Testing Strategy for Species Sensitivity Distribution (SSD) Tests with Aquatic Plants

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The guidelines OECD 238 and 239 with the aquatic plant *Myriophyllum spicatum* were setup to cover toxicity of chemicals on rooted macrophytes in addition to the OECD guideline 221 which covers free floating species. By now, it became obvious that the focus on one submerged rooted, and one free floating species might under, or overestimate effects based on the mode of action of test items. Therefore, further tests and ring-tests with additional macrophyte species were setup to refine risk assessment. In most cases the species sensitivity distribution (SSD) approach with at least eight species is used.

This leads to the general question which species show good growth under laboratory conditions, are relevant for the ecosystem, represent different growth forms and finally, can close missing data gaps. Also, it is essential to follow the standard test protocols as far as possible in order to compare data of non-standard species with standard species. We present non-standard macrophyte test species which fulfilled validity criteria according to OECD 239 or 221, respectively and recommend a test strategy based on handling, growth form and sensitivity of test plants.

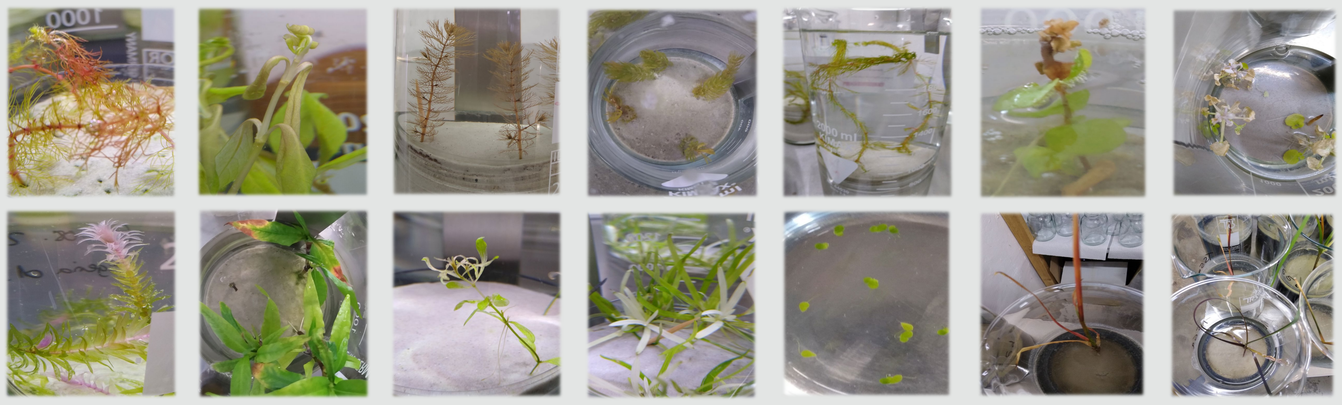
Material & Methods

Test Duration: 14 days test based on OECD 239, 7 days test based on OECD 221

Validity Criteria:
- two-fold increase in fresh weight of the controls (mean)
- mean coefficient of variation for yield based on measurements of shoot fresh weight (i.e. from test initiation to test termination) in the control cultures does not exceed 35% between replicates

Test Design: based on OECD Guideline 239 and/or OECD 221

Different Effects on Macrophytes during testing



Summary of Findings

Depending on the species different visual observations and sensitivity could be evaluated. To quantify negative effects 6 parameters for decrease in biomass were measured. Endpoints were calculated based on shoot fresh weight, shoot dry weight and shoot length or number of leaves from yield and growth rate, respectively. These endpoints were already evaluated during the ring-testing and selected and confirmed during establishment of the current OECD guidelines 238, 239 and 221.

It should be noted that visual effects on aquatic plants can also be divers and also could been observed under different environmental conditions in control plants. Ongoing discussion to add a score for visual effects in aquatic plants should be handled with care. There is less data available on relevance. Therefore, a full validated round robin test with at least three different test items with different mode of action and at least 4 non-standard species (including free floating, emergent, submerged species and species with different leaf forms) is needed, to clarify if visual endpoints can be used for refined risk assessment. However, in most cases visual injury is related to effects on plant growth and therefore covered with the determined endpoints. Until further clarification, non-standard species tests should follow as strict as possible to the recent guidelines, to allow comparison of endpoints.

Testing Strategy

Results show that testing based on the mode of action developed for terrestrial plants is not comparable to aquatic plants. Especially, selection between monocotyl and dicotyl plants doesn´t work to evaluate sensitive species in all cases. Sensitivity mostly seems to be related to habitat and growth forms. The general assumption that emergent species are always less sensitive compared to submerged species not always ends with the right approach. To cover toxicity in the environment it is important to select species from different taxa with different growth forms. 10 species including *Lemna gibba* and *Myriophyllum spicatum* should be screened. Based on these data, 8 sensitive species which show a clear dose response and fulfil validity criteria should be chosen.

Species with low sensitivity, as well as sensitive species with no clear dose response are not useful for further testing. Based on our research the following species showed good dose responses and fulfilled the validity criteria of the OECD 239 and/or 221:

Ceratophyllum demersum, Egeria densa, Elatine hydropiper, Heteranthera zosterifolia, Hygrophila polysperma, Limnophila sessiliflora, Ludwigia repens, Lysimachia nummularia, Mentha aquatica, Nasturtium officinale, Persicaria hydropiper, Rotala rotundifolia, Spirodela polyrhiza, Veronica beccabunga, .

It should be noted, that based on the experience of the laboratories different species can be chosen. If mode of action to aquatic macrophytes is unclear a previous screening is needed. Also, data from microcosm and mesocosm studies could be used for selection of species.

Species	Group	Adapted to Guideline	Handling under Laboratory Conditions	Sensitivity
Callitriche palustris	*Dicotyledons*	OECD 239	fragile; handling is difficult; damaging of shoots is likely	high sensitivity; one of he most sensitive species; possibly because of the fragile habitus
Ceratophyllum demersum	*basal form*	OECD 239	high care should be taken on lab culture; only plants with strong growing apical should be used	depending on mode of action sensitivity could be quite low
Crassula recurva	*Dicotyledons*	OECD 239	further review needed, doubling time is critical	no data available
Egeria densa	*Monocotyledons*	OECD 239	easy to handle; showed sufficient growth	depending on mode of action sensitivity could be quite low
Elatine hydropiper	*Dicotyledons*	OECD 239	showed sufficient growth	few data available; expected to be less sensitive compared to *M. spicatum*
Eleocharis acicularis	*Monocotyledons*	OECD 239	slow growth; may be modification needed	sensitivity not clear; further testing needed
Elodea canadensis	*Monocotyledons*	OECD 239	different hybrid forms exist; growth is less compared to *Egeria densa*	sensitivity comparable to *Egeria densa*
Glyceria maxima	*Monocotyledons*	OECD 239 adapted	high effort on stock culture to select uniform material	in most cases less sensitive compared to *M. spicatum or L. gibba*
Heteranthera zosterifolia	*Monocotyledons*	OECD 239	doubling of fresh weight could be difficult	high sensitivity, comparable to *M. spicatum;* one of the most sensitive monocot species
Hippuris vulgaris	*Dicotyledons*	OECD 239	higher control mortality compared to others; different growth forms	growth forms show different sensitivity
Hottonia palustris	*Dicotyledons*	OECD 239	doubling of fresh weight could be difficult	comparable to *M. spicatum* but less sensitive in most cases
Hydrocotyle leuccocephala	*Dicotyledons*	OECD 239	difficult to select uniform material; creeping species	sensitive species
Hygrophila polysperma	*Dicotyledons*	OECD 239	easy to handle; showed sufficient growth	comparable to *M. spicatum* but less sensitive in most cases
Iris pseudacorus	*Monocotyledons*	OECD 239	size often to big for lab testing; high effort on selection of uniform material	sensitivity seems to be lower compared to most other species
Lemna gibba	*Monocotyledons*	OECD 221	standard species	sensitive species
Lemna minor	*Monocotyledons*	OECD 221	standard species	sensitive species
Limnophila sessiliflora	*Dicotyledons*	OECD 239	easy handling and selection of uniform material	sensitive species
Littorella uniflora	*Dicotyledons*	OECD 239	slow growth; might be not sufficient without modifications of test medium	sensitive species
Ludwigia repens	*Dicotyledons*	OECD 239	easy to handle; showed sufficient growth	sensitive species
Lysimachia nummularia	*Dicotyledons*	OECD 239	easy to handle; showed sufficient growth	emergent species; sensitive species; in most cases less sensitive compared to *M.spicatum*
Lythrum salicaria	*Dicotyledons*	OECD 239	good growth; testing of new grown shoots in spring	less sensitive compared to others
Mentha aquatica	*Dicotyledons*	OECD 239	easy to handle; showed sufficient growth	emergent species; sensitive species; in most cases less sensitive compared to *M. spicatum*
Myosotis palustris	*Dicotyledons*	OECD 239	further review needed; selection of uniform material has to be modified	further evaluation needed
Myriophyllum sibiricum	*Dicotyledons*	OECD 239	comparable to *M. spicatum*	comparable to *M. spicatum*
Myriophyllum spicatum	*Dicotyledons*	OECD 239	standard species	high sensitivity
Nasturtium officinale	*Dicotyledons*	OECD 239	showed the strongest growth during lab testing, easy to cultivate	emergent species; in most cases higher sensitivity compared to other emergent species
Nymphoides peltata	*Dicotyledons*	OECD 239	difficult to generate uniform material; has to be cutted several times before testing	moderate sensitivity
Oenanthe aquatica	*Dicotyledons*	OECD 239	size often to big for lab testing; high effort for selection of uniform material	further evaluation needed
Persicaria amphibia	*Dicotyledons*	OECD 239	develops up to three growth forms (floating leaves, submerged and emergent); difficult to test	depends on the growth form; less sensitive compared to others
Persicaria hydropiper	*Dicotyledons*	OECD 239	showed good growth	emergent species; sensitive species; sensitivity comparable to other emergent species
Phalaris arundinacea	*Monocotyledons*	OECD 239	validity criteria could not be fulfilled	further evaluation needed
Potamogeton natans	*Monocotyledons*	OECD 239	difficult to test and generate uniform material	sensitivity depends on quality and growth form of test material
Ranunculus aquatilis	*Dicotyledons*	OECD 239	quite bad quality for lab tests available; high effort to generate clean and healthy stock culture	further evaluation needed
Ranunculus inundatus	*Dicotyledons*	OECD 239	slow growth under test conditions	moderate sensitivity
Rotala rotundifolia	*Dicotyledons*	OECD 239	showed sufficient growth	sensitive species
Sagittaria subulata	*Monocotyledons*	OECD 239	care should be taken to select young plants with healthy leaves; old leaves showed necrosis	further evaluation needed
Sparganium natans	*Monocotyledons*	OECD 239	selection of uniform material is difficult; only new grown shoots show doubling	further evaluation needed
Spirodela polyrhiza	*Monocotyledons*	OECD 221	comparable to *Lemna gibba;* but growth is stronger compared to *Lemna gibba*	in most cases slightly more sensitive compared to *Lemna gibba*
Vallisneria spiralis	*Monocotyledons*	OECD 239	high risk for invalid test in accordance to OECD 239	could be quite sensitive but comparable to other submerged monocots
Veronica beccabunga	*Dicotyledons*	OECD 239	easy to handle	emergent species; sensitive species; sensitivity comparable to other emergent species
Wolffia arrhiza	*Monocotyledons*	OECD 221	comparable to *Lemna gibba;* but growth is slower; validity according to OECD 221 could faile	in most cases slightly less sensitive compared to *Lemna gibba*
Other taxon				
Azolla filiculoides	*ferns*	OECD 221/239	growth is slower compared to *Lemna* species, therefore validity criteria of OECD 239 has to be applied	data set is low; less sensitive compared to *Lemna gibba* in most cases
Chara globularis	*charophyte green algae*	OECD 239	fragile species; difficult to handle; best testing time is spring	really sensitive species in some cases
Riccia fluitans	*moss*	OECD 221/239	is important to weight the start material to be sure to have uniform plants for all replicates	sensitive species
Salvinia natans	*ferns*	OECD 221/239	growth is slower compared to *Lemna* species, therefore validity criteria of OECD 239 has to be applied	less sensitive compared to *Lemna gibba* in most cases

Discussion

Using young shoots and uniform healthy test material, several macrophytes met the validity criteria according to OECD 239 or 221. It became clear that water changes have an impact on plant growth. Therefore, at least one water change after 7 days could be helpful for rooting species to achieve a doubling of biomass. In addition, different growth was observed between seasons, plant source or shoot age. Previous studies have also shown that shoot length and fresh weight are more sensitive than dry weight for most species. However, for some other species, a contrary finding could be found depending on the test item. Healthy cultures have the greatest influence on the results. Seasonal influence on final endpoints is less for healthy plants, which stock cultures were handled equal to the test conditions year-round. In any case plants must be well adapted to laboratory conditions. If there are several growth forms, the form of the plant with the best growth under laboratory conditions is preferred. To cover a wide range of natural systems, it is important to study plants from different taxa and littoral zones (e.g., floating, emergent, submerged).

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