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Research Paper

Quantifying the interactions among metal mixtures in toxicodynamic process with generalized linear model

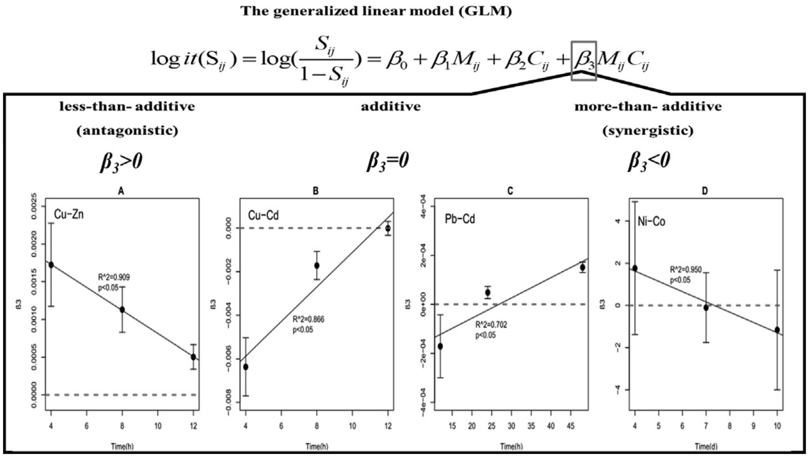
Jianfeng Feng[a,*,1], Yongfei Gao[b,1], Yijun Ji[a], Lin Zhu[a]

[a] *Key Laboratory of Pollution Process and Environmental Criteria of Ministry of Education and Tianjin Key Laboratory of Environmental Remediation and Pollution Control, College of Environmental Science and Engineering, Nankai University, Tianjin 300071, China*
[b] *Institute of Grassland Research, Chinese Academy of Agriculture Sciences, Hohhot 010010, China*

HIGHLIGHTS

- GLM was used to quantify the metal interactions in toxicodyamics process.
- Joint actions occur among Cu–Zn, Cu–Cd, Cd–Pb, and Ni–Co in toxicodyamics process.
- The interaction types among Cu–Zn, Cu–Cd, Cd–Pb, and Ni–Co were time dependent.
- GLM is a powerful tool for assessing the toxicities of interacting chemical mixtures.

GRAPHICAL ABSTRACT



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ABSTRACT

Predicting the toxicity of chemical mixtures is difficult because of the additive, antagonistic, or synergistic interactions among the mixture components. Antagonistic and synergistic interactions are dominant in metal mixtures, and their distributions may correlate with exposure concentrations. However, whether the interaction types of metal mixtures change at different time points during toxicodynamic (TD) processes is undetermined because of insufficient appropriate models and metal bioaccumulation data at different time points. In the present study, the generalized linear model (GLM) was used to illustrate the combined toxicities of binary metal mixtures, such as Cu–Zn, Cu–Cd, and Cd–Pb, to zebrafish larvae (*Danio rerio*). GLM was also used to identify possible interaction types among these method for the traditional concentration addition (CA) and independent action (IA) models. Then the GLM were applied to quantify the different possible interaction types for metal mixture toxicity (Cu–Zn, Cu–Cd, and Cd–Pb to *D. rerio* and Ni–Co to Oligochaeta *Enchytraeus crypticus*) during the TD process at different exposure times. We found different metal interaction responses in the TD process and interactive coefficients significantly

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Abbreviations: TK-TD model, toxicokinetic–toxicodynamic model; TK model, toxicokinetic model; TD model, toxicodynamic model; GLM, generalized linear model; CA, concentration addition model; IA, independent action model.

[*] Corresponding author.
E-mail address: fengjf@nankai.edu.cn (J. Feng).

[1] Equal contribution.

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changed at different exposure times ($p < 0.05$), which indicated that the interaction types among Cu–Zn, Cu–Cd, Cd–Pb and Ni–Co were time dependent. Our analysis highlighted the importance of considering joint actions in the TD process to understand and predict metal mixture toxicology on organisms. Moreover, care should be taken when evaluating interactions in toxicity prediction because results may vary at different time points. The GLM could be an alternative or complementary approach for BLM to analyze and predict metal mixture toxicity.

1. Introduction

Contaminants in the actual environment usually exist in a mixed state. Thus, predicting the toxicity of metal mixtures is a key but challenging task in the assessment of environmental risk and toxicology [1,2]. The results of studies on metal mixture were inconsistent [2–7], and thus assessing and regulating ecological risks remain difficult. This condition consequently increases the difficulty of understanding critical toxic mechanisms and developing useful approaches for the prediction of combined toxicity of mixtures. As part of the Metal Mixture Modeling Evaluation (MMME) project, several models were developed to predict the effects of metal mixtures on aquatic organisms [2,4,5,7]. Two traditional models, namely, concentration addition (CA, i.e., assumption of similar joint action) and independent action (IA, i.e., assumption of dissimilar joint action), are often used to categorize the main interactive types between metals in a mixture and predict their combined toxicities [8]. However, the CA and IA approaches often ignore the uncertainty associated with the time endpoint estimate of survival, which may lead to unreliable conclusions on chemical interactions [5]. Thus, some metal mixtures of the CA and IA models may overestimate or underestimate joint effects and increase the uncertainty of risk assessment. Literature reported that CA or IA fails to describe the joint toxicity of metals because of existing interactions [9–14].

The joint action of metals may arise in the following two related processes, namely, toxicokinetic (TK) and toxicodynamic (TD) processes. In the TK process (from free ion concentrations to internal concentration), the inhibition or promotion of absorption between two metals frequently occur [3,6,15–19] and result in different internal concentrations, thereby becoming a confounding factor when the interactions between two metals at an exposure time point during the uptake phase are considered. In the TD process, metals may interact at target sites within an organism possibly through the molecular toxicology of the toxicants, including different modes of action (MOA) or adverse outcome pathways (AOPs). Most studies used the relationship involved in exposure concentration response to determine the type of interaction; moreover, the uncertainty of the results (dose- or occupational faction -related, etc.) might be attributed to the TK and TD processes [6,19]. Previous work on the interactions in the TK or TD processes mainly focused on the effective concentration (EC50) or median lethal concentration (LC50) level of the median on the basis of a single time-point toxicity [3,6,15,16]. Our previous work showed that the metal concentration in zebrafish can be predicted effectively through the TK model, which considered the possibility of competitive absorption between two metals regardless of their combined effects [15,16]. Our results suggested that the joint effect of these metals is not significant in the TK process. By contrast, the TD process overestimates or underestimates the toxicity of metal mixtures because metal interactions are not considered in the TD model [15]. Moreover, the TD process is directly correlated with the MOA between two metals, and this correlation can result in toxicity via AOP and produce a combined effect at this stage, especially of heavy metals [20].

At present, evaluating metal interaction types is generally based on toxicity data at fixed exposure time points (24 h or 48 h). For example, the prediction of metal interactions through metal mixture toxicity tests were found expressed by the toxicity function model for organisms exposed to Cd and Zn mixture from 24 h to 96 h of exposure [7,21]. However, the type of interaction depends on the metals involved, their external concentration, availability and length of exposure and tested species [22,23]. Some recent studies suggested that the toxicities of metal mixtures may depend on exposure duration because different metals may have different toxic actions at different time points [15,16,24–26]. However, no direct evidence was found for the possibility of interactions among different metals at different time points.

Here, we first demonstrates an application of the three different effect analysis models, namely, CA, IA, and generalized linear model (GLM), to analyze the survival data from toxicity tests for Cu–Zn, Cu–Cd, and Cd–Pb mixtures in zebrafish larvae (*Danio rerio*) [15,16]. Cu, Zn, Cd, and Pb are common heavy metals in surface water and they generally differ in uptake mechanism for aquatic organisms [27–29]. Cu and Zn play an important role in cellular metabolism, and their body concentrations can be regulated by the organisms. Cd and Pb are toxic even at low concentrations and tend to accumulate in the body [28,29]. Then the internal concentrations of metals were tested as predictors of metal toxicity on the survival of zebrafish larvae and addressed the following questions: (1) Can the GLM model be a better supplementary method than the CA and IA models in describing the joint action between metal mixtures? (2) Can the joint action between two metals occur in the TD process when internal concentration is used as an input parameter? (3) Are there time-varied metal interaction patterns for metal mixtures toxicity?

2. Materials and methods

2.1. Data collection and inclusion

The accumulation and survival data used in the present study were extracted from our previous research on the effects of Cu, Zn, Cd, or Pb or binary metal mixtures (Cu–Zn, Cu–Cd, and Cd–Pb) [15,16]. The toxicity test with zebrafish larvae was conducted at 26 °C ± 0.5 °C with a cycle of 12 h light and 12 h dark. The larvae were reared in sterile six-well cell culture plates (Cellstar, Greiner Bio-one, Germany) at a density of 30 larvae per well. Each well contained 10 mL of test solutions in triplicate. During exposure, the pH of each test solution was approximately controlled at pH 7.0 with 10−2 M MOPS [3-(N-morpholino) propanesulfonate, >99%, Sigma] and measured using a pH meter (S20P-K SevenEasy Plus, Mettler Toledo, Switzerland). Larvae were collected and then digested in a 1 mL mixture of 1:1 concentrated HNO_3 (Kermel, Ultra Pure, 70%) and H_2O_2 (Kermel, Ultra Pure, 30%) by 80 °C ± 2 °C water bath method (thermostat water bath cauldron, Jinxiang, Shanghai, China) and filtered with a 0.45 μm membrane filter (Whatman). Metal concentrations in animals or test solutions were measured by Inductively Coupled Plasma-Mass Spectrometry (ICP-MS, ELAN DRC-e, PerkinElmer, USA).