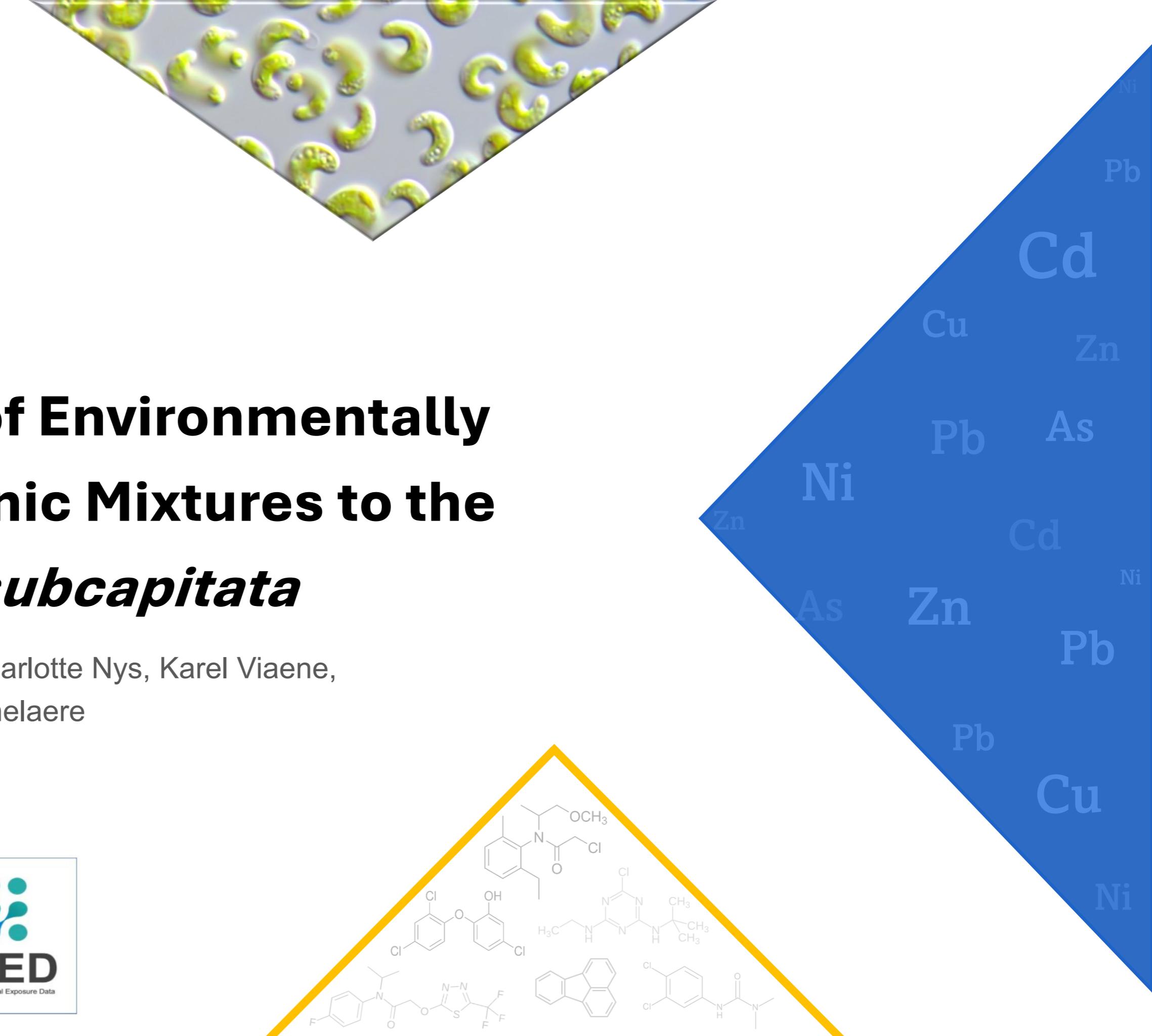


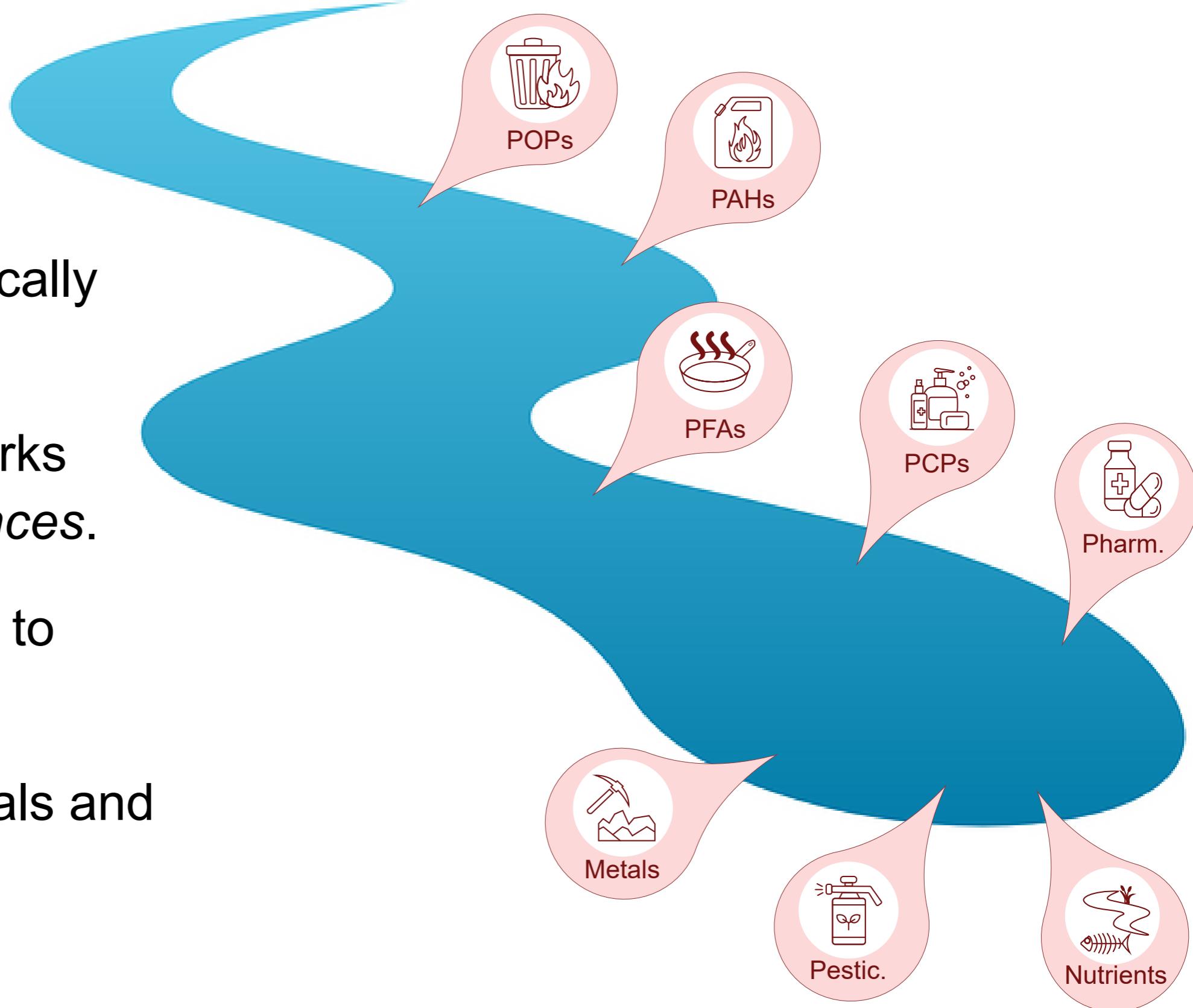
# Aquatic Ecotoxicity of Environmentally Realistic Metal-Organic Mixtures to the *Algae Raphidocelis subcapitata*

Laura De Donno, Franz Marius Schmitt, Charlotte Nys, Karel Viaene,  
Hugo Waeterschoot and Karel De Schamphelaere



# BACKGROUND

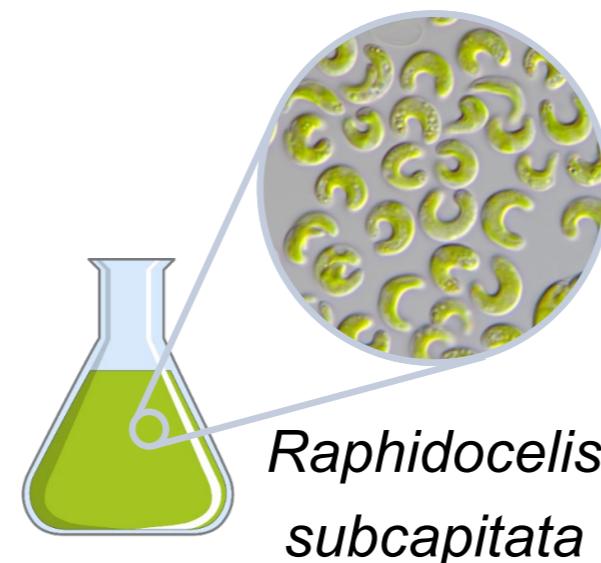
- Chemicals in the environment typically occur as *complex mixtures*.
- Existing risk assessment frameworks mostly focus on *individual substances*.
- Current lack of consensus on how to best assess *mixture risk*.
- Lack of studies of mixtures of metals and organics.



# RESEARCH QUESTIONS

1. Independent action (IA) vs Concentration Addition (CA) : which mixture model is most accurate for predicting mixture toxicity?
2. Do organics <EC10<sub>Org</sub> affect the toxicity of metals?
3. Do metals <EC10<sub>Me</sub> affect the toxicity of organics?

In regulatory frameworks, should metals and organics be assessed separately or together?



- 72h algae growth inhibition test
- OECD 201 guidelines



# TEST DESIGN

## Selection of the metal-organic combinations



*Which **organic substances** occur most frequently in hazardous and **environmentally relevant binary mixtures** with metals in European freshwater?*

**Waterbase:**  
**Data filtering**

**Risk Quotient** based  
PNEC prioritization

**Toxic unit** based  
toxicity prioritization



# TEST DESIGN

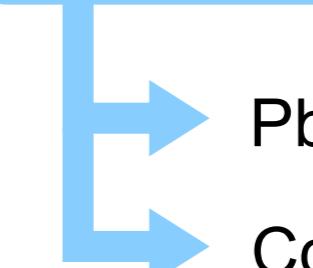
Diuron



Metolachlor



Triclosan



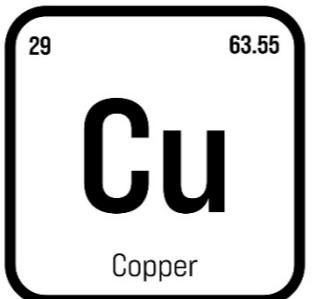
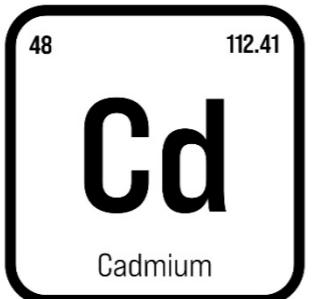
Terbutylazine



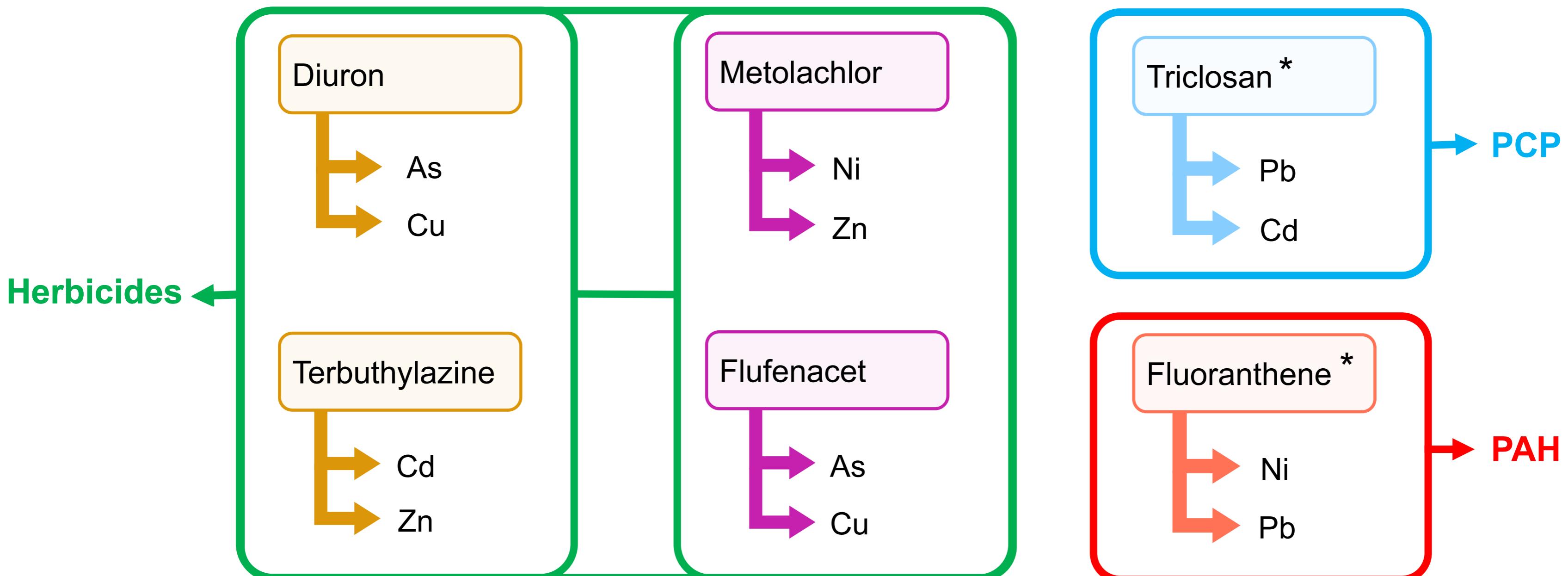
Flufenacet



Fluoranthene



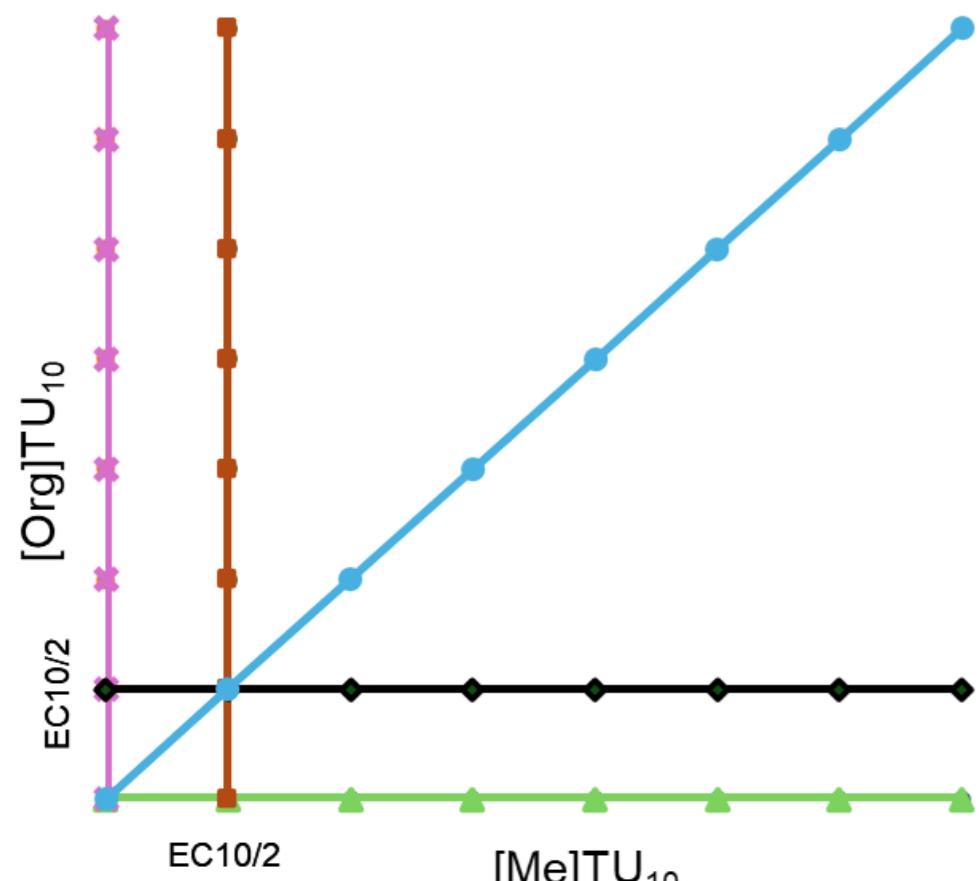
# TEST DESIGN



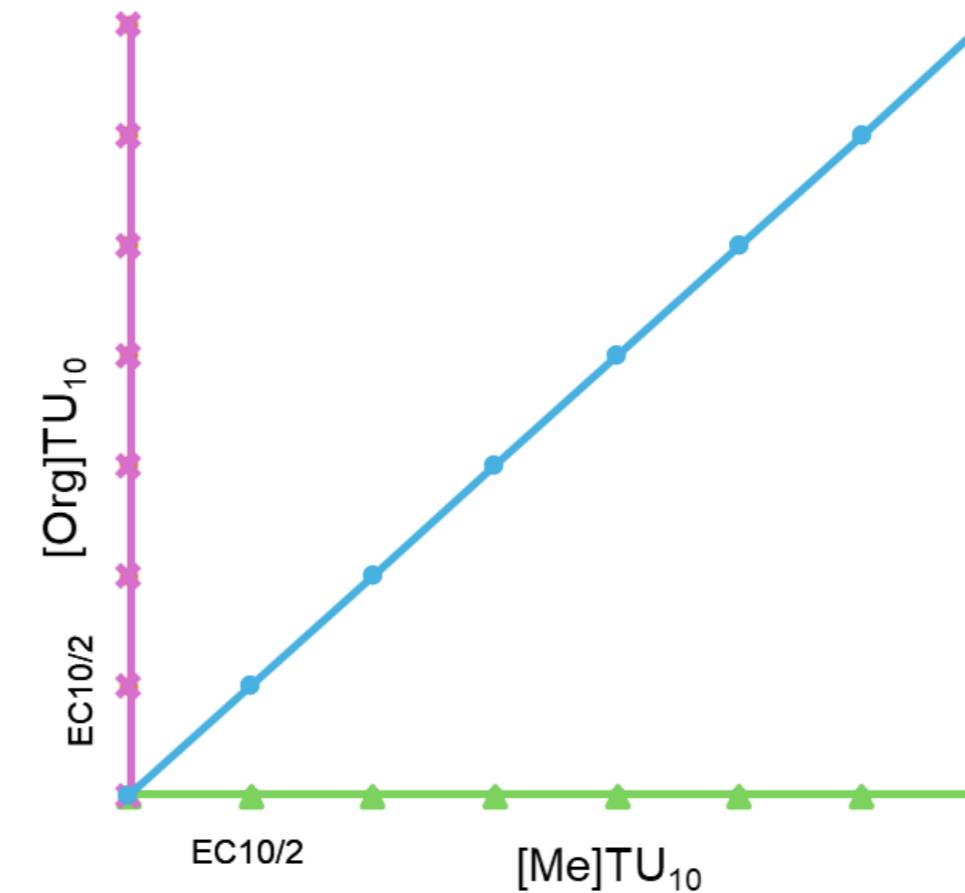
*MoA: Inhibition of  
Photosynthesis at PSII*

*MoA: Inhibition of Very  
Long-Chain Fatty Acid  
Synthesis*

# TEST DESIGN

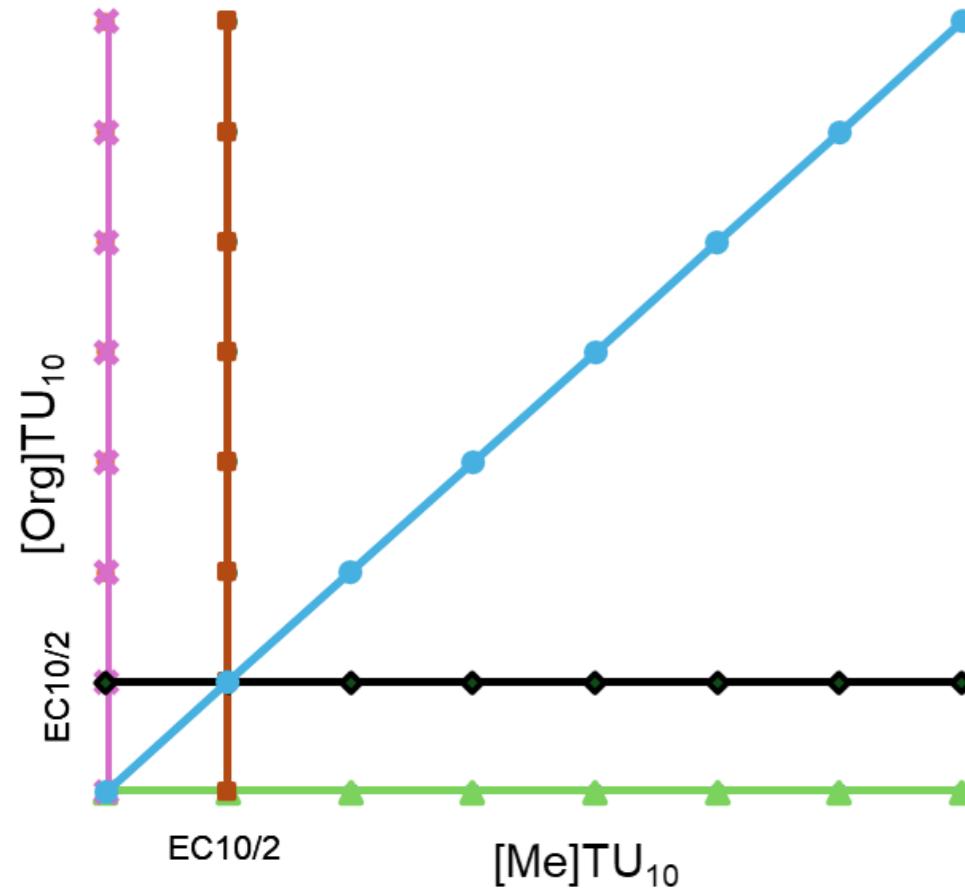


3 test designs for each metal-organic combination

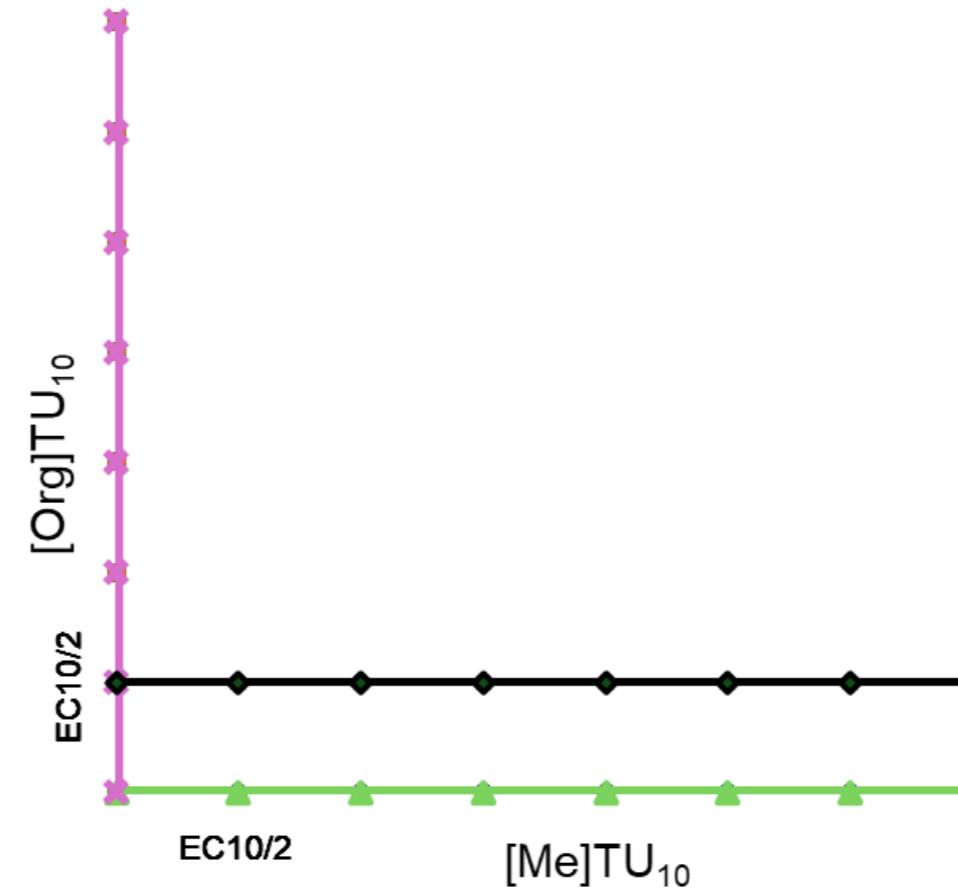


1. CA vs IA: Which mixture model is most accurate for predicting the mixture toxicity?

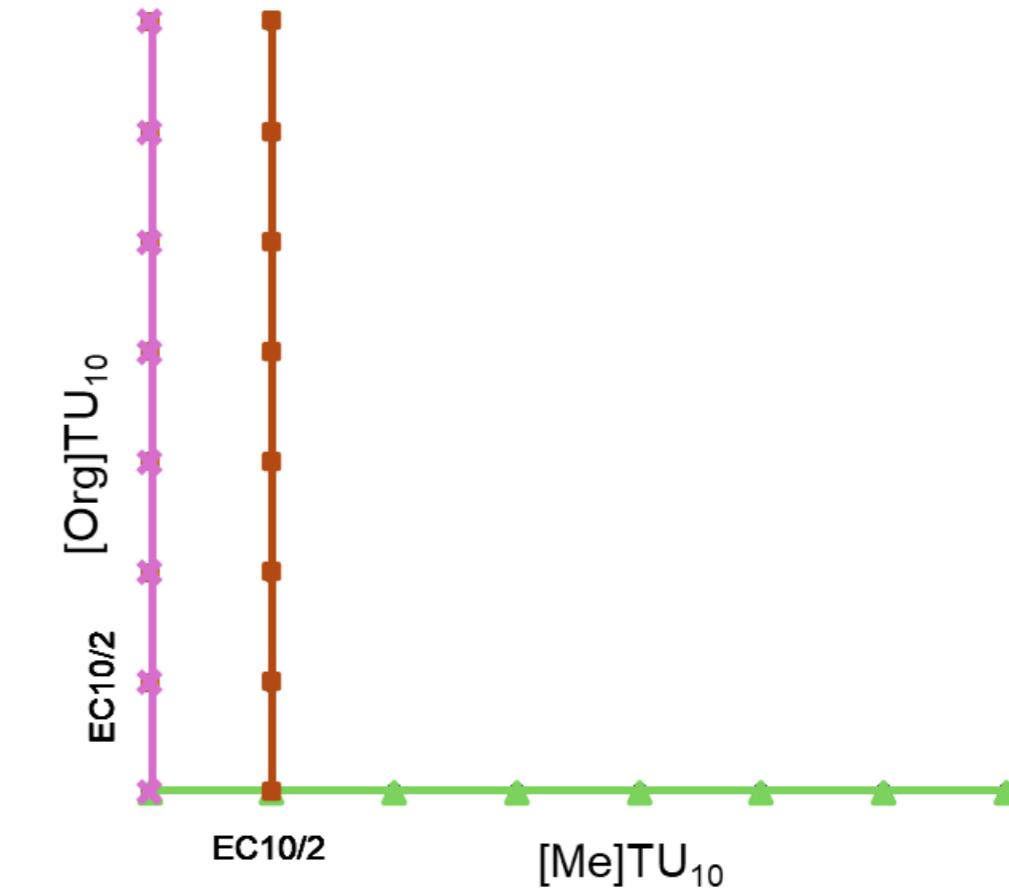
# TEST DESIGN



3 test designs for each metal-organic combination



2. Do organics  $< EC10_{Org}$  affect the toxicity of metals?



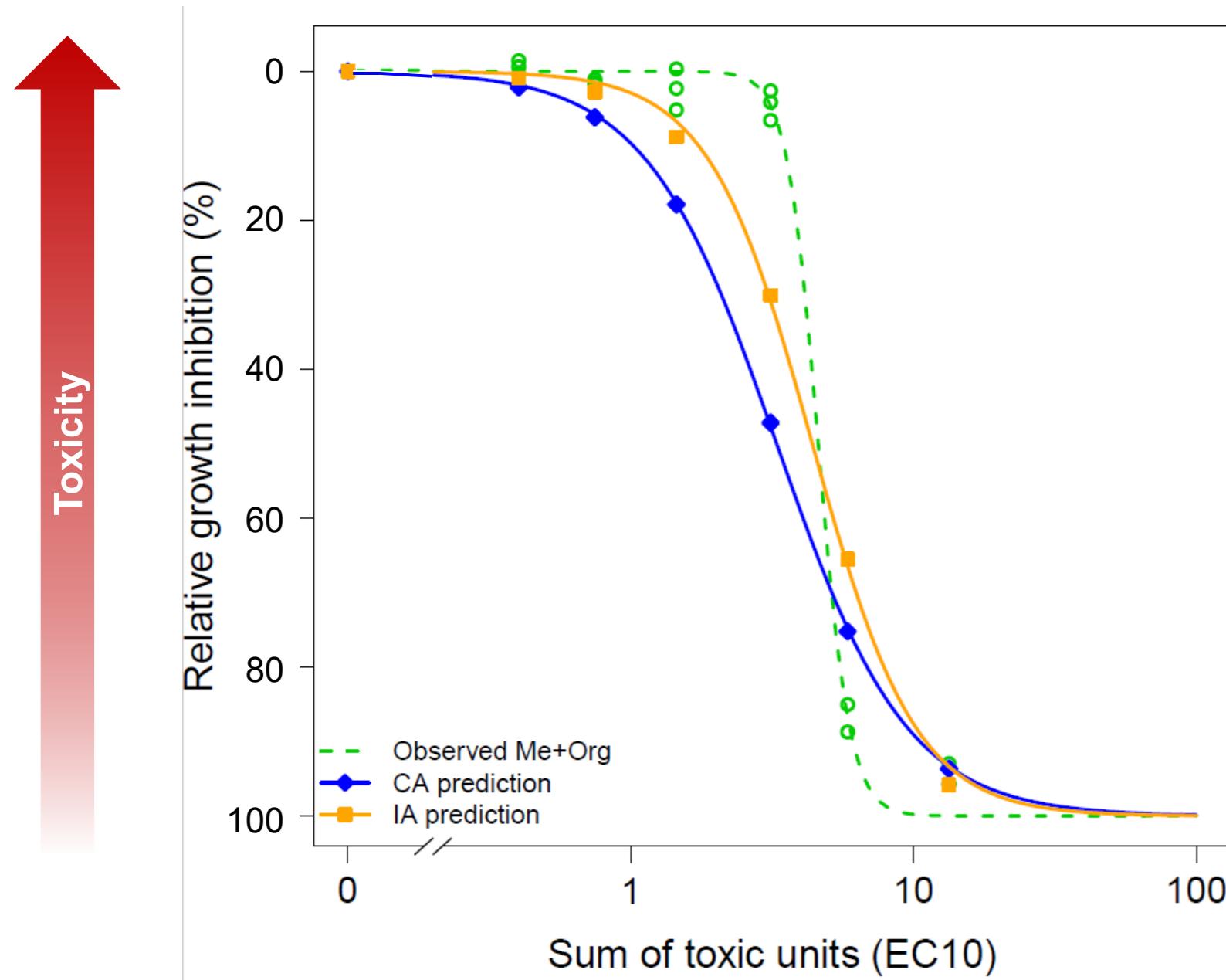
3. Do metals  $< EC10_{Me}$  affect the toxicity of organics?

Keeping **constant one variable** (i.e.,  $EC_{10/2}_{Org}$  or  $EC_{10/2}_{Me}$ ) and **changing the other** (i.e., the toxicity of the metal or organic)

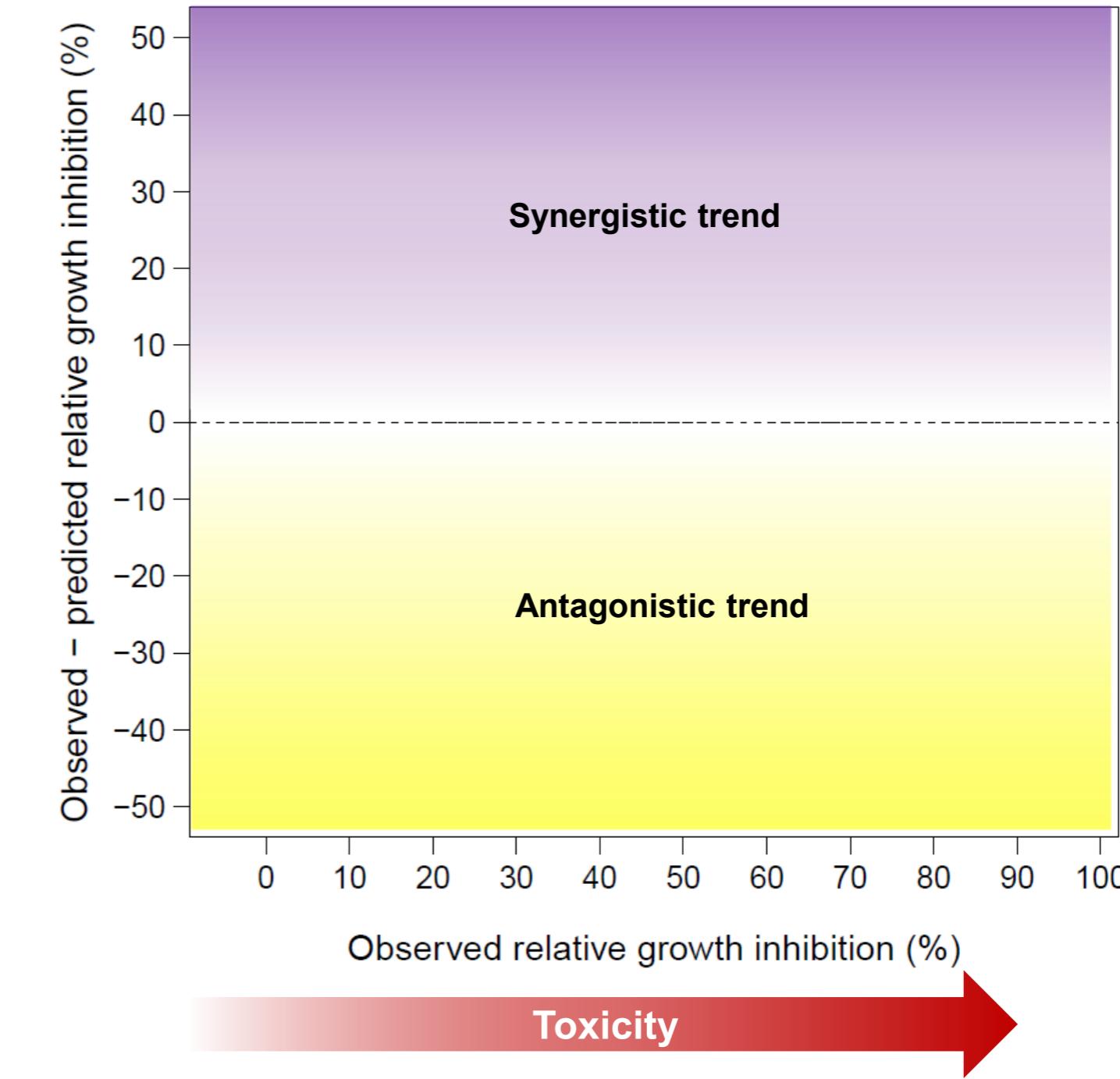
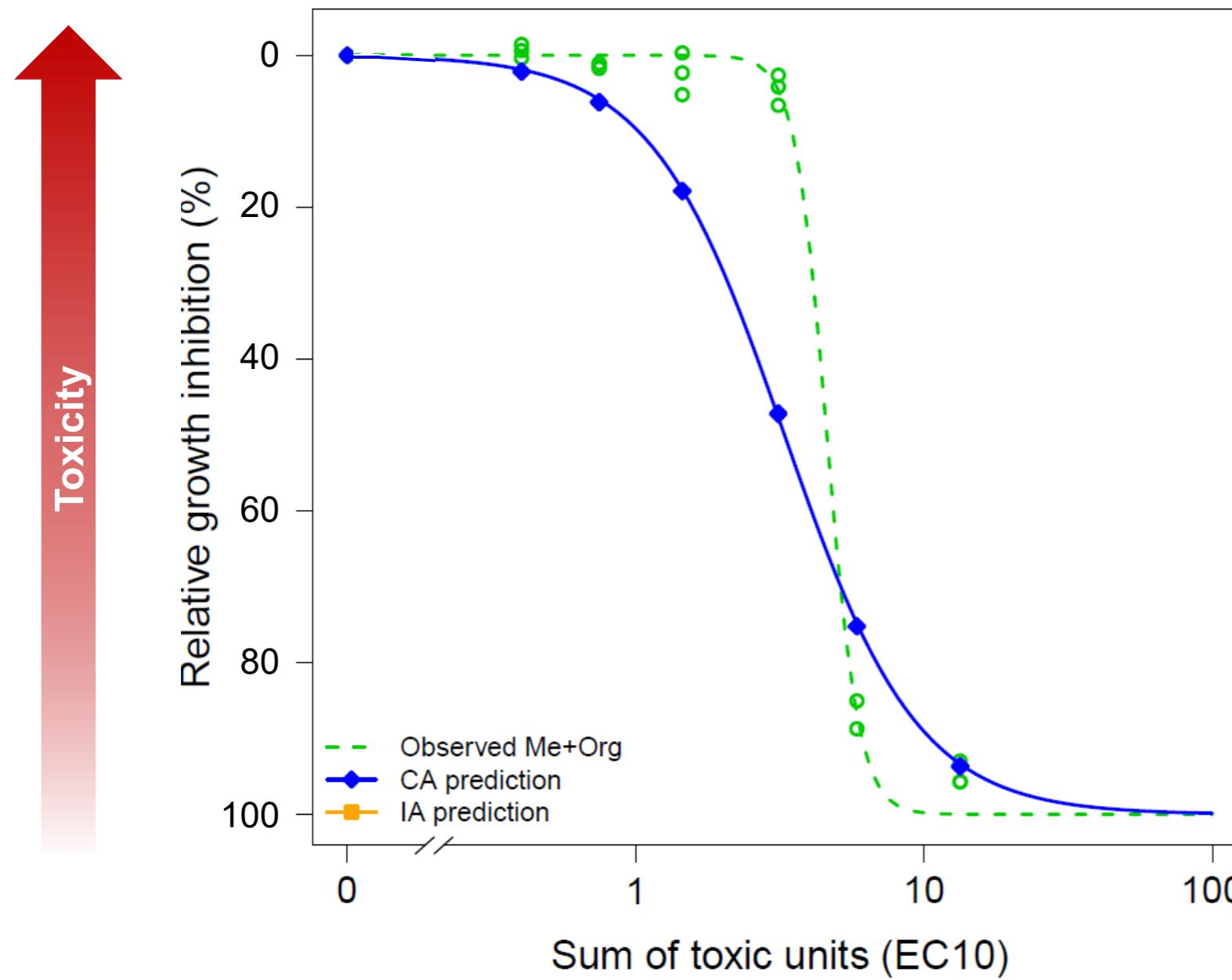
# RESULTS - CA vs IA

1. Which mixture model is most accurate for predicting the mixture toxicity?

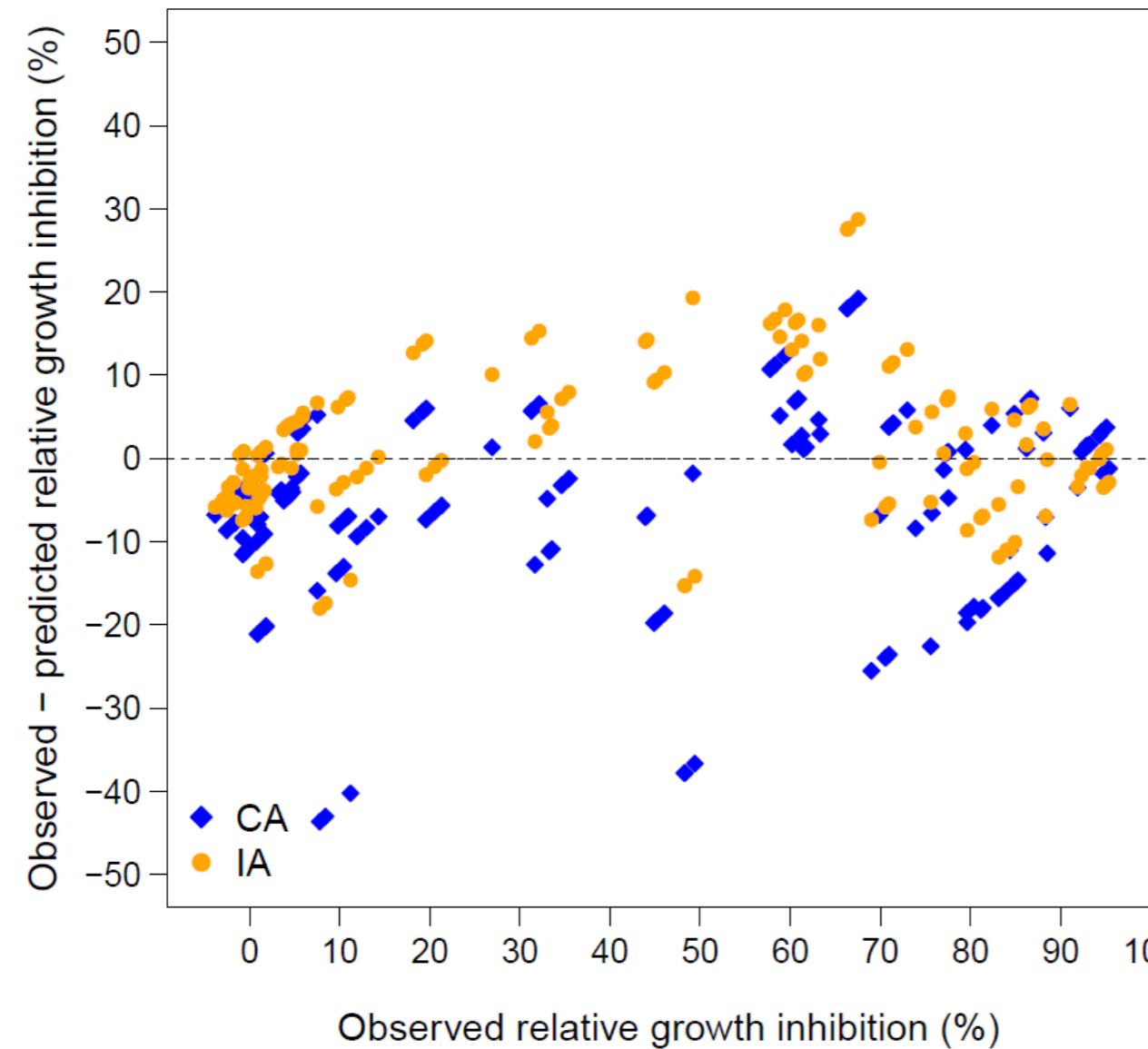
# RESULTS - CA vs IA



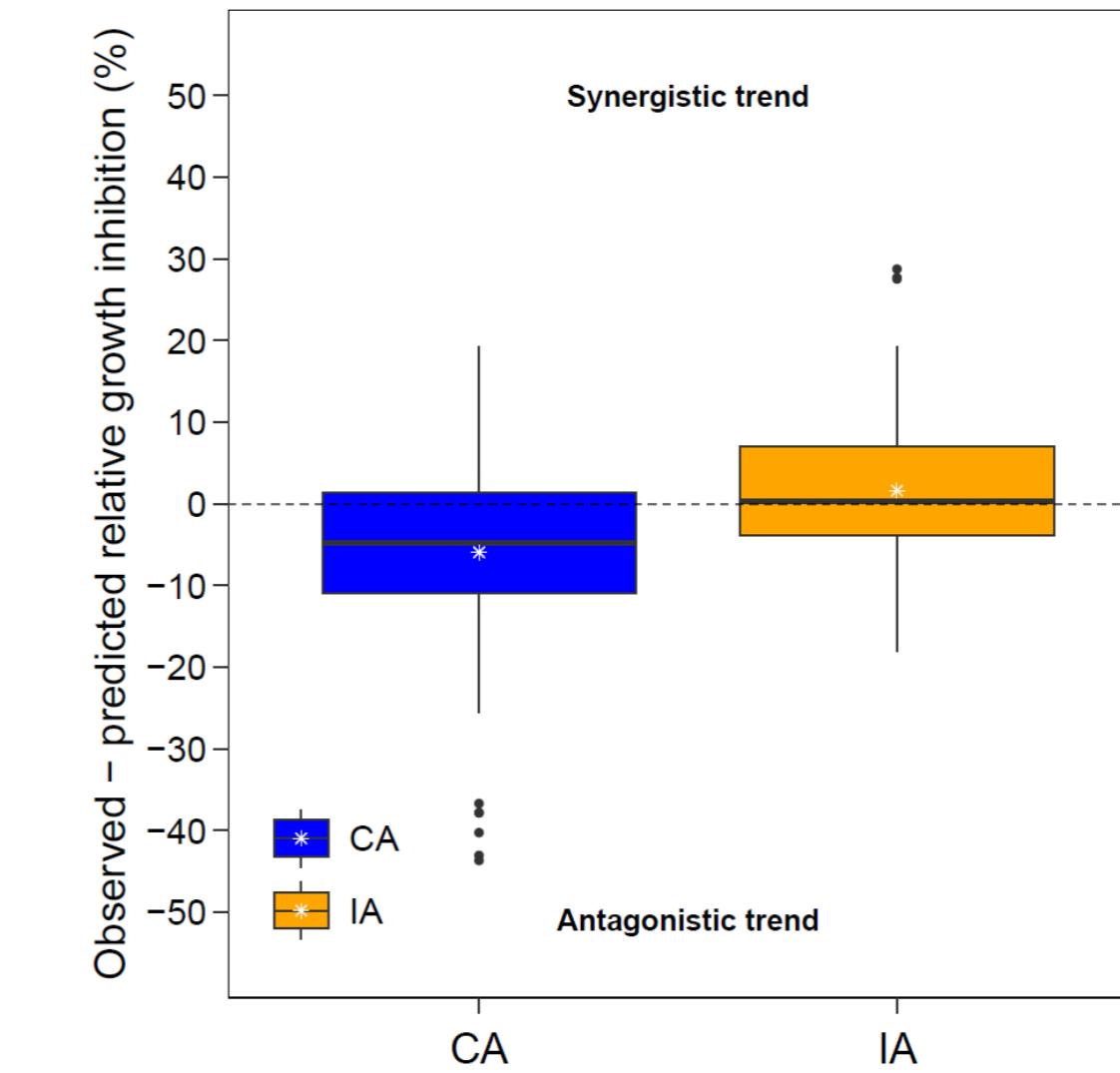
# RESULTS - CA vs IA



# RESULTS - CA vs IA



\*based on measured values



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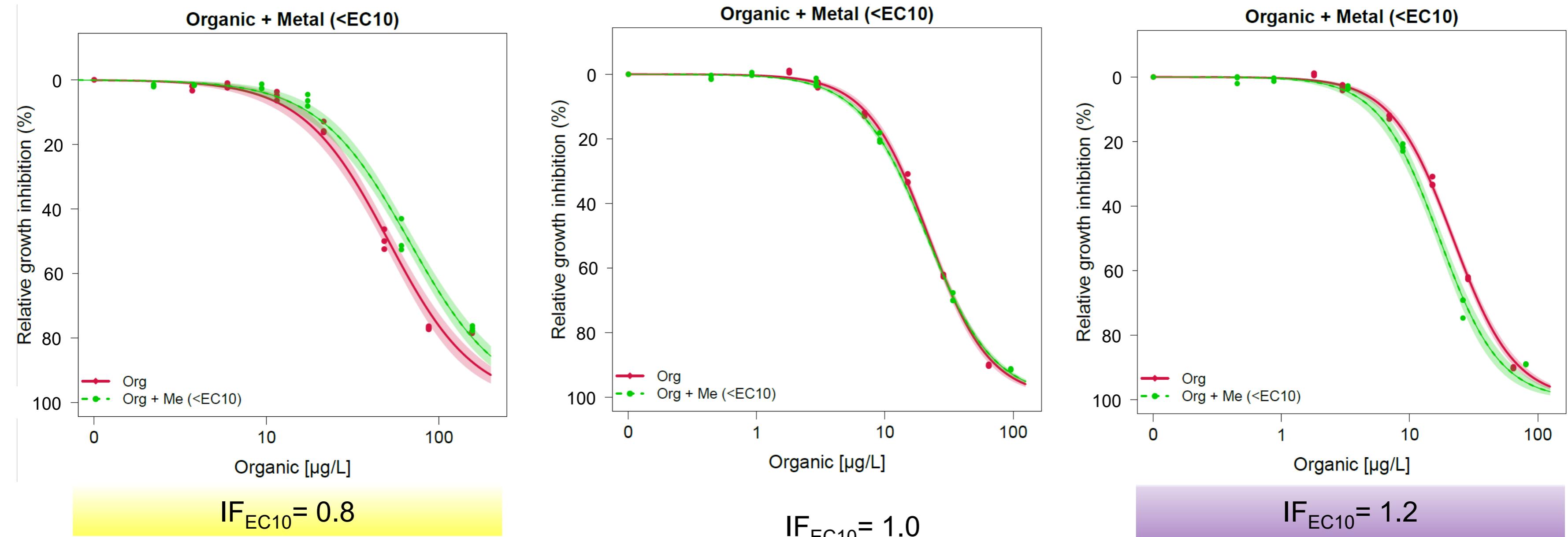
**On average:**

- IA was a *more accurate predictor than CA.*
- CA showed a *trend toward antagonism.*

# RESULTS

2. Do organics <EC10<sub>Org</sub> affect the toxicity of metals?
3. Do metals <EC10<sub>Me</sub> affect the toxicity of organics?

# METAL-ORGANIC INTERACTIONS

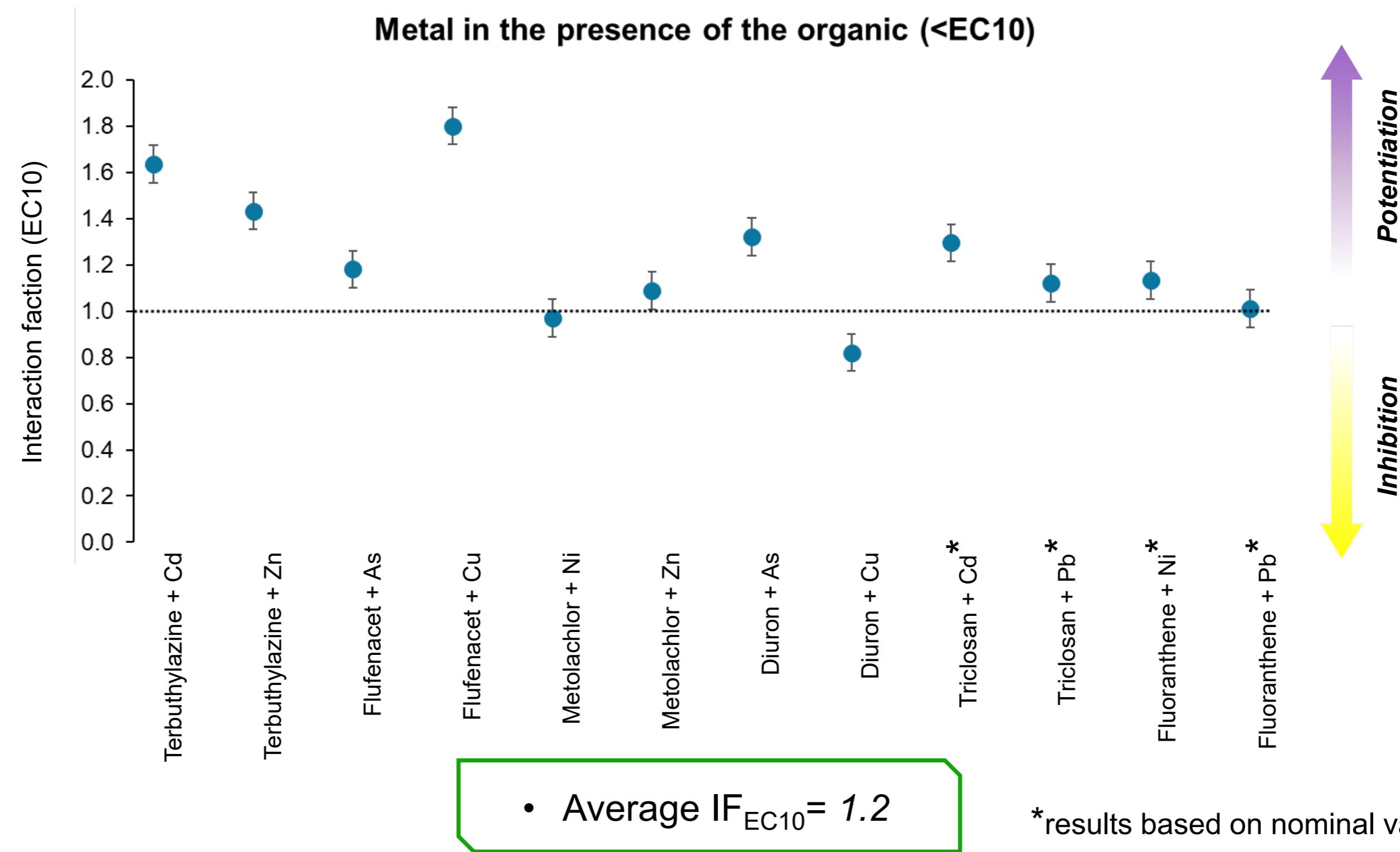


$$Interaction\ factor_{EC10} = \frac{EC10_{Alone}}{EC10_{Mixture}}$$

$IF_{EC10} < 1.0 \rightarrow$  Toxicity inhibition  
 $IF_{EC10} = 1.0 \rightarrow$  No interaction  
 $IF_{EC10} > 1.0 \rightarrow$  Toxicity potentiation

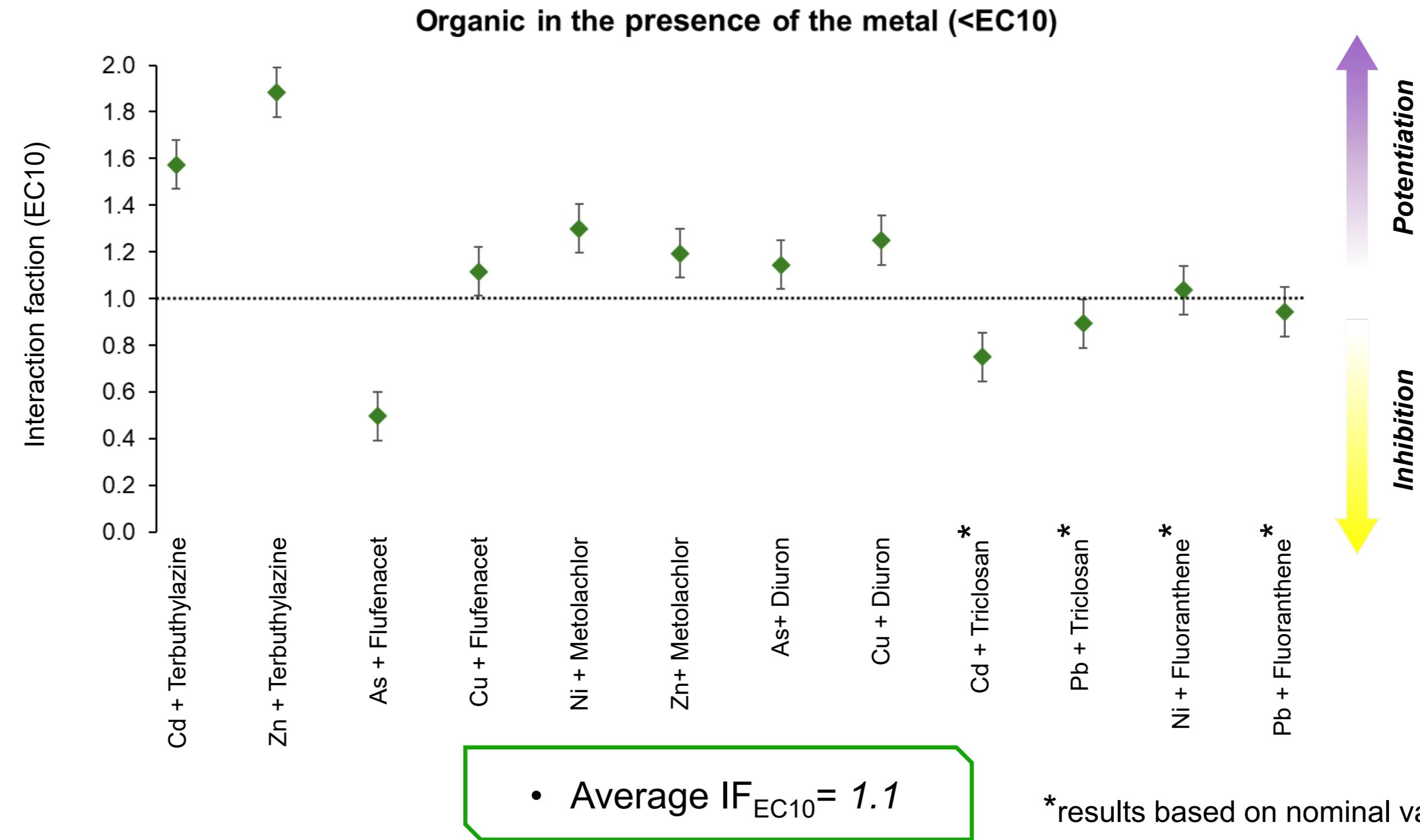
# METAL-ORGANIC INTERACTIONS

$$IF_{EC10} = \frac{EC10_{Metal}}{EC10_{Metal+Organic}(<EC10)}$$



# METAL-ORGANIC INTERACTIONS

$$IF_{EC10} = \frac{EC10_{Organic}}{EC10_{Organic+Metal}(<EC10)}$$



# CONCLUSIONS

On  
average:  
**average:**



The two models showed rather similar results.

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No clear trend toward synergistic or antagonistic interactions.

Trend toward antagonistic interactions relative to both models.

**Future  
directions:**

At low mixture effect (i.e., 10%) CA tends to be protective for mixture toxicity.  
The presence of low concentrations (<EC10) of a compound can have an impact on the toxicity of another one.

No continuous increase of MIF with the number of metals in the mixture.  
Tests with more complex and field-realistic metal-organic mixtures.

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