17 January 2024

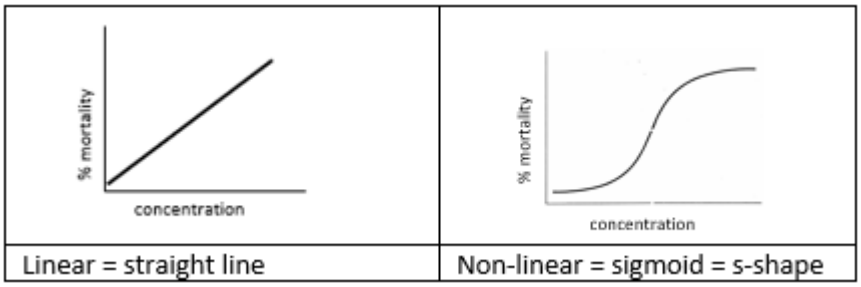
Some thoughts about slope, LD50, dose response functions and calculation of PIEs as discussed in the EFSA Bee Guidance Document[1]

A central idea of the EFSA Bee Guidance Document (2023) is the determination of a predicted individual effect (PIE) for a predicted environmental quantity (PEQ) by 'back-calculating' the function for the regression. The EFSA Bee Guidance Document (2023) uses non-linear regression for this. However, the implementation according to the EFSA document raises a number of questions and we think that the objective - determining a PIE for a PEQ - can be achieved at least as well with a linear regression, as offered by ToxRat.

Given the large scope of the EFSA document including supplementary information, we may have missed some aspects. Therefore, please consider the statements below as a basis for discussion.
In view of future discussions, all references to the EFSA Documents are written in purple, to find the passages more quickly later on.

First of all, let´s clarify a few terms which in fact often get mixed up.

dose response curve means the visual shape of <variable> vs concentration
here: variable = %mortality.



Linear = straight line	Non-linear = sigmoid = s-shape

There is no question that mortality in bees follows a non-linear *dose-response curve.*

regression is a mathematical procedure to fit a dose-response-curve to the data; thereby, a certain *dose-response-function* is used

dose-response-function is a mathematical *model* or an *equation* to describe a dose-response-curve.
There are linear and non-linear functions - the difference is very simple:
a linear function looks always like y = bx + a , i.e. there are two parameters: (1) slope b and (2) y-axis-intercept a; --> results in a straight line
a non-linear function is - simply speaking - anything that is not a straight-line-equation, i.e. with an equation different from y=bx+a. The graph of a non linear function is a curve. A non-linear function can (but must not) have more than two parameters.

[1] Revised guidance on the risk assessment of plant protection products on bees (Apis mellifera, Bombus spp. and solitary bees), EFSA Journal 2023;21(5):7989, 133 pp. https://doi.org/10.2903/j.efsa.2023.7989)

So, *linear regression makes use of a linear function, non-linear regression makes use of a non-linear function*.
But - and that is probably a reason for confusion:
It is possible to model a non-linear dose-response curve with linear regression.
This is done by transforming the data in an intermediate step and then back-transforming the results. This is exactly how the classical Probit or Logit analysis works, which has been developed specifically for quantal data, such as mortality.

The EFSA document exclusively recommends non-linear regression for modelling mortality data. As justification, why the classical linear regression for quantal data should not be used, it is stated (EFSA doc. p66):

'A non-linear dose–response relationship allows a much more accurate calculation of effects as compared to linear dose–response relationships. In the years since the publication of the previous guidance (EFSA, 2013a,b), evidence has been collected that observed dose response data follow sigmoidal rather than linear dose–response relationships."

Here, obviously the terms 'dose response relationship" and 'regression" are mixed up. There is no question, that mortality follows a nonlinear dose response relationship. But it is also beyond doubt, that the classical method to model such non-linear dose response relationships for quantal data is the linear regression, e.g. Probit or Logit. Hence, the EFSA document does not explain at any point why the classical linear regression should not be suitable for quantal data, especially in view of the fact that linear regression is commonly found in bee study reports.

Usually, the resulting LDx values from both regression types (linear vs non-linear) are very similar. Nevertheless, it is important to be aware of the fact that the used functions are completely different. *Hence, the parameter 'b' = slope obtained for a linear dose-response function must not be used in a non-linear dose-response function and vice-versa.*

To make it even more difficult, the names of the different mathematical functions are often very similar:
Functions used for linear regression: 'Probit', 'Logit'
Functions used for non-linear regression: 'log-normal', 'log-logistic'
And if there are only two parameters used for non-linear functions, then they are sometimes called 'log-probit' or 'log-logit'.

In the following, let´s focus on the recommendations in the EFSA document - and the possibilities and limitations of using ToxRat for these purposes.

A key idea of the EFSA document is the calculation of a PIE (predicted individual effect) to a PEQ (predicted environmental quantity). For this purpose, the function describing the dose-response relationship is to be calculated 'backwards', i.e. the PEQ of interest is entered together with the LD50 and the slope into the equation (parameter 'c' = control mortality is set to zero, see supporting document p 102). A percent effect (the PIE) is then obtained for the PEQ of interest, EFSA doc p 66:

Formally, the calculation of the predicted effect, in unit of percentage, is given by

$$PIE_j = 100 \cdot f(DRC_j, PEQ_j) \qquad (18)$$

The equation of the DRCj function is found out somewhat indirectly in various places, and in fact, there are several possible functions available (supplementary information, p. 102, Table 37), but in the end it comes down to the log-logistic function (Table 29, p69):

Log-logistic

$$c + (1-c)\left(1 - \frac{1}{1+\left(\frac{x}{e}\right)^b}\right)$$

with b=slope, e=inflection point (=LD50), c= minimum (=control mortality).

The *log-logistic function* (and also the log-normal function) are non-linear functions, they are fitted to the data using non-linear regression. In ToxRat 330, non-linear regression is only offered for metric data, but not (yet) for quantal data. This will come with ToxRat 4.0.

So, to make it clear:

ToxRat 330 fits a so-called *logit function* to the data using *linear regression.* Thereby, a slope 'b' is provided. But: this slope 'b' only applies to the underlying linear function. **Using the slope 'b' from the linear logit function for the non-linear log-logistic function to calculate a PIE is like using "apples in a pears-equation": it is incorrect. The resulting error in the PIE can be orders of magnitude.**

But: there is multiple justification to use (ToxRat´s) linear regression instead.

The key advantage of non-linear regression for quantal data over linear regression is that if a 3-parameter-function is used, control mortality is considered by means of a function parameter. Therefore, it does not require Abbott´s correction to account for control mortality (see also OECD 2006 Statistical Guidance Document).

However - following the supporting document p 102, in the suggested non-linear functions, the parameter for control mortality ('lower limit', 'c') is apparently set to zero:

'When fitting dose-responses to experimental data, the lower limit (d in Ritz[2], 2010 and a in the BMD guidance) can in practice be higher than zero, as some mortality can occur in the control. However, note that the real value of this parameter is unimportant in the context of the present risk assessment scheme, which is focusing on the effects triggered by the pesticide exposure."

It is not really clear here, whether this actually means that control mortality is already set to zero in the non-linear regression, i.e. that only two parameters are used for regression, namely 'b' = slope and 'e' = inflection point (=ED50) - or whether this only means that the parameter 'c' is not used for calculation of the PIE (as demonstrated in Table 29 (p 69, EFSA document).

There are two arguments supporting the interpretation that only two parameters are used for regression:

(1) In Figure 13, EFSA doc. p 67, which illustrates the PIE approach, no control mortality is shown at all. If control mortality were considered for regression, the curve should start somewhat higher than at 0%

[2] 'd' is obviously a typing error in the EFSA document, in Ritz (2010), and in supporting document p102, Table 37, respectively, the lower limit is parameter c

(unless the control mortality in that test was actually zero. This, however, then should have been stated).

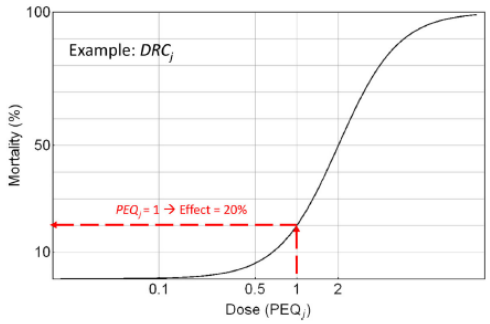


Figure 13: Graphical illustration of the proposed calculation for the effect on a specific endpoint using a non-linear dose–response curve (DRC_j). The resulting mortality (%) can be interpreted as probability of one individual to die on exposure to a certain dose, which can also be interpreted as a percentage of a cohort of individual bees to die after exposure to the identical dose

(2) In the Draft Guidance Document (July 2022), the corresponding section explicitly states that a 2-parameter log-logistic non-linear regression is meant (Draft Doc p 76):

2458 As explained in Chapter 6, the dose-response is assumed to be a 2-parameter log-logistic dose-
2459 response, described by the two hazard parameters $LD_{50,j}$ and $slope_j$. A non-linear dose-response
2460 relationship allows a much more accurate calculation of effects as compared to linear dose-
2461 response relationships (see Chapter 6). In the years since the publication of the previous guidance
2462 (EFSA, 2013), evidence has been collected that observed dose response data rather follow
2463 sigmoidal, not linear dose-response relationships (see Section 6.3 and Supplementary document
2464 Section 6.3).

2465 Formally, the calculation of the predicted effect, in unit of percentage, is given by

2466
$$PIE_j = 100 \cdot f(LD_{50,j}, slope_j, PEQ_j) = 100 \cdot \frac{1}{1 + \left(\frac{PEQ_j}{LD_{50,j}}\right)^{slope_j}}$$

2467 where *PIE* is the predicted individual level effect following the application and exposure of a PPP,
2468 *j* refers to a risk case as assessed in an experimental test such as acute-contact, acute-dietary,
2469 chronic-dietary or repeated-dose-larvae; $LD_{50,j}$ and $slope_j$ are the relevant hazard parameters
2470 that parameterise a log-logistic dose-response function *f*, and PEQ_j is a realistic worst-case
2471 exposure estimate for the respective exposure assessment Tier, which could be screening, or

In the Final Guidance Document (2023) p 66, the wording has been changed to a more general recommendation of non-linear models (rather than only the 2-parameter log logistic). However - it is not stated anywhere that more than two parameters should be used:

As explained in Chapter 6, the dose–response used in this guidance document is limited to four non-linear models. A non-linear dose–response relationship allows a much more accurate calculation of effects as compared to linear dose–response relationships. In the years since the publication of the previous guidance (EFSA, 2013a,b), evidence has been collected that observed dose response data follow sigmoidal rather than linear dose–response relationships (see Section 6.3 and supplementary document Section 6.3).

Formally, the calculation of the predicted effect, in unit of percentage, is given by

$$PIE_j = 100 \cdot f(DRC_j, PEQ_j) \qquad (18)$$

where PIE is the predicted individual level effect following the application and exposure of a PPP, j refers to a risk case as assessed in an experimental test such as acute-contact, acute-dietary, chronic-dietary or repeated-dose-larvae; DRC_j is the dose–response function f; and PEQ_j is a realistic worst-case exposure estimate for the respective exposure assessment Tier, which could be screening, or

ToxRat Solutions GmbH & Co. KG
Naheweg 15 D-52477 Alsdorf
Amtsgericht Aachen / County Court Aachen HRA 8916
Persönlich haftende Gesellschafterin / Personally liable partner:
ToxRat Solutions Verwaltungs GmbH
Amtsgericht Aachen / County Court Aachen HRB 20820
Geschäftsführerin / Managing director: Dr.Monika Ratte

Ust.-IdNr. / VAT No DE 813358923

Bank details:
Sparkasse Aachen, IBAN DE22 3905 0000 0047 6739 42
SWIFT-BIC AACSDE33

Provided that parameter ‘c’ (= control mortality) indeed is not used for non-linear regression, this negates the main advantage of non-linear regression over linear regression.

Last but not least:

The EFSA document recommends to make use of Abbott´s correction when deriving a ‘surrogate dose response’, see EFSA doc p 57:

> For example, considering a limit test where the control mortality was equal to 3% and the mortality in the only treatment level (100 µg a.s./bee) was 9% (corrected mortality = 6.2%). As the (corrected) effect of the treatment was below 10%, the extrapolated LD50 should use the factor of 4.6 from Table 23, thus: surrogate LD50 = 100 µg a.s./bee × 4.6 = 460 ug a.s./bee. This value together with the

Here, the term ‘Abbott’ is not used - but the presented calculations to obtain the corrected mortality is nothing else than the application of the formula for Abbott´s correction:

$$\text{Corrected \%} = \left(\frac{\text{Mortality \% in treated plot - Mortality \% in control plot}}{\text{100 - Mortality \% in control plot}} \right) * 100$$

Using the data of EFSA doc p 57: corrected% = ((9 -3)/(100-3))*100 = 6.2%

To sum up:
If only two parameters are used with the log-logistic function anyway, and in view of the fact that the EFSA document makes use of Abbott´s correction, we regard it as justified, to use classical linear regression (including Abbott´s correction in case of any control mortality) instead of non-linear regression.

In order to ‘back-calculate’ the linear functions used in ToxRat 330 to determine a PIE, we have developed a tool in which the slope and intercept parameters determined with ToxRat and the desired PEQ are entered and the PIE is obtained as the output. This is exactly what the EFSA document suggests - but is based on parameters from linear regression instead of based on non-linear regression.
We will be offering the tool for PIE determination from Probit, Logit and Weibull functions as a browser application on our website.

Additionally, we have described how you can determine a PIE for any exposure value (PEQ) in ToxRat without a ‘back calculation’, even including a measure of uncertainty.

https://www.toxrat.com/files/toxrat/documents_info/How%20to%20get%20a%20PIE%20for%20PEQ%20using%20ToxRat_Jul23.pdf