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Ecotoxicological assessment of pesticide mixtures on aquatic non-target species

Abrantes et al.

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Introduction

This deliverable is part of WP4 activities under the SPRINT project, titled "Ecotoxicological assessment of Plant Protection Products (PPP) mixtures." Its primary aim is to assess the impact of pesticide mixtures on aquatic non-target species by applying a whole-mixture approach, addressing the European Union's priorities for sustainable pesticide management and protection of aquatic ecosystems.

The assessment focuses on understanding the combined effects of multiple PPP typically found in agricultural runoff, which may exhibit additive or synergistic toxicity not captured in single-compound studies. Selection of PPP mixtures for testing was based on (i) the occurrence and risk quotients of individual pesticides in multiple case study sites (CSS) across Europe and Argentina, (ii) ecotoxicological data sourced from standardized databases and predictive modeling (e.g., PEC and RAC values), and (iii) prioritization outcomes from regional surveys and pesticide ranking metrics (for more information please see Abrantes et al, 2024; DOI: https://doi.org/10.1016/j.scitotenv.2024.177322). This result in a total of 11 distinct PPP mixtures that were tested (corresponding to the 11 CSS). The ecotoxicological effects were assessed not only on standard organisms specified by EFSA guidelines but also on alternative native species, with a focus on capturing both indirect and chronic effects. This broader testing approach allows SPRINT to provide a more comprehensive assessment of the environmental impacts of PPP mixtures.

A complete overview of all planned tests is outlined in the Description of Action (DoA), with additional details available in Milestone 5, produced in December 2021. Table 1.1 summarizes all tests conducted from 2022 to 2024, detailing the methodologies and results across multiple species and exposure conditions.

Table 1.1 Overview of all aquatic ecotoxicology tests performed during 2022 – 2024.

Test	**Endpoints**	**Duration**	**Number of pesticide mixtures tested**	**Concentrations tested**	**Number of replicates**	**Publication status (Aug 2024)**	**SPRINT partner mainly involved**
Freshwater algae *Raphidocelis subcapitata*	Growth inhibition	96h	11	CTR, MEC, PEC, 3PEC, 5PEC	6	In preparation	UAV
Freshwater plant *Lemna minor*	Growth inhibition	7 days	11	CTR, MEC, PEC, 3PEC, 5PEC	3	In preparation	UAV
Freshwater crustacean *Dahnia magna**	Immobilization	48h	11	CTR, MEC, PEC, 3PEC, 5PEC	4	In preparation	UAV
Freshwater insect *Chironomus riparious*	Immobilization Gene expression[a,b] Enzimatic activity[a,b]	48h	11 3 3	CTR, MEC, PEC, 3PEC, 5PEC	4	In preparation	UAV
Fish *Danio rerio*	Lethality Behaviour[b] Cholinesterase activity (ChE)[b] Gene expression[a,b]	4	11 11 11 3	CTR, MEC, PEC, 3PEC, 5PEC	6 6 3 9	In preparation	UAV
Native freshwater crustacean *Daphnia longispina*[a, b]	Immobilization[a,b] Reproduction[a,b]	48h 21 days	11 9	CTR, MEC, PEC, 3PEC, 5PEC	4 10	In preparation	UAV

[a.] Additional test performed that were not included in the GA.
[b.] New SPRINT indicators.

This deliverable is structured as follows:

Chapter 1 – Introduction

This chapter provides an overview of pesticide use in agriculture, the environmental impact of PPP mixtures on aquatic ecosystems, and the rationale behind the ecotoxicological assessment of these mixtures.

Chapter 2 – Selection of pesticide mixtures for testing

This chapter describes the selection process for PPP mixtures, including the distribution of pesticides in aquatic ecosystems, the calculation of Predicted Environmental Concentrations (PEC), the collection of ecotoxicological data, and the establishment of Regulatory Acceptable Concentrations (RAC). It concludes with the ranking and prioritization of pesticides based on their risk quotient and frequency of occurrence.

Chapter 3 – Experimental methods for each species

This chapter outlines the methodologies used for testing the effects of PPP[a] mixtures on various aquatic species, including microalgae, macrophytes, crustaceans, insects, and fish embryos. Details are provided for each test, including growth inhibition and immobilization protocols.

Chapter 4 – Results and discussion by species

In this chapter, the outcomes of the ecotoxicological tests are presented, focusing on the observed effects of the 11 PPP mixtures on each species at different concentration levels.

Chapter 5 – Final considerations

This chapter summarizes the key findings of the ecotoxicological assessment, discusses the implications for policy and regulatory practices, and offers a future outlook on advancing research in the area.

Summary of Findings

The SPRINT project's ecotoxicological assessment revealed that pesticide mixtures affect all tested aquatic species, with varying impacts depending on the mixture's composition. Key findings include:

- **Microalgae (*Raphidocelis subcapitata*)** experienced moderate to significant growth inhibition at higher concentrations in several countries, while some regions showed only slight effects.
- **Macrophyte (*Lemna minor*)** tests indicated high growth inhibition from most mixtures, except those from France and Denmark, which showed minimal toxicity.
- **Crustaceans (*Daphnia magna*)** were significantly affected by PPP mixtures from Spain, France, and other regions, but not in Portugal or Argentina.
- **Aquatic insects (*Chironomus riparius*)** showed high immobilization rates, particularly in PPP mixtures from Argentina, Italy, and France.
- **Fish embryos (*Danio rerio*)** had low toxicity overall, though some mixtures affected behavior and enzyme activity, suggesting potential long-term effects.
- **Native crustacean (*Daphnia longispina*)** tests showed variable impacts, with some mixtures affecting immobilization and reproduction, particularly at higher concentrations.

Implications for Policy and Regulation

These findings indicate a need to shift pesticide risk assessments to consider the combined effects of mixtures rather than individual chemicals. Regulatory bodies like EFSA should adopt whole-mixture approaches and enforce localized assessments to mitigate impacts on aquatic ecosystems.

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