Centro Internazionale per gli Antiparassitari e la Prevenzione Sanitaria
International Centre for Pesticides and Health Risk Prevention (ICPS)

Ospedale Luigi Sacco
AZIENDA OSPEDALIERA - POLO UNIVERSITARIO

European Food Safety Authority

WAGENINGEN UR
For quality of life

From NOEC to EC_x: a large scale data analysis on ecotoxicological studies with pesticides

Flavio Marchetto [(1)], Francesco Galimberti [(1)], Waldo de Boer [(2)], Hilko van der Voet [(2)], Angelo Moretto [(1)(3)]

[(1)] ICPS - International Centre for Pesticides and Health Risk Prevention, Milan, IT; [(2)] Wageningen University and Research Centre, Wageningen, NL; [(3)] Università degli Studi di Milano, Milan, IT

flavio.marchetto@icps.it, francesco.galimberti@icps.it

Introduction

Historically chronic or long-term studies performed for the authorisation of plant protection products (PPP) result in the reporting of endpoint values in terms of No-Observed Effect Concentration (NOEC). NOECs are however criticized since their values strongly depends on the experimental study design, and nowadays the extrapolation of Effect Concentrations (EC) from the dose-response curve is considered more appropriate also for long-term studies. The new Regulation [A] for the authorisation of PPPs and the related data requirements [B,C] suggest that ecotoxicological endpoint data from chronic or long-term studies provided by the Applicant are reported as EC_{10} or EC_{20} values together with the NOEC. However, there is no systematic comparison available to compare NOEC values to EC_{10} and EC_{20} values derived from the same study.

Objectives

In the present work long term and chronic studies with pesticides on aquatic organisms are re-analysed in order to calculate NOEC, EC_{10}, EC_{20}, EC_{50} and their limit of confidence (95%) using appropriate statistical analyses. A comparison of NOEC with EC_x values and their lower limit of confidence is performed by analysing the distribution of the NOEC/ EC_x ratios. Considerations are made on studies based on the same organisms and on the study design (appropriately developed to calculate EC_{50} for algae and macrophytes and NOEC for fish and daphnids).

Materials and methods

Data selection:

- Dataset: 200 studies on fish, daphnids, algae and aquatic macrophytes. Studies not resulting in a dose/response curve and studies without no-response level are not considered further.
- Only effects related to the endpoint(s) of ecotoxicological relevance (lowest NOEC or EC_x reported in the original reports) are taken into account; in case of two or more effects resulting in equal endpoints (same NOEC or EC_x) all the effects are considered.
- The corresponding raw data are used to re-calculate NOEC, EC_{10}, EC_{20}, EC_{50} , and the lower confidence limits (LL, 95%) of the EC_x.

Data analysis:

NOEC Calculation:

- Count and continuous data: significance is calculated using Dunnett's procedure for multiple comparisons, and using a one-sided test with confidence probability 95%.
- Quantal data: Fisher's exact test, in particular useful when dealing with small counts, for 2 x 2 contingency tables based on the hypergeometric distribution is used.

Dose-Response Models:

- Count and continuous dose-response data: a sequence of models from the exponential and the Hill model family are applied. This sequence of models are nested and differ in complexity and number of parameters [D].
  The log-likelihood ratio test is used to determine the optimal model(s) within each family of models.
- Quantal data, the logistic, log-logistic, complementary log-log and logistic with mortality are applied.

Count and Continuous data				
	Exponential family		**Hill family**	
M1	y = a	M1	y = a	b = 0, c = 0, d = 0
E2	y = a*exp(bx)	H2	y = a*[1 - x/(x+b)]	c = 0, d = 1
E3	y = a*exp(bx^d)	H3	y = a*[1 - x^d / (b^d + x^d)]	c = 0
E4	y = a*[c - (c - 1)*exp(-bx)]	H4	y = a*[1 + (c - 1) x / (b + x)]	d = 1
E5	y= a*[c - (c - 1)*exp(-bx^d)]	H5	y = a*[1 + (c - 1) x^d / (b^d + x^d)]	

Quantal data	
Logistic	y = 1 / (1 + exp(-b(x - e)))
Log-Logistic	y = 1 / 1 + exp(b(log(x) - log(e))
Complementary log-log	y = exp(-exp(a + bx))

- The choice of the final model between non-nested alternative models is based on Akaike information criterion.
- The EC_{10}, EC_{20} and EC_{50} are calculated together with a 95% confidence interval using the parametric bootstrap method with 100 uncertainty runs. For zero responses, the detection limit is set to 10% of the minimum non-zero response.

Comparison NOEC-EC_x:

- Ratios between NOEC and EC_{10}, EC_{20}, EC_{50}, and the lower confidence limits of the EC_x, are calculated for the four taxa. The distribution of the results are reported in box-plots.

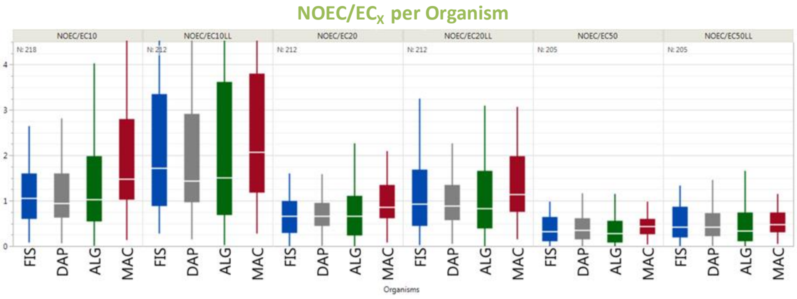
EC_{50}/EC_{10} slope:

- Ratios between EC_{50} and EC_{10} are reported to estimate the slope of the dose response curves.

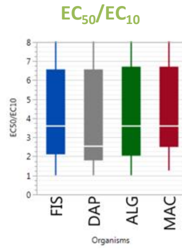
Results and discussion

- Graphical ratios between NOEC and EC_x show strong similarities among fishes, daphnids and algae. Macrophytes shows a slightly different pattern.
- Fish, daphnids and algae have a resulting median value of NOEC/EC_{10} ratio close to 1 as expected, while for aquatic macrophytes a similar ratio is shown for NOEC/EC_{20}.
- As also expected, NOEC/EC_{20}LL ratio for all the four organisms are similar to NOEC/EC_{10} ratio, with a value for macrophytes slightly higher than others.
- Slope of curves, estimated by the EC_{50}/EC_{10} ratio, shows similar results for fish, algae and macrophytes, while for daphnids the median value is lower.
- Dispersion of the NOEC/EC_xLL (difference between 1st and 3rd quartiles)is wider than the dispersion of corresponding NOEC/EC_X. No taxa relation can be observed in the wide of dispersion of the resulting ratios even if generally daphnids show a lower dispersion among taxa.
- An evaluation of the percentage of EC_X values lower than NOEC is presented in order to assess the protection level of the endpoint. By comparing EC_{10} and EC_{20}LL, whose NOEC/EC_X ratios are close to one, the percentages of EC_{20}LL are slightly lower than the EC_{10} ones. EC_{20} shows even lower percentages.

NOEC/EC_X per Organism

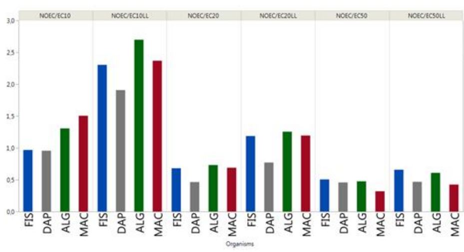


EC_{50}/EC_{10}



(JMP software)

Quartile 3 – Quartile 1



NOEC > EC_X (%)

	NOEC>EC_{10}(%)	NOEC>EC_{10}LL(%)	NOEC>EC_{20}(%)	NOEC>EC_{20}LL(%)	NOEC>EC_{50}(%)	NOEC>EC_{50}LL(%)
ALG	51%	60%	26%	43%	10%	13%
DAP	45%	75%	25%	44%	5%	9%
FIS	56%	73%	24%	49%	2%	14%
MAC	77%	86%	36%	58%	2%	7%

Conclusions

- Results obtained from algae (derived from a study design aiming to the EC_{50} extrapolation) show a close similarity to fish and daphnids (derived from a study design aiming to NOEC extrapolation) instead to macrophytes as expected. This suggests a stronger influence of experimental data on obtained results instead on a dependence of the study design.
- Median values for EC_{10} and EC_{20}LL result close to NOEC for fish, daphnids and algae, while for aquatic macrophyte the EC_{20} (together with EC_{20}LL) results closer to the NOEC than EC_{10}.
- In assessing the pesticide risk for aquatic organisms, the protection level gained by selecting EC_{10} as long-term/chronic endpoint, is greater than the NOEC one for macrophytes and similar among the other three taxa. For EC_{20}LL the protection level is lower.

References

A - Regulation (EC) No 1107/2009 of the European Parliament and of the Council of 21 October 2009 concerning the placing of plant protection products on the market and repealing Council Directives 79/117/EEC and 91/414/EEC.

B - Commission Regulation (EU) No. 283/2013 setting out the data requirements for active substances, in accordance with Regulation (EC) No. 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

C - Commission Regulation (EU) No 284/2013 of 1 March 2013 setting out the data requirements for plant protection products, in accordance with Regulation (EC) No 1107/2009 of the European Parliament and of the Council concerning the placing of plant protection products on the market.

D - EFSA (2009). Guidance of the Scientific Committee on a request from EFSA on the use of the benchmark dose approach in risk assessment. The EFSA Journal 2009: 1150, pp1-72

The present poster is part of a project which received funding from EFSA (GP/EFSA/PRAS/2013/01). This publication reflects only the author's view and EFSA is not responsible for any use that may be made of the information here contained.

Sistema Sanitario Regione Lombardia