AN UPDATED UNIFIED ZINC BIOTIC LIGAND MODEL FOR PROTECTION OF FRESHWATER AQUATIC LIFE AND ITS APPLICATION FOR SITE-SPECIFIC WATER QUALITY OBJECTIVES

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AUTHOR CONTRIBUTION

Adam C. Ryan: Conceptualization; data curation; formal analysis; investigation; methodology; visualization; writing-original draft; writing-review & editing; **Robert C. Santore:** Conceptualization; writing-original draft; writing-review & editing; **Kenneth C. Schiff:** Conceptualization; writing-original draft; writing-review & editing.

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No disclaimer

DATA ACCESSIBILITY STATEMENT

All data used in this evaluation are publicly available and can be accessed in the supplementary materials or can be provided by the authors. All tools used in this evaluation (i.e., blm_245.exe provided with BLM Research Version 3.41.2.45; PEST Version 15; R Version 4.2.1) are publicly available. Please contact corresponding author Adam Ryan with data requests (acryan@zinc.org).

AN UPDATED UNIFIED ZINC BIOTIC LIGAND MODEL FOR PROTECTION OF FRESHWATER AQUATIC LIFE AND ITS APPLICATION FOR SITE-SPECIFIC WATER QUALITY OBJECTIVES

ABSTRACT—A unified biotic ligand model (BLM) was developed to predict acute Zn toxicity to four invertebrates and two fish, and chronic toxicity to three invertebrates and a fish. Developed using a comprehensive ecotoxicity database, this unified BLM represents the first update to the unified Zn BLM in nearly 14 years. For comparative purposes, a unified Zn multiple linear regression (MLR) model was also developed. Both models are unified because they each use a single set of parameters to characterize the effects of toxicity modifying factors (e.g., pH, dissolved organic carbon [DOC], and major ions) on acute and chronic Zn toxicity. While both models were capable of accurately predicting Zn toxicity, the unified BLM performed marginally better based on quantitative model performance scores (MPS) and qualitative single-variable pH and DOC evaluations. The unified Zn BLM, which also performed better than the U.S. Environmental Protection Agency's hardness equation, was then used to normalize separate acute and chronic species sensitivity distributions (SSDs) to develop acute and chronic 5th percentile hazardous concentrations (HC5s) analogous to USEPA's aquatic life ambient water quality criteria (WQC). The unified Zn BLM-based WQC were shown to be protective of threatened and endangered species in California and appear to be protective of chemosensory endpoints for salmonids. Using monitoring data for California as a test case, chronic unified BLM-based WQC were lower than hardness-based WQC in 77% of samples, yet fewer WQC exceedances were observed. Implementation of the Zn BLM for site-specific water quality objectives (SSWQOs) using the fixed monitoring benchmark (FMB) approach indicates

that for the California dataset, dry weather and wet weather samples should be considered separately to develop SSWQOs for each condition.

KEY WORDS

Zinc, Bioavailability, Biotic Ligand Model , Water Quality Criteria, Implementation

INTRODUCTION

The bioavailability and toxicity of zinc (Zn) in freshwater systems are influenced by multiple water chemistry characteristics. However, current U.S. Environmental Protection Agency (USEPA) acute and chronic ambient water quality criteria (WQC) for Zn are adjusted solely as a function of water hardness. This approach reflects recognition of hardness as a toxicity-modifying factor (TMF; e.g., USEPA 1987; Spry and Wood 1989; Hogstrand et al. 1998), but it does not account for several additional water chemistry variables now known to influence Zn bioavailability. Since the development of the existing hardness-based WQC equations in the late 1980s (USEPA 1987, 1996), nearly four decades of research have expanded understanding of the roles of pH, dissolved organic carbon (DOC), and major ions in modifying Zn toxicity.

Numerous laboratory studies demonstrate that DOC can significantly reduce Zn bioavailability by complexing dissolved Zn and limiting uptake at biological surfaces. Across a range of taxa, Zn effect concentrations (ECx, where x denotes the effect level) generally increase by approximately 1.5- to 3-fold as DOC concentrations increase from <1 mg/L to >10 mg/L (e.g., Heijerick et al. 2003, 2005; Hyne et al. 2005; Bringolf et al. 2006; Clifford and McGeer 2009; Hoang and Tong 2015). The magnitude of DOC effects appears to increase at concentrations above approximately 6 mg/L, which are common in many freshwater systems, including wetlands and forested watersheds (Liu and Wang 2022). Collectively, these results indicate that reliance on hardness alone can mischaracterize Zn bioavailability under environmentally relevant conditions.

The influence of pH on Zn toxicity is more variable and appears to depend on species, exposure duration, and interactions with other water chemistry characteristics. Acute Zn toxicity has been reported to either decrease with increasing pH or remain relatively constant across moderate pH ranges, while chronic toxicity is generally less responsive to pH, particularly in waters with >5 mg/L DOC (Cusimano et al. 1986; Schubauer-Berigan et al. 1993; Heijerick et al. 2002, 2003; Hyne et al. 2005; Hoang and Tong 2015). These apparent differences in responses on the basis of dissolved Zn underscore the importance of considering pH in conjunction with other TMFs, rather than as an isolated predictor of toxicity.

In contrast, the ameliorative effects of major cations on Zn toxicity are well established. Calcium (Ca), magnesium (Mg), and sodium (Na) consistently reduce Zn bioavailability through competitive interactions at biological uptake sites, with Ca generally exerting the strongest influence (Heijerick et al. 2002; De Schamphelaere and Janssen 2004; Clifford and McGeer 2009). Potassium (K) has not been shown to provide similar protection. The combined effects of these cations form the mechanistic basis for hardness adjustments, but their individual contributions and interactions with other TMFs are not fully captured by hardness alone.

Recognizing the limitations of hardness-based criteria, several approaches have been developed to incorporate multiple TMFs into Zn WQC derivation, including the water-effect ratio (WER) method (USEPA 1994; Diamond et al. 1997), the biotic ligand model (BLM; Di Toro et al. 2001; Santore et al. 2002; Heijerick et al. 2002; DeForest and Van Genderen 2012), and multiple linear regression (MLR) models (e.g., Brix et al. 2021; DeForest et al. 2023). While WERs can provide site-specific adjustments, their cost and sensitivity to reference water selection limit widespread application. In contrast, BLMs and MLR models provide cost-effective, science-based alternatives that explicitly incorporate key water chemistry drivers of metal bioavailability.

For Zn, several BLMs have been developed, beginning with separate acute models (Santore et al. 2002; Heijerick et al. 2002) and later expanding to include chronic applications. DeForest and Van Genderen (2012) demonstrated that a single unified Zn BLM, using one set of biotic ligand parameters, could successfully predict both acute and chronic ECx values across a range of water chemistries. More recently, DeForest et al. (2023) developed separate acute and chronic Zn MLR models and corresponding BLMs using an expanded ecotoxicity database and demonstrated that both modeling approaches outperformed hardness-based criteria. However, despite the substantial growth of the underlying database since development of the unified Zn BLM in DeForest and Van Genderen (2012), unified BLM or MLR models were not evaluated in that effort.

Several considerations provide strong motivation for revisiting and updating a unified Zn BLM. First, the unified Zn BLM has not been updated in more than a decade, during which time >100 additional Zn ECx values have become available, substantially expanding the database supporting its development. Second, from a mechanistic perspective, the influence of water chemistry on Zn bioavailability is not expected to depend on exposure duration associated with acute vs. chronic exposures. The fundamental processes governing Zn bioavailability in the BLM framework (i.e., speciation and competition with major cations) are shared across acute and chronic exposures, suggesting that separate models for different effect durations may be conceptually unnecessary. Third, unified models offer practical advantages for environmental assessment and management, including reduced implementation complexity and avoidance of internal inconsistencies (e.g., chronic hazardous concentrations exceeding acute values) that can arise when separate models are applied across diverse water chemistries. Finally, unified BLMs have been successfully developed and applied for other metals, including copper (USEPA 2007; ECCC 2019) and nickel (B.C. Ministry of Water, Land, and Resources Stewardship 2024), demonstrating the feasibility and regulatory utility of a single-model approach.

In parallel with model development, the adoption of bioavailability-based WQC highlights the need for clear implementation frameworks to translate time-variable criteria into site-specific water quality objectives (SSWQOs). The fixed monitoring benchmark (FMB; Ryan et al. 2018) approach provides a robust method for applying BLM- or MLR-based criteria using routine monitoring data in a manner consistent with USEPA (1985). Importantly, many implementation challenges are shared across modeling approaches. Although MLR models offer simplicity and ease of incorporation into regulatory standards, application of separate acute and chronic MLR-based criteria has led to implementation challenges in some jurisdictions, including cases where acute criteria are numerically lower than chronic criteria across wide ranges of water chemistry conditions (e.g., Washington State Department of Ecology 2024). Such issues are less likely to arise with unified modeling frameworks.

Biotic ligand models also offer several advantages for implementation, including mechanistic treatment of interacting TMFs, applicability across media, and the ability to support forensic evaluations of bioavailability drivers in complex exposure scenarios. Recent advances have further streamlined BLM application by reducing input requirements to a subset of commonly measured parameters (e.g., pH, DOC, and hardness; Windward 2019) and enabling deployment within open-source computational environments (e.g., the {BLMEngineInR} package; Santore and Croteau 2025). These developments are anticipated to facilitate broader adoption by regulatory agencies and the regulated community thereby improving transparency in decision-making.

The present study updates the unified Zn BLM originally developed by DeForest and Van Genderen (2012) using an updated comprehensive ecotoxicity database and evaluates its application for derivation and implementation of Zn WQC. For comparative purposes, a unified Zn MLR model was also developed using methods consistent with DeForest et al. (2023). The objectives of this work were to: (1) develop an updated unified Zn BLM applicable to both acute and chronic exposures; (2) develop a unified Zn MLR model for comparison; (3) apply the unified Zn BLM in a manner consistent with USEPA guidelines for WQC development (USEPA 1985); and (4) demonstrate an implementation approach for BLM-based SSWQOs using monitoring data from California.

MATERIALS AND METHODS

Toxicity database

We regularly maintain and update a comprehensive Zn ecotoxicity database to provide a resource that can be used for ecological risk assessment and to support regulatory jurisdictions during development of water quality criteria/guidelines/objectives. The data used in the current study (Table S1) is a subset of the parent database and is consistent with the data used by DeForest et al. (2023). With the exception of removing 11 observations from the acute dataset for *O. mykiss* which were inconsistent with the other data (i.e., immobilization endpoint, and fish age/size of 1 day post hatch or ≤0.1 g]), all changes since publication of DeForest et al. (2023) involved adding water chemistry data or making estimates for missing BLM inputs. These changes generally affected the data used for development of species sensitivity distributions (SSDs) because information for each BLM input was needed for development of the acute and chronic BLMs described by DeForest et al. (2023). Because the focus of this application was to derive BLM-based WQC, this analysis focused on toxicity data that met USEPA (1985) guidelines for WQC development.

While our ecotoxicity database is comprehensive due to the inclusion of all types of reported ECx (e.g., no observed effect concentrations [NOECs], lowest observed effect concentrations [LOECs], maximum acceptable toxicant concentrations [MATCs], 10% inhibitory/effect/lethal concentrations [IC/EC/LC10s], 20% inhibitory/effect/lethal concentrations [IC/EC/LC20s], 50% inhibitory/effect/lethal concentrations [IC/EC/LC50s], etc.), exposure durations, and organism life stages, the data were curated and screened appropriately for the two components of WQC development. As in DeForest et al. (2023), data for refinement of the Zn BLM were constrained to acute and chronic toxicity data that represented consistent effect levels (i.e., 50% effect for acute and generally 20% effect level or MATC for chronic), exposure durations, and life stages. Additionally, for Zn BLM refinement, only those organisms for which Zn toxicity was evaluated over a sufficient range of water chemistry conditions were considered (i.e., as described in Brix et al. [2017]).

In contrast to the data subsets used to refine the Zn BLM, the data used to develop the SSDs were less constrained, while still generally meeting USEPA (1985) guidelines for WQC development (see DeForest et al., (2023) for details and exceptions). In short, only acute toxicity data expressed as LC50s or EC50s from 48- to 96-h exposures (some exceptions for species-specific exposure duration) and chronic toxicity data from life cycle exposures for invertebrates and early life stage, partial life cycle, or full life cycle exposures for fish were considered for SSD development. Chronic data expressed as EC20s were preferentially used, although chronic values such as MATC, LOEC, and NOEC were considered in lieu of EC20s for relatively insensitive organisms. Although algae and aquatic plants are not directly incorporated into calculation of USEPA's WQC (USEPA 1985), they are considered in the criteria development process (via an evaluation of the Final Plant Value). The ecotoxicity database (Table S1) includes studies on algae and aquatic plants to support this evaluation and to be consistent with other jurisdictions that directly consider plant data in setting WQC.

Estimating missing BLM inputs

Many toxicity tests considered for BLM development and SSD development were missing data for BLM inputs. For BLM calibration, toxicity tests with reported pH, hardness (or Ca and Mg), and DOC were preferred, but to expand the scope of the dataset source water information was used to make estimates where data were missing. Estimations of DOC from source water were conducted according to Appendix C in USEPA (2007) or, in some cases, using professional judgment based on water recipes or the source of the dilution water. For example, in chronic tests with daphnids, DOC will increase with addition of food. If the DOC concentration was reported only in the dilution water prior to testing, or if DOC was estimated (e.g., for tests conducted in synthetic water), a DOC concentration of 2 mg/L was assumed in chronic daphnid tests (Besser et al. 2021).

For toxicity tests with missing data for major ions (e.g., Ca, Mg, Na, K, SO_4, Cl, and alkalinity), information about laboratory dilution waters was used to estimate concentrations. Laboratories generally use consistent water sources over time and often report enough information to provide a reasonably complete picture of their exposure water conditions (e.g., USEPA Great Lakes Toxicology and Ecology Division Laboratory, USEPA Western Ecology Division Laboratory, Colorado Parks & Wildlife Aquatic Toxicology Laboratory, USGS Columbia Environmental Research Center). That type of information was used to fill data gaps prior to using the BLM to normalize ECx values for the SSDs. When alkalinity was not reported, we assumed equilibrium with the atmosphere and estimated dissolved inorganic carbon concentrations using pH and pCO_2.

Generally, we required that pH be reported, but five studies requiring pH estimates were retained. Three of the studies (Carlson and Roush 1985; Norberg-King 1989; Sibley et al. 1996) used Lake Superior water from the USEPA Duluth, MN laboratory as their dilution water, and the other two studies (Cairns et al. 1978; Chadwick Ecological Consultants 2005) provided the recipes for their dilution waters. Because these water types have been repeatedly characterized, we were reasonably confident in estimating pH for these waters. In the database used for BLM development and WQC derivation (Table S1), all estimates for missing BLM inputs are indicated. All BLM calculations were performed with the collated reported and estimated values. Table S2 provides a summary of how many estimates were made and describes the rationale for the limited number of pH estimates.

Update of the unified Zn BLM

Consistent with the approach taken in DeForest et al. (2023) and recommended by Brix et al. (2017), we used data for organisms tested over a minimum DOC range of 5 mg/L, a minimum hardness range of 100 mg/L, and a minimum pH range of 1.5 pH units. For acute toxicity, *Ceriodaphnia dubia*, *Daphnia magna*, *D. pulex*, *Oncorhynchus mykiss*, *Pimephales promelas*, and *Pomacea paludosa* met these criteria. For chronic toxicity, *C. dubia*, *D. magna*, *Lymnaea stagnalis*, *O. mykiss*, and *Raphidolcelus subcapitata* met these criteria. In DeForest et al. (2023), acute and chronic BLMs were developed for individual species and then separate pooled acute and pooled chronic models were developed. Here, the primary objective was to develop a unified

BLM. However, given that there were some updates to the database, we also updated the species-specific BLMs.

Parameter Estimation Software (PEST, Version 15; Doherty 2018) was used to develop a unified set of BLM parameters for the BLM refinement dataset. The unified BLM was developed with two permutations of the dataset: (1) all acute (EC50) and chronic (EC20) fish and invertebrate data, (2) the same data considered in (1) except acute *P. promelas* data and chronic *C. dubia* data were excluded. In the second permutation, the *P. promelas* and *C. dubia* data were excluded because of excessive variability in observed ECx that could not be explained by previous MLR models or BLMs (DeForest et al. 2023). Additionally, separate acute and chronic species-specific BLM optimizations were performed to provide an indication of how much the unified BLM differed from the species-specific models, and to provide direct comparisons to the models described in DeForest et al. (2023).

The PEST program performs parameter optimization by an iterative, least squares procedure, and provides a mechanism for objectively developing BLMs with optimum sets of parameters, given the provided dataset and a set of user-developed instructions/constraints. Conceptually, application of PEST for this purpose is analogous to using analysis of covariance (ANCOVA) to develop a pooled MLR model. The difference is that the BLM executable is a standalone software tool that must be run iteratively while interacting with PEST so that PEST can determine the optimum set of BLM parameters that minimizes the difference between predicted and observed ECx in the calibration dataset. To develop a unified BLM, we instructed PEST to assign and optimize a consistent set of biotic ligand (BL) parameters (i.e., binding strengths of bioavailable Zn species [e.g., Zn^{2+} and $ZnOH^{+}$] and competing cations at the BLs) for acute and chronic effects for all organisms in the dataset simultaneously.

The PEST optimization procedure was conducted with log10-tranformed dissolved Zn ECx. Only the BL parameters and the critical accumulation level of Zn at the BL were optimized using PEST. During each iteration of PEST, log10-transformed predicted ECx are compared with the corresponding log10-transformed observed ECx and based on the residual error of the predictions, PEST makes subsequent changes to the BL parameters until the prediction error is minimized. For all PEST optimizations, the thermodynamic database specifying the bulk solution chemical reactions was not modified and was identical to the thermodynamic database provided with the BLM software (i.e., USEPA 2007, and https://www.windwardenv.com/biotic-ligand-model/). Similarly, this optimization procedure did not modify any dissolved organic matter reactions used by the BLM. All BLM calculations were performed using the BLM executable (i.e., blm_245.exe which is distributed with the Biotic Ligand Model Windows® Interface, Research Version 3.41.2.45 (Windward 2019). We used the ranges of BL log K values identified in DeForest and Van Genderen (2012) to guide definition of the ranges over which PEST would search for optimum BL log K values. The ranges for each log K were expanded by one log unit at the lower and upper ends of the range. For example, the range of BL-Ca log K values reported for BLMs in DeForest and Van Genderen (2012) was 3.2 to 4.9. Therefore, the range we used for PEST optimizations (for all fish and invertebrates) was 2.2 to 5.9. Table 1 provides the ranges and starting values used for the PEST optimizations.

For all PEST optimizations, two of the BL parameters (BL-Zn and BL-H) were used to anchor the optimization procedure and were therefore held constant in all optimizations. The BL-Zn log K had a narrow range (5.3-5.6) in all BLMs summarized in DeForest and Van Genderen (2012), so it was fixed at 5.4. To accommodate a pH effect, while including both BL-H and BL-

ZnOH, the log K for BL-H was fixed at 6.4 (which is the mean value from literature, ranging from 5.9-6.7, as summarized in DeForest and Van Genderen [2012]).

Development of a unified Zn MLR model

For comparative purposes, a unified Zn MLR model was also developed using the BLM development dataset described here. Given that pH, DOC, and hardness were identified as the most important TMFs in the MLR models developed by DeForest et al. (2023), it was logical to develop a unified Zn MLR model using those TMFs as independent variables. In a manner consistent with developing an acute or chronic pooled MLR model, we used the combination of species+endpoint (e.g., *C. dubia* Acute EC50, *D. magna* Chronic EC20, etc.) as the categorical variable in the MLR model. The unified MLR model was fitted to the calibration dataset using the lm() function in R (R Version 4.2.1; R Core Team 2022). The form of the model fitted was the same as used by DeForest et al. (2023), where the dependent variable was ln(ECx) and the independent variables were pH, ln(DOC), and ln(hardness).

Model comparison

The unified BLM and unified MLR model were compared in a manner consistent with the approach used by DeForest et al. (2023). In short, plots of predicted vs observed ECx, plots of residuals vs. TMFs, and model performance scores (MPS) were used to qualitatively and quantitatively evaluate model performance. For additional comparisons, these same evaluations included predictions made by the unified BLM from DeForest and Van Genderen (2012), the separate acute and chronic MLR models from DeForest et al. (2023), and the hardness equation (USEPA 1996). The MPS calculation performed here (Equation 1) was modified slightly from DeForest et al. (2023) to use an r^2 calculation (Equation 2) that corresponds to the variability explained by the 1:1 line of perfect agreement on the predicted vs. observed plots (consistent with the MPS calculations in Besser et al. [2021]). For this calculation, *SSR* is the sum of squared residuals, *SST* is the total sum of squares, and *i* identifies the *i*th observation in the dataset. In this formulation, if the predictions agree exactly with the observations, the sum of squared residuals (SSR) = 0, so $r^2 = 1$, which is the desired case. Residual scores (RS_{pH}, RS_{DOC}, $RS_{Hardness}$) were calculated from the slopes of linear regressions of the residuals [i.e., log10(observed ECx/predicted ECx)] vs. pH, log10(DOC), and log10(hardness), respectively. The RS calculations are described by Equation 3, where s_i is the slope of the relationship with the *i*th independent variable, and p_i is the p-value associated with the *i*th slope. Finally, $RF_{x,2.0}$ is calculated by determining the fraction of the predictions that are within 2-fold of the observed ECx.

$$MPS = \frac{r^2 + RF_{x,2.0} + RS_{pH} + RS_{DOC} + RS_{Hardness}}{5} \qquad \text{Equation 1}$$

$$r^2 = 1 - \frac{SSR}{SST} = 1 - \frac{\sum_i (predicted\ ECx_i - observed\ ECx_i)^2}{\sum_i (observed\ ECx_i - mean\ observed\ ECx)^2} \qquad \text{Equation 2}$$

$$RS_i = \frac{2}{\left(1 + 10^{\left|s_i \times (1 - p_i)\right|}\right)} \qquad \text{Equation 3}$$

Additionally, data from tests separately evaluating the effects of pH and DOC without varying other water chemistry conditions were used to qualitatively assess unified BLM and unified MLR model performance. For these evaluations, plots of predicted vs. observed ECx were used to assess model performance under conditions where either pH or DOC was the only variable. Additionally, observed ECx were plotted against either pH or DOC with simulated ECx representing predicted pH or DOC effects under the reported water chemistry conditions. These types of plots provide additional insight into predicted model behavior under varying TMF conditions. Appropriate data for these plots were available for either acute or chronic ECx for several species (e.g., *C. dubia*, *D. magna*, *D. pulex*, *P. promelas*, *P. paludosa*, and *O. mykiss*).

Ambient WQC

Acute and chronic Zn HC5s were developed by incorporating BLM normalization into the USEPA procedure for deriving WQC (USEPA 1985). Application of the Zn BLM for WQC in the US is straightforward, and the approach has been described previously for Cu, Zn, and Pb (USEPA 2007; DeForest and Van Genderen 2012; DeForest et al. 2017, respectively). After selecting data appropriate for developing acute and chronic sensitivity distributions (described above), the first step in determining the 5th percentiles of the genus sensitivity distributions (i.e., the final acute value [FAV] and final chronic value [FCV]) was to use the unified Zn BLM in speciation mode to estimate the Zn critical accumulations for each ECx in the acute and chronic WQC datasets. This step uses the reported/estimated water chemistry conditions associated with each reported Zn ECx to estimate how much Zn would need to be present at the BL (i.e., the critical accumulation) to result in the reported Zn ECx. For both the acute and chronic WQC datasets, geometric mean critical accumulation values were calculated for each species. For the acute WQC (i.e., criteria maximum concentration [CMC]) dataset, the resulting values were the critical accumulations associated with the species mean acute values (SMAVs), and for the chronic WQC (i.e., criteria continuous concentration [CCC]) dataset, they were the species mean chronic values (SMCVs). Using the critical accumulations for the SMAVs and SMCVs, critical accumulations associated with the genus mean acute values (GMAVs) and genus mean chronic values (GMCVs) were calculated by determining the geometric means at the genus level. Finally, the critical accumulations associated with the 5th percentiles of the GMAVs and GMCVs were used as the FAV and FCV critical accumulations, which can be used by the Zn BLM to derive acute and chronic WQC under specified water chemistry conditions.

Sensitivity of BLM and WQC to estimated inputs

A sensitivity analysis was performed to evaluate the potential consequences of uncertainty in estimated BLM inputs on optimized BL parameters and HC5 determination (see details in Supplementary Text 1). This was accomplished by simultaneously increasing or decreasing all estimated inputs by 1.25- to 2-fold to understand potential effects on optimized BL parameters and HC5s. The choice of which factor to increase or decrease each estimated input was driven by the standard deviations of TMF concentrations from reconstituted laboratory waters with reported measured TMF concentrations. The joint probability of independent events (Zar 1999) was calculated for each scenario to provide an indication of the likelihood of making estimates that are either high or low by up to ~2 standard deviations based on the variation in the type of data used to inform the estimates (e.g., data from related studies or from studies using similar dilution waters).

Application of the Zn BLM to California surface waters

To demonstrate application of the unified Zn BLM, the model was applied to a dataset compiled for California from data retrieved from the water quality portal (Water Quality Portal 2021). California was used as an example application because of interest from the State Water Resources Control Board in using the BLM as an option for developing SSWQOs. All data were screened to include only freshwater samples taken from rivers, streams, lakes, and reservoirs, and data were retained only if sufficient information for BLM inputs was provided. Dissolved inorganic carbon (DIC) concentrations were estimated from pH and alkalinity in samples for which alkalinity was reported. For cases in which alkalinity was not reported, pH and atmospheric CO_2 partial pressure ($pCO_2 = 10^{-3.42}$) were used to calculate DIC. To evaluate the effect of migrating from hardness-based to BLM-based WQC, BLM-predicted WQC and hardness-based WQC were calculated for each sample and then hazard quotients (HQs) were calculated as the quotient of reported Zn concentration divided by the calculated WQC. These calculations were performed on a per sample basis. Therefore, each hazard quotient represents an instantaneous indication as to whether the BLM- or hardness-based WQC was exceeded.

Application of the Zn BLM to develop SSWQOs

The unified Zn BLM and the fixed monitoring benchmark (FMB) approach (Ryan et al. 2018) were used with data collected from the Los Cerritos Channel to demonstrate how SSWQOs could be developed for locations with time variable WQC. The data used for this evaluation were provided by Richard Watson & Associates on behalf of the Los Cerritos Channel Watershed Group and represented the freshwater portion of the Los Cerritos Channel and several tributaries near Los Angeles, CA (Table S3). Zinc and Cu concentrations and all necessary BLM inputs have been collected for the Los Cerritos Channel and 4 tributaries since 2018 in anticipation of BLM adoption by the state of California as statewide WQC or through state provisions allowing the use of the BLM for SSWQOs (Richard Watson, Richard Watson & Associates, Inc., personal communication, 18 January 2024). The dataset includes monitoring data for routine compliance sampling (which occurs during dry weather), additional dry weather sampling, and wet weather sampling. The data included in our analysis extend from 2018 to the first quarter of 2023 and provide sufficient data for each site to calculate Zn BLM-based FMBs. The Zn BLM was used to develop acute and chronic FMBs for various permutations of the dataset (e.g., combining and separating wet and dry sampling events).

RESULTS

Toxicity database

The complete acute and chronic toxicity database considered in this evaluation is provided in Table S1 (n = 998 observations). The database includes test details and identifies all observations used in the development of the current unified Zn BLM, as well as the observations used to develop the genus sensitivity distributions (GSDs) for HC5 calculations. To transparently indicate cases for which estimates were made for BLM inputs, three blocks (grouped sets of columns, with appropriate headers) of water chemistry information are provided. The first block represents collated reported and estimated BLM inputs, the second block represents reported BLM inputs, and the third block represents estimated BLM inputs. Data included in Table S1 also represent algae, plants, macrophytes, and cyanobacteria; however, they were removed from the definitive evaluation due to historical USEPA exclusion (n = 78 observations; retained as “other” data). With the exception of data for *R. subcapitata*, most of these “other” data were incomplete with respect to BLM input data. Additionally, 86 observations were removed due to incomplete information with respect to BLM input data, resulting in a definitive dataset which included a total of 834 observations.

Initially, 325 of these observations were used to update the unified Zn BLM, and the remainder of the data were used for acute and chronic SSDs and GSDs. However, as described above, acute *P. promelas* data (n = 18) and chronic *C. dubia* data (n = 18) were ultimately excluded from the definitive BLM refinement dataset. As a result, 289 observations were included in the definitive unified Zn BLM dataset. The definitive acute GSD dataset included 623 observations, and the definitive chronic dataset considered 211 observations. For the chronic GSD, an additional analysis was performed to identify the most sensitive endpoints for each species, and only those data were used for construction of the chronic GSD.

Unified Zn BLM and unified Zn MLR model

Six species from acute tests (*C. dubia*, *D. magna*, *D. pulex*, *O. mykiss*, *P. paludosa*, and *P. promelas*) and five species from chronic tests (*C. dubia*, *D. magna*, *L. stagnalis*, and *O. mykiss*) met minimum DOC, hardness, and pH requirements for inclusion in unified BLM and unified MLR model development . The ranges of DOC, hardness, and pH are provided for each exposure type and species in Table S4. Table 2 summarizes the optimized BLM parameters and fitted unified MLR model parameters and Table S5 provides parameters for the species-specific BLMs. Critical accumulations and critical intercepts determined for each species used in the optimization of the unified Zn BLM and fitting of the unified MLR model are also provided in Table 2. For the species-specific BLMs, the PEST *r* values range from 0.68 for chronic *D. magna* to 0.97 for acute *P. paludosa* (Table S5). Removal of acute *P. promelas* data and acute *C. dubia* data from the optimization dataset for the unified BLM had relatively minor effects on the log K values for the BL reactions, with the largest relative change occurring for Na, the least important of the TMFs. The largest differences in log K values between the unified BLM and the species-specific BLMs are also for Na, which is shown to be relatively unimportant with log K values at the lower bound of the optimization space (Table S5).

The unified BLM (r^2 = 0.75; Figure 1A) performs similarly to the unified MLR model (r^2 = 0.71; Figure 1D), and both models perform better than current nationally recommended hardness equation (USEPA 1996; r^2 = 0.60; Figure 1E). Also performing similarly are the unified BLM from DeForest and Van Genderen (2012; Figure 1B) and the separate acute and chronic MLR models from DeForest et al. (2023; Figure 1C). All BLMs and MLR models predict a higher percentage of ECx values within a factor of 2 than the hardness equation (Figure 1F). It should be noted that 86% of the observations in the BLM development dataset reported

DOC concentrations <5 mg/L. Therefore, much of the water chemistry-associated variability in Figure 1 is due to changes in major ion concentrations (e.g., water hardness) and pH.

While r^2 and $RF_{x,2.0}$ were calculated for the entire unified BLM development dataset to give an overall sense of performance, the r^2, $RF_{x,2.0}$, and RS values used to calculate the MPS were determined for each individual dataset and then were used to calculate the mean MPS (Table 3). Table S6 provides a summary of MPS calculated for the unified BLM and unified MLR model for each species+endpoint combination included in the model development dataset. On the basis of MPS, the unified BLM (this study) slightly outperforms the other BLMs and MLR models. All BLMs and MLR models outperform the hardness equation.

The unified BLM and unified MLR model perform show similar overall performance with acute and chronic toxicity tests varying either DOC or pH while holding other water chemistry conditions constant (Figure 2; Figure S1), with a few notable differences. Both models predict the majority of ECx within 2-fold of observed ECx, but the BLM underpredicts 2 ECx and overpredicts 1 ECx in acute *P. promelas* tests (Figure 2A) and the MLR model overpredicts 1 ECx and underpredicts 1 ECx in chronic *C. dubia* tests (Figure 2C). The single variable plots in Figure S1 suggest that the slope of the BLM-based DOC predictions appears to be too steep for *P. promelas* tests by Bringolf et al. (2006), and that the MLR-based DOC slope appears to be too shallow for most of the *C. dubia* tests by Heijerick et al. (2003). On the basis of pH, the BLM and the MLR model over- or underpredict 8 observations. The notable difference is that the BLM is more accurate with the low acute *O. mykiss* ECx values from Cusimano et al. (1986). Figure S1 indicates that for the acute tests the slopes of the relationships are different for the different data series and that the BLM tracks many, but not all these data series. In contrast, the MLR model generally splits the difference with a constant pH slope. Regarding the effects of pH in chronic tests, the data suggest that ECx are largely unaffected by increases in pH or that they increase slightly. Similarly, the BLM predicts that under the conditions of these tests, ECx should largely be unaffected by pH or slightly increase with pH. Conversely, the unified MLR model predicts slight decreases in ECx. While both models appear to be capable of accurately predicting these results, the BLM tends to better track the shape of the relationships.

Ambient WQC

Aggregation of BLM-normalized Zn EC50s at the species level resulted in an SSD including 121 SMAVs and a GSD including 88 GMAVs. The 20 most sensitive genera included in the BLM-normalized GSD are shown in Table 4, and the GSD is shown in Figure 3A. Table S7 provides a complete summary of all BLM-normalized SMAVs and GMAVs for all 88 genera for which BLM normalizations could be performed. The most acutely sensitive species was the baetid mayfly *Neocloeon triangulifer*, followed by the rotifer *Euchlanis dilatata*, the snail *Leptoxis ampla*, the rotifer *Lecane quadridentata*, the amphipod *Hyalella azteca*, and the genus for *Ceriodaphnia* water fleas. The calculated FAV (i.e., HC5) critical accumulation level for the acute GSD was 1.011 nmol/gw (Table 2). Using the USEPA moderately hard water recipe (provided in Table 4), the BLM-calculated HC5 is 152 μg/L. Following USEPA (1985), dividing the FAV by two provides a CMC of 76 μg/L. For this same water composition, the current USEPA hardness-based CMC would be 102 μg/L.

After screening the dataset to include only the most appropriate chronic ECx associated with each individual toxicity test, the chronic SSD included 161 observations and consisting of 136 EC20s, 23 MATCs, and 2 NOECs (Table S8). The 2 NOECs were retained in tests for which no LOECs were available. Of the 161 observations considered for development of the chronic SMCVs and GSD, 150 observations were from flow-through or static renewal tests with fish or invertebrates. The remaining 11 observations were from static tests conducted with rotifers or bivalves.

Aggregation of BLM-normalized chronic values resulted in 38 SMCVs and 30 GMCVs. The BLM-normalized GSD is summarized in Table 5, and the chronic GSD is shown in Figure 3B. The most chronically sensitive species was the baetid mayfly *N. triangulifer*, followed by the bivalve *Lampsilis siliquoidea*, the snail *Potamopyrgus jenkinsi*, the water flea *C. dubia*, and the invasive clam species *Corbicula*. The calculated FCV (i.e., HC5) critical accumulation level for the chronic GSD was 0.1076 nmol/gw. Using the USEPA moderately hard water recipe (provided in Table 5), the BLM-calculated FCV corresponds to 17.4 µg/L. For this same water composition, the current USEPA hardness-based WQC is 103 µg/L.

Application of the Zn BLM to California surface waters

Figure 4 provides a summary of the comparisons of BLM- and hardness-based HQs for freshwaters in California. The dataset shown in Figure 4 includes 1,271 individual samples collected from surface waters across the state of California. Figures 4a and 4c represent BLM and hardness equation application to the California dataset, with no modification, and Figures 4b and 4d represent BLM and hardness equation application to the same dataset, but with inputs constrained to ranges informed by the prescribed ranges for BLM inputs (Windward 2019) and the ranges of inputs in the calibration and SSD datasets for BLM and hardness equation application (Table S4). Specifically, inputs were constrained to $0.1 \leq$ DOC ≤ 35 mg/L, $5.5 \leq$ pH ≤ 9.0, $3.2 \leq$ Ca ≤ 120 mg/L, $0.6 \leq$ Mg ≤ 52.0 mg/L, $0.2 \leq$ Na ≤ 240 mg/L, $2.0 \leq$ Alkalinity ≤ 360 mg/L as $CaCO_3$, and $10 \leq$ hardness ≤ 500 mg/L as $CaCO_3$. Given that the BLM bases its predictions on chemical speciation, alternative constraints can be applied. However, to avoid interpretation issues, constraints should not extend far beyond the calibration range and should not extend to conditions that are likely to be considered physiologically stressful. When constraining input values, the constraints were used as ceiling and floor values to avoid omitting data. For application of the hardness equation, the hardness constraint was applied after calculating hardness from constrained Ca and Mg concentrations. Despite the hardness values in the BLM development dataset extending to >800 mg/L as $CaCO_3$, the hardness ceiling was set to 500 mg/L as $CaCO_3$ as a compromise between this dataset and the typical hardness ceiling of 400 mg/L as $CaCO_3$ (e.g., as used in the "California Toxics Rule": 40 CFR § 131.38).

On the basis of CMCs, the BLM- and hardness-based HQs agreed in terms of WQC attainment and exceedance for 1,056 samples under the unconstrained scenario (Figure 4A; sum of lower left and upper right quadrants) and for 1,059 samples under the constrained scenario (Figure 4B). In both scenarios, the BLM identified a single sample as a CMC exceedance that was not also a hardness-based CMC exceedance. In the unconstrained scenario, the hardness equation identified 214 samples as CMC exceedances that were not also BLM-based CMC exceedances. In the constrained input scenario, the hardness equation identified 211 samples as CMC exceedances that were not also BLM-based CMC exceedances. For this dataset, BLM-based CMCs are higher than the hardness-based CMCs in 673 samples when inputs are not constrained (53% of samples; Figure S2a). When inputs are constrained, the BLM-based CMCs are higher than the hardness-based CMCs in 667 samples (52% of samples; Figure S2b).

On the basis of CCCs, the BLM- and hardness-based HQs agreed in terms of WQC attainment and exceedance for 1059 samples under the unconstrained scenario (Figure 4C) and for 1077 samples under the constrained scenario (Figure 4D). In the unconstrained scenario, the BLM identified 61 samples as CCC exceedances that were not also hardness-based CCC exceedances, whereas the hardness equation identified 151 samples as CCC exceedances that were not also BLM-based CCC exceedances. In the constrained input scenario, the BLM identified 63 samples as CCC exceedances that were not also hardness-based CCC exceedances, and the hardness equation identified 131 samples as CCC exceedances that were not also BLM-based CCC exceedances. For this dataset, the hardness-based CCCs are higher than the BLM-based CCCs in 979 samples (77% of samples; Figure S2c). When inputs are constrained, the hardness-based CCCs are higher than the BLM-based CCCs in 981samples (77% of samples; Figure S2d).

Application of the Zn BLM to develop SSWQOs

To explore how time-variable instantaneous WQC (IWQC) could be evaluated to derive SSWQOs, acute or chronic FMBs were calculated on separate datasets for wet and dry weather using data from the Los Cerritos Channel in southern California (Figure 5; Table 6). The Zn BLM-based chronic FMB for combined dry weather (i.e., dry weather samples + compliance samples) and wet weather samples for site LCC1 was 78.6 µg/L and the associated 50th percentile hardness-based IWQC was 138 µg/L (Figure 5A,D; Table 6).The FMBs and hardness-based WQCs both change substantially when the dry weather and wet weather samples are separated. The hardness-based median IWQC for the dry weather samples is 211 µg/L, and the BLM-based FMB is 268 µg/L (Figure 5B,E; Table 6). For wet weather samples, the acute FMB for LCC1 is 297 µg/L, while the hardness-based median IWQC is 30 µg/L (Figure 5C,F; Table 6). For the other sampling locations (i.e., SB4, SB8, SB9, and SB10), only wet weather sampling data were available, but they provided similar results as were observed for the wet weather samples from LCC1.

DISCUSSION

Comparison of the unified Zn BLM and unified Zn MLR with other models

The pH response of the optimized BLM in the present study (log $K_{ZnBL\text{-}ZnOH}$ of -2.45) was very similar to DeForest and Van Genderen (2012; log $K_{ZnBL\text{-}ZnOH}$ of -2.4). This provides a strong indication that the pH response characterized by the Zn BLM, now including 107 new data points, is conserved across freshwater taxa and endpoints. The results of the species-specific model optimizations (i.e., BL log K values) are similar to those reported for the nearly identical dataset used in DeForest et al. (2023).

Aside from the pH response, the hardness response in the unified BLM (this study) is characterized by stronger binding constants for both Ca and Mg than those used in DeForest and Van Genderen (2012). Except for the chronic species-specific model for *O. mykiss*, the optimized Ca binding constants for the species-specific BLMs in the present study (Table S5) are similar to or somewhat higher than that determined in DeForest and Van Genderen (2012) (Tables 1). For fish and invertebrates, only the acute species-specific BLMs for the cladocerans in the present study have lower optimized Mg binding constants than the unified BLM in DeForest and Van Genderen (2012). The highest Mg binding constant in the present study is for the chronic species-specific BLM for *O. mykiss*, for which the Ca binding constant is set equal to the lower PEST constraint.

The unified BLM (this study) also has a slightly higher Na binding constant than the one described in DeForest and Van Genderen (2012). Examining the species-specific Na binding constants, it is clear that there is a wide range of variability in the Na binding constants with Na appearing to be very important for some species, but much less so for other species. For example, acute species-specific BLMs for *C. dubia*, *D. pulex*, and *P. paludosa* and the chronic species-specific BLM for *L. stagnalis* were fixed at the lower log K boundary (i.e., 0.9) for the PEST optimizations. Yet the PEST optimized log K in the acute *O. mykiss* species-specific model was 4.4. The Na log K value for unified model indicates that overall, Na does appear to affect Zn bioavailability, so it was retained in the unified BLM.

As might be expected, the unified MLR slopes for pH, ln(DOC), and ln(hardness) (Table 3) are intermediate to the slopes for the separate acute and chronic models developed by DeForest et al. (2023). The pH slope in the present study is -0.2, while the acute and chronic pH slopes from DeForest et al. (2023) were -0.12 and -0.646, respectively. The ln(DOC) slope in the present study is 0.176, while the acute and chronic ln(DOC) slopes from DeForest et al. (2023) were 0.127 and 0.376, respectively. The ln(hardness) slope in the present study is 0.553, while the acute and chronic ln(DOC) slopes from DeForest et al. (2023) were 0.600 and 0.237, respectively. A less negative pH slope for acute ECx would improve MLR performance for some of the data in Figure S1 but would make it worse for Hyne et al. (2005) and Cusimano et al. (1986). While data from Cusimano et al. (1986) were not included in the calibration dataset used by DeForrest et al. (2023), data for Hyne et al. (2005) were included. A more negative slope for chronic ECx would make the predictions worse for all the data included in Figure S1, suggesting that data from tests in which pH covaried with other TMFs had a greater influence on the fitted pH slope. Similarly, a shallower slope for the DOC term would likely make predictions worse for the acute studies in which DOC concentrations were tested above 5 mg/L (Figure S1). Conversely, a higher slope for chronic tests in which DOC was varied would improve MLR predictions for the chronic DOC tests in Figure S1. It is worth noting that data from Heijerick et al. (2003) were not used in the development of the MLRs described by DeForest et al. (2023), nor were they used in the update of the unified BLM (this study).

Unified Zn BLM and unified Zn MLR model performance

The unified Zn BLM and unified Zn MLR model were shown to perform comparably based on qualitative and quantitative evaluations (Figures 1 and 2, Table 3). Despite the BLM having slightly higher MPS than the unified MLR model (0.78 vs. 0.75), both models predicted 84% of ECx to within 2-fold of observed ECx. However, the unified BLM (this study) arguably better approximates the behavior (i.e., shape of response) of ECx across ranges of DOC and pH when other water chemistry conditions are held constant (Figure S1). For example, the unified BLM generally predicts the shape of the DOC response in chronic *D. magna* tests, whereas the unified MLR model shows a much shallower slope that is inconsistent with the effect of DOC on ECx at DOC concentrations greater than 10 mg/L. Conversely, the unified BLM tends to underpredict ECx at DOC <5 mg/L. In terms of pH responses, the ability of the unified BLM to predict different pH responses under different conditions (i.e., in the very soft waters in the *O. mykiss* tests by Cusimano et al. 1986, and in the very hard *P. pomacea* tests by Hoang and Tong 2015) is clearly an advantage over the unified MLR model. This is also true in the chronic tests, where the data indicate either no effect of pH on ECx or that ECx slightly increases with increasing pH. It is worth noting that application of the unified BLM and unified MLR model to data from Cusimano et al. (1986) represents a true validation test, because those data were not included in the calibration dataset.

The primary data series for which the unified MLR model more accurately predicts the response to varying DOC concentrations is the acute results for *P. promelas* (Bringolf et al. 2006), The only difference between the Bringolf et al. (2006) tests and the other DOC tests is that Na concentrations were comparatively low (~4 mg/L), except in two of the high DOC tests where both Na and Cl concentrations were elevated (up to ~420 mg/L and 700 mg/L, respectively). It is also worth noting that the species-specific BLM and MLR models developed for *P. promelas* in DeForest et al. (2023) were exceedingly poor (with $r^2 < 0.05$), suggesting either that TMFs affect their response differently than for other organisms, or more likely that inter-lab variability has introduced noise to the dataset. The inability of TMFs to explain variation in EC50s for *P. promelas* in DeForest et al. (2023) was the reason we excluded from the dataset used to update the unified Zn BLM and unified MLR model.

From the perspective of WQC, the unified BLM (this study), the unified MLR (this study), and the separate acute and chronic MLR models (DeForest et al. 2023) offer potentially effective alternatives to hardness-based WQC. Figure 1 and Table 3 clearly indicate that all the BLMs and MLR models considered here are superior to the hardness equation based on r^2, $RF_{x,2.0}$, and MPS. It should be recognized that marginal differences in the MPSs for the BLMs and MLR models (e.g., 0.75 vs. 0.78) may to some extent be reflective of inherent variability in ecotoxicity data, but that the BLMs and MLR models consistently perform better than the hardness equation based on all quantitative metrics. Unified models would be the most parsimonious, and the unified MLR model offers the simplest model, but the qualitative evaluation with the single variable pH and DOC tests highlights some potential limitations. Use of a unified model would also prevent the potential issue of CCCs being higher than FAVs. For example, applying the unified BLM to nearly 100,000 freshwater sampling events compiled from the water quality portal (Water Quality Portal 2021), almost all (i.e., 99.98%) chronic criteria would be lower than acute criteria. Although the BLM-derived FAV is always higher than the BLM-derived FCV, there is a small percentage (0.02%) of sampling events for which the CMC is reduced to a lower value than the CCC due to the applied safety factor (i.e., the CMC = FAV/2). With the unified MLR model, this would never occur if the effective acute-to-chronic ratio is >2. In comparison, when applying the separate acute and chronic Zn MLR models

(DeForest et al. 2023), the acute and chronic criteria are inverted (chronic higher than acute) in 5% of the sampling events. Using the same dataset to evaluate this phenomenon for potential MLR-based Cu WQC (Brix et al. 2021) indicates an even higher inversion rate, but this issue does not occur with the current nationally recommended Cu BLM (USEPA 2007) which uses an acute-to-chronic ratio of 3.22.

Sensitivity of WQC to estimated BLM inputs

Many of the studies requiring estimates were also used by DeForest et al. (2023) to develop and apply Zn MLR models for WQC. Overall, we viewed the inclusion of studies that required some level of input estimation as beneficial because it expanded the scope of the acute and chronic SSDs and retained several sensitive genera that otherwise would have been omitted because one or more estimates were needed to establish BLM inputs. To emphasize this point, of the 10 most sensitive genera included in the acute GSD, only two species did not require some level of estimation for at least one BLM input (see Table 4 and Table S2). Similarly, for the chronic GSD, only one of the five most sensitive species (*Lampsilis siliquoidea*) did not require some level of estimation for at least one BLM input. Given that we are reasonably confident in the exposure chemistry estimates, omitting these data because of missing information would have neglected to include important sensitive taxa.

Even under reasonable worst-case scenarios, where all estimated BLM inputs were increased or decreased by as much as 2-fold for DOC concentration and 1.5-fold for major ions (ranges based on ~2 standard deviation variability observed for targeted water chemistries in the ecotoxicity database), the resulting change in BL binding constants was less than 5% in all cases and acute and chronic WQC determinations changed by generally less than 4% from the original input estimation scenario (Table S9 and Supplementary Text 1). It should also be noted that simultaneously modifying all estimated TMF inputs to an extreme value (i.e., increased or decreased by 2-fold) is a highly unlikely scenario. As an example, there is about a 2.3% chance that a single estimated DOC concentration would be 2-fold lower or higher (or approximately 2 standard deviations) than the mean estimate based on reported water chemistry from similar source waters, but the joint probability of even 5 estimated DOC concentrations simultaneously being 2-fold higher (or simultaneously 2-fold lower) than the estimate is $0.023^5 = 6.1E-09$. Even under these unlikely worst-case scenarios, a maximum deviation in CCC of 20.8% (or change from 17.7 µg/L to 14.0 µg/L) and a maximum deviation in CMC of 13.7% (or change from 75.8 µg/L to 65.4 µg/L) – with a median change of 1.2% for CCC and 3.2% for CMC for all scenarios is remarkable. Much of this can be traced to the pragmatism of the USEPA (1985) approach for deriving FAVs and FCVs, by which only the data for the 4 genera that are nearest to the 5th percentile are directly needed to calculate the FAV and FCV. Our evaluation demonstrated that unlikely worst-case estimation scenarios do not have much influence on the fits of BLM parameters or the identity of the 4 genera that are closest to the 5th percentile. This is an extremely important revelation that should temper concerns about the number of estimates present in the Zn ecotoxicity dataset.

Retaining data from studies requiring estimates for BLM inputs facilitated the development of a comprehensive ecotoxicity dataset that included many sensitive organisms that would have been omitted had the estimates not been made. Because several sensitive species were included in the final acute and chronic SSDs, the resulting CMC and CCC appear to be protective of threatened and endangered species in California, as well as likely protective of adverse impacts on salmonid chemosensory endpoints (Supplementary Text 2).

Applicable chemistry ranges for unified Zn BLM-based WQC

The dataset used to develop the unified Zn BLM covers a wide range of water chemistry conditions (Table S4). The ranges of pH, DOC, and hardness considered in the unified Zn BLM dataset were 5.42-8.6 S.U., 0.09-29 mg/L, and 6.7-826 mg/L as $CaCO_3$, respectively. All other major ions covered a similarly wide range (see Table S4). Approximately 98% of the pH values in the Zn ecotoxicity database were reported as measured, including the minimum and maximum reported values. Roughly half of the DOC concentrations used in the unified Zn BLM calibration dataset were estimated, and the vast majority of those estimates were for laboratory derived waters (i.e., well water, dechlorinated municipal water, or reconstituted waters). The highest and lowest DOC concentrations in the calibration dataset were based on measured values, although the lowest concentration (Hansen et al. 2002) was associated with total organic carbon concentrations that were near or below the detection limit of 0.09 mg/L. Therefore, we can be reasonably confident that the DOC concentration was no higher than 0.09 mg/L. Regarding the highest and lowest water hardness values, both values were measured and the highest value of 826 mg/L as $CaCO_3$ was from an acute *D. magna* test, and the lowest value of 6.7 mg/L as $CaCO_3$ was from a chronic *D. magna* test. The water chemistry ranges are quite wide and encompass >90% of conditions observed in US surface waters (e.g., Table 1 in Brix et al. 2020 indicates that 5th to 95th percentiles for pH, DOC, and hardness in US waters are 6.5 to 8.5, 1.1 to 9.9 mg/L, 21 to 420 mg/L as $CaCO_3$, respectively). Therefore, the unified BLM is widely applicable and given that the BLM framework defines bioavailability on the basis of bioavailable Zn species (determined through equilibrium-based chemical speciation calculations), application to conditions outside these ranges may be defensible.

Effects of TMFs on Zn BLM-based WQC calculations and comparisons with the hardness equation

Applying the unified Zn BLM to a number of potential freshwater chemistry scenarios provides an indication of how the BLM calculations change with changing water chemistry conditions (Figure 6). Such an evaluation also provides an indication of how BLM-based WQC calculations compare with the existing hardness equation. Because the acute and chronic hardness-based WQC for Zn are essentially the same equation (i.e., acute-to-chronic ratio = 2, so CMC and CCC are equal, except for the conversion factor that translates from total Zn to dissolved Zn), we show a single hardness-based WQC for each hardness indicated in Figure 6.

The effect of DOC concentration on Zn BLM-based WQC (Figure 6A) is similar for both acute and chronic WQC for waters with varying DOC at pH 6.5, but the effect of DOC concentration on acute and chronic WQC differs somewhat at pH 7.5 and 8.5. For both acute and chronic WQC, the effect of DOC concentration is minimal for DOC concentrations below approximately 2 mg/L. This is especially true for waters with a pH of 6.5. For waters with pH 7.5 and 8.5, the effect of DOC is greater than in waters with pH of 6.5. The apparently interactive effect of pH and DOC concentration can largely be explained by the effect of protons on dissolved organic matter (DOM) binding sites. At lower pH, more sites will be occupied by protons and therefore will be less likely to accept Zn. The result is that DOM is not as effective at binding Zn as pH decreases. It is also apparent that an increase of DOC concentration under higher pH conditions has a greater effect on Zn bioavailability than at lower pH. The crossing of the different pH series also somewhat reflects the interplay between speciation effects and competition effects at the biotic ligand. The apparent difference in slope between the CMC and CCC also demonstrates that the DOC response is stronger for the CCC than for the CMC, which agrees with the difference in DOC slope for the two different MLR models described by DeForest et al. (2023). Conceptually, this can be explained by the importance of stronger binding sites on DOM in the lower Zn concentrations ranges that are relevant for chronic effect concentrations. In contrast to an MLR-based approach using separately fitted acute and chronic MLR models, the unified BLM characterizes this difference with a single model.

The effect of hardness on BLM-based WQC appears to be consistent for both acute and chronic exposures, and the slopes of the relationship increase slightly with decreasing pH (Figure 6B). Additionally, the BLM-based hardness response is very similar to the hardness equation (USEPA 1996) for hardness greater than approximately 40 mg/L as $CaCO_3$. At lower hardnesses, the BLM-predicted hardness effect is slightly lower than that indicated by the hardness equation. In Figure 6B, the BLM predicted WQC are shown for a DOC of 0.5 mg/L, and it is clear that all WQC are lower than the hardness equation across the entire range of hardness considered. Figure 6B emphasizes the major inadequacy of the hardness equation, with respect to CCC. The BLM-based CCC would be more appropriately protective, given the information we have on the sensitivity of aquatic organisms to chronic exposures to Zn. At a higher DOC concentration (i.e., at 10 mg/L), the slopes of the hardness response become much shallower (Figure S3), and the BLM-based CMC exceed the current hardness-based CMC. The increase in magnitude relative to the hardness equation is expected, but the decrease in hardness slope with increased DOC concentration indicates that DOC has a large relative effect on bioavailability at hardness less than ~80 mg/L.

The effect of pH on BLM-predicted WQC indicates that at a DOC concentration of 0.5 mg/L, Zn tends to be slightly more toxic as pH increases (Figure 6C). This is consistent with the MLR models developed by DeForest et al. (2023). Figure 6C also indicates that the current hardness equation is not protective of chronic effects at any pH, and that it is generally not protective of acute effects as pH increases. When DOC concentration is increased to 10 mg/L

(Figure S3), the pH response changes as a result of the pH-dependent properties of DOM. These changes in pH response across ranges of DOC concentrations and hardnesses are also evident in Figure S1. At high hardness (i.e., 500 mg/L as $CaCO_3$), the Zn BLM predictions are consistent with the current hardness equation predictions. However, when hardness decreases with 10 mg/L DOC in the exposure water, the Zn BLM-predicted WQC generally increases as pH increases above pH 6.3. Unlike the MLR-based predictions, the BLM-based predictions are not linearly related to pH.

Application of the Zn BLM to California surface waters

The BLM results in higher CMC estimates than the hardness equation in slightly more than half of the samples considered (Figure S2a,b). On the basis of exceedance frequency, where the measured dissolved Zn concentration is also considered, the BLM indicates fewer CMC exceedances than the hardness equation (Figure 4A,B). However, when CCCs are considered, the BLM is much more likely to provide lower CCC estimates than the hardness equation (Figure S2c,d). Interestingly, the BLM identifies fewer CCC exceedances (Figure 4C,D) than the hardness equation. This happens despite the generally lower BLM-based CCCs, which suggests that hardness-based CCC exceedances tend to occur in samples with lower Zn bioavailability conditions (i.e., lower pH or higher DOC) that are not properly characterized with the hardness equation. The high rate of hardness-based WQC exceedances, despite generally higher WQC estimates, indicates that hardness is not the most dominant TMF in the samples that exceed the hardness-based WQC, but not the BLM-based WQC. Importantly, the chronic BLM-based WQC identify many water samples in which Zn concentrations might not necessarily be safe to aquatic life, whereas the hardness-based HQs for the same samples incorrectly indicate that they are safe. We can come to this conclusion based on the improved accuracy of the BLM relative to the hardness equation. Therefore, use of the unified zinc BLM for WQC in areas such as southern California – where stormwater runoff can contribute to elevated zinc and DOC concentrations in urban waterways (e.g., Kayhanian et al. 2007; Kayhanian et al. 2012) – would improve identification and management of impaired waters.

In addition to being more accurate than hardness-based WQC, BLM-based WQC appear to be protective of all aquatic species listed as threatened and endangered in California (Supplementary Text 2). Although, it should be noted that some uncertainty exists for a single species (vernal pond tadpole shrimp), for which a sensitive surrogate species could only be identified at the class level. Additionally, BLM-based WQC appear to be protective of predator detection in Coho salmon (Supplementary Text 2), though there is some uncertainty in the reported effect concentrations for this endpoint because of an unbounded LOEC of 30 μg/L from a 3-hour exposure (Smith 2023). However, the CMC of 22 μg/L is below this LOEC, and the CCC is so low (5 μg/L) that it is approaching background concentrations. Given the short exposure time, the CMC would be considered the more applicable criterion for comparison. Our evaluations also indicated that the Zn BLM-based CCCs also appear to be protective of Zn detection in Atlantic salmon and rainbow trout (Supplementary Text 2).

Application of the Zn BLM to develop SSWQOs

The FMB approach takes into consideration the site-specific conditions that influence metal bioavailability, including metal concentration, the effects of TMFs on IWQC, the correlation between metal concentrations and IWQC, and their relative variability to identify a concentration that shall not be exceeded more frequently than the allowable exceedance frequency to ensure attainment of WQC. The interplay among these variables changes from site to site, so that it is virtually impossible to arbitrarily select an appropriately protective IWQC percentile (e.g., 5th, 10th, or 50th) at any given location. For example, in the analysis conducted by Ryan et al. (2018), the calculated Cu BLM-based FMBs for 61 different surface waters across the US ranged from the 1st to 94th percentile of the site-specific IWQC distributions. The conditions driving that range of percentiles were identified as the correlation between the metal concentration and IWQC and their relative variability. Arbitrarily choosing a low percentile (e.g., 5th or 10th), as previously suggested (e.g., USEPA 2015, 2021; Idaho DEQ 2017), may be overprotective in many cases, and not protective enough in other cases.

The FMB approach could be used to correctly implement any bioavailability-based WQC in a manner consistent with USEPA (1985) guidelines, including the hardness equation. When considering all sample types (i.e., compliance, dry weather, and wet weather) for the LCC1 location, the FMB approach gives a much lower SSWQO than the hardness equation, and this low FMB can be explained largely by the low correlation (i.e., Pearson's $r = 0.039$) between Zn concentration and IWQC (Table 6). This low correlation is likely explained by combining data from two very different populations of samples. Indeed, when the FMB approach is applied separately to the dry weather and wet weather samples for LCC1, the FMBs are much higher than when all data are combined. The correlation between Zn concentration and IWQC is much higher when FMBs are calculated on the dry weather and wet weather samples separately, and this along with the relative variability in the Zn concentrations and IWQCs is an important determinant of the IWQC percentile at which the FMB occurs (Ryan et al. 2018). The FMB approach implicitly makes use of these site-specific attributes by using sample-specific HQs to evaluate if Zn concentrations exceed IWQC. The higher positive correlations between Zn concentration and IWQC indicate that relatively high Zn concentrations are observed when Zn bioavailability conditions are relatively low.

The low hardness-based median WQC for wet weather (Table 6) indicates that the wet weather samples are relatively soft compared to the dry weather samples, and this can be verified by examining the LCC dataset (Table S3). These results also suggest that during wet weather events, hardness is not the dominant TMF. For all locations under wet weather conditions, there is a large difference between the BLM-based FMBs and the acute hardness-based median WQCs. Table 6 shows that for all wet weather sampling events, there is a high correlation between Zn concentration and IWQC. These high correlations contribute to the high FMBs, which all occur well above the 50^{th} percentile of each IWQC distribution. Importantly, it should be recognized that all FMBs presented in Table 6 and Figure 6 represent a literal interpretation of the USEPA (1985) guidelines in that the FMB would be the concentration that should not be exceeded at a given location more frequently than once every three years to attain WQC. It is also important to note that because dry weather and wet weather samples have such different attributes, they should be separated (at least at this location) for the purpose of defining SSWQOs.

CONCLUSIONS

The Zn ecotoxicity dataset by DeForest et al. (2023) for development of Zn MLR models and further refined by this study is robust and fully supports development of a unified Zn BLM and a unified MLR model for freshwaters. The unified Zn BLM performs better than the hardness equation by explicitly considering the effects of multiple TMFs (in addition to hardness) on Zn toxicity to aquatic life. Additionally, using quantitative and/or qualitative evaluation metrics, the unified Zn BLM performs marginally better than the unified Zn MLR model, similarly to separate acute and chronic Zn MLR models, and marginally better than the 14 year-old version of the unified Zn BLM. Marginal differences in MPSs for the BLMs and

MLRs may to some extent reflect inherent variability in the ecotoxicity datasets, but the qualitative single-variable pH and DOC evaluations appear to better support BLM behavior over a wide range of conditions. Given these considerations, the unified Zn BLM, unified Zn MLR model, and separate acute and chronic Zn MLR models offer potentially effective alternatives to the hardness equation, but each comes with advantages and limitations.

Biotic ligand model-based CMCs are lower than current hardness-based CMCs when DOC concentrations are less than ~3 mg/L, and Zn BLM-based CCC are lower than current hardness-based CCC when DOC concentrations are less than ~10 mg/L. These outcomes are due to the Zn BLM more accurately characterizing the effects of TMFs on Zn toxicity than the hardness equation and due to the update of the Zn WQC databases used to derive the WQC. Biotic ligand model-based WQC appear to be protective of aquatic species listed as threatened and endangered in California, and they also appear to be protective of olfactory responses in salmonids.

Application of Zn BLM-based WQC to monitoring data from the State of California indicates that fewer statewide exceedances of both acute and chronic Zn WQC are likely to occur, despite the chronic Zn BLM-based WQC being lower than the hardness-based WQC in 77% of samples. Implementing the Zn BLM using the FMB approach in the Los Cerritos Channel in southern California indicates that dry weather and wet weather samples should be considered separately when developing SSWQOs.

FIGURE CAPTIONS

Figure 1. Performance of the unified biotic ligand model (BLM) (this study) (A), unified BLM from DeForest and Van Genderen (2012) (B), separate acute and chronic multiple linear regression (MLR) models from DeForest et al. (2023) (C), unified MLR model (this study) (D), and hardness equation (E) with the calibration dataset. The percentage of predictions within a specified residual factor difference from observed ECx is shown in (F). ECx = Effect concentration at 'x' effect level, generally 20% and 50% for the data presented; r^2 = the coefficient of determination in the most general sense, where $r^2 = 1$ - (residual sum of squares/total sum of squares).

[Alt text: Model performance comparison showing predicted versus observed zinc toxicity. Compared with the previous BLM and MLR models, the unified BLM most closely matches observations across species and exposure durations, while the hardness equation shows the greatest deviation. Overall, the unified BLM produces the highest proportion of predictions within approximately two-fold error.]

Figure 2. Comparison of unified biotic ligand model (BLM) (A and B) and unified multiple linear regression (MLR) model (C and D) performance with data from acute (circles) and chronic tests (squares) where dissolved organic carbon (DOC) concentrations (A and C) and pH (B and D) were varied. See Figure S1 for predicted and observed responses plotted vs. pH or DOC. ECx = Effect concentration at 'x' effect level. For acute tests, all ECx are for 50% effect (i.e., EC50) and for chronic tests all ECx are for 50% effect (i.e., EC50). Organisms tested in each study were: Hyne et al. (2005) – *C. dubia*; Clifford and McGeer (2009) – *D. pulex;* Bringolf et. al. (2006) – *P. promelas*; Hoang and Tong (2015) – *P. paludosa*; Heijerick et al. (2002) – *D.*

magna; Schubauer-Berigan et al. (1993) (a) – *P. promelas*; Schubauer-Berigan et al. (1993) (b) – *C. dubia*; Cusimano et al. (1986) – *O. mykiss*; Heijerick et al. (2005) – *D. magna*; Heijerick et al. (2003) – *D. magna*. The different data series for Heijerick et al. (2003) represent different water chemistry conditions as follows: (a) Hardness = 240 mg/L as $CaCO_3$, pH = 7.25; (b) Hardness = 240 mg/L as $CaCO_3$, pH = 7.25; (c) Hardness = 240 mg/L as $CaCO_3$, pH = 7.25; (d) Hardness = 240 mg/L as $CaCO_3$, pH = 7.25; (e) Hardness = 240 mg/L as $CaCO_3$, pH = 7.25; (f) Hardness = 240 mg/L as $CaCO_3$, DOC = 21 mg/L; (g) Hardness = 110 mg/L as $CaCO_3$, DOC = 9.7 mg/L; (h) Hardness = 370 mg/L as $CaCO_3$, DOC = 32.3 mg/L; (i) Hardness = 370 mg/L as $CaCO_3$, DOC = 9.7 mg/L; (j) Hardness = 110 mg/L as $CaCO_3$, DOC = 32.3 mg/L.

[Alt text: Predicted versus observed zinc toxicity for datasets where DOC or pH varies. Except for a single acute *Pimephales promelas* study testing the effects of DOC, the unified BLM consistently tracks observed changes for both acute and chronic tests, whereas the unified MLR generally shows greater scatter, especially for pH effects.]

Figure 3. Unified biotic ligand model (BLM)-normalized genus mean acute values (GMAVs) (A) and genus mean chronic values (GMCVs) (B). Black diamonds represent GMAVs in (A) and GMCVs in (B). For genera representing multiple species, box-and-whisker plots are provided for the normalized species mean acute values (SMAVs) (A) and species mean chronic values (SMCVs) (B), where the edge of the boxes represent the 25th and 75th percentiles and the whiskers represent the range of data points within 1.5-fold times the interquartile range from the median. Normalization conditions are provided in the footnote for Table 3. Criteria maximum concentrations (CMCs) and criteria continuous concentrations (CCCs) calculated using three different models are shown for reference.

[Alt text: Acute and chronic normalized genus sensitivity distributions for zinc with criteria benchmarks. Biotic ligand model-derived criteria align with the lower portion of the sensitivity distribution, and those derived in this study are lower than previous BLM-derived criteria and current hardness-based criteria.]

Figure 4. Comparison of unified Zn biotic ligand model (BLM)-based and hardness-based hazard quotients for California fresh surface waters. Dashed lines represent hazard quotients of 1 for each criteria calculation method. Calculations were performed using unconstrained input data for both acute (A) and chronic (C) criteria comparisons, and with constrained input data for acute (B) and chronic (D) criteria comparisons. The constraints for model application were applied to provide minimum and maximum dissolved organic carbon (DOC), calcium (Ca), magnesium (Mg), and sodium (Na) concentrations, alkalinity and hardness values, and pH for inputs to the BLM and hardness equation (i.e., floor and ceiling values). If reported values for inputs were below the minimum value, the minimum value was used for input. Similarly, if reported values for inputs were above the maximum value, the maximum value was used for input. The constraints were: $0.1 \leq DOC \leq 35$ mg/L, $5.5 \leq pH \leq 9.0$, $3.2 \leq Ca \leq 120$ mg/L, $0.6 \leq Mg \leq 52.0$ mg/L, $0.2 \leq Na \leq 240$ mg/L, $2.0 \leq$ alkalinity ≤ 360 mg/L as $CaCO_3$, and $10 \leq$ hardness ≤ 500 mg/L as $CaCO_3$. Data used to develop these comparisons were from the water quality portal and the Los Cerritos Channel (LCC) Watershed Group.

[Alt text: Comparison of acute and chronic hazard quotients calculated using hardness-based and unified BLM criteria for California waters. Biotic ligand model-based hazard quotients are generally lower, indicating fewer exceedances, and this pattern remains after applying input constraints.]

Figure 5. Unified Zn biotic ligand model (BLM)-based fixed monitoring benchmark (FMB) evaluation and hardness-based criteria continuous concentrations (CMC) and criteria continuous concentrations (CCC) for data from a location within the Los Cerritos Channel (LCC1). Specifically chronic FMB calculations (A) and chronic hardness-based criteria (D) were determined for combined dry, compliance, and wet sampling events for LCC1, combined dry and compliance samples for LCC1 (B, E), and wet weather samples (C, F). The dashed horizontal black line represents a hazard quotient (HQ) of 1, the dashed horizontal blue line represents the FMB and is drawn to aid in visualizing the instantaneous water quality criteria (IWQC) percentile that corresponds to the FMB, and the solid grey vertical line represents the allowable criteria exceedance frequency of once in three years.

[Alt text: Biotic ligand model-based fixed monitoring benchmark results for a monitoring location in the Los Cerritos Channel in southern California show much higher fixed monitoring benchmarks when dry-weather and wet-weather samples are evaluated separately than when combined. Conversely, median hardness-based criteria increase in dry-weather samples but decrease by more than 4-fold in wet-weather samples.]

Figure 6. Comparison of unified Zn biotic ligand model (BLM)-based criteria maximum concentrations (CMC) and criteria continuous concentrations (CCC) with current hardness-based criteria across a range of dissolved organic carbon (DOC) concentrations (A), hardness (B), and pH (C). The grey dot dash line represents hardness-based criteria, and the colored lines represent unified Zn BLM-based criteria. Figure S3 shows how unified BLM predicted CMC and CCC change when 10 mg/L DOC is included in the simulations for (B) and (C).

[Alt text: Acute and chronic zinc criteria predicted by the unified biotic ligand model vary with dissolved organic carbon concentration and pH. The unified BLM shows a hardness-dependent slope similar to the hardness equation, and in moderately hard water acute biotic ligand model-based criteria remain below hardness-based criteria if dissolved organic carbon concentrations are less than 3 mg/L.]

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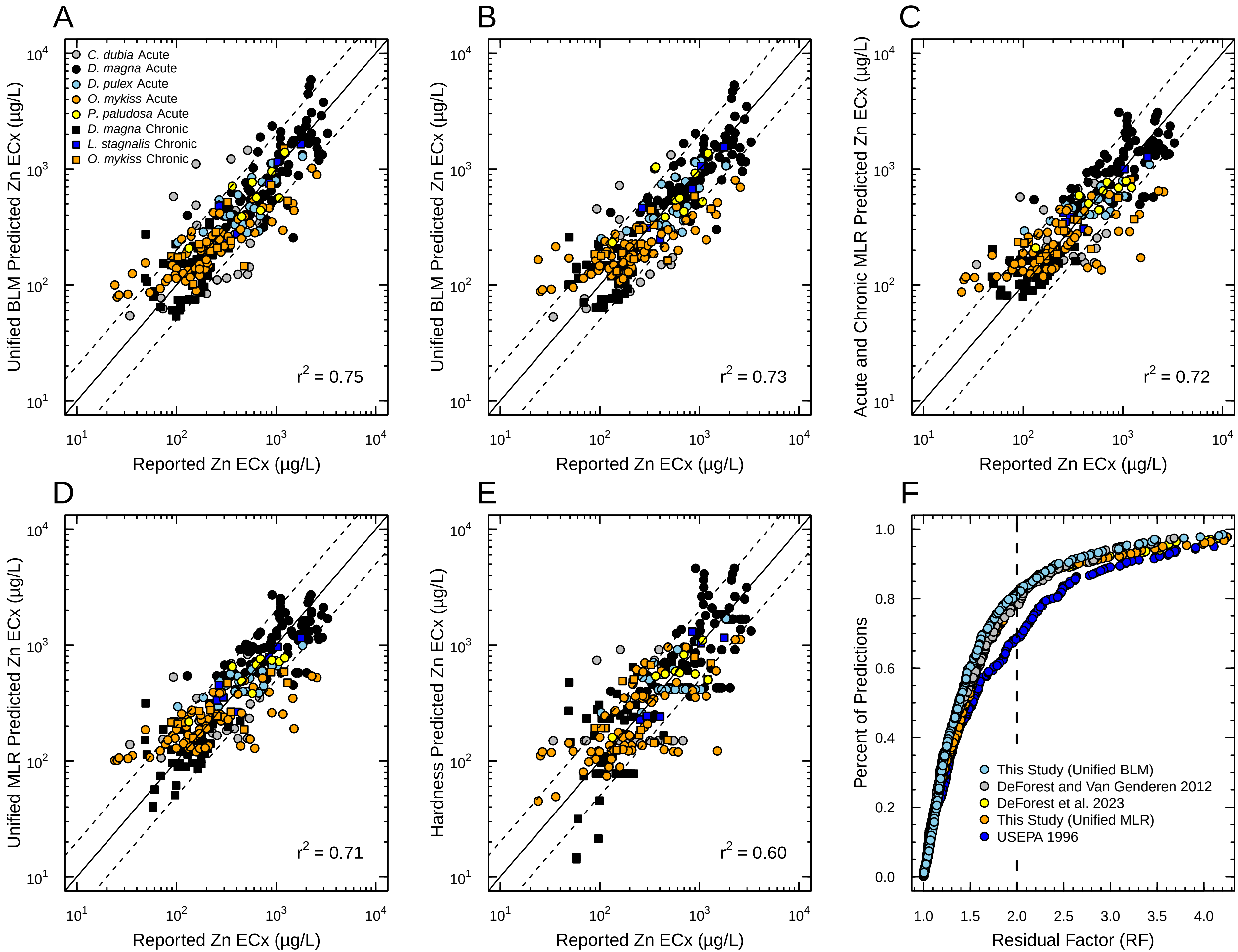
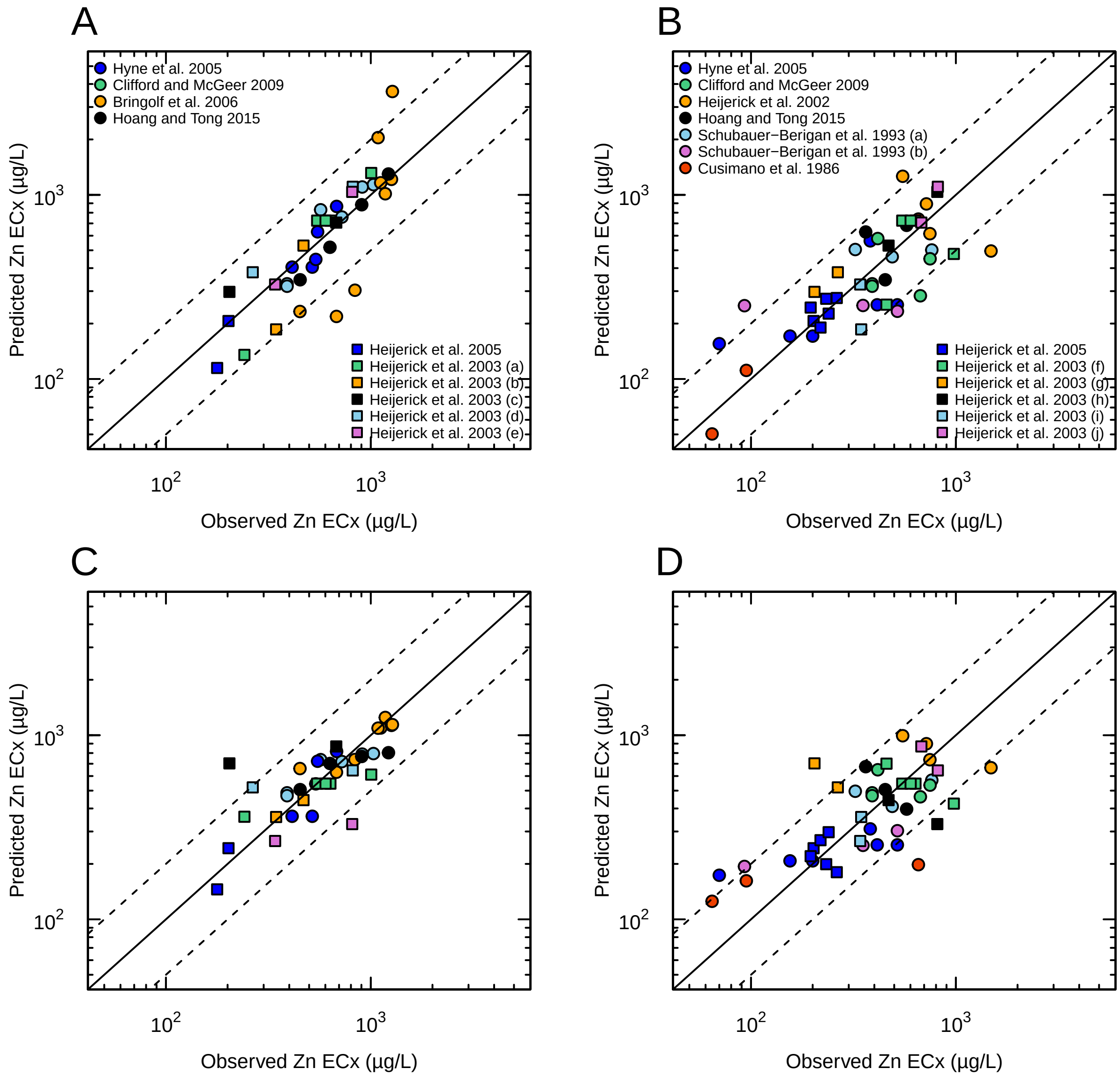
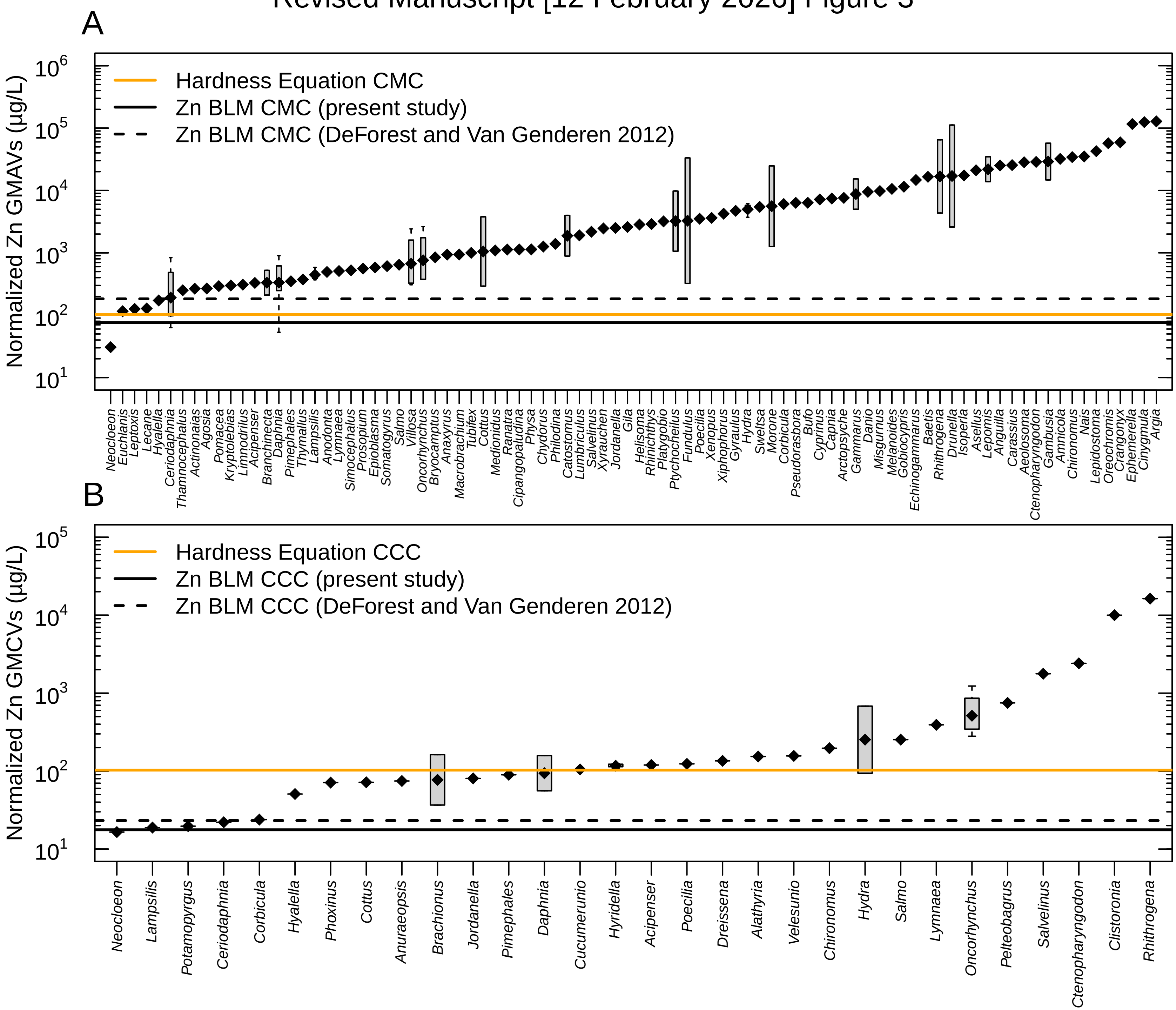
Revised Manuscript [12 February 2026] Figure 1



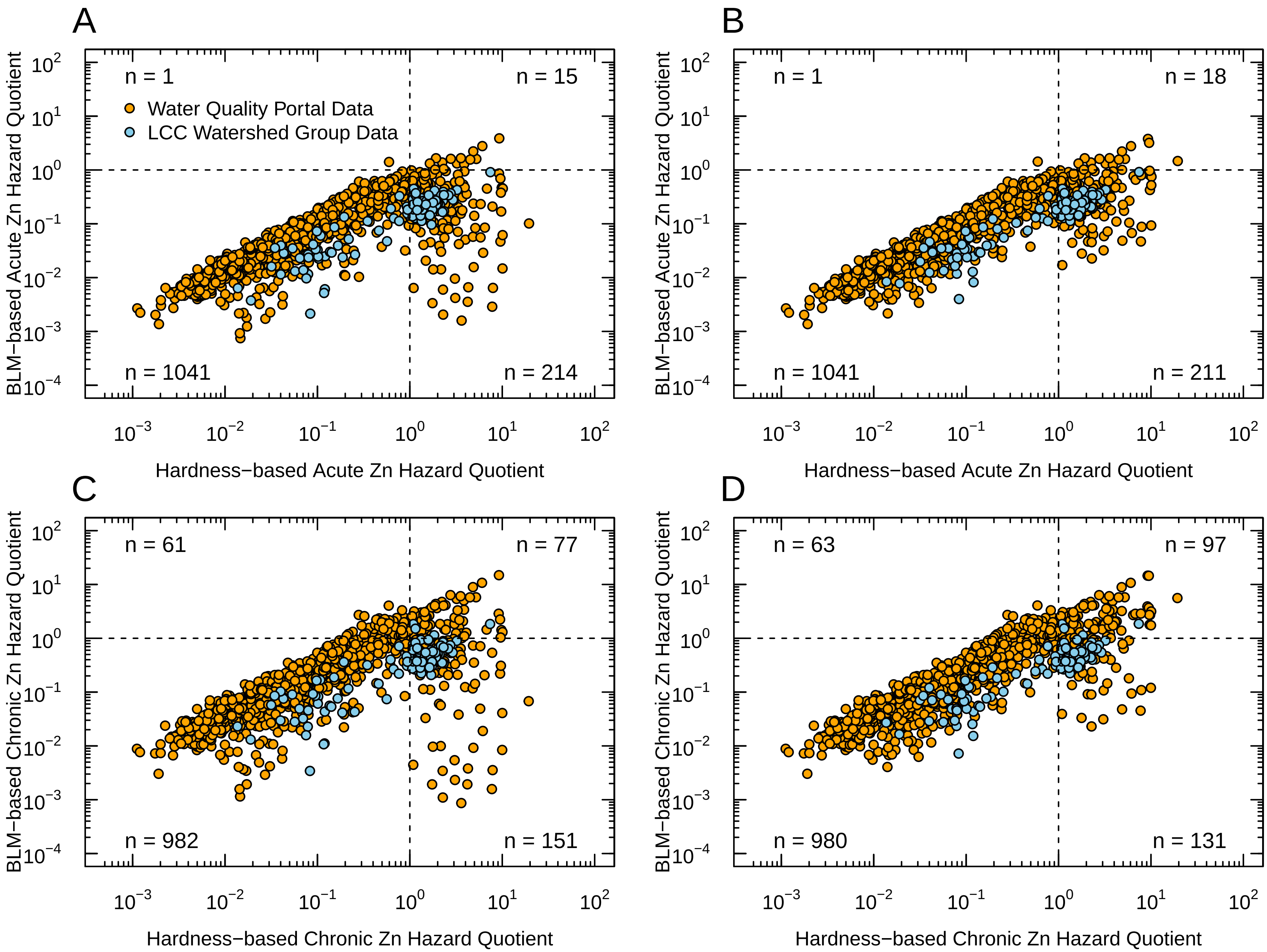
Revised Manuscript [12 February 2026] Figure 2



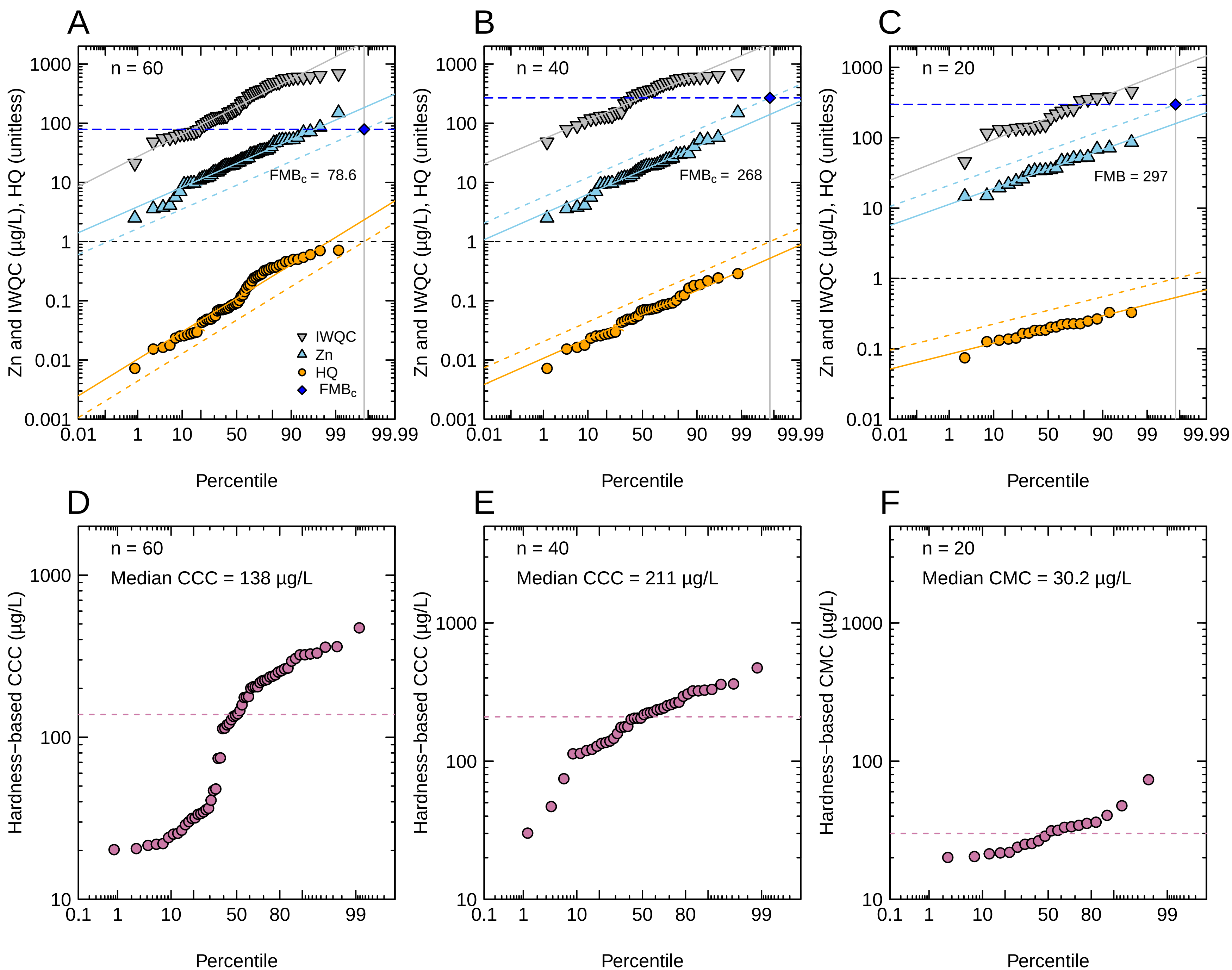
Revised Manuscript [12 February 2026] Figure 3



Revised Manuscript [12 February 2026] Figure 4



Revised Manuscript [12 February 2026] Figure 5



Revised Manuscript [12 February 2026] Figure 6

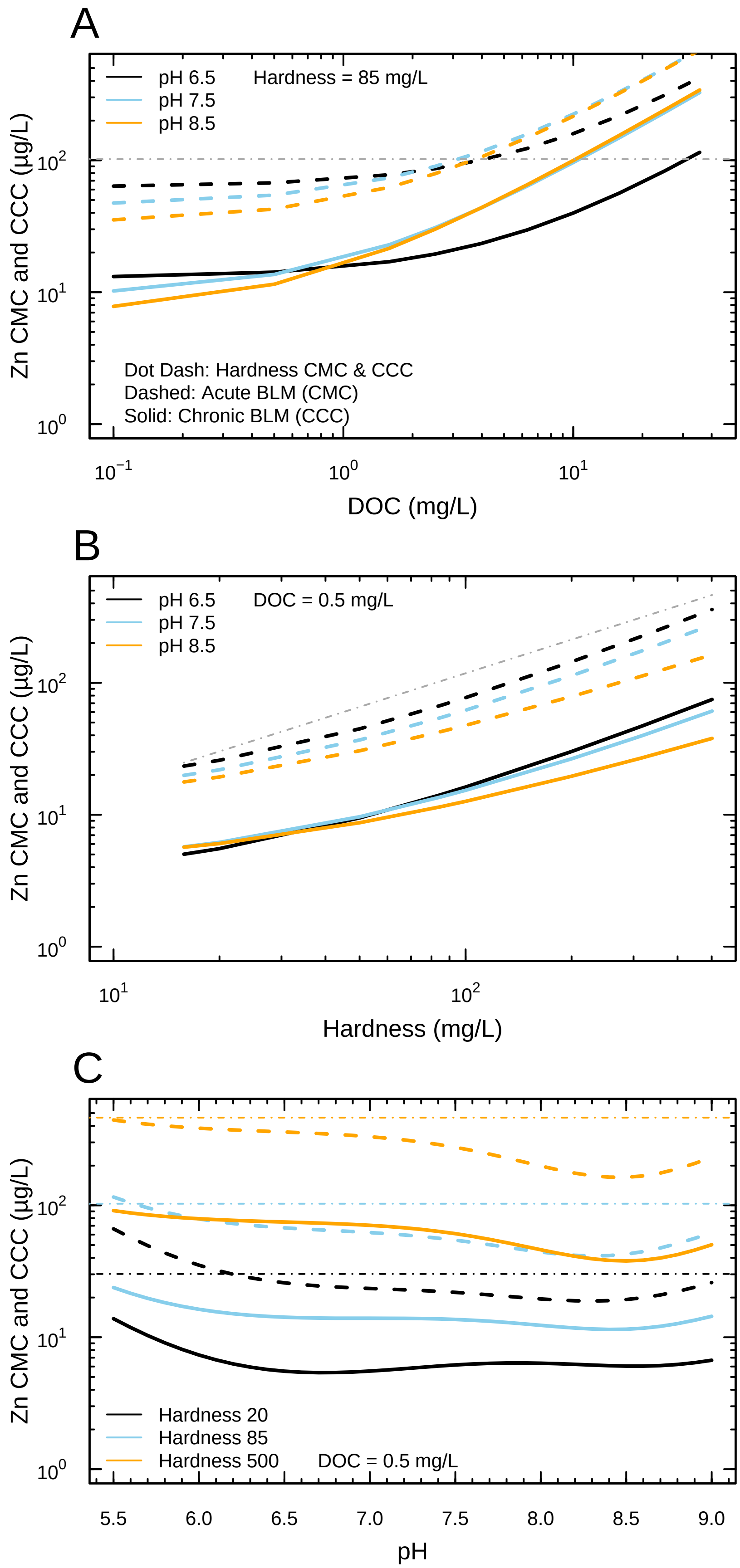


Table 1. Starting values and constraints for parameters in PEST (Doherty 2019) application for Zn biotic ligand model (BLM) optimizations.

Taxa	Parameter	Starting Value[1]	Minimum Value	Maximum Value
Invertebrates and Fish	log K BL-ZnOH	-2.4	-4.8	-1
	log K BL-Ca	3.8	2.2	5.9
	log K BL-Mg	3.3	1.7	5.4
	log K BL-Na	2.6	0.9	4.5
	Critical Accumulation (nmol/g ww)	1.27	0.01	30

[1]Starting values taken from the unified Zn BLM described in DeForest and Van Genderen (2012)

BL = biotic ligand; ww = wet weight

Table 2. Fitted parameters for unified zinc biotic ligand model (BLM) and unified multiple linear regression (MLR) model.

Unified Zn BLM			Unified Zn MLR Model		
Parameter		Value	Parameter		Value
Log K[a]	ZnOH	-2.447	Slope	pH	-0.200
	Ca	4.216		ln(DOC)	0.176
	Mg	3.772		ln(Hardness)	0.553
	Na	3.216			
Critical Accum. (nmol/g ww)[b]		FAV: 1.016 FCV: 0.1076	Intercept[c]		ND

[a]Log K values for binding with biotic ligand; Log K values for BL-Zn and BL-H fixed at 5.4 and 6.4, respectively

[b]Species-specific critical accumulations: *C. dubia* acute EC50: 1.3079; *D. magna* acute EC50: 4.2809; *D. pulex* acute EC50: 6.0497; *O. mykiss* acute EC50: 1.9804; *P. promelas* acute EC50: 2.2196; *P. paludosa* acute EC50: 1.985; *C. dubia* chronic EC20: 0.1366; *D. magna* chronic EC20: 0.3689; *L. stagnalis* chronic EC20: 2.6364; *O. mykiss* chronic EC20: 1.8458.

[c]Species-specific intercepts: *C. dubia* acute EC50: 4.659; *D. magna* acute EC50: 5.777; *D. pulex* acute EC50: 5.744; *O. mykiss* acute EC50: 4.731; *P. promelas* acute EC50: ND; *P. paludosa* acute EC50: 5.101; *C. dubia* chronic EC20: ND; *D. magna* chronic EC20: 3.599; *L. stagnalis* chronic EC20: 5.096; *O. mykiss* chronic EC20: 4.811.

DOC = dissolved organic carbon; nmol/gw = nanomoles/gram wet weight; FAV = final acute value; FCV = final chronic value; ND = not determined; FAV and FCV intercepts not determined for unified MLR model.

Table 3. Model performance metrics for the unified biotic ligand model (BLM), unified multiple linear regression (MLR) model, separately parameterized MLR models, and the hardness equation.

		Entire Dataset[c]		
Model Source[a]	**Model Type**	**r^2**	**$RF_{x,2.0}$**	**Mean MPS[d]**
This study	Unified BLM	0.75	0.82	0.78
[1]	Unified BLM	0.73	0.78	0.75
This study	Unified MLR	0.71	0.82	0.75
[2]	MLR[b]	0.72	0.81	0.78
[3]	Hardness	0.60	0.69	0.67

[a]References: 1 = DeForest and Van Genderen 2012; 2 = DeForest et al. 2023; USEPA 1996.
[b]Separately parameterized acute and chronic MLR models.
[c]r^2 and $RF_{x,2.0}$ shown for the entire combined dataset. See Table S6 for a full tabulation of r^2 and $RF_{x,2.0}$ by each species/endpoint.
[d]Model performance score (MPS) shown as the mean MPS of the individual organism/endpoint data series. See Table S6 for a full tabulation of MPS by each species/endpoint.
ECx = Effect concentration corresponding to ‘x’ effect level (e.g., 50% for acute and 20% for chronic).
$RF_{x,2.0}$ = Fraction of predicted ECx within 2-fold of observed ECx.
DOC = dissolved organic carbon.

Table 4. Unified Zn biotic ligand model (BLM)-normalized species mean acute values (SMAVs) and genus mean acute values (GMAVs) for the 20 most sensitive genera for which BLM normalization could be performed. Table S6 provides a complete summary for all 88 genera for which BLM normalizations could be performed. All data were normalized to synthetic US EPA moderately hard water conditions[a].

Genus species	**Family**	**GMAV Rank**	**GMAV (μg/L)**	**SMAV (μg/L)**
Lymnaea stagnalis	Lymnaeidae	20	510.9	510.9
Anodonta imbecillis	Unionidae	19	493.4	493.4
Lampsilis straminea	Unionidae	18	443.9	583.8
Lampsilis rafinesqueana	Unionidae			405.9
Lampsilis siliquoidea	Unionidae			369.2
Thymallus arcticus	Salmonidae	17	373.9	373.9
Pimephales promelas	Leuciscidae	16	349.8	349.8
Daphnia pulex	Daphniidae	15	333.7	901.1
Daphnia magna	Daphniidae			697.8
Daphnia ambigua	Daphniidae			536.1
Daphnia galeata	Daphniidae			500.8
Daphnia carinata	Daphniidae			348.7
Daphnia lumholtzi	Daphniidae			147.1
Daphnia longispina	Daphniidae			53.3
Branchinecta lynchi	Branchinectidae	14	331.7	524.2
Branchinecta lindahli	Branchinectidae			209.9
Acipenser transmontanus	Acipenseridae	13	329.2	329.2
Limnodrilus hoffmeisteri	Naididae	12	308.8	308.8
Kryptolebias marmoratus	Rivulidae	11	299.3	299.3
Pomacea paludosa	Ampullariidae	10	292.5	292.5
Agosia chrysogaster	Leuciscidae	9	267.9	267.9
Actinonaias pectorosa	Unionidae	8	266.1	266.1
Thamnocephalus platyurus	Thamnocephalidae	7	250.7	250.7
Ceriodaphnia pulchella	Daphniidae	6	191.3	837.2
Ceriodaphnia dubia	Daphniidae			132.4
Ceriodaphnia reticulata	Daphniidae			63.2
Hyalella azteca	Hyalellidae	5	172.2	172.2
Lecane quadridentata	Lecanidae	4	128.0	128.0
Leptoxis ampla	Pleuroceridae	3	126.0	126.0
Euchlanis dilatata	Euchlanidae	2	115.7	115.7
Neocloeon triangulifer	Baetidae	1	30.7	30.7

[a]Normalization Conditions:

Temperature (C): 25, pH: 7.5, dissolved organic carbon (DOC; mg/L): 0.5, Ca (mg/L): 13.97, Mg (mg/L): 12.12, Na (mg/L): 26.3, K (mg/L): 2.1, SO_4 (mg/L): 81.3, Cl (mg/L): 1.9, Alkalinity (mg/L as $CaCO_3$): 60.5, Hardness (mg/L as $CaCO_3$): 84.79

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Table 5. Unified Zn biotic ligand model (BLM)-normalized species mean chronic values (SMCVs) and genus mean chronic values (GMCVs). All data were normalized to synthetic US EPA moderately hard water conditions[a].

Genus species	Family	GMCV Rank	GMCV (μg/L)	SMCV (μg/L)
Rhithrogena hageni	Heptageniidae	30	16292.7	16292.7
Clistoronia magnifica	Limnephilidae	29	10003.1	10003.1
Ctenopharyngodon idella	Xenocyprididae	28	2411.9	2411.9
Salvelinus fontinalis	Salmonidae	27	1772.4	1772.4
Pelteobagrus fulvidraco	Bagridae	26	749.9	749.9
Oncorhynchus tshawytscha	Salmonidae	25	513.0	1233.1
Oncorhynchus nerka	Salmonidae			488.8
Oncorhynchus clarkii	Salmonidae			410.4
Oncorhynchus mykiss	Salmonidae			280.0
Lymnaea stagnalis	Lymnaeidae	24	392.2	392.2
Chironomus tentans	Chironomidae	23	322.7	322.7
Salmo trutta	Salmonidae	22	253.2	253.2
Hydra vulgaris	Hydridae	21	253.1	681.9
Hydra viridissima	Hydridae			94.0
Velesunio ambiguus	Hyriidae	20	156.6	156.6
Alathyria profuga	Hyriidae	19	153.8	153.8
Dreissena polymorpha	Dreissenidae	18	135.4	135.4
Poecilia reticulata	Poeciliidae	17	123.8	123.8
Acipenser transmontanus	Acipenseridae	16	119.7	119.7
Hyridella drapeta	Hyriidae	15	117.1	124.4
Hyridella depressa	Hyriidae			120.8
Hyridella australis	Hyriidae			106.8
Cucumerunio novaehollandiae	Hyriidae	14	105.0	105.0
Daphnia pulex	Daphniidae	13	93.9	157.8
Daphnia magna	Daphniidae			55.8
Pimephales promelas	Leuciscidae	12	89.7	89.7
Jordanella floridae	Cyprinodontidae	11	80.6	80.6
Brachionus calyciflorus	Brachionidae	10	77.2	162.5
Brachionus rubens	Brachionidae			36.7
Anuraeopsis fissa	Brachionidae	9	74.7	74.7
Cottus bairdi	Cottidae	8	71.8	71.8
Phoxinus phoxinus	Leuciscidae	7	71.2	71.2
Hyalella azteca	Hyalellidae	6	50.8	50.8
Corbicula sp.	Cyrenidae	5	23.9	23.9
Ceriodaphnia dubia	Daphniidae	4	22.1	22.1
Potamopyrgus jenkinsi	Tateidae	3	19.7	19.7
Lampsilis siliquoidea	Unionidae	2	18.9	18.9
Neocloeon triangulifer	Baetidae	1	16.5	16.5

[a]Normalization Conditions:

Temperature (C): 25, pH: 7.5, dissolved organic carbon (DOC; mg/L): 0.5, Ca (mg/L): 13.97, Mg (mg/L): 12.12, Na (mg/L): 26.3, K (mg/L): 2.1, SO_4 (mg/L): 81.3, Cl (mg/L): 1.9, Alkalinity (mg/L as $CaCO_3$): 60.5, Hardness (mg/L as $CaCO_3$): 84.79

Table 6. Summary of unified Zn biotic ligand model (BLM)-based acute and chronic fixed monitoring benchmark (FMB) calculations for the Los Cerritos Channel (LCC) monitoring dataset

Location ID	**Sample Type**	**Criteria Type**	**BLM-based FMB (µg/L)**	**Hardness-based median WQC (µg/L)**	**IWQC Percentile at FMB**	$r_{Zn\ vs.\ IWQC}$
LCC1	Combined Dry Weather, Compliance, and Wet Weather Samples	Chronic	78.6	138	14	0.039
LCC1	Combined Dry Weather and Compliance Samples	Chronic	268	211	49	0.41
LCC1	Wet Weather Samples	Acute	297	30.2	79	0.78
SB4	Wet Weather Samples	Acute	387	27.2	95	0.82
SB8	Wet Weather Samples	Acute	334	22.7	83	0.91
SB9	Wet Weather Samples	Acute	403	31.1	84	0.94
SB10	Wet Weather Samples	Acute	232	23.6	59	0.64

WQC = Water quality criteria

IWQC = Instantaneous water quality criteria

r = Pearson's correlation coefficient