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Brief Communication

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

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Editor's note: This article is part of the special series "Statistical Analysis of Ecotoxicology Data for Regulatory Purposes." The series provides an overview of approaches in statistical ecotoxicology and reflects recent developments, processes, and debates. The papers represent viewpoints from academia, industry, and government.

Abstract

In ecotoxicology, EC_x usually refers to the concentration that causes *x*% effect. This seems to be a precise definition, but is typically only clear for EC_x values calculated for inhibitions of metric variables from two- and three-parameter regressions, as they assume that the minimum of the affected variable is zero. In contrast, some four-parameter regression (4PR) assume that the maximum achievable effect levels off at a value of the affected variable greater than zero. As a consequence, two types of EC_x can then be calculated. While the absolute EC_x considers effects always as a change compared to the control level, the relative EC_x is related to the maximum achievable response to a stressor. In literature and in common software such as the *drc* package of R, the question whether absolute or relative EC_x should be calculated is not handled in a uniform way. Based on a sensitivity analysis, it is shown, that depending on the steepness of the curve and the level of the lower limit, a relative EC_x from 4PR can be considerably lower than the corresponding absolute EC_x. Thus, the question of whether to use absolute or relative EC_x should not be left to the preference of the user or arbitrary settings of the used software, but requires consistent and binding regulatory guidance. This paper does not advocate for absolute or relative EC_x from 4PR, but outlines the characteristics and consequences of each approach. The objective is to highlight the need for discussion and to provide information for an informed decision. Future guidelines should address this issue in detail to ensure consistency, clarity, and transparency in data interpretation.

Keywords: nonlinear regression, maximum achievable effect, absolute EC_x, relative EC_x, risk assessment

Introduction

Ecotoxicity data from laboratory test systems are generally evaluated by fitting regression models as concentration–response curves to calculate concentrations or doses at which a particular magnitude of effect is expected (Organisation for Economic Co-Operation and Development, 2006a). A frequently used regression model for continuous or count variables to describe the concentration–response relationship is the log-logistic function (Ritz et al., 2015) using three parameters: the control response as an upper limit of the curve, the slope, and the inflection point of the curve. The inflection point corresponds to the EC_{50}, the concentration where the response is reduced by 50% compared to the control. Such a three-parameter regression (hereafter referred to as 3PR) assumes a maximum inhibition of 100%, which means that the minimum of the concentration response curve is assumed to be zero, regardless of whether the response variable has been reduced to zero in the measured dataset.

If the data from a biotest do not reach values close to zero in the highest test concentration(s), the 3PR may result in a visually unsatisfactory fit. This can often be improved when an additional parameter is introduced which describes a minimum response reached above zero, i.e., if a four-parameter regression (4PR) is used instead. However, the choice of either 3PR or 4PR has strong implications on the resulting EC_x, as two types of EC_x can be calculated: absolute and relative EC_x. So far, it is not clearly specified in relevant guidance documents which type of EC_x should be reported for use in ecological risk assessment.

The objectives of this brief communication are (a) to clarify when it makes sense to use 4PR at all—and when it does not, (b) to demonstrate and analyze how absolute and relative EC_x can differ and what the magnitude of the difference depends on, and (c) to present characteristics and consequences of absolute and relative EC_x from 4PR.

Theoretical context

In the following, we will briefly explain the 4PR regression model and function discussed in the present paper. The features which lead to calculation of either absolute or relative EC_x values and

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the fundamental difference between these two types of EC_x will be outlined. Subsequently, we will give a brief review of how EC_x terminology and definitions are handled in the literature and the software R. We will use the term "concentration" to characterize exposure levels but this should cover doses and dose–response curves.

Statistical background and framework

In ecotoxicology, nonlinear regression is a widely accepted standard for deriving dose–response curves of continuous (and count) data (Green et al., 2018; Organisation for Economic Co-Operation and Development, 2006a; Ritz, 2010). Among the most commonly applied models for nonlinear regression of concentration–response data are those belonging to the log-logistic, log-normal, and Weibull families, which are also implemented in the well-used *drc* package (Ritz et al., 2015) of the software R. In this paper, we use the log-logistic model as an exemplary application due to its frequent use in practice (Environment Canada, 2007; European Food Safety Authority, 2023; Ritz et al., 2015). The conclusions and implications are equally valid for all families of models mentioned. The log-logistic function is defined as follows (parametrization shown from the *drc* package):

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

where x = concentration (set to small value >0 for the control), b = slope, c = lower limit, d = upper limit (value of control), and e = concentration at inflection point.

The parameter e represents the concentration at which the response is halfway between the upper limit d and the lower limit c. It defines the x-coordinate of the inflection point of the curve and is often referred to in publications as the effective concentration at 50% effect (EC_{50}; Ritz et al., 2019). The slope parameter b determines the steepness of the curve at the EC_{50}. If the lower limit c is set to zero, the 4PR reduces to a 3PR.

Absolute versus relative EC_x values

In a 4PR it is assumed that the maximum achievable inhibition in the observed test system is smaller than 100% compared to the control, i.e., the lower limit of the curve (parameter c) is assumed to be higher than zero. As a consequence, two types of EC_x can be calculated, absolute and relative EC_x. While the absolute EC_x considers effects always as a change compared to the control level, the relative EC_x is related to the maximum achievable response to a stressor (Noel et al., 2018; Sebaugh, 2011). A relative EC_{50} is the concentration which shows the half maximal inhibition (corresponding to the inflection point in symmetrical dose–response curves), whereas an *absolute* EC_{50} is the concentration which shows 50% effect related to control and corresponds to the inflection point only, if the maximum inhibition is 100%.

This is demonstrated in Figure 1. If the minimum of a variable is, e.g., limited at slightly below 200, i.e., the maximum reduction corresponds to slightly more than 50% of the control value (which is about 400), the maximum absolute response in terms of difference to control corresponds to about 400−200 = 200. The relative EC_{50} is the concentration at which the variable is reduced by 50% of the maximum response, i.e., by 50% of 200 = 100. Thus, the relative EC_{50} corresponds to a variable value of 400−100 = 300, equivalent to only about 25% reduction compared to the control. In contrast, the absolute EC_{50} is the concentration at which the variable is reduced by 50% compared to the control, i.e., by 200.

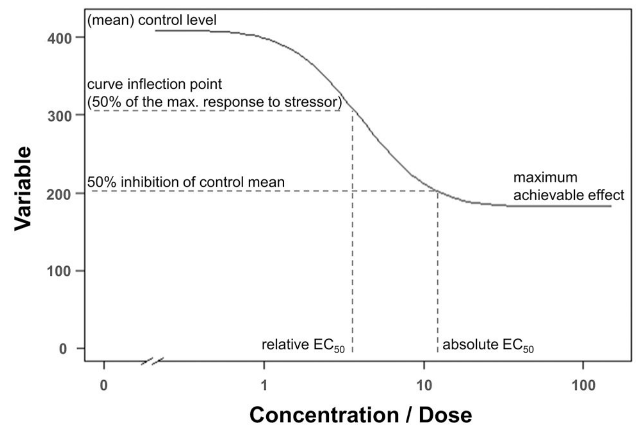

Figure 1. Scheme of a four-parameter nonlinear regression model showing the difference between the relative EC_x and absolute EC_x. Response values from 0 to 400 are fictitious numbers. EC = effect concentration.

For three-parameter functions within the log-logistic, log-normal, or Weibull families, when the lower limit (c in *Equation 1*) is fixed at zero, there is no difference between absolute and relative EC_x values. When using the four-parameter models, where the lower limit c is not constrained to zero, discrepancies between absolute and relative EC_x values can arise. These differences are especially relevant in the analyses of continuous data, where the 4PR is commonly employed.

Our brief communication specifically addresses concentration–response curves where the lower limit c is not fixed at zero. This excludes special regression functions, such as 4PR hormesis models, where the lower limit is explicitly set to zero. On the other hand, it also includes models for continuous or count variables where only three model parameters are used, but the upper limit is set at a certain value instead of the lower limit. However, such cases are uncommon in ecotoxicological practice, which is why we focus here on 4PR.

The definition of EC_x in ecotoxicology

In the literature on the evaluation of ecotoxicity data, differences between absolute and relative EC_x are recognized, and both approaches are considered justified depending on the reference. The OECD Statistical Guidance to Application (Organisation for Economic Co-Operation and Development, 2006a) gives a definition of EC_x, stating that "x is defined as a percent change in the (average) level of the endpoint considered, e.g., a 10% decrease in weight" (p. 76, section 297). Although not explicitly mentioned, it is likely to assume that this means that EC_x should be calculated solely related to the control, i.e., as absolute EC_x. However, when functions for nonlinear regressions are discussed (p. 79, section 312), the presented formula for EC_x results in a relative EC_x if the parameter of the lower limit is >0. Thus, even in the OECD Statistical Guidance to Application (OECD, 2006a), the definition is ambiguous and not straightforward.

The same applies to other references and common software: Green et al. (2018) clearly state: An "EC_x refers to the concentration at which there is an x% effect relative to the model estimated control mean." But there are also references that make different suggestions. Brain and Cousens (1989) state: "The ED50, defined as the dose that gives 50% of the total achievable effect." This clearly means the relative EC_x. Van der Vliet and Ritz (2013) demonstrate that from a 4PR, the EC_{50} as the x-coordinate of the inflection point is related to the maximum achievable effect (=relative EC_x) and argue that if one wants to calculate an absolute EC_{50}, one should use a 3PR, because then the lower limit is fixed at zero. Ritz et al. (2019) define ED_{50} as “the dose resulting in a 50% reduction in the average response relative to the lower and upper limits of f,” i.e., as relative EC_x. They note that “such relative effect doses are mostly suitable for continuous responses.”

In the commonly used *drc* package from the R software (Ritz et al., 2015), the default setting calculates EC_x from 4PR (function *ED()*) as relative EC_x values. Many users are probably unaware of the consequences of this setting and may be unaware of the existence of two EC_x types at all. As the EC_x type can be changed to absolute EC_x by the user, this can lead to confusion when interpreting and comparing results. This fact triggered Noel et al. (2018) to investigate the effect of EC_x type on EC_x estimation for fungicides. They concluded that “future studies should pay careful attention to model selection and interpretation in EC_{50} estimation and clearly indicate which model and EC_{50} measure (relative vs absolute) was used.” We see parallels between the situation in phytopathology, as described by Noel et al. (2018), and the current situation in ecotoxicology. When considering 4PR, transparency is important and guidance is required.

Case study

In the following, we present a case study from the literature to demonstrate the difference between absolute EC_x and relative EC_x from 4PR and to analyze which factors affect the magnitude of this difference. Given the potentially significant differences between absolute and relative EC_x, the data example is also used to make some fundamental considerations about the variables and data situations for which 4PR is a suitable model—and those for which it may be not. However, as this is not the central theme of the paper, this discussion is not intended to be exhaustive. All following calculations were performed using the *drc* package (ver. 3.0-1) and R software (ver. 4.4.2).

We used a dataset available in the *drc*Data package (ver1.1-3, function ryegrass2()) and previously described by Ritz et al. (2019). It originates from an experiment on perennial ryegrass plants (*Lolium perenne*) treated with seven concentrations of a mixture of herbicides. These substances prevent the production of growth-enabling compounds. As a result, while the plants' growth is inhibited, they remain visually viable for an extended period.

When a 4PR is appropriate

Figure 2A shows the original biomass data at the end of the test on day 15 from the ryegrass dataset. Start biomass was recorded using three representative plants, resulting in a mean of 76.83 (*SD* ± 1.61) g per plant. After 15 days of exposure to seven treatment levels (1.5625–100 g.a.i/ha), biomass values showed a clear dose–response pattern, ranging from ∼214 g in the control group to 71–85 g at the highest herbicide concentrations.

In this case, the use of a 4PR model is justified by the variable which is assessed, i.e., biomass: even if growth is reduced to zero, the start biomass at day 0 is maintained during the test. Thus, for the analyzed variable, biomass, the lower limit is above zero. It is therefore plausible to include this information in the regression model, where the lower limit reflects the initial biomass on day 0. The same would apply if the biomass of the tested individuals decreased to a verifiable minimum during the experiment but did not reach zero.

Figure 2A illustrates this rationale, with the dashed horizontal line indicating the biomass at day 0, which corresponds to the lower limit of the 4PR model. For comparison, the 3PR result is also shown (dashed curve), which gives a less visually appealing fit. Consequences for absolute and relative EC_x will be discussed in the section on “Absolute or relative ECx from 4PR?”.

When a 4PR is inappropriate

In contrast to biomass, the yield, defined as the change of biomass over the test duration, is reduced to ∼0 in the ryegrass example, with a maximum inhibition of 99% in the highest treatment. Therefore, a 4PR would be unnecessary, as a 3PR model provides an excellent fit to the data (Figure 2B). Following the Parsimony principle (Ockham's Razor, Organisation for Economic Co-Operation and Development, 2006a), the simpler model should be used.

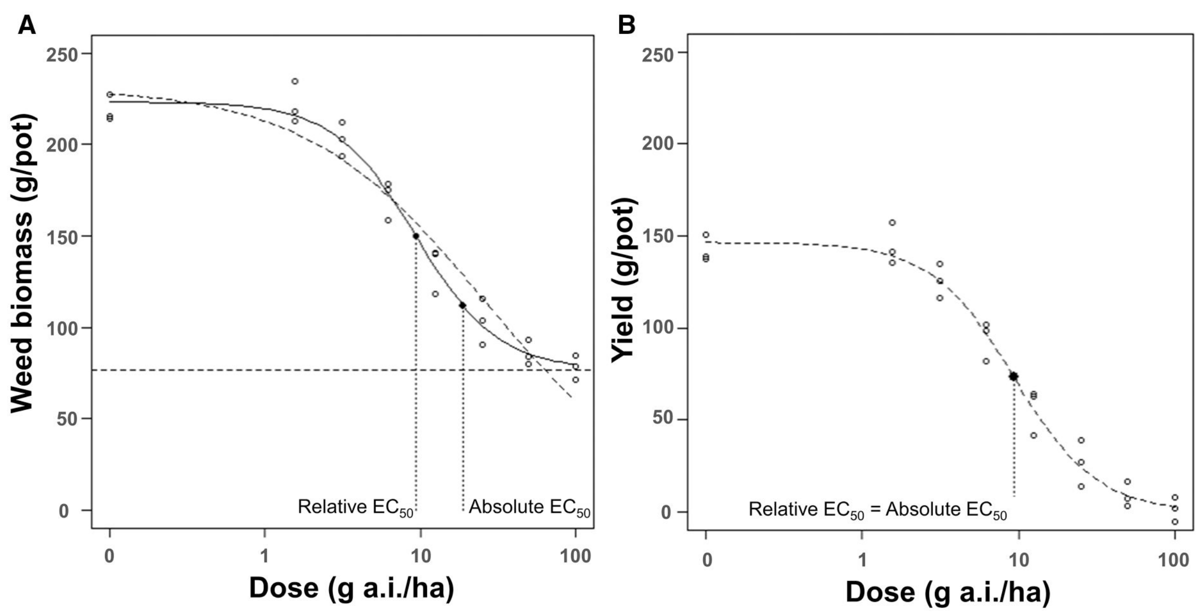

Figure 2. Fitted three-parameter log-logistic (dashed line) and four-parameter (solid line) log-logistic function for biomass at the end of the test (A), and fitted three-parameter log-logistic function for yield (B) calculated from the ryegrass2 dataset (Ritz et al., 2019) with the *drc* package (ver 3.0-1). The dashed horizontal line in Figure 2A indicates biomass on day 0. EC = effect concentration; a.i. = active ingredient.

If, as in this example, the analyzed variable (here: yield) is reduced to zero, the situation is obvious. But what if this is not the case at the highest test concentration? Range finding is challenging and it is not uncommon for the highest concentration in biotests not to reduce the variable close to zero. Is 4PR generally appropriate in such a case if it enables a visually better fit?

In our opinion, the decision for or against a 4PR should not just be motivated by the visual quality of the fit or results of a formal lack of fit test. Applying a 4PR makes an assumption about the response at untested concentrations, namely that the variable would not decrease further but level off at a lower limit above zero even at high concentrations. Thus, it should be considered whether a plausible explanation exists that can support a maximum inhibition below 100% compared to control. And it goes without saying, that the tested concentration range and measured effects should provide clear evidence for the existence of a maximum inhibition below 100% to allow most accurate parameter estimation. A statistical indication of a biologically meaningful nonzero maximum effect could be a lower plateau across multiple high concentrations. This highlights the need for careful concentration selection to encompass both partial and strong inhibitory effects, ensuring a complete characterization of the concentration–response relationship.

Yields and growth rates can often be inhibited down to zero if a sufficiently high concentration is tested. So, applying a 4PR model to yield or growth rate will probably be biologically unsound in most cases. Exceptions may be possible, and should then generally show clear lower plateaus at the highest test concentrations, as seen, e.g., in the publication by Schmitt et al. (2013) for growth of the duckweed *Lemna gibba* inhibited by an herbicide. Such data situations should be discussed to justify the use of 4PR. Potential reasons for not reaching 100% inhibition include limited solubility of test substance, interference with lethal effects, or slow uptake of the substance which would allow normal performance until a sufficient internal concentration is reached. Otherwise, if no plausible explanation for a lower limit above zero is available, 3PR can be regarded the more appropriate model.

Last, but not least, the objective of ecotoxicological tests is to draw conclusions about potential effects on populations in the field, and not just about the tested sample of organisms. Thus, the assumptions of the lower limit in the dose–response curve should at best reflect a fundamental characteristic of the biological variable or properties of the test substance, and not just provide a visually appealing fit to a possible random result for a single dataset.

Absolute or relative EC_x from 4PR?

When the assumption of a maximum achievable effect is plausible, a 4PR can be used in a meaningful way. In this case, the question appears whether relative or absolute EC_x from 4PR should be reported and used in the risk assessment. In the following, we will present the results for absolute EC_x and relative EC_x from 4PR for the ryegrass biomass dataset, discuss the parameters that affect the difference between absolute and relative EC_x, and provide an overview of the characteristics and consequences of either relative or absolute EC_x.

Factors affecting the ratio of absolute EC_x/relative EC_x

The relative EC_x from 4PR for the ryegrass biomass (Figure 2A) are lower than the absolute EC_x (EC_{10}: 2.46 compared to 3.29; EC_{50}: 9.3 compared to 18.9 g a.i./ha). Thus, the absolute EC_{50} is about twice the relative EC_{50} in this example. This is not surprising, given that the maximum inhibition achieved compared to the control is only about 66%. This raises the question of whether the observed differences between absolute and relative EC_x can be generalized, and what the absolute EC_x/relative EC_x ratio depends on.

To further address this question, we analyzed the critical role of slope and lower limit—respectively, the maximum achievable response—for the ratio between both EC_x types. For this analysis, we once again applied the 4PR model to our ryegrass example (biomass as the response variable, see above). The slope parameter, which determines the steepness of the dose–response curve, and the lower limit of the 4PR model were systematically varied while keeping the other parameters constant. The original slope parameter of 1.6 was varied between $b_{\min} = 0.5$ and $b_{\max} = 5$ in steps of 0.1 (Figure 3A). Note that the slope of the log-logistic curve is decreasing with a positive parameter value b, which is a direct consequence of the parametrization shown above (*Equation 1*). The original maximum effect of 66% was varied in steps of 1% between 100% and 50% effect in case of EC_{50} (as for lower maximum effects, absolute EC_{50} cannot be determined) and 100% and 10%, respectively, for EC_{10} (Figure 3B).

Figure 4 shows how the ratio of absolute versus relative EC_{10} and EC_{50} depends on both slope and lower limit. The solid lines apply to a change in the slope parameter (Figure 4A and B) or in the lower limit parameter (Figure 4C and D) with constant values of the respective other parameter. The colored areas show the range when the other parameter varies. The constants were taken from the curve fit with data for ryegrass biomass (slope = 1.6 and maximum achievable effect = 66%).

Overall, the results of the simulations clearly indicate that differences between absolute and relative EC_x values increase as the dose–response curves become shallower (i.e., with lower slope parameters) and the maximum achievable effect becomes lower. Thereby, the EC_{10} ratio is less sensitive to slope than the EC_{50} ratio. In general, the lower the maximum achievable response, the more the EC_x ratio depends on the slope. The differentiation between relative and absolute ECx is particularly relevant when the concentration–response curves have a shallow slope. According to the European Food Safety Authority (EFSA) criteria for reliability of EC_{10} (European Food Safety Authority, 2019), this is the case with an EC_{10}/EC_{50} ratio <0.33, which applies to a slope parameter $b < 2$ in our simulation study. Thus, the original curve with a slope of 1.6 also represents a shallow curve according to EFSA criteria. In the simulations, the ratio abs. EC_{50}/rel. EC_{50} becomes higher than 2 when curves show such a shallow appearance. Shallow dose–response curves are not uncommon in ecotoxicology; 63.8% of analyzed dose–response curves from ecotoxicity studies were classified as shallow in a recent meta-study (European Food Safety Authority, 2019).

These findings can help to raise awareness of data situations where in the case of 4PR the differences between absolute and relative EC_x would be particularly pronounced and thus the type of EC_x needs to be chosen with particular care.

Absolute or relative EC_x: characteristics and implications

In the following, we highlight some key aspects that should be considered when deciding in favor of one or the other EC_x type. Overall, the choice between absolute and relative EC_x includes biological, statistical, and regulatory aspects.

The absolute EC_x corresponds to what is generally associated with the meaning of EC_x, namely the concentration which leads to x% reduction compared to control. The extent of inhibition in

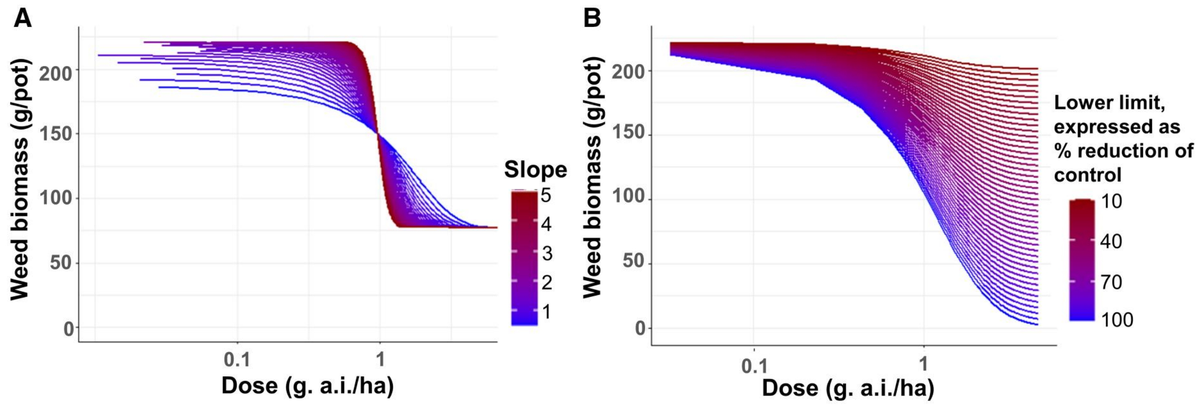

Figure 3. Simulated concentration–response curves (4PR log-logistic model) of the ryegrass dataset (variable biomass), with varying slope parameter *b* (A) and varying lower limit (expressed as % reduction of control) (B), while keeping the other parameter constant. a.i. = active ingredient.

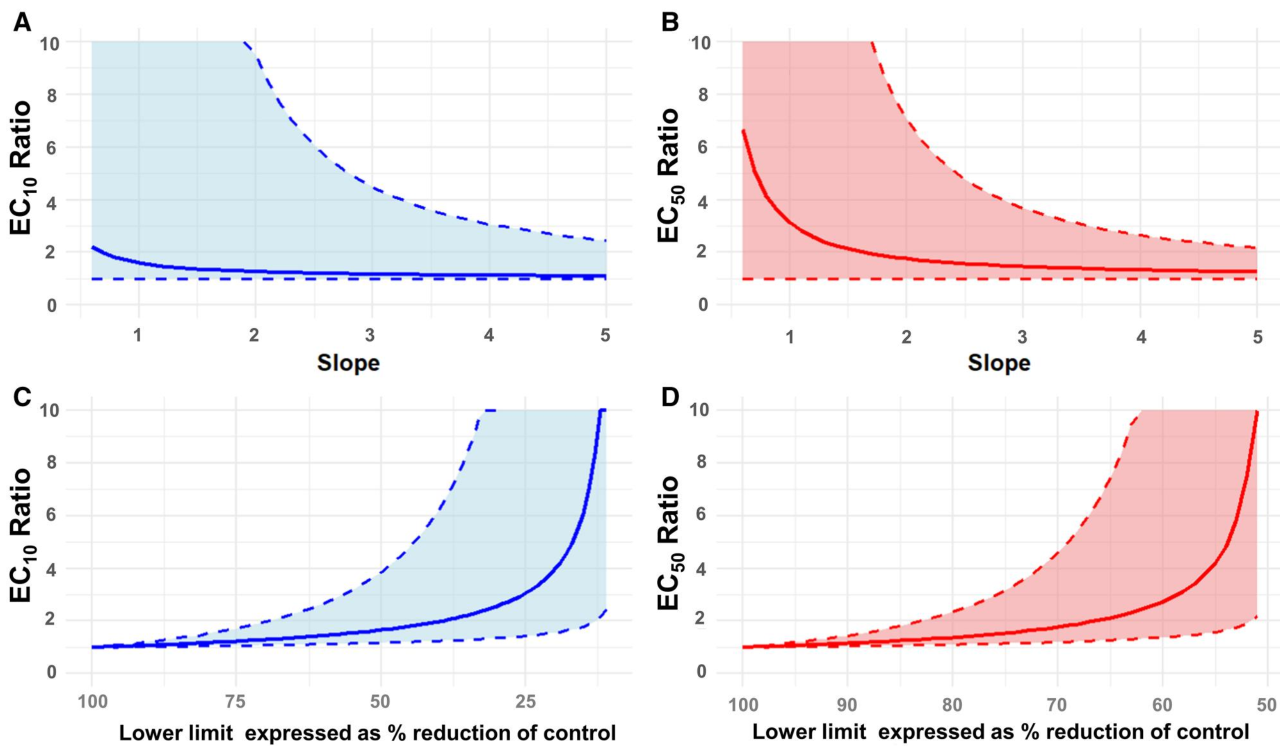

Figure 4. Ratios of absolute versus relative EC_{10} (left, A and C) and EC_{50} (right, B and D) for the 4PR of the ryegrass biomass dataset. Upper curves (A and B): ratios depending on slope, solid line: lower limit fixed at 66% reduction compared to control, colored area: maximum reduction of control varied between 100% and 10% (A) or 100% and 50% (B). Lower curves (C and D): ratios depending on lower limits, solid line: slope fixed at 1.6, colored area: slope varied between 0.5 and 5. Reading example: at a given maximum reduction of 66% compared to the control, the ratio abs. EC_{50}/rel.EC_{50} becomes higher than 2, if the slope is lower than 1.6 (Figure 4B, solid line). At a given slope of 1.6, the ratio abs. EC_{50}/rel.EC_{50} becomes higher than 2, if the maximum achievable effect decreases below 66% (Figure 4D). EC = effect concentration.

relation to the unexposed control is usually considered the relevant measure in ecotoxicology and ecological risk assessment, as this is what is assessed to be acceptable or not acceptable. Absolute EC_x from 4PR can also be directly compared to EC_x from 3PR or 2PR and between different stressors. With similar control levels, organisms exposed to absolute EC_x show the same absolute performance while organisms exposed to the same relative EC_x of different stressors can show different levels of absolute performance.

Thereby, derivation of absolute EC_x values by 4PR is only possible for x up to the maximum achievable effect, i.e., certain EC_x values may become undefined. For instance, if the highest observed reduction compared to control is only 40%, an absolute EC_{50} cannot be determined at all. Thus, the absolute EC_x may have a partially defined inverse function. However, situations with a plausible maximum effect <50% are probably rare.

The relative EC_x, in contrast, is fully defined via the inverse function of the concentration–response curve because it is inherently linked to the maximum achievable effect. This allows the calculation of any EC_x value within the entire possible response range (between 0% and 100%).

It could be argued that biological consequences of an effect solely depend on the difference to the unaffected control, rather than on reached proportion of the maximum achievable effect. However, whether this statement is true depends on the variable under consideration. To illustrate this, we revisit the ryegrass example: at the highest test concentration, biomass remains at the level observed at test initiation. In terms of relative EC_x for variable biomass, the highest treatment would be defined as a concentration approaching approximately EC_{100}. In contrast, using absolute EC_x, the effect in the highest test concentration would be quantified solely in relation to final biomass achieved in the control, corresponding to an "EC_{66}." This is correct regarding the total biomass of the individuals. However, the vigor or performance of the plant does not depend solely on its biomass, but also on its ability to grow. In this case, an absolute EC_{66} for biomass corresponds to "zero growth" rather than to "only 34% growth." Hence, the absolute EC_x for variable biomass (in contrast to the EC_x of the variable growth rate or yield, see Figure 2B) would underestimate the biological consequences for the organism. Thus, if biomass is evaluated at the end of the test but growth should be assessed, an absolute EC_x will underestimate the effect and a relative EC_x will better reflect the effects on growth. In our ryegrass example, this is immediately evident from the fact that the relative EC_{50} for biomass is almost identical to the absolute = relative EC_{50} for yield biomass (Figure 2A and B). As the variable biomass is still required as a variable for reporting EC_x values in several biotest guidelines, even if the initial biomass is greater than zero (Organisation for Economic Co-Operation and Development, 2006b, 2007), this aspect is of practical relevance. It could be argued that variables with a maximum achievable effect of less than 100% should be replaced by those with an achievable maximum of 100%. For instance, biomass or length could be replaced by growth rate. Although variable substitution may not always be possible (e.g., if high treatment levels not only inhibit growth but result in loss of weight), it would reduce the need for 4PR and avoid the complications of interpreting relative versus absolute EC_x and its implications for protection.

Mathematically, the relative EC_{50} corresponds to the inflection point of symmetrical dose–response functions, such as log-logistic and log-normal. The relative EC_{50} therefore has some appealing mathematical properties: it is a model parameter and thus directly fitted. The inflection point—and thus the relative EC_{50}—is less sensitive to outliers and thus results in a more robust derivation than EC_x for other effect sizes. This might be the reason why it is also the default setting in the *drc* package of R.

It could also be argued that relative EC_x should be preferred because they are lower than the absolute EC_x and thus more protective. Alternatively, if protection should be increased this could also be achieved by using a smaller effect size x or by using a larger assessment factor applied to the EC_x. As shown above, the increase in conservatism by using relative instead absolute EC_x depends on slope and maximum achievable effect.

Summary and conclusion

In ecotoxicology, where biological systems are analyzed, it is crucial to ensure that the statistical model is consistent with the biological processes in the test system. In other words, biological systems require biological interpretation. Therefore, the selection of the model (e.g., 3PR or 4PR) should not be based solely on achieving a visually appealing fit but must be biologically or physiochemically justified. 4PR can be justified for variables such as absolute biomass or length, when the starting values are clearly larger than zero, but are a priori rarely valid for rates or yields. If the data suggest a lower limit above zero, it should be justified that this is not merely a random outcome of a single experiment, but rather reflects a fundamental characteristic of the test item's effects. A robust dataset should clearly display a plateau (e.g., multiple concentrations yielding similar responses). Overfitting must be avoided by ensuring sufficient data points. In cases of uncertainty, 3PR should be preferred. Applying these criteria consistently would likely limit the use of 4PR to only a few cases.

If 4PR is used, relative EC_x tend to be lower than absolute EC_x values. The differences between absolute and relative EC_x values increase when the dose–response curves become shallower (lower slope parameters) and the maximum achievable effect becomes lower.

Absolute EC_x values more closely correspond with what is sought for regulatory purposes, because they quantify and standardize an x% change solely in comparison to the control. Absolute EC_x from 4PR can also be directly compared to EC_x from 3PR or 2PR and between different stressors. Thereby, calculation of absolute EC_x in 4PR is limited to x up to the maximum achievable effect, i.e., certain EC_x values may become undefined. Moreover, depending on the variable, absolute EC_x values may underestimate the biological implications of an effect in some cases, e.g., if biomass is used but growth should be assessed.

The relative EC_{50} has the advantage that it is directly derived from the fitted inflection point (similar to the absolute EC_{50} in the 3PR). Additionally, since the relative EC_x values account for both the fitted maximum and minimum, they can be calculated for any x close to 100%. Thus, the relative EC_x is fully defined via the inverse function of the concentration–response curve, whereas the absolute EC_x may have a partially defined inverse function. In some cases, e.g., if only biomass is analyzed but growth inhibition needs to be assessed, relative EC_x can be more suitable for risk assessment.

How to decide?

This paper does not advocate for either absolute or relative EC_x derived from 4PR, but highlights the implications of each approach. The choice between absolute and relative EC_x includes biological, statistical, and regulatory considerations, meaning also the selection of variables.

Currently, the selection of either absolute or relative EC_x is often dedicated by default software settings or the preference of a statistically experienced user. The use of custom-built calculation tools can introduce transparency issues, functioning as "black boxes" that hinder reproducibility and interpretation. To ensure transparency and prevent misinterpretation, studies which use 4PR should explicitly specify whether and why absolute or relative EC_x values are reported and should clearly state the assumed lower limit (i.e., the level of maximum achievable effect). Future guidelines should address this issue in detail to promote consistency, transparency, and reliability in data interpretation.

Data availability

The data of the example dataset are available in the *drc*Data package (ver1.1-3, function ryegrass2()) and were previously described by Ritz et al. (2019).

Author contributions

Benjamin Daniels (Conceptualization, Formal analysis, Methodology, Visualization, Writing—original draft, Writing—review & editing), Udo Hommen (Conceptualization, Writing—original draft, Writing—review & editing), and Monika Ratte (Conceptualization, Writing—original draft, Writing—review & editing)

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Conflicts of interest

ToxRat Solutions offers commercial software for the statistical evaluation of standardized ecotoxicological tests. B.D. is also in charge of a time-limited in-house research project at the German Environment Agency (FKZ 3723 67 4010).

Disclaimer

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