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# Ecotoxicological assessment of pesticide mixtures on terrestrial non-target species

Tourinho et al.

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<b>Project coordinator</b>	Wageningen University
<b>Overall Project coordinator</b>	Prof. Dr. V. Geissen +31317485144 (violette.geissen@wur.nl)
<b>Scientific Project Manager</b>	V. Felix da Garca Silva, MSc (vera.felixdagracasilva@wur.nl)
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<b>Principle Author(s)</b>	Paula da Silva Tourinho (MU), Kayode Jegede (WU), Erin Henry (WU), Franziska Ditterich (UH), Philipp Mäder (UH), Fabian Stache (UH), Ellen Kandeler (UH), Frank van Langevelde (WU), Štěpánka Skudová (MU), Mária Janošíková (MU), Zuzana Hochmanová (MU), Violette Geissen (WU), Vera Felix da Garca Silva (WU) & Jakub Hofman (MU)
<b>Principle Author e-mail</b>	jakub.hofman@recetox.muni.cz
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## Introduction

SPRINT aims to provide tools and novel data to assess integrated risks and impacts of plant protection products (PPP; pesticides) on the environment and human health. Using this, SPRINT wants to accelerate the adoption of innovative transition pathways towards more sustainable plant protection.

A significant part of the SPRINT work package four (WP4) goals lies in the ecotoxicity testing of pesticide mixtures on terrestrial organisms (i.e. terrestrial non-target species). The focus of that effort is not only on standardized single-species low-tier assays with EFSA test organisms but also on native species, new endpoints, and higher-tier testing systems.

In Chapter 2, this deliverable summarizes the testing concept chosen for the SPRINT project, which was based on a whole-mixture approach – i.e., organisms are exposed to the pesticide mixtures and the effects are evaluated (in contrast to the calculation approach based on single substances testing).

In Chapter 3, the prioritization approach for the selection of the pesticide mixture is described. This approach used the pesticide residue data based on the SPRINT survey 2021 performed at case study sites (CSS) and hazard data.

Chapters 4 – 11 report the methodologies, results, discussion, and conclusions for individual tests performed.

The ecotoxicological effects were tested not only on current EFSA organisms and tests but also on alternative (native) species and in multi-species systems, highlighting indirect and chronic effects. In this way, SPRINT proposes and validates a novel and/or improved strategy for testing the integrated effects of pesticide mixtures. The overview of all tests performed during 2022 - 2024 is provided in Table 1.

Chapter 12 describes the methodology for determination of the pesticides in soil samples and compare the results to the nominal concentrations. And finally, Chapter 13 summarizes the significant findings, limitations, and outlook.



**Table1:** Overview of all terrestrial ecotoxicology tests performed during 2022 – 2024.

Test	Endpoints	Duration	Number of pesticide mixtures tested	Chapter
<b>"EFSA tests"</b>				
<b>Soil microbial activity</b>	C and N mineralization	28 (- 84 days)	11	<b>4</b>
<b>Collembola</b>	Mortality, reproduction	28 days	11	<b>5</b>
<b>Earthworms</b>	Mortality, growth, reproduction	28 and 56 days	11	<b>6</b>
<b>Beneficial insects</b>	Mortality, reproduction	7 and 14 days	11	<b>7</b>
<b>Terrestrial plants</b>	Root growth, seedling emergence and early growth	4 and 14 days	11	<b>8</b>
<b>Honey bees</b>	Acute: mortality Colony: mortality, food, brood	Acute: 48 hours Colony: 42 days	Acute: 11 Colony: 1	<b>9</b>
<b>"New SPRINT indicators"</b>				
<b>Soil microbiome - Functional and structural biodiversity</b>	Phospholipid fatty acids Enzyme activities involved in C-, N- and P-cycles Functional genes based on qPCR Carbon use efficiency	28 and 56 days	4 11 + 2 11 + 4 2	<b>10</b>
<b>Native earthworm species</b>	Mortality, growth, reproduction for <i>L. rubellus</i> Mortality, cast production, burrowing activity for <i>L. terrestris</i>	28 and 74 days 74 days	11	<b>6</b>
<b>Soil microcosms</b>	Earthworm survival and reproduction, plant growth, C and N mineralization	14, 28, and 56 days	1	<b>11</b>
<b>Wild bees</b>	Colony mortality, food, brood	42 days	1	<b>9</b>

## Highlights

- The use of alternative (native) species and the testing in the multi-species systems were found to be extremely important when assessing the effects of pesticide mixtures.
- Higher-tier testing was shown as more sensitive compared to single species tests.
- The microbial activity tests and the novel microbial indicators suggested that prolonged exposure (>28 days) should be recommended.
- In a few cases, 'more than additive' effects were suggested.
- Significant effects were observed at relevant environmental concentrations in several cases of our testing.



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## Contact

Jakub Hofman: [jakub.hofman@recetox.muni.cz](mailto:jakub.hofman@recetox.muni.cz)

Paula Tourinho: [paula.tourinho@recetox.muni.cz](mailto:paula.tourinho@recetox.muni.cz)