Science of the Total Environment 466–467 (2014) 788–799

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Predicting the toxicity of metal mixtures

Laurie S. Balistrieri [a,*], Christopher A. Mebane [b,1]

[a] U.S. Geological Survey, School of Oceanography, University of Washington, Box 355351, Seattle, WA 98195, United States
[b] U.S. Geological Survey, 230 Collins Road, Boise, ID 83702, United States

HIGHLIGHTS

- A new approach that predicts the toxicity of metal mixtures is presented.
- The approach is evaluated using single metal toxicity tests of trout.
- Theoretical data sets illustrate differences in toxicity among metal solutions.
- Toxicity is predicted at a field site and compared with toxicity test data.
- Multiple factors influence the toxicity of metal mixtures.

ARTICLE INFO

Article history:
Received 5 April 2013
Received in revised form 10 July 2013
Accepted 11 July 2013
Available online 25 August 2013

Editor: Mark Hanson

Keywords:
Metal mixtures
Metal toxicity
Biotic ligand model
BLM
Trout

ABSTRACT

The toxicity of single and multiple metal (Cd, Cu, Pb, and Zn) solutions to trout is predicted using an approach that combines calculations of: (1) solution speciation; (2) competition and accumulation of cations (H, Ca, Mg, Na, Cd, Cu, Pb, and Zn) on low abundance, high affinity and high abundance, low affinity biotic ligand sites; (3) a toxicity function that accounts for accumulation and potency of individual toxicants; and (4) biological response. The approach is evaluated by examining water composition from single metal toxicity tests of trout at 50% mortality, results of theoretical calculations of metal accumulation on fish gills and associated mortality for single, binary, ternary, and quaternary metal solutions, and predictions for a field site impacted by acid rock drainage. These evaluations indicate that toxicity of metal mixtures depends on the relative affinity and potency of toxicants for a given aquatic organism, suites of metals in the mixture, dissolved metal concentrations and ratios, and background solution composition (temperature, pH, and concentrations of major ions and dissolved organic carbon). A composite function that incorporates solution composition, affinity and competition of cations for two types of biotic ligand sites, and potencies of hydrogen and individual metals is proposed as a tool to evaluate potential toxicity of environmental solutions to trout.

Published by Elsevier B.V.

1. Introduction

Evaluating the toxicity of chemical mixtures is an enduring problem in aquatic ecotoxicology and ecological risk assessment (Altenburger et al., 2013). Chemicals invariably occur in mixtures in natural waters and the complex composition of these solutions reflects the weathering of mineral assemblages within the watershed, plus any anthropogenic inputs. In contrast to these environmental mixtures, aquatic toxicity testing is commonly conducted with individual chemicals in order to unambiguously attribute causality. In turn, water quality guidelines are developed for individual chemicals and have the potential to misrepresent toxicity of chemical mixtures (Mount et al., 2003).

Previous approaches for evaluating the toxicity of mixtures revolve around the concept of additivity (Bliss, 1939; Drescher and Boedeker, 1995; Feron and Groten, 2002; Rider and LeBlanc, 2005). Concentration addition (also known as dose addition) assumes that each chemical in the mixture contributes to toxicity through a common site of action; i.e., the same effect can be produced by replacing one chemical with an equally toxic amount of a different chemical (Altenburger et al., 2000). In contrast, response addition (also called independent action) assumes that chemicals in mixtures act statistically independent of each other; i.e., chemicals with different sites of action may affect different physiological systems (Backhaus et al., 2000). In each addition approach, biological responses to individual chemicals are combined to predict responses in mixtures of the chemicals (Olmstead and LeBlanc, 2005; Rider and LeBlanc, 2005). One important limitation of these addition approaches is that chemicals in a mixture do not interact (Vijver et al., 2010, 2011). Non-interaction for metal mixtures means that competition among multiple metals for organic and inorganic ligands in solution or during accumulation and uptake by organisms is not considered. Despite this limitation, literature reviews of metal mixture studies for a wide range of organisms have shown that the majority (70–75%) of

* Corresponding author. Tel.: +1 206 543 8966.
E-mail addresses: balistri@usgs.gov (L.S. Balistrieri), cmebane@usgs.gov (C.A. Mebane).
[1] Tel.: +1 208 387 1308.

0048-9697/$ – see front matter. Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.scitotenv.2013.07.034

toxicological results are consistent with less or equal toxicity relative to addition methods (Norwood et al., 2003; Vijver et al., 2011).

Within the past 15 years, the biotic ligand model (BLM) has been proposed as an approach for modeling the toxicity of metals (Paquin et al., 2002; Erickson, 2013). The BLM evolved from the gill surface interaction and free ion activity models (Pagenkopf, 1983; Playle, 1998; Sunda and Guillard, 1976) and links solution composition and chemical speciation of ions to the health of aquatic organisms (Niyogi and Wood, 2004). The model uses thermodynamic calculations to evaluate competitive interactions among dissolved major and minor cations, inorganic and organic ligands, and biological receptors (or biotic ligands) (Di Toro et al., 2001). These equilibrium calculations predict the chemical speciation of ions, including biotic ligands, for unique solution compositions. The model also relates predicted or measured accumulation of metal on the biotic ligand to acute or chronic toxicity (De Schamphelaere and Janssen, 2002; Paquin et al., 2002). BLMs typically are specific for an individual metal and organism and are used to predict dissolved metal concentrations that correspond to lethal accumulations of that metal on biotic ligands for unique water compositions (U.S. Environmental Protection Agency, 2007).

BLMs generally consider the toxicity of metals individually. However, Playle (2004) theoretically explored the loading or accumulation of multiple metals by biotic ligands using a BLM. He compiled binding constants for biotic ligand–metal interactions determined in single metal systems and combined them to model metal accumulation on biotic ligand sites in multiple metal solutions. One key simplifying assumption of this multiple metal modeling approach is that there are common sites of toxic action among different metals. That is, Playle (2004) assumed that Ag, Cd, Co, Cu, Pb, and Zn interrupt Ca homeostasis in fish, even though Ag and Cu previously were shown to interrupt Na homeostasis and Cd and Pb likely bind to different high affinity sites on the gill (Paquin et al., 2002; Winter et al., 2012). Another key assumption of the Playle (2004) approach is that 50% of organisms die at 50% metal loading of the gill.

To move from these theoretical calculations to real world conditions, the assumptions of a single type of biotic ligand site and 50% mortality at 50% total gill-metal load need to be re-considered. Multiple sites of toxic action (e.g., metal uptake through Ca or Na receptors) could be simulated by considering several types of biotic ligand sites with different binding characteristics. While the assumption of gill-metal load is reasonable for single metal systems where accumulation of an individual metal on the biotic ligands (LA50) is *directly* related to a given biological response (50% mortality), total gill-metal load in multiple metal solutions could result from very different distributions of metals on the gill. For example, the same total metal load could be distributed among one, two, or more metals, depending on metal affinities for biotic ligands and solution composition. In turn, differences in speciation of biotic ligands at the same total metal load could result in different toxicities because each metal may have a unique toxicity or potency. Stockdale et al. (2010) addressed these issues by considering humic acid with multiple types of binding sites as an analog for biological receptors and by developing a toxicity function that not only sums the amount of hydrogen and metal ions on the biotic ligand for a given solution composition, but also weights those loads using cation-specific potency coefficients. Stockdale et al. (2010) then used piecewise regression to relate the toxicity function, rather than metal loads or dissolved metal concentrations, to the health or diversity of benthic macroinvertebrates in natural aquatic systems. This approach is very different from evaluating the quality of water in the environment based on single metal concentrations (e.g., LC50 or lethal concentration at 50% mortality) or ratios of observed metal concentrations to water quality criteria (e.g., toxic unit (TU) = metal concentration/LC50). Rather, the toxicity function incorporates the composition of the solution as well as accumulation and potency of each metal on the biotic ligands, and, thereby, allows for a direct comparison of the relative toxicity of a wide range of solutions containing different pH, hardness, and concentrations of dissolved organic carbon, major ions, and single or multiple metals. In Balistrieri et al. (2012), we explored the approach of Stockdale et al. (2010) with a small toxicity dataset from metal-mixture field tests using binding constants for biotic ligand–metal interactions from Playle (2004) and a toxicity function called "Tox". We further develop and generalize our approach in the present work.

The three main objectives of this work are to:

(1) Present a quantitative approach that predicts impacts to the health of aquatic organisms (e.g., mortality) upon exposure to solutions containing single and multiple metals. Model parameters are determined using data compiled from previously conducted laboratory and field studies on survival of fish in synthetic and natural solutions containing various combinations and concentrations of Cd, Cu, Pb, and Zn (Farag et al., 2003; Hagler Bailly, 1996; Hansen et al., 2002; Marr et al., 1998, 1999; Mebane et al., 2012; Naddy et al., in preparation; Nimick et al., 2007; Stratus, 1999).
(2) Use the approach to predict and examine metal accumulation and associated toxicity in single metal and binary, ternary, and quaternary mixtures of Cd, Cu, Pb, and Zn. This portion of the work uses theoretical data sets that have variable metal concentrations but constant background solution composition.
(3) Demonstrate how the model can be used to compare the relative toxicity of natural samples and identify major contributors to metal toxicity at field sites. Previously collected data at the Elizabeth Copper Mine in Vermont is used in this part of the work (Balistrieri et al., 2007; Hathaway et al., 2001).

2. Approach for modeling the toxicity of metal mixtures

Our conceptual model is based on the biotic ligand model, and is a hybrid of existing models. The model includes (1) defining the equilibrium chemical speciation of the solution, (2) evaluating the accumulation of cations (H, Ca, Mg, Na, Cd, Cu, Pb, and Zn) by biological receptors, (3) defining a toxicity function, and (4) using a dose–response equation to relate the toxicity function to the survivability of aquatic organisms. The primary components of the approach include:

- Windermere Humic Aqueous Model 7 (WHAM 7) that predicts the chemical speciation of the solutions (Lofts, 2012; Tipping et al., 2011);
- Biological receptor–cation interaction model that defines the chemical speciation of biotic ligands, including toxicant accumulation by biota in single metal solutions and metal mixtures;
- Tox function that weights the accumulation of hydrogen ions and metal toxicants on biotic ligands; and
- Dose–response equation that links Tox to biological response (i.e., mortality of fish).

Each of these components is discussed below.

2.1. WHAM 7

WHAM 7 is incorporated into a computer program that is used to determine the chemical speciation of a solution at equilibrium (Lofts, 2012; Tipping et al., 2011). WHAM 7 considers both inorganic and organic complexation with cations. Several assumptions are used in the calculations. First, the average composition and metal-reactivity of dissolved organic matter (DOM) is used. DOM is assumed to be 50% dissolved organic carbon (DOC) (Thurman, 1985), 100% of DOM is fulvic acid, and 65% of DOC is reactive or complexes with metals (Bryan et al., 2002). Second, the importance of binding between organic matter and Al^{+3} and Fe^{+3} ions in natural waters is included by assuming that the solutions are in equilibrium with amorphous iron and aluminum hydroxides [$Fe(OH)_3$ and $Al(OH)_3$] (Tipping et al., 2008). The activity of dissolved Al^{+3} is calculated using a solubility product of $10^{8.5}$ at 25 °C for aluminum oxide [$Al(OH)_3 + 3H^+ = Al^{+3} + 3H_2O$], which is corrected for temperature using an enthalpy of −107 kJ/mol