The accuracy of the Concentration Addition and Independent action model to predict the toxicity of complex metal-metal mixtures to *Daphnia magna* – Is IA the better model?

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Chemical risk assessment is predominantly designed around the regulation of single substances. However, along their lifecycle, chemicals will end up in the environment, mix with other substances and form unintentional mixtures that are not regulated by legislations like REACH. To manage these unintentional mixtures, the European Union has proposed to introduce a generic MAF (Mixture Allocation Factor) into chemicals policy. However, the magnitude of the MAF value is currently being debated.
Chemical mixtures are mainly assessed according to the concentration addition (CA) model which generally assumes that the compounds present in the mixture follow the same Mode of Action. At low effect concentrations, CA seems to overestimate metal mixture toxicity at the species level.
For complex metal-metal mixtures, we tested the hypothesis that the independent action model (IA) is in general a more accurate predictor of metal mixture toxicity than CA. Deviations of the "observed toxicity" from the "predicted toxicity" by CA can be quantified with the MIF (mixture interaction factor), which indicates either additive (MIF ≈1), synergistic (MIF<1) or antagonistic (MIF>1) interactions, relative to CA.
Our second hypothesis was that the MIF increases with an increasing number of metals present in the mixture.
Both hypotheses were tested in three large mixture experiments using *Daphnia magna,* which assessed chronic mixture effects of different combinations of Ag, As, Ba, Cd, Cr, Cu, Mn, Ni, Pb and Zn. These metals and their mixture combinations were previously selected in another study, based on their environmental relevance in European freshwater. This study also revealed that 90% of the mixture toxicity pressure to *D. magna* (expressed as sum of toxic units, STU) is driven in most freshwaters by five metals or less.
Each experiment tested simultaneously each single metal and a binary, ternary, quaternary and quinary mixture combination. All mixtures followed an equitoxic ray design and an additional quinary combination was included based on environmentally relevant mixture ratios.
Preliminary results of the first metal mixture experiment support our hypothesis that the MIF increases with an increasing number of metals in the mixtures and that IA is a more accurate predictor of metal toxicity. Once completed, this study will add valuable experimental data to the ongoing discussion on how to assess and estimate the potential risks of combined metal exposure.

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

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1. Introduction

The European Commission calls in the Chemicals Strategy for Sustainability for a systematic investigation of the impact of combined exposure into chemical risk assessments under REACH [1]. To cover for such effects a default Mixture Allocation Factor (MAF) of 5 will be introduced, which most probably will imply that the safety limit of single substances will be reduced. The implementation of a MAF will have a large impact on the environmental risk assessment of inorganics (including metals and metalloids). This has been demonstrated by an extensive review of REACH exposure scenarios for metals, for which it was shown that with a MAF of 5, 68% of existing local exposure scenarios for the metals sector would need revisions [2]. Additionally, given that inorganics are naturally occurring, the implementation of a default MAF may result in exceedances of safety limits at natural background concentrations, suggesting a combined risk.

To anticipate the mixture toxicity correction under REACH, a dedicated research program on chronic metal mixture toxicity in the aquatic environment has been set up as part of the Metals Environment Exposure Data (MEED)-program. The research program aims to deliver robust scientific evidence on the mixture assessment of metals, with the focus on those inorganics that contribute most to the predicted overall risks of unintentional mixtures in Europe, i.e., the Inorganic-Priority Contributing Substances (I-PCS). In a previous exercise which characterized (mixture) pressure of inorganics using European monitoring data, the following elements have been identified as I-PCS for the aquatic ecosystem: Ag, As, Ba, Cd, Co, Cr, Cu, Hg, Mn, Ni, Pb, Se, V, and Zn and a series of rare earth elements Ce, Dy, Er, Gd, La and Y [3]. The current study discusses the progress made over the first two years of the MEED-mixture research program and gives an outlook on how the gathered information may be potentially used to refine the default MAF-setting for inorganic substances.

2. Materials and Methods

The following two key questions with respect to combined risk assessment have been evaluated (following the methodology described in [4]):

I) Can mixture effects of aquatic I-PCS at regulatory and environmentally relevant exposure levels be predicted based on standard models? This was evaluated by considering the prediction performance of the two most common mixture reference models, Concentration Addition (CA) and Independent Action (IA).

II) How accurate is CA for predicting mixture effects at low effect concentrations? To quantify the prediction performance of CA at low effect levels (i.e., 10% mixture effect), the Mixture Interaction Factor (MIF) has previously been proposed [4]. The MIF quantifies the prediction performance of CA at these low effect levels, and is indicative of additive (MIF≈1), antagonistic (MIF>1) or synergistic (MIF<1) interactions relative to CA.

These questions were evaluated in a quantitative reappraisal that considered the data obtained from an extensive literature search on chronic metal mixture toxicity to aquatic organisms. In addition, we considered the newly generated experimental data gathered within the MEED-program, which focusses on chronic mixture effects of I-PCS to *Raphidocelis subcapitata* and *Daphnia magna* in mixture combinations covering metals with data gaps, in varying complexity (up to quinary combinations). As such, our dataset represents a comprehensive overview of chronic metal mixture toxicity to aquatic organisms.

3. Results and Discussion

3.1. Chronic metal mixture effects to aquatic organisms.

Results show that chronic metal mixture effects can be predicted with the standard mixture reference models, CA and IA, whereby CA results in more conservative predictions compared to IA. In addition, the best performing model depends on the trophic group considered. In general, chronic metal mixture toxicity is most accurately predicted by the independent action model for invertebrates, while there is less difference between both models for algae. The available evidence show that non-interactive effects or antagonistic effects are most frequently observed in chronic metal mixture experiments, while significant synergisms are less frequently observed, certainly when evaluated relative to the regulatory most preferred model CA. For the dataset resulting from the literature review, a median MIF of 1.3 has been derived, indicating that on average CA overestimates metal mixture toxicity at these regulatory (and environmentally) relevant low effect levels. The first results from the experimental program on algae, seem to confirm the conclusions from the quantitative reappraisal of the existing literature data.

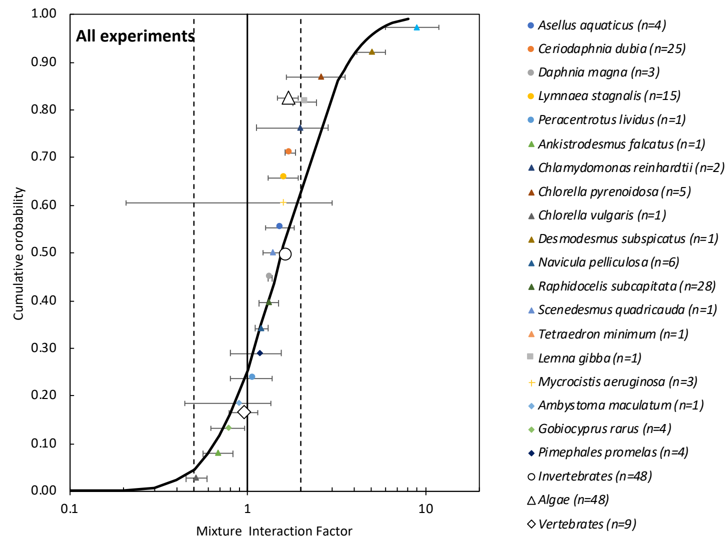

Figure 1 Overview of Mixture Interaction Factors (MIF) in chronic metal mixture experiments for different species. The MIF quantifies the prediction performance of concentration addition at low effect levels: MIF≈1 indicates additivity, MIF>1 antagonism, MIF<1 synergism

3.2. Regulatory refinements and research outlooks

MAF impact assessment strategies have traditionally been based on the application of the concentration addition model at the regulatory community-level threshold (i.e., considering either predicted no-effect concentration or 5% hazardhous concentrations). As such, the following implicit assumptions are made: I) at the species-level it is assumed that all substances have the same mode of action, and that all substances contribute to the mixture presure, and II) at the community-level that for all substances the same species drive the regulatory threshold and are performing equally well in all EU ecosystems. These assumptions lead to additional conservatism in risk assessment, which may lead to overly protective situations when a default MAF is used. In that respect, the Mixture Interaction Factor directly quantifies the level of conservatism at the species level, and may potentially be used to increase the scientific relevancy of MAF-values for inorganic substances. Future research efforts will focus on quantifying the conservatism at the community-level, by evaluating the margin of safety provided by applying the concentration addition method directly on the community-treshold. In addition, we also aim to cover data-gaps regarding the combined exposure of inorganics and organic substances as these have rarely been studied at environmentally and regulatory relevant concentrations.

4. Conclusions

Based on a comprehensive dataset of chronic metal mixture toxicity data to aquatic organisms it can be concluded that chronic metal mixture effects can be predicted with the standard mixture reference models, CA and IA, whereby CA results in more conservative predictions compared to IA. For the extensive dataset, a median MIF of 1.3 has been derived, indicating that on average CA overestimates mixture toxicity at regulatory relevant low effect levels. The MIF may increase the scientific relevancy of MAF-values for inorganic substances. Future research efforts within the MEED project will further focus on quantifying the conservatism provided by a regulatory MAF-setting and developing tools for scientifically based refinements of the proposed mixture risk assessment methodology for inorganic substances.

References - [1] European Commission. 2020. Chemicals Strategy for Sustainability. [2] ARCHE Consulting 2021. Report prepared for Eurometaux. [3] ARCHE Consulting 2023. Report prepared for Eurometaux. [4] Nys et al. 2018. *Environ Toxicol Chem* 37: 623-642.

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EU-WIDE EXPOSURE ASSESSMENT OF METALS IN MUNICIPAL SEWAGE TREATMENT PLANTS.

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Releases from consumer and professional widespread uses to the aquatic environment are based on default assumptions leading to precautionary assessments in the REACH dossiers. Around 80-90% of urban wastewater is collected and treated in the EU. Hence metal emissions from e.g. consumer use of products via STP effluents are not to be neglected. Emissions from municipal sewage treatment plants (STP) can provide a better estimate, given they collect metal emissions from these combined sources.

Providing a realistic estimate of metal concentrations in the effluent of municipal STPs is part of Eurometaux's ongoing comprehensive "Metals Environmental Exposure Data gathering" (MEED), covering today's and tomorrow's need to comply with the REACH combined effects, Zero Pollution Ambition and biodiversity objectives.

The EU's Urban Wastewater Treatment Directive sets requirements for urban wastewater quality but lacks specific regulations for monitoring metals. As a result, there is no centralized database for metal concentration data in the EU. To address this gap and collect data, environmental and water agencies across EU countries were contacted for metal emission data.

Data sets for 20 metals, covering up to 12 countries (availability of data varied between countries), were compiled, and represented the basis for the derivation of reasonable worst-case STP effluent concentration.

The methodology included an outlier analysis to minimize the impact of extreme local point sources (e.g. due to contamination with small industrial sources) when deriving a representative STP effluent concentration.

The EU PEC_{STP} was represented by the median of all 90th percentile values from the different countries. The comparison of the EU PEC_{STP} value with the $PNEC_{STP}$ was made to derive the risk characterization ratios (RCR) at EU scale.

All RCRs for the individual metals were below 1 for the STP. The RCRs for the subsequent environmental compartments after STP discharge were all below 1 except for two metals with RCRs > 1 for the sediment compartment. When also including the regional background concentrations, a significant portion of the RCRs are larger than 0.2 hence will require an assessment of the combined risk in the EU.

Nonetheless, this does not imply the existence of an actual risk. These values could potentially undergo further refinement by incorporating the bioavailability concept, although such refinement lies beyond the scope of this project.