



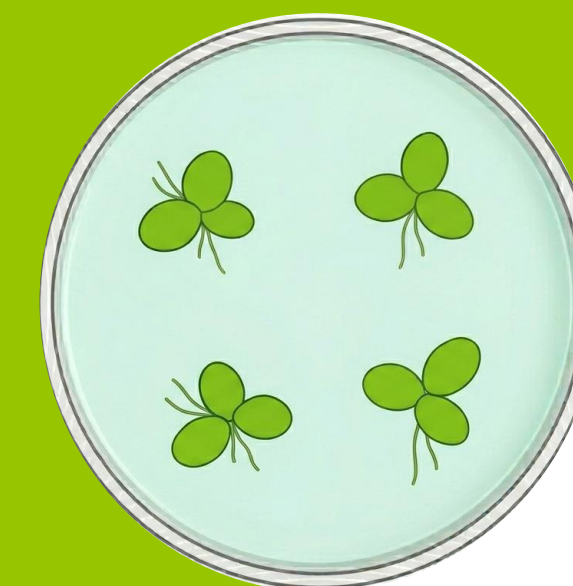
Development and relevance of overspray tests for aquatic plants

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OECD test does not cover overspray of contact herbicides

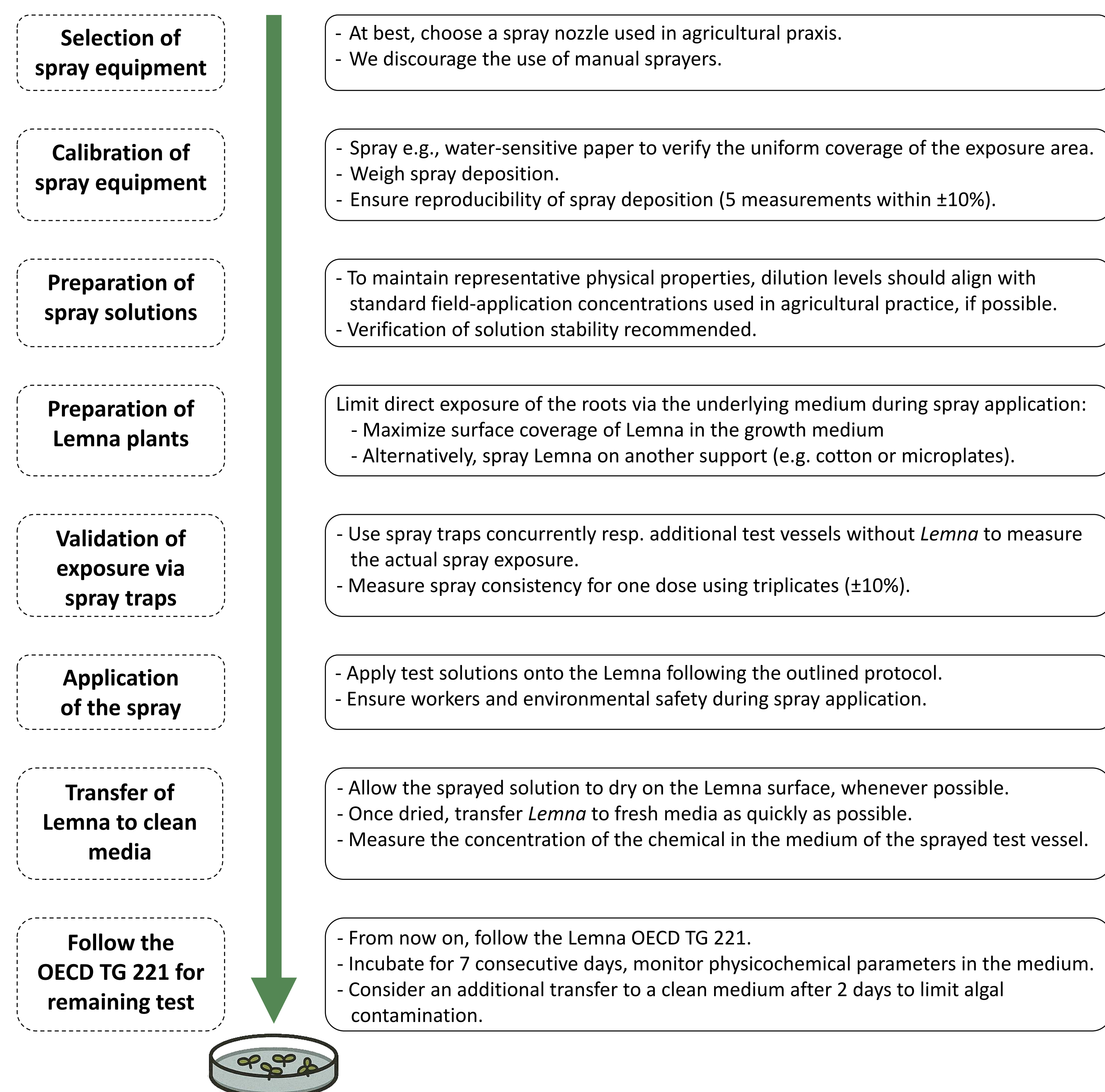
Current ecotoxicological studies on floating plants, such as *Lemna* spp., focus on exposure via the water phase, e.g., OECD TG 221, and may therefore **fail to capture effects of contact herbicides**. When floating and emergent aquatic plants are exposed to spray drift in the field, these substances are taken up by the leaves (see Renewal Report for the active substance glyphosate, PLAN/2023/1497 RR - Rev 2 - 13 October 2023).

Within our SETAC Working Group we tackle this scenario

To clarify this issue and to help addressing the pending regulatory gap, we established a dedicated SETAC working group to:

- (1) develop a reproducible and economically feasible **standardized protocol** for an overspray test for the standard species *Lemna* sp., and
- (2) determine to what extent this exposure scenario is already **covered by current NTT** risk assessment.

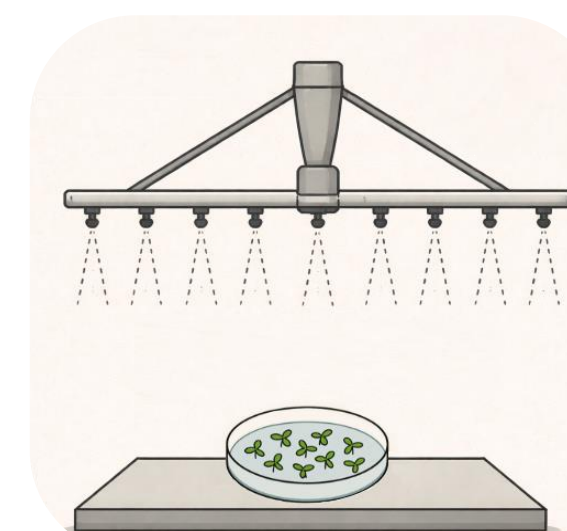
Flow chart for the overspray test protocol



Adapting and improving existing test guidelines

While OECD TG 221 for *Lemna* only refers to Lockhart *et al.* (1989) for overspray scenarios, guidelines for terrestrial plants, i.e. OECD TG 208 and 227, provide valuable recommendations for spray applications.

Our protocol development builds on these guidelines, but several **challenges unique to Lemna** remain.



Due to its floating habit and smaller fronds compared to NTTs, *Lemna* experiences a distinct spray deposition. Specifically, plants may be simultaneously exposed via foliar deposition and, incidentally, via the growth medium.

Currently, we are addressing these challenges ranging from spray application parameters, post-treatment handling, and accurate quantification of actual exposure.

Here, we **present the current status** of our protocol and **seek your feedback** on unresolved issues.

Main challenges identified

- Overspray of *Lemna* cultures (e.g., in Petri dishes) can lead to **root exposure**, due to pesticide entering the medium via drip-off from fronds or direct spray. This unintended exposure cannot be assumed to be negligible.
- Monitoring chemical concentrations throughout every procedural step entails a **substantial analytical workload**.
- Is **quantification of the actual deposition** (i.e., effective foliar dose) required, and if so, which approach should be used?

Methodologies agreed upon

Scenario

Although developed to assess the effects of spray drift, a **direct overspray** is simulated at Tier 1.

Calibration

Filter paper or spray traps should be used and weighed to calibrate the spray system (using water) and to verify the **effective dose** delivered during testing.

Mixed exposure

Where **root exposure is unavoidable** by design, it must be analytically quantified.

Discussions ongoing

- Which exposure parameters should be used to calculate the endpoints in mg a.s./ha?
- Shall additional specifications for spray devices or the sprayed drops be defined?

Once a test protocol has been agreed upon ...

it will serve as a basis for further experimental tests with various active substances and plant protection products.

Endpoints from these tests will be compared with the non-target terrestrial plant (NTTP) risk assessment to determine if the NTTP risk assessment covers the aquatic overspray scenario.

Depending on the outcome of this comparison, further validation of the protocol may be required in view to amending existing test guidelines.

References

Lyle Lockhart, W., Billeck, B.N. & Baron, C.L. Bioassays with a floating aquatic plant (*Lemna minor*) for effects of sprayed and dissolved glyphosate. *Hydrobiologia* 188, 353–359 (1989). <https://doi.org/10.1007/BF00027800>
OECD (2006), *Test No. 221: Lemna sp. Growth Inhibition Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, <https://doi.org/10.1787/9789264016194-en>.
OECD (2006), *Test No. 208: Terrestrial Plant Test: Seedling Emergence and Seedling Growth Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, <https://doi.org/10.1787/9789264070066-en>.
OECD (2006), *Test No. 227: Terrestrial Plant Test: Vegetative Vigour Test*, OECD Guidelines for the Testing of Chemicals, Section 2, OECD Publishing, Paris, <https://doi.org/10.1787/9789264067295-en>.