Aquatic Macrophyte Toxicity: Water Phase vs. Overspray Application

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Introduction

Following the data requirements (Commission Regulation (EU) 284/2013) for plant protection products (PPP) with an herbicidal mode of action, effects on emergent (or floating) macrophytes shall be assessed following e.g., test guideline OECD 221 [1]. As most herbicides are applied by spraying, (direct) exposure by spray drift should be considered as an additional scenario in the risk-assessment in case of unexpected low toxicity (E_rC_{50} > 1 mg/L) for the aqueous route [2]. Spray drift exposure can be recreated by an overspray-design in the laboratory. While this design is nowadays a common experimental procedure for terrestrial ecotoxicity studies (e.g., for active substances with a physical MoA), no guideline or validated test protocols are available for aquatic macrophytes. We herein present a suitable experimental setup to address the aforementioned requirement and an exemplarily investigation of effects of a glyphosate-containing PPP towards *Lemna gibba* when exposed to direct spray application(s).

Method / Test Design / Assessment

A growth inhibition test with *Lemna gibba* was conducted in style of test guideline OECD 221, with a laboratory overspray application re-enacting conditions for spray drift exposure scenarios (see Fig. 1). The experimental setup includes parallel testing of replicates under static and semi-static exposure conditions for five concentrations in a geometric series (SF = 2.5). Five runs were used. Application was performed using a conveyor belt at a constant velocity transporting the exposure vessels (n = 3 per concentration) underneath a static nozzle with a fixed flow to reach the target exposure rates. Growth inhibition was assessed by determining the average specific growth rate, among others, from frond numbers which were recorded at test start and at the end of the 7-day exposure period and finally compared to control replicates for a static and a semi-static procedure. Analytical verification included measurements of the application solution and monitoring the exposure concentration over time in the static setup (but can be complemented by analyses of sacrifice replicates, i.e., same vessels with identical dimensions and amount of AAP medium sprayed in the same run, but without *Lemna gibba*).

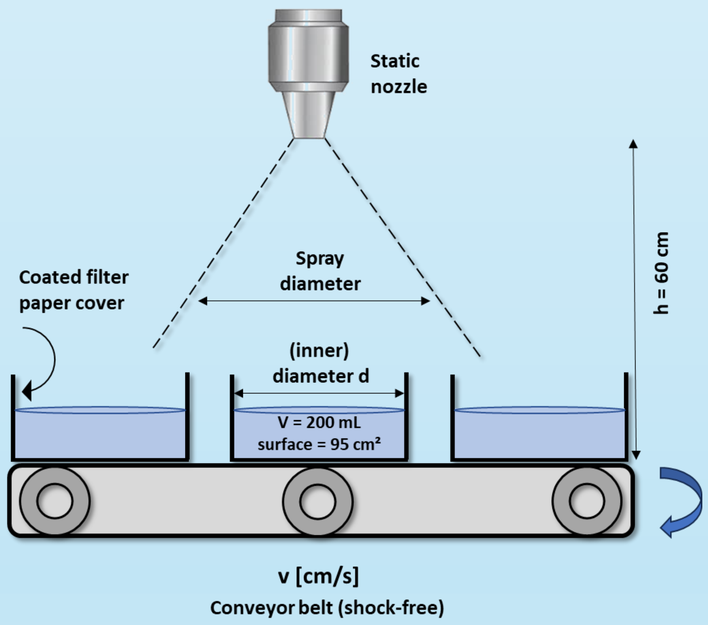
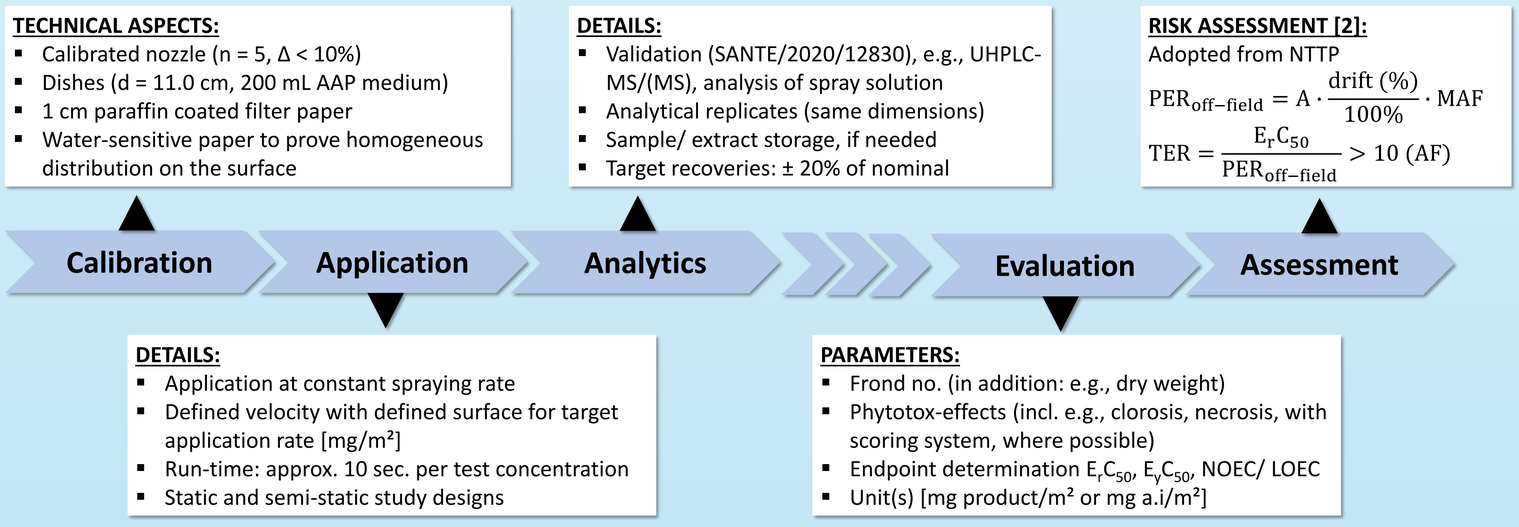


Fig. 1: Experimental setup for overspray exposure



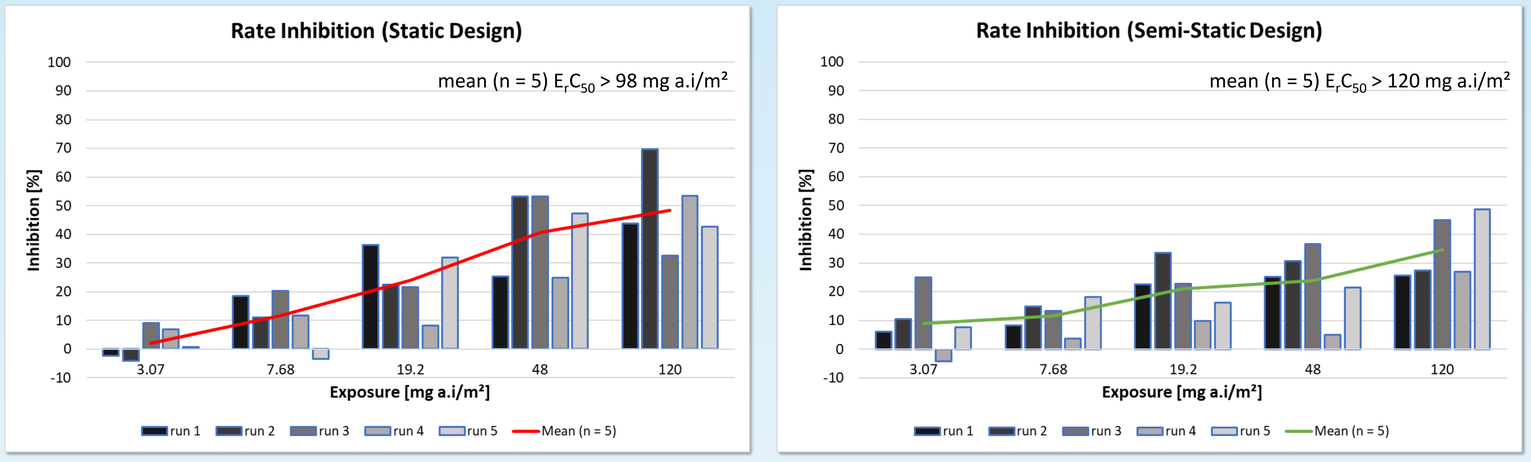
A: Application rate [L/ha or g a.i/ha]; **MAF:** Multiple Application Factor (ESCORT 2 [4]); **Drift:** Drift Rate according to Rautman *et al* [3].
AF: Assessment Factor (10) for inter-species variability; **semi-static:** Replacement of water directly after application and on days 2 and 5.

Fig. 2: Timeline and milestones for study conduct.

Results and Discussion

Growth rate inhibition and increase in frond numbers (Fig. 3) were measured for five separate runs (three replicates each) per concentration and compared to the results of control replicates after seven continuous days of incubation under static and semi-static conditions. Phytotoxic effects were determined qualitatively (Fig. 4) at study end.

Quantitative effects (static vs. semi-static exposure)



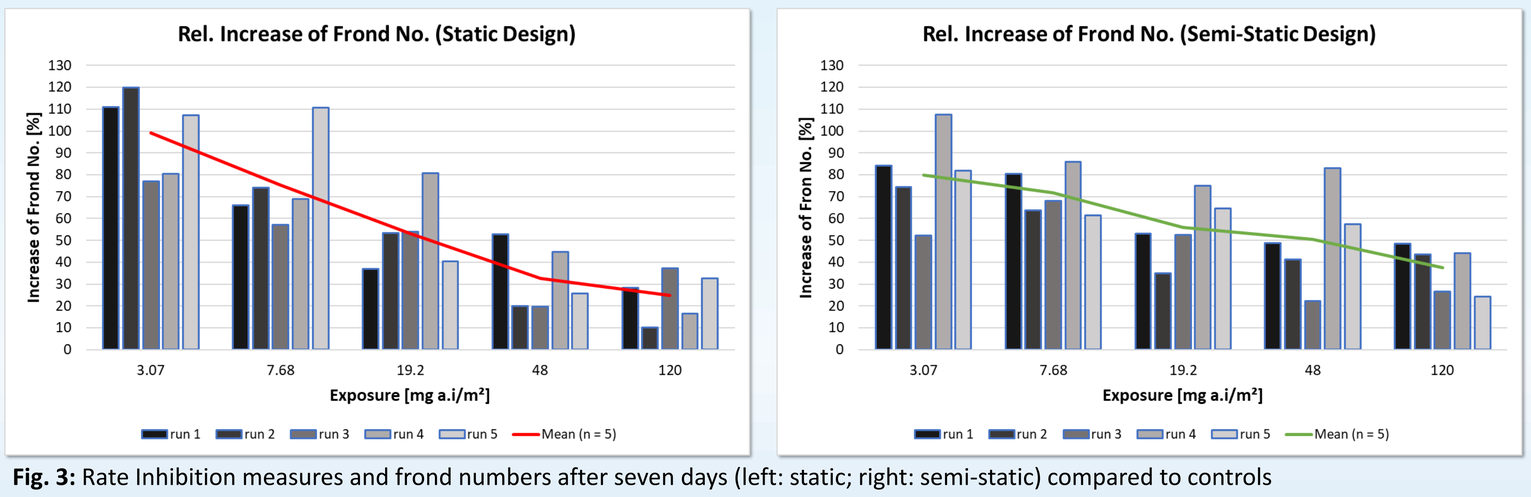


Fig. 3: Rate Inhibition measures and frond numbers after seven days (left: static; right: semi-static) compared to controls

Phytotoxic effects

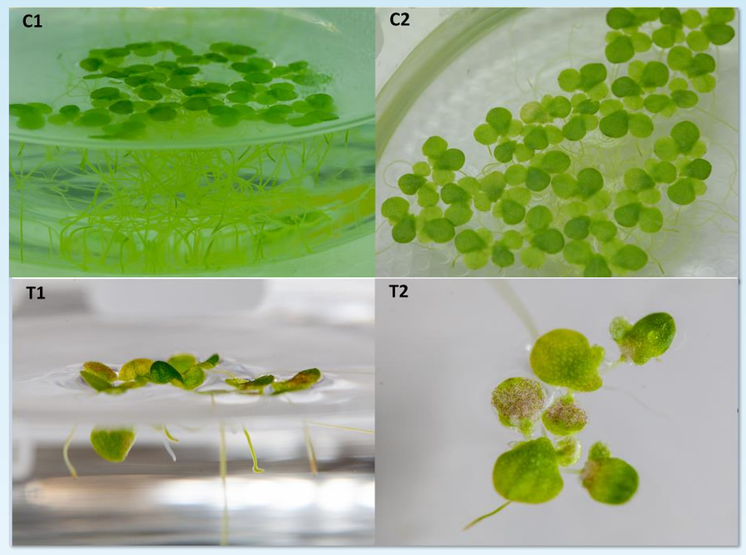


Fig. 4: Control (**C**) and highest exposure (**T**) after 7 days

Exposure [mg a.i/m²]	Rate Inhibition [%]		Frond No. [% rel.]	
	Static	Semi-static	Static	Semi-static
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
3.07	1.3 (11.3)	8.5 (13.3)	99.0 (30.8)	80.0 (25.7)
7.68	12.7 (13.2)	11.6 (7.7)	63.8 (26.8)	71.9 (15.3)
19.2	24.1 (13.5)	21.0 (11.3)	53.1 (22.5)	56.0 (19.6)
48.0	40.4 (13.5)	23.8 (14.6)	33.1 (18.7)	52.8 (22.0)
120	48.5 (16.4)	34.7 (13.3)	24.9 (14.0)	37.2 (13.5)

Fig. 5: Summary of assessed parameters after 7 days. Mean of five (5) studies with each three (3) replicates per concentration.

Analytical recovery 98-102% in spray solution ☑; Deviation in spray output < 10% (n = 5) ☑; Concentration-depending reduction in growth rate and frond numbers ☑. Significant phytotoxic effects with dose-response observable (in particular chlorosis, necrosis and valuted fronds).
Static design: Stronger quantitative and qualitative effects upon exposure indicate overlaying effects from both exposure routes, i.e., overspray and aqueous exposure.
Semi-static design: Quantitative and qualitative effects apparently smaller compared to static design. Considered as effects only caused by single (overspray) route exposure.

Conclusion

- Application successful proven by spray output of nozzle (Δ < 10%), homogeneous wetted water-sensitive paper and recovery in spray solutions within ± 10%.
- Quantitative and qualitative effects observable with clear differences for static and semi-static exposure. Drift-only exposure is exclusively realized by a semi-static design.
- Some variability between runs can be attributed to natural asynchrony in frond development, despite comparable growth stages at test start. Increasing the number of replicates may improve statistical robustness.
- Exposure routes of classical methodology for aquatic risk assessment (drift + run-off + drainage = PEC_{SW}) considers contamination of non-target water bodies but does not distinguish for single types of exposure. Both routes of exposure can be assessed, but require a separate approach in their evaluation even the AF is considered both sides. Depending on their MoA, unintentional exposure by drift could but do not necessarily have to cause significant effects on non-target species.
- The presented design and followed methodology provide a technical setup to address latest requirements [2] and may serve as valuable basis for developing a standardized testing protocol for regulatory purposes.

References:
[1] OECD Guidelines for Testing of Chemicals, Sec. 2, Test No. 221: Lemna sp. Growth Inhibition Test, 11 July 2006. [2] Working Document on Risk Assessment of Plant Protection Product in the Central Zone Ecotoxicology, v. 3.0, December 2024.
[3] Ganzelmeier, H., Rautmann, D., Spangenberg, R., Streloke, M., Herrmann, M., Wenzelburger, H-J., Walter H-F; Studies on the spray drift of plant protection products. Mitt. Biol. Bundesanst. Land-Forstwirtsch. Berlin-Dahlem, Vol 30, 1995.
[4] Candolfi, MP. *et al.*, 'Guidance Document on regulatory testing procedures for plant protection products with non-target arthropods'. From the workshop, European Standard Characteristics of Non-target Arthropod Regulatory Testing (ESCORT 2) 21-23 March 2000.