Metal Mixture Effects in the Aquatic Environment: Evaluating the Potential for the Derivation of a Mixture Allocation Factor (MAF) Based on Experimental Data

Charlotte Nys[1], Laura De Donno[2], Marius Schmitt[2], Karel Viaene[1], Koen Oorts[1], Marnix Vangheluwe[1], Hugo Waeterschoot[3], Violaine Verougstraete[3], Karel De Schamphelaere[2]

[1]ARCHE Consulting, Belgium; [2]Ghent University, GhEnToxLab, Belgium; [3] Eurometaux, Belgium
Contact: charlotte.nys@arche-consulting.be

Introduction & Methodology

The **Mixture Allocation Factor (MAF**) has been proposed by the European Commission (EC) to integrate unintended combined exposure into REACH[a] which reduces the safety limit of single substances. The EC plans to implement the MAF concept later also in other EU environmental legislation. To inform the upcoming mixture correction, a research project was set up as part of the Metals Environment Exposure Data (MEED) program focusing on **chronic metal mixture toxicity in the aquatic environment.** The research project aims to **quantify conservatism built into default mixture calculations** (if any), **and to deliver a science-based MAF-value for metals.**

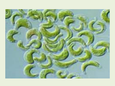
Set-up of the MEED-metal mixture research project

Literature review
- Chronic metal mixture toxicity
- Existing meta-analysis[b-c] + literature search

Data-gap analysis
Main data-gaps:
- Anionic metals
- Complex mixtures (n>3)

Development of dedicated test program
- Fill data-gaps
- Focus on environmentally and regulatory relevant mixtures (Waterbase[d])
- Retest strong synergism (Cd, Ni, Cr[c])

Testing phase - Chronic mixture toxicity



Ba, Cd, Cr, Mn, Zn
As, Cd, Cu, Pb, Ni
Ag, As, Cu, Pb, Zn
Cd, Cr, Ni

Co, Cu, Mn, Ni, Zn
Ba, Mn, Ni, Pb, Se
*(Ag, Cd, Cu, Ni, Zn)**

Tested in binary to quinary mixtures
*No analytics yet – not included here

Quantitative reappraisal
How can we use the data gathered to derive environmentally realistic MAF-values for metals?

Results

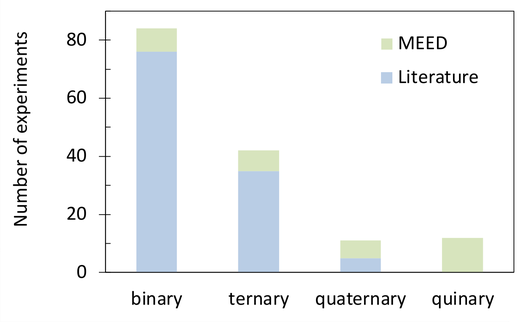
1. Overview of the quantitative reappraisal dataset

Summary of data in the quantitative reappraisal dataset describing chronic metal mixture toxicity to aquatic organisms

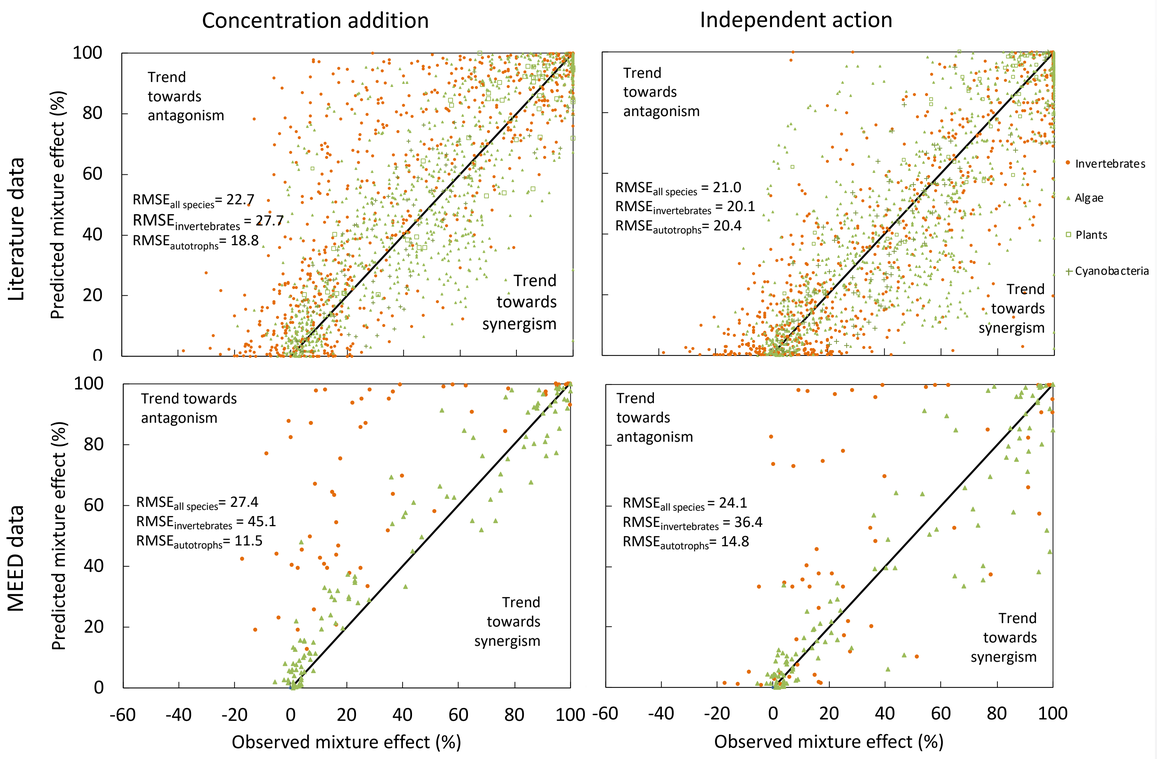
	(34 studies)	MEED	Total
# of experiments	116	28	144
# of treatments	1865	176	2041
# of metals	15	12	18*
# of species	24	2	24

* Ag, Al, As, Ba, Cd, Ce, Co, Cu, Cr, Fe, Gd, Lu, Mn, Ni, Pb, Se, V & Zn

Overview of the complexity of mixtures in the quantitative reappraisal database



2. Which model predicts metal mixture toxicity most accurately?



The data obtained within the MEED-project is largely in line with the data from literature: **chronic metal mixture toxicity is – on average - slightly better predicted with independent action** (lowest Root Mean Square Error; RMSE, when all species are considered).
- For invertebrates, independent action is the more accurate model compared to concentration addition.
- For autotrophs, there is less difference between mixture reference models.

Concentration addition is – on average - a more conservative model than independent action.

3. How accurate is concentration addition (CA) for predicting mixture effects at low effect concentrations?

Mixture Interaction Factor (MIF)[b]
*= degree of conservatism that **concentration addition** provides relative to observed mixture effects at low effect levels (i.e. 10% mixture effect)*

$$MIF = EC10_{\sum TU_{EC10}}$$

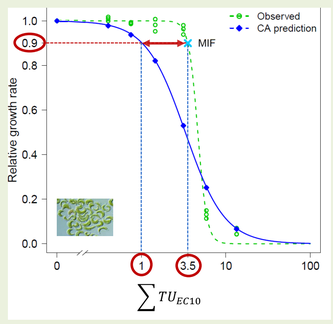
with $$\sum TU_{EC10} = \sum \frac{c_{Me_i}}{EC10_{Me_i}}$$

MIF << 1 = synergism
MIF >> 1 = antagonism
MIF ∽ 1 = additive / non-interactive

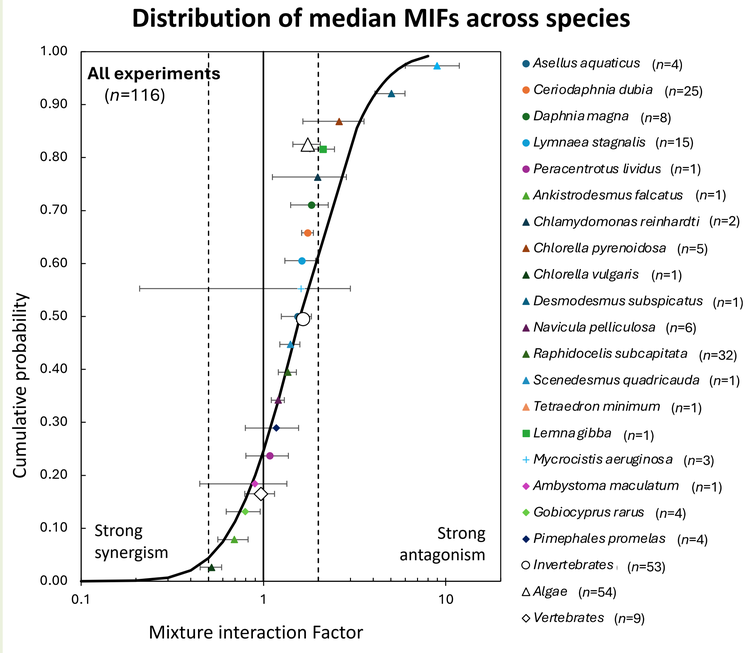
MIF is calculated for each mixture experiment separately: e.g., As-Cu-Ni-Pb mixture of MEED-program with *Raphidocelis subcapitata*

MIF=3.5

CA overestimates mixture toxicity in this example by 3.5-fold



Distribution of median MIFs across species



- MIFs range between 0.5 (*Chlorella vulgaris)* and 8.9 (*Tetraedron minimum)*
- MIFs obtained within the MEED-program are in line with those of literature (MIF: 1.1-3.6)
- No strong synergisms (MIF<0.5)
- Median MIF (=1.3) is independent of data quality or environmental and regulatory relevance of tested concentrations

Median MIF=1.3
Concentration addition overestimates metal mixture toxicity at low effect levels on average by 1.3-fold

4. Next steps in quantifying the conservatism of MAF-approaches

MAF for metals

Most MAF-calculations include mixtures of organic substances, but metals are rarely considered. What is the **MAF for metals** based on European monitoring databases[d]?

$MAF_{ceiling}$ vs. MAF_{factor}[e]

Metal-organic mixture interactions

Dedicated testing program

Potential refinements of a metal MAF

Mixture Interaction Factor (MIF): expresses the conservatism at the **species-level** associated with the concentration addition-assumption that substances have the same mode of action.

Margin of Safety (MoS): expresses the conservatism resulting from applying concentration addition at the PNEC or HC5-level rather than applying concentration addition at the species-level (EC10).

For mixtures with 5 metals the median MoS is equal to 1.4[b].

MoS for more complex mixtures?

$$MAF_{metals,refined} = \frac{MAF_{metals}}{(MIF \times MoS)}$$

Conclusions

- Chronic metal mixture effects can be predicted with the standard mixture reference models, independent action and concentration addition.
- Concentration addition results in more conservative predictions compared to independent action.
- Across the dataset a median MIF of 1.3 was derived, indicating that on average concentration addition somewhat overestimates mixture toxicity at regulatory relevant low effect levels. The MIF may increase the scientific accuracy of MAF-values for metals.
- Future research efforts within this MEED-project will focus on quantifying the conservatism provided by a regulatory MAF-setting for metals and developing tools for scientifically based refinements as an alternative of the implementation of a default MAF

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References: [a] European Commission. 2020. https://environment.ec.europa.eu/strategy/chemicals-strategy_en; [b] Nys et al. 2018. *Environ Toxicol Chem* 37: 623-642; [c] Martin et al. 2021. *Environ Int* 146: 106206; [d]https://sdi.eea.europa.eu/data/bdeadea2-cfaf-4724-b002-816d71c7e361; [e] Backhaus. 2024. *Curr Opin Toxicol* 100460.

Liefkensstraat 35D ▼ B-9032 Gent, Belgium ▼ T. +32 9 216 70 38 ▼ info@arche-consulting.be ▼ www.arche-consulting.be