

# Is the *Lemna* test appropriate to predict effects of the antifouling biocide Irgarol on macrophytes in the aquatic environment?

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## Background

Up to now, only the standard laboratory test with the monocot, emerge *Lemna* sp. is required for risk assessment of pesticides. However, it is doubtful whether this species is representative for dicot plants especially for those with different growth types like submerged rooted macrophytes.

Mesocosm studies offer the possibility to test several types of macrophytes simultaneously. The submerged rooting macrophyte species *Myriophyllum verticillatum* and *Potamogeton nodosus* (Fig. 2) were introduced into indoor pond mesocosms. Additionally, an *in-situ* modification of the standard test with the emerged duckweed *Lemna* sp. was applied (Fig. 1). The related species *Spirodela* was used instead of *Lemna* since *Spirodela* can cope with the mesotrophic nutrient levels that occur in the pond mesocosms (Fig. 1, 3).

## Methods

### Pond design

- Length 690 x width 325 x height 250 cm (Fig. 3)
- 15 m<sup>3</sup> water volume
- Artificial light, mean 13,000 lx
- Nutrient regime of 0.045 mg/L TP and 1.5 mg/L TN (mean)
- Sand, natural fine sediment, littoral zone as ground

### Experimental design

- Single application of Irgarol (11-04-05) in 6 mesocosms at different concentrations (1 x 0.04, 2 x 0.2, 1 x 1, 2 x 5 µg/L nominal).
- Two systems served as controls.

### Analysis of Irgarol

- Analysis of Irgarol in water taken from a hourly to a fortnightly sampling interval, analysis of macrophytes at the end of the experiment (Fig. 4 and Tab. 1)
- Sample analysis with GC-MS

### In-situ test with *Spirodela*

- Introduction of 4 floating silicon rings (Fig. 6) into each pond mesocosm
- Exposure of 20 fronds prior to Irgarol application (Fig. 1)
- Weekly photo-documentation until day 31



Fig. 1: *Spirodela polyrrhiza* (left) and *Lemna minor* (right)

### Macrophyte sampling

- Harvesting of macrophytes at the end of the experiment (150 days)
- Determination of freshweight after 5 min of centrifugation (2800 rpm)
- EC50 calculation with log logistic model using Graph Pad Prism V 4.00
- Modified bioconcentration factor "BCF" calculation according to OECD 305 (Ref. 5)



Fig. 2: *Myriophyllum verticillatum* (left) and *Potamogeton nodosus* (right)



Fig. 3: Photo of an indoor pond mesocosm

## Conclusions

Submerged rooted plants especially with high surface area seem to be far more sensitive than the standard test organism *Lemna* or related species. This can be explained by the rapid decrease of Irgarol in the water phase and its high sorption potential to sediment and organic material. Including submerged sediment rooting macrophytes into risk assessment for chemicals with high log K<sub>oc</sub> is therefore considered appropriate.

## Results and discussion

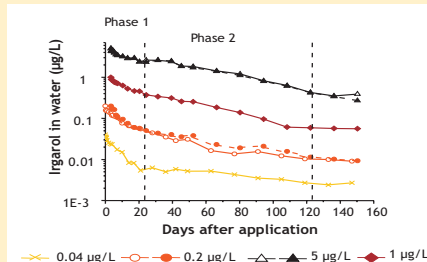


Fig. 4: Decrease of Irgarol in the water column in the contaminated ponds

- Irgarol concentrations decreased strongly in the water column directly after application due to sorption to the sediment and organic material such as macrophytes (phase 1; Fig. 4 and Tab. 1).
- In the second phase, the decrease of Irgarol is mainly caused by biodegradation with calculated DT50 up to 113 days (Fig. 4).
- Myriophyllum* and *Potamogeton* were almost completely eliminated in the 5 µg/L ponds (Fig. 6 A1 and B1).
- The strong decline of macrophytes in the highly contaminated ponds led to a decrease of nutrient uptake and therefore to an increase of nutrients as shown for nitrogen (Fig. 5).
- In contrast, growth of *Spirodela* increased with increasing Irgarol concentrations (Fig. 6 A-D). The lack of competition for nutrients in the highly contaminated ponds combined with a lower sensitivity may have caused this increase.
- Myriophyllum* was by factor 8 to 49 times more sensitive to Irgarol than *Lemna* and *Spirodela* (Tab. 1).
- Irgarol absorbed to macrophytes and the "BCF" values seem to increase with increasing surface area of the plants (*Myriophyllum* > *Potamogeton*; Tab. 1).

### References

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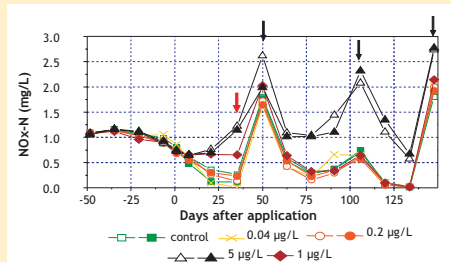


Fig. 5: NOx-N concentration over time in the 8 pond mesocosms. Red arrow: day 31, black arrows: nutrient pulses

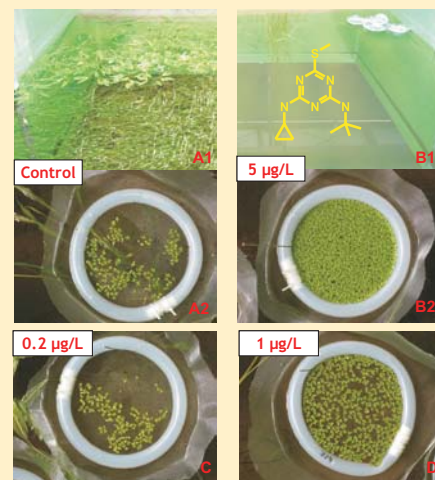


Fig. 6: View of one control and 5 µg pond (A1 and B1), *Spirodela* rings on day 31 (A2, B2, C and D)

Tab. 1: EC 50 and calculated "BCF" for the tested macrophytes

Species	Exposure time (d)	EC50 (µg/L)	"BCF" (L/kg)
<i>Myriophyllum verticillatum</i>	150	0.21	1520
<i>Potamogeton nodosus</i>	150	0.92	284
<i>Spirodela polyrrhiza</i>	31	> 5	n.a.
<i>Lemna</i> sp. (Ref. 3, 6-8)	7	9.8 ± 4.6	-
<i>Lemna</i> sp. (Ref. 9)	14	1.6	-

n. a. = not analysed