A comprehensive laboratory comparison for generating data for green algae response to time-variable exposures

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Introduction and methods

The use of Toxicokinetic-Toxicodynamic (TKTD) modelling in European risk assessments for plant protection products is rapidly gaining momentum – especially following the 2018 publication of the EFSA opinion on TKTD modelling which states that several models are now ready for use in risk assessment [1]. This report reviewed a number of TKTD models for primary producers, including the SAM-X model for green micro-algae. This model was considered not ready for use in risk assessment due to the lack of a robust and ring tested methodology for generating calibration and validation studies. In response, the present work funded by CropLife Europe (CLE) aims to establish robust and standardized guidelines for generating data for algae responses to time-variable exposures.

The CLE project tackles two distinct methodologies – a flow-through system (method A) and a semi-static system (method B). Each of these methods are evaluated by conducting a laboratory comparison on comprehensive parameters across six and eight independent laboratories, respectively. We here provide a detailed overview of the project results for method B. The work for method A is still ongoing and will be presented later. Green algae of the species *Raphidocelis subcapitata* were exposed to time-variable concentrations of the test item flurtamone.

Method B is based on OECD TG 201 [2]. Variable exposure patterns are achieved using a semi-static system. The exposure and non-exposure periods are achieved by transferring subsets of algal cells to new flasks using a filtration and resuspension step. The concentration in the sequence of flasks was varied to achieve the desired variable exposure. Each laboratory tested two pulsed exposure patterns, with two 24-hour lasting peaks. Each pattern was tested for three peak concentrations of 25, 50 and 100 µg test item/L in comparison to an untreated control.

Results and Discussion

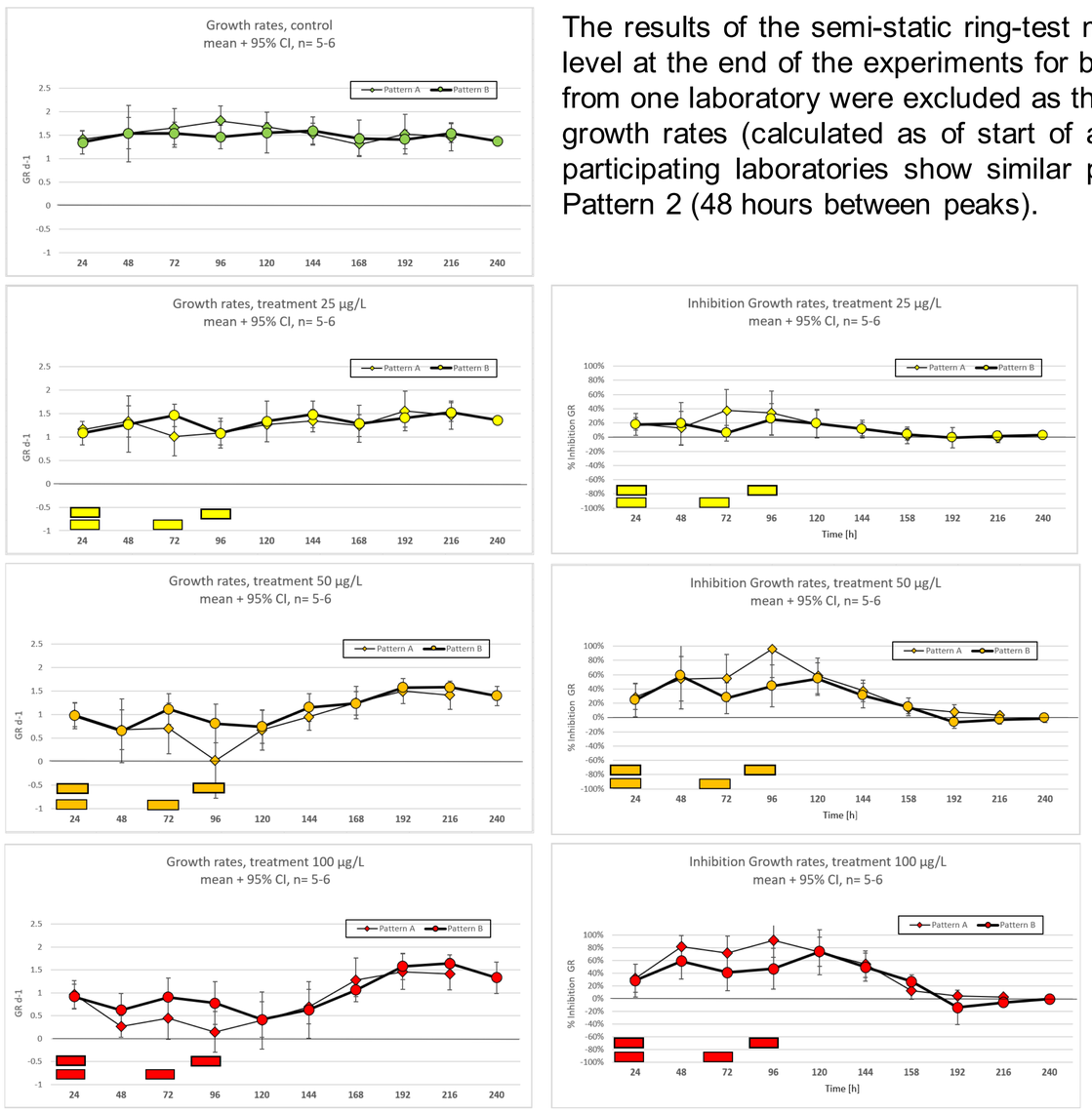

Figure 1: *Growth rates calculated as of start of a certain growth phase and inhibitions of growth rates for both exposure patterns; semi-static test. Pattern 1 (24h interval) = Pattern A; Pattern 2 (48h interval) = Pattern B*

The results of the semi-static ring-test method are presented alongside with statistical evaluations. Growth rates reached control level at the end of the experiments for both exposure patterns. Results of different laboratories show a consistent pattern. Results from one laboratory were excluded as the biological as well as the analytical results did not fulfil the requirements. In **Figure 1**, the growth rates (calculated as of start of a certain growth phase) and their inhibitions over time are presented. The results of the participating laboratories show similar patterns. Pattern 1 (24 hours between peaks) resulted in higher growth inhibitions than Pattern 2 (48 hours between peaks).

The chemical analysis confirmed that the test item concentrations were within ± 20% of nominal concentrations with very few exceptions. Thus, the test item flurtamone proved to be stable. Considering the few exceptions, measured concentrations were considered for statistical analyses.

Table 1 provides information on the inter- and intra-laboratory variability. The data presented in this table refer to the growth in controls in all initial 24h-sections during the study – comprising the study start as well as the 24-hour time intervals after each filtration/resuspension step. The data demonstrate a very good comparability of control growth within the different labs as well as between the participating laboratories.

Table 1: *Results for relative repeatability and -reproducibility of control growth rate in all initial 24-hour sections*

	Control growth rate in all initial 24-hour sections	
	Pattern 1 (24h interval)	**Pattern 2** (48h interval)
number of laboratories with tests performed	7	7
number of laboratories with tests considered *	6	6
number of tests considered *	6	6
number of initial GR considered per test	5	5
number of laboratories for statistics	**6**	**6**
number of GR for statistics	**30**	**30**
repeated measurements for statistics	6x5	6x5
laboratories identified as outlier (not excluded)	2 (a)	2 (b)
min / max GR [d^{-1}]	1.052 / 2.393	0.752 / 2.002
mean GR [d^{-1}]	**1.574**	**1.462**
95% CI GR [d^{-1}]	1.226 - 1.929	1.174 - 1.751
sr % (relative repeatability - standard dev.)	17.9	18.3
sL % (relative between-laboratory - standard dev.)	11.2	4.3
sR % (relative reproducibility - standard dev.)	**21.1**	**18.8**
sR / sr	**1.2**	**1.0**

GR = initial-24h-sectional-growth rate [d-1], 95% CI = 95% Confidence Interval, sr%, sL% and sr% = relative repeatability- / relative between-laboratory- / relative reproducibility - standard deviation. No outliers excluded.
* One lab excluded; for another lab, only second test run considered.
(a) One lab, Mandels k test significant --> points to intralaboratory variability; another lab = Mandels h test significant --> points to laboratory mean deviating from the overall mean
(b) Two labs, Mandels k test significant --> points to intralaboratory variability

Please see also the following related posters:

4.09.P Th316 “Statistical analysis of Laboratory Comparison data: how we demonstrate robustness”
4.09.P Th315 “A framework for algae modelling in regulatory risk assessment”

Conclusion

The presented data suggest the usability and robustness of the proposed method in general and for model validation. Based on the results and their statistical analysis, validity criteria will be proposed. The use of such data for TKTD modelling as derived from method B is discussed on the related poster “A framework for algae modelling in regulatory risk assessment”. The outcome of the laboratory comparison test will be published with complete transparency in peer reviewed scientific journals including the experimental protocols, data, and statistical analysis.

Participating Laboratories

References

[1] EFSA PPR Panel (EFSA Panel on Plant Protection Products and their Residues), 2018. Scientific Opinion on the state of the art of Toxicokinetic/Toxicodynamic (TKTD) effect models for regulatory risk assessment of pesticides for aquatic organisms EFSA Journal 2018;16(8):5377 DOI: 10.2903/j.efsa.2018.5377

[2] OECD Guidelines for testing of chemicals. No. 201 Freshwater algae and cyanobacteria, growth inhibition test. Adopted March 2006; corrected July 2011.