ELSEVIER
Comparative Biochemistry and Physiology Part C 133 (2002) 271–285
CBP
www.elsevier.com/locate/cbpc

Review

Application of the biotic ligand model to predicting zinc toxicity to rainbow trout, fathead minnow, and *Daphnia magna*☆

Robert C. Santore[a,]*, Rooni Mathew[b], Paul R. Paquin[b], Dominic DiToro[b,c]

[a]*HydroQual Inc., 4914 West Genesee Street, Camillus, NY 13031, USA*
[b]*HydroQual Inc., Mahwah, NJ, USA*
[c]*Manhattan College, Bronx, NY, USA*

Received 24 December 2001; received in revised form 14 June 2002; accepted 20 June 2002

Abstract

The Biotic Ligand Model has been previously developed to explain and predict the effects of water chemistry on the toxicity of copper, silver, and cadmium. In this paper, we describe the development and application of a biotic ligand model for zinc (Zn BLM). The data used in the development of the Zn BLM includes acute zinc LC50 data for several aquatic organisms including rainbow trout, fathead minnow, and *Daphnia magna*. Important chemical effects were observed that influenced the measured zinc toxicity for these organisms including the effects of hardness and pH. A significant amount of the historical toxicity data for zinc includes concentrations that exceeded zinc solubility. These data exhibited very different responses to chemical adjustment than data that were within solubility limits. Toxicity data that were within solubility limits showed evidence of both zinc complexation, and zinc-proton competition and could be well described by a chemical equilibrium approach such as that used by the Zn BLM.

Keywords: Zinc; Toxicity; Biotic ligand model; Fathead minnow; Rainbow trout; *Daphnia magna*

1. Introduction

The recently developed Biotic Ligand Model (BLM) describes and quantifies the bioavailability of metals and can be used to calculate the chemical speciation of a dissolved metal, including complexation with inorganic, organic, and biotic ligands (Di Toro et al., 2001). The term 'biotic ligand' refers to a discrete receptor or site of action on an organism where accumulation of metal leads to acutely toxic effects. The BLM can provide an estimate of the amount of metal accumulation at this site for a variety of chemical conditions and metal concentrations and, by relating a fixed level of accumulation to an observed effect, can thereby be used to predict metal toxicity to aquatic organisms. Previous applications of the BLM have successfully predicted acute copper toxicity (Santore et al., 2001) and silver toxicity (Paquin et al., 1999) to aquatic organisms. Both copper and silver are known to inhibit active transport of sodium ions in gill membranes of freshwater fish. The application of the BLM in understanding the toxicity of metals via inhibition of ion regulatory mechanisms has recently been extended to include a time-dependent analysis of sodium uptake and depuration (Paquin et al., in press).

The importance of developing a tool such as the BLM is to both emphasize the importance of metal bioavailability, and to explain and predict bioa-

☆ This paper is the outcome of discussions on the Biotic Ligand Model held during the November 2001 SETAC Annual Meeting in Baltimore, MD, USA.

*Corresponding author. Tel.: +1-315-484-6220; fax: +1-315-484-6221.
E-mail address: rsantore@hydroqual.com (R.C. Santore).

1532-0456/02/$ - see front matter © 2002 Elsevier Science Inc. All rights reserved.
PII: S1532-0456(02)00106-0

vailability effects on metal toxicity. Metal toxicity, even to the same organism, can vary widely in different waters because of the influence of water chemistry and metal speciation on metal bioavailability. As a result, it is difficult to develop sensible and defendable regulatory criteria for metals unless bioavailability is explicitly considered. Given the wide range of toxic effects that are routinely observed for metals to aquatic organisms, regulatory frameworks must consider the factors that can explain this variability, or risk being over- or underprotective for many conditions. The BLM is an attempt to explain and predict the variability in metal toxicity that results from these bioavailability considerations, and as such can serve as the basis for an improved regulatory approach.

Most of what is known about the physiological effects of zinc on aquatic organisms is based on acute studies with fish where it has been linked to inhibition of Ca ion regulation. Several reviews are available including one that pertains specifically to zinc (Hogstrand and Wood, 1996), and several others that are more general in nature (McDonald et al., 1989; Olsson et al., 1998 and Wood, 2001). It is well known that zinc is both an essential nutrient and a toxicant to fish. At low levels it is taken up actively from water via the gill and from dietary sources via the intestine. Within limits, it is possible for these two routes of uptake to be maintained in balance, such that one source may compensate for a deficiency in the other. For example, Spry et al. showed that branchial uptake was able to compensate when the diet was deficient in zinc (Spry et al., 1988). The uptake of zinc has been shown to occur actively via a saturable uptake process that conforms to Michaelis–Menten kinetic formulation (Spry and Wood, 1989).

At low concentrations, zinc uptake is well regulated by fish (Hogstrand and Wood, 1996). However, zinc has been shown to inhibit calcium uptake even at levels that are considerably less than the 96-h LC50 values (Hogstrand et al., 1994, 1995). Hogstrand and Wood (1996) have hypothesized that impaired reproduction reported for rainbow trout exposed to as little as 50 $\mu g/l$ zinc may be an indirect effect due to inhibition of calcium uptake. These effects are likely due to the fact that oocyte production requires the synthesis of vitellogenin, and this requires considerable amounts of calcium. If inhibition of calcium uptake is the mechanistic basis for reproductive effects, the success of BLM applications in other cases where toxicity is due to inhibition of ion uptake suggests that a BLM approach may also be applicable for prediction of chronic zinc effects.

At intermediate exposure levels (e.g. 800 $\mu g/l$, approximately the 96-h LC50 values in soft water) zinc can exert a toxic effect on fish by inhibiting the uptake of calcium (Spry and Wood, 1985). The inhibition of calcium uptake creates an imbalance in the uptake and loss rates, leading to a net loss of calcium. The gradual net loss of calcium can result in decreased plasma calcium concentrations, and eventually to hypocalcemia. This condition may be lethal if sufficiently high levels of zinc are sustained for an extended period of time.

Zinc and calcium appear to compete for the same ion channel, such that while elevated levels of zinc inhibit calcium uptake, the converse is also true. Hence, calcium can produce a strong protective effect against zinc toxicity. Limited data suggest that calcium may be more protective than magnesium against zinc toxicity, at least for invertebrates such as *D. magna* (Heijerick et al., in press). This difference may reflect the fact that calcium and zinc share a common uptake pathway. While increasing either calcium or magnesium in the water may provide a competitive benefit by reducing the degree of interaction of zinc at the gill, an increase in calcium may have the additional benefit of facilitating calcium uptake when this saturable process is operating at a less than maximum rate.

Exposure to sub-lethal concentrations of zinc can also result in the disturbance of acid–base regulation (Spry and Wood, 1985). This may be due to the inhibition of carbonic anhydrase activity, which in turn would have an impact on CO_2 excretion. It is possible that disruption of acid–base regulation is partly responsibility, in combination with effects on calcium regulation, for the toxicological effects of zinc.

The loss of sodium and chloride in response to zinc exposure has also been reported in some situations (Heath, 1995). Spry and Wood (1985) showed that both sodium and chloride influx and efflux increased after several days of exposure to zinc at concentrations near the 96-h LC50 value for the test water that was used (800 $\mu g/l$ in soft water). The increase in sodium and chloride efflux was attributed to displacement of calcium from the paracellular junctions, which increases gill permeability and hence the rate of loss of these ions by passive diffusion from the gill. It is possible that the increase in influx rate of these ions was a protective response that was triggered by the ionic deficit caused by the increase in efflux. The net effect was a loss of both sodium and chloride over the course of the 60-h experiment.

Zinc is not nearly as toxic to fish as are copper and silver. Although the reason for this difference is not clear, it may be related to different mechanisms of toxicity. The primary effect of copper and silver is on sodium and chloride uptake and efflux (e.g. refer to Lauren and McDonald, 1985, 1987 for copper and Wood et al., 1996a for silver). Since plasma calcium levels represent a much smaller fraction of the plasma ionic composition, the resulting loss of calcium ions would be expected to exert much less of an osmotic stress than would losses of sodium or chloride during lethal exposures to copper or silver. In the case of copper and silver, the proximate cause of death has been described as cardiovascular collapse, resulting from an increase in the viscosity of the blood (Wilson and Taylor, 1993 for copper, Wood et al., 1996b; Hogstrand and Wood, 1998 for silver). It is unlikely that this same mechanism would apply for zinc.

The differences in the relative toxicity of silver, copper and zinc are consistent with the finding in this study that the gill binding constant for Zn is lower than that of either Cu or Ag (Santore et al., 2001; Paquin et al., 1999; McGeer et al., 2000). They are also consistent with the results of Galvez et al. who have also measured a gill binding constant for Zn which is lower than the binding constants for either of these other two metals as well (Galvez et al., 1998). One might also infer from the ordering of the acute water quality criteria (WQC) for these same metals (i.e. Zn > Cu > Ag) that, on a qualitative basis at least, a similar conclusion applies to other aquatic organisms generally.

Exposure to relatively high levels of Zn, considerably higher than the 96-h LC50 value, induces an adverse respiratory response by fish due to an acute inflammatory reaction manifested by physical damage to the branchial surface (Skidmore and Tovell, 1972; Tuurala and Soivio, 1982; Spry and Wood, 1984) For purposes of the BLM, these extremely high exposure levels are of less interest, as they are not representative of ambient environmental levels and they are also well in excess of the acute and chronic WQC.

This paper details the initial development of a BLM for zinc. Versions of the BLM have previously been developed for silver and copper and are currently under review by EPA as a means for developing site-specific WQC (Paquin et al., 1999; US EPA, 1999; Santore et al., 2001). Initial BLM development relies on chemical and toxicological information available in the scientific literature. The goal of this work is to develop a preliminary BLM for zinc and to identify data gaps in the current literature that should be addressed in subsequent BLM research. This paper will summarize information relevant to understanding the effects of water chemistry on zinc toxicity to aquatic organisms and to consider these effects within the conceptual framework of the BLM. The process of model development and comparison of model results with selected toxicity literature for rainbow trout, fathead minnows, and *Daphnia magna* will be described.

2. Methods

2.1. Model development

The BLM includes metal interactions with organic, inorganic, and biotic ligands (Fig. 1). Previous versions of the BLM have used a description of metal interactions with natural organic matter (NOM) developed for the Windermere Humic Aqueous Model (WHAM version 1.0, Model V, Tipping, 1994). The use of the WHAM representation of metal-organic matter interactions is advantageous due to the significant calibration of this model with metal speciation datasets based on diverse sources of organic matter over a wide range of chemical conditions. As with previous versions of the BLM the WHAM reactions will be incorporated within the zinc BLM and thermodynamic information will be obtained from the WHAM database.

Thermodynamic constants for inorganic speciation reactions relevant to the zinc BLM were compared with values in the NIST database (Smith et al., 1995). The NIST database is a compilation of thermodynamic constants from the chemical literature screened through rigorous selection criteria and is generally viewed as a definitive source for thermodynamic information. Also listed for comparison are thermodynamic constants from the MINEQL+ model (Schecher and McAvoy, 1992). The MINEQL+ model will be also used in this analysis to screen toxicity data for reported zinc concentrations that exceed solubility limits. Although most of the thermodynamic constants in these databases agree quite well there are discrepancies for values for two of the inorganic zinc species (Table 1). The thermodynamic constant for the $Zn(OH)_2$ species in both models is about a factor of 10 higher than the value in the NIST database. A summary of inorganic zinc speciation predicted using these values shows that $Zn(OH)_2$ exists at very low concentrations at pH values below 8 but becomes important at pH values greater than 8.5. The use of the NIST constant would result in shifting the predicted concentration of this species to even lower concentrations. The use of either parameter value, then, is not likely to effect predictions of zinc speciation except at pH values at 8.5 or above. At these high pH values, however, the use of the value in the WHAM or MINEQL database results in predictions of declining concentrations of free zinc ion (Zn^{2+}). The pH at which the shift away from Zn^{2+} being the major inorganic species would be higher if the NIST value were used. There is a potential then for the choice of this parameter value to be important for predicting reductions in zinc bioavailability at high pH.

Also of concern is the difference in the thermodynamic values for the formation of the $ZnHCO_3$ species. The value in the WHAM database is approximately 100 times higher than the NIST value (Table 1). The value in the MINEQL database is smaller, but still much higher than the NIST value. Predictions of the inorganic speciation for zinc using thermodynamic values in the WHAM database shows that the major inorganic Zn species over most pH values is the free zinc ion (Zn^{2+}). Although $ZnHCO_3$ is among the most important inorganic zinc complexes over a pH range from approximately 6 to 8, it accounts for

Table 1
Comparison of log *K* values for inorganic reactions in WHAM 1.0 and MINEQL 4.06 with those in the NIST database (5.0)

Species formation reactions	WHAM	MINEQL	NIST
$CO_3+H=HCO_3$	10.329	10.33	10.329
$CO_3+2H=H_2CO_3$	16.681	16.681	16.681
$Mg+H+CO_3=MgHCO_3$	11.4	11.4	11.339
$Mg+CO_3=MgCO_3$	2.98	2.98	2.92
$Mg+SO_4=MgSO_4$	2.37	2.25	2.26
$Ca+H+CO_3=CaHCO_3$	11.44	11.33	11.599
$Ca+CO_3=CaCO_3$	3.22	3.15	3.20
$Ca+SO_4=CaSO_4$	2.3	2.309	2.36
$Zn+OH=ZnOH$	5.04	5.038	5.00
$Zn+2OH=Zn(OH)_2$	*11.1*	*11.097*	10.2
$Zn+SO_4=ZnSO_4$	2.38	2.37	2.34
$Zn+CO_3=ZnCO_3$	4.76	5.3	4.76
$Zn+Cl=ZnCl$	0.4	0.43	0.4
$Zn+H+CO_3=ZnHCO_3$	*13.12*	*12.4*	11.829

Parameter values that are considerably different than NIST values are in italics.

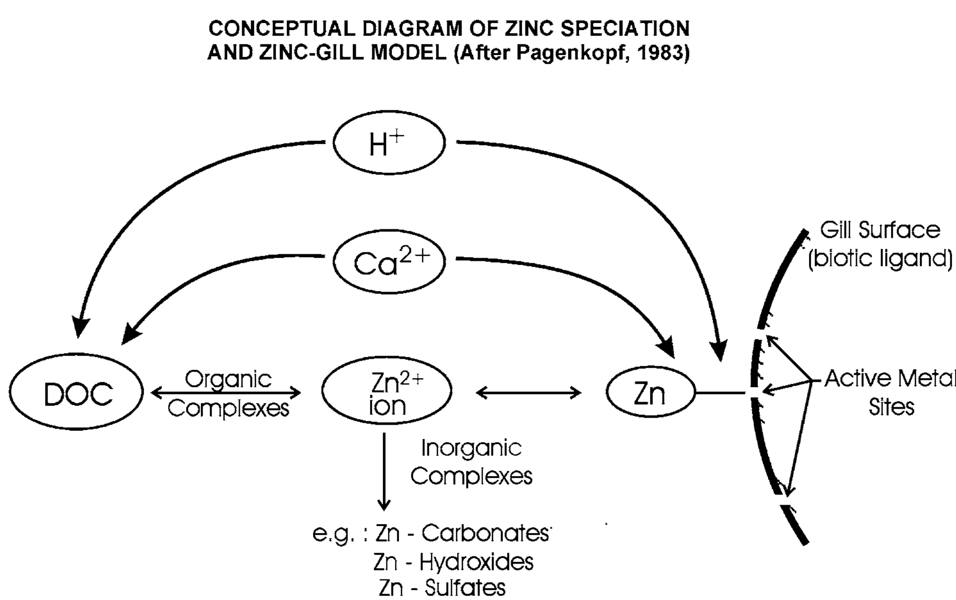

Fig. 1. Zinc BLM conceptual framework.

less than 2% of total dissolved zinc. Use of the NIST value would result in even lower predictions of the concentration of this species. The choice of WHAM, MINEQL, or NIST values in these simulations, therefore, would have little impact on BLM predictions of zinc bioavailability.

Despite these discrepancies, the WHAM database was used for development of the zinc BLM. The primary advantage of using WHAM, is the extensive calibration of zinc interactions with NOM. Since the calibration of organic matter binding constants may be dependent on the inorganic speciation, the entire database was adopted here. The discrepancies between the WHAM database and the NIST database are noted and additional testing of the WHAM predictions of zinc speciation should be conducted in subsequent research to verify that this model is accurate, especially at high pH values where these discrepancies are likely to produce different results.

2.2. BLM parameters and calibration

Thermodynamic parameters that describe interactions with the biotic ligand were chosen from literature values whenever possible. Alsop and Wood (2000) measured the binding strength of zinc to gill surfaces in freshwater fish. Since both zinc and cadmium toxicity affect the regulation of calcium ion uptake, the mechanisms leading to toxic effects of these two metals may involve the same biotic ligand. This assumption would suggest that the strength of interactions of the biotic ligand with calcium and protons in the zinc and cadmium models should be similar since they both involve the same membrane components. In the development and calibration of the zinc and cadmium versions of the BLM, the values for binding of other cations to the biotic ligand were chosen to maintain consistency between these models and with published information on competition at the gill surface (Playle et al., 1993; Cadmium BLM, HydroQual results of unpublished analyses). While BLM versions that have been developed for copper and silver benefited from the availability of suitable accumulation datasets (e.g. Playle et al., 1993; Janes and Playle, 1995), this was not an option for zinc. Hence, it was necessary to rely more on calibration to toxicity datasets and on the use of a cadmium accumulation dataset, since cadmium is believed to have the same site of action as zinc. The BLM thermodynamic parameter values are summarized in Table 2. As with previous version of the BLM, the LA50 value was adjusted to match the sensitivities of different organisms to zinc (Table 3).

Table 3
Lethal zinc accumulation on the biotic ligand associated with 50% mortality (LA50)

Organism	LA50 (nmol/g_{wet})
Rainbow trout—adult	2.29
Rainbow trout—juvenile	0.63
Fathead minnow—2 to 3 week	8.14
Fathead minnow—24-h	0.33
D. magna	0.33

Values were determined by calibration to datasets used in this study.

2.3. Acute zinc toxicity datasets

The zinc BLM will be used to explain and predict the effects of water chemistry on the

Table 2
Parameter values for gill species in BLM formulations for several metals

Gill species	Zn BLM[a] (log *K* values)	Cd BLM[b] (log *K* values)	Cu BLM[c] (log *K* values)	Ag BLM[d] (log *K* values)
Gill-Metal	5.5	8.6	7.4	7.3
Gill-Metal OH	–	–	6.2	–
Gill-Metal Cl	–	–	–	10
Gill-Ca	4.8	4.5	3.6	2.3
Gill-H	6.7	6.7	5.4	4.3
Gill-Na	–	3	3	2.3
Gill-Mg	–	3.5	–	–

[a] Gill-Zn constant from Alsop and Wood (2000), Gill-Ca and Gill-H constant from Playle et al. (1993), and calibration (this study).
[b] Playle et al. (1993) and HydroQual (unpublished Cd BLM).
[c] Playle et al. (1993) and Santore et al. (2001).
[d] Paquin et al. (1999).

Table 4
Toxicity datasets selected for model development and testing

Species	Reference	*N*	Parameters measured
Rainbow trout			
	Cusimano et al. (1986)	3	pH, H, Ca, Mg, SO_4, Cl, CO_3
	Bradley and Sprague (1985)	10	pH, H, CO_3
	Pagenkopf (1976)	2	pH, H, Ca, Mg, SO_4, Cl, CO_3
	Holcombe and Andrew (1978)	6	pH, H, CO_3
	Goettl et al. (1972–1975)	6	pH, H, CO_3
Fathead minnow			
	Mount (1966)	18	pH, H, CO_3
	Schubauer-Berigan et al. (1993)	3	pH, H, CO_3
	Pickering and Henderson (1966)	4[a]	pH, H, CO_3
	Judy and Davies (1979)	2	pH, H, Ca, Mg, CO_3
	Parkerton et al. (1988)	10	pH, H, Ca, Mg, Na, K, SO_4, Cl, CO_3
D. magna			
	Chapman et al. (1980)	3	pH, H, Ca, Mg, Na, K, SO_4, Cl, CO_3
	Paulaskis and Winner (1988)	9[a]	pH, H, Ca, Mg, CO_3

[a] LC50 values are based on nominal metal concentrations.

bioavailability and toxicity of zinc. Development and calibration of the model requires comparison with suitable datasets that illustrate the effects of water chemistry on zinc toxicity. Suitable datasets were selected from a previous review of metal toxicity datasets (Meyer, 1999), plus several others that were identified for use. Datasets were selected for three freshwater organisms including fathead minnow, rainbow trout, and *D. magna*. The sources of data for these organisms are listed in Table 4. Individual datasets were selected based on the quality of chemical information that was available to characterize the exposure conditions. At a minimum, datasets selected for this analysis had to measure pH in the exposure water. Preference was given to datasets where LC50 value calculations were based on measured rather than nominal metal concentrations. Preference was also given to datasets with more complete chemical characterizations including measurements for as many BLM input parameters as possible. A total of 12 datasets were selected for these three organisms and included a total of 76 LC50 values (Table 4).

While the toxicity information used in this study were screened to select studies where the water chemistry was documented as much as possible, almost none of the studies summarized here included measured information about dissolved organic carbon (DOC) concentrations. It was necessary to assume values that were consistent with the water sources used in the study. In general, very low organic carbon concentrations were assumed for studies that used purified water sources, while appreciable higher concentrations were assumed for studies that used natural waters. Whenever possible, assumed DOC concentrations were based on other reported measurements (such as other published reports or USGS data) for the same water source. In the absence of any other information we assumed default concentrations of 0.1 mgC/l for synthetic waters developed from purified sources, and 1.0 mgC/l for treated natural or tap waters. The DOC concentrations assumed in this analysis are summarized in Table 5.

3. Results

3.1. Rainbow trout results

Data for zinc toxicity to rainbow trout were summarized from the five studies listed in Table 4. Data from these various sources were used to look for relationships to determine which chemical parameters could explain trends in observed zinc toxicity. In these data there was a notable trend of increasing zinc LC50 value with increasing hardness. In the context of the BLM framework, competitive effects between hardness cations (such as Ca) and zinc on the biotic ligand would explain the increase in zinc LC50 value with increasing hardness (Fig. 1). Few of these rainbow trout studies reported measurements of calcium or magnesium. In most cases calcium and magnesium were calculated from measured hardness with

Table 5
Assumed DOC concentrations used in this analysis

Reference	Species	Water source	Assumed DOC concentration (mg/l)
Paulaskis and Winner (1988)	DM	Pond water diluted with distilled, deionized, Carbon-filtered, Organex-Q filtered water. Humic acid added in nominal concentrations of 0.0, 0.75, and 1.5 mg/l (assume 50% DOC by weight).	1.00, 1.38, or 1.75
Chapman et al. (1980)	DM	Well (WFTS) water described and characterized in Chapman, 1978	1.30
Cusimano et al. (1986)	RT	Well water used in Chapman et al., 1980 blended with reverse osmosis water.	1.30
Bradley and Sprague (1985)	RT	Deionized water	0.10
		Well water blended with deionized water	1.00
Pagenkopf (1976)	RT	Borehole water	1.00
Holcombe and Andrew (1978)	RT	Lake Superior water (DOC assumed from Erickson et al., 1996)	1.00
Goettl et al. (1972–1975)	RT	No information on water source	1.00
Mount (1966)	FM	Natural limestone spring water mixed with carbon filtered deionized tap water	1.00
Schubauer-Berigan et al. (1993)	FM	Very hard reconstituted water	1.00
Pickering and Henderson (1966)	FM	Natural limestone spring water	1.00
		Natural limestone spring water mixed with distilled, demineralized water	0.50
Judy and Davies (1979)	FM	No information on water source	1.00
Parkerton et al. (1988)	FM	Elm Fork River site water, DOC assumed from USGS records	3.00
		Tap water	1.00

either the reported Ca:Mg ratio or an assumed Ca:Mg ratio from reported values in the literature for that source water. Zinc LC50 values did not appear to be correlated with other chemical parameters (including alkalinity, sulfate, and DOC). Co-variation between chemical parameters in these data would tend to obscure the effects of all but the most important parameters. Since these data are from existing literature sources, the range of values observed for any parameter are due to the coincidental conditions that exist in the various source waters. As a result of these two limitations (co-variation, and lack of suitable ranges), the lack of an observed correlation does not necessarily mean that there is no effect.

Fortunately, several of these studies manipulated the pH of the exposure water while keeping other chemical concentrations constant. The subset of results in pH adjusted exposures are plotted in Fig. 2. Three groups of data are shown representing different hardness conditions including ~8.5 mg/l as $CaCO_3$ (circles), 31 mg/l as $CaCO_3$ (open square), and 390 mg/l as $CaCO_3$ (filled square). The effects of changing hardness in these different exposures has an obvious effect on measured toxicity values that results in a nearly 100-fold increase in measured zinc LC50 value from lowest to highest hardness conditions. The chemistry of the water within each group of points is the approximately the same with the exception that the pH modification also resulted in a modification of alkalinity. The trends of zinc toxicity with changing pH in this subset of toxicity data are evident in Fig. 2. The trends within each group of data show a U-shaped pattern with higher LC50 values at low (<6) and high (>8) pH and low LC50 values at intermediate pH values. It should be noted that these LC50 data are plotted on a logarithmic scale and the LC50 values at low and high pH are elevated by a factor of 10 or more. These effects can be interpreted within the BLM framework as a combination of both complexation and competition. Competitive effects between zinc and protons at low pH values would result in displacement of zinc from the biotic ligand. Although competition with protons at low pH would also tend to displace zinc from NOM making it more bioavailable, the net effect under

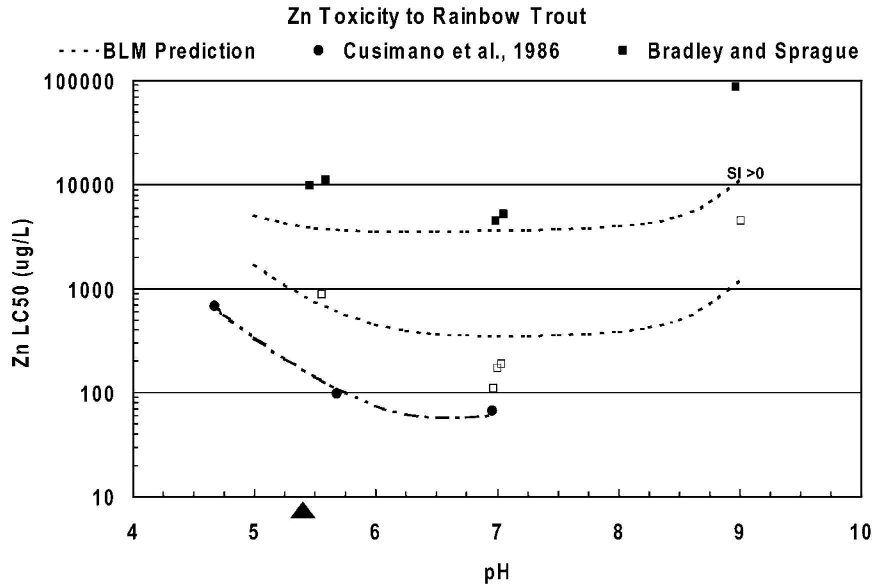

Fig. 2. Comparison of measured and BLM predicted zinc toxicity response to changing pH.

these low organic matter conditions is to reduce zinc bioavailability and zinc binding to the biotic ligand. Hence, toxicity is reduced and LC50 values are higher. This competitive effect diminishes at intermediate pH values and toxicity increases (LC50 values are lower). However, as pH increases further the formation of zinc to form hydroxide complexes becomes important thereby reducing zinc ion activity and resulting in reduced binding of zinc to the biotic ligand and decreased zinc toxicity (higher LC50 values).

Predictions of these competition and complexation effects were made using the zinc BLM. The zinc BLM predicted that LC50 values would be elevated at high and low pH values and the overall patterns are similar to those observed in these studies (Fig. 2). Zinc BLM predictions agree very well with some of the measured toxicity data (especially Cusimano et al., 1986). Comparison with the Bradley and Sprague results suggests that although the model has a similar overall functional response, the model response is flatter overall than the data suggest. The model especially seems to be under-predicting effects at high pH where complexation effects are important in reducing the bioavailability of zinc. The discrepancies noted between the WHAM and NIST database for the formation of $Zn(OH)_2$ would impact the amount of free zinc (and therefore zinc bioavailability) in this pH range. However, use of the NIST value would predict that even lower concentrations of $Zn(OH)_2$ should form and therefore increased bioavailability (and lower LC50 values). Another factor that needs to be considered is that organic zinc complexes would also decrease zinc bioavailability at high pH. None of these studies reported measured DOC values and the extent to which organic matter complexation would be important in these studies is unknown. These questions emphasize the importance of complete chemical analyses in toxicity studies.

Another possibility, which will be explored in greater detail subsequently, is that the zinc toxicity in these high pH conditions departs from the pattern predicted by the BLM because under these conditions the measured LC50 value exceeds the solubility for aqueous zinc. In fact, the two LC50 values that were determined at pH 9 were measured in exposures noted by the researchers as cloudy, possibly due to the precipitation of a zinc solid phase. It is not clear how the measured LC50 value should be interpreted in this case since it likely corresponds to a mixture of dissolved and precipitated forms of zinc. If the toxicity is entirely related to dissolved zinc, then the measured LC50 values at pH 9 should be lower on a dissolved basis since they include both dissolved and precipitated zinc. In that case the discrepancy between the predicted and the measured LC50 values would be reduced but by an unknown amount. It is also

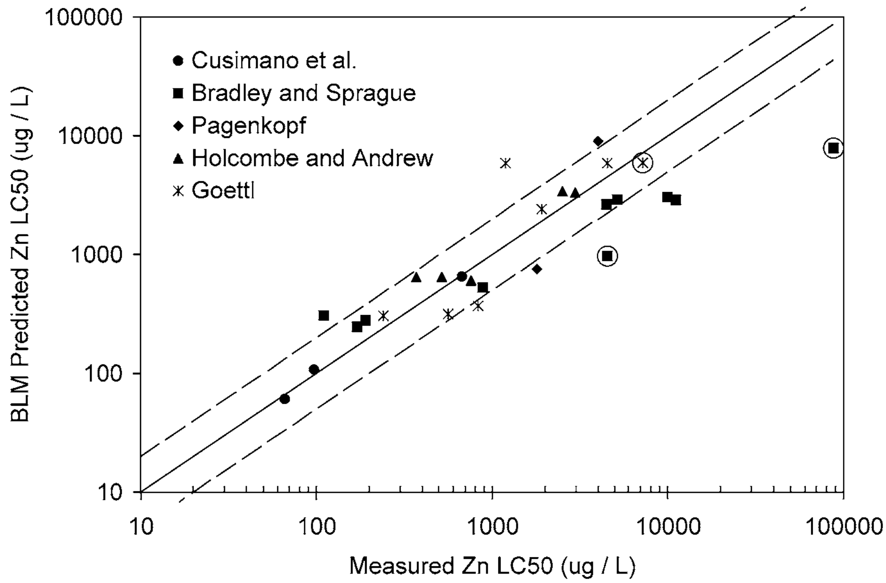

Fig. 3. Comparison of measured and predicted zinc toxicity (LC50) to rainbow trout. Also shown in the line of perfect agreement (solid line) and a range of plus or minus a factor of two (dashed lines) for all rainbow trout toxicity datasets.

possible that an additional mechanism of zinc toxicity would be operative when organisms are exposed to water that is cloudy with precipitated zinc. The lack of agreement between the predicted and measured LC50 values at pH 9, therefore, is not necessarily an indication that the mechanism or model formulation in the zinc BLM is in error.

Despite these problems the BLM LC50 value predictions agree favorably with measured values. The BLM predictions for all of rainbow trout studies are plotted versus measured LC50 values in Fig. 3. The solid line in Fig. 3 represents the line of perfect agreement. The dashed lines on either side represent plus or minus a factor of two. Agreement within a factor of two is an approximate estimate of the variability inherent in toxicity tests. For example, US EPA requires that bioassay tests are checked with a reference toxicant and shown to agree within a factor of two of standard values (US EPA, 1994). The overall range in measured LC50 values is significant covering more than a 1000-fold range in zinc concentrations. Over this range, the BLM predictions compare favorably although the tendency to predict several LC50 values that are too low result from the previously noted discrepancies at high pH. The reported LC50 values that exceeded theoretical solubility limits for dissolved zinc in these high pH exposures are identified with circles drawn around the symbols in Fig. 3.

3.2. Fathead minnow results

Zinc toxicity data to fathead minnow were summarized from the five studies listed in Table 1. As with the rainbow trout data, it is fortunate that a subset of the fathead minnow data can be isolated to look at effects of pH on zinc toxicity. As can be seen in Fig. 4, the response of zinc toxicity to fathead minnow does not conform to the U-shaped pattern that was evident for rainbow trout, or that would be predicted by the BLM (solid lines). In contrast, fathead minnow LC50 values tended to decrease with increasing pH in the data reported by Mount (1966). It is less obvious if the data reported by Schubauer-Berigan et al. (1993) fit either the overall decreasing trend seen in the Mount (1966) data, or the U-shaped pattern predicted by the BLM.

As with the high pH data for rainbow trout, the fathead minnow results are likely to be confounded by LC50 measurements that exceed the solubility for zinc (shown in Fig. 4 with a dashed line for average conditions in the Mount, 1966 exposures). To further illustrate the relationship between zinc solubility and pH, the MINEQL+ model (Schech-

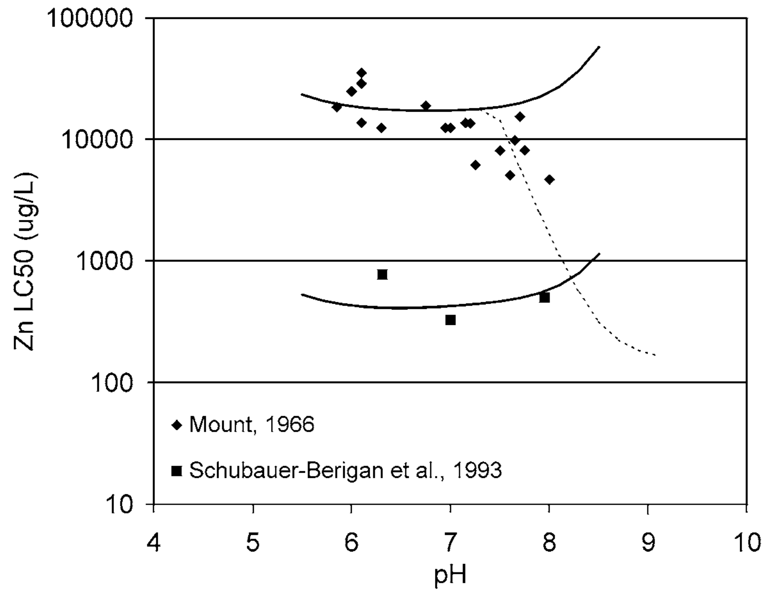

Fig. 4. Zinc toxicity to fathead minnow over a range of pH values. Also shown are the BLM predictions for average conditions in these two datasets (solid lines) and solubility limits (dashed line).

er and McAvoy, 1992) was used to predict the maximum dissolved zinc concentration that would be expected to occur under average conditions typical for these toxicity tests. Zinc solubility decreases with increasing pH to a minimum at approximately pH 8.5 where the solubility begins to increase due to the formation of soluble aqueous hydroxide species (Fig. 5). All of the zinc LC50 values from the studies listed in Table 1 are also plotted on Fig. 5. It is clear in Fig. 5 that a majority of the fathead minnow LC50 values and the rainbow trout LC50 values measured at pH 9 exceeds the zinc solubility. In addition, LC50 values that were determined in exposures where the investigator noted cloudiness in the water are plotted with hollow (not filled) symbols. There is a good correspondence between LC50 values that exceed solubility and cloudiness in the exposure waters suggesting that zinc was precipitating in these exposures and the fresh precipitate was of a colloidal nature that remained in suspension and turned the exposure water cloudy. The fact that the pattern of measured LC50 values in Fig. 4 do not fit what would be predicted by the BLM, therefore, is likely due to a combination of uncertainty in dissolved zinc concentrations and potentially different toxicity mechanisms that would result from exposure to precipitated zinc.

It is not surprising then, that application of the BLM to predict zinc LC50 values for fathead minnow do not agree as well with these measured data. Shown in Fig. 6 is a comparison between predicted and measured zinc LC50 value for fathead minnow. There are results for two different age fish on this figure. Results using larval fish (24 h) are shown with stars (*) and the data plotted with all other symbols are from studies using 2–3-week-old fish. In particular the model predictions for the data reported by Parkerton et al. (1988) as well as those reported by Mount (1966) do not compare favorably. The larval fish are considerably more sensitive to zinc toxicity and BLM predictions for larval fish use a different LA50 value than the predictions made for older fish (Table 3). The results for the 2–3-week-old fish suggest that within each group of data, measured LC50 values cover a greater range than the model would predict. Since the pattern of declining zinc LC50 values with increasing pH is not predicted by the model, the predicted LC50 values are too high at high pH and too low at low pH. There is not enough data for the younger 24-h fish to evaluate the performance of the model and the data that are included here correspond to a relatively narrow range of conditions.

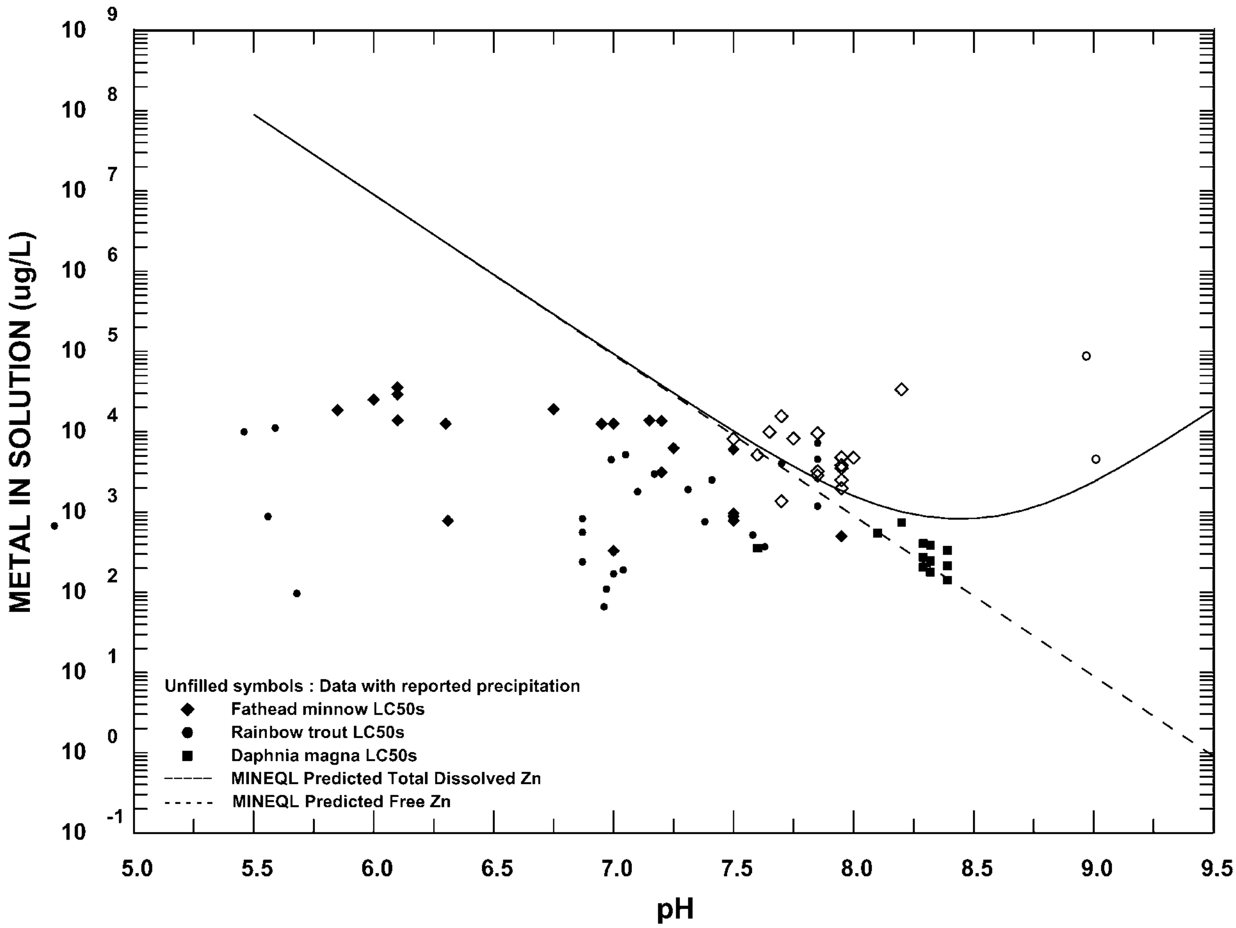

Fig. 5. Theoretical solubility line for aqueous zinc given average chemical conditions in toxicity tests compared with measured zinc LC50 values. Hollow symbols indicate measurements from cloudy exposures.

3.3. *Daphnia magna* results

Measured zinc LC50 values for *D. magna* were lower overall and did not exceed the zinc concentrations that would be expected from solubility considerations (Fig. 5). As with the other organisms, measured zinc LC50 value was observed to be correlated with hardness, while the effects of other parameters are weaker and are likely confounded by co-varying hardness concentrations. Nevertheless, BLM predictions for this organism match measured LC50 values reasonably well given the limited data available for this organism (Fig. 7). Only three of these LC50 concentrations (reported by Chapman et al., 1980) were based on measured zinc concentrations and the remaining values (reported by Paulaskis and Winner, 1988) were from nominal metal concentrations. The use of nominal concentrations is problematic, since any loss of metal, such as by sorption on the container, will result in reported LC50 values that are higher than they should be. Conversely, background metal concentrations or possible contamination in the exposure would not be accounted for and could result in nominal concentrations that are too low. The nominal values in this comparison, plotted with hollow symbols with a central dot in Fig. 7, are all plotting on one side of the 1 to 1 line, while the measured values are all on the other side. This suggests that the nominal concentrations may be biased and the model calibration is attempting to account for this bias. Although the comparison of the model to the measured results is actually quite good, it would be preferable to evaluate the model with a dataset based on measured values. However, it is important to emphasize that these predictions, as with those for rainbow

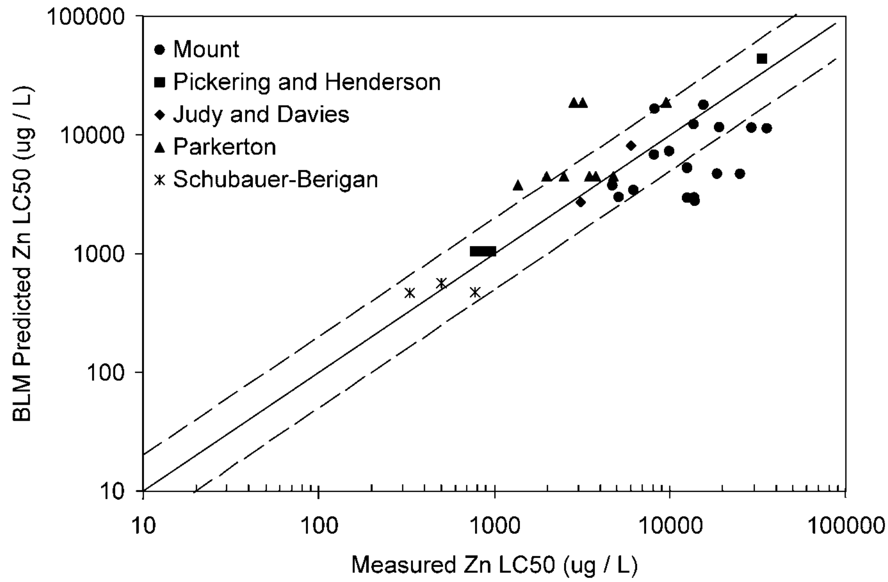

Fig. 6. Comparison of measured and predicted zinc toxicity (LC50) to fathead minnow. Also shown in the line of perfect agreement (solid line) and a range of plus or minus a factor of two (dashed lines).

trout, compare very favorably when measured zinc toxicity was below theoretical solubility limits. Further, that the adjustment of the model to account for the different sensitivities of different organisms is accomplished with the adjustment of a single parameter. Nearly all of the parameters in the model are based on thermodynamic information and are invariant between different organisms.

4. Summary and conclusions

The BLM for zinc was developed and used to predict the effects of water chemistry on zinc

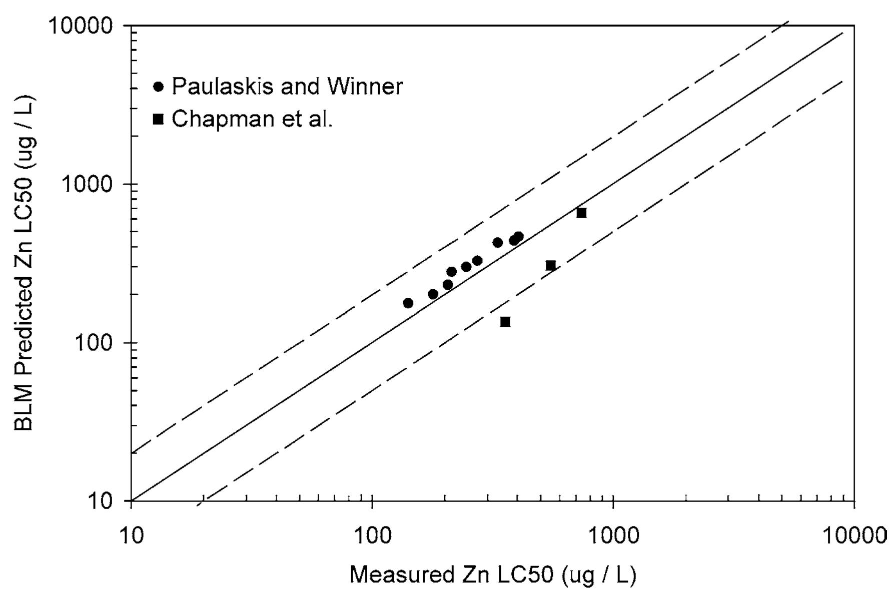

Fig. 7. Comparison of measured and predicted zinc toxicity (LC50) to *D. magna*. Also shown in the line of perfect agreement (solid line) and a range of plus or minus a factor of two (dashed lines).

toxicity to aquatic organisms including rainbow trout, fathead minnow, and *D. magna*. Important chemical effects were observed that influenced the measured toxicity data for these organisms. Hardness effects were evident in toxicity data for each of these organisms. Hardness effects, in general, require that organism interactions are incorporated in predictive models in addition to metal speciation calculations in order to simulate metal toxicity to aquatic organisms (Meyer et al., 1999).

The rainbow trout data suggested that pH effects were also important and resulted in a U-shaped response of measured LC50 value to changes in pH. At low pH (<6) zinc LC50 values were elevated due to competition with protons at zinc binding sites. At high pH (>8) LC50 values were elevated due to complexation reactions. Measured zinc LC50 values were lowest at intermediate pH values. This pattern was consistent with what would be predicted by the BLM and the model was able to predict a wide variation in zinc LC50 values to rainbow trout that were in very good agreement with measured values with the exception of several LC50 values measured at pH 9.

The rainbow trout LC50 values measured at pH 9 and nearly all of the fathead minnow LC50 values exceeded the concentration of zinc that would be expected given solubility constraints. Theoretical solubility calculations that showed that conditions of over-saturation were corroborated with reports of cloudy water during the exposures that was likely the result of precipitated zinc solids. In these cases the BLM predictions did not agree well with reported LC50 values. It is not clear what fraction of the measured LC50 values would have corresponded to dissolved zinc and therefore the LC50 values themselves are likely to be inaccurate. The mechanism of acute toxicity in exposures with colloidal suspensions of zinc may also be different than the mechanism that is assumed in this modeling analysis. For these data, therefore, lack of agreement between the predicted and measured LC50 values does not reflect on the usefulness of the BLM in predicting zinc toxicity.

Limited data were available for testing the ability of the model to predict zinc toxicity to *D. magna*, and most of the available data were based on nominal rather than measured zinc concentrations. Nevertheless, the model was able to predict zinc LC50 values that agreed well with measured values given the limited data available. These predictions were made with the adjustment of a single model parameter (the LA50 value) leaving every other parameter in the model invariant for applications to different organisms.

Acknowledgments

The authors would like to gratefully acknowledge the support of the International Lead Zinc Research Organization for the support of this work.

References

Alsop, D.H., Wood, C.M., 2000. Kinetic analysis of zinc accumulation in the gills of juvenile rainbow trout: effects of zinc accumulation and implications for biotic ligand modelling. Env. Toxicol. Chem. 19, 1911–1981.

Bradley, R.W., Sprague, J.B., 1985. The influence of pH, water hardness, and alkalinity on the acute lethality of zinc to rainbow trout (*Salmo gairdneri*). Can. J. Fish Aquat. Sci. 42.

Chapman, G.A., 1978. Toxicities of cadmium, copper, and zinc to four juvenile stages of chinook salmon and steelhead. Trans. Am. Fish Soc. 107.

Chapman, G.A., Ota, S., Recht, F., 1980. Effects of Water Hardness on the Toxicity of Metals to *Daphnia Magna*. US EPA, Corvallis, OR.

Cusimano, R.F., Brakke, D.F., Chapman, G.A., 1980. Effects of pH on the toxicities of cadmium, copper, and zinc to steelhead trout (*Salmo gairdneri*). Can. J. Fish Aquat. Sci. 43.

Di Toro, D.M., Allen, H.E., Bergman, H.L., Meyer, J.S., Paquin, P., Santore, R.C., 2001. Biotic ligand model of the acute toxicity of metals. I. Technical basis. Environ. Toxicol. Chem. 20, 2383–2396.

Erickson, R.J., Benoit, D.A., Mattson, V.R., Nelson, H.P., Leonard, E.N., 1996. The effects of water chemistry on the toxicity of copper to fathead minnows. Env. Toxicol. Chemi. 15, 181–193.

Galvez, F., Webb, N., Hogstrand, C., Wood, C.M., 1998. Zinc binding to the gills of rainbow trout: the effect of long-term exposure to sub-lethal zinc. J. Fish Biol. 52, 1089–1104.

Goettl, J.P., Davies, P.H., and Sinley, J.R., Colorado Fisheries Research Review 1972–1975 (Water Pollution Studies), Fisheries Research Section, Colorado Division of Wildlife, Fort Collins, CO, USA.

Heath, A.G., 1995. Water Pollution and Fish Physiology. second ed.. Lewis Publishers, New York, pp. 359.

Heijerick, D.G., De Schamphelaere, K.A.C., and Janssen, C.R., Predicting acute zinc toxicity for *Daphnia magna* as a function of key water chemistry characteristics: development and validation of a biotic ligand model. Env. Toxicol. Chem., in press.

Hogstrand, C., Wilson, R.W., Polgar, D., Wood, C.M., 1994. Effects of zinc on the kinetics of branchial calcium uptake in freshwater rainbow trout during adaptation to waterborne zinc. J. Exp. Biol. 186, 55–73.

Hogstrand, C., Reid, S.D., Wood, C.M., 1995. Ca^{2+} versus Zn^{2+} transport in the gills of freshwater rainbow trout and the cost of adaptation to waterborne Zn^{2+}. J. Exp. Biol. 198, 337–348.

Hogstrand, C., Wood, C.M., 1998. Towards a better understanding of the bioavailability, physiology, and toxicity of silver in fish: implications for water quality criteria. Env. Toxicol. Chem. 17, 547–561.

Hogstrand, C., Wood, C.M., 1996. The physiology and toxicology of zinc in fish. In: Taylor, E.W. (Ed.), Toxicology of Aquatic Pollution: Physiological, Cellular and Molecular Approaches, Society for Experimental Biology, Seminar Series: 57. Cambridge University Press, Cambridge, pp. 61–84.

Holcombe, G.W., and Andrew, R.W., 1978. The acute toxicity of zinc to rainbow and brook trout-comparisons in hard and soft water. Environmental Research Laboratory, Office of Research and Development, US EPA.

Janes, N., Playle, R.C., 1995. Modeling silver binding to gills of rainbow trout (*Oncorhynchus mykiss*). Environ. Toxicol. Chem. 14, 1847–1858.

Judy, R.D., Davies, P.H., 1979. Effects of calcium addition as $Ca(NO_3)$ on zinc toxicity to fathead minnows, *Pimephales promelas* Rafinesque. Bull. Environ. Contam. Toxicol. 22.

Lauren, D.J., McDonald, D.G., 1985. Effects of copper on branchial ionoregulation in the rainbow trout, *Salmo gairdneri* Richardson. J. Comp. Physiol. B 155, 635–644.

Lauren, D.J., McDonald, D.G., 1987. Acclimation to copper by rainbow trout, *Salmo gairdneri*: physiology. Can. J. Fish Aquat. Sci. 44, 99–104.

McDonald, D.G., Reader, J.P., Dalziel, T.R.K., 1989. The combined effects of pH and trace metals on fish ionoregulation. In: Morris, R., Taylor, E.W., Brown, D.J.A., Brown, J.A. (Eds.), Acid Toxicity and Aquatic Animals. Soc. Exp. Biol. Seminar series 34. Cambridge University Press, Cambridge, UK, pp. 221–242.

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

Meyer, J.S., 1999. Literature review on the bioavailability and toxicity of selected metals in the aquatic environment relative to natural water chemistry. International Lead–Zinc Research Organization, Research Triangle Park, NC.

Meyer, J.S., Santore, R.C., Bobbitt, J.P., et al., 1999. Binding of nickel and copper to fish gills predicts toxicity when water hardness varies, but free ion activity does not. Environ. Sci. Technol. 33, 913–916.

Mount, D.I., 1966. The effect of total hardness and pH on acute toxicity of zinc to fish. Int. J. Air Water Pollut. 10.

Olsson, P.E., Kling, P., Hogstrand, C., 1998. Mechanisms of heavy metal accumulation and toxicity in fish. In Metal Metabolism in Aquatic Environments. Chapman and Hall, London, pp. 321–349.

Pagenkopf, G.K., 1976. Zinc speciation and toxicity to fishes. Proceedings of Toxicity to Biota of Metal Forms in Natural Water (ISC).

Paquin, P.R., Di Toro, D.M., Santore, R.C. Trivedi, D. Wu, K.-B., 1999. A biotic ligand model of the acute toxicity of metals. III. Application to fish and daphnia exposure to fish. p. 3–59 to 3–102 In Integrated approach to assessing the bioavailability and toxicity of metals in surface waters and sediments, the EPA Science Advisory Board. Office of Water, Office of Research and Development, Washington, DC.

Paquin, P.R., Zoltay, V., Winfield, R.P., Wu, Kb., Mathew, R., Santore, R.C., and Di Toro, D.M., Extension of the biotic ligand model of acute toxicity to a physiologically-based model of the survival time of rainbow trout (*Oncorhynchus mykiss*) exposed to silver. Comp. Biochem. Physiol., in press.

Parkerton, T.F., Stewart, S.M., Dickson, K.L., Rodgers, J.H., Saleh, F.Y., 1988. Evaluation of the indicator species procedure for deriving site-specific water quality criteria for zinc. Aquat. Toxicol. Hazard Assess. 10, 423–435.

Paulaskis, J.D., Winner, R.W., 1988. Effects of water hardness and humic acid on zinc toxicity to *Daphnia magna*. Straus. Aquat. Toxicol. 12, 273–290.

Pickering, Q.H., Henderson, C., 1966. The acute toxicity of some heavy metals to different species of warmwater fishes. Int. J. Air Water Pollut. 10,.

Playle, R.C., Dixon, D.G., Burnison, K., 1993. Copper and cadmium binding to fish gills: estimates of metal-gill stability constants and modelling of metal accumulation. Can. J. Fish Aquat. Sci. 50, 2678–2687.

Santore, R.C., Di Toro, D.M., Paquin, P.R., Allen, H.E., Meyer, J.S., 2001. A biotic ligand model of the acute toxicity of metals. II. Applicaiton to acute copper toxicity in freshwater fish and daphnia. Environ. Toxicol. Chem. 20, 2397–2402.

Schecher, W.D., McAvoy, D.C., 1992. MINEQL+: a software environment for chemical equilibrium modeling. Comput. Env. Urban Syst. 16, 65–76.

Schubauer-Berigan, M.K., Dierkes, J.R., Monson, P.D., Ankley, G.T., 1993. pH dependent toxicity of Cd, Cu, Ni, Pb and Zn to *Ceriodaphnia dubia*, *Pimephales promelas*, *Hyalella azteca* and *Lumbriculus variegatus*. Env Toxicol. Chem. 12.

Skidmore, J.F., Tovell, P.W.A., 1972. Toxic effects of zinc sulphate on the gills of rainbow trout. Water Res. 6, 217–230.

Smith, R.M., Martell, A.E., Motekaitis, R.J., 1995. NIST Critically Selected Stability Constants of Metal Complexes Database. US Department of Commerce, Gaithersburg, MD.

Spry, D.J., Wood, C.M., 1984. Acid–base, plasma ion and blood gas changes in rainbow trout during short term toxic zinc exposure. J. Comp. Physiol. B 154, 149–158.

Spry, D.J., Wood, C.M., 1985. Ion flux rates, acid–base status, and blood gases in rainbow trout, *Salmo gairdneri*, exposed to toxic zinc in natural soft water. Can. J. Fish Aquat. Sci. 42, 1332–1341.

Spry, D.J., Hodson, P.V., Wood, C.M., 1988. Relative contributions of dietary and waterborne zinc in the rainbow trout, *Salmo gairdneri*. Can. J. Fish Aquat. Sci. 45, 32–41.

Spry, D.J., Wood, C.M., 1989. A kinetic method for the measurement of zinc influx in vivo in the rainbow trout, and the effects of waterborne calcium on flux rates. J. Exp. Biol. 142, 425–446.

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. Comp. Geosci. 20, 973–1023.

Tuurala, H., Soivio, A., 1982. Structural and circulatory changes in the secondary lamellae of *Salmo gairdneri* gills after sub-lethal exposures to dehydroabietic acid and zinc. Aquat. Toxicol. 2, 21–29.

United States Environmental Protection Agency, 1994. Water Quality Standards Handbook. United States Environmental Protection Agency. Office of Water, Washington, DC.

United States Environmental Protection Agency April 6–7, 1999. Integrated Approach to Assessing the bioavailability and toxicity of metals in surface waters and sediments, a report to the EPA Science Advisory Board, Office of Water, Office of Research and Development, Washington, DC, US EPA-822-E-99-001.

Wilson, R.W., Taylor, E.W., 1993. The physiological responses of freshwater rainbow trout, *Oncorhynchus mykiss*, during acutely lethal copper exposure. J. Comp. Physiol. B 163, 38–47.

Wood, C.M., Hogstrand, C., Galvez, F., Munger, R.S., 1996a. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*): 1. The effects of ionic Ag. Aquat. Toxicol. 35, 93–109.

Wood, C.M., Hogstrand, C., Galvez, F., Munger, R.S., 1996b. The physiology of waterborne silver toxicity in freshwater rainbow trout (*Oncorhynchus mykiss*) 1. The effects of silver thiosulfate. Aquat. Toxicol. 35, 111–125.

Wood, C.M., 2001. Toxic responses of the gill. In: Schlenk, D., Benson, W.H. (Eds.), Target Organ Toxicity in Marine and Freshwater Teleosts, vol. 1, Organs. Taylor and Francis, London and New York, pp. 1–89.