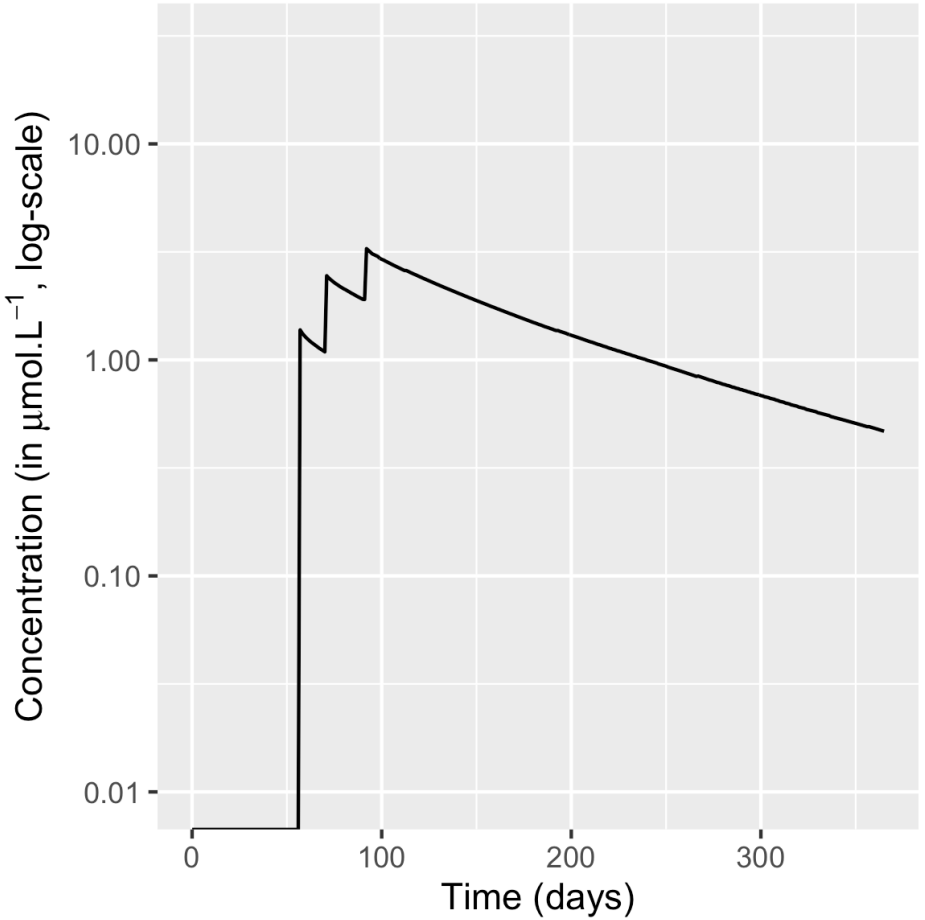
Step 3: the LPx concept
Example with scenario 01



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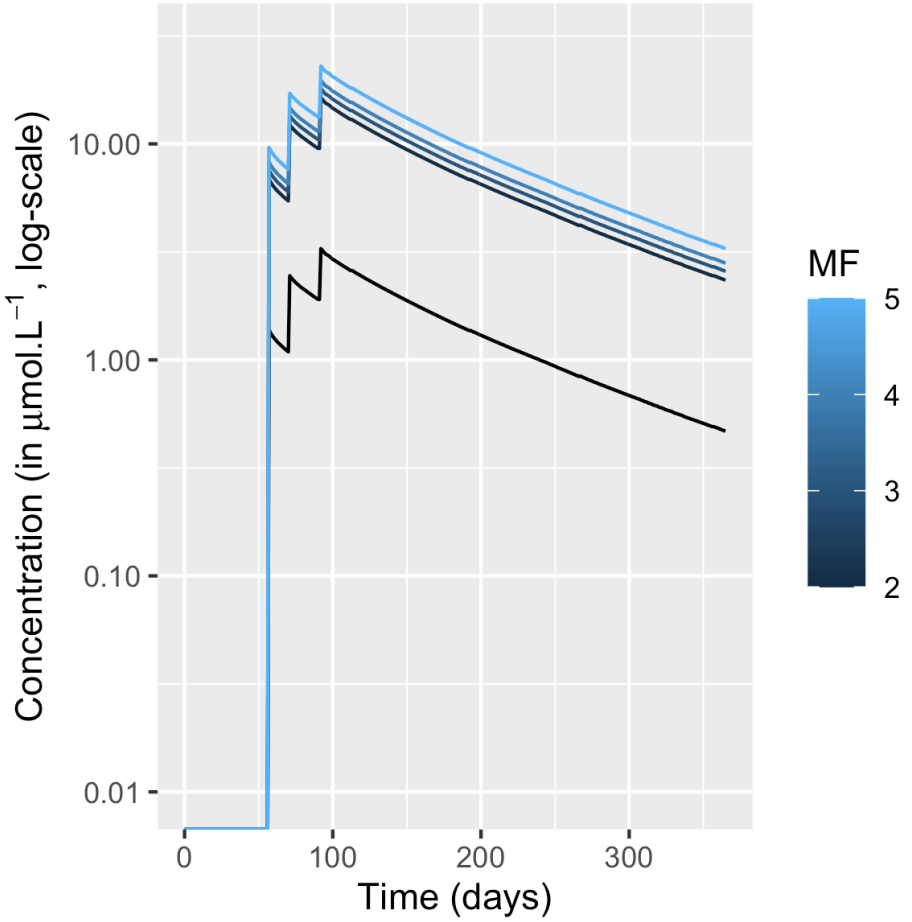
Step 3: the LPx concept
Example with scenario 01



MF	Final survival (%, model SD)	Final survival (%, model IT)
1	100	100
4	100	100
5	84.3	100
6	18.9	3.10^-8
7	0.013	0

LP_{50} (SD) = 5.2

LP_{50} (IT) = 5.4

qQ

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Step 3: application of the LPx

- LPx are multiplication factors that can be compared to Toxicity Exposure Ratios (TER)
- $TER = LC_{50} / PEC_{max}$
- LC_{50} = the one estimated from a classical dose-response curve
- PEC_{max} = maximal concentration of the exposure profile

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Step 3: application of the LPx
Results with the 10 scenarios

LC_{50} = 20.44 µmol.L^{-1} in [18.41 ; 23.31]

Scenario	01	02	03	04	05	06	07	08	09	10
PEC_{max}	3.3	5.9	30.9	23.6	26.5	4.9	6.6	4.9	7.0	8.8
1st tier TER	**6.6**	**3.5**	**0.66**	**0.87**	**0.77**	**4.2**	**3.1**	**4.2**	**2.9**	**2.3**
LP_{50} (SD)	5.2	5.2	< 1	1.2	1.1	3.6	5.3	3.6	5.1	3.9
LP_{50} (IT)	5.4	8.5	1.1	1.5	2.7	3.7	32.7	3.7	99.6	7.4

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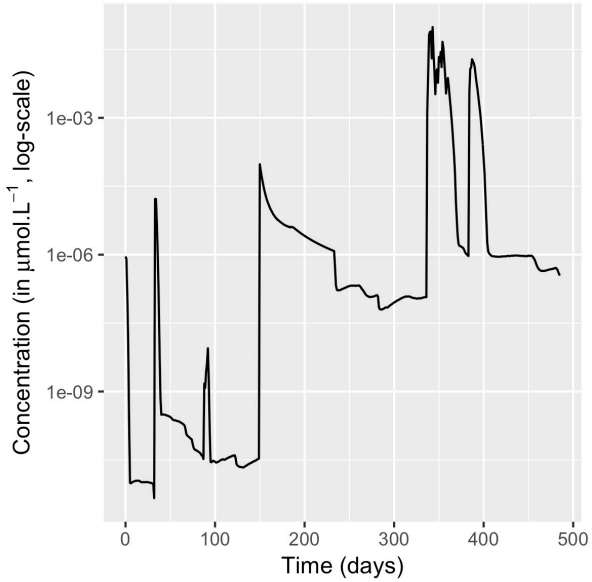
Step 3: application of the LPx

LP_{50} values differentiate risk between almost constant and highly variable exposure profiles!



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TDTK models: GUTS tools for ERA

The **MOSAIC** web-platform: http://mosaic.univ-lyon1.fr/

Calibration

- 'Surv' menu, then 'GUTS-fit': http://mosaic.univ-lyon1.fr/guts/

Validation & Prediction (LPx calculation)

- 'Surv' menu, then 'GUTS-predict': http://lbbe-shiny.univ-lyon1.fr/guts-predict/

Calibration, validation and prediction

- The R-package 'morse': https://CRAN.R-project.org/package=morse

→ Our GUTS implementation has been ring-tested: Jager and Ashauer, 2018 (Chap. 7)

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