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Concentration addition and independent action model: Which is better in predicting the toxicity for metal mixtures on zebrafish larvae

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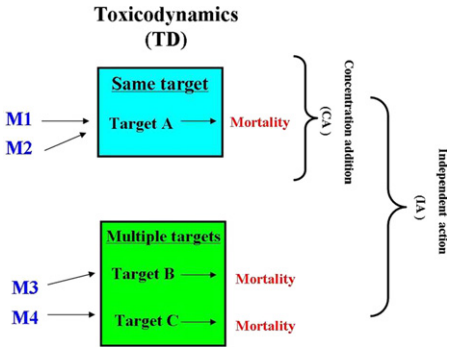
HIGHLIGHTS

- Joint toxicity was predicted by concentration addition (CA) and independent action (IA) models.
- The IA model simulated the Cu-Pb mixture toxicity significantly better than the CA model.
- The CA model simulated the Cu—Cd and Cd—Pb mixture toxicity better than the IA model.
- For the Cu-Zn mixtures, it was difficult to recognize which of the two models was better.
- CA and IA model are consistent in specifying interaction patterns of binary metal mixtures.

GRAPHICAL ABSTRACT



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ABSTRACT

The joint toxicity of chemical mixtures has emerged as a popular topic, particularly on the additive and potential synergistic actions of environmental mixtures. We investigated the 24 h toxicity of Cu–Zn, Cu–Cd, and Cu–Pb and 96 h toxicity of Cd–Pb binary mixtures on the survival of zebrafish larvae. Joint toxicity was predicted and compared using the concentration addition (CA) and independent action (IA) models with different assumptions in the toxic action mode in toxicodynamic processes through single and binary metal mixture tests. Results showed that the CA and IA models presented varying predictive abilities for different metal combinations. For the Cu–Cd and Cd–Pb mixtures, the CA model simulated the observed survival rates better than the IA model. By contrast, the IA model simulated the observed survival rates better than the CA model for the Cu–Zn and Cu–Pb mixtures. These findings revealed that the toxic action mode may depend on the combinations and concentrations of tested metal mixtures. Statistical analysis of the antagonistic or synergistic interactions indicated that synergistic interactions were observed for the Cu–Cd and Cu–Pb mixtures, non-interactions were observed for the Cd–Pb mixtures, and slight antagonistic interactions for the Cu–Zn mixtures. These results illustrated that the CA and IA models are consistent in specifying the interaction patterns of binary metal mixtures.

1. Introduction

Humans and wild animals are simultaneously exposed to numerous environmental pollutants via multiple exposure pathways. The adverse effect of a single chemical is occasionally ignored due to such chemical's low environmental concentrations (Brock et al., 2010; Traas and van

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Leeuwen, 2007; Liu et al., 2016). However, the effects of co-existing multiple chemicals cannot be ignored because of the so-called "something from nothing" phenomenon (Silva et al., 2002; Jin et al., 2014; Cobbina et al., 2015). Therefore, the joint toxicity of a mixture has emerged as a popular topic, particularly on the additive and potential synergistic actions of environmental mixtures (Farley et al., 2015; Lopes et al., 2016).

In current regulatory approaches, metal mixture toxicity is generally predicted by the concentration addition (CA; i.e., dose or response addition) or independent action (IA) models (Farley et al., 2015; Baas et al., 2007; Nagai and De, 2016). The CA model is often used as a traditional model to predict the mixture toxicity of metals with the same mode of action or metals with unknown mode of action; by contrast, the IA model is frequently applied to assess the mixture toxicity of metals with different action modes (Baas et al., 2007; Jonker et al., 2005). Several studies have described the currently available toxicokinetic and toxicodynamic (TK–TD) models explicitly formulated as the CA and IA models for the TD simulations of different organic chemicals, thereby clarifying and comparing their underlying TD assumptions (Ashauer and Brown, 2008; Nyman et al., 2012; Jager et al., 2011). Such analysis supports the selection of the appropriate model that depends on the toxicant under its toxic action mechanism.

Our previous study determined the joint toxicity of binary metal to zebrafish larvae and used the CA approach in the TD models to evaluate the toxic effects of metal mixtures (i.e., Cu + Zn, Cu + Cd, Cu + Pb, and Cd + Pb) at different exposure times (Gao et al., 2016, 2017). Cu, Zn, Cd, and Pb were selected because they generally differ in uptake mechanism for aquatic organisms. Cu^{2+} is a known Na^{+} uptake inhibitor (Grosell and Wood, 2002). The inhibition of the Cd uptake by Zn is in agreement with well-documented competitive interactions between two metals for cell entry via Ca^{2+} channels (Paquin et al., 2002). Cd^{2+} is known to be a Ca^{2+} uptake inhibitor (Rainbow and Black, 2005). Lastly, Pb^{2+} has been observed to inhibit the Ca^{2+} and Na + uptake pathways (Rogers et al., 2005). The interaction between binary metals was mainly antagonistic based on the free ion concentrations in exposure solutions, and concentration additive based on the body concentrations (Gao et al., 2016). Therefore, the IA approach assumed that metals in a mixture do not affect each other in terms of mode of toxic action and the interactions among metals that diminish or enhance bioavailability and toxicity are ignored with no clear patterns in additive and non-additive behavior (Farley et al., 2015). Only a few studies have extended the CA and IA approaches, as well as incorporated two assumptions to estimate the metal mixture toxicity in TD processes (Baas et al., 2007; Nagai and De, 2016).

The objective of the present study is to investigate the toxicity of binary metal mixtures (i.e., Cu + Zn, Cu + Cd, Cu + Pb, and Cd + Pb) on the survival of zebrafish larvae (*Danio rerio*) and to test the applicability of the CA and IA models in predicting metal joint toxicity. Moreover, applying the mixture effect models is necessary to understand the mechanisms of toxic action for different metals in the TD processes. Therefore, we interpreted mixture toxicity with different modes of toxic action for each metal at the target level.

2. Materials and methods

2.1. Toxicity assays

Experimental research was conducted in previous studies (Gao et al., 2016, 2017) and acute toxicity was analyzed for zebrafish larvae that were exposed to binary metal mixtures (Cu + Zn, Cu + Cd, Cu + Pb, and Cd + Pb). Ultrapure water (Milli-Q, R > 18.2 MΩ cm) was used as the medium. The Cu, Zn, Cd, and Pb stock solutions (10, 20, 5, and 50 g/L, respectively) were prepared by adding copper chloride, zinc sulfate, cadmium chloride, and lead nitrate salts (>99%; Kermel Ultra Pure), respectively. The stock solution was diluted in ultrapure water to create a graded series of metal test solutions. Acute toxicity experiments that involve zebrafish larvae were conducted based on the guidelines of the Organisation for Economic Co-operation and Development (OECD, 1992). Westerfield (1995) described the culture conditions of the zebrafish larvae prior to the test. For all exposure experiments, the larvae were maintained at 26 ± 0.5 °C under a 12 h:12 h light:dark cycle and reared in sterile six-well cell culture plates (Cellstar, Greiner Bio-one, Germany) at a density of 30 larvae per well with each well containing 10 mL of test solutions in triplicate. The metal concentrations (Table S1 of the Supporting Information) were chosen based on the environmental metal concentrations in polluted waters(e. g. 15–470 nM Cu, 0.7–34 μM Zn, 1–110 nM Cd, and 0.5–5 nM Pb)(Bervoets and Blust, 2003; Reinders et al., 2008) and the 24 h median lethal concentration values (LC50) for the metals individually (Cu:0.358 μM, Zn:3.65 μM, Cd:0.44 μM, and Pb:2.98 μM) (Gao et al., 2016, 2017). The larvae were exposed to a constant concentration of the first metal and various concentrations of the competing metal with three negative controls (ultrapure water) in each binary metal combination (Gao et al., 2016, 2017). Survival was assessed as recommended by the OECD guideline, and 15 sets of time (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 48, 72, and 96 h) were selected as recording times in each treatment. At recording times, mortality was recorded in triplicate. All data were extracted from previous studies and predicted thereafter in the following model.

2.2. Model concept

Metal toxicity varied reasonably because the water chemistry (e.g., hardness, pH, and dissolved organic matter) can affect metal speciation, uptake of metals at the biological uptake sites equivalent to biotic ligands (BLs), and result in organ diseases equivalent to the target sites (Di et al., 2001; Niyogi and Wood, 2004). Gao et al. (2016) considered the effect of H + in exposure on metal toxicity and integrated the BL models into the TK–TD model to predict the toxicity of binary metal mixtures on zebrafish. In all treatments, over 99% (in terms of free metal ion M^{2+}) of the total Cu, Zn, Cd, or Pb concentrations existed as bio-available species (e.g., for Cu: Cu^{2+}, $CuOH^{+}$, $Cu2(OH)_2^{+2}$, and dissolved $Cu(OH)_2$) (Gao et al., 2016, 2017). Consequently, we inputted 99% of the total metal concentration (in terms of M^{2+}) into the modeling equations and conceptually interpreted the possible mixture effects of metals through the BL concept (see Fig. 1). The differences between the CA and IA models are described as follows. In the CA model, we considered that different metals share the same uptake pathway (single site) at TK (uptake level) and the modes of toxic action are the same among the metals at TD (target level) (Altenburger et al., 2012). In the IA model, we assumed that different metals share the same uptake pathway (single site) at TK, the modes of toxic action are different among the metals at TD, and the target sites are independent among metals (Altenburger et al., 2012).

2.3. Toxicity analysis

Our present and previous studies (Gao et al., 2016) were conducted on the same organisms with the same test condition. A time-course TK model from Gao et al. (2016) was used for an improved understanding of the joint effects of the mixture by considering the processes of competitive uptake and independent elimination.

In the CA model, the TD model for metal mixtures is a function that hazard ($H(t)$, dimensionless) caused by the internal toxic equivalent quantity (TEQ_{inter}) of the metal mixtures and the probability of survival decreases when the metal concentration in organisms exceeds the safe threshold concentration (threshold, amount $mass^{-1}$) (Gao et al., 2016).

$$\frac{dH(t)}{dt} = k_k \times \ max[TEQ_{\ inter}(t) - threshold, 0], \tag{1}$$

$$S(t) = e^{-H(t)} \times S_{control}(t), \tag{2}$$