



Reassessing Exposure Design: Exploring Overspray in Toxicity Tests for Herbicides with unexpectedly low Toxicity to Macrophytes

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Environmentally relevant spray drift scenario is not considered in risk assessment of aquatic plants

- Pesticides enter aquatic systems via spray drift, potentially affecting floating plants via spray deposition on top of their leaves
- Standard lab tests, e.g. OECD 221, expose herbicides via the media to floating macrophytes, mainly *Lemna sp.*
- Exposure to herbicides can result in low toxicity values for floating macrophytes, despite their intended adverse effects.

Comparison of two exposure scenarios

We compared the exposure of an herbicide **via spray and via medium** (water column) to *Lemna minor*.

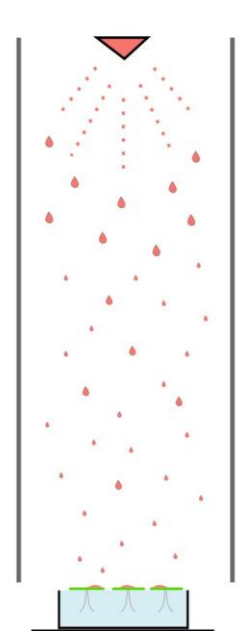
We **hypothesized a higher toxicity via spray** exposure on top of the leaves of *Lemna minor*.

Adapted OECD protocol 221 with *Lemna minor*

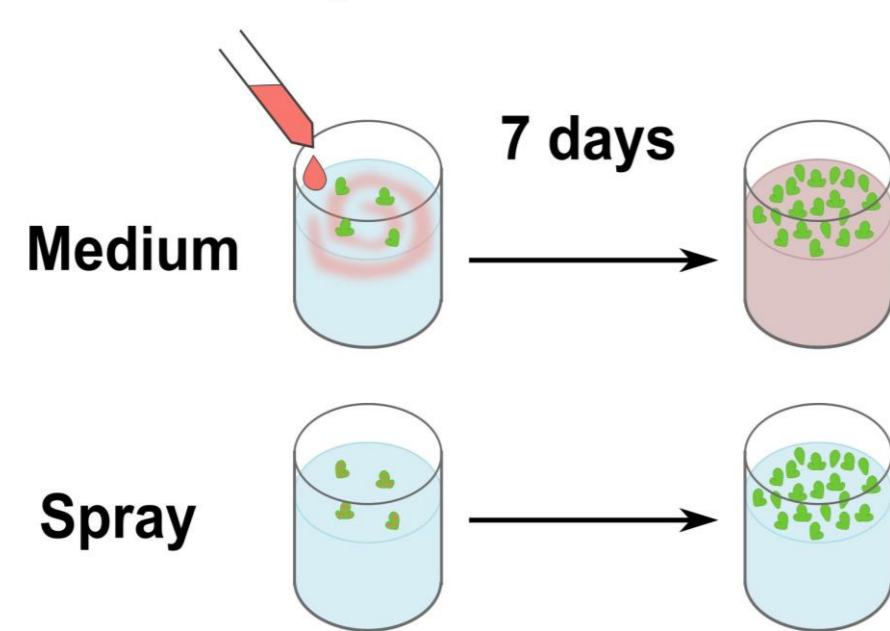
- Use of the *Potter Spray Tower* for standardized spray exposure
- Exposure of the herbicide *Basagran 480*[®] with the active ingredient **Bentazone** (PSII inhibitor)
- Duration: 7 days
- Dose-response scheme:
Control + 5 Concentrations: 0, 0.5, 1, 2, 4, 8 mg L⁻¹
- Biological endpoints (relative growth rate):
Dry weight, frond number, total frond area



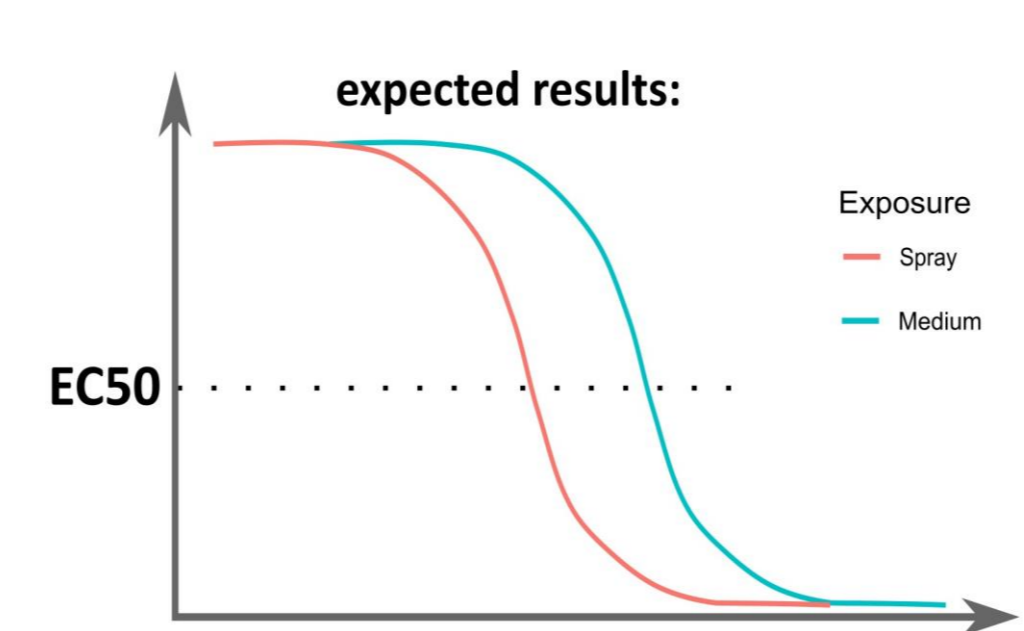
Spray exposure using the "Potter Tower"



Comparing exposure via Medium and Spray over 7 days and 5+1 concentrations



Modelling dose-response curves based on Relative Growth Rate



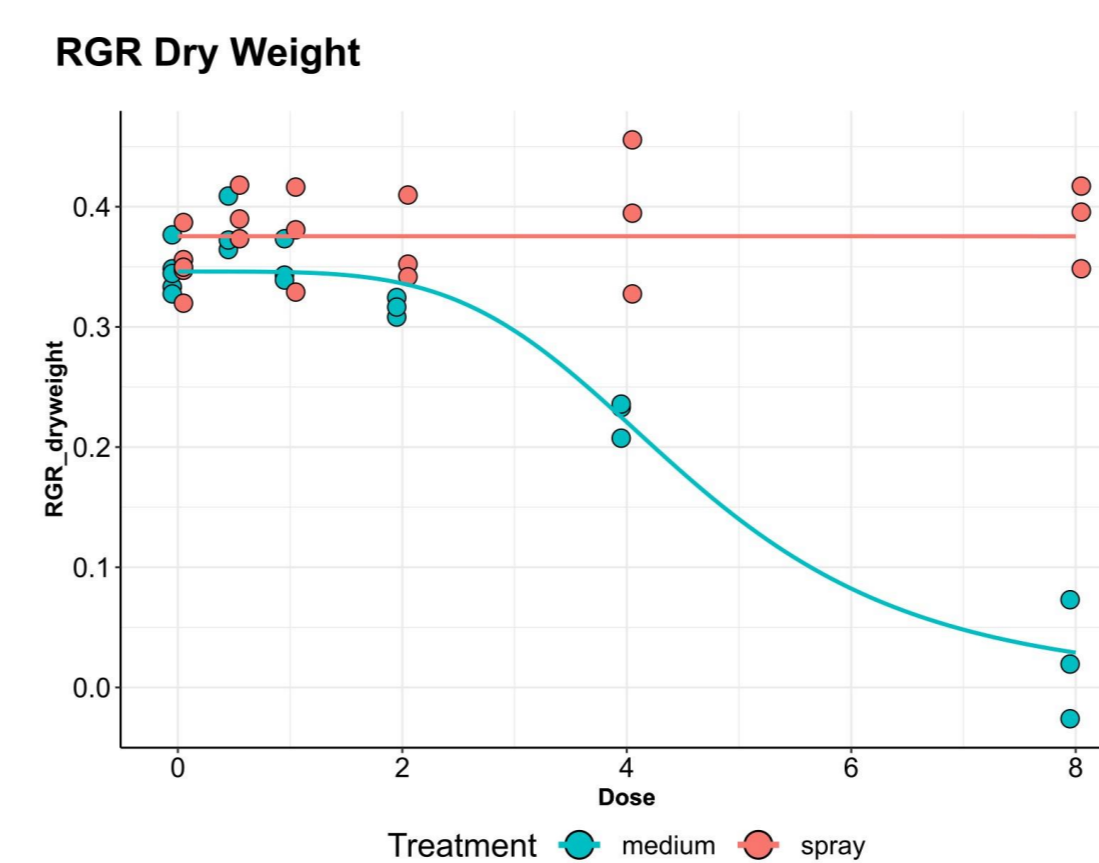
The Bentazone concentration was measured in both exposure scenarios. **To quantitatively compare the exposure** via spray (surface area) and the medium (volume), **we also sprayed test vessels without *Lemna minor***, measured the Bentazone concentration in the medium, and used this data as a proxy for quantifying the spray exposure.

Comparability of both scenarios: chemical analysis

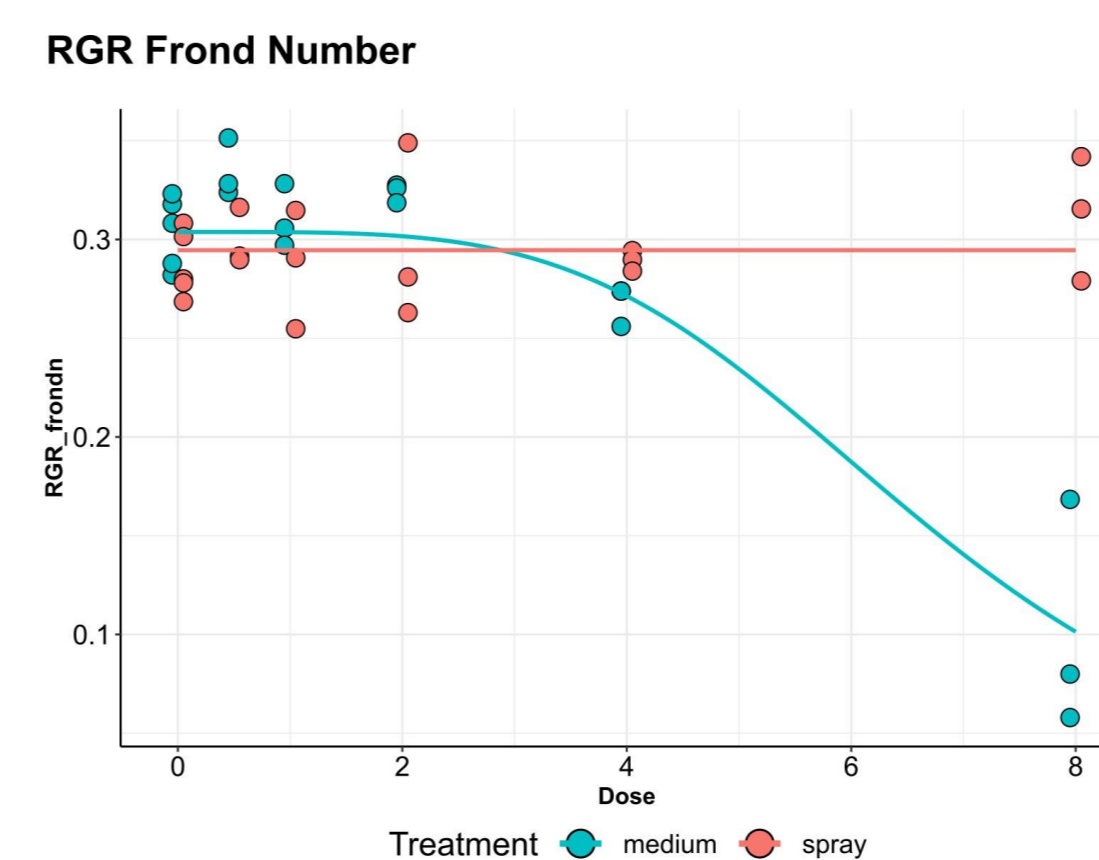
- We reached approx. **90% of the nominal** concentrations for the exposure via the medium
- **Exposure via spray** resulted in an approx. **4.5x higher** concentration than via the medium, e.g., for the highest dose:

Dose	Medium	Spray
nominal	mean measured	mean measured
8 mg L ⁻¹	7.31 mg L ⁻¹	32.72 mg L ⁻¹

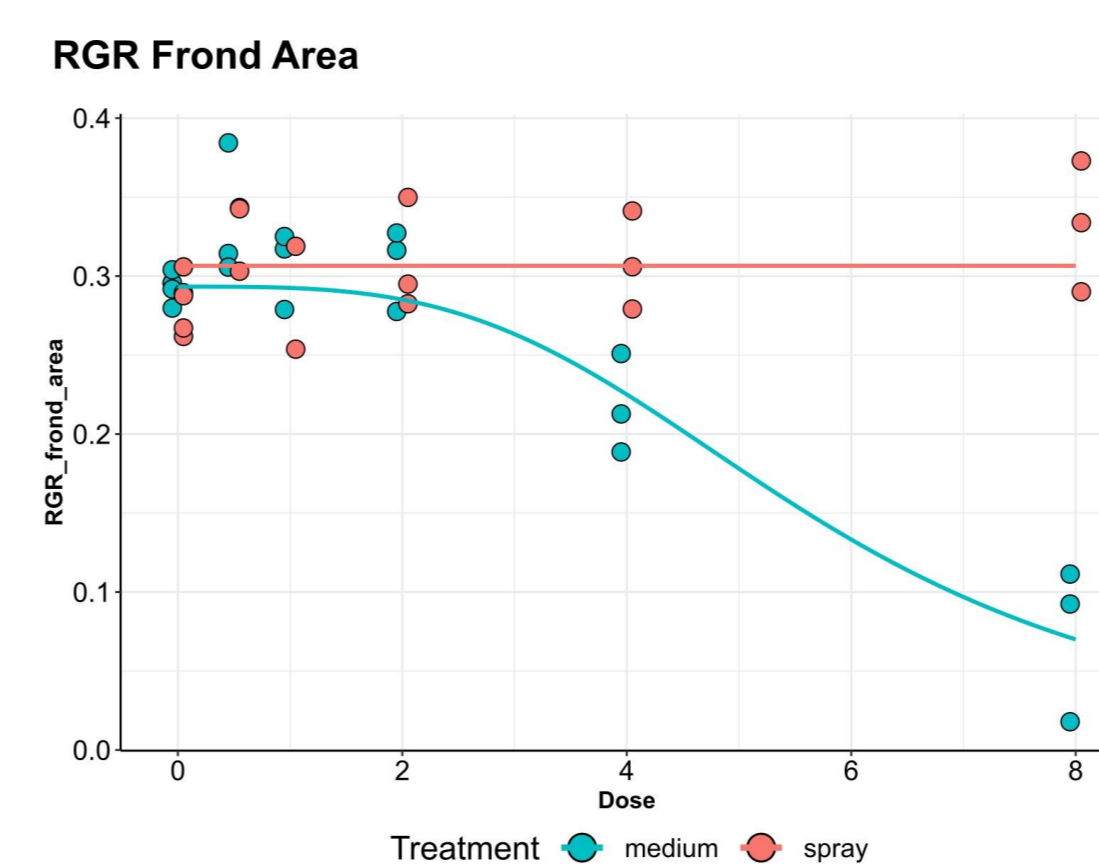
The exposure scenario matters!



Dry weight is reduced when exposed via the medium but not when exposed via spray.



Frond number is reduced when exposed via the medium but not when exposed via spray.



Total frond area is reduced when exposed via the medium but not when exposed via spray.

Our own EC50-values for the exposure via the medium are comparable to literature:

Species	ErC50 [mg L ⁻¹]	Endpoint	Reference
<i>L. gibba</i>	12.0	na	EFSA (2015)
<i>L. gibba</i>	9.3	na	EFSA (2015)
<i>L. gibba</i>	7.2	na	EFSA (2015)
<i>L. minor</i>	2.6	Frond Area	Cedergreen & Streibig (2005)
<i>L. minor</i>	4.6	Dry Weight	our own study
<i>L. minor</i>	6.7	Frond Number	our own study
<i>L. minor</i>	5.7	Frond Area	our own study

Exposure via spray did not affect growth of *Lemna minor*

Although we managed to overachieve on our comparable scenario.

Some open questions remain:

- Did *Lemna* actually **uptake** the sprayed herbicide?
- Was the exposure **duration** long enough for the sprayed herbicide to affect the plants?
- Did other **unaccounted factors** contribute to the lack of effects via the spray, such as pH on top/within the leaves?
- Are other floating or emerging aquatic plants **equally resistant** to spray exposure of herbicides?

Acknowledgements

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