Take two – Uncertainties with EC_x calculations are reduced by assessing confidence limits as well as performing the "good old" visual inspection of data

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Introduction

The use of EC_x (Effect Concentration with an effect of x %) instead of the No-Observed Effect Concentration/Lowest Effect Concentration (NOEC/LOEC)-approach is the preferred endpoint in many OECD guidelines (e.g. OECD 201 (2011)). For the assessment of the quality of the calculated EC_x and the reliability of the used models many parameters are suitable:

- **Chi²-value** for the correspondence between the distribution of the calculated values and the experimental data.
- **F-value** from the F-test for the statistical significant difference of the slope of concentration/dose-effect curve from zero,
- **Coefficient of determination r^2** for the goodness of fit of the concentration/dose-effect curve.
- **Normalized width (NW) of confidence limits of an EC_x** (i.e. difference between upper/lower 95 % CI, divided by EC_x (EFSA (2019)).
- **Overlap of EC_{10} and the lower 95 % confidence interval of EC_{20}** (EFSA (2019)).

Problem

All these parameters can demonstrate a strong reliability of the one selected concentration-effect curve but a careful visual inspection of this curve is sometimes essential to identify the unsatisfactory fit for the whole range of concentrations. This can be observed when:
1) the effect increases slowly at the lower test concentrations and increases rapidly at the highest test concentrations (**Figure 1 & 2**).
2) the effect does not follow a continuous concentration-effect relationship but shows a saturation in one range of concentrations and increases afterwards again (Figure 3 & 4).
Therefore, the use of only one concentration-effect in the risk assessment might over-/underestimate the EC_{10}, EC_{20} and EC_{50}.

Example 1 (Algal growth inhibition test, growth after 72 hours, OECD 201)

Figure 1: Concentration-effect curve of growth rate with all nine test concentrations

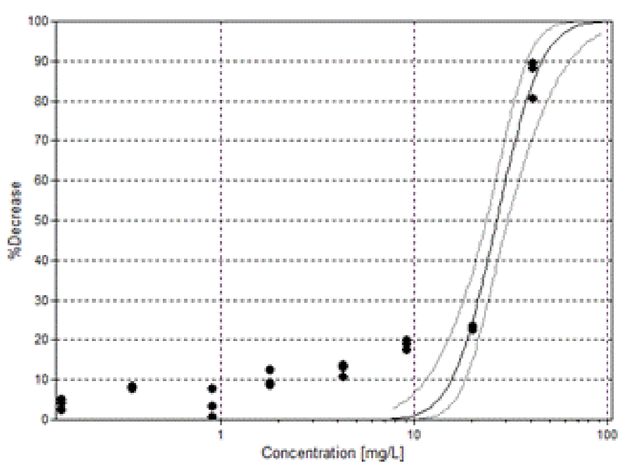
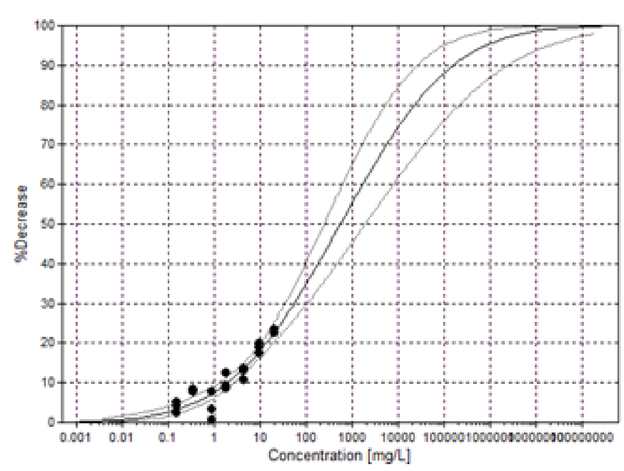


Figure 2: Concentration-effect curve of growth rate excluding the highest test concentration of mean measured 41 mg/L



	EC_5	EC_{10}	EC_{20}	EC_{25}	EC_{50}
Value [mg/L]	13.3	15.5	18.7	20.0	26.7
lower 95% CI	12.5	14.8	18.0	19.4	26.0
upper 95% CI	14.0	16.2	19.3	20.7	27.4
Normalised width of CI	0.11	0.09	0.07	0.07	0.05
	excellent				
Overlap EC_{10} with lower 95 % CI of EC_{20}:	no				

	EC_5	EC_{10}	EC_{20}	EC_{25}	EC_{50}
Value [mg/L]	0.386	1.92	13.4	27.9	n.d.
lower 95% CI	0.174	1.26	10.1	19.5	n.d.
upper 95% CI	0.660	2.63	19.1	46.1	n.d.
Normalised width of CI	1.3	0.71	0.67	0.95	-
	poor	fair			
Overlap EC_{10} with lower 95 % CI of EC_{20}::	no				

Table 1: Probit Analysis using linear maximum likelihood regression and Fieller´s theorem for confidence intervals

Parameter	Fig. 1	Fig. 2
Slope b:	5.453	0.522
Intercept a:	-7.774	-1.429
Variance of b:	14.914	0.687
Degrees of freedom:	22	19
Chi² (Goodness of Fit):	1.196	0.077
p(Chi²):	1.000	1.000
Log EC50:	1.426	2.738
SE Log EC50:	0.107	3.218
g-Criterion:	0.006	0.045
r²:	0.625	0.837
F:	36.675	97.89
p(F) (df: 1;13):	<0.001	<0.001
Slope function:	1.525	82.418

SE: Standard Error
CI: Confidence Interval
n.d.: Not determined

Example 2 (Adult honey bee acute oral toxicity test, mortality after 48 hours, OECD 213)

Figure 3: Dose-effect curve of survival with all five test doses

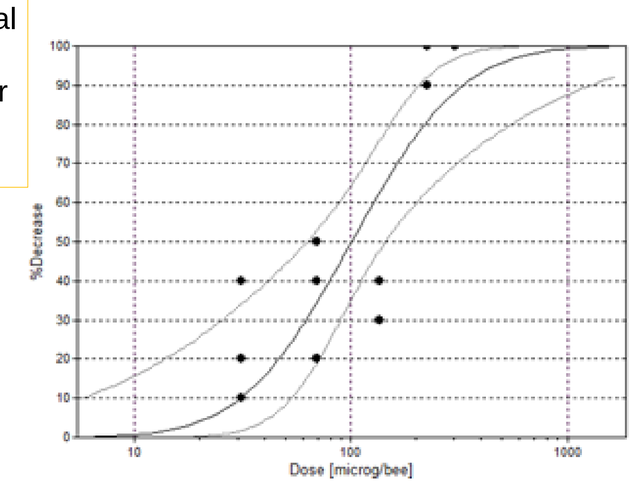
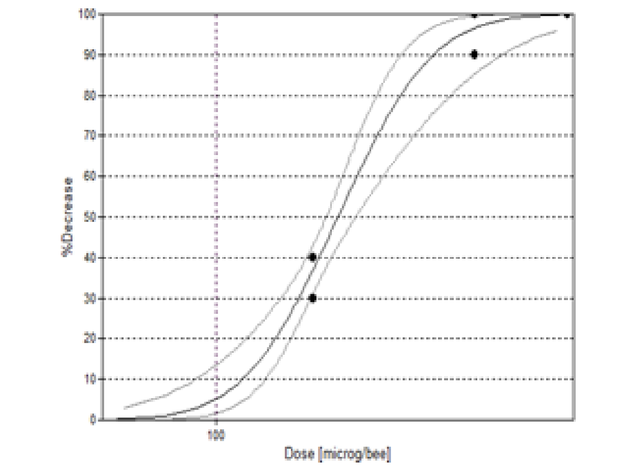


Figure 4: Dose-effect curve of survival excluding the two lowest test doses



	ED_5	ED_{10}	ED_{20}	ED_{25}	ED_{50}
Value [mg/L]	22.1	31	46.4	54.2	101
lower 95% CI	2.84	5.86	13.9	19.2	62.8
upper 95% CI	42.2	53.2	71.3	80.2	144
Normalised width of CI	1.78	1.53	1.24	1.23	0.80
	poor				fair
Overlap EC_{10} with lower 95 % CI of EC_{20}:	yes				

	ED_5	ED_{10}	ED_{20}	ED_{25}	ED_{50}
Value [mg/L]	99.6	109	120	125	147
lower 95% CI	81.9	93.6	110	116	141
upper 95% CI	109	117	126	131	155
Normalised width of CI	0.09	0.21	0.13	0.12	0.10
	excell.	good	excellent		
Overlap EC_{10} with lower 95 % CI of EC_{20}::	no				

Table 2: Probit Analysis using linear maximum likelihood regression and Fieller´s theorem for confidence intervals

Parameter	Fig. 3	Fig. 4
Slope b:	2.497	9.775
Intercept a:	-5.003	-21.177
Variance of b:	1.738	196.25
Degrees of freedom:	13	7
Chi² (Goodness of Fit):	2.985	0.082
p(Chi²):	0.998	1
Log EC50:	2.004	2.167
SE Log EC50:	0.145	0.072
g-Criterion:	0.299	0.135
r²:	0.546	0.855
F:	15.617	41.372
p(F) (df: 1;13):	0.002	<0.001
Slope function:	2.515	1.266

SE: Standard Error
CI: Confidence Interval
n.d.: Not determined

Conclusion

Based on the assumption that one concentration/dose-effect curve does not reliably represent the observed pattern of effects over the whole range of tested concentrations/doses, two different concentration/dose-effect calculations in two different concentration/dose ranges can improve the estimation of reliable EC/D_{10} and EC/D_{20} as well as EC/D_{50} with an acceptable representation of the experimental data for the whole data set.

Acknowledgements

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Sources

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