



Ecotoxicity of Environmentally Realistic Metal-Organic Mixtures to the Freshwater Algae *Raphidocelis subcapitata*

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Objective

Investigating whether metals and organics should be assessed separately or together in environmental risk assessment frameworks for mixture toxicity

Research questions

- Do *organic* compounds that highly contribute to organic mixture toxicity at low effect concentrations affect *metal* toxicity?
- Do *metals* that highly contribute to metal mixture toxicity at low effect concentrations affect *organic* toxicity?

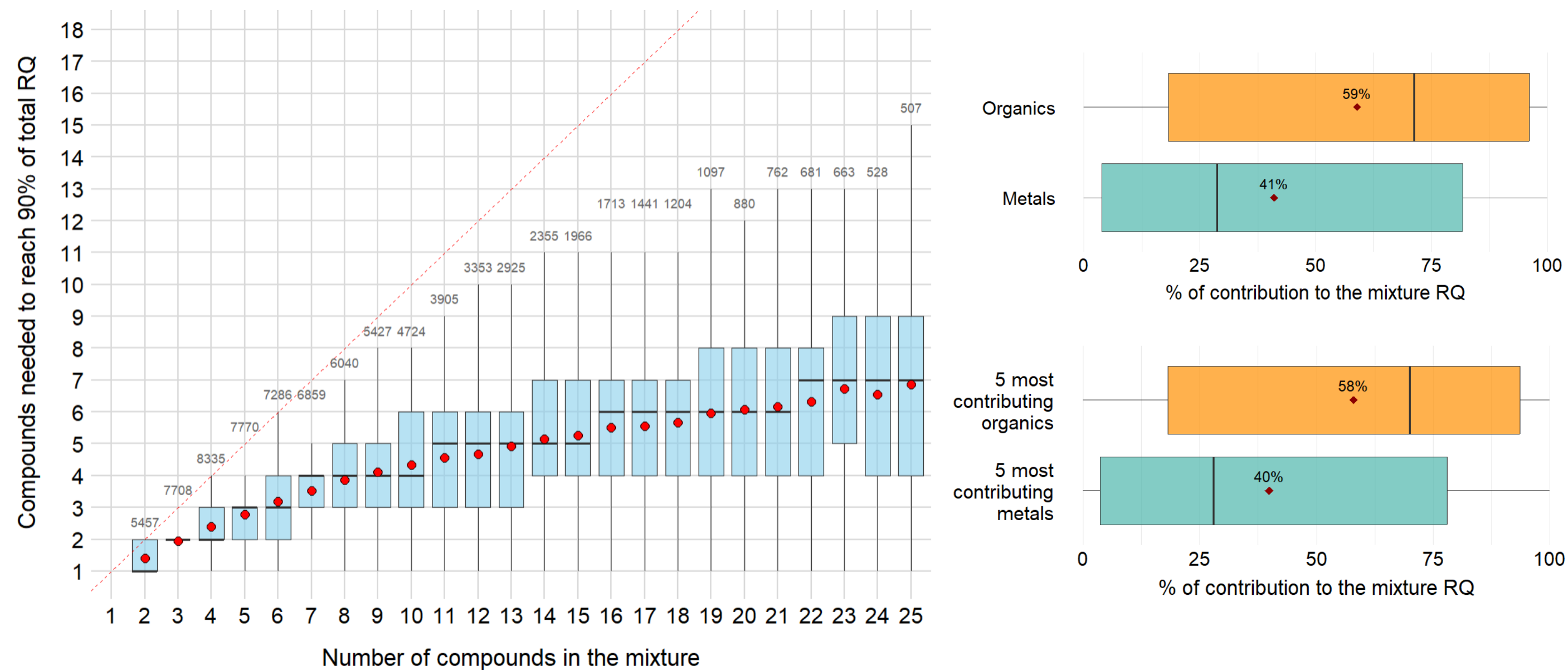
Materials & Methods

Analysis of the *European freshwater monitoring data database (Waterbase)*



Mixture complexity → five metals and five organics explain ≥90% of the overall mixture risk in ≥90% of samples

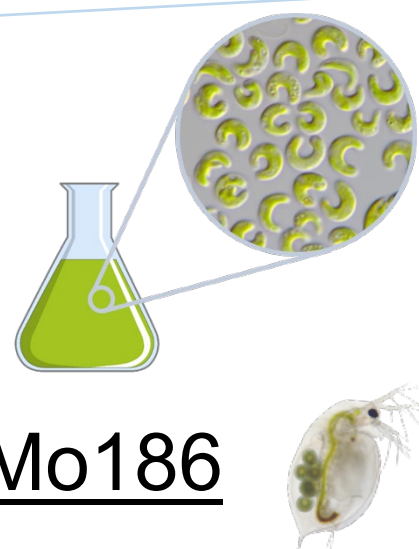
- Risk Quotient (RQ) based PNEC prioritization



Test organism → *Raphidocelis subcapitata*

72h algal growth inhibition test, OECD 201 guideline

For results with the test organism *Daphnia magna*, see poster [Mo186](#)



Mixture combinations → selection based on toxicity contribution

Metals: Toxic Unit (TU) based toxicity prioritization

- Metals with ≥1% average contribution to the metal mixture toxicity, presented in order of % of contribution to *R. subcapitata*: Zn, As, Cu, Pb, Mn, Ba, Ni, Cd and Cr

Organics: selection based on TU-derived toxicity contribution of organic chemical classes to *R. subcapitata*

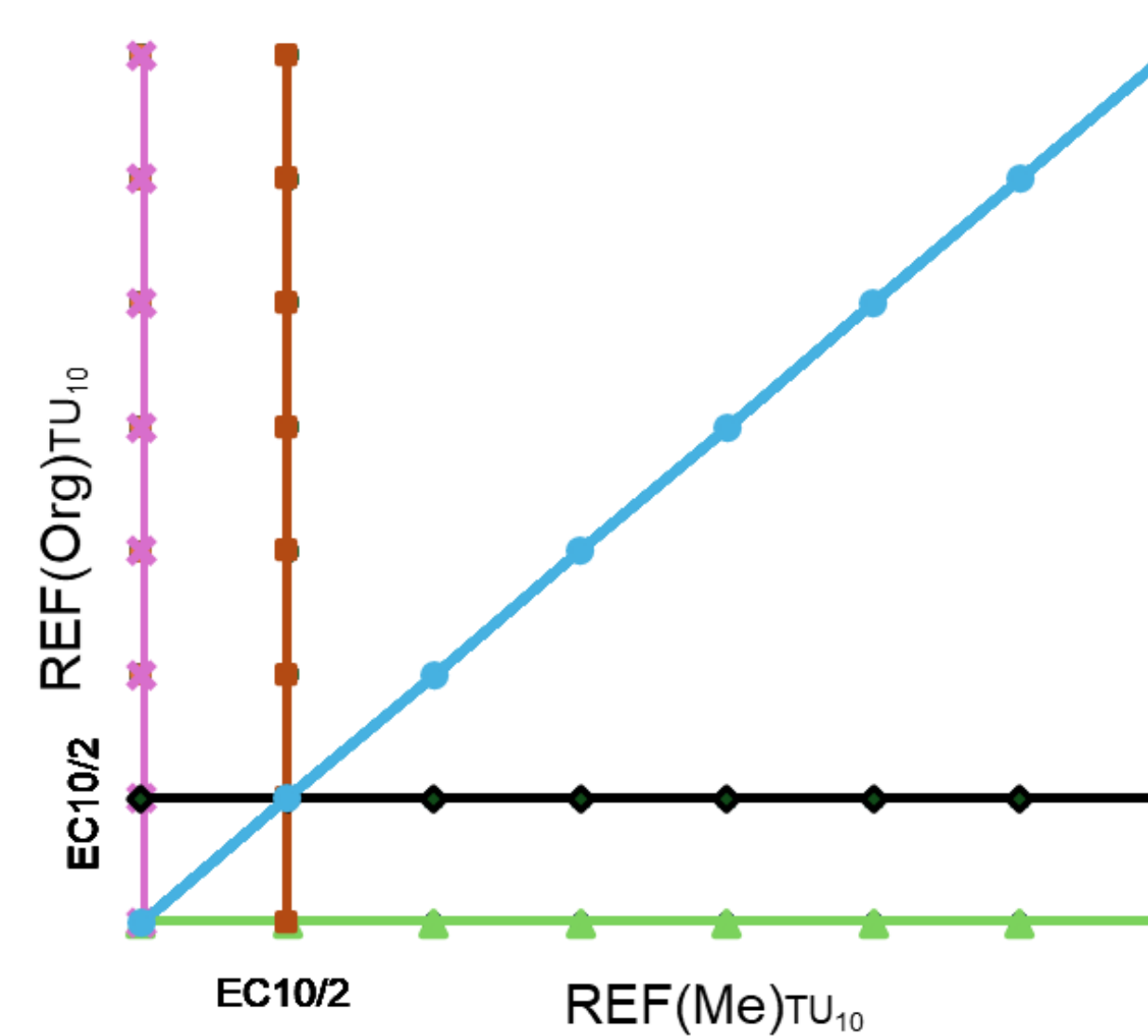
- Herbicides accounted for 96% of the organic mixture toxicity
- The selected herbicides represented the highest contributing herbicidal MoAs
- Organics selected: 10 herbicides of 4 herbicidal MoA (terbutylazine, metamitron, lenacil, metobromuron, chlortoluron, metazachlor, dimethachlor, flufenacet, diflufenican, pendimethalin) and 1 fungicide (azoxystrobin)

Test design

4 mixture tests of 10 compounds each (5 metals and 5 organics)



For each test, the organics (pink) and metals (green) mixtures were tested *simultaneously* individually and in 3 mixture designs:



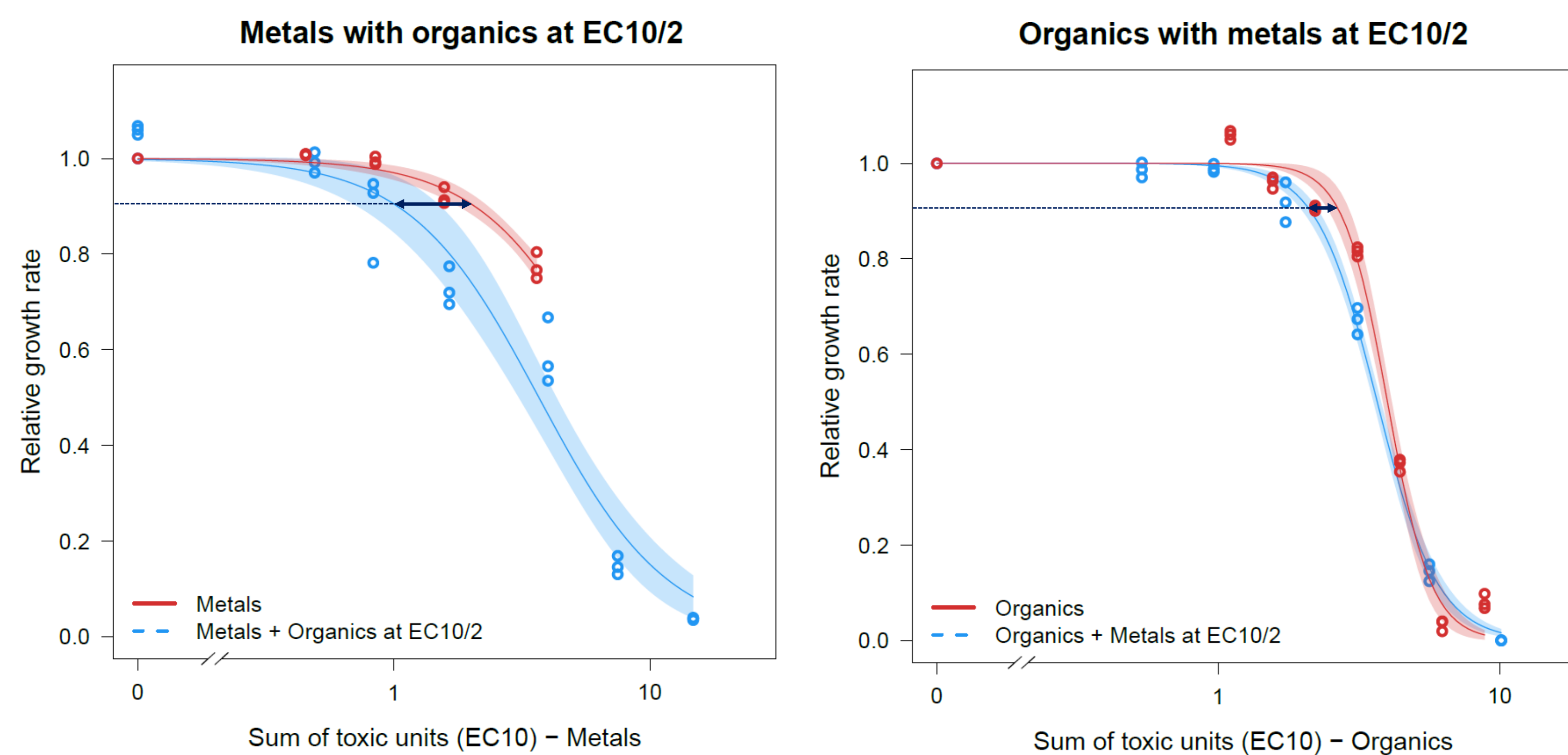
- Equitoxicity (i.e., the metals and the organics contribute equally to the mixture toxicity; light blue)
- Keeping the organics mixture constant at an EC10/2 while increasing the concentration of the metal mixture and vice versa (black and red)

Results

Preliminary results of the first mixture test:

metamitron, metazachlor, metobromuron, pendimethalin, terbutylazine + As, Ba, Cd, Ni, Zn

Results based on nominal values for the organic compounds



$$\frac{EC10_{Metals + Organics(EC10/2)}}{EC10_{Metals}} = 0.45$$

$$\frac{EC10_{Organic + Metal(EC10/2)}}{EC10_{Organic}} = 0.79$$

Conclusion

Based on these preliminary results, there may be added value in assessing metals and organics together in the mixture toxicity effects assessment for regulatory framework purposes.

Outlook

- Performing the remaining three mixture toxicity tests
- Quantification of the metal and organic compounds in the exposure media of the four mixture tests
- More in-depth statistical analysis of the results
- Comparison of the experimental results with the Independent Action (IA) and Concentration Addition (CA) predictive models

