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RELATIONSHIP BETWEEN BIOTIC LIGAND MODEL-BASED WATER QUALITY CRITERIA AND AVOIDANCE AND OLFACTORY RESPONSES TO COPPER BY FISH

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Abstract—The U.S. Environmental Protection Agency's (U.S. EPA) water quality criteria for Cu were tested to determine whether they protect fish against neurophysiological impairment. From published studies with rainbow trout (*Oncorhynchus mykiss*), Chinook salmon (*Oncorhynchus tshawytscha*), coho salmon (*Oncorhynchus kisutch*), and fathead minnows (*Pimephales promelas*), 20% inhibition concentrations (IC20s) were calculated for avoidance of Cu-containing water and for impairment of electroencephalogram (EEG) and electro-olfactogram (EOG) responses to natural odorants in Cu-containing water. Additionally, a Cu-olfactory biotic ligand model (BLM) that fits the coho salmon EOG data was parameterized by changing the sensitivity parameter in the ionoregulatory-based BLM. The IC20s calculated from reported Cu avoidance, EEG, and EOG data and IC20s predicted by the olfactory BLM were compared with acute and chronic Cu criteria calculated using U.S. EPA's BLM 2007 or hardness-adjustment equations. The BLM-based chronic criteria were protective in all 16 exposure water–species combinations used in avoidance and olfaction experiments. Additionally, the BLM-based acute criteria were protective in all 11 exposure water–species combinations in which comparisons could be made with olfactory BLM-predicted IC20s but not in two of the 16 exposure water–species combinations in which comparisons could be made with the reported IC20s (which were $\leq 8\%$ lower than but did not differ significantly from the BLM-based acute criteria; $p > 0.05$). In effect, the olfactory BLM factored out the relatively high variability in the reported IC20s. It is concluded that the U.S. EPA's BLM-based water quality criteria for Cu protect against these types of neurophysiological impairment in the six species–endpoint combinations analyzed in this paper. However, the U.S. EPA's hardness-based criteria for Cu sometimes were considerably underprotective and sometimes were much less protective than the BLM-based criteria. Environ. Toxicol. Chem. 2010;29:2096–2103. © 2010 SETAC

Keywords—Biotic ligand model Electroencephalogram Electro-olfactogram Fathead minnow Salmonid fishes

INTRODUCTION

At elevated concentrations, Cu can decrease survival, growth, and reproduction of aquatic organisms [1]. Copper can also interact with the olfactory system of fish and aquatic invertebrates [2–9], causing them to avoid Cu-containing water while their olfactory system is not impaired and to lose important functions such as attraction to food odors and reproductive pheromones, or avoidance of predators, when olfaction is impaired [9–16]. At relatively low Cu concentrations that do not kill olfactory neurons, olfactory impairment in fish is caused by depression of a variety of crucial genes within the olfactory signal transduction pathway and elsewhere in olfactory tissues [17], and, at higher Cu concentrations that kill olfactory neurons, olfactory signals cannot be stimulated and transduced to the brain [4,16]. Recent studies have demonstrated avoidance of Cu or impairment of olfaction in salmonid fishes exposed to concentrations as low as approximately 1 to 2 μg Cu/L (lake whitefish, *Coregonus clupeaformis* [10]; Chinook salmon, *Oncorhynchus tshawytscha*, and rainbow trout, *Oncorhynchus mykiss* [11]; coho salmon, *Oncorhynchus kisutch* [5,15,18,19]), prompting public concern that important activities such as spawning and predator avoidance might be impaired in Cu-contaminated streams in the Pacific Northwest of the United States [[20,21]; see http://oregonstate.edu/ua/ncs/archives/2007/mar/osunoaa-study-copper-autos-other-sources-increases-predation-risk-salmon]. Therefore, an important question is, do the U.S. Environmental Protection Agency's (U.S. EPA) aquatic life criteria for Cu protect for avoidance and olfactory responses by salmonids and other fish in Cu-contaminated water?

Because water chemistry such as pH, dissolved ligands, or competing cations modifies the acute and chronic toxicity of Cu to aquatic organisms [22,23], the concentration of Cu alone is not adequate to establish defensible water quality criteria. Instead, algorithms such as the gill ionoregulatory-based biotic ligand model (BLM) for acute toxicity [24] and chronic toxicity [25,26] of cationic metals are needed to account for toxicity-modifying effects of water chemistry. For example, the U.S. EPA incorporated an acute-toxicity BLM into its recently revised freshwater life criteria for Cu [1], replacing the previous hardness-based criteria for Cu ([27]; see http://www.epa.gov/waterscience/criteria/wqctable/). As a consequence, the question of whether aquatic life criteria for Cu are protective for olfactory-related responses by salmonids and other fish should be addressed only in the context of the exposure-water chemistry, without which Cu concentrations alone are difficult to interpret. Therefore, another way of stating the earlier question is, do these neurophysiological responses occur at Cu concentrations lower than the U.S. EPA's BLM-based criteria continuous concentration ([CCC]; the chronic criterion) or criteria maximum concentration ([CMC]; the acute criterion), implying that those criteria are not protective enough? An analogous

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All Supplemental Data may be found in the online version of this article.

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question can also be asked regarding the U.S. EPA's previous hardness-based criteria for Cu, which are still used by most states in the United States.

Similar to the toxicity-modifying effects of water chemistry on survival, growth, and reproduction of aquatic organisms, water chemistry also appears to modify olfactory impairment by Cu ([18]; see also pp 131–132, 141, 143–144 in Meyer et al. [23]). Therefore, in concept, it should be possible to develop an olfactory-based BLM analogous to the current gill ionoregulatory-based BLM and thus predict threshold Cu concentrations that will protect against olfactory impairment and behavioral avoidance in a wide variety of Cu-contaminated waters. However, because the current BLM, as parameterized for ionoregulatory toxicity at the fish gill, did not accurately predict olfactory neurotoxicity [18,19], the parameterization of an olfactory BLM might have to differ from the parameterization of the gill ionoregulatory-based BLM.

In the present study, the 20% inhibitory concentrations (IC20s) for Cu avoidance and for impairment of olfactory responses to Cu in three salmonid fish and the fathead minnow (in four published laboratory studies) were compared with the U.S. EPA acute and chronic criteria for Cu determined for the chemistry of the exposure waters in those studies, using both the BLM-based criteria and the hardness-based criteria. Additionally, a freshwater olfactory BLM for Cu based on electro-olfactogram (EOG) responses of coho salmon was parameterized and used to factor out the variability in comparisons of the coho salmon IC20s to the Cu criteria.

MATERIALS AND METHODS

Data sources

Although many studies have reported avoidance of Cu or impairment of olfactory responses by fish in Cu-containing waters, insufficient water chemistry was measured to allow reliable BLM-based calculations of the CMCs and CCCs for the laboratory exposure waters in most of those studies [2,3,5–8,10,12,14–16]. Sufficient water chemistry data are available for only four studies [4,9,11,18]. The endpoints for those studies are percentage avoidance of Cu-containing water by juvenile rainbow trout and Chinook salmon (calculated from data in Hansen et al. [11]); percentage impairment of electrophysiological activity in the olfactory bulb (the odor-processing region) in the brains of juvenile rainbow trout and Chinook salmon, as measured by electroencephalogram (EEG) response to an L-serine stimulus in Cu-containing water (calculated from data in Hansen et al. [4]); percentage impairment of electrophysiological activity recorded from the epithelium of the olfactory rosette (an odor-sensing organ in the nares, or nostril) of juvenile coho salmon, as measured by EOG response to an L-serine stimulus in Cu-containing water (calculated from data in McIntyre et al. [19]); and percentage impairment of EOG response by adult fathead minnows (*Pimephales promelas*) to an L-arginine stimulus in Cu-containing water (reported in Green et al. [9]). Overviews of those studies and details of the methods used to calculate the percentage responses, median inhibitory concentrations (IC50s), and IC20s are presented in the Supplementary Methods and Supplementary Results.

Benchmark calculations

As in Sandahl et al. [6], the IC20 was chosen as the benchmark index for avoidance of Cu and impairment of olfactory responses, because the uncertainty in control EOG and EEG responses typically is approximately 20%. For example, two times the average standard error of the EOG and EEG responses of control coho salmon to L-serine and taurocholic acid (TCA; another natural odorant to which fish respond) in Sandahl et al. [6] ranged from 15 to 28% of the average control EOG amplitude (where two times the standard error is a lower-bound estimate of the half-width of a 95% confidence interval); two times the standard error of the EOG response of fathead minnows to L-arginine during a 30-min pre-exposure period in Green et al. [9] ranged from 8 to 68% (average of 31%); the lower 95% confidence limits for average relative EOG responses of control coho salmon to L-serine and TCA in Baatrup [3] were 0.73 and 0.77, respectively (i.e., 27% and 23% deviations from the mean values); and the lower 95% confidence limit for the average relative EOG response of control coho salmon to L-serine in McIntyre et al. [18] was approximately 0.82 (i.e., an 18% deviation from the mean value). Hansen et al. [4] did not report variability in their EEG measurements with rainbow trout and Chinook salmon; however, the 95% confidence intervals for avoidance of a randomly chosen challenge side of the avoidance chamber by control rainbow trout and Chinook salmon in Hansen et al. [11] were ±12% and ±13% of the means, respectively.

Criteria calculations

With the water chemistries reported by Hansen et al. [4,11], Green et al. [9], and McIntyre et al. [18] as inputs (Supplemental Data, Table S1), the HydroQual Cu BLM version 2.2.3 (available at http://www.hydroqual.com/wr_blm.html) was used to calculate the U.S. EPA acute (CMC) and chronic (CCC) water quality criteria for Cu in each exposure water in the four studies. The U.S. EPA's BLM 2007 (available at http://www.epa.gov/waterscience/criteria/copper/2007) contains the same algorithms as the HydroQual Cu BLM version 2.2.3 but reports only Cu criteria concentrations in its output (i.e., BLM 2007 cannot be operated in speciation or toxicity mode, as is needed in the *BLM parameterization* section below).

Similarly with the water hardness values reported by Hansen et al. [4,11], Green et al. [9], and McIntyre et al. [18] as inputs, the U.S. EPA hardness-adjustment equations [27] were used to calculate the U.S. EPA CMC and CCC water quality criteria for Cu in each exposure water in the four studies. Those equations are $\mathrm{CMC} = 0.96 \cdot e^{0.9422 \cdot \ln(H) - 1.700}$ and $\mathrm{CCC} = 0.96 \cdot e^{0.8545 \cdot \ln(H) - 1.702}$, where H is hardness (expressed as mg/L as $CaCO_3$).

BLM parameterization

To parameterize an olfactory BLM to predict EOG IC50s for Cu at any water chemistry combination (analogous to the LC50 predictions that would be made using the current gill ionoregulatory-based BLM), Cu BLM version 2.2.3 was first run in speciation mode for all 11 corrected IC50s that McIntyre et al. [19] reported for EOG impairment of coho salmon, using the water chemistry McIntyre et al. [18] reported for each treatment (Supplemental Data, Table S1). That allowed us to calculate the ΣCu-BL concentration (i.e., the sum of the concentrations of Cu and CuOH bound to the biotic ligand [the BL-Cu and BL-CuOH concentrations reported by the BLM]) for each of the 11 exposure waters. Then, a ΣCu-BL concentration was iteratively chosen so that, when entered as the critical Cu accumulation in the Cu_Rainbow_Trout_06-10-07.DAT BLM parameter file, it produced (BLM-predicted IC50):(reported IC50) ratios for which the geometric mean was 1.0 among the 11 exposure waters. A geometric mean of 1.0 for that ratio is equivalent to constraining the sum of the percent deviations of the BLM-predicted IC50s from the reported IC50s to equal zero among the 11 exposure waters (i.e., the sum of the 100 · [predicted–observed]/observed values is constrained to equal zero).

In these calculations of BLM-predicted IC50s, the critical Cu accumulation (the sensitivity parameter) was changed because it seems logical that fish would be more sensitive to Cu for a sublethal, olfactory-related endpoint than they would be for the lethality endpoint for which the current gill ionoregulatory-based BLM was parameterized. In concept, one could instead alter the binding constants for Cu and the other cations to the biotic ligand with approximately equal modeling effectiveness, although more than one binding constant might have to be changed to achieve the same solution as is produced when the sensitivity parameter is changed. In fact, altering all the biotic-ligand-binding constants and the sensitivity parameter would allow one to find the best fit of the BLM-predicted IC50s to the reported IC50s. However, none of the thermodynamic constants for binding of cations to the biotic ligand (i.e., log $K_{\text{BL-Cu}} = 7.4$, log $K_{\text{BL-CuOH}} = -1.3$, log $K_{\text{BL-Ca}} = 3.6$, log $K_{\text{BL-Mg}} = 3.6$, log $K_{\text{BL-H}} = 5.4$, log $K_{\text{BL-Na}} = 3.0$) was changed because those default cation-binding constants provided a satisfactory fit to the data (see below under *Olfactory BLM*). The equations that define these thermodynamic constants are shown in Supplemental Data, Equations S6 to S11.

Alternatively, one could use other estimators as the critical Cu accumulation in the BLM. For example, in addition to the optimized ΣCu-BL value, the geometric mean ΣCu-BL calculated for each of the 11 exposure waters, the arithmetic mean of the ΣCu-BL values, the median of the ΣCu-BL values, and the ΣCu-BL calculated for the water chemistry in the low-ion treatment (the base water to which $CaCl_2$, $NaHCO_3$, or dissolved organic matter [DOM] was added in McIntyre et al. [18]) were tried as the critical Cu accumulation. However, none of those alternate estimators of the IC50 fit the reported IC50 data as well as the optimized ΣCu-BL value (see below under *Olfactory BLM*).

RESULTS AND DISCUSSION

Concentration–response curves

Sigmoid concentration–response curves were a good fit to the Cu-avoidance data from Hansen et al. [11] for rainbow trout and Chinook salmon, in which avoidance increased as Cu concentration increased up to approximately 10 μg Cu/L (Fig. 1). In fact, if the outlier at 0.7 μg Cu/L in the Chinook salmon data (Fig. 1b) is omitted, the concentration–response relationships for the two species are almost identical (i.e., the IC50s were 2.1 and 2.5 μg Cu/L for rainbow trout and Chinook salmon, respectively; and the IC20s were 0.84 and 0.91 μg Cu/L, respectively). However, at concentrations greater than 11 μg Cu/L for rainbow trout and greater than 6 μg Cu/L for Chinook salmon (data shown in Fig. 1 in Hansen et al. [11]), avoidance of Cu by both species began decreasing as Cu concentration increased, presumably because of structural damage to olfactory epithelia at those high concentrations [4].

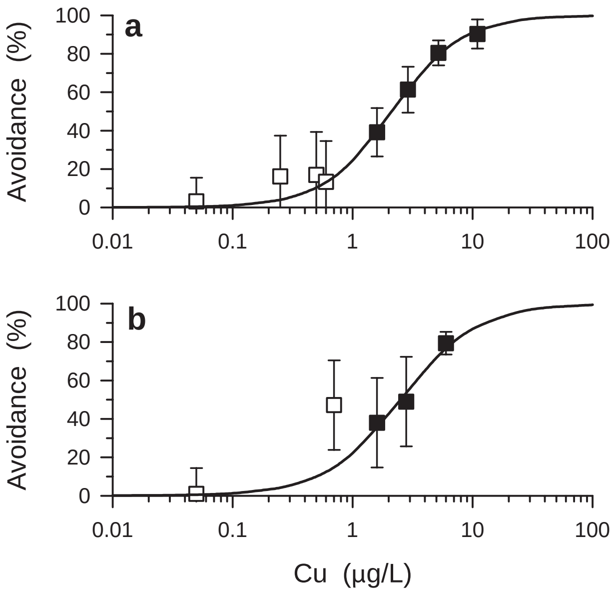

Fig. 1. Avoidance of Cu by rainbow trout (*Oncorhynchus mykiss*; **a**) or Chinook salmon (*Oncorhynchus tshawytscha*; **b**) in laboratory water (10.2°C, pH 7.5, 28.0 mg/L as $CaCO_3$ alkalinity, 25.3 mg/L as $CaCO_3$ hardness; calculated from data in Hansen et al. [11]). Open squares had Cu concentrations less than or equal to the detection limit (0.7 μg Cu/L); error bars are 95% confidence intervals. The curve is a logistic-regression fit to the data that had Cu concentrations above the detection limit.

Sigmoid concentration–response curves were also a good fit to the EEG data from Hansen et al. [4] for rainbow trout and Chinook salmon (Fig. 2) and to the EOG data from Green et al. [9] for fathead minnows (Fig. 3), in which EEG or EOG amplitude decreased as Cu concentration increased. The IC50s for rainbow trout, Chinook salmon, and fathead minnows were 43.0, 27.8, and 7.1 μg Cu/L, respectively; and the IC20s were 5.1, 10.7, and 5.0 μg Cu/L, respectively. Therefore, the EOG response of fathead minnows was more sensitive than the EEG responses of rainbow trout and Chinook salmon (compare Fig. 3 vs. Fig. 2a,b), and, at concentrations greater than approximately 30 μg Cu/L, the EEG response was impaired considerably more in Chinook salmon than in rainbow trout (compare

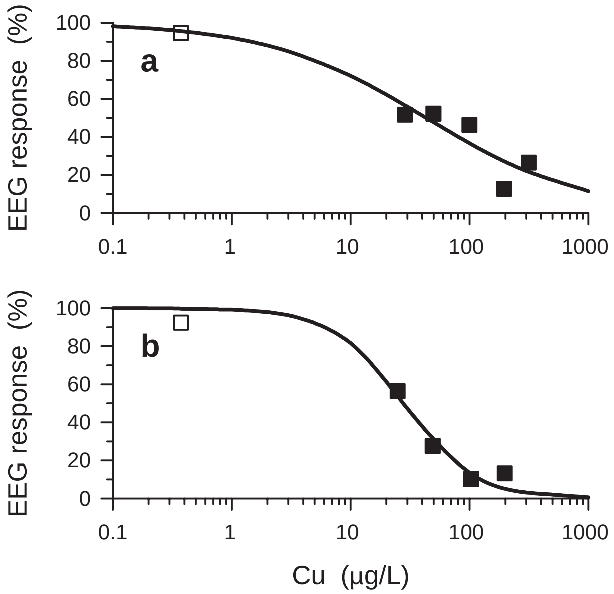

Fig. 2. Electroencephalogram (EEG) responses by rainbow trout (*Oncorhynchus mykiss*; **a**) or Chinook salmon (*Oncorhynchus tshawytscha*; **b**) exposed to Cu in laboratory water (12.3°C, pH 7.67, 24.5 mg/L as $CaCO_3$ hardness; calculated from data in Hansen et al. [4]). Open squares had Cu concentrations less than or equal to the detection limit (0.7 μg Cu/L). The solid curve is a logistic-regression fit to the data that had Cu concentrations above the detection limit.

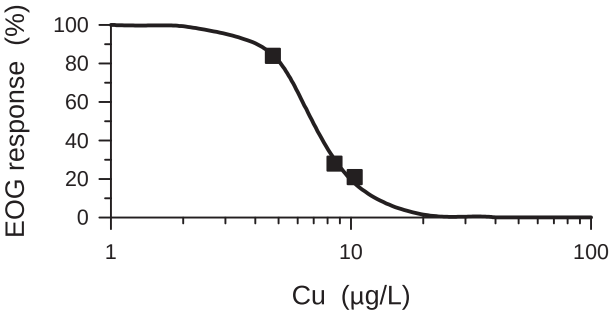

Fig. 3. Electro-olfactogram (EOG) responses by fathead minnows (*Pimephales promelas*) exposed to Cu for 10 min in laboratory water (19°C, pH 6.82, 23.1 mg/L as $CaCO_3$ hardness; calculated from data in Green et al. [9]). The curve is a logistic-regression fit to the data.

Fig. 2b vs. a). Analogous concentration–response curves for EOG impairment versus dissolved Cu concentration cannot be plotted for the McIntyre et al. [18] study, because only one dissolved Cu concentration was tested in each of their exposure waters.

Olfactory BLM

The ΣCu-BL concentrations calculated by Cu BLM version 2.2.3 for the 11 water chemistries in McIntyre et al. [18] ranged from 0.0530 to 1.3726 nmol/g wet weight (Supplemental Data, Table S3). Although that range of concentrations is high, no trend was apparent as $CaCl_2$, $NaHCO_3$, or DOM concentration was increased in the exposure waters (recognizing that the low-ion water is the first member in each of those series of chemical gradients).

A good fit of Cu BLM version 2.2.3 to the reported IC50s was obtained by inserting the optimized ΣCu-BL concentration of 0.1988 nmol/g wet weight as the critical Cu concentration in the BLM parameter file (i.e., replacing the default median lethal accumulation [LA50] of 3.70 nmol/g wet weight for rainbow trout with the optimized ΣCu-BL), without changing any of the cation-BL binding constants. The resulting BLM-predicted IC50s agreed well with the corrected IC50s reported by McIntyre et al. [19] (Fig. 4), for which the (BLM-predicted IC50):(reported IC50) ratios ranged from 0.32 to 2.74 (Supplemental Data, Table S3). For the four treatments to which $NaHCO_3$ was added (including the base-water treatment) and for the five treatments to which DOM was added (including the base-water treatment), the slope of the regression of ln(BLM-predicted IC50) versus ln(reported IC50) did not differ significantly from 1.0 (slope = 1.786, $p > 0.05$, $r^2 = 0.852$ for the $NaHCO_3$ treatments; slope = 1.032, $p > 0.05$, $r^2 = 0.702$ for the DOM treatments). In contrast, for the four treatments to which $CaCl_2$ was added (including the base-water treatment), the slope of the regression of ln(BLM-predicted IC50) versus ln(reported IC50) differed significantly from 1.0 (slope = 0.225, $p < 0.05$, $r^2 = 0.701$ for the $CaCl_2$ treatments), suggesting that the BLM fit to those coho salmon EOG data might be improved by additional adjustments to the parameterization of the model. However, the BLM-predicted IC50s in the Ca-concentration series tended to be lower than the reported IC50s (Fig. 5a). Therefore, the olfactory BLM developed in the present study is conservative from a regulatory perspective (i.e., the olfactory BLM predicts olfactory impairment at lower Cu concentrations than were reported by McIntyre et al. [19] for the Ca-concentration series).

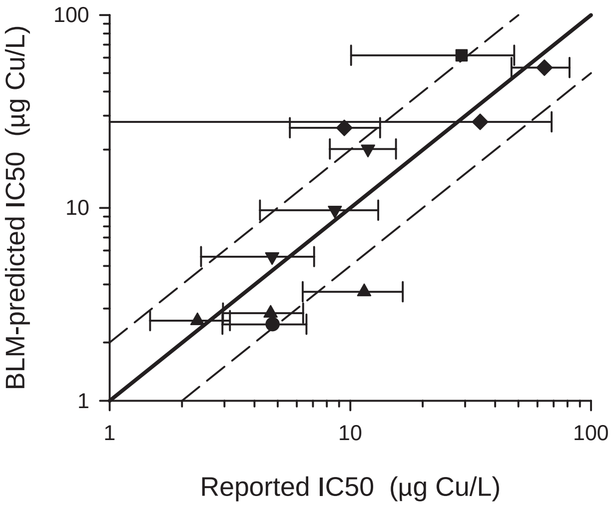

Fig. 4. Median inhibitory concentrations (IC50s) for electro-olfactogram responses of coho salmon (*Oncorhynchus kisutch*) exposed to Cu in various water chemistries (see Supplemental Data, Table S1, for water chemistries in the 11 different treatments) as reported by McIntyre et al. [19] (error bars are 95% confidence intervals), compared with IC50s calculated using version 2.2.3 of the HydroQual Cu biotic ligand model (BLM; http://www.hydroqual.com/wr_blm.html) for rainbow trout (*O. mykiss*), but with 0.1988 nmol Cu/g wet weight used as the critical Cu accumulation in the BLM. Circle is low-ion base water; upward-pointing triangles are $CaCl_2$ added; downward-pointing triangles are $NaHCO_3$ added; diamonds are Suwannee River fulvic acid added; square is Suwannee River natural organic matter added.

A generally accepted range of good agreement for a predicted:observed toxicity ratio is 0.5 to 2.0 [28]. Only three of the 11 ratios were outside that acceptable range, and those three predicted IC50s were less than or equal to three times greater than or less than the observed IC50s (Supplemental Data, Table S3). Variability in the EOG responses of the coho salmon might have contributed to those extreme values. Moreover, because the geometric mean of the (BLM-predicted IC50):(reported IC50) ratios was optimized at 1.0, the ratios for the 11 exposure waters were approximately evenly distributed above and below the ratio of 1.0 that represents perfect agreement between predicted and observed IC50s (Fig. 4).

Alternative ways of calculating the critical Cu accumulation for the EOG response [geometric mean ΣCu-BL (0.2129 nmol Cu/g wet weight), arithmetic mean ΣCu-BL (0.3448 nmol Cu/g wet weight), median ΣCu-BL (0.1649 nmol Cu/g wet weight), and ΣCu-BL of the low-ion base water (0.7121 nmol Cu/g wet weight)] produced BLM-predicted IC50s that fit the reported IC50s less adequately (Supplemental Data, Table S4). For all those alternative methods, three or more of the (BLM-predicted IC50):(reported IC50) ratios were outside the range of 0.5 to 2.0, and the geometric means of the 11 ratios (1.04, 1.40, 0.87, and 2.14, respectively) indicated a less acceptable fit than when the optimized ΣCu-BL of 0.1988 nmol Cu/g wet weight was used in the BLM.

Therefore, the parameterization of Cu BLM version 2.2.3 was adjusted to predict the coho salmon EOG data relatively well, simply by changing the sensitivity parameter (i.e., changing the default LA50 to 0.1988 nmol Cu/g wet weight), without changing any of the cation-BL binding constants. Although the slopes of BLM-predicted IC50 versus Ca or HCO_3^- concentration might appear to differ from the slopes of reported IC50 versus Ca or HCO_3^- concentration (i.e., a shallower BLM-predicted slope in Fig. 5a and a steeper BLM-predicted slope

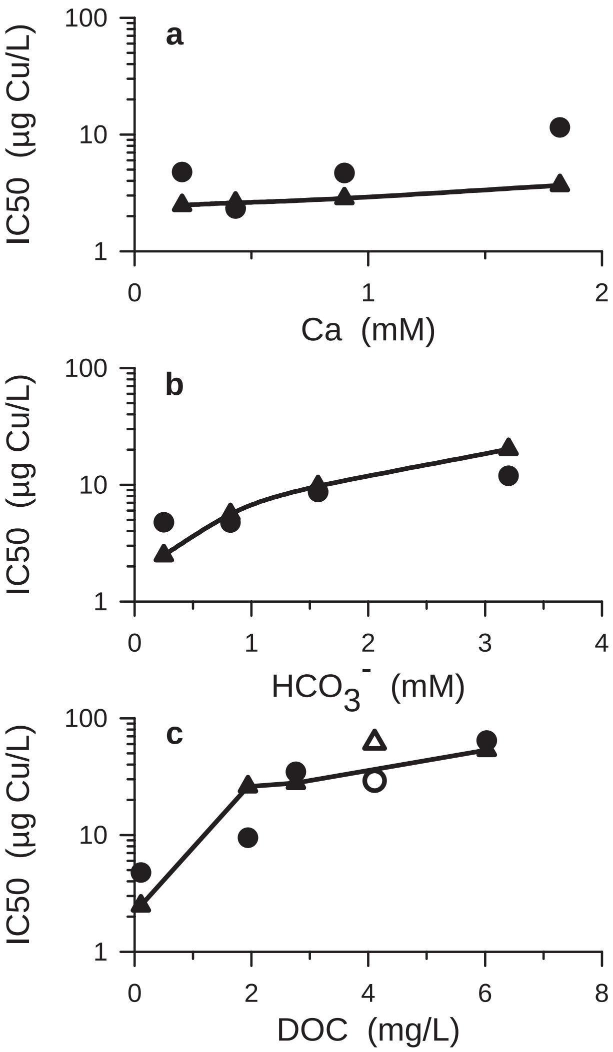

Fig. 5. **a–c**: Median inhibitory concentrations (IC50s) for electro-olfactogram responses of coho salmon (*Oncorhynchus kisutch*) to an L-serine stimulus in the presence of a nominal 20 µg Cu/L. The exposure waters were low-ion water to which $CaCl_2$, $NaHCO_3$, or dissolved organic matter was added (see Supplemental Data, Table S1, for water chemistries in the 11 different treatments). Circles are IC50s reported in McIntyre et al. [19]; triangles are IC50s predicted by the olfactory biotic ligand model parameterized in the present study (using 0.1988 nmol Cu/g wet wt as the critical Cu concentration). In c, solid symbols are treatments to which fulvic acid was added, and open symbols are treatment to which Suwannee River natural organic matter was added. DOC = dissolved organic carbon.

in Fig. 5b), those slopes are not significantly different ($p > 0.05$; Supplemental Data, Table S5), because the apparent differences disappear in the noise of the variability of the reported IC50s. Effectively, the BLM factored out that variability to produce monotonic averaged trends of expected IC50 versus Ca or HCO_3^- concentration (Fig. 5a and b).

The trend of BLM-predicted IC50 versus DOC concentration (Fig. 5c) is not monotonic like the trends for Ca and HCO_3^- concentration. The trend is not monotonic because pH, alkalinity, and DOC concentration concurrently changed in counteracting directions when DOM (either as fulvic acid [FA] or as natural organic matter [NOM]) was added to the low-ion base water and because the three FA treatments (1.94, 2.76, and 6.03 mg DOC/L) were entered into the BLM slightly differently from the NOM treatment (4.11 mg DOC/L) in this study and in McIntyre et al. [18] (i.e., 0.01% humic acid was specified for the FA treatments and 10% humic acid [the default value recommended in BLM version 2.2.3] was specified for the NOM treatment in both studies).

In concept, the fit of the BLM-predicted IC50s to the reported IC50s could be improved slightly by altering one or more of the cation-BL binding constants in the BLM. For example, log $K_{\text{BL-Ca}}$ might be increased slightly to increase the BLM-predicted IC50s closer to the reported IC50s in Figure 5a, and log $K_{\text{BL-CuOH}}$ might be decreased slightly to decrease the slope of BLM-predicted IC50 versus HCO_3^- in Figure 5b to match more closely the slope of reported IC50 versus HCO_3^-. However, it is not believed that such manipulations will be justified until additional EOG-response data sets can be tested with our simple reparameterization of the gill ionoregulatory-based BLM to determine whether similar trends occur among independent data sets.

Modification of a BLM that predicts lethality (for which the underlying physiological mechanism is ionoregulatory disruption) to fit olfactory-related responses of fish does not imply that metal-induced processes occurring on or in olfactory tissue (e.g., the olfactory rosette) are the same as metal-induced processes occurring on or in ionoregulatory tissue (e.g., gills). In fact, olfactory tissue contains molecular receptors, ion channels, transporters, and gene expression different from those of the ionoregulatory tissue [17,18,29]. However, from a coarse modeling perspective, the geochemical basis of the BLM appears to be flexible enough to predict olfactory-related responses relatively well in a variety of water chemistries, even though the BLM does not explicitly include information about metal-induced processes occurring on or in olfactory tissue, just as it predicts lethality relatively well, even though it does not explicitly include information about metal-induced processes occurring on or in ionoregulatory tissue.

IC20:Criteria ratios

BLM-based criteria. In all exposure waters used by Hansen et al. [4,11], Green et al. [9], and McIntyre et al. [18,19], the ratios of the IC20 calculated from the reported data to the U.S. EPA's BLM-based chronic criterion (CCC) were greater than 1.0 (ranging from 1.5 to 36.9), and the (olfactory BLM-predicted IC20):(BLM CCC) ratios (calculated only for the McIntyre et al. [18,19] study, because the olfactory BLM was only parameterized for that study) also were always greater than 1.0 (ranging from 2.7 to 4.7; Table 1, Supplemental Data, Table S6). Additionally, in all exposure waters used by Hansen et al. [4,11] and Green et al. [9], and in all but two exposure waters used by McIntyre et al. [18,19], the ratios of the IC20 calculated from the reported data to the U.S. EPA's BLM-based acute criteria (CMC) were greater than 1.0 (ranging from 1.4 to 23.3). All the (olfactory BLM-predicted IC20):(BLM CMC) ratios also were always greater than 1.0 (ranging from 1.7 to 2.9; Fig. 6, Table 1, Supplemental Data, Table S6). All the IC20:BLM CCC ratios were 1.61 times greater than their IC20:BLM CMC ratios, because the BLM CCC for Cu is always 1.61 times lower than the corresponding BLM CMC [1]. These calculations indicate that U.S. EPA BLM-based criteria for Cu are also protective for olfactory and avoidance responses in these six species-endpoint combinations.

In the two exposure waters in which the ratio of the IC20 calculated from the reported data to the BLM-based CMC did not exceed 1.0 (treatments FA-1 and NOM in McIntyre et al. [18,19]), coho salmon were exposed to Cu in water to which Suwannee River FA was added at 1.94 mg DOC/L (treatment FA-1) or Suwannee River NOM was added at 4.11 mg DOC/L

Table 1. IC20s (20% inhibitory concentrations) for avoidance of Cu by or impairment of olfactory responses in rainbow trout (*Oncorhynchus mykiss*) and Chinook salmon (*Oncorhynchus tshawytscha*; calculated from data in Hansen et al. [4,11]) or for impairment of olfaction in fathead minnows (*Pimephales promelas*) exposed to waterborne Cu (calculated from data in Green et al. [9]), compared with the U.S. Environmental Protection Agency's (U.S. EPA) acute and chronic aquatic life criteria for Cu (calculated using either the hardness-adjustment equations in U.S. EPA [27] or version 2.2.3 of the HydroQual Cu biotic ligand model [BLM; see http://www.hydroqual.com/wr_blm.html; additional results are presented in Supplemental Data, Tables S6 and S7])

Species	Behavior/ olfactory response[a]	IC20 (μg Cu/L)	Hard CMC[b,c] (μg Cu/L)	Hard CCC[b,d] (μg Cu/L)	IC20:hard CMC ratio	IC20:hard CCC ratio	BLM CMC[b,e] (μg Cu/L)	BLM CCC[b,f] (μg Cu/L)	IC20:BLM CMC ratio	IC20:BLM CCC ratio
Rainbow trout	Avoidance	0.84	3.68	2.77	0.23	0.30	0.38	0.23	2.2	3.7
	EEG (L-serine)	5.1	3.57	2.69	1.43	1.90	0.46	0.29	11.1	17.6
Chinook salmon	Avoidance	0.91	3.68	2.77	0.25	0.33	0.38	0.23	2.4	4.0
	EEG (L-serine)	10.7	3.57	2.69	3.01	3.98	0.46	0.29	23.3	36.9
Fathead minnow	EOG (L-arginine)	5.0	3.38	2.56	1.47	1.94	1.53	0.95	3.2	5.2

[a] Measured responses were avoidance of Cu-containing water; electroencephalogram (EEG; the electrical potential measured in the olfactory lobe of the brain after 30 min of exposure to Cu-containing water, when the olfactory rosette was challenged with L-serine); and electro-olfactogram (EOG; the transepithelial electrical potential measured at the surface of the olfactory rosette after 10 min of exposure to Cu-containing water, when challenged with L-arginine).

[b] Calculated using water chemistry in Supplemental Data, Table S1.

[c] Hard CMC = U.S. EPA hardness-based criteria maximum concentration (the acute criterion).

[d] Hard CCC = U.S. EPA hardness-based criteria continuous concentration (the chronic criterion).

[e] BLM CMC = U.S. EPA BLM-based criteria maximum concentration (the acute criterion).

[f] BLM CCC = U.S. EPA BLM-based criteria continuous concentration (the chronic criterion).

(treatment NOM; Fig. 6, Supplemental Data, Table S6). However, those IC20s were only 7 and 8% lower than (i.e., the IC20:BLM CMC ratios were 0.93 and 0.92, respectively) and not significantly different from ($p > 0.05$) the acute criteria for those exposure waters, and the acute Cu criteria were protective at the two other FA concentrations (2.76 and 6.03 mg DOC/L in treatments FA-2 and FA-3, respectively). Given the high variability in the IC20 estimates for treatments FA-1, FA-2, FA-3, and NOM (the 95% confidence intervals were ±27–104% of the mean IC20s; Supplemental Data, Table S6), the reported IC20s for treatments FA-1 and NOM might have been underestimates.

Because the olfactory BLM factors out the variability in the reported IC50s (and, thus, in the back-calculated IC20s), the higher IC20s predicted by the olfactory BLM probably are more reliable estimates of the real IC20s in the FA-1 and NOM exposure waters than are the IC20s back-calculated from the reported IC50s. Therefore, with the BLM-predicted IC20s, it is concluded that 20% avoidance of Cu and 20% impairment of olfaction (as measured by EEG or EOG responses) always occurred at Cu concentrations greater than the U.S. EPA's BLM-based acute and chronic criteria, demonstrating that the Cu criteria were protective for those sublethal endpoints in rainbow trout, Chinook salmon, coho salmon, and fathead minnows. However, additional olfactory studies could help to decrease the uncertainty in the EOG data and thus increase confidence in this conclusion.

Hardness-based criteria. In two of the four exposure water–species combinations used by Hansen et al. [4,11] (for Cu avoidance by rainbow trout and Chinook salmon) and in six of the 11 exposure waters used by McIntyre et al. [18,19] (for EOG responses by coho salmon), the ratios of the IC20 calculated from the reported data to the U.S. EPA's hardness-based chronic criteria (CCC) were less than 1.0 (ranging from 0.14 to 0.87, with the other ratios ranging from 1.0 to 6.7), and, in the same six exposure waters in McIntyre et al. [18,19], the (olfactory BLM-predicted IC20):(hardness CCC) ratios also were less than 1.0 (ranging from 0.07 to 0.98, with the other ratios ranging from 2.0 to 6.6; Table 1, Supplemental Data, Table S7). The IC20:hardness-based CCC ratio in the exposure water used by Green et al. [9] was 1.9 (Table 1). Olfactory BLM-predicted IC20s were not calculated for the Cu-avoidance and EEG responses by rainbow trout and Chinook salmon in Hansen et al. [4,11] and for the EOG responses by fathead minnows in Green et al. [9], because the olfactory BLM was parameterized only for the coho salmon EOG responses reported by McIntyre et al. [18,19].

The IC20:hardness-based CMC ratio in the exposure water used by Green et al. [9] was 1.5 (Table 1). However, in two of the four exposure water–species combinations used by Hansen et al. [4,11] (again, for Cu avoidance by rainbow trout and Chinook salmon) and in eight of the 11 exposure waters used by McIntyre et al. [18,19], the ratios of the IC20 calculated from the reported data to the U.S. EPA's hardness-based acute criteria (CMC) were less than 1.0 (ranging from 0.10 to 0.87, with the other ratios ranging from 1.4 to 5.0); and the (olfactory BLM-predicted IC20):(hardness-based CMC) ratios also were less than 1.0 (ranging from 0.05 to 0.73, with the other ratios ranging from 1.5 to 5.0; Fig. 6, Table 1, Supplemental Data, Table S7). All the IC20:hardness CCC ratios were 1.3 to 1.6 times greater than their IC20:hardness CMC ratios, because, within the hardness range used by Hansen et al. [4,11], Green et al. [9], and McIntyre et al. [18] (23–190 mg/L as $CaCO_3$), the hardness-based CCC for Cu is 1.3 to 1.6 times lower than the corresponding hardness-based CMC.

These calculations indicate that U.S. EPA's hardness-based criteria for Cu sometimes are considerably underprotective for olfactory and avoidance responses and sometimes are much less protective than the BLM-based criteria, whereas, in other water quality conditions, the hardness-based criteria are more protective than the BLM-based criteria (compare Figs. 6a–c with d–f). When the hardness-based criteria were more protective than the BLM-based criteria in these studies, the DOC concentration was >4 mg/L. Water quality leading to underprotection by the hardness-based criteria included low to high hardness alone (27–190 mg/L as $CaCO_3$), low to high alkalinity alone (11–160 mg/L as $CaCO_3$), and low to intermediate DOC concentration alone (0.11–1.94 mg/L). Combinations of these water quality parameters at intermediate concentrations were not tested in these studies.

Limitations

This analysis was confined to water quality in Cu-avoidance and neurophysiology studies conducted in laboratory waters.

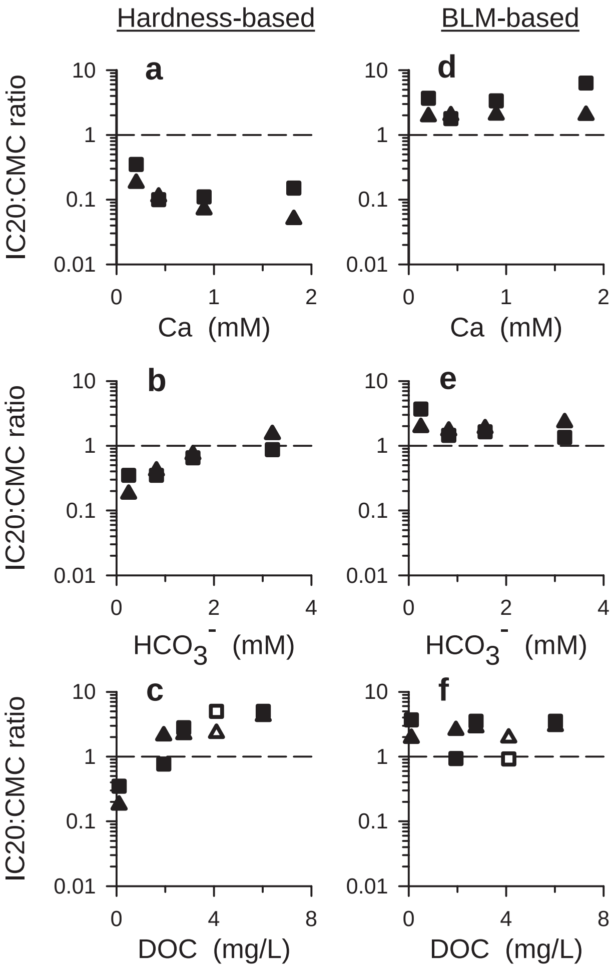

Fig. 6. IC20s (20% inhibitory concentrations) for electro-olfactogram responses of coho salmon (*Oncorhynchus kisutch*) exposed to waterborne Cu, compared with the acute aquatic life criteria for Cu (calculated using the hardness-adjustment equations in U.S. Environmental Protection Agency [27]; [**a–c**] or version 2.2.3 of the HydroQual Cu biotic ligand model [BLM; http://www.hydroqual.com/wr_blm.html; **d–f**]). CMC = criteria maximum concentration (i.e., the acute criterion); DOC = dissolved organic carbon. Squares are IC20:CMC ratios calculated from IC50s in McIntyre et al. [19]; triangles are IC20:CMC ratios calculated from IC50s predicted by the olfactory BLM parameterized in the present study (using 0.1988 nmol Cu/g wet weight as the critical Cu concentration). In **c** and **f**, solid symbols are treatments to which fulvic acid was added, and open symbols are the treatment to which Suwannee River natural organic matter was added. Some triangles are overlapped by squares.

The analysis could be extended to a variety of surface water chemistries, using the olfactory BLM parameterized in the present study to predict IC20s and using the U.S. EPA Cu BLM or the U.S. EPA hardness-adjustment equations to calculate the CMCs and CCCs. Such an analysis would test whether the Cu criteria protect for olfactory-related responses across a much wider range of water chemistries than have thus far been tested in the laboratory. Furthermore, such an analysis could help refine estimates of the degree to which episodic elevated concentrations of Cu due to nonpoint-source pollution might impair olfactory-related function in salmonid fishes. Nonpoint-source Cu loading to receiving waters sometimes is relatively high in, for example, urban stormwater runoff, mine drainage, atmospheric deposition, and accidental spills (see, e.g., pages 16–21 in Meyer et al. [23],[30]).

Although the U.S. EPA's new BLM-based Cu criteria were the focus of this analysis, those criteria have not yet been adopted by any of the states in which migration and spawning of Pacific salmonids are major concerns (i.e., Alaska, California, Idaho, Montana, Oregon, and Washington, USA). Instead, many states still use hardness-based Cu criteria. Therefore, another relevant concern for salmonid conservation is whether the criteria currently used by individual states protect against olfactory-related responses to Cu. Addressing that concern would require a state-by-state analysis beyond the scope of this article. However, if a state's current criteria are not protective, results of the present study suggest that adoption of the BLM-based Cu criteria would provide adequate protection against olfactory-related responses in salmonid fishes exposed to Cu.

SUMMARY

By adjusting only the LA50 in the current gill ionoregulatory-based Cu BLM version 2.2.3, the relatively sensitive EOG responses of coho salmon reported by McIntyre et al. [19] could be predicted well. That result is surprising, suggesting the BLM is a flexible tool that, when appropriately parameterized, can be used to predict biological responses of seemingly disparate tissues across a wide range of water-chemistry conditions. This olfactory BLM was used to factor out the variability of EOG responses in the McIntyre et al. [19] data and demonstrate that the BLM-based U.S. EPA acute and chronic water quality criteria for Cu protected against at least 20% avoidance of Cu (by rainbow trout and Chinook salmon) and at least 20% olfactory impairment (to rainbow trout, Chinook salmon, coho salmon, and fathead minnows) in the 16 exposure water–species combinations tested by Hansen et al. [4,11], Green et al. [9], and McIntyre et al. [18,19]. However, the U.S. EPA hardness-based criteria for Cu would have been considerably underprotective against olfactory-related responses in many of those same exposure waters. Although less than 20% olfactory-related impairment might be considered important for some species of concern, the variability in the available EOG data do not justify a benchmark olfactory-impairment index lower than approximately 20%. Therefore, it is concluded that the U.S. EPA's current BLM-based water quality criteria for Cu [1] would not have to be adjusted to provide additional protection against avoidance of Cu or impairment of olfactory responses in the six species–endpoint combinations analyzed in the present study, but the U.S. EPA's previous hardness-based water quality criteria for Cu [27] would have to be adjusted.

SUPPLEMENTAL DATA

Supplementary Methods. Overviews of the four studies whose results are used in the present study; details of the methods used to calculate the percentage avoidance, EEG, and EOG responses in those studies; and equations for the thermodynamic constants for binding of cations to the biotic ligand in the HydroQual BLM.

Table S1. Water chemistry used to calculate U.S. EPA acute and chronic water quality criteria for Cu in the exposure waters.

Table S2. Chemistry of well water from Red Buttes Environmental Biology Laboratory used in Cu titrations of its DOM.

Table S3. Comparison of the reported IC50s for impairment of EOG responses of coho salmon and the IC50s predicted using the olfactory BLM parameterized in the present study.

Table S4. Comparison of the reported IC50s for impairment of EOG responses of coho salmon and the IC50s predicted using alternate ways of parameterizing the olfactory BLM in the present study.

Table S5. Comparison of the slopes of linear regressions of reported or BLM-predicted IC50s versus Ca or HCO_3^- concentration for EOG responses of coho salmon exposed to Cu.

Table S6. Comparison of the IC20:CMC and IC20:CCC ratios calculated for avoidance of Cu or impairment of olfactory responses of rainbow trout, Chinook salmon, coho salmon, and fathead minnows exposed to waterborne Cu, using CMCs and CCCs calculated by the BLM.

Table S7. Comparisons analogous to those in Table S6, but instead using CMCs and CCCs calculated from the U.S. EPA's hardness-adjustment equations.

Fig. S1. Cu^{2+} concentrations measured in well water from Red Buttes Laboratory during Cu titrations of its DOM.

Appendix A. Concentration–response equations used in the present study (163 KB PDF).

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