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# The role of "maximum achievable effect" in ECx calculation.

Recently, the issue of the "maximum achievable effect" in the calculation of ECs from non-linear regressions with metric variables has been raised by several users and it has been claimed that ToxRat calculates incorrectly and not in accordance with current guidelines. In the following, we will clarify how this issue is addressed in guidelines and literature, what it means for 4-parameter non-linear regressions, and what the consequences would be in terms of ECx definition if the maximum achievable effect were not considered.

With non-linear regression, it is important to distinguish between
(1) a 3-parameter function, which generally assumes a minimum equal to zero, and
(2) a 4-parameter-function, which explicitly assumes a minimum greater than or equal to zero - depending on the data.
**When a 3-parameter function** is used, ECx values refer to zero as "maximum achievable effect" (even if zero is not achieved in the corresponding data set). This results in ECx values where "x" is the percentage inhibition observed compared to the control.
**When a 4-parameter function is used**, the ECx values refer to the "maximum achievable effect" achieved in the corresponding data set (which may be either zero or greater than zero). This results in ECx values where "x" does not necessarily correspond to the observed percentage reduction compared to the control, and which at first glance can appear to be "too low". This is, how it is handled also in ToxRat – the reasons are given below.

Let's start with the following consideration:
The use of a 4-parameter function means that it is assumed that at some point the effect may level off at a value greater than zero. This is then the maximum effect on the observed organism, i.e. EC100. Therefore, increasing concentrations beyond this EC100 will not result in increasing effects. Consequently, an EC50 must indicate the concentration at which there is a 50% effect on the organism. This means that the EC50 must be related to the maximum achievable effect, which in this case is greater than zero. Otherwise, increasing concentrations could be calculated as increasing "ECx values", which in reality would not result in increasing effects.

**(1) Statements in Statistical Guidance Documents**
*The OECD Statistical Guidance Document*[1] just gives a very general definition of ECx, stating that “x is defined as a percent change in the (average) level of the endpoint considered, *e.g.*, a 10% decrease in weight“ (p76). This could be interpreted to mean that ECx should always be calculated relative to the control - regardless of what the maximum achievable effect is relative to the control. Unfortunately, the issue of “maximum achievable effect” is not addressed at all.
In contrast, the *Environment Canada Statistical Guidance Document*[2] states on p 140, that comparing EC50 values is "valid only when the dose effect curves [...] show the *same* maximum achievable effect".
This implicitly means that the EC50 is related to the maximum achievable effect - and that the maximum achievable effect is not necessarily the same (namely zero) in all cases.

So unfortunately, in current guidelines, it remains unclear how to address the issue of maximum achievable effect.

[1] Current Approaches in the Statistical Analysis of Ecotoxicity Data. A Guidance to Application, OECD Series on Testing and Assessment, No 54, 2006

[2] Guidance Document on Statistical Methods for Environmental Toxicity Tests. Environmental Protection Series, Report EPS 1/RM/46, March 2005 (with June 2007 amendments).

**(2) Statements in literature**

In contrast, references from literature clearly confirm, that an ECx from a 4-parameter-nonlinear regression must be related to the obtained maximum achievable effect, rather than to zero.

**(2a)** In the paper of Brain P, Cousens R (1989)[3] it is stated:
"The ED50 is the dose at which there is 50% of the achievable yield reduction".

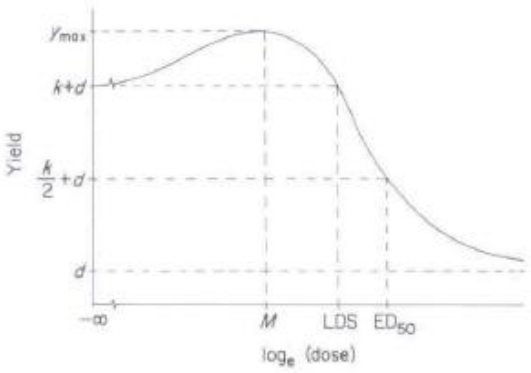
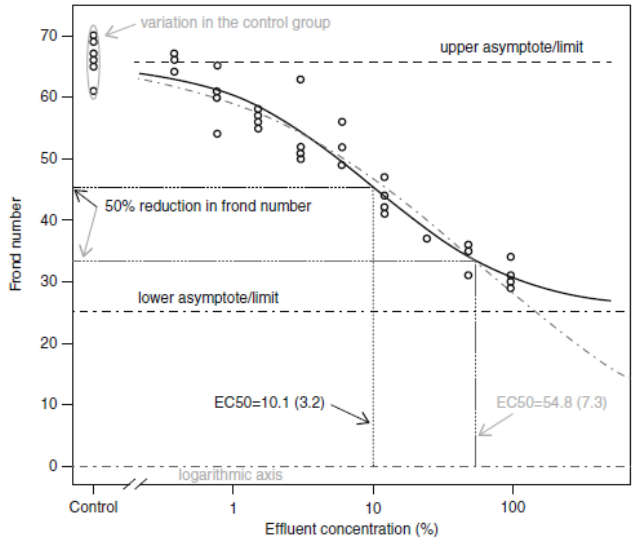



Fig. 1 Representation of the salient features of the peaked dose–response curve. ymax is the maximum yield, LDS is the limiting dose for stimulation, below which all doses increase yield, $ED_{50}$ is the dose at which there is 50% of the achievable yield reduction, and $M$ is the dose for maximum yield; $k$ and $d$ are as defined in the text.

In the *ToxRat output*, there is even a corresponding text included, to make the user aware of this specific feature of the 4-parameter function:

"The 4-param. normal CDF is asymptotic to a minimum value greater than zero. Therefore, the ECx relates to the maximum achievable effect, max - min (min > 0 | max = b0 | min = b3; b0, b3: parameter 1 and 4 of the function)."

**(2b)** Van de Vliet and Ritz (2013)[4] explicitly discuss the difference between ECx calculation from a 3-parameter regression and a 4-parameter regression .
It is clearly stated, that with a 4-parameter-function, the EC50 is calculated related to the obtained maximum achievable effect. And that if one wants to calculate the ECx related to zero, *one should use the 3-parameter-function instead.*




**Statistics for Analyzing Ecotoxicity Test Data, Fig. 1** Original data from the Lemna test and two fitted curves corresponding to the three- and four-parameter log-logistic models (shown as *dashed* and *solid lines*, respectively). The corresponding estimated EC50s and their standard errors (given in *brackets*) are also given

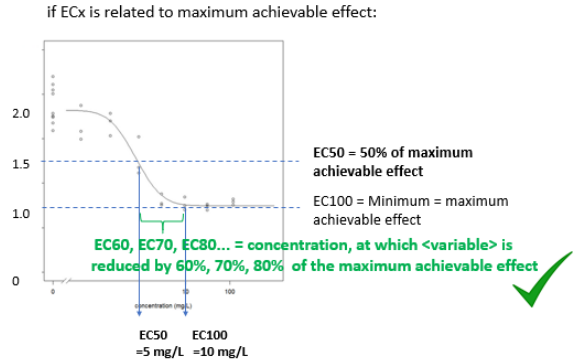
"For instance, by choosing the above four-parameter log-logistic model, we implicitly assume or at least do not rule out the possibility of a nonzero lower limit being attained for large concentrations. On the other hand, it might have been natural to assume that the frond number tends to 0 as the concentration gets very large.
Following this line of reasoning, we should then instead use the long-dashed fitted dose-response curve seen in Fig. 1, corresponding to a log-logistic model where the lower limit is in advance fixed at 0 (i.e., not being estimated).
However, Fig. 1 shows that the resulting estimated EC50 is roughly five times as large as the estimated EC50 based on the four-parameter model. Thus, initial model assumptions may have a substantial impact on the resulting estimate of the parameter of interest."

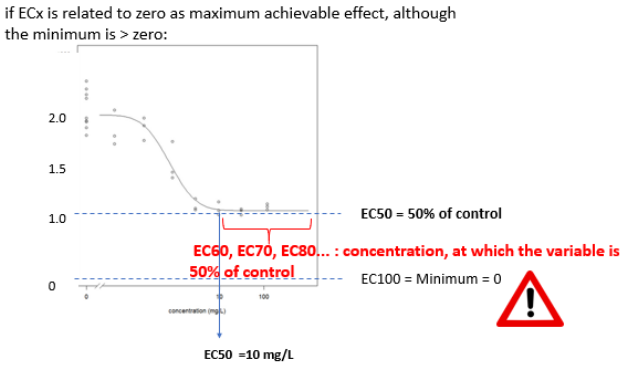
[3] Brain P, Cousens R (1989): An equation to describe dose responses where there is stimulation of growth at low doses. Weed research, Vol 29, 93-96,
[4] (Van der Vliet, L., Ritz, C. (2013). Statistics for Analyzing Ecotoxicity Test Data. In: Férard, JF., Blaise, C. (eds) Encyclopedia of Aquatic Ecotoxicology, Springer, Dordrecht)

**(3) Contradicting the biological definition of ECx**

If a 4-parameter non-linear regression is performed, it is assumed that the minimum can be greater than zero, i.e. that 100% effect on the measured variable does not necessarily mean 100% reduction compared to the control. However, if ECx is related to zero rather than the observed minimum, then the definition that ECx = the concentration at which <variable> is reduced by x% is contradicted.

This becomes clear when a data situation is "thought through to the end", see figures below:




In the example above, the maximum value for the variable is “2”. From a test concentration of 10 mg/L, the measurement variable levels off at a value of “1”. Following the definitions from literature (see above), the test concentration of 10 mg/L corresponds to 100% effect. A 100% effect is therefore equivalent to a reduction of 1 and a 50% reduction is equivalent to a reduction of 0.5. So, the EC50, i.e. a 50% reduction, is achieved at a variable value of 2 minus 0.5 = 1.5, which corresponds to 5 mg/L (green part of the figure above). If the EC50 were related to zero, rather than to the observed minimum, the EC50 were achieved at a variable-value of 50% of 2 = 1) and calculated as 10 mg/L (i.e. related to the control) (red part of the figure above).

But - and this is the key point: Not only the EC50, but also the EC60, EC70, EC80... would correspond to a 50% effect rather than a 60%, 70%, 80%... effect.

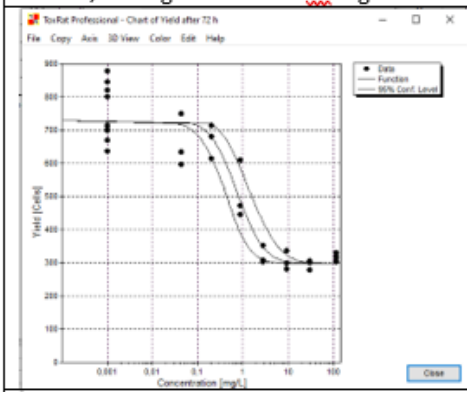
The definition of ECx is therefore violated by this calculation method. This is even more obvious if the maximum effect that can be achieved is, let's say, 10%. In this case, no EC20, EC50, etc. could be calculated. Or, if they are derived, the EC20 and EC50 would still be equivalent to a 10% effect.

In other words: *If the ECx derived from a 4-parameter non-linear regression is not related to the maximum achievable effect, then you cannot calculate any ECx, but only those up to the maximum achievable effect. I.e. the definition of ECx is violated.*

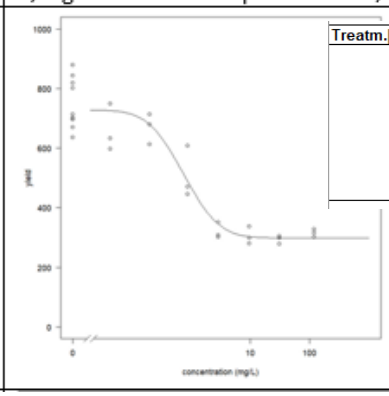
**(4) Same calculation method used in R standard procedure**

We evaluated a sample dataset with the maximum achievable effect about 60% reduction compared to the control both in ToxRat and in R:

ToxRat, 4 P-log-normal non lin regression



R, lognormal dose-response model, "*LN.4*"



| Treatm.[mg/L] | Mean | Std. Dev. | n | %Decrease |
|---|---|---|---|---|
| Control | 751,0 | 85,68 | 9 | |
| 0,043 | 659,3 | 79,35 | 3 | 12,2 |
| 0,200 | 668,7 | 51,12 | 3 | 11,0 |
| 0,870 | 508,0 | 87,89 | 3 | 32,4 |
| 2,870 | 320,0 | 26,96 | 3 | 57,4 |
| 9,550 | 305,0 | 28,81 | 3 | 59,4 |
| 30,900 | 293,8 | 13,51 | 3 | 60,9 |
| 119,000 | 315,0 | 13,00 | 3 | 58,1 |

| Toxicity Metric | EC10 | EC20 | EC50 |
|---|---|---|---|
| Value [mg/L] | 0,183 | 0,297 | 0,745 |
| lower 95%-cl | 0,097 | 0,160 | 0,341 |
| upper 95%-cl | 0,348 | 0,558 | 1,589 |

```
Estimated effective doses

       Estimate Std. Error     Lower     Upper
e:1:10 0.183574   0.133712 -0.091276  0.458423
e:1:20 0.296920   0.163177 -0.038494  0.632334
e:1:50 0.744984   0.204489  0.324650  1.165317
```

The results for EC10, EC20 and EC50 using ToxRat and using the drc package in R (functions: drm () and ED()) exactly agree - and especially the EC50 (0.745 mg/L) is clearly lower than the concentration showing 50% decrease compared to control (see data table). This clearly indicates, that also *in the standard procedure in R*, the ECx values are related to the maximum achievable effect, i.e. to control minus minimum, rather than to zero.

**To sum up:**
In current guidelines, it remains unclear how to address the issue of maximum achievable effect when calculating ECx values from 3- and 4-parameter non-linear regression.
In contrast, in literature, there is clear evidence, that ECx values should be calculated related to the maximum achievable effect (maximum – minimum), *with "minimum" depending on the used function:*
*With a 3-parameter function*, the minimum of the function is assumed to be zero. This results in ECx values where x = percentage effect relative to the control.
*With a 4-parameter function*, the minimum of the function is assumed to be the observed minimum. This results in ECx values where x = percent effect relative to the maximum achievable effect, rather than the control.

Calculating ECx requires to stay with the assumed function for regression. If one regards the resulting ECx from a 4-parameter function too low, *the correct alternative is to apply a 3-parameter regression*. If the data do not support this assumption (e.g. because of an inappropriate range of tested concentrations), this probably is at the expense of accuracy, i.e. the confidence range can become wider.

Some users prefer to modify the standard procedure and suggested an "alternative approach": they use a 4-parameter function with minimum = maximum achievable effect to produce a visually well-fitting fit, and subsequently set the minimum = zero to calculate a so called "ECx". This results in higher "ECx" values compared to those obtained by the standard procedure suggested in literature. A modification of standard procedures may well be justified - but it is essential that such changes are clearly stated and immediately apparent.
Moreover: If the ECx derived from a 4-parameter non-linear regression is not related to the maximum achievable effect, then you cannot calculate any ECx, but only those up to the maximum achievable effect. I.e. the definition of ECx is violated.

As R is open source, it is possible to implement non-standard calculation methods, e.g. using a 4-parameter function with minimum = maximum achievable effect to produce a visually well fit, and setting the minimum = zero to calculate a so-called "ECx". This illustrates the problem of the lack of standardisation in open source applications.

We therefore consider it essential that the procedure for calculating ECx values based on regressions is clearly addressed in future guidance documents to avoid erroneous conclusions and non-standardized procedures.