Beyond default Mixture Allocation Factor (MAF)-settings: how can we increase the applicability and scientific-relevance of mixture risk assessments for metal(loid)s?

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Introduction & Methodology

The European Commission calls in the Chemicals Strategy for Sustainability[a] to cover the impact of combined exposure into chemical risk assessments under REACH with a **Mixture Allocation Factor (MAF). For environmental compartment endpoints a default MAF of 5 has been proposed**.

Analysis shows that the implementation of a MAF will have a large impact on the environmental risk assessment of inorganics (including metals and metalloids) in soils and waters and may set safety limits below natural background levels.

However, to our knowledge, a specific MAF for metal(loid)s has not yet been derived, as most existing studies do not consider metal(loid)s.

As part of the Metals Environmental Exposure Data (MEED)-research programme, we evaluated:

- **Relevance of the proposed default MAF for metal(loid)s**.
- **Conservatisms** build in the default MAF for metal(loid)s.

MAF for metal(loid)s

$MAF_{ceiling}$ [b]

- Concentration addition (CA)-based algorithm, solved until:

$$HI_{MAF(ceiling)} = \sum RQ_{MAF(ceiling),i} = 1$$

$$\text{where } RQ_{MAF(ceiling),i} = \begin{cases} \text{if } RQ_i > \dfrac{1}{MAF_{ceiling}}, RQ_i = \dfrac{1}{MAF_{ceiling}} \\ \text{else } RQ_i \leq \dfrac{1}{MAF_{ceiling}}, RQ_i \text{ remains unchanged} \end{cases}$$

$$\text{with } RQ_i = \frac{c_i}{HC5_i}$$

$HI_{MAF(ceiling)}$=Hazard Index after MAF
$RQ_{MAF(ceiling)}$= Risk Quotient after MAF
RQ_i= Risk Quotient for metal *i*

c= dissolved concentration for metal *i*
HC5=5% hazardous concentration for metal *i*

- Focus on inorganic-priority contributing substances (I-PCS[c]) with species sensitivity distribution approach in PNEC derivation: HC5 and toxicity data extracted from REACH dossiers
- Applied on Flemish freshwater monitoring database[d] – years 2010-2024 – Cd, Co, Cu, Ni, Pb & Zn bioavailability corrected.

I-PCS = inorganics most contributing to mixture pressure
Ag, As, Ba, Cd, Co, Cr, Cu, Hg, La, Mn, Ni, Pb, Se, V & Zn

Quantifying conservatism in MAF

I) MAF algorithm is CA-based, assumes that **all substances have the same mode-of-action and all substances contribute**, conservatism quantified by

Mixture Interaction Factor (MIF)[e]

$$MIF = EC10_{\sum TU_{EC10}} \qquad \text{with} \sum TU_{EC10} = \sum \frac{x_{Me_i}}{EC10_{Me_i}}$$

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

→ Calculated from chronic toxicity data on metal(loid) mixtures

II) MAF assumes that **for all substances the same species drives the HC5,** conservatism quantified by

Margin of Safety (MoS)[e] Hazard Index at which 5% of the species is predicted to be affected when CA is applied at the species-level (EC10).

→ Calculated for the Flemish monitoring database

Driving species for HC5 (e.g., Pb-Zn)

Pb

Lymnaea stagnalis

Zn

Raphidocellis subcapitata

MAF derivation

hybrid species between *L. stagnalis* & *R. subcapitata* (©DALL-E)

Results & Discussion

MAF for metal(loid)s

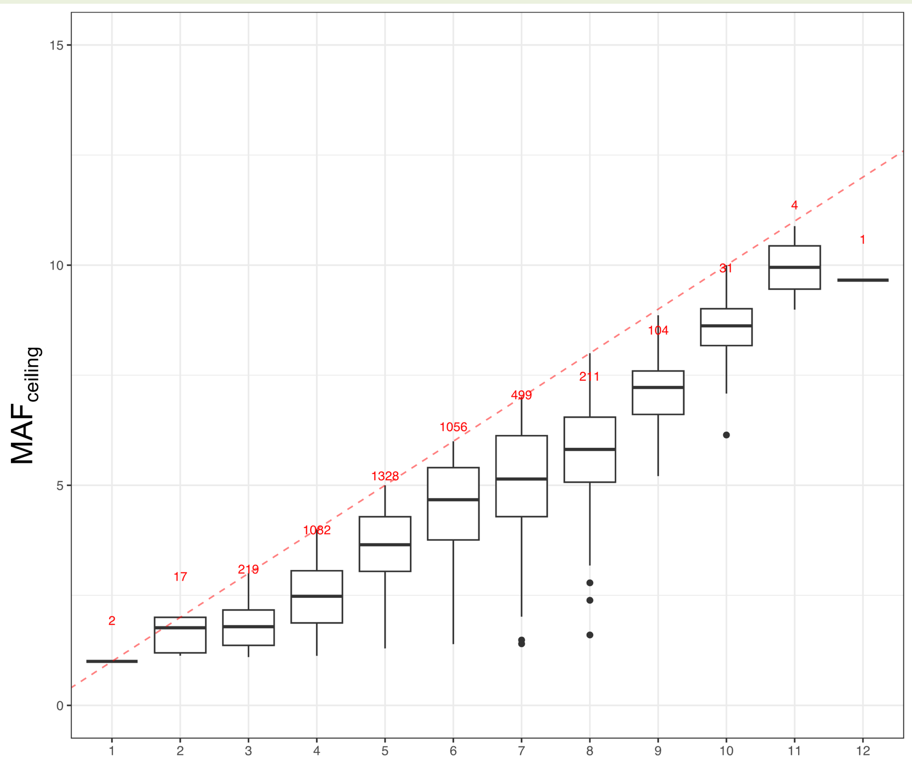

Figure 1. $MAF_{ceiling}$ as a function of number of metals measured in a sample. Only those samples for which the original Hazard Index>1 are shown. Numbers indicate the number of samples.

- For the samples where a mixture risk is predicted, the $MAF_{ceiling}$ increases with increasing number of metal(loids) measured in the mixture, and the 90th percentile of $MAF_{ceiling}$ equal to exactly 6.
- However, 41% of the samples in the Flemish monitoring database[d] show no mixture risks, i.e., for these samples the $MAF_{ceiling}$=1.
- Across all samples (i.e., with and without mixture risks predicted), the 90th percentile of $MAF_{ceiling}$ is equal to 4.6.

Overall, this case shows the **protectiveness of the proposed MAF of 5** for mixture risks of metal(loids) in a highly industrialized region, such as Flanders.

Quantifying conservatisms in MAF-calculations

CA predictions of mixture effects at low effect levels

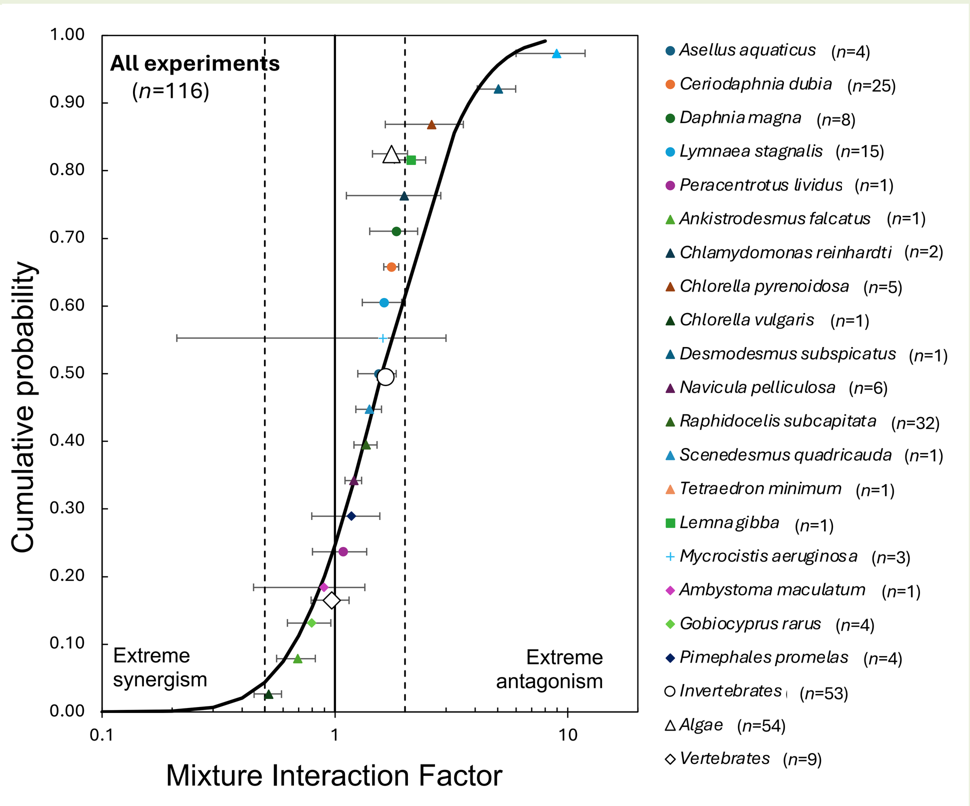

Figure 2. Cumulative distribution of Mixture Interaction Factors across species for which data on chronic metal(loid) mixture toxicity to aquatic organisms is available.

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

- An extensive literature search of available chronic metal(loid) mixture experiments with aquatic organisms was conducted. 34 studies covering 116 mixture experiments with 24 species were identified. MIF was calculated for each experiment and averaged per species.
- MIFs range across species between 0.5 (*Chlorella vulgaris)* and 8.9 (*Tetraedron minimum)* (Fig. 2).

Median MIF = 1.3
→ **CA is not the perfect model** and **overestimates** metal mixture toxicity at low effect levels on average by 1.3-fold.

Conservatism of applying CA at the HC5-level

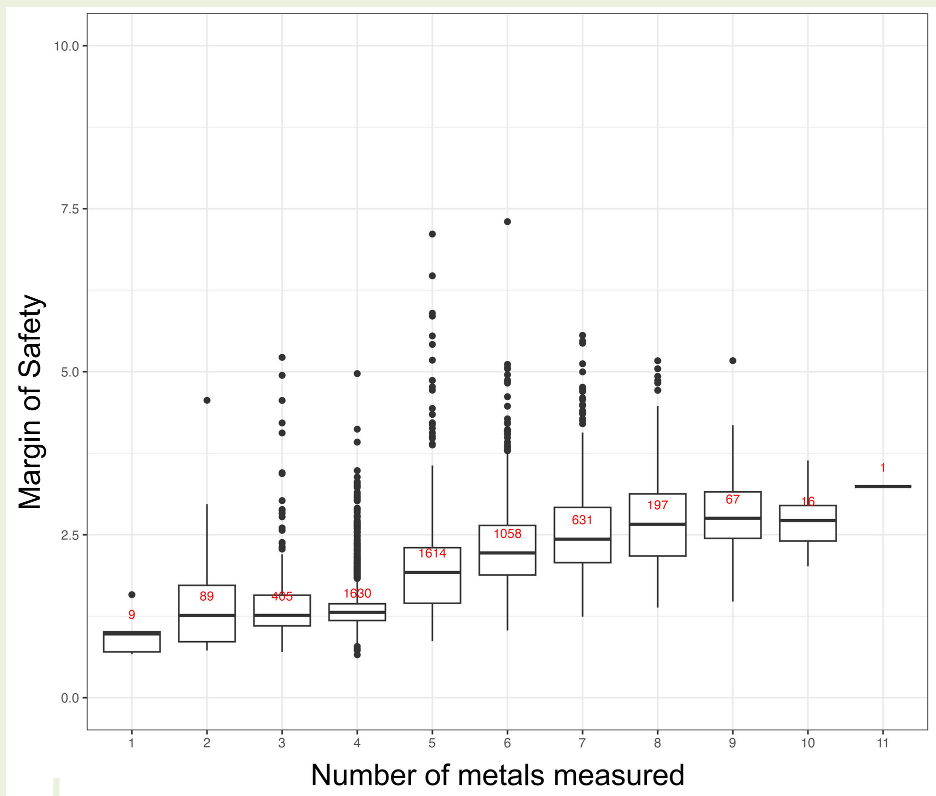

Figure 3. Margin of Safety as a function of the number of metals measured in a sample in the Flemish monitoring database.

Margin of Safety (MoS) [e]
degree of conservatism that is associated with applying **CA** at the HC5-level compared to the theoretically more consistent method of applying CA first at the species-level (EC10)

- MoS increases with the number of metals measured (Fig. 3). This indicates that the conservatism in hazard index-based mixture risk estimation approaches, such as MAF-calculations, increases for more complex mixtures.
- The median MoS across samples is 1.8 (10^{th} – 90^{th} percentile: 1.2-2.9).

Median MoS = 1.8
→ Mixture predictions which apply CA at the HC5-level instead of the EC10-level overestimate mixture risks by 1.8-fold

Conclusions & Outlook

Based on $MAF_{ceiling}$ calculations for the Flemish freshwater monitoring database, our study indicates that a **MAF of 5 is protective against risks of metal(loids)** a highly industrialized region. As such, a **default MAF could serve as a baseline tier for covering risks of unintentional mixtures**.

The **conservatism embedded in default MAF calculations** - a concentration addition (CA)-based approach - was **quantified using the Mixture Interaction Factor (MIF) and the Margin of Safety (MoS):**

- The MIF indicates that CA overestimates mixture toxicity of metals by 1.3-fold (median MIF).
- The MoS indicates that applying CA at the HC5-level instead of at the EC10-level results in an additional overestimation of mixture risks by 1.8-fold (median MoS).

Outlook: In a next step, a refined MAF will be derived, by taking into account the conservatisms quantified by the MIF and MoS in the MAF algorithm. The refined MAF can increase the scientific relevance under REACH in higher tiers of mixture risk assessments as it takes mixture evidence into account.

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References: [a] European Commission. 2020. https://environment.ec.europa.eu/strategy/chemicals-strategy_en; [b] Backhaus. 2024. *Curr Opin Toxicol* 100460; [c] ARCHE Consulting. 2023. I-PCS identification and MAF level 2-refinement. Report for Eurometaux; [d]www.vmm.be; [e] Nys et al. 2018. *Environ Toxicol Chem* 37: 623-642.