Challenges with Algae Growth Inhibition Test including Recovery based on OECD Guideline 201 with Non-Standard Algae Species

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Goals of the Study

Find green algae species, which
- are not regularly used for OECD 201 studies
- fulfill the validity criteria of the OECD 201
- are suitable for recovery studies as well.

Check toxicity of a herbicide using the algal growth inhibition test according to OECD 201 including recovery phase and determine the No Observed Ecologically Adverse Effect Concentration (NOAEC).

Why?

Refinement of Risk Assessment for herbicidal plant protection products. More details regarding the background of the study can be found in the abstract book.

Material & Methods

We followed the methodology described in the OECD 201. For more details, please contact the authors.

Results, Discussion & Conclusion

Test Conditions

- 3 - 6 replicates/conc. + 6 repl. for controls
- Start: 10’000 cells/mL (exposure and recovery, if possible)
- Static design
- Duration 72h (exposure + recoveries, each)
- Solvent: DMF
- Test Item Type: Herbicide
- Scheme of test design available on request

Selection of Test Species

The following species were tested as well, but were less or not suitable:
- *Chlamydomonas monadina* CCAP 11/27
- *Chlorella luteoviridis* CCAP 211/3
- *Scenedesmus quadricauda* CCAP 276/16
- *Scenedesmus bernardii* CCAP 276/34
- *Chloromonas typhlos* SAG 26.86
- *Closterium cornu* SAG 132.80

Results & Discussion

- Time-consuming preselection of test species
- Good concentration/response relationship during exposure phase (EP) for both used algae species (Figures 1,4, Tables 1,3)
- *A. falcatus* slightly more sensitive
- Growths inhibition in same range as other green algae (DAR data EFSA, not shown)
- Same recovery pattern for both species (Figures 2,3,5,6; Tables 1,3)
- Complete recovery after 6d for both species
- No Observed Ecologically Adverse Effect Concentration (NOAEC) >20 fold higher after recovery compared to NOEC
- Recovery data comparable with other literature data (DAR data EFSA, not shown)
- Test item concentrations between 89 and 125% of nominal in both tests. No test item during recovery phase (RP, one exception very close to the LOQ of 20 ng/L, data not shown in detail)
- All validity criteria fulfilled (Tables 2,4)

Conclusion

- Several months are required to conduct OECD 201 studies including recovery under GLP conditions in case non-standard algae have to be tested
- Best available equipment and very experienced personnel in both areas, biology and analytics, is prerequisite for a successful performance in time
- Every algae species behaves differently (growth, culture conditions etc.)

We thank all members of the Aquatic Ecotoxicology Group of IES for their support for this project.

Scenedesmus obliquus CCAP 276/7

Figure 1-3: Growth curves for *S. obliquus*; conc. in µg/L

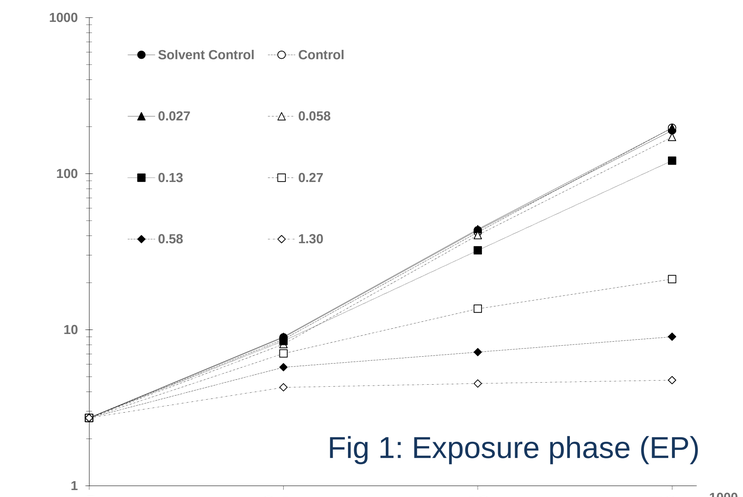

Fig 1: Exposure phase (EP)

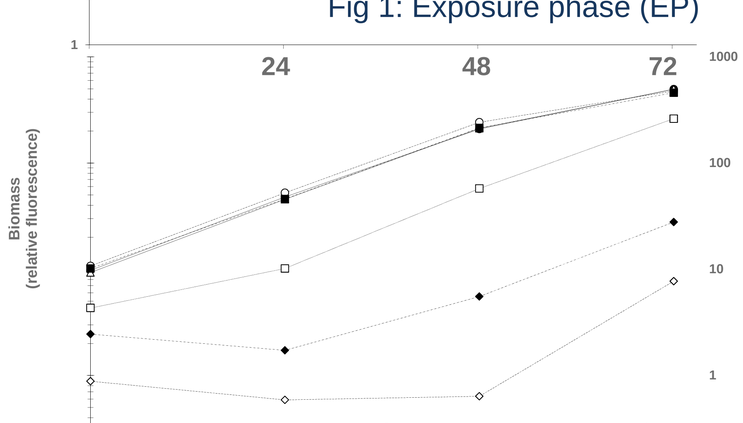

Fig 2: Recovery phase (RP) 1

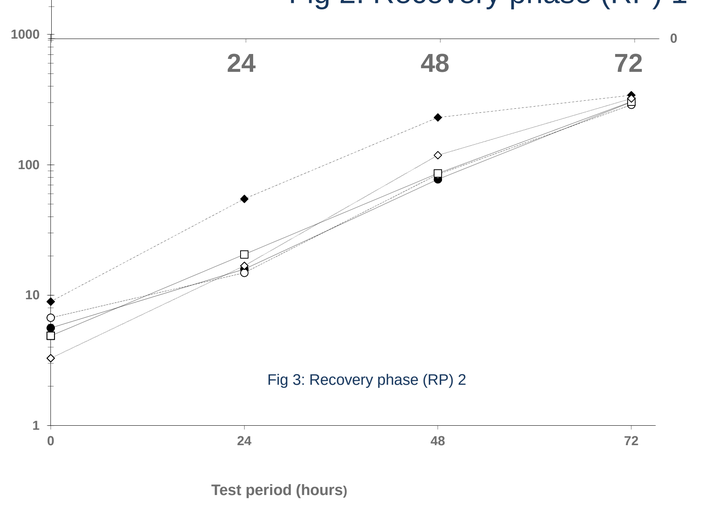

Fig 3: Recovery phase (RP) 2

Table 1: Summary Toxicity Data *Scenedesmus obliquus*

Nominal test item concentration [µg/L]	Inhibition of average specific growth rate [%]		
	EP (3d)	RP1 (3d)	RP2 (3d)
0.027	-0.5	--	--
0.058	**2.6**	-2.3	--
0.13	11	1.9	--
0.27	52	**-5.7**	-5.2
0.58	72	37	6.1
1.3	87	44	**-17**
NO(A)EC [µg/L]	0.06	0.27	1.3

Table 2: Validity Criteria and Result for *S. obliquus*

Parameter	OECD201	Control	Solvent Control
Biomass increase [factor of]	≥16	70	70
Daily growth rates CV [%]	≤35	18	15
Average specific growth rate CV [%]	≤10	0.9	1.9

Ankistrodesmus falcatus CCAP 202/15A

Figure 4-6: Growth curves for *A. falcatus*; conc. in µg/L

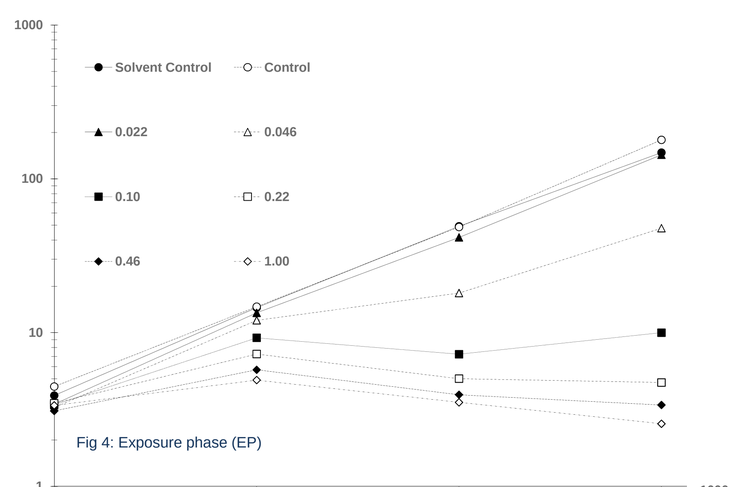

Fig 4: Exposure phase (EP)

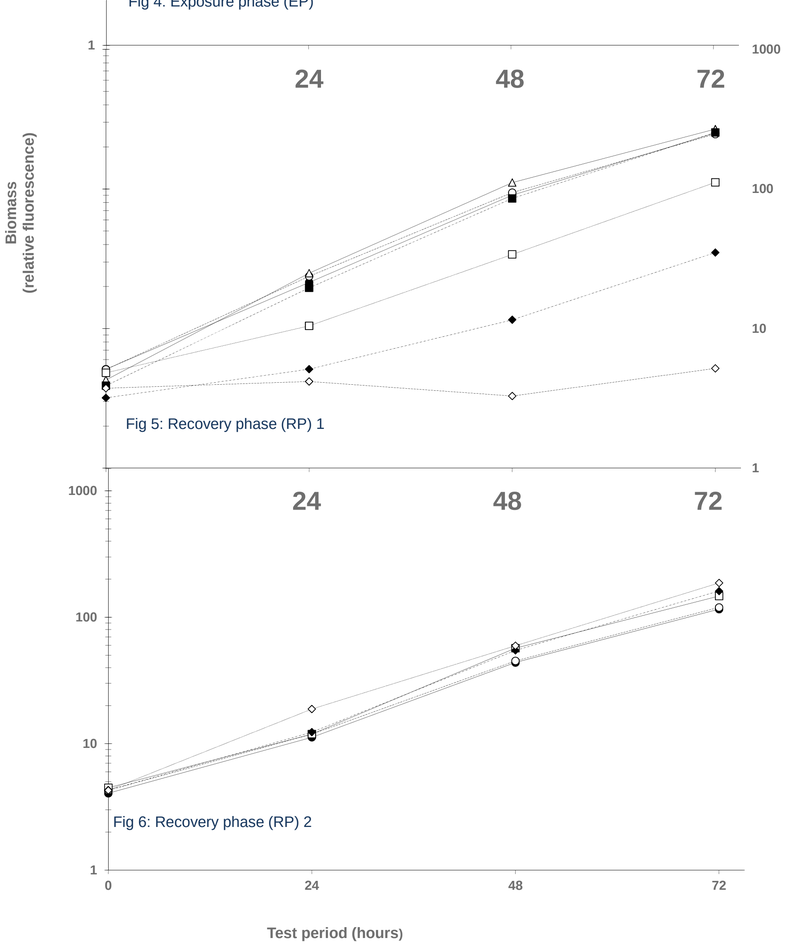

Fig 5: Recovery phase (RP) 1

Fig 6: Recovery phase (RP) 2

Table 3: Summary Toxicity Data *Ankistrodesmus falcatus*

Nominal test item concentration [µg/L]	Inhibition of average specific growth rate [%]		
	EP (3d)	RP1 (3d)	RP2 (3d)
0.022	-2.3	--	--
0.046	**27**	-6.4	--
0.10	70	-7.2	--
0.22	92	**19**	-4.1
0.46	98	39	-7.8
1.0	107*	92	**-13**
NO(A)EC [µg/L]	0.046	0.22	1.0

Table 4: Validity Criteria and Result for *A. falcatus*

Parameter	OECD201	Control	Solvent Control
Biomass increase [factor of]	≥16	38	38
Daily growth rates CV [%]	≤35	8.5	15
Average specific growth rate CV [%]	≤10	5.0	6.4

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