Further Evaluation of Macrophytes for Species Sensitivity Distribution (SSD) Tests

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The OECD guideline 239 assessing the rooted aquatic plant *Myriophyllum spicatum* and the OECD guideline 221 assessing free-floating species *Lemna* spec. are followed to evaluate toxicity to macrophytes in the Tier 1 risk assessment. In addition to one dicotyledon submerged rooted and one monocotyledon free floating species, studies on further species might be needed to refine the risk assessment. In case higher Tier approaches refinements are needed for aquatic plants, the Species Sensitivity Distribution (SSD) is a commonly applied approach.

This leads to the general question: which non-standard test species show good growth under lab condition and can be tested based on the available guidelines? Following this question, we performed tests with non-standard species, including monocotyledons and dicotyledons, emergent, submersed, and free-floating species. We evaluated data of non-standard macrophyte test species based on growth factor/doubling time and coefficient of variation which fulfilled validity criteria according to OECD 239 or OECD 221.

Material & Methods

Test Duration: 14 days (OECD 239); 7 days (OECD 221)

Test Design: based on OECD 239 or 221; without test item

Validity Criteria:

OECD 239
- CV (Yield Fresh Weight) ≤35.0%
- Growth Factor (Fresh Weight) ≥2.0
- Growth Factor (Total Shoot Length) ≥2.0

OECD 221
- Doubling Time (Frond Number) ≤2.5 days
- Growth Rate (Frond Number) ≥0.275 day^{-1}

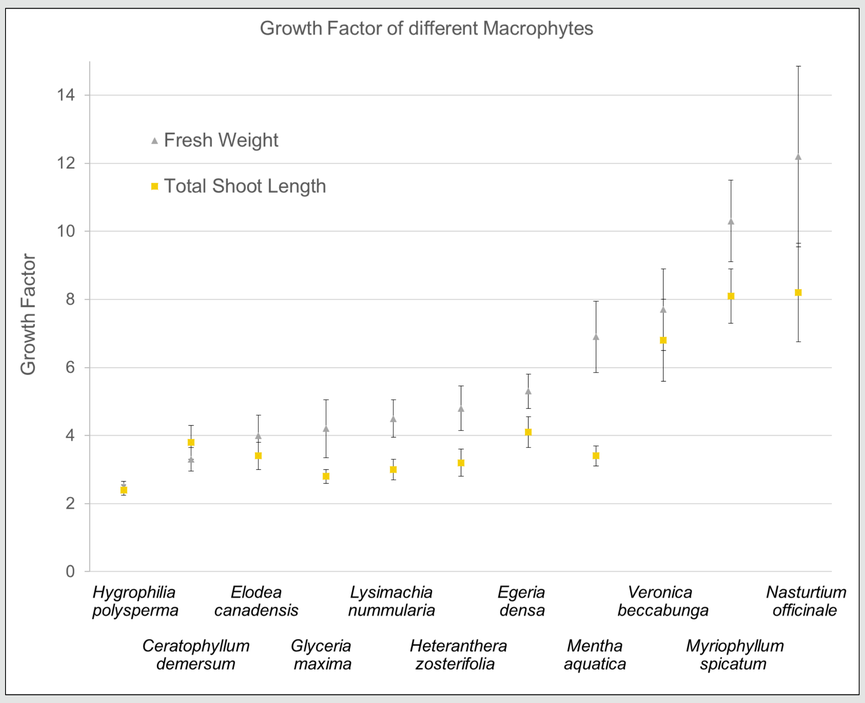
List of species showing good growth under lab conditions

Species	Group	Species	Group
Ceratophyllum demersum	basal form	*Myriophyllum spicatum*	Dicotyledons
Egeria densa	Monocotyledons	*Nasturtium officinale*	Dicotyledons
Elatine hydropiper	Dicotyledons	*Nymphoides peltata*	Dicotyledons
Elodea canadensis	Monocotyledons	*Persicaria amphibia*	Dicotyledons
Glyceria maxima	Monocotyledons	*Persicaria hydropiper*	Dicotyledons
Heteranthera zosterifolia	Monocotyledons	*Ranunculus inundatus*	Dicotyledons
Hottonia palustris	Dicotyledons	*Rotala rotundifolia*	Dicotyledons
Hydrocotyle leucocephala	Dicotyledons	*Spirodela polyrhiza*	Monocotyledons
Callitriche palustis	Dicotyledons	*Vallisneria spiralis*	Monocotyledons
Lemna gibba	Monocotyledons	*Veronica beccabunga*	Dicotyledons
Lemna minor	Monocotyledons	*Wolffia arrhiza*	Monocotyledons
Limnophila sessiliflora	Dicotyledons	*Azolla filiculoides*	fern
Ludwigia repens	Dicotyledons	*Chara globularis*	charophyte green algae
Lysimachia nummularia	Dicotyledons	*Riccia fluitans*	moss
Mentha aquatica	Dicotyledons	*Salvinia natans*	fern

Microcosm Study with 10 different species (emergent, submersed and free floating)

Single Species Tests (*Callitriche palustris; Myriophyllum spicatum; Glyceria maxima*)

Results - Growth factor and CV of various Macrophytes; Test Design based on OECD 239



Listed (in the figure and table) is a selection of ten species [1] beeing used several times in tests which makes a representative evaluation of data possible:

- All species show a Growth Factor > 2.0 for Fresh Weight and Total Shoot Length during the 14-day period.
- All species show a CV of Fresh Weight (Yield) < 35%.

Further species which were used rarely within the last 3 years but showed valid tests, are:
Pogostemon erectus, *Ranunculus inundatus*, *Ludwigia repens*, *Limnophila sessiliflora*, *Hydrocotyle leucocephala*, *Callitriche palustris*, *Hottonia palustris* and *Rotala rolundifolia*

Species for which valid tests are difficult to achieve and optimization is needed, are:
Persicaria amphibia and *Hippuris vulgaris*

Note: For data evaluation focus was set on monocotyl and dicotyl plants. Data for other taxa like ferns, moss and macroalgae are under review.

[1] additionally to *Myriophyllum spicatum*

	CV (Yield Fresh Weight) [%]	Std. Dev.	Growth Factor (TSL)	Std. Dev.	Growth Factor (FW)	Std. Dev.
Myriophyllum spicatum	7.4	4.3	8.1	1.6	10.3	2.4
Heteranthera zosterifolia	10.7	3.2	3.2	0.8	4.8	1.3
Lysimachia nummularia	12.9	3.1	3.0	0.6	4.5	1.1
Veronica beccabunga	13.1	1.4	6.8	2.4	7.7	2.4
Nasturtium officinale	13.9	4.2	8.2	2.9	12.2	5.3
Elodea canadensis	14.8	4.2	3.4	0.8	4.0	1.2
Mentha aquatica	15.0	4.6	3.4	0.6	6.9	2.1
Egeria densa	18.3	3.8	4.1	0.9	5.3	1.0
Ceratophyllum demersum	19.5	5.5	3.8	1.0	3.3	0.7
Glyceria maxima	23.3	6.2	2.8	0.4	4.2	1.7
Hygrophilia polysperma	23.4	1.6	2.4	0.3	2.5	0.3

Doubling Time and Growth Rate of free-floating Macrophytes; Test Design based on OECD 221

	Doubling Time Frond Number [d]	Std. Dev.	Growth Rate (Frond Number)	Std. Dev.
Wolffia arrhiza	2.4	0.55	0.297	0.052
Spirodela polyrhiza	2.0	0.13	0.348	0.028

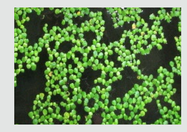
Beside *Lemna gibba* two fast growing free-floating species mainly beeing tested based on OECD 221 are: *Wolffia arrhiza* and *Spirodela polyrhiza*

- With both species a Doubling Time of ≤2.5 days for Frond Number during the 7-day period can be achieved.
- In most of the performed tests the Growth Rate of Frond Number is ≥0.275 day^{-1}.
- *Wolffia arrhiza* showed a slower growth compared to *Spirodela polyrhiza* and the number of fronds was increased to generate robust data for dry weight.

Free Floating Species

S. polyrhiza, W. arrhiza and *L. gibba*

S. polyrhiza



W. arrhiza

Discussion

Many aquatic plants can be tested under laboratory conditions according to OECD 239 and 221. Some results indicate that the tests developed for terrestrial plants are sometimes not comparable to those for aquatic plants on the basis of mode of action. In particular, the choice between monocotyledonous and dicotyledonous plants is not in all cases suitable for assessing sensitive species. It should be noted that different species can be selected depending on the experience of the laboratories. If toxicity to aquatic macrophytes is unclear, prior screening is recommended. Data from microcosm and mesocosm studies could also be used for the selection of species. Care should be taken regarding species with different growth forms, e.g. *Persicaria amphibia*, *Hippuris vulgaris* and *Hygrophilia polysperma*, as the coefficient of variation can significantly increase and growth can be slower. A higher variability of results also can be expected when using plants from commercial suppliers. Here, the plants are often grown in hydroponics with different types and amounts of fertilizers and under different light and temperature regimes. Therefore, care should be taken to adapt plants to the laboratory conditions to ensure good growth during the tests.

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