# Method and performance evaluation in a laboratory comparison test:
# Algal growth inhibition test with time-variable exposures

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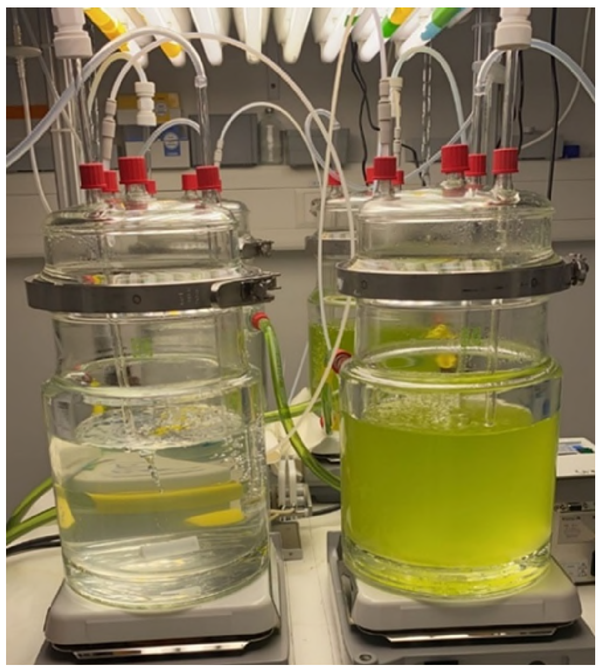
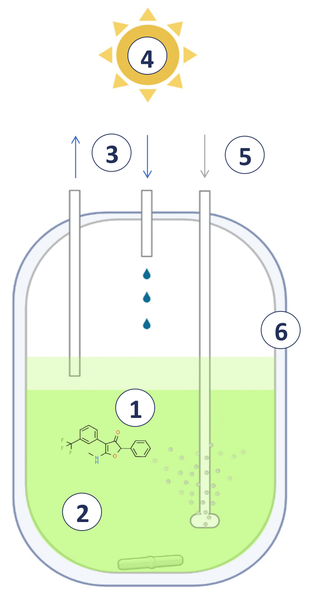
## Introduction

The Algal Growth Inhibition Test according to OECD guideline 201 is an essential part of the ecotoxicological risk assessment of plant protection products. However, the test system is limited in different exposure scenarios and does not reliably reflect real environmental conditions due to the static single-exposure. The innovative test setup of a combination of *in vitro* experimental data and the SAM-X *in silico* toxicokinetic/toxicodynamic (TK/TD) model for green-micro algae give a deeper insight into population effects and thus might refine future Tier 2C risk assessments for primary producers. Based on comments from EFSA on this test setup CropLife Europe wanted to investigate the general robustness and reproducibility of two methods (semi-static and flow through) for the validation and calibration of the algae TK/TD models. As part of their laboratory comparison test, we performed algae growth inhibition tests as semi-static test designs and in flow-through bioreactors with multiple peaks of the test substance and time variable intervals between exposures. We present the feasibility and some exposure scenarios of the flow-through system using *Raphidocelis subcapitata* and an herbicide.

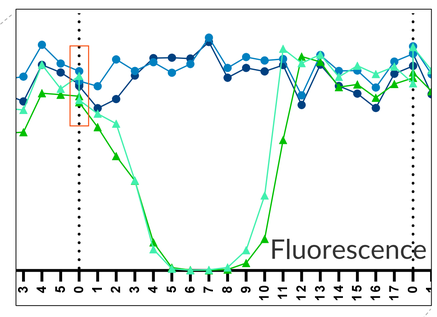




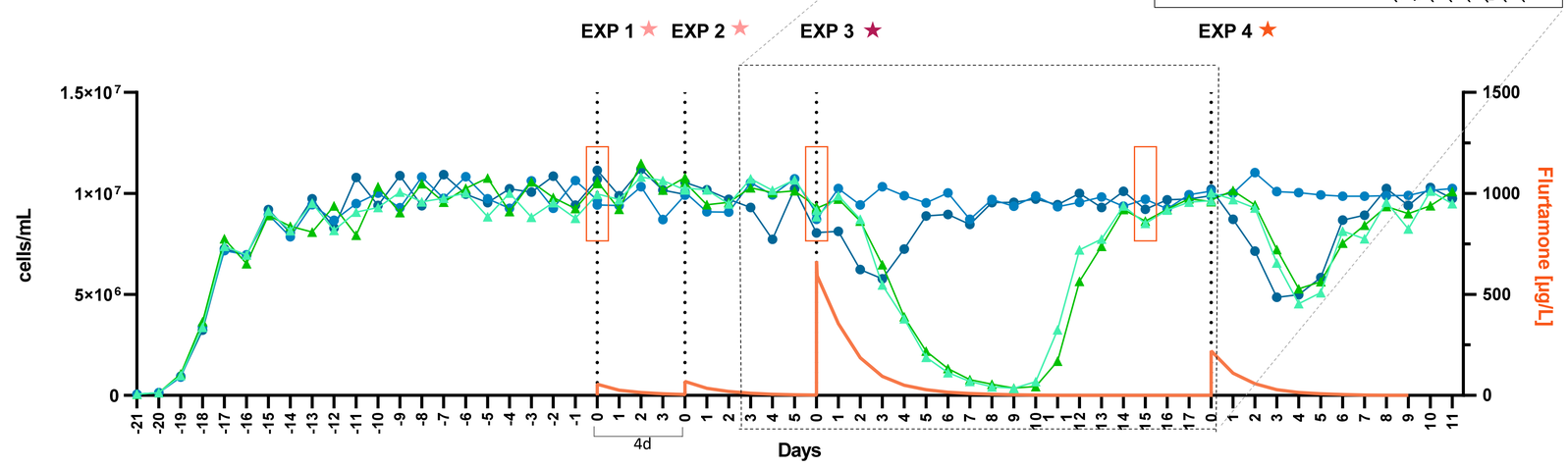

## Materials & Methods

| Parameters | |
|---|---|
| Test vessels | 4 x Bioreactors * (2x solv. controls, 2x test replicates) |
| Test substance (1) | Herbicide: Flurtamone* |
| Species | *Raphidocelis subcapitata* SAG 61.81 (Inoculum: 60'000 cells/mL) |
| Medium (2) | OECD 201, stirred |
| Flow rate (3) | 42 mL/h; Dilution rate = 0.5/day |
| Light (4) | 8 kLux ± 0.3 (108 µE $m^{-2}$ $s^{-1}$) |
| Air (5) | 1 L/min; Sterile |
| Temperature (6) | 24°C ± 1°C |
| Phosphate | 0.364 mg P/L |
| pH | pH= 8.1 |
| Biomass via | Cell number; Fluorescence* |

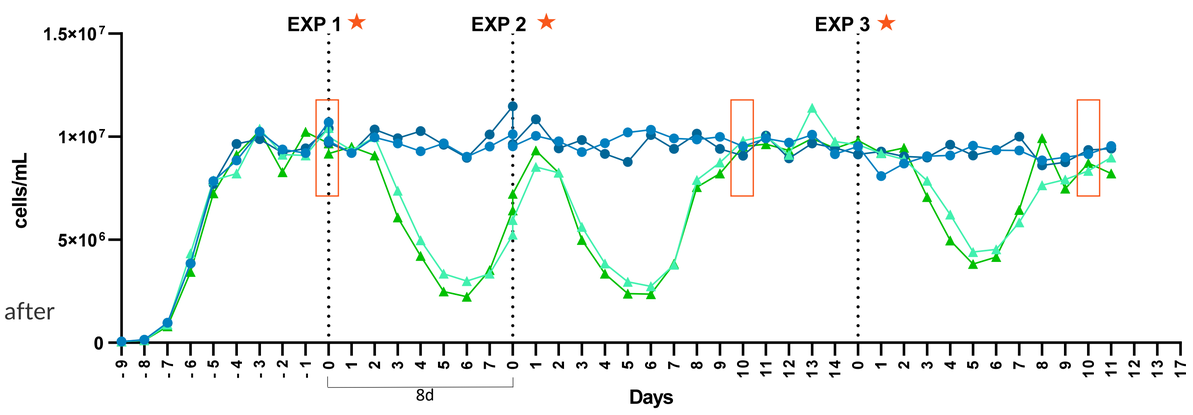
*Provided by CLE incl. methods and test protocols ( for EXP1 & EXP2)




**Design 1: Time-variable exposure with 4 peaks**




**Design 2: Time-variable exposure with 3 peaks**




**EXPOSURES**

- 60 µg/L Flurtamone
- 200 µg/L Flurtamone
- 600 µg/L Flurtamone

Control 1 / Replicate 1
Control 2 / Replicate 2
Flurtamone [µg/L]
Steady state

## Results & Discussion

- **Steady state** reached around **9-10 days** after inoculum and after exposure of test chemical
- **Control culture stays in steady state ( ~1x10$^7$ cells/mL)**
- **Cell density returned to steady state** after different exposure conc., even after exposure with high test substance concentrations.
- Concentration-effect curve could be **reproduced. Potential tolerance arises** after repetitive exposure.
- **Course of the effect varies depending on biomass measurement method.** For reaching the steady state: Fluorescence changes less during growth phases, therefore the steady state is earlier reached compared to cell number. After exposure: Fluorescence react faster compared to cell number.

## Conclusion

1. **System establishment (3-6 months)**
   Requirements are:
   1) Lab technician with algae expertise
   2) Project coordinator
   3) In the beginning: Technical expert and general lab equipment incl. pumps, thermostat etc.
   → Key to success: System optimization with risk analysis based on accurate documentation
2. **Choice of Biomass detection method**
   Physico - chemical properties and mode of action of test substance need to be considered
3. **Retarded Effect** of Flurtamone could be observed
4. **No accumulation of toxic effect** after short interval between exposure peaks, **but potential resistance effect** oberservable in later exposure.

This work is one small part of a larger laboratory comparison test funded, designed, and organized by CropLife Europe. The general robustness and reproducibility of the method will be assessed as part of the CropLife Europe Laboratory comparison project.

For more information or continuing the conversation, scan the QR code to visit our website

## References & Acknowledgement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- *Weber, D., 2012. Measuring and predicting the effects of time-variable exposure of pesticides on populations of green algae: Faculty for mathematics, informatics and nature science RWTH University of Aachen.*


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