Reproductive Endpoints and Phenological Stage Exposed in Non-Target Terrestrial Plants Studies

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Introduction

Currently, tests on Non-Target Terrestrial Plants (NTTP) are typically conducted at the juvenile stage and only vegetative endpoints (EP) are evaluated in NTTP risk assessment under the European Regulation (EU) 1107/2009. However, assessing effects across the entire life cycle — including reproductive stages or exposing other phenological stages— is crucial to accurately evaluate the impacts of pesticides on natural NTTP populations. EFSA (2014) highlights this gap, noting that reproductive stages might be more sensitive; however, this conclusion is based on only five studies, as it relies solely on studies following OECD 227-like and prolonged test designs.

Several meta-analyses have since expanded the dataset for more robust results, by including studies with more variable test conditions. Usually, these analyses are based on the mean of ratios of paired endpoints across studies (e.g., Christl et al. 2019), which capture only the central tendency of species responses. Moreover, such approaches may mask differences in sensitivity due to variation in study design, test substances, environmental conditions. From a regulatory perspective, the protection of the more sensitive species is crucial for regulatory purposes. Indeed, regulatory frameworks typically rely on percentile-based approaches—such as the 10th or 5th percentile—to ensure protection for 90% to 95% of the species.

Objectives

- Perform an accurate literature research to expand the data set used in EFSA 2014.
- Extract vegetative and reproduction EP and phenological stages at exposure.
- Explore possible statistically analyses and evaluate the advantages and disadvantages of each method.
- Provide recommendations for coming Terrestrial Guidance Document.

Methodology

- A systematic literature review was conducted to identify new scientific knowledge, following the EFSA Journal 2011; 9(2):2092 methodology. **See Poster Byers et al. (P-Tu473)**
- Additionally, it was completed using Inciteful (https://inciteful.xyz/), an AI-assisted citation mapping tool.
- Studies identified were evaluated for their reliability and suitability for data extraction, i.e. ERx available for vegetative and reproductive EP and assessed from experiments conducted under similar conditions; intra- and inter-species competitions were excluded.

Reproduction Endpoints

To expand the dataset, different BBCH growth stages for both time of exposure and EP recording have been considered, but comparisons were only considered reasonable with comparable study conditions. Our literature search identified **70 new studies** potentially relevant. But only 7 studies were evaluated reliable & suitable for data extraction. We have created an **Excel data based with 1649 entries**.

Applying a percentile-based approach - whether empirical or model-based, such as SSDs – requires a sufficient number of comparable data points. Although the aim was to extend the approach used in EFSA (2014), Appendix A, by using the additional data, none of the studies identified provided enough relevant EP to enable a robust percentile-based comparison. As a result, the **EFSA methodology could not be meaningfully expanded**.

However, the consideration of single study independently, can support EFSA (2014), suggesting that **reproductive EPs (especially seed production) are in more than 10% cases more sensitive than vegetative EPs**.

Phenological Stage Exposed

- Studies cited in EFSA 2014 were quantitatively re-evaluated. **Only three studies were suitable** for comparison of effects after exposition during vegetative and exposition during reproductive stage (Strandberg et al. 2012, Pfleeger et al. 2011 and Olszyk 2009).
- 34 and 45 pairwise comparisons were possible between early and late exposure, for vegetative and reproductive Eps, respectively.
- **In 20% for vegetative EPs and 37.7% for reproductive EPs, a higher sensitivity was observed when the plants were exposed in their reproductive stage** (BBCH stages 5-6).
- **This was especially true for sulfonylurea herbicide**, but higher sensitivity of plants following an exposure during the reproductive stage was also observed for other herbicides.
- This highlights the importance of the phenological stage at the time of exposure and suggests that standard test designs - as proposed in OECD 208 and 227 - may not represent the worst-case scenario for all herbicides. **Herbicide specificity and Mode of Action should also be considered**.

Conclusions and Recommendation

- **Expanding the data set to various study designs introduces bias and can mask effects** - particularly at the tails of the distribution, where the most sensitive species are found. This presents a major drawback for regulatory purposes, as these species are especially relevant for the risk assessment.
- Given this variability across studies, the approach outlined in Appendix A of EFSA (2014)—using similar study designs, extended reproductive EPs, and **percentile-based approaches such as SSD modelling—remains the most suitable for regulatory purposes**. However, suitable data for such analyses remain limited.
- **More studies following OECD 227-like and prolonged test design should be performed in the future.**

Für Mensch & Umwelt

References

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Christl et al. (2019): DOI: 10.1002/ieam.4218
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