FACULTY OF BIOSCIENCE ENGINEERING

Concentration Addition vs Independent Action
Which model predicts complex metal-metal mixtures best?

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Introduction

This work is part of the comprehensive Eurometaux "Metals Environmental Exposure Data" program (MEED) as project 4.

Hypotheses:

- Independent Action (IA) is a more accurate model to predict metal-metal mixture toxicity than Concentration Addition (CA)
- the Mixture Interaction Factor (MIF) will increase with an increasing number of metals present in the mixture

Mixture Allocation Factor (MAF):

- A MAF will be introduced in REACH following the EU Chemicals Strategy for Sustainability
- The MAF should protect against unintended mixture effects of chemicals

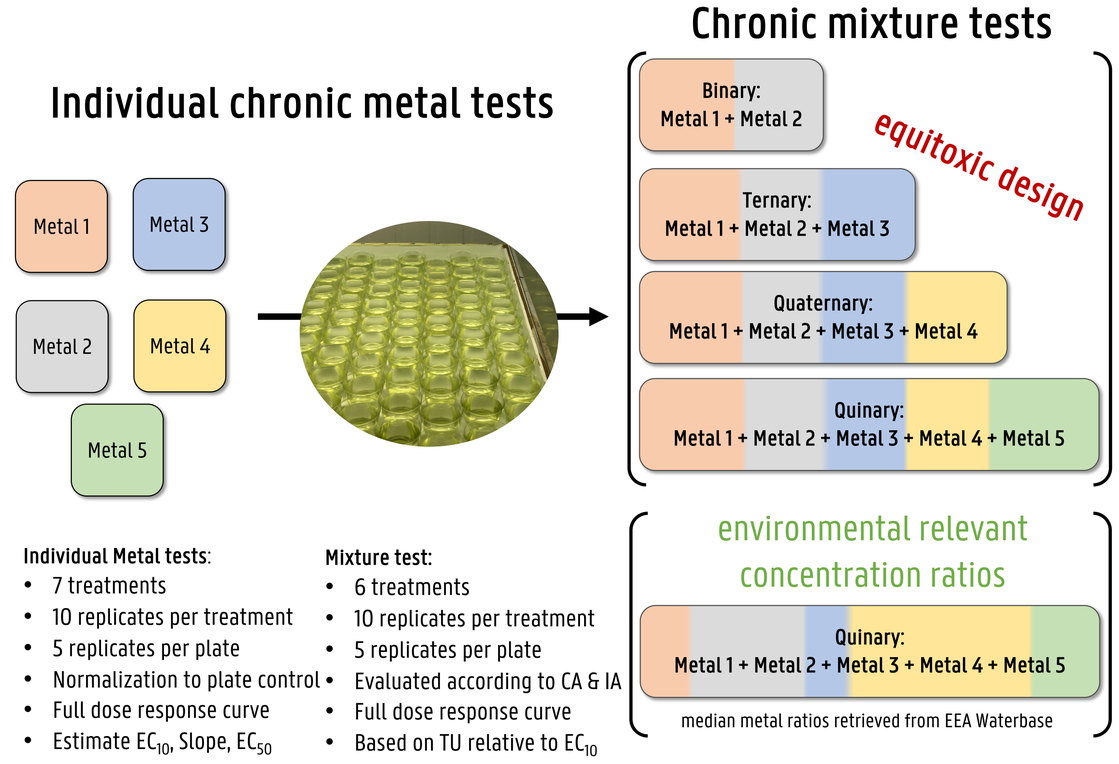
Mixture Interaction Factor (MIF):

- The MIF is a quantifier used to assess the deviations of observed toxicity from toxicity predicted with CA.
- It indicates additivity (MIF = 1), or synergistic (MIF<1) and antagonistic (MIF>1) interactions, relative to CA.

Mixture Assessment:

- CA is widely used in chemical risk assessment
- CA tends to overestimate metal mixture effects at low effect concentrations (EC_{10})[1,2]

Methods



Individual Metal tests:

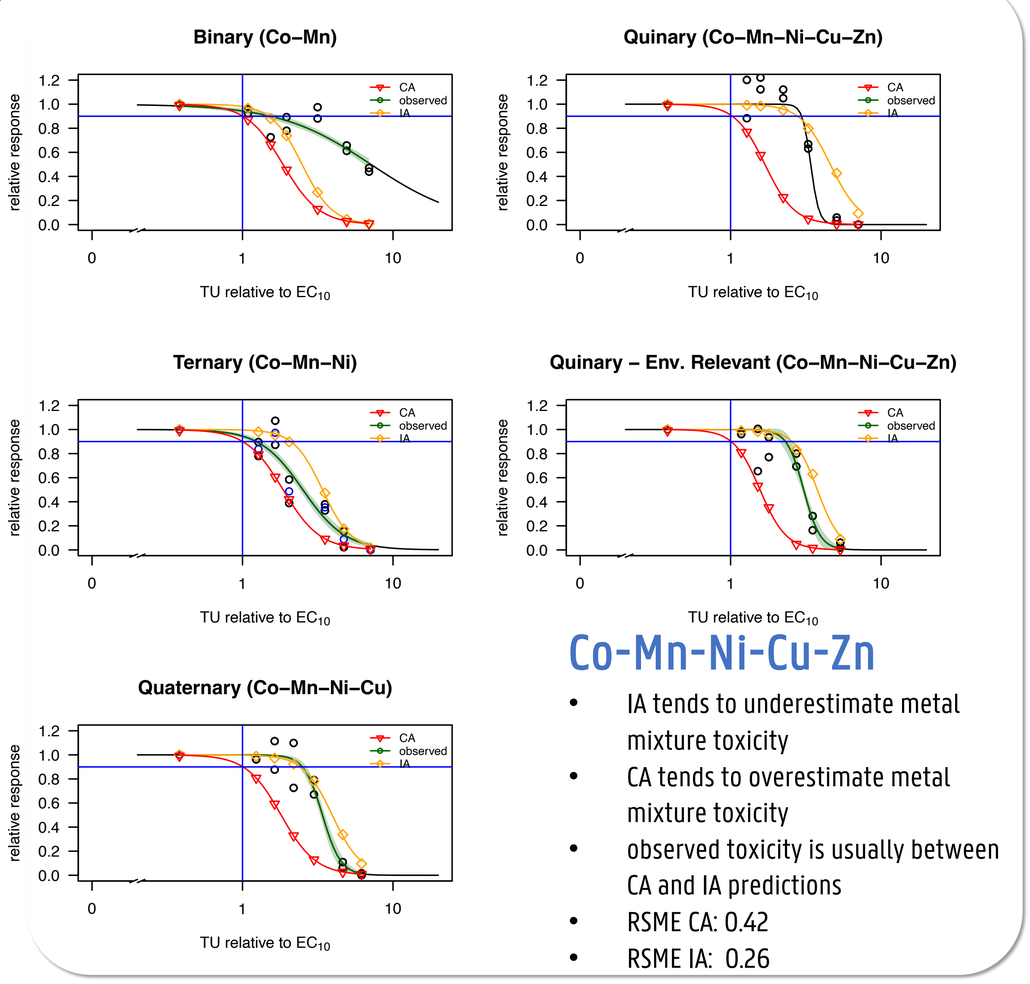
- 7 treatments
- 10 replicates per treatment
- 5 replicates per plate
- Normalization to plate control
- Full dose response curve
- Estimate EC_{10}, Slope, EC_{50}

Mixture test:

- 6 treatments
- 10 replicates per treatment
- 5 replicates per plate
- Evaluated according to CA & IA
- Full dose response curve
- Based on TU relative to EC_{10}

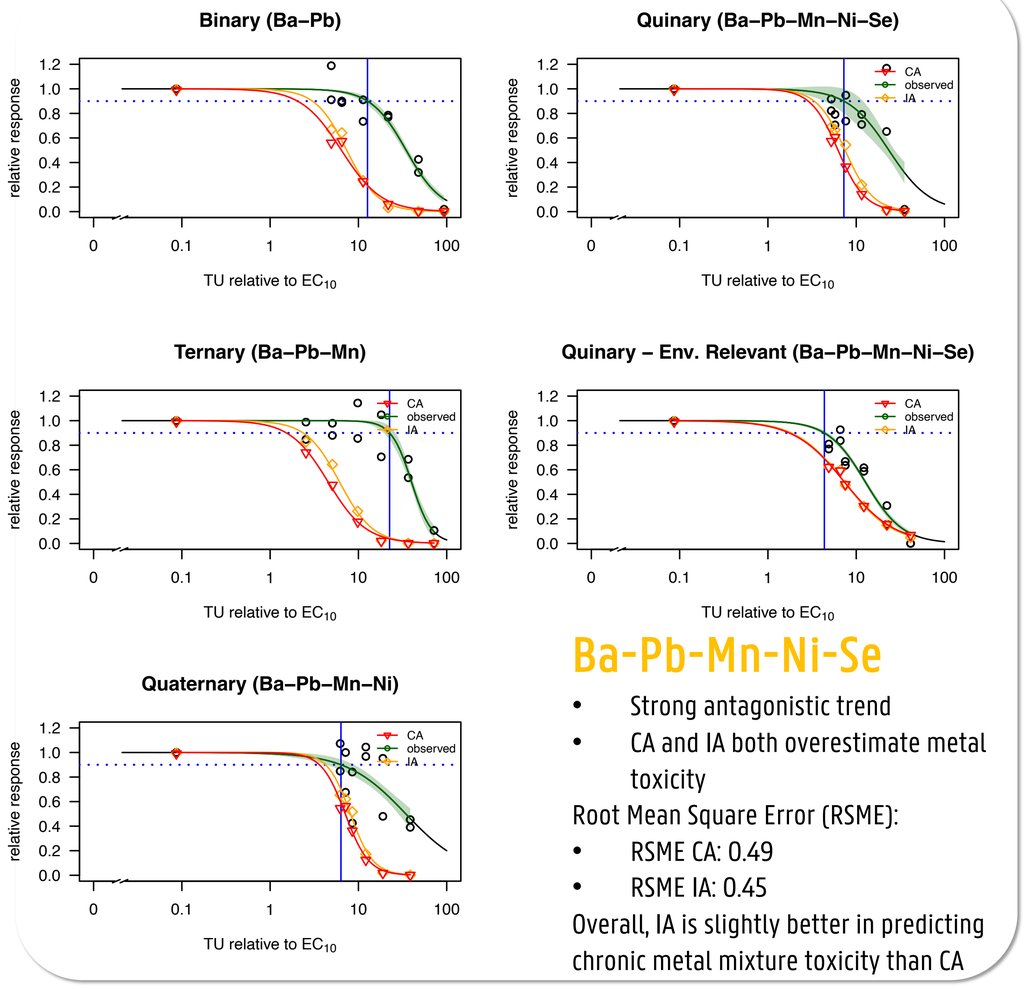
All single and mixture experiments ran simultaneously and were conducted according to OECD no. 211

Results



Co-Mn-Ni-Cu-Zn

- IA tends to underestimate metal mixture toxicity
- CA tends to overestimate metal mixture toxicity
- observed toxicity is usually between CA and IA predictions
- RSME CA: 0.42
- RSME IA: 0.26



Ba-Pb-Mn-Ni-Se

- Strong antagonistic trend
- CA and IA both overestimate metal toxicity

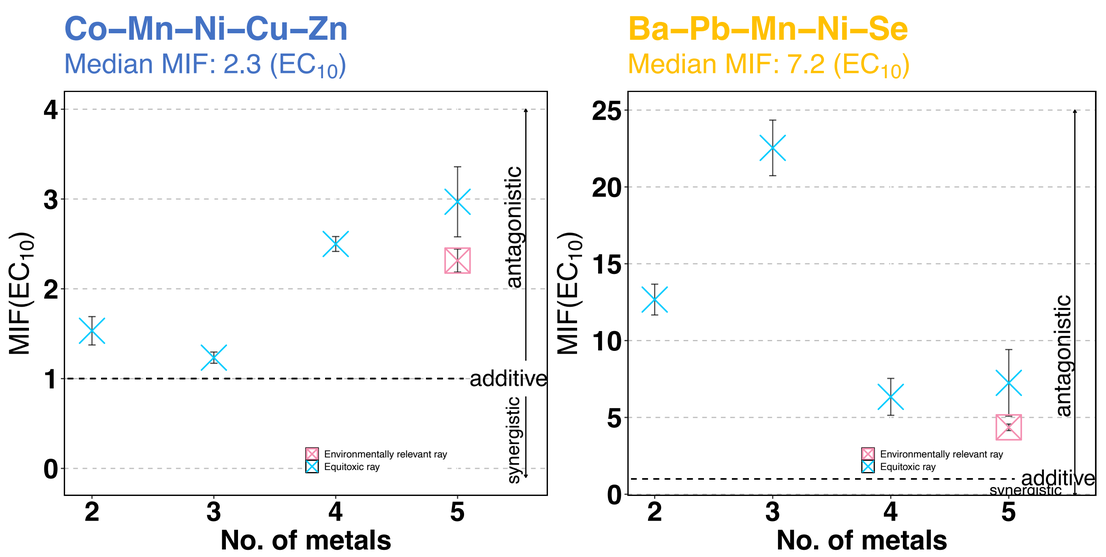
Root Mean Square Error (RSME):

- RSME CA: 0.49
- RSME IA: 0.45

Overall, IA is slightly better in predicting chronic metal mixture toxicity than CA

Conclusion

- **CA** overestimates chronic metal mixture toxicity to *D. magna* and is **protective at low** effect concentrations
  - both in equitoxic **and environmentally relevant metal ratio mixtures**
- In some specific combinations IA tends to underestimate metal-mixture toxicity but **is in general a better predictor than CA**
  - Next step: evaluate whether this indicates a significant synergism[3]
- **Ba-Pb-Mn-Ni-Se** showed strong **antagonism**
- No synergistic metal-combinations found relative to CA
- MIF does not increase with mixture complexity: Median MIF at EC_{10} is 2.3 or higher
  → CA overestimates metal mixture toxicity by 2.3 fold or more



- MIF does not increase with increasing number of metals
- Lower MIF at environmental relevant metal ratios

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2. Nys C, et al. "A framework for ecological risk assessment of metal mixtures in aquatic systems." *Environmental toxicology and chemistry*, 37.4 (2018): 623-642
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