Application of the biotic ligand model to predict copper acute toxicity to Medaka fish in typical Chinese rivers

Chunyan Wang, Hao Chen, Kuen Benjamin Wu, Lihui An
and Binghui Zheng

ABSTRACT

LA_{50}, the Lethal Accumulation of Cu on the Medaka fish (*Oryzias latipes*) gills that results in 50% mortality during a toxicological exposure (96 hours) in synthetic water was assessed by use of the biotic ligand model (BLM). The LA_{50} was employed to predict the 96 h Cu toxicity (LC_{50}) to this fish in different natural surface waters in China. The LC_{50} values were predicted with errors of no more than 1.55 for the river water except for two water samples, one of which was from a tidal river and the other of which was from a river that was subject to joint metal pollution and possibly affected by other pollutants.

Key words | biotic ligand model, copper, Medaka fish

Chunyan Wang
School of Resource and Environmental Science,
Wuhan University,
Wuhan,
China

Chunyan Wang
Hao Chen (corresponding author)
Kuen Benjamin Wu
Lihui An
Binghui Zheng (corresponding author)
State Key Laboratory of Environmental Criteria and Risk Assessment,
Chinese Research Academy of Environmental Sciences,
Beijing,
China
E-mail: *chenhao@craes.org.cn*; *zhengbh@craes.org.cn*

Kuen Benjamin Wu
HydroQual Inc.,
New Jersey,
USA

INTRODUCTION

It is well known that free metals are responsible for toxicity to aquatic species (e.g. fish) (Paquin *et al.* 2002). Metal toxicity can be highly variable and dependent on ambient water chemistry, because this affects the free metal concentrations. Consequently, a lethal metal concentration, when expressed as dissolved metal concentration (referred to as LC_{50}) resulting in 50% mortality of an aquatic organism during 96 h of exposure, has been observed to vary significantly with water chemistry. Metal speciation (including complexation with organic and inorganic ligands), rather than total concentration of the metal, has been taken into consideration to account for metal bioavailability in metal risk assessment in several countries and regions (e.g. the US and European Union). For risk assessment, the biotic ligand model (BLM) may be considered the most widely accepted tool, in which organism organs are considered to be biotic ligands that adsorb metals (Pagenkopf 1983; Playle *et al.* 1993; Campbell 1995; MacRae *et al.* 1999; Di Toro *et al.* 2001; EPA 2007). The BLM was originally developed to account for observed copper toxicity to fish, and this toxicity has now been well accepted to originate from the gills-adsorbed copper speciation (i.e. Cu(II) and $Cu(OH)^+$), which blocks the pathway for sodium exchange between fish and water. The lethal accumulation of metal speciation on the gills resulting in 50% mortality within 96 h of exposure is referred to as LA_{50}, and this parameter is usually assumed to be metal-specific and invariant for given organisms of the same age.

The last two decades have seen a wide application of the BLM in predicting metal toxicity (e.g. Cu, Zn, Cd, Ag, Ni, Pb) to a variety of aquatic organisms (McGeer *et al.* 2000; Paquin *et al.* 2002; De Schamphelaere *et al.* 2005; Kozlova *et al.* 2009; Chen *et al.* 2010). Extensive work has been conducted in natural waters in the US and Europe, but much less has been done in China and many other countries. For example, the BLM has been recommended for establishment of a Cu aquatic toxicity standard by the Environmental Protection Agency (2007). However, it is not clear whether the BLM can be applied for predicting Cu aquatic toxicity under the conditions in China, because China and the US

doi: 10.2166/wst.2011.728

differ markedly in water quality. Additionally, the BLM has been verified with fishes that are native in North America, e.g. rainbow trout (*Oncorhynchus mykiss*) but not with typical fishes in China. Little information has been documented as to the applicability of the BLM in China. Given the diversity and complexity of natural watersheds in China, it is worth verifying the BLM in different natural waters before this model is ultimately employed in water quality standard establishment in China.

Oryzias latipes, also known as Medaka fish and Japanese killifish is a member of Oryzias. A mature fish is 20–40 mm in length. This fish is easy to feed, and can survive in a wide range of temperature and salinity (i.e. freshwater and brackish water), widely present in the north, south and east of China. However, little information is available in the literature for predicting Cu toxicity data for *Oryzias latipes* with use of the BLM. The present work aimed at verifying the BLM with Cu toxicity to *Oryzias latipes* in typical rivers in China.

EXPERIMENTAL

Experimental design

Acute toxicity experiments were first undertaken in laboratory-made synthetic water that had well-controlled water quality parameters. A series of LC_{50} of Cu were experimentally obtained, and the LC_{50} were used in BLM software to estimate LA_{50}. After obtaining the LA_{50}, the LC_{50} for fishes in river water was predicted with use of the BLM, and the values were compared to the values obtained by direct toxicity experiments. The validity of the BLM for each river was evaluated using the ratios of the two LC_{50} values (predicted to observed) of that river.

River water sampling and synthetic solutions

One river water sample was collected in the upper reach of the Three-Gorge Dam, which is the largest dam for hydropower in the world and is located in the middle reach of the Yangtze River, the largest river in China. Another water sample was collected in the same river but at a different cross-section, which was close to Nanjing, a city located in the lower reach of the Yangtze River.

Two samples were collected in the Yellow River, the second largest river in China which is also a sediment-laden river currently containing significant amounts of suspended solids (e.g. sands and clays); one was sampled in a section close to Zhengzhou City and the other was sampled in a place close to Jinan City, the former and latter cities being in the middle and lower reach of the river, respectively.

Xiang Jiang River is one of the main tributaries of the Yangtze River, and has been reported to be subject to high metal pollution due to mining and industrial activities in the draining area. One sample was collected at the SongBai Bridge in the upper reach of the river, while another sample was collected at Chang Sha City in the lower reach.

Gan Jiang River is the second largest branch of the Yangtze River and is subject to increasing water pollution in the last decade due to rapid industrialization and urbanization. One sample was collected in Gan Zhou City located in the upper reach and another sample was collected in the Ba Yi Bridge in the lower reach.

Da Liao River is a typical tidal river that enters the Bohai Sea in the north-east of China. Three different locations, i.e. San Cha Kou (upper reach), Tian Zhuang Tai (middle reach) and Liao River Park (lower reach) were involved for the sample collections; the distances to the estuary were 92, 34 and 9 km, for these three sampling locations, respectively. For each location, two samples were collected in the rising tide and ebb tide, respectively. All waters were immediately transported back to laboratories and filtered through 0.45-μm filter membranes (Millipore).

Synthetic solutions were prepared by dissolving analytical grade chemicals ($CaCl_2{\cdot}2H_2O$, $MgSO_4$, $NaHCO_3$, KCl and $CuCl_2{\cdot}2H_2O$ in proper concentrations) in ultrapure water (Milli-Q, Millipore), which was thoroughly aerated by air bubbling for more than 2 h.

Preliminary studies in our work and earlier research by others (De Schamphelaere & Janssen 2002) had showed that the measured concentrations of Ca^{2+}, Mg^{2+}, Na^{+}, K^{+}, Na^{+}, Cl^{-}, $SO_4{}^{2-}$ were not apparently different from the nominal concentrations in this synthetic water. Therefore, we did not measure these ion concentrations in the present work but reported the concentrations as prepared. Parameters such as pH, temperature and alkalinity were monitored. Sulfide and DOC were not detectable. Given the possibility of slight contamination of the reference water (e.g. dissolution of airbone organic molecules in the water when exposed to air) and the requirement of input parameters being non-zero in the BLM software, the concentrations of S^{2-}, DOC and the percentage of HA-related DOC in the total DOC pool were assumed to be 1×10^{-10} mg/L, 0.05 mg/L and 10% in the synthetic water, respectively.

Ninety per cent of the total DOC was assumed to be related to fulvic acid, which is a default assumption in the current BLM model and is in line with the literature report that fulvic acid is the dominant DOC fraction in natural surface waters (Thurman 1985).

For the collected natural surface waters, temperature, pH, DOC, alkalinity, Ca^{2+}, Mg^{2+}, K^+, Na^+, Cl^-, SO_4^{2-} were all monitored (Table 2). Ca^{2+}, Mg^{2+}, K^+, Na^+ were measured by an atomic absorption spectrometer (WFX-210, BRAIC, China), Cl^- and SO_4^{2-} were measured by ion chromatography (DX-300, DIONEX, US). DOC was determined on a Shimadzu TOC analyzer (TOC-VCPN, Japan). Alkalinity was analyzed by titration as reported by Nollet (2000). The concentrations of sulfide in filtered natural waters were lower than the detection limit and were assumed to be 1×10^{-10} mg/L.

Acute toxicity reference

The synthetic solutions (4 L) without containing Cu were stored in jars (10 L) at 23 ± 1° for 1 day prior to the toxicity experiments. Three groups of synthetic solutions were prepared to have different concentrations of $CaCl_2$ and $MgSO_4$ but fixed concentrations of $NaHCO_3$ and KCl. The chemical compositions of these solutions are summarized in Table 1.

Fish were acclimated to a laboratory environment for several generations prior to toxicological exposure. Fish selected for toxicity exposure were 30 ± 5 mm in length and 0.3 ± 0.1 g in weight. Before toxicological exposure to copper, the selected fish was acclimated for 7 days in the above-mentioned synthetic water. The settled sludge and food leftover in the jars were frequently removed. Feeding of the fish was stopped within 24 h prior to addition of copper to the synthetic water for toxicity exposure. The mortality was less than 10% during the period time of acclimation in the absence of added Cu.

Preliminary studies had been conducted to find a suitable concentration range for the Cu that would give a fish mortality rate between 20% and 80% during 96 h exposure. Cu was spiked (as a stock solution) into the above-mentioned synthetic solutions to give final Cu concentrations of 0, 0.10, 0.20, 0.30, 0.40, 0.50 and 0.60 mg/L, respectively. The total number of solutions was therefore 21 (3 groups × 7 solutions). Eight fish were placed in each solution. Experiments were carried out under continuous and uniform illumination at near constant temperature (23 ± 1 °C). The number of live fish was counted after 24, 48, 72 and 96 h. Fish were considered dead if they were not responsive when touched by a glass rod. All the toxicity experiments were undertaken in duplicate.

Toxicity experiments in natural surface waters were conducted using similar procedures except that the waters were passed through 0.45-μm membrane filters (Millipore) and no chemicals but Cu were added. Preliminary studies showed that the Cu concentration in the filtered natural waters was quite low, and on the order of magnitude of 1 ~ 10 μg/L. Given the difficulty of precisely measuring the low metal concentration of μg/L and the relatively large spiked Cu concentration for acute toxicity, the waterborne Cu concentration was not taken into account when measuring the LC_{50}, in contrast to chronic toxicity exposure in which relatively low Cu concentrations were used (McGeer *et al.* 2002).

Data treatment and statistics

The 96-h LC_{50} was evaluated with SPSS 16.0 (Statistical Program for Social Sciences, SPSS Inc., US). Given the water quality parameters of the synthetic waters and the above-obtained LC_{50}, a 'speciation' mode in the BLM software of version 2.2.3 (HydroQual Inc., US) was used to compute the concentrations of BL-Cu and BL-CuOH, which refer to the concentrations of Cu and CuOH adsorbed onto the fish gill, respectively. LA_{50} was the sum of BL-Cu and BL-CuOH (Table 3). A 'userdefined' mode in the BLM software was used to predict the LC_{50} of copper toxicity to the fish in real waters based on LA_{50} and the water quality parameters (Table 3).

Table 1 | Relevant water quality parameters of the synthetic water

No	T (°C)	pH	Ca^{2+} (mg/L)	Mg^{2+}(mg/L)	Na^+ (mg/L)	K^+ (mg/L)	Cl^- (mg/L)	SO_4^{2-} (mg/L)	S^{2-} (mg/L)	DOC (mg/L)	Alkalinity (mg/L)
1	23.0	7.84	40.0	12.03	17.25	2.88	73.48	48.12	0	0.05	45.54
2	23.0	7.88	80.12	12.03	17.25	2.88	144.54	48.12	0	0.05	41.83
3	23.0	7.57	80.12	30.0	17.25	2.88	144.54	120.0	0	0.05	40.46

Table 2 | Relevant water quality parameters of the natural water

No	River		T (°C)	pH	Ca^{2+} (mg/L)	Mg^{2+} (mg/L)	Na^{+} (mg/L)	K^{+} (mg/L)	SO_4^{2-} (mg/L)	Cl^{-} (mg/L)	Alkalinity (mg/L)	DOC (mg/L)	S^{2-} (mg/L)
1	Yangtze River	Upper reach of the Three-Gorge Dam	25.5	7.74	42.51	8.407	15.33	0.015	35.26	17.24	103.07	3.6	0
		Nanjing City	21	7.79	28.91	6.287	8.71	0.015	25.23	15.5	82.45	8.9	0
2	Yellow River	Zheng Zhou City	24.2	8.10	46.40	15.73	9.04	7.47	62.84	37.29	135.59	2.885	0
		Ji Nan City	23.8	8.21	42.95	19.24	9.42	8.70	96.07	63.93	142.73	4.348	0
3	Xiang Jiang River	Song Bai Bridge	24.0	8.03	19.32	2.279	3.71	2.09	10.77	4.84	85.64	0.863	0
		Chang Sha City	24.6	7.85	20.20	2.686	6.13	3.16	20.25	15.01	64.23	0.904	0
4	Gan Jiang River	Gan Zhou City	29	6.79	19.86	2.329	7.00	0.015	13.29	20.83	21.17	1.8	0
		Ba Yi Bridge	31	7.62	22.83	1.711	4.84	0.015	28.07	14.05	37.47	1.6	0
5	Da Liao River	San Cha River (rising tide)	22.0	7.56	2.56	0.65	4.47	2.92	56.73	18.17	80.15	2.56	0
		San Cha River (ebb tide)	23.0	7.41	2.42	0.65	4.48	2.98	59.34	20.78	78.35	2.42	0
		Tian Zhuang Tai (rising tide)	27.9	7.47	2.39	0.65	4.48	2.96	47.52	23.66	80.34	2.39	0
		Tian Zhuang Tai (ebb tide)	24.2	7.34	2.36	0.65	4.50	3.05	51.52	31.76	80.15	2.36	0
		Liao River park (rising tide)	20.2	7.35	2.35	0.65	4.50	3.06	51.84	30.57	84.5	2.35	0
		Liao River park (ebb tide)	23.0	7.45	2.34	0.65	4.48	2.91	61.08	45.24	86.92	2.34	0

Table 3 | The values LC_{50} and LA_{50} for the synthetic water in duplicate experiments. The series 1, 2 and 3 refer to different synthetic water samples for the exposure experiments

Series	LC_{50} (μg/L)	LC_{50} (95% confidence interval) (μg/L)	Avg. LC_{50} (μg/L)	BL-Cu (nmol/gw)	BL-CuOH (nmol/gw)	LA_{50} (nmol/gw)	Avg. LA_{50} (nmol/gw)
1	337 366.92	271.09–394 299.0–424.90	351.96	11.36	1.592	12.952	12.43
2	360.99 360.81	296.86–419.91 273.22–475.25	360.9	9.614	1.472	11.086	
3	414.06 457.0	348.10–480.22 386.08–537.19	435.56	12.32	0.9214	13.2414	

RESULTS AND DISCUSSION

Relevant water quality parameters of three synthetic waters are listed (Table 1), while the relevant water quality parameters in the natural waters are detected in Table 2. As presented in Table 2, water quality parameters varied substantially across the sampling sites. The Yellow River samples had the highest alkalinity amongst all the samples while the Gan Jiang River samples had the lowest alkalinity. The LC_{50} values obtained for synthetic waters with varied concentrations of solute are listed in Table 3. As indicated (Table 3), the apparent LC_{50} values increase in the order of No. 1 < No. 2< No. 3 for the three synthetic waters, but the computed LA_{50} values fluctuate in a narrow range without showing an obvious variability trend. Given the same type of synthetic water, gill-bound Cu has larger quantities than gill-bound CuOH (Table 3). It is evident that gill-bound Cu is the dominant copper speciation leading to 50% mortality. The calculated LA_{50} values are 12.95, 11.09 and 13.24 nmol metal/g of wet gill tissue (nmol/gw) for the three types of synthetic water, and the average value is 12.43 nmol/gw (Table 3).

The predicted LC_{50} and experimentally observed LC_{50} for the fish in each type of river water were compared (Table 4). The Yangtze River and Yellow River had the highest LC_{50} amongst all the tested samples, while the Gan Jiang

Table 4 | Comparison of experimentally observed and model predicted values of LC_{50}

No.	River names	Sampling sites	LC_{50} (μg/L) SPSS	BLM	Ratio of LC_{50} obtained from SPSS and BLM (high value divided by low value)	Characteristic of river
1	Yangtze River	Upper reach of the Three-Gorge Dam	1086.23	980.48	1.11	The biggest river in China having the Three-Gorge Dam
		Nanjing City	1313.93	1242	1.06	
2	Yellow River	Zheng Zhou City	1142.69	1544	1.35	Sediment-laden river
		Ji Nan City	1326.42	1916	1.44	
3	Xiang Jiang River	Song Bai Bridge	296.41	459.84	1.55	Metals pollution
		Chang Sha City	305.94	347.52	1.14	
4	Gan Jiang River	Gan Zhou City	192.82	216.83	1.12	Metals pollution
		Ba Yi Bridge	141.16	270.40	1.92	
5	Da Liao River	San Cha River (rising tide)	565.28	487.87	1.16	Tidal river
		San Cha River (ebb tide)	517.13	523.58	1.01	
		Tian Zhuang Tai (rising tide)	468.89	480.27	1.02	
		Tian Zhuang Tai (ebb tide)	455.19	440.51	1.03	
		Liao River Park (rising tide)	616.51	433.6	1.42	
		Liao River Park (ebb tide)	819.76	360.96	2.27	

River had the lowest LC_{50}. For each natural water sample, the ratio of two LC_{50} values (the bigger value divided by the smaller value) was computed (Table 4). Except for the largest ratio (2.27) at the Liao River Park, two relatively large ratios, 1.92 and 1.55, occurred at the Ba Yi Bridge and at the Song Bai Bridge, respectively. The other ratios ranged between 1.02 and 1.44.

It is noteworthy that the three largest LC_{50} ratios occurred to the natural surface waters that were either close to bridges or in the lower reach of a tidal river at ebb tide. A large ratio indicates the high discrepancy between the predicted LC_{50} and experimental LC_{50}. In addition, the BLM overestimated the LC_{50} relative to the experimental values for these two bridge water samples. In these cases, copper toxicity to the fish in these bridge waters was actually more severe than expected, which may be caused by the multiple pollutants in these waters from anthropogenic activities in the local environment.

The most severe deviation of the BLM prediction from the experimentally observed values occurred at the Liao River Park, which was within 10 km of the estuary and therefore subject to marked influence of the saline waters. In addition, severe overestimation of the copper toxicity by the BLM occurred for this water. To our best knowledge, application of the BLM to tidal rivers has not been reported until now. The exact cause for the large discrepancy between predicted and measured LC_{50} values in the Liao River Park is not well understood.

The Cu speciation in the waters was evaluated by the Windermere Humic Aqueous Model (WHAM v.5, Tipping 1994) in the the BLM. It was reported previously that saline waters affected the BLM prediction (Arnold *et al.* 2005), and the bias was suggested to be related to the limitation of WHAM v. 5 for which the generic parameters of copper binding by DOC were only calibrated for freshwaters. Therefore, as suggested by Arnold *et al.* (2005), there is a need to correct the WHAM for saline waters, and related work is under way in our laboratory. It should be noted that the BLM assumes that 90% of the DOC is due to fulvic acid, while 10% is assumed to be humic acid. The characteristics and composition of the DOC in natural water however are likely quite different for freshwaters and tidal rivers. Although it has been reported that the 'active fulvic acid' portion could be optimized to give best fit for the metal binding by DOC in waters (Vulkan *et al.* 2000; Tipping 2002), this 'optimization' procedure was not implemented in the BLM. Should the metal-DOC binding data be available, it may be used to optimize the DOC composition rather than assume it, and the BLM may give better fit to the toxicity data. Additionally, the state-of-the-art version of WHAM (version 6.0) instead of the version 5.0 should have been incorporated into the current BLM (version 2.2.3) to improve the modeling accuracy.

In contrast, other sites showed relatively good agreement between prediction and experiments, although some overestimation or underestimation of the toxicity was made by the use of the BLM (Table 4). The best agreement was observed for the samples of Da Liao River collected in the river upper and middle reaches. The Yellow River samples showed worse ratios than the Yangtze River, although these two rivers in general showed relatively good results. An early study (Lv *et al.* 2006) used the BLM to study the Cu toxicity to Rainbow trout in the Yellow River water and found that the experimental LC_{50} ranged between 0.21 and 0.33 mg/L, lower than the predicted values ranging between 0.42 and 1.00 mg/L. In addition, the experimental and predicted values in that study were lower than the counterparts in the present study. In contrast to Rainbow trout, *Oryzias latipes* are well adapted to fresh water in China, which may account for the higher tolerance to Cu toxicity of this fish than Rainbow trout in the Yellow River water. The Yellow River is a sediment-laden river, and such a river has received very limited attention in the application of the BLM in the literature. Application of the BLM to predict the Cu acute toxicity in sediment-laden water to a fish that has not yet been included as a 'model' fish in the current BLM software adds to the understanding of the applicability of the BLM.

It should be noted that the BLM worked relatively well for predicting the LC_{50} for some water samples from the Xiang Jiang River and Gan Jiang River that are subject to joint metal pollution. Predicting the joint metal toxicity is a difficult task and has been rarely explored in BLM studies. However, more recent work by Chen *et al.* (2010) demonstrated use of the BLM for metal mixtures of Pb and Cu (Chen *et al.* 2010), in line with the findings in the present work.

CONCLUSIONS

The present work employed the BLM to predict Cu toxicity to *Oryzias latipes* in filtered waters collected from several typical rivers in China. Good agreement between the model prediction and the experimental values were achieved, except for a tidal river water sample and two water samples, the former being under strong influence of nearby estuarine environment and the latter (near traffic bridges) being presumably polluted by non-metal toxicants (e.g. organic pollutants). The effect of non-metal toxicants on BLM prediction of metal acute toxicity should be taken into consideration to modify the current BLM. The direct application of the BLM to Cu toxicity testing for unfiltered river waters is more relevant to real-world problems, and is worth further investigating.

ACKNOWLEDGEMENTS

This work was supported by China National Programme of Water Pollution Control and Treatment (project no. 2009ZX07528-003-05).

REFERENCES

Arnold, W. R., Santore, R. C. & Cotsifas, J. S. 2005 Predicting copper toxicity in estuarine and marine waters using the biotic ligand model. *Mar. Pollut. Bull.* **50**, 1634–1640.

Campbell, P. 1995 Interactions between trace metals and aquatic organisms: a critique of the free-ion activity model. In: *Metal Speciation and Bioavailability in Aquatic Systems* (A. Tessier & D. R. Turner, eds). Vol. 3, pp. 45–102. John Wiley, New York.

Chen, Z., Zhu, L. & Wilkinson, K. 2010 Validation of the Biotic ligand model in metal mixtures: bioaccumulation of Lead and Copper. *Environ. Sci. Technol.* **44** (9), 3580–3586.

De Schamphelaere, K. & Janssen, C. 2002 A biotic ligand model predicting acute copper toxicity for *Daphnia magna*: the effects of calcium, magnesium, sodium, potassium, and pH. *Environ. Sci. Technol.* **36** (1), 48–54.

De Schamphelaere, K., Lofts, S. & Janssen, C. 2005 Bioavailability models for predicting acute and chronic toxicity of zinc to algae, daphnids, and fish in natural surface waters. *Environ. Toxicol. Chem.* **24** (5), 1190–1197.

Di Toro, D., Allen, H., Bergman, H., Meyer, J., Paquin, P. & Santore, R. 2001 Biotic ligand model of the acute toxicity of metals. 1. Technical basis. *Environ. Toxicol. Chem.* **20** (10), 2383–2396.

EPA, U. 2007 Aquatic life ambient freshwater quality criteria—copper EPA 822/R07/001. Washington, DC. Available from: http://www.epa.gov/waterscience/criteria/copper/2007/criteria-full.pdf (accessed 20 February 2011).

Kozlova, T., Wood, C. & McGeer, J. 2009 The effect of water chemistry on the acute toxicity of nickel to the cladoceran *Daphnia pulex* and the development of a biotic ligand model. *Aquat. Toxicol.* **91** (3), 221–228.

Lv, Y., Li, G., Gong, Z. & Wang, Q. 2006 To predict copper toxicity in China 5 main rivers using biotic ligand model (BLM). *Acta Scientiae Circumstantiae* **26** (12), 2080–2085 (abstract in Chinese).

MacRae, R., Smith, D., Swoboda-Colberg, N., Meyer, J. & Bergman, H. 1999 Copper binding affinity of rainbow trout (*Oncorhynchus mykiss*) and brook trout (*Salvelinus fontinalis*) gills: implications for assessing bioavailable metal. *Environ. Toxicol. Chem.* **18** (6), 1180–1189.

McGeer, J., Playle, R., Wood, C. & Galvez, F. 2000 A physiologically based biotic ligand model for predicting the acute toxicity of waterborne silver to rainbow trout in freshwaters. *Environ. Sci. Technol.* **34** (19), 4199–4207.

McGeer, J., Szebedinszky, C., McDonald, D. & Wood, C. 2002 The role of dissolved organic carbon in moderating the bioavailability and toxicity of Cu to rainbow trout during chronic waterborne exposure* 1. *Comp. Biochem. Physiol.–C: Toxicol. Pharmacol.* **133** (1–2), 147–160.

Nollet, L. M. L. 2000 *Handbook of Water Analysis*. CRC Press, Taylor & Francis, Boca Raton, FL.

Pagenkopf, G. 1983 Gill surface interaction model for trace-metal toxicity to fishes: role of complexation, pH, and water hardness. *Environ. Sci. Technol.* **17** (6), 342–347.

Paquin, P., Gorsuch, J., Apte, S., Batley, G., Bowles, K., Campbell, P., Delos, C., Di Toro, D., Dwyer, R. & Galvez, F. 2002 The biotic ligand model: a historical overview* 1. *Comp. Biochem. Physiol.–C: Toxicol. Pharmacol.* **133** (1–2), 3–35.

Playle, R., Dixon, D. & Burnison, K. 1993 Copper and cadmium binding to fish gills: Modification by dissolved organic carbon and synthetic ligands. *Can. J. Fish. Aquat. Sci.* **50** (12), 2667–2677.

Thurman, E. 1985 *Organic Geochemistry of Natural Waters*. Martinus Nijhoff/Dr W. Junk Publishers, Dordrecht.

Tipping, E. 1994 WHAM–A chemical equilibrium model and computer code for waters, sediments, and soils incorporating a discrete site/electrostatic model of ion-binding by humic substances. *Comput. Geosci.* **20**, 973–1023.

Tipping, E. 2002 *Cation Binding by Humic Substances.* Cambridge University Press, Cambridge.

Vulkan, R., Zhao, F., Barbosa-Jefferson, V., Preston, S., Paton, G., Tipping, E. & McGrath, S. 2000 Copper speciation and impacts on bacterial biosensors in the pore water of copper-contaminated soils. *Environ. Sci. Technol.* **34** (24), 5115–5121.

First received 1 March 2011; accepted in revised form 6 June 2011