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# *Myriophyllum* growth inhibition tests

## Multiple designs – multiple uses?

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on behalf of the SETAC AMEG Steering Committee

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Version without photos

# Reasons to develop *Myriophyllum* test protocols

- Standard risk assessment for aquatic plants based on algae and *Lemna* test (OECD 201 and 221)
- No consideration of **dicotyledonous** species in standard risk assessment
- Indication that for some **modes of action** algae and *Lemna* seem to be not sufficiently sensitive for protective standard risk assessment
- Most macrophytes are rooted in the sediment, **exposure via sediment** not considered in *Lemna* test
- In summary: Aim of *Myriophyllum* tests is to **reduce uncertainty on assessments based on *Lemna* and alga tests alone**

# Development of different *Myriophyllum* test protocols

- ‘**UBA-design**’: sediment-free test based on ASTM Standard E 1913-04
- ‘**AMRAP design**’: water–sediment test, developed as follow-up of the SETAC AMRAP workshop
- Sediment-only test with *M. aquaticum* (U. Feiler, BfG, Koblenz, Germany) for the assessment of sediment quality: not discussed here (focus on risk assessment for plant protection products)

# Test system and test species

	UBA	AMRAP
Test system	Water only, sterile conditions, addition of sucrose (3%)	Smart-Barko medium (without N & P) OECD 219 sediment (plus N & P)
Test species	<i>M. spicatum</i> ( <i>M.s.</i> )	<i>M.s.</i> or <i>M. aquaticum</i> ( <i>M.a.</i> ) (method in principle suitable for testing submersed and emergent macrophytes)

# Standard test conditions and design

	UBA	AMRAP
Light	100- 150 $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 16 : 8 h light – dark cycle	140 (+/- 20) $\mu\text{E}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ 8.8 -11.8 klux 16 : 8 h light dark cycle
Temperature	23 +/- 2 C	20 +/- 2 C
pH, O <sub>2</sub>	pH measured at start, end and twice in between	pH and O <sub>2</sub> measured at start, end and twice in between
Test duration	14 d	14 d ( <i>M.s.</i> ) or 7 d ( <i>M.a.</i> ) (extension possible depending on MoA)
Shoots per vessel	1	3
Control replicates	10	6
Treatment levels	8	5
Treatment replicates	5	3
Total plants per test	50	63

# Measurements & observations

	UBA	AMRAP
During the test	<ul style="list-style-type: none"> <li>• Every 3-4 days main shoot length and number of unfolded whorls documentd via photos</li> </ul>	<ul style="list-style-type: none"> <li>• Visual inspection at least twice (day 3 and 5 or 5 and 10)</li> <li>• total shoot length, number of site shoots once, on day 3 or 5</li> </ul>
Measurement at test termination	<ul style="list-style-type: none"> <li>• total wet and dry weight,</li> <li>• main shoot length</li> <li>• number and lenght of lateral shoots</li> <li>• number of unfolded whorls</li> <li>• number and length of roots</li> <li>• visual inspection on growth anomalies</li> </ul>	<ul style="list-style-type: none"> <li>• total wet and dry weight,</li> <li>• main shoot length</li> <li>• number and lenght of lateral shoots</li> <li>• visual inspection (and rating) of root development</li> <li>• visual inspection on growth anomalies</li> </ul>

# Measurements & observations

	UBA	AMRAP
Validity criteria	<ul style="list-style-type: none"> <li>• doubling of mean main shoot length of controls</li> <li>• at least 50 % of controls and 50 % replicates per treatment without visible contamination</li> </ul>	<ul style="list-style-type: none"> <li>• doubling of mean total shoot length of controls*</li> <li>• coefficient of variation &lt; 33%*</li> </ul>
Assessment endpoints	<ul style="list-style-type: none"> <li>• Value at end of test</li> <li>• Yield (increase over test)</li> <li>• Growth rates (assuming exp. growth of shoots)</li> </ul>	<ul style="list-style-type: none"> <li>• Value at end of test</li> <li>• Yield (increase over test)</li> <li>• Growth rate (assuming exp. growth of shoots)</li> </ul>
Statistics (applied in ring-test)	<ul style="list-style-type: none"> <li>• EC50 and EC20 via probit analysis for metric data</li> <li>• NOECs via Williams-test (if appropriate)</li> </ul>	<ul style="list-style-type: none"> <li>• EC50 and EC20 via probit analysis for metric data</li> <li>• NOECs via Williams-test (if appropriate)</li> </ul>

\*first proposal, will be defined following the full evaluation of the ring-tests; final protocol may include more criteria

# Ring test overview

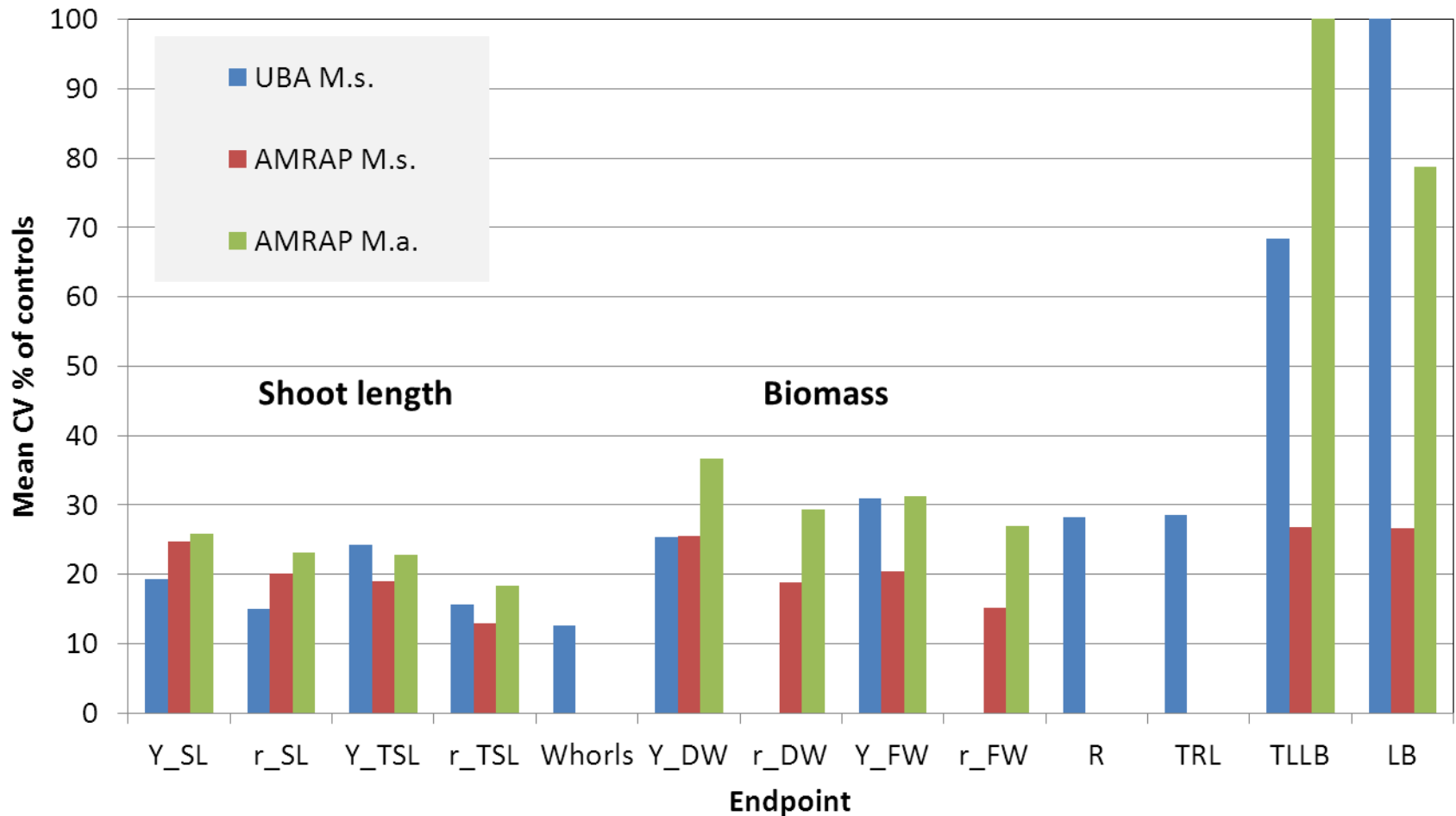
	UBA	AMRAP M.s.	AMRAP M.a.
Laboratories	12	15	
Total tests	30	25	26
Total valid tests	23 (77%)*	21 (84 %) <sup>#</sup>	18 (69 %) <sup>#</sup>
3,5-DCP	11	10	9
2,4,-D	6	0	0
Isoproturon	6	9	10
Trifluralin	0	6	7

\* in 26 out of 30 tests sufficient growth of controls, 86% of control replicates without visible contamination

# based on first proposals for quality criteria

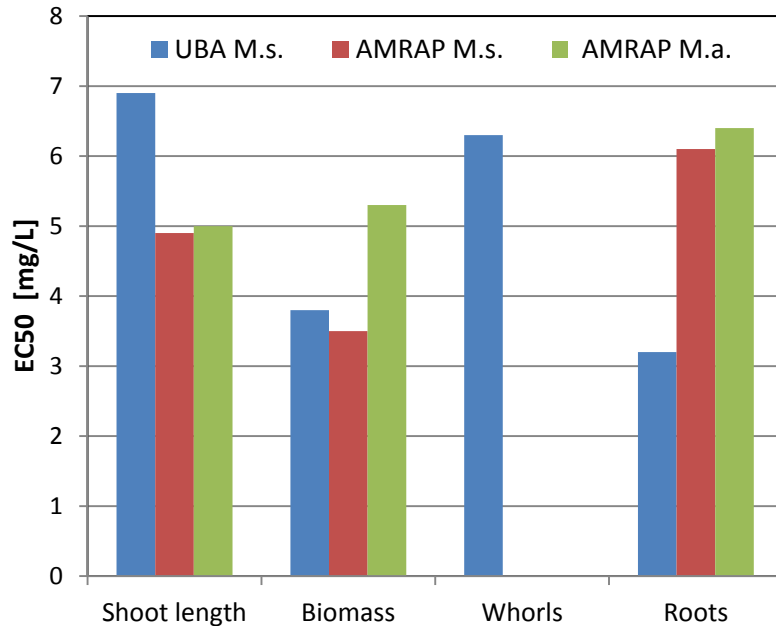


# Variability of controls in the ring-tests (intra laboratory variability)

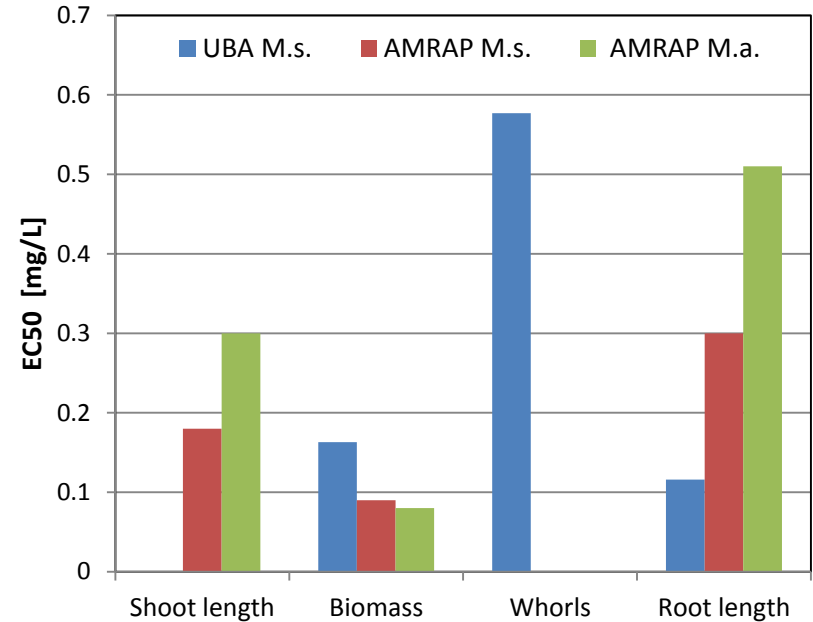


# EC50 for test items

## 3.5 DCP



## IPU



Differences between the tests below a factor of 2 (except for root related endpoint for IPU where however, the lowest EC50 are comparable).  
IPU resulted in increased shoot growth in UBA test (no EC50).

# Preliminary conclusions

- Proportion of valid tests in the ring tests seems to be sufficiently high considering low experience with the tests
  - Coefficient of intra-lab variation between 10 and 30 % allows sufficiently reproducible estimation of EC50.
  - The sensitivity to 3,5 DCP and IPU was comparable in both ringtests
  - Independent of MoA Total Root Length was the most sensitive endpoint in the UBA ring test. In the AMRAP test, root growth was not quantitatively measured. Biomass parameters (mainly biomass yield) were found to be the most sensitive endpoints
  - In general the recommended endpoints according to the ring tests are:
    - Yield Fresh Weight and / or Yield Dry Weight,
    - Yield and Growth Rate of Main / Total Shoot Length (could not be evaluated IPU UBA test)
    - Root development (Total Root Length in the UBA-test, semi-quantitative root assessment in sediment-water test)
  - Less important or useful seem to be:
    - Yield of Whorls only measured in UBA-test, low CV, but not the most sensitive endpoint)
    - Number and length of lateral branches seem to be to variable
  - In summary, both protocols seem to allow assessing effects on *Myriophyllum* growth with sufficient precision and reproducibility in a standardised test.
  - Interpretation and relevance of some effects to be discussed .
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# Acknowledgements

Dirk Maletzki (UBA)

Peter Dohmen (BASF)

Johanna Kubitzka (BASF)

Gertie Arts (Alterra)

Jo Davies (Syngenta)

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