Are additional macrophyte tests necessary for a safe risk assessment of auxins and fatty acid synthesis inhibitors?

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Introduction & Objectives

Fig. 1: Indoor pond microcosms

In the current risk assessment (RA) of plant protection products, the estimation of the toxicity of herbicides on aquatic macrophytes is based on laboratory tests with the monocot duckweed species *Lemna.* However, it is known that *Lemna* is not sensitive to some herbicides with specific modes of action such as synthetic auxins and fatty acid synthesis inhibitors (Maltby *et al.* 2010).

For that reason, an additional test with the submerged dicot species *Myriophyllum sp.* has been proposed, which is known to be sensitive to auxins (Maltby *et al.* 2010). In contrast to *Myriophyllum* and *Lemna*, grasses like the emerse swamp species *Glyceria maxima* seem to be affected by fatty acid synthesis inhibitors.

A microcosm experiment was conducted to further elucidate the differences in sensitivity of these three aquatic macrophytes to the synthetic auxin Fluroxypyr and the fatty acid inhibitor Clodinafop-propargyl. It was investigated, whether *Myriophyllum* and *G. maxima* are suitable test organisms for herbicides with these modes of action and which endpoints are sensitive.

Fig. 2: Application of herbicides

Material & Methods

Experimental design:

- 13 indoor pond microcosms (fibreglass, 3 m x 1 m, 1.4 m³, Fig. 1 + 3); mesotrophic nutrient status

Active substances:

- EC_x design with single application (Fig. 2) of 5 concentrations per herbicide, no replicates
- auxin (growth regulator): Fluroxypyr nominal conc. 32 – 156 – 781 – 3,905 – 19,540 µg/L
- fatty acid inhibitor: Clodinafop-propargyl nominal conc. 4.8 – 20 – 77 – 310 – 1,239 µg/L
- 3 microcosms as controls
- test duration: 10 weeks (mid-May to July 2010)

Aquatic macrophytes:

- duckweed (monocot): *Landoltia punctata* in swimming rings (Fig. 3)
- monocot grass *Glyceria maxima* + dicot *Myriophyllum spicatum* in single pots (sand–sediment mixture, Fig. 3)

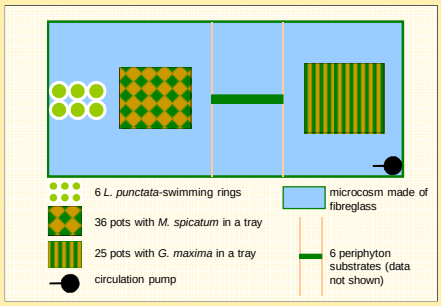

Fig. 3: Scheme of a pond microcosm

Sampling and endpoints:

***M. spicatum* & *G. maxima*:**

- weekly removal of 4 or 5 plants, respectively
- longest shoot and total length of shoots, fresh weight, dry weight
- *for G. maxima* additionally offshoots + leaf numbers

***L. punctata*:**

- photo-documentation of leaf area (weekly) and root lenghth (at the end of the experiment), dry weight

Statistical analysis:

- One-way ANOVA, Dunnet's test, coefficient of variation (CV) of endpoints, Fisher´s exact test, calculation of effect concentration 50% (EC_{50}, based on nominal concentrations and normalized on mean of controls) and half life (DT_{50}) with PRISM 5.03
- determination of leaf area and root length with software WinDias and WinRhizo, respectively

References:

- European Commission (1999): Review report for the active substance fluroxypyr.
- EPA (2000): Clodinafop-propargyl Pesticide Fact Sheet.
- Knauer, K., Mohr, S. & Feiler, U. (2008): Comparing growth development of *Myriophyllum spp.* in laboratory and field experiments for ecotoxicological testing. *Environmental science and pollution research international*, 15 (322-331).
- Maltby, L. *et al.* Aquatic macrophyte risk assessment for pesticides, SETAC Press, Florida, 2010.

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Results

Fluroxypyr

L. punctata

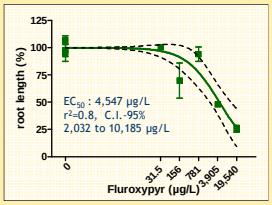

Fig. 4: *L. punctata* root length after 36 days of Fluroxypyr exposure (n=30)

- Concentration dependent decrease of *L. punctata* root length (Fig. 4).
- Leaf area and dry weight: only a significant decrease at highest Fluroxypyr concentration (19,540 µg/L, Dunnet's test, p=0.05).

M. spicatum

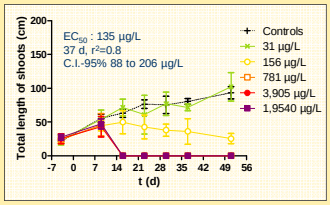

Fig. 5: Development of total length of *M. spicatum* shoots (n=6, mean ± SD)

- *M. spicatum* is most sensitive for the auxin Fluroxypyr (Fig. 5), with root length being the most sensitive endpoint (EC_{50} 37 d, 62 µg/L, confidence intervals 95% (C.I.-95%) 14 to 267 µg/L).

G. maxima

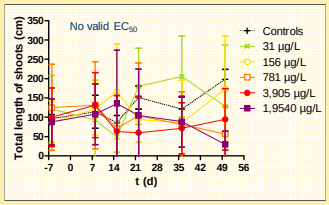

Fig. 6: *G. maxima*, development of total length of shoots (n=5, mean ± SD)

- No significant effects of Fluroxypyr on *G. maxima* (Fig 6.)

DT_{50} of Fluroxypyr in the microcosms:
283-358 d

Clodinafop-propargyl

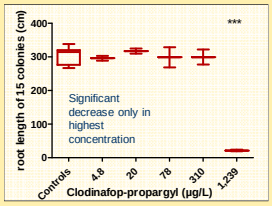

Fig. 7: *L. punctata* root length after 36 days of Clodinafop-propargyl exposure (boxplot, n=6)

- Significant decrease of *L. punctata* root length (Fig. 7), leaf area and dry weight in highest Clodinafop-propargyl concentration (1,239 µg/L, Dunnet´s multiple comparison test, p=0.05).

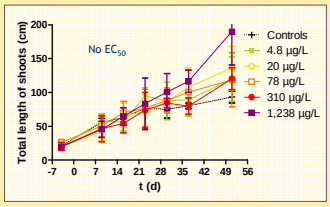

Fig. 8: Development of total length of *M. spicatum* shoots (n=6, mean ± SD)

- No effects of Clodinafop-propargyl on all measured endpoints of *M. spicatum* (Fig. 8).

DT_{50} of Clodinafop-propargyl in the microcosms:
76-205 d

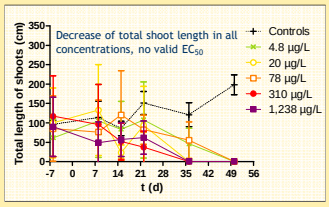

Fig. 9: Development of total length of *G. maxima* shoots (n=5, mean ± SD)

- Significant higher mortality of *G. maxima* compared to controls in all microcosms with Clodinafop-propargyl on day 50 (Fisher´s exact test, p=0.0027, Fig. 9).

Endpoints

- low CV (<17%) for leaf area, root length and dry weight
- root length most sensitive

- lowest CV for total shoot length and longest shoot (26 and 28%)
- fresh weight of roots most sensitive, but high CV (79%)

- high CV for all endpoints
- high variability between individuals
- high mortality even in controls

Discussion & Conclusions

→The monocot species *L. punctata* as well as the standard test organism *Lemna* sp. was less sensitive to the auxin and the grass herbicide (EC_{50}: 12,300 µg/L, European Commission 1999; EC_{50}: 2,400 µg/L, EPA 2000) than the dicot species *M. spicatum* and the monocot grass species *G. maxima*, respectively.
→Hence, in the risk assessment (RA) there is a demand for additional test species to estimate the risks of herbicides with these modes of action

→ *M. spicatum* was a suitable dicot test organism for auxins with total length of shoots being a very good endpoint with low CV, as also found by Knauer *et al.* (2008).

→ The high variability and mortality of *G. maxima* was possibly due to the different size of rhizomes at planting and water stress
→ Other grass species may be more appropriate for the testing in microcosm studies.

→ For *L. punctata,* the endpoint root length seems to be a promising and very sensitive endpoint and may also be a sensitive one for *Lemna sp.*
→ This endpoint should be routinely evaluated in the standard *Lemna* test (OECD 221) for the RA.

→ The EC_{50} of *L. punctata* and *M. spicatum* strongly decreased with increasing exposure time (data not shown). This indicates, that the proposed test durations in the guidance documents (7 d *Lemna* test, OECD 221; 14 d *Myriophyllum* test, Maltby *et al.* 2010) might be too short. This should be considered in more detail in the RA for substances with long half lives as turned out to be the case for Fluroxypyr and Clodinafop-propargyl in this study.