# Four-parameter nonlinear regression and maximum achievable effect in ecotoxicology: Just visually appealing or relevant for risk assessment?

**Benjamin Daniels[1], Udo Hommen[2], Monika Ratte[1]**

[1] ToxRat Solutions GmbH & Co. KG, Alsdorf, Germany [2] Fraunhofer IME, Schmallenberg, Germany
*Email: benjamin.daniels@toxrat.com

## 1. Motivation and Objective

- Ecotoxicity data from lab tests are often evaluated by fitting regression models to calculate $EC_X$ values (OECD, 2006). Some models, e.g. the log-logistic **4-parameter-regression (4PR),** assume that a **maximum achievable effect** levels off above zero, i.e. the max. inhibition is smaller than 100 % (Fig. 1).
- From such a 4PR, two types of $EC_X$ can be calculated (Noel et al. 2018):
  **absolute $EC_X$ :** Considers effects solely in comparison to control levels.
  **relative $EC_X$ :** Relates to the maximum achievable effect.
  -> Results can vary significantly (Fig. 1).
- Guidelines, literature and common software do not uniformly address whether to report abs. $EC_X$ or rel. $EC_X$.
- **Decision criterion for $EC_X$:** The choice between absolute and relative ECX should not depend on user preference or arbitrary software settings but requires clear regulatory guidance.
- This presentation does not advocate for either absolute or relative $EC_X$ derived from 4PR, but highlights the implications of each.

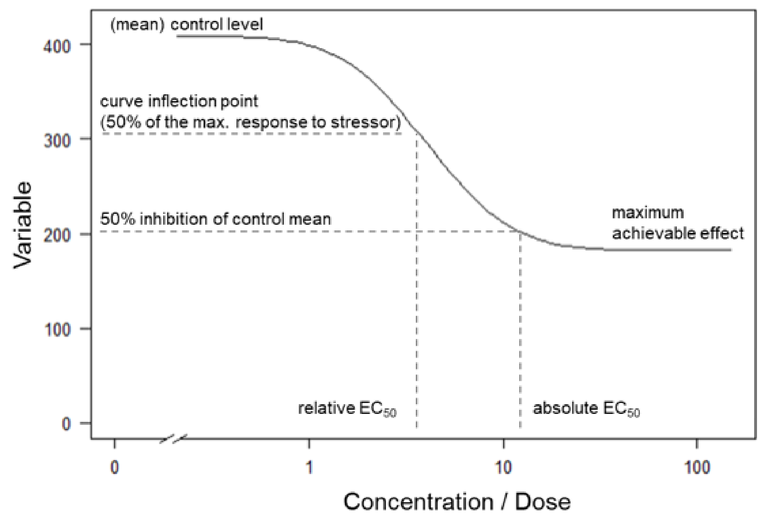


***Figure 1***
*Scheme of a 4-parameter nonlinear regression model showing the difference between the relative $EC_X$ and absolute $EC_X$. abs. $EC_{50}$ = concentration at 50% reduction compared to control; rel. $EC_{50}$ = concentration at half maximal response*

**Questions:**
1) When it is appropriate to use 4PR and when it is not?
2) Which factors influence how far the relative $EC_X$ is below the absolute $EC_X$?
3) What are consequences of using absolute or relative $EC_X$ for risk assessment?

## 2. Statistical background

- We use the commonly applied log-logistic model as defined by Ritz et al. (2015) and included in the *drc* package in R.
- The inflection point e is the dose at which the response is halfway between upper limit and lower limit, often referred to as $EC_{50}$ or $ED_{50}$.
- Thus, such an $EC_{50}$ is the relative $EC_{50}$.

$$f(x,(b,c,d,e)) = c + \frac{d-c}{1+\exp(b(\log(x)-\log(e)))}$$

with
x= concentration / dose, d = upper limit (control value),
c = lower limit, b = slope, e = inflection point

## 3. A Case Study: When 4PR – and when not?

Ryegrass plants (*Lolium perenne*) treated with a mixture of herbicides which prevent the production of growth-enabling compounds.
Data: Biomass (Ritz et al. 2019), Yield (own calculations)

**Biomass:** lower limit = biomass at test start → **4PR justified** → abs. $EC_X$ > rel. $EC_X$

**Yield**: lower limit = zero → **4PR inappropriate,** 3PR instead → abs. $EC_X$ = rel. $EC_X$

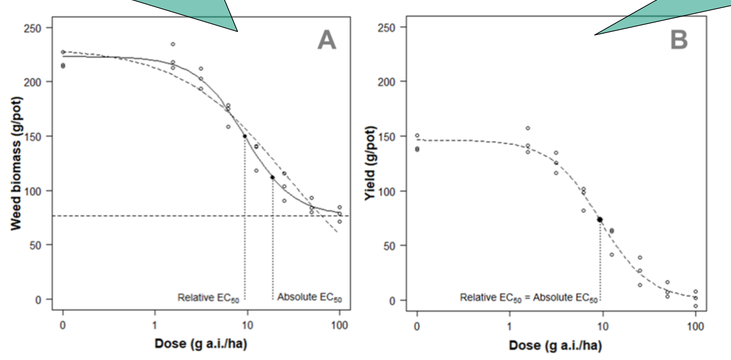


***Figure 2*** *Fitted 3P log-logistic function (dashed line) and 4P log-logistic function (solid line) for Biomass (2A), fitted 3P log-logistic function for Yield (2B).*
*The dashed horizontal line in Fig. 2A indicates biomass on day 0.*

Biomass, 4PR: abs. $EC_{50}$=18.9 g/ha,
rel. EC50=9.3 g/ha
Yield, 3PR: abs. $EC_{50}$=rel. $EC_{50}$=9.3 g/ha

Is 4PR generally appropriate if lower limit > 0 and 4PR enables a visually better fit?

No. **4PR should not just be used because of visual quality of the fit, but only if**

- **plausible biological or physiochemically justification** that maximum achievable effect reflects a fundamental characteristic of the test item's effects on analysed variable
- **data shows a clear plateau** above zero across multiple concentrations
- **overfitting is avoided,** i.e. sufficient data points

→ In case of uncertainty, 3PR should be preferred.
→ In the case study, the rel. $EC_{50}$ is factor 2 below the abs. $EC_{50}$, i.e. ratio abs. $EC_{50}$ /rel. $EC_{50}$ =2

Can this magnitude of difference be generalized? → **Sensitivity Analysis**

## 4. Factors affecting abs. $EC_X$/rel. $EC_X$-ratio

### Sensitivity Analysis

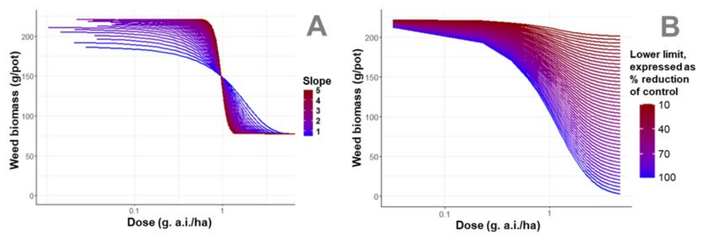


***Figure 3*** *Simulated dose response curves of the ryegrass- dataset with **varying slope parameter b** (0.5 to 5) and fixed lower limit at 66% (3A), and **varying lower limits** (100% reduction of control down to 10%) and fixed slope of 1.6 (3B). Constants were taken from the curve fit with Ryegrass data.*

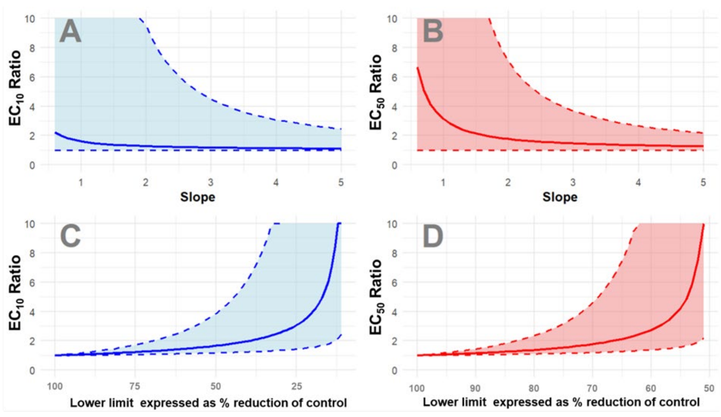


***Figure 4***: *Ratios of absolute vs. relative $EC_{10}$ (left, A and C) and $EC_{50}$ (right, B and D) for the 4PR of the Ryegrass biomass dataset.*
*A and B: ratios depending on slope, solid line: lower limit fixed at 66% reduction compared to control, coloured area: maximum reduction of control varied.*
*C and D: ratios depending on lower limits, solid line: slope fixed at 1.6, colored area: slope varied.*

**Reading example Fig. 4**

4B, solid line:
"At a given maximum reduction of 66% compared to the control, the ratio abs.$EC_{50}$/rel.$EC_{50}$ exceeds 2, if the slope is below 1.6".

4D, solid line:
At a given slope of 1.6, the ratio abs.$EC_{50}$/rel.$EC_{50}$ exceeds 2, if the maximum achievable effect decreases below 66%."

**Conclusions**

- Relative $EC_X$ from 4PR are lower than absolute $EC_X$.
- The lower the maximum achievable effect and the shallower the dose response, the higher the difference between absolute and relative $EC_X$.
- $EC_{10}$ ratio is less sensitive to slope than the $EC_{50}$ ratio.

## 5. Absolute or relative $EC_X$?

The choice between absolute and relative $EC_X$ involves biological, statistical and regulatory considerations, meaning also the selection of variables.

**Absolute $EC_X$**
- quantify an effect as % reduction compared to control → usual regulatory goal
- can be compared to $EC_X$ from 2PR and 3PR and between stressors
- are limited to the maximum achievable effect, $EC_X$ for higher x are not defined
- assessment variable is critical: abs. $EC_X$ of biomass underestimates biological implications for growth (Fig. 2); $EC_X$ biomass still prescribed in OECD 225, 227

**Relative $EC_X$**
- allow for calculation of any $EC_X$ between 1% and 99%
- are fully defined via the inverse function
- $EC_{50}$ corresponds to a fitted parameter
- if biomass is evaluated but growth should be assessed, relative $EC_X$ can be more suitable for environmental risk assessment

## 6. Take home messages

**Do not use 4PR** simply because the fit is visually appealing or when 3PR shows significant lack of fit.

**Use 4PR if:**
- there is a plausible physical mechanism or biological explanation behind AND
- Data shows a clear plateau AND
- there is no overfitting

**Absolute $EC_X$ are higher than relative $EC_X$**. The lower the maximum achievable effect and the shallower the dose response, the greater the difference.

**Explicitly state** whether relative or absolute $EC_X$ are reported. Include the level of the maximum achievable effect.

**Future guidelines** should specify which type of $EC_X$ should be reported for each variable to ensure consistency and clarity in risk assessments.

**References:**
- Noel ZA, Wang J, Chilvers MI. Significant Influence of EC50 Estimation by Model Choice and EC50 Type. Plant Dis. 2018 Apr;102(4):708-714. doi: 10.1094/PDIS-06-17-0873-SR. Epub 2018 Feb 14. PMID: 30673399.
- OECD (2006) Current Approaches in the Statistical Analysis of Ecotoxicity Data. A Guidance to Application, OECD Series on Testing and Assessment, No 54, 2006
- Ritz C, Baty F, Streibig JC, Gerhard D. Dose-Response Analysis Using R. PLoS One. 2015. 10(12):e0146021. doi:10.1371/journal.pone.0146021
- Ritz, C., Jensen, S.M., Gerhard, D., & Streibig, J.C. (2019). Dose-Response Analysis Using R (1st ed.). Chapman and Hall/CRC. https://doi.org/10.1201/b21966