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Application of biotic ligand and toxicokinetic–toxicodynamic modeling to predict the accumulation and toxicity of metal mixtures to zebrafish larvae☆

Yongfei Gao [a], Jianfeng Feng [a, **], Feng Han [b], 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] Bureau of Hydrology and Water Resources of Henan Province, Zhenzhou, China

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Abbreviations:
TK-TD model
toxicokinetic–toxicodynamic model
TK model
toxicokinetic model
TD model
toxicodynamic model
BLM
biotic ligand model
TEF
toxic equivalent factor
CBR
the dynamic Critical Body Residue
TEQ
the toxic equivalent quantity
BL
biotic ligand
TK1
toxicokinetic model 1
TK2
toxicokinetic model 2

ABSTRACT

Predicting the accumulation and toxicity of mixtures of metals to aquatic organisms is a key challenge in ecotoxicological studies. In this study, the accumulation and toxicity of mixed essential (Cu) and nonessential (Cd and Pb) metals in zebrafish larvae exposed to a binary mixture of these elements at environmentally relevant concentrations were predicted using a refined toxicokinetic (TK)–toxicodynamic (TD) model aided with biotic ligand model (BLM) and toxic equivalent factor (TEF) approach. Competitive inhibition and non-competitive interaction/inhibition were observed in biouptake. Both Pb and Cd behaved as competitive inhibitors of Cu uptake at high Cu concentrations (>0.1 μM). By contrast, Cu uptake was independent of Cd or Pb when the Cu concentrations were below 10^{-7} M. Furthermore, low concentrations of Cu had an adiaphorous effect on Cd or Pb uptake. Cd uptake was inhibited by Pb, and the Pb uptake rates consistently decreased in the presence of Cd. The accumulation processes of Cd–Pb, Cu–Cd, and Cu–Pb were accurately predicted by the BLM-aided TK models. The traditional TD model could successfully predict the toxicity of Cd–Pb mixtures, but not those of Cu–Cd or Cu–Pb mixtures. The revised TD model, which considered the possible different killing rates (K_k) above or below the threshold, offered better prediction for the toxicity of Cu–Cd or Cu–Pb mixtures. The overall findings may be of key significance in understanding and predicting metal uptake, accumulation, and toxicity in binary or multiple metal exposure scenarios.

1. Introduction

Aquatic organisms are often exposed to mixtures of metals with elevated concentrations caused by anthropogenic activities

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* Corresponding author.
** Corresponding author.
E-mail addresses: fengjf@nankai.edu.cn (J. Feng), zhulin@nankai.edu.cn (L. Zhu).

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(Borgmann et al., 2008). However, metals are still generally regulated with a strong single-chemical basis because of the complexity of assessing the toxicity of mixtures and difficulties in modeling and predicting metal mixture toxicity. Mixture toxicity is usually investigated in a descriptive manner, with fixed durations and constant external exposure (Norwood et al., 2003; Vijver et al., 2011). However, this approach does not explicitly consider the temporal dimension of toxicity and obscures the fact that mixture toxicity effects depend on exposure time (Baas et al., 2007). Process-based models, such as toxicokinetic (TK)–toxicodynamic (TD) models, are increasingly used in predicting the toxicity of single metal exposure (Tan and Wang, 2012; Veltman et al., 2014; Ashauer et al., 2007); however, only a few studies have addressed mixture toxicity (Margerit et al., 2015).

A major challenge in modeling metal mixture toxicity is the need to consider the competition of metals and cations in binding to inorganic ligands and dissolved organic matter. Trace metals may enter organisms through common uptake pathways and interact with each other, thereby affecting uptake, bio-accumulation, and toxicity. Quantification of metal mixture toxicity is based on measurements of external exposure. However, internal concentrations of individual metals are more suitable for understanding, interpreting, and extrapolating joint toxicological effects (Niyogi and Wood, 2004; Di et al., 2001). Therefore, understanding the relationship between external and internal concentrations of individual metals in organisms is necessary. As part of an effort to address metal mixture toxicity, biotic ligand models (BLMs) have been developed to evaluate responses of aquatic organisms to metal mixtures and provide a priori predictions of toxicity (Farley et al., 2015). The assumptions of BLM potentially allow considering the interactions between different metal ions in the assessment of mixture toxicity (Borgmann et al., 2008; Niyogi and Wood, 2004; Norwood et al., 2003). For example, two model structures presented by Le et al. (2013) indicated that Cu^{2+}-Zn^{2+} bound to the same transporters and Cu^{2+}-Ag^{+} bound to the different transporters, based on assumptions and model structures of BLM (Niyogi and Wood, 2004). Moreover, the dynamic Critical Body Residue (CBR) approach indicated that the accumulation of metal ions in organisms is a better indicator of the toxicity of metal mixtures compared with metal concentrations or activities in solutions (Matida, 1960; Kooijman, 1981; Mackay et al., 1992). Consequently, the toxic equivalent factor (TEF) of metals in mixtures and the toxic equivalent quantity (TEQ) of mixtures have been determined based on the total concentration of metals in organisms, overcoming the disadvantages of the conventional TEF approach based on metal concentrations in solutions (Birnbaum and DeVito, 1995; Cheng and Allen, 2001).

Some essential metals, such as Cu and Zn, play an important role in cellular metabolism (Lutsenko and Petris, 2003; Puig and Thiele, 2002; De Feo et al., 2007). However, an excess of these metals may damage marine and freshwater organisms (Tao et al., 1999; Hassler et al., 2004; Komjarova and Blust, 2009). Other nonessential metals, such as Cd and Pb, are toxic even at low concentrations and tend to accumulate in the body (Rainbow, 1997, 2002). Metal mixtures of essential Cu and non-essential Pb exhibit non-competitive bioaccumulation in fish (Tao et al., 1999; Komjarova and Blust, 2009). However, metal mixtures of non-essential Cd and Pb show competitive bioaccumulation in zebrafish (Komjarova and Blust, 2009).

The present study sought to predict the toxicity of binary metal mixtures (Cu–Cd, Cu–Pb, and Cd–Pb) to zebrafish larvae, *Danio rerio*, by combining the refined TK–TD model and TEF approach with the premise validation whether two metals compete for the same or different biotic ligand (BL) sites. In particular, the accumulation of each metal in organisms following single metal exposure, which determines the toxicity of the single metal according to the dynamic CBR, was used to determine the TEF of the metals in mixtures. The accumulation of metals in the whole body is influenced by interactions between the metals and other competing cations. Therefore, interactions between different metal ions in mixtures at the BL sites can be integrated in an attempt to model the toxicity of mixtures with the premise validation whether two metals bind to the same or different BL sites.

2. Materials and methods

2.1. Chemicals

Ultrapure water (Milli-Q, R > 18.2 MΩ cm) was used as medium. The Cu, Cd and Pb stock solutions (Cu: 10 g/L, Cd: 5 g/L, and Pb: 50 g/L) were prepared by adding copper chloride, cadmium chloride, and lead nitrate salts (>99%; Kermel Ultra Pure), respectively. The stock solution was diluted into working solution as Cu 1 g/L, Cd 1 g/L, and 10 g/L. The working solution was diluted in ultrapure water to create a graded series of metal test solutions.

2.2. Test organisms

AB strain zebrafish (*D. rerio*) were reared in 5 L glass tanks containing 10–15 fish per tank and kept under a 12 h light and 12 h dark photoperiod at 26 ± 0.5 °C (Westerfield, 1995). The fish were fed with brine shrimp (*Artemia* nauplii) twice a day. The fish were maintained, and their embryos were collected from tanks as described by Westerfield (1995). The embryos were reared for hatching at 72 h post-fertilization, and the larvae were prepared for experimental treatments. 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. Before animal exposure, the test solutions were automatically aerated for 24 h. The plates were covered with a preservative film floating on the surface of the six-well cell culture plates to prevent excess evaporation and subsequent inaccurate metal concentration in the test solutions. 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). The larvae were not fed during the experiment to eliminate the influence of food on metal accumulation in the body. This study was conducted in accordance with the national and institutional guidelines for the protection of human subjects and animal welfare.

2.3. Biouptake experiments between metals

The larvae were exposed in triplicate to a constant concentration of the first metal and various concentrations of the competing metal (Table S2 of the Supporting Information). A sampling time of 2 h was selected for the competition experiments. The initial and final test solutions (5 mL) were collected in duplicate.

2.4. Accumulation and toxicity of metal mixtures

The larvae were exposed to a constant concentration of the first metal at 10^{-8}, 10^{-7}, 10^{-6}, and 10^{-5} M and various concentrations of the competing metal from 0 M to 10^{-5} M. Nine exposure times (2,4,8, 12, 18, 24, 48, 72, and 96 h) were selected as sampling times, whereas 15 times (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 sampling times, surviving animals and test solutions were