

Analytics in Soil Ecotoxicological Studies: Challenges and Approaches from a Regulatory, Biological and Analytical Perspective



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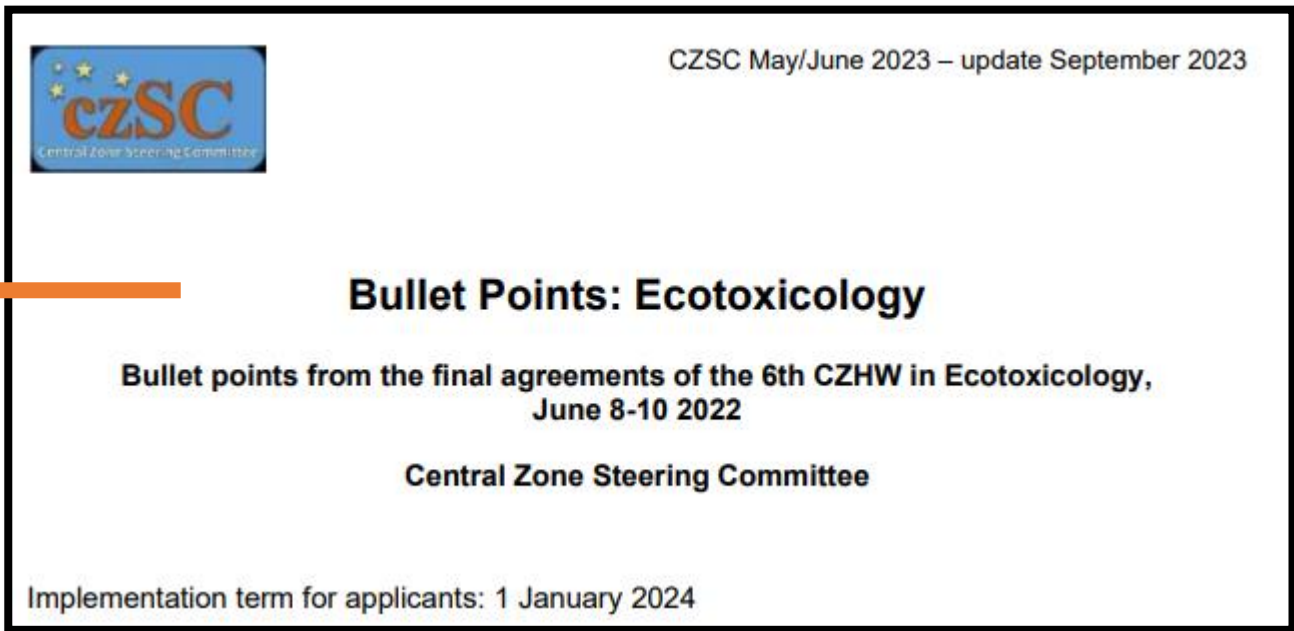
Background

- For the assessment of plant protection products in the **European Central Zone**, analytical measurements are requested for soil organism studies for substances where the DT₉₀ does not cover the exposure phase of the laboratory Tier 1 tests. These substances are considered as “unstable” [1, 2].
- Analytical dose verification must be performed at least at the start, middle and end of a study [1, 2].
- Calculation of time-weighted average / mean or geometric mean and use of these if the concentration falls below 80% of nominal [1, 2]

Working document on Risk Assessment of Plant Protection Products in the Central Zone

Ecotoxicology

Version 3.0, December 2024



Challenges

- Currently, there are only limited (technical) guidance available
- The selection for timepoints of samplings is a challenge
 - Suitable time points for soil samplings depend on the substance degradability → start, middle and end of a study might be too short-sighted
 - Exposure phase differs between test organisms (14 days for predatory mite, 28 days for springtails, 56 days for earthworms OECD 226, 232, 222)
 - DT₉₀ and DT₅₀ derived in natural soils can differ from that in OECD artificial soil.
- Test organism biology of earthworms might play a role for biodegradation patterns in comparison to plain soil → earthworms increase microbiological soil activity by incorporating their feed / manure into the soil
- Subsampling from running biological replicates is technically not possible
- 2 sampling points with concentrations above LOQ are required to be allowed for calculating mean concentrations
- How to consider new endpoints in the risk assessment (i.e. lack of semi-static, flow-through approaches)?
- Which PEC_{SOIL} should be used in the Risk Assessment (i.e. Initial, time-weighted average)?

Approaches

	Earthworm <i>Eisenia fetida</i>	Springtail <i>Folsomia candida</i>
Exposure phase	56 days	28 days
Destructive soil samplings	<ul style="list-style-type: none">Minimum at day 0, 28, 56At least 27 additional test units for samplings for combined NOEC/ECx-Design with 8 concentrations and controlAdditional replicates with test organisms → earthworms increase microbiological soil activity by incorporating their feed / manure into the soil	<ul style="list-style-type: none">Minimum at day 0, 14, 28At least 27 additional test units for samplings for combined NOEC/ECx-Design with 8 concentrations and controlAdditional replicates without test organisms
Analytics	<ul style="list-style-type: none">Ensure homogenous distribution of test item in soil before extraction e.g. using whole amount of soil or milling the soil before extraction and use aliquots for extractionControl samples which correspond to the current test design needed for each sampling point and used for blank matrix and procedural recoveriesFor extraction of soil an extraction method used for E-Fate or residue studies can be usedAnalysis with LC-MS/MS (known metabolite can also be investigated)	
Calculation of time-weighted/geomean	<ul style="list-style-type: none">Calculation using the measured concentrations following Annex 2 of OECD guidance 23 (2019) for static-renewal exposure systems [3] → Actually aquatic toxicity guidance	
Regulatory	<ul style="list-style-type: none">Impact of using time-weighted/geomean: non-realistic endpoint, since there is no test design adapted to soil organisms (i.e. Semi-static, flow-through)Risk assessment is not mirroring the exposure in a real case scenario, in which substances will degrade	

Summary

- Proposal of a realistic test design and endpoint to be used in the Risk Assessment
- Alignment on PEC_{SOIL} to be used

Biological

Analytical

Regulatory

SCAN ME



References

[1] Working document on Risk Assessment of Plant Protection Products in the Central Zone – Ecotoxicology. Version 3.0. December 2024
[2] Bullet Points from the final agreements of the 6th Central Zone Steering Committee in Ecotoxicology, June 8-10 2022 (adopted by the CZSC May/June 2023 – update September 2023)
[3] OECD (2019): Guidance Document on Aqueous-Phase Aquatic Toxicity Testing of Difficult Test Chemicals. OECD Series on Testing and Assessment, No. 23 (2nd Edition). ENV/JM/MONO(2000)6/REV1. OECD Publishing, Paris.

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