The biotic ligand model: a historical overview

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Abstract

During recent years, the biotic ligand model (BLM) has been proposed as a tool to evaluate quantitatively the manner in which water chemistry affects the speciation and biological availability of metals in aquatic systems. This is an important consideration because it is the bioavailability and bioreactivity of metals that control their potential to cause adverse effects. The BLM approach has gained widespread interest amongst the scientific, regulated and regulatory communities because of its potential for use in developing water quality criteria (WQC) and in performing aquatic risk assessments for metals. Specifically, the BLM does this in a way that considers the important influences of site-specific water quality. This journal issue includes papers that describe recent advances with regard to the development of the BLM approach. Here, the current status of the BLM development effort is described in the context of the longer-term history of advances in the understanding of metal interactions in the environment upon which the BLM is based. Early
developments in the aquatic chemistry of metals, the physiology of aquatic organisms and aquatic toxicology are reviewed first, and the degree to which each of these disciplines influenced the development of water quality regulations is discussed. The early scientific advances that took place in each of these fields were not well coordinated, making it difficult for regulatory authorities to take full advantage of the potential utility of what had been learned. However, this has now changed, with the BLM serving as a useful interface amongst these scientific disciplines, and within the regulatory arena as well. The more recent events that have led to the present situation are reviewed, and consideration is given to some of the future needs and developments related to the BLM that are envisioned. The research results that are described in the papers found in this journal issue represent a distinct milestone in the ongoing evolution of the BLM approach and, more generally, of approaches to performing ecological assessments for metals in aquatic systems. These papers also establish a benchmark to which future scientific and regulatory developments can be compared. Finally, they demonstrate the importance and usefulness of the concept of bioavailability and of evaluative tools such as the BLM.

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1. Introduction

There is a general consensus among the scientific, regulatory, and regulated communities that the process of developing sound environmental regulations, regulations that are protective of aquatic life, is one that should be based on what is commonly referred to as ‘good science’. The basis for such regulations is derived from several scientific disciplines, including chemistry, physiology and toxicology. The level of understanding in these disciplines is evolving, with steady advances made in each area over recent years. Unfortunately, while ‘regulatory science’ is also an evolving discipline, it is evolving via somewhat of an incremental process, one that is necessarily intertwined with legislative cycles, changes in political climate and other events that can only impede the continuous updating of regulations as advances in scientific understanding occur.

Despite the somewhat sporadic nature of the regulatory process, advances in the sciences have continued to occur over time, and these advances tend to become eventually incorporated into the regulations. Over the last 30 years or more, the efforts of environmental chemists have been directed at gaining improved understanding of the forms of the chemicals that are present in aquatic systems. This is particularly important in the case of metals, as the form of the metal species that is present (i.e. its ‘speciation’) has been shown to have a direct bearing on the degree to which the metal is available to the organism and to which it is reactive. Environmental chemists have developed an array of analytical techniques to determine the speciation of trace metals in aqueous systems, including various electrochemical (e.g. potentiometry with ion selective electrodes and voltammetry) and spectroscopic techniques. These techniques have provided ways to measure directly the concentration of the free metal ion, an important form, if not necessarily the only bioreactive form. The efforts of physiologists over this same period of time have been directed at studying how aquatic organisms respond to a stressor, such that the nature and degree of the response and the mechanism by which it is caused could be better understood. An important tool of physiologists has been the use of radiolabeled tracers as probes. These tracers have provided a way to measure changes in the fluxes of essential ions into and out of the test organism, giving a direct indication of the degree of effect of the stressor on the organism.

Unfortunately, up until the mid-1990s, neither of these scientific disciplines, chemistry nor physiology, succeeded in having much influence on the evolving environmental regulations. This was probably because the level of scientific understanding was not far enough advanced or, at least, not well enough understood, verified or accepted by the regulatory agencies, for these disciplines to have had an important bearing on the development of water quality criteria (WQC) and environmental regulations in general. The toxicologists took a practical and rather direct approach to bridging this gap. Simply stated, they placed the organism in the test water and measured the dissolved metal concentration that resulted in a fixed effect. The essence of this approach was very much analogous
to chemists’ use of ion selective electrodes, electrochemical sensors that were selectively responsive to specific metal species and which could be used to measure the concentrations of those metal species. That is, the toxicologists used the test animal itself as an ‘ion selective organism’, a biological sensor that was responsive to the relevant bioreactive metal species. In so doing, they were able to make the requisite connection between dissolved metal exposure level and effect, without necessarily understanding all of the intricate details about what was occurring either chemically or physiologically. This link between exposure level and effect was the link that was needed to develop regulatory criteria. Unfortunately, while this approach of conducting laboratory toxicity tests has proven to be of great practical utility with regard to the development of regulatory criteria, it was not an entirely satisfactory solution for several reasons. First, it was becoming increasingly clear over time that test results obtained in laboratory reference waters were not necessarily indicative of results that would be obtained in natural waters. Furthermore, there was a considerable body of scientific information (i.e. chemistry and physiology) that was available for use, but this information was scattered and neglected. It was recognized that there was room for improvement, but the way to do so was not clearly defined.

This is the point at which models come into the picture. Models, whether a conceptual model of a physiological process (i.e. simply a detailed description of the process), a chemical equilibrium model of chemical reactions (i.e. a set of algebraic equations that can be solved using standard mathematical techniques), or a kinetic model of a toxicological response (i.e. one or more differential equations that describe the response of the organism to a chemical exposure), have been available for some time. They are useful in part because they provide an organized framework within which it is possible to gain improved understanding of the underlying processes involved, whether chemical, physiological or toxicological in nature. In addition, they often provide a quantitative basis for evaluating the net effect of the many complex interactions that must often be considered in environmental systems. The biotic ligand model (BLM), although not the first model of this type, is a relatively recent example of just such a model. Perhaps as much because the time is right, and with the level of understanding in each of the underlying disciplines being well advanced over what it was 20–30 years ago, the BLM is currently being considered for use in the regulatory arena.

The BLM framework, illustrated in Fig. 1, has become a focal point for quantitatively considering the information that is provided by each of the key disciplines mentioned previously. At the same time, it is progressively gaining acceptance by regulatory agencies, notably in North America, Europe and elsewhere, as a way to integrate ‘good science’ into a comprehensible and quantitative tool, one that has practical utility in the development of water quality regulations and in performing risk assessments. The BLM is fundamentally a chemical equilibrium-based model. However, one of the components of this model is the site of action of toxicity of the organism. This site of action of toxicity, which corresponds to the biotic ligand, is shown at the center of Fig. 1. The BLM is used to predict the degree of metal binding at this site of action, and this level of accumulation is in turn related to a toxicological response. The link between exposure and effect is computationally made, in much the same way that the ‘ion-selective organism’ would make this link in a laboratory toxicity test. That is the intent or, at least, the hoped-for objective, and just as the effect level for an organism may vary with the characteristics of the site water, the BLM-predicted effect level will also vary. However, in the case of the BLM, the toxicological response will vary in a predictable, systematic manner, one that reflects the current understanding of both the chemistry of the ambient water and the physiology of the organism.

The level of development of the BLM currently varies for different metals and for different organisms. As such, numerous researchers are currently carrying out BLM-related development efforts. While much work remains to be completed before the BLM is ready for use for all metals, the overall results that have been achieved thus far are encouraging.

Perhaps as much as its utility in the regulatory arena, an important benefit of the BLM is that, having been developed on the basis of information from each of the scientific disciplines referred to above, it has provided a common ground for
technical exchange among these disciplines. It is believed that this has fostered the interaction of chemists, physiologists, toxicologists, and ‘regulatorists’ in a way that would not otherwise have been possible, such that a truly synergistic benefit has been realized. It is expected that the need for use of the ‘Precautionary Principle’, as a way to protect the environment, will be reduced as improved understanding of the processes that control metal availability and toxicity is gained from these interactions. At the same time, situations in which inadequate precautionary measures have been implemented will be identified and corrected. A direct benefit of this will be that the limited financial resources that are available for implementation of environmental control measures will be efficiently utilized in ways that will maximize the overall benefit to the environment and to society as a whole.

While many BLM-related development efforts are ongoing, as detailed in the remainder of this issue, it is important to recognize that the BLM is founded on the substantial efforts of many earlier investigators. As such, it is appropriate to first provide a historical overview of some of the important investigations that have led to the current level of understanding. These developments are categorized, to the degree possible, in accordance with the following disciplines: chemistry, physiology, toxicology, and regulatory science. However, because the lines of demarcation between these areas are not always easy to define, the discussion may not strictly conform to this outline in all cases. The historical overview section is followed by a review of some of the more recent events that have led to the development of the BLM, which is then briefly described. Finally, an attempt is made to look forward, to identify what some of the future needs are, given the state of the science as it exists today. The actual state of the science with regard to the overall BLM development effort is highlighted in detail in the companion articles that are included in this journal issue.
2. Historical overview: the BLM, the past

As is natural for men, engaged in the same inquiries, to be defruous of ascertaining their priority in the discoveries, even when there is little merit in the making them, I therefore take this opportunity of doing justice to those gentlemen, and at the same time of mentioning that I first saw those vessels, as near as I can recollect, in September or October 1763, which, as far as I know, was before they had been seen by either of them.

William Hewson (1769), 'An Account of the Lymphatic System in Fish', Read to the Royal Society on November 16, 1769.

The foregoing account illustrates an early attempt by the honorable William Hewson, in a presentation made to the Royal Society, to stake his claim to having been the first to discover the lymphatic system at a time earlier than that of two of his contemporaries. Our purpose here is not to correct Hewson and his contemporaries with regard to the current view of fish physiologists that fish do not have a true lymphatic system, but instead that they have a 'secondary circulation'. Rather, it is to recognize that most of the advances made by scientists and researchers are founded upon the work of their predecessors, and that continuing advances would proceed slowly without this foundation. It is in this spirit, and to provide a background for the results that follow in this issue, that a review of past advances that have led to the current state of development of the BLM is presented. While acknowledging that much of what is known today is based on the work of pre-1900 scientists, in the interest of meeting page limitations, this review focuses on developments that have occurred since the mid- to late 20th century.

2.1. Chemistry

The ability to measure the concentrations of metals in natural waters has significantly advanced over the last 50 years. This has largely been due to the development of instrumental techniques, such as atomic absorption spectrometry (AAS), inductively coupled argon plasma emission spectrometry (ICP-AES), and inductively coupled argon plasma mass spectrometry (ICP-MS). Trace metals in fresh waters can now be determined routinely and accurately to low µg/l concentrations in most analytical laboratories, and can be measured at even lower levels, providing appropriate care is taken. The development of sensitive instrumental analytical techniques is only one part of the story. As detection limits dropped, workers became increasingly aware of the problems of contamination during both sample collection and analysis. Clean sampling and analysis protocols, along with the use of clean room facilities to minimize atmospheric contamination (essential for ultratrace analysis), are now in widespread use. Over the last 15–20 years, the combined use of clean techniques and sensitive instrumental methods has led to the generation of reliable data on metal distributions in the world’s oceanic, estuarine and freshwater systems.

As soon as ecotoxicologists availed themselves of these developments in trace analysis, it became clear that metal toxicity was poorly correlated with total metal concentrations. This stimulated an interest in measuring metal speciation, with the goal of determining the elusive ‘metal fraction’ that correlated with toxicity. A number of operationally defined procedures have been developed to measure labile metal fractions in natural waters. These include voltammetry, ligand competition and resin equilibration (Tessier and Turner, 1995). The advent of the free ion activity model (FIAM; Morel, 1983; Campbell, 1995) further crystallized the need for methods capable of measuring ambient-free metal ion concentrations in natural waters. Ion-selective electrodes are the only technique currently available for the direct measurement of free metal ion concentrations. Commercially available, solid-state electrodes were developed in the 1960s, largely through the efforts of the Orion Instrument Company (Frant, 1993) and are available for cadmium, copper, lead, and silver. Detection limits are typically \(10^{-6}\text{-}10^{-8}\) M, suitable for use in toxicity and modeling studies where metal concentrations are relatively high. The direct measurement of the free metal ion at concentrations typically found in natural waters remains an elusive goal.

The advent of low-cost, accessible computing facilities in the 1960s and beyond led to the development of computer codes for calculating solution equilibria. These were soon applied to the calculation of trace metal speciation in natural waters. Programs such as MINEQL (Westall et al., 1976; Schecher and McAvoy, 1994) and MINTEQA2 (Brown and Allison, 1987) are now widely used by aquatic scientists. The effective use of such computational models requires critical knowl-
edge of key reactions and judicious selection of the appropriate stability constants. Recent research endeavors in this area have been directed toward describing metal interactions with natural organic matter (NOM). This is an immense challenge, due to the polydisperse, heterogeneous nature of NOM. The Windermere Humic Aqueous Model (WHAM), model V (Tipping, 1994), and the Non-Ideal Competitive Adsorption (NICA) model (Benedetti et al., 1995), which incorporate multiple binding sites and competition between cations, represent some of the most significant advances over the last decade.

The current understanding of how water quality characteristics affect the toxicity of metals to aquatic life has steadily developed over time. Zitko et al. (1973) demonstrated the importance of organic matter in mitigating the toxicity of metals to fish and provided one of the earliest demonstrations that the free metal ion, the cupric ion activity measured in this case, was more indicative of the potential for toxicity than was the dissolved metal concentration. Zitko (1976) subsequently identified competition of the hardness cations (Ca$^{2+}$ and Mg$^{2+}$) with metal ions, for binding at sites where the metals exert a toxic effect, as the mechanism by which hardness mitigates toxicity. Pagenkopf et al. (1974) employed a chemical equilibrium model to explain how water chemistry affects the form of the metal that is present, and how this is related to the toxicity of the metal.

One of the more important results of these early investigations of the 1970s was the finding that the free ion activity of the metal was directly related to metal availability and toxicity (Fig. 2; refer to caption for a description). This concept has subsequently been the subject of considerable interest, with numerous examples, including some notable exceptions having been reported in the scientific literature (see Campbell, 1995 for a review). These examples include results for copper (Sunda and Guillard, 1976; Sunda and Lewis, 1978; Sunda and Gillespie, 1979; Anderson and Morel, 1978), cadmium (Sunda et al., 1978), zinc...
(Allen et al., 1980) and silver (LeBlanc et al., 1984; Bury et al., 1999a,b). During this same time period, researchers first began to appreciate that acidification can affect not only metal complexation equilibria in solution, but also metal interactions at biological surfaces, i.e. that protons can exert a competitive benefit in much the same way as the hardness cations (Campbell and Stokes, 1985; Cusimano and Brakke, 1986).

The earliest of the studies noted above led to the formulation of what is now commonly referred to as the free-ion activity model (FIAM). The FIAM is a conceptual model that describes how variations in the effect levels of metals can be explained on the basis of metal speciation and metal interactions with the organism. Morel (1983), in what is perhaps the earliest formal exposition on the FIAM, describes these metal–organism interactions in terms of a fairly general chemical equilibrium formulation. This early conceptual description of the FIAM incorporates the binding of both the free metal ion and other metal complexes to cellular sites at the site of action of toxicity, and also considers competition among these metal species and other cations, including protons and the hardness cations, for binding at these cellular sites. It was proposed that the response, or percentage inhibition of some unspecified process, would be directly related to the fraction of the total number of sites at the critical site of action of toxicity that are deactivated by the reactive metal species. Morel’s description of the FIAM was rather generic and primarily a conceptual description of these metal–organism interactions. While examples were presented showing that free ion activity was correlated to effects, a specific description of how the FIAM would be applied in practice was not provided.

It was at approximately the same time that the FIAM was first formalized by Morel, and nearly 10 years after the initial investigations of Pagenkopf and co-workers, that the gill surface interaction model (GSIM) for trace metal toxicity to fish was proposed (Pagenkopf, 1983). The GSIM framework accounted for the previously observed decreasing metal toxicity with increasing hardness by competition between the metal stressor and hardness cations for binding at the physiologically active gill sites. Whereas Morel’s description of the FIAM was primarily a conceptual one, Pagenkopf actually used the GSIM to interpret toxicity test results for both individual metals (Cd, Cu, Pb, and Zn) and mixtures of metals. Although Morel neglected the effect of metal complexation by organic matter in these GSIM analyses of toxicity test results obtained with laboratory waters, the potential significance of this interaction in natural waters was recognized.

Although the FIAM and the GSIM actually contain many of the features that are currently incorporated in the BLM, and they are very similar to it in principle, they were not readily accepted by regulatory agencies for use in the establishment of WQC for metals. As such, the full potential of these models as tools for use in the regulatory arena was not realized, at least not at the time that they were originally proposed. While the reasons for the limited use of the FIAM and the GSIM for regulatory purposes are not entirely clear, the important role of these models in the evolutionary development of the BLM (described in more detail below) is clearly recognized today.

### 2.2. Physiology

At this point it is appropriate to consider how advances in physiology have led to an improved understanding of the interactions of metals with aquatic organisms. It will be shown that one of the most important effects of many metals on aquatic life is the disruption of the ability of an aquatic organism to regulate its internal ion pools, with significant departures from the norm leading to adverse consequences, including death. While not the only cause of adverse effects due to metals, this mode of action of toxicity is particularly important. As such, it warrants careful consideration in the review that follows.

The literature pertaining to ionoregulatory processes is a rich and extensive one, with key studies having been performed as long ago as the 1920s and 1930s. It was at that time that Homer Smith, a renal physiologist and the head of the Department of Physiology, New York University and Bellevue Hospital Medical College, New York City, conducted some of the earliest detailed efforts to ascertain the nature and magnitude of the influx and efflux of ions in fish. In what was a critical finding at that time, he determined that the principal route of transfer of ions between the ambient water and the internal fluids of a marine teleost occurred via the gill, rather than the kidney (Smith, 1930). A short time thereafter, Keys (1931), as described by Keys and Wilmer, (1932), determined...
that chloride was actively secreted by the gills of eels in seawater and that the process required a significant expenditure of energy. Detailed morphological studies were subsequently performed in order to gain improved understanding of this process. As a result of these studies, ‘chloride-secreting cells’ were identified and described in detail (Keys and Wilmer, 1932).

Pursuant to their finding that freshwater species also contained chloride cells (note that the osmotic gradient between the blood and the outer medium is reversed in freshwater), Keys and Wilmer drew what was a truly insightful conclusion for that time, that the chloride cells do not necessarily secrete chloride out of the blood. Rather, they always transport it in opposition to the concentration gradient. August Krogh (1938, 1939), a Nobel Prize-winning Danish physiologist, was the first to show experimentally that there was also a net transport of ions across the gills of freshwater teleosts (goldfish) and to suggest the possibility that the uptake of Na\(^+\) and Cl\(^-\) by freshwater aquatic animals involved independent active transport systems for both Na\(^+\) and Cl\(^-\). He also recognized that when one ion is taken up while its counterion is not, it is not possible to maintain electroneutrality unless there is efflux of another ion of like charge. In this regard, he hypothesized that Na\(^+\) uptake is accompanied by NH\(_4\)\(^+\) or H\(^+\) efflux and Cl\(^-\) uptake by HCO\(_3\)\(^-\) efflux. These results were the first of many studies since that time, studies that have shown the importance of the ambient water chemistry including, but not limited to, pH and the concentrations of Na\(^+\), Cl\(^-\), HCO\(_3\)\(^-\) and Ca\(^{2+}\) to the ionoregulatory needs and capabilities of aquatic life. The chloride cell itself has also continued to be a focal point of research activity by aquatic physiologists through to the present time (see Laurent, 1984; Perry, 1997 for reviews).

Given that the research physiologists, and everyone else for that matter, were preoccupied with other worldly matters, there was somewhat of a lull in research activity during the late 1930s and early 1940s. As a result, it was not until the late 1940s that further advances in understanding were again routinely reported in the scientific literature. At this time researchers began to make use of isolated membranes, frog skin being the most popular membrane of choice, to explore further the mechanisms of ionoregulatory processes (e.g. Ussing, 1949).

Many of the investigations of ionoregulation that were conducted during the late 1940s and 1950s were also performed with freshwater crustaceans and amphibians. The use of radiolabeled tracers to study the one-way fluxes of ions was a key development that occurred around this time, one that provided a way to perform detailed investigations of the mechanisms of ionoregulation that would have been impossible to do otherwise. Jorgensen et al. (1946) conducted some of the earliest measurements of one-way fluxes, using isotopes of sodium and chloride, in an attempt to ascertain the mechanism of the active uptake process for a fully grown, unmetamorphosed axolotl (Ambystoma mexicanum). Later, they determined that frogs and toads that lost salt after an extended period in distilled water took up more NaCl via the skin from dilute solutions than do tap water-adapted animals (Jorgensen, 1950). They postulated that this increased uptake and a concomitant decrease in urinary losses were the result of enhanced delivery of cortical hormones. Jorgensen et al. (1954) also demonstrated that sodium and chloride were independently taken up by frogs that had been previously depleted of these ions. Jean Maetz (1956) of France eventually used radiolabeled tracers to study ionoregulation by fish. Use of tracers in this way is now a widely used and invaluable experimental technique for the study of ionoregulation by aquatic organisms.

Kirschner (1955), working with isolated frog skin, was one of the first to observe that the uptake of sodium was a function of the external sodium concentration. Kirschner proposed a model that incorporated saturation kinetics for sodium uptake as a function of the external sodium concentration. The expression for sodium uptake (J\(_{\text{Na}}\)) that was derived by Kirschner, often referred to as the Kirschner Equation in the early literature on osmoregulation, was actually equivalent to the more well-known Michaelis–Menten expression (Michaelis and Menten, 1913). Kirschner also represented the rate of loss of sodium from the gill (J\(_{\text{Na}}\)) as being proportional to the concentration gradient across the gill. Shaw (1961) used Kirschner’s formulation to define a steady-state condition with regard to the ion balance, where

\[\text{influx} = \text{efflux}.\]

The resulting relationship high-

\(^{1}\) Any of several salamanders (genus Ambystoma) native to Mexico and the western United States that, unlike most amphibians, often retain their external gills and become sexually mature without undergoing metamorphosis.
highlighted the importance of the maximum uptake rate \( (J_{in}) \), the half-saturation constant \( (K_{M}) \), and the permeability constant \( (P) \), to the maintenance of ionic equilibrium.

Since the initial observations of Kirschner, saturation kinetics for uptake of sodium and/or chloride have been observed in a variety of organisms, including intact goldfish (Maetz, 1956) and crayfish (Shaw, 1959; Bryan, 1960). Goss and Wood (1990a,b) provided a summary of values of Michaelis–Menten kinetic parameters for sodium and chloride that have been reported for rainbow trout.

The electron microscope was another important technological development that has helped physiologists to gain improved understanding of metal toxicity. The electron microscope, developed around 1960, has been of use in morphological investigations by providing highly detailed electron micrographs of the ultra-fine structure (i.e. at the cellular level and finer) of the ionoregulatory epithelia, including the gill (e.g. Copeland, 1967; Degnan et al., 1977; Kikuchi and Matsumasa, 1993). This has provided researchers with a much clearer image of gill structure, including that of the chloride cell. Use of these techniques, in combination with radiotrace methods, came into common use around this time, and increased reliance on in vivo sampling methods (e.g. Mayer and Nibelle, 1969; Wood and Randall, 1971) for repetitive sampling and monitoring of plasma ion levels have led to an improved understanding of the processes that were first envisioned by Smith, Keys and Wilmer, Krogh, and others during the 1930s.

It is appropriate at this point to redirect attention to more recent developments in the understanding of physiology and how metals exert a toxic effect, particularly as they relate to the BLM. The BLM is a conceptual framework in which the binding of metals to defined sites (i.e. biotic ligands) on or within an aquatic organism is associated with acute toxicity. Furthermore, the BLM assumes that the other water quality constituents that are present can influence the extent of metal binding to the biotic ligand. These substances may either form complexes with the metal, thus reducing its free ion activity (e.g. dissolved organic carbon), or compete with the metal for binding at the biotic ligand (e.g. calcium). Both complexation and competition reactions typically limit metal binding to the biotic ligand, with metal accumulation beyond a critical concentration associated with toxicity. Research has shown that the gill is the primary site of toxic action for most metals, certainly for freshwater organisms during acute exposure. Consequently, understanding the physiology of the gills of aquatic organisms is critical to understanding the mechanisms of metal toxicity in these organisms. Although it is not possible to provide a comprehensive review of the physiology of aquatic organisms herein, it is useful to highlight some of the general principles of the subject, especially as they relate to the BLM.

As indicated above, research in comparative physiology over the last 70 years has identified the role of gills in a large number of physiological processes that include, but are not limited to, ion and acid–base regulation, gas exchange and nitrogenous waste excretion (Perry, 1997; Goss et al., 1998). In essence, the functions performed by the gills are as diverse as those associated with the lungs and kidneys in mammals. These functions are mediated by a complex suite of negatively charged proteins on the surface of membranes, proteins that bind with positively charged metals. It is for this reason that the physiological processes performed at the gills may be particularly sensitive to acute metal exposure. During the 1970s and early 1980s, evaluation of toxic responses was performed using relatively high concentrations of metals that were of limited environmental relevance. Evidence suggested that acute metal toxicity in freshwater fish produced gross morphological damage to gills, resulting in either impaired gas exchange at the gills leading to suffocation (Fig. 3, point 1), an increase in loss of ions \( (J_{out}) \) or efflux; Fig. 3, point 2), or both (Mallatt, 1995). Experiments evaluating physiological mechanisms of toxicity at more environmentally relevant concentrations of metals have more recently addressed the importance of water chemistry on toxicity and adopted a number of sensitive diagnostic tools. It has become clear that metals (as for protons), including cadmium, copper, silver, zinc and others, tend to bind to the gill at very specific sites. At concentrations at the 96-h \( LC_{90} \) value and below, metals and protons tend to interfere with the ability of the organism to regulate both ion uptake and efflux across the gill (e.g. McDonald et al., 1989; Wood, 1992; Wood et al., 1996, 1999). The physiological mechanisms of toxicity for most metals can generally be divided into three categories:
monovalent metals (Ag⁺, Cu⁺) affecting Na⁺ transport (Fig. 3, points 3 and 4); divalent metals (e.g. Cd²⁺, Zn²⁺) disrupting Ca²⁺ metabolism (Fig. 3, point 5); and metals that cross the gill and act centrally (e.g. Pb²⁺, Hg²⁺; Fig. 3, point 6). In the case of copper, it is worth noting that, although it predominantly exists as a divalent metal (Cu²⁺) in bulk water, it is probably reduced to the Cu⁺ form before it is transported across biological membranes. This highlights how physiological properties often dictate the ‘reactivity’ of a waterborne metal once it interacts with an aquatic organism. Since the BLM has been more extensively developed to date for silver and copper than for other metals, the focus here is on silver and copper and their influence on Na⁺ transport mechanisms.

The disturbance of internal ion balance was originally found to be the direct cause of acutely toxic effects that result from exposure to acidic pH levels (Milligan and Wood, 1982; McDonald, 1983a,b). It was subsequently shown that elevated levels of certain metals could have similar effects in freshwater fish. With regard to copper and silver in particular, a first-order effect is the inhibition of the active uptake of sodium (Fig. 3, point 3) (Morgan et al., 1997). In addition, at high enough concentrations, they may also affect the efflux of sodium (Fig. 3, point 2), but this effect would mainly be mediated by a general disruption in gill epithelial integrity. The consequence of this ionoregulatory dysfunction is that there is a re-distribution of ions and water between the internal fluid compartments of the fish, a response that is known to have adverse consequences for the organism. Specifically, these effects on ionoregulation result in a decrease in levels of plasma sodium, chloride, and other ions, which in turn triggers a sequence of events that potentially leads to cardiovascular collapse and death (Milligan and Wood, 1982; McDonald, 1983b; Wood, 1989 for effects of pH; Wilson and Taylor, 1993a; Taylor et al., 1996 for copper; Wood et al., 1996; Hogstrand and Wood, 1998 for silver). Although other factors may be important, including the rate of loss of ions, it was found that, as a first approximation, an overall decrease of approximately 30% in plasma sodium levels was associated with death (McDonald et al., 1980; Wood, 1989; Wood et al., 1996; Webb and Wood, 1998; Hogstrand and Wood, 1998; Grosell et al., 2000).

While the loss of sodium from plasma is only one of the steps leading to acute silver and copper toxicity in freshwater fish, it serves as a useful marker for modeling purposes. At the same time, it should be understood that while similar concepts
apply to marine organisms, the details are somewhat different. Perhaps most importantly, the ionic concentration gradients and fluxes tend to be in the reverse direction in marine waters, a result of the much higher salinity of the external medium. As a result, exposure to metals such as copper and silver results in a net inward flux of ions (Wilson and Taylor, 1993b; Wood et al., 1999), and the proximate cause of death is necessarily different and not as well understood as it is for freshwater organisms. In addition, exposure to metals via drinking water and the role of the gastrointestinal tract in the water balance increase in importance in the case of marine organisms.

 Playle et al. (1993a,b), in the first in a key series of experiments, demonstrated how water chemistry affects metal interactions at the gills of fish and simulated these results using a chemical equilibrium program. As discussed in a later section, these studies provided the impetus that was needed for the ongoing development of a chemical equilibrium-based model that could be used to predict effect levels in aquatic organisms. At the same time, the physiological developments described above on how aquatic organisms regulate the levels of fluids and ions in internal compartments have provided a physiological basis for the chemically based interactions that are represented in the BLM (McGeer et al., 2000). Such advances in understanding should continue to facilitate the interpretation of the results of experimental investigations that are being performed as part of ongoing BLM development efforts. They will also serve as a basis for the ongoing development of the BLM as its applicability is extended to other areas of toxicological interest in the future (e.g. Grosell et al., 2002b; Paquin et al., 2002).

2.3. Toxicology

Laboratory toxicity testing has provided a wealth of empirical data upon which predictive models, such as the BLM, are based. Since the time of early developments in the field of ecotoxicology in the 1960s, tremendous progress has been made in our understanding of the toxicology of metals to aquatic organisms. These developments were, in turn, based on steady progress in the development of toxicity testing methods and on improved understanding of chemical and environmental factors that modify metal toxicity.

Although the study of toxicology dates back to the late 1880s, toxicity testing with non-human organisms flourished in the 1940s–1950s as concerns surfaced over the potential hazards from industrial chemicals to the environment (for a thorough discussion of the history of aquatic toxicology, see Rand et al., 1995). These postwar years saw the development of the most basic acute (i.e. short-term or lethal) toxicity test methods for fish and cladocerans, methods that were mostly static in design (sometimes termed ‘pickle-jar bioassays’). The acute toxicity test became the most widely used and applied method, ultimately leading to the development of ‘standardized’ methods published by the American Society for Testing and Materials (ASTM) in 1954, and the American Public Health Association (APHA) Standard Methods in 1960 (Rand et al., 1995). The use of standard methods was a significant advance, as it enabled the consistent screening of numerous chemicals for use in basic research or for regulatory purposes. Throughout the 1960s, the field of applied aquatic toxicity testing rapidly grew, leading to the development of standard methods for testing a variety of aquatic species.

Up until the 1960s, toxicity test methods were largely based on static exposure to single chemicals and rarely entailed analytical verification of chemical exposure concentrations. This situation improved as a result of the development of the continuous flow diluter (Fig. 4) by Mount and Brungs (1967) and the increased use of analytical measurements of chemical exposure concentrations. Quantifying organism responses to accurate and consistent chemical exposure, by using flow-through systems, was an essential requirement if the mechanisms of toxicity were to be better understood, and if reliable regulatory threshold data were to be generated (USEPA, 1985a). Further developments around this time included the study of modifying factors, analysis of the toxicity of chemical mixtures, and improvements in experimental design and methods for derivation of toxicity test endpoints (Sprague, 1969, 1970, 1971; Rand et al., 1995).

As acute toxicity test methods became more widely accepted in the 1970s, increased emphasis began to be placed on chronic (i.e. long-term, sublethal) toxicity, multi-species tests (including microcosms), improved statistics for endpoint determination, the development of predictive models (e.g. quantitative structure–activity relation-
Fig. 4. An example line diagram of a continuous flow diluter, a device that was developed for use in continuous-flow toxicity studies. It is used to achieve serial dilutions of a chemical stressor while at the same time maintaining relatively constant exposure conditions over time. (Courtesy of J. Russell Hockett.)

ships or QSARs) and a refined understanding of the chemistry of exposure, including both waterborne and tissue-based exposure (Rand et al., 1995). Aquatic toxicology rapidly became a multidisciplinary field, with study advancing at the biochemical, individual, population, community and ecosystem scales of biological complexity. The dramatic expansion of the fields of aquatic toxicology and ecotoxicology followed in the 1980s and to the present day, with several new journals and scientific societies being created to facilitate both scientific and regulatory advances in this field.

A thorough understanding of the toxicology of metals would not be complete without an evaluation of which chemical form is most toxic and how toxicity might be modified by various environmental factors. For metals, this is a daunting task, because they exist in several chemical forms in the environment, including the ‘free’ aquo ion (e.g. Cu$^{2+}$), dissolved inorganic (e.g. simple hydroxides) and organic (e.g. humic and fulvic acids) complexes, and a variety of particulate forms (Schnoor et al., 1997). The relative distribution of these forms as a function of environmental conditions is of significant concern from both a scientific and regulatory point of view. Early research focused on simple manipulations of single factors that modify the toxicity of metals to aquatic organisms. For many metals (e.g. Cu, Ag, Zn, Pb and Cd), these factors include the distribution of dissolved vs. particulate metals, complexation by dissolved organic matter, factors that control dissolved ion speciation (e.g. pH and alkalinity) and hardness (Sprague, 1985; Wood et al., 1997).

Perhaps one of the first and most well recognized of the modifying factors for metals is water hardness, with consistent empirical relationships between toxicity and hardness first being published in the late 1960s (Sprague, 1985). The significance of these relationships was so widely accepted that hardness–toxicity equations are often used to directly modify regulatory water quality standards on the basis of site-specific hardness conditions (USEPA, 1985a, 1999b). While hardness–toxicity relationships have been shown to be remarkably consistent for both acute and chronic metal toxicity to a wide range of aquatic organisms, tests conducted under a range of different water quality conditions (e.g. pH and alkalinity) showed the situation to be far more complex. Hardness-dependent metal toxicity often varied when tested under different conditions of hardness, pH and alkalinity. These patterns could be reconciled in most cases by the important assumption that the free metal cation was in equilibrium with the biological surface and was thus the best predictor of metal toxicity. The hypothesis was that pH and alkalinity functioned to change the relative degree of complexation of the metal ion in solution, with hardness ameliorating toxicity by ‘protecting’ the organism against metal toxicity via several possible mechanisms (Sprague, 1985; Wood et al., 1997). For copper, multifactorial experimental designs were an important means of testing these hypotheses and of elucidating quantitative relationships.
between toxicity and the concentrations of the different metal species that were present, in association with varying water quality characteristics (Erickson et al., 1996a,b; De Schamphelaere and Janssen, 2002). Similarly, the ameliorative effects of hardness itself have since been shown to be more complex than the simple hardness–toxicity relationships would suggest. In particular, recent studies have shown that calcium and magnesium can modify the toxicity of metals by different degrees, with hardness-dependent toxicity thus varying as a function of calcium, magnesium, or the ratio between the two for some species (Welsh et al., 2000b; Naddy et al., 2002).

Along with advances in aquatic chemistry and physiology described elsewhere in this paper, toxicity studies have clearly helped to improve our mechanistic understanding of metal toxicity to aquatic organisms. However, even this body of work cannot yet elucidate and predict all of the possible variability in metal toxicity under natural conditions. For example, natural variability in the quality and quantity of dissolved organic carbon may modify metal toxicity in ways that cannot yet be predicted by current models (Wood et al., 1997; Campbell et al., 1997). Empirical approaches can be used, however, to quantify the difference between the toxicity of a metal in a natural water compared to that in the more standard laboratory waters typically used in toxicity tests developed to measure water quality criteria. In the US, this approach is called a ‘water-effect ratio’ (WER; USEPA, 1994, 2001), and involves a series of paired acute or chronic toxicity tests in both site waters and in standard laboratory test waters (Fig. 5). The resulting WER is intended to ‘correct’ a regulatory water criterion on the basis of the relative toxicity of the metal in site water vs. that in laboratory reference waters that are free from many modifying factors (e.g. organic matter, suspended solids). This approach has significant applied benefits, in that regulatory criteria can be modified even without an adequate understanding of all site-specific modifying factors (Wood et al., 1997). However, caution must sometimes be applied to data interpretation, depending on differences between the chemical characteristics of site and laboratory waters (Welsh et al., 2000a; Naddy et al., 2002). In addition, WER studies have provided important empirical data used to help validate BLM predictions under site-specific conditions (Santore et al., 2001).
2.4. Regulatory science

The development of methods to evaluate water quality criteria in the US and Canada, and North America, generally, has been an evolving process. While there are some differences in the approaches that are used to define the desired level of protection, they also have much in common. Similarly, while various approaches have been used in Europe, Australia and elsewhere, considering the brief time span over which scientifically based WQC have been developing, the various approaches are remarkably similar, if not perfectly synchronized in this regard. A few of the highlights of the approaches that are used are described below, along with a discussion of some of the important considerations, similarities and differences that exist among them.

2.4.1. Regulatory approach in North America

Around 1980, the US Environmental Protection Agency (EPA) began to formulate WQC which recognized that the toxicity of metals depends on interactions with other substances that are present in the water. One of the first things recognized was that, for toxicity tests to be acceptable for use in deriving WQC, the tests had to be run with soluble metal salts and in comparatively clean water (i.e. low in particulate matter and dissolved organic carbon (DOC)) to minimize the binding of metal. The rationale for these conditions was to produce WQC that were protective in nearly all waters. There was some acknowledgment that such a criterion had the potential to be overly protective in the majority of waters (Stephan et al., 1985).

Hardness was another factor that was recognized as being important for establishing WQC for metals. The EPA WQC for several metals were formulated as functions of hardness, based on a regression of log LC50 value (or EC50 value) with log hardness. The reason for this was that increasing hardness had been found to mitigate metal toxicity. In addition, hardness was often correlated with other water quality parameters such as pH and alkalinity, and these other variables also affected metal availability and toxicity.

As of 1980, all WQC for metals in the US were expressed as total recoverable metal measurements. At the time, total recoverable analyses for metals were viewed as a stable and reproducible method that could be reliably implemented on a nationwide basis. The preferred analytical measurement, that of measuring the metal species that produced the toxic response in organisms, was recognized at that time, but the procedure was viewed as being too complex to implement, and its use for regulatory purposes was deferred. Subsequently, in 1984, the EPA began to express its WQC for metals in terms of an acid-soluble measurement method (extraction at pH 1.75 for 16 h, followed by filtration; USEPA, 1991), although the evidence that this corresponded to the toxic fraction was somewhat limited:

Because of the variety of forms of copper and the lack of definitive information about their relative toxicities, no analytical measurement is known to be ideal for expressing aquatic life criteria for copper...acid-soluble copper...is probably the best measurement... (USEPA 1985b)

Subsequent testing indicated that this little-used procedure yielded results that were fairly similar to the total recoverable metal measurement method.

In contrast to the US, the Canadian water quality regulatory system is organized as a series of initiatives that are linked by key pieces of legislation, the most important of which are the Fisheries Act and the Canadian Environmental Protection Act (CCME, 1999). One of the central authorities related to standards for water quality is the Canadian Council of Ministers of the Environment (CCME). The CCME is the agency that has responsibility for publishing Canadian Environmental Quality Guidelines (CEQG), the Canadian analog to WQC in the US. The CCME guidelines are nationally consistent, environmental standards designed to achieve a high level of environmental quality across the country.

The water quality criteria for metals were seldom applied in the US immediately following their initial implementation in the 1980s. However, this changed with the passage of amendments to the Clean Water Act, which mandated the adoption and application of water quality standards for toxic substances. As a result, the limitations of the acid-soluble method came into sharp focus in the early 1990s. This perception was accentuated by the rigidity of the US regulatory system, in which the standards represented mandatory goals that were not readily modified. As such, the substantial overprotective bias that was inherent in the regulations, at least in some situations, caused major problems in public acceptance of the regulatory program. This situation in the US was in contrast
to the situation elsewhere, such as in Canada, where water quality standards were viewed as desirable goals, but goals that could be modified in light of the feasibility and practicality of their actually being achieved. Many of the early CCREM (1987) Canadian Water Quality Guidelines (CWQG) were derived from the US EPA water quality criteria of the early 1980s. While the CCME does not have the authority to impose its guidelines, the CEQG are influential with regard to decision-making and in setting permissible criteria within Canadian jurisdictions. The decisions on regulatory criteria and discharge limits are made at a variety of governmental levels, from municipal to provincial and up to the national level.

After seeking advice from an expert panel (58 FR 32131, 9 July 1993), the US EPA revised the basis of its WQC for metals from the total recoverable metal to that of dissolved metal on 4 May 1995 (60 FR 22229). The Panel recognized that:

Metals criteria for ambient waters should be based on bioavailable metal rather than either total recoverable or soluble concentrations. This will achieve the desired goal of protection of aquatic life without over-regulation of dischargers. (USEPA, 1993a,b,c)

This provided a more representative measure of the available metal form, one that more closely corresponded to the laboratory test conditions upon which the WQC were based. However, while this revision improved the overall situation for metals, this interim change to criteria that were based on dissolved concentrations had only a limited effect on the regulatory problems associated with metals such as copper and silver. The reason was that, for such metals, the DOC-bound fraction was still being included in the dissolved measurement used for comparison to the WQC. Copper, in particular, was experiencing widespread compliance problems in municipal wastewater effluents because of its presence in the drinking water distribution system and, subsequently, in the wastewater collection system, as a result of corrosion.

Resolution of the ongoing regulatory problems for copper generally required site-specific adjustments of the copper criterion. The approach used was to apply an empirically measured factor that was evaluated as the ratio between (a) the LC50 value in site water and (b) the LC50 value in low-DOC water (i.e. water customarily used for toxicity testing). This so-called ‘water-effect ratio’ (WER; see Fig. 5), reflecting the ‘effect’ or influence of the test water on the measured LC50 value, involved a relatively cumbersome laboratory procedure that was originally envisioned for use in exceptional cases. When used on a widespread basis, however, the WER approach to setting site-specific WQC was viewed as being inefficient to apply (USEPA, 2001). Furthermore, unless the test species had an LC50 value equal to the criterion (an uncommon situation), the results were likely to be inaccurate (Allen and Hansen, 1996).

The EPA views the BLM as a more appropriate general solution to the problems associated with the evaluation of WQC for metals. Nevertheless, the BLM inherits certain problems from the empirical hardness normalization and the WER approaches. Specifically, the results from acute tests still need to be applied to the chronic WQC via an acute/chronic ratio. The EPA default assumption, which is only overridden by unequivocal data, is that chronic effects respond in the same manner as acute effects, as far as the influences of other water quality parameters are concerned. Feasibility constraints, likewise, necessitate that, for the present, the acute BLM serves as the basis for determination of chronic criteria via the application of an empirically derived acute/chronic ratio (ACR).

The diversity of the Canadian guideline/regulatory system makes it difficult to implement a broad-based and uniform strategy for new approaches to water quality, such as the BLM. However, the FIAM concepts are recognized, and leadership in this regard has been shown in recent environmental risk assessments conducted by Environment Canada and Health Canada (i.e. ‘Releases from Primary and Secondary Copper Smelters and Copper Refineries’ and ‘Releases from Primary and Secondary Zinc Smelters and Zinc Refineries’; see Priority Substances List Assessments Reports, http://www.ec.gc.ca/CEPAreports/subs_list/PSL2_cfm). As far back as the early 1990s, a free ion speciation model for predicting the potential impacts of metals was developed by the British Columbia Ministry of Energy, Mines and Petroleum Resources in conjunction with the mining industry (CB Research, 1992). One of the upcoming opportunities for determining if it will be possible to implement BLM approaches more broadly within Canada will come through the CWQG. An addendum to the CWQG Protocol, which will address issues for metals, is currently being developed. The adden-
dum may eventually lead to updates of CWQG for some metals. While the addendum to the protocol will maintain the original principles, a number of scientific issues and approaches are under consideration, including the FIAM, the BLM and bioaccumulation.

The BLM approach for setting WQC does have some limitations, some of which are currently being addressed. Because the BLM involves so many input parameters, all of which vary over time at a specific site, it would be desirable to characterize how their combined variability affects the time-variability of the resulting criteria. Again, this is not a new problem that is unique to the BLM. Hardness levels also vary on a daily basis, as do WER values, even after correcting for hardness; and, while seasonal variation is considered in EPA guidance, daily variations are not addressed. In addition, it is typically the case that there are not enough samples at any one site to reliably characterize the inherent variability over a higher-resolution time scale. While the variability of hardness over time could be readily characterized, the EPA has not yet provided guidance on how to account for this variability in determining site-specific WQC. As a result, nearly all of the states use a design-condition approach, with the method for selecting the hardness value for the design condition varying from state to state. The EPA is currently conducting an evaluation of how to refine WQC for metals, and is considering use of Monte Carlo methods as one possible solution to the problem of addressing water quality variability in the setting of WQC.

2.4.2. Regulatory approach in Europe

While North America has approached the regulation of metal discharges through the development of WQC, Europe has addressed the issue of releases of metals to the environment through the risk assessment approach, with the exception of several metals classified as hazardous substances, which are being addressed through the derivation of WQC. The European Union Council Regulation (EEC) No 793/93 on the evaluation and control of existing substances (European Commission, 1993a) under Article 10 requires the assessment of the real or potential risk for man and environment of priority substances, using principles that have been laid down in Commission Regulation No 1488/94 on risk assessment of existing substances (European Commission, 1993b). The technical guidance for performing risk assessments for new and existing substances has been harmonized and published in one document: Technical Guidance Documents (TGD) in support of Commission Directive 93/67/EEC on risk assessment for new notified substances and Commission Regulation (EC No1488/94) on risk assessment for existing substances (European Commission, 1996). The risk assessment, as laid down in the technical guidance document, entails exposure and effects assessment leading to the derivation of predicted environmental concentrations (PEC) and predicted no-effect concentration(s) (PNEC). Currently, the potential risk to humans and the environment of Cd, Zn, Ni and Cr are being (or have been) assessed. During these exercises, it has been recognized that, for metals, standard TGD procedures may not be appropriate to accurately assess their true impact on the ecological quality of terrestrial and aquatic ecosystems. The Water Framework Directive is another important EU regulatory action aimed at deriving water quality standards (WQS) for substances classified as priority hazardous substances (PHS). Cd, Hg, Ni and Pb are at present identified as PHS.

Pursuant to environmental legislation for risk assessment and WQC, it has been recognized that accurate assessment of the bioavailable metal fraction is crucial. The way to incorporate bioavailability into these procedures is still under discussion. In the EU risk assessment for zinc, for example, the dissolved metal is currently being considered as the bioavailable, bioreactive form (this is often referred to as the Precautionary Principle, in that any material is always potentially available in a toxic form). As discussed in the concluding section, the further integration of bioavailability within the dissolved phase, including the possible implementation of the BLM approach for use in considering the bioavailability of metals in water, is at present being examined by European regulatory agencies.

2.4.3. Regulatory approach in Australia and New Zealand

In Australia and New Zealand, recent revision of water quality guidelines has resulted in a tiered evaluation approach to metal regulation (ANZECC/ARMCANZ, 2000). Guideline trigger values are largely derived from statistical distributions of (NOEC) no-observed-effect concentration data for a range of species, modified in
freshwater settings using published hardness algorithms. If total (acid-soluble) metal concentrations in the test sample exceed the guideline value, the next tier is triggered, which involves measurement of dissolved metal concentrations (0.45-μm membrane filtration). If the dissolved metal concentration still exceeds the guideline value, metal speciation is considered. This may involve modeling or measurement, with confirmation by toxicity testing using an appropriate sensitive organism (bacteria, algae or daphnids). Models such as PHREEQE, MINTEO or WHAM can be used to calculate speciation based on solution measurements of DOC, pH, etc., while measurements might include the use of anodic stripping voltammetry (ASV), diffusive gradients in thin films (DGT), or chelating resin columns. It is acknowledged that both modeling and measurement methods are imperfect, but derived bioavailability can be readily confirmed by toxicity testing.

The approach recognizes the importance of bioavailability, with the measurement from each successive tier better approximating the bioavailable fraction, and more reliably predicting impact. Measurements may commence at any tier, dictated by the anticipated level of compliance with the guideline. A major advantage of the tiered approach is that the cost penalty of speciation measurements, modeling or toxicity testing is only applied when dissolved metal concentrations exceed guideline values.

2.4.4. Regulatory approaches elsewhere

In South America, the regulation of metals in ambient waters draws worldwide attention, particularly because of the prevalence of mining activity. Current regulatory approaches are largely derivatives of the US scheme. Recently, short courses on metals bioavailability and the BLM approach were presented in Argentina and Chile. These sessions were well attended by government regulators, and the approach has received preliminary favorable review.

In China, water quality standards are set by the national government (the State Environmental Protection Administration, or SEPA). The current Chinese Water Quality Standards for metals appear to be largely modeled on the US EPA Ambient Water Quality Criteria approach. Development of permit limitations, and enforcement of these limitations, is largely delegated to Environmental Protection Bureaus in the provinces and major cities. Currently, the provincial Bureaus are struggling with setting effluent limitations that can feasibly be achieved with available treatment technology. After attending recent academic presentations on the concept of bioavailability and its practical impacts on regulatory programs, the provincial and national water quality managers requested more information on the BLM and its applicability to Chinese waters. Thus, the value of the bioavailability approach for assessing the potential toxicity of metals and the potential use of the BLM as a cost-effective tool have been recognized in areas where government resources for environmental protection are scarce.

3. A convergence of ideas: the BLM, the present

By the early 1990s, developments of the previous 60 years or more had led to greatly improved understanding of the chemistry, physiology and toxicology of metals. Even so, the regulatory standards that were being adopted in most countries were still based on either total recoverable metal or total dissolved metal concentrations. While it was recognized that this was not an ideal situation, a clear path for rectifying this situation had yet to be defined.

Playle et al. (1992), in a key series of experiments, investigated metal–gill interactions and showed that cations in solution compete for a limited number of binding sites at the gill, and that this competition affects the degree of metal accumulation at the gill. These experiments were used to evaluate gill binding site densities and conditional stability constants, parameters that could be incorporated into chemical equilibrium models and used to predict the interactive effects of concentration, competition and complexation on metal accumulation at the fish gill (Playle et al., 1993a,b for Cu and Cd; Janes and Playle, 1995 for Ag). This was an important advance, because the degree of metal accumulation at the gill reflects the integration of effects resulting from both complexation of the metal in the water and competition of the metal with other cations for binding sites at or near the gill surface. Perhaps of equal, if not greater, importance was that these studies provided a clear and tangible demonstration that metal accumulation at the site of action of toxicity was truly a function of metal complexation and competitive interactions, as indicated in the earlier conceptual models (e.g. Pagenkopf, 1983; Morel,
Fig. 6. Short-term rainbow trout gill silver accumulation data (mean with 95% CI) showing the effects of silver concentration, natural organic matter (NOM) complexation, inorganic complexation by Cl⁻ and cation competition by Na⁺ on gill Ag accumulation. (Data: Janes and Playle, 1995.) (a) Gill Ag level as a function of total dissolved silver concentration in low-DOC, soft water. (b) The effect of adding Luther Marsh NOM (measured as DOC) on gill Ag accumulation in water with total dissolved Ag = 18.3 μg/L. The data show decreasing gill Ag as DOC increases because NOM complexation reduces the concentration of Ag⁺. (c) The similar effect of inorganic complexation of Ag⁺ by Cl⁻ on gill silver levels at total dissolved Ag = 11.9 μg/L. (d) The effect of cation competition between dissolved Ag species and Na⁺ on gill Ag level at total dissolved Ag = 11.9 μg/L.

1983), and that these features could be verified with actual measurements of metal accumulation (Fig. 6).

While Playle and co-workers showed that accumulation of metal at the gill was associated with a physiological effect, an important element that was not addressed by them, and one that would be needed to enhance the utility of their results to regulatory agencies, was how to relate the level of metal accumulation at the site of action, the gill, to acute toxicity. In addition, because WQC are typically compared to the dissolved metal concentration, it would be a matter of practical utility if the focus could be shifted from both the free metal ion activity and the level of accumulation at the site of action of toxicity to the more readily measured and reported total dissolved concentration. This total dissolved metal concentration includes not only the free metal ion, but also the organic and inorganic metal complexes.

An interdisciplinary meeting of international experts at a SETAC Pellston Workshop was subsequently held in early 1996. The consensus of those in attendance was that a generalized framework was needed to concurrently and quantitatively evaluate the significance of these important factors that affect metal availability and toxicity (Bergman and Dorward-King, 1997). Around the same time, Allen and Hansen (1996) proposed the use of a chemical equilibrium model for the purpose of, focusing on the effect of complexation of copper by organic matter and its effect on bioavailability and toxicity and, of equal importance, how to relate these analyses to the needs of
regulatory agencies. Di Toro and co-workers then proposed the biotic ligand model (BLM) of acute toxicity as a way to establish a connection between accumulation level and effect, and, at the same time, to relate this effect to the total dissolved concentration in the water column (USEPA, 1999a; Di Toro et al., 2000, 2001). This implementation of the BLM is an adaptation of the GSIM of Pagenkopf (1983).

Central to the BLM is the notion that the chemistry of the system is at equilibrium. This allows the use of thermodynamic and conditional binding constants to calculate the concentrations of metal species in the system, including [M–BL], the metal bound to the biotic ligand. The conceptual model can be considered in terms of three separate components (Fig. 1). One component involves the solution chemistry in the bulk water, which allows prediction of the concentration of the toxic free metal ion ([M^{n+}]; Fig. 1). A second component involves the binding of the toxic metal to the BL. The final component is the relationship between metal binding to the BL and the toxic response. Within the context of this chemical equilibrium-based framework, the ‘biotic ligand’ represents the site of action of toxicity, where binding of the metal results in the manifestation of a toxic effect. Because the computations are conducted within a chemical equilibrium framework, the division of the conceptual model into three components is carried out merely for explanatory purposes. In reality, all processes are simulated as simultaneous transformations.

Generally, the free metal ion (M^{n+}) is considered to be the most toxic metal species, but the BLM allows for toxicity caused by other species as well (e.g. MOH^{n-1}, MCl^{n-1}) (Fig. 1). The toxicity of metal species other than M^{n+} is in accordance with the FIAM, as long as the other metal species are in equilibrium with the free ion (Morel, 1983). Regardless of how the available metal species are defined, a key component of published versions of the BLM is to predict the concentration of [M^{n+}], albeit using different modeling strategies. All versions of the BLM consider that M^{n+} binds to inorganic ligands and to dissolved organic matter (DOM). The complexation of M^{n+} by inorganic ligands is calculated using geochemical speciation codes, such as CHESS (Santore and Driscoll, 1995) or MINEQL+ (Schecher and McAvoy, 1994), with stability constants for inorganic reactions obtained from well-accepted, published databases.

The prediction of the organic complexation of M^{n+} presents major challenges as a result of the enormously varied nature of DOM in aquatic systems. Consequently, the BLM framework of Di Toro et al. (2001) is based on chemical equilibrium computations made with CHESS. It includes the simultaneous solution of a standard set of metal–inorganic matter reactions in combination with the metal–organic matter reactions that were developed for WHAM, model V (Tipping, 1994). This formulation of WHAM includes a detailed representation of DOM chemistry, including proton and metal binding on numerous heterogeneous binding sites and a continuum of binding affinities suitable for a range of metal concentrations that are typical of natural waters. The model also considers competition with protons and calcium on metal binding sites within DOM. McGeer et al. (2000), in their Ag BLM, used the MINEQL+ model to calculate the binding of Ag^+ to organic matter based on a single binding constant derived from experiments with Luther Marsh DOM, isolated by tangential flow ultrafiltration (Janes and Playle, 1995). Also considered is competition for binding sites on the DOM by H^+, but not Ca^{2+} (McGeer et al., 2000). The use of WHAM V is the more sophisticated of these approaches in terms of the input parameters and complexity of assumptions regarding organic matter complexation of metals. While one of its
advantages is that it has been calibrated for a number of metals over a range of concentrations, it continues to undergo refinement and further testing as warranted. This is particularly important, because the BLM is routinely being applied to evaluate metal speciation at increasingly lower metal effect levels, levels that are below the range for which WHAM V was originally calibrated. Input parameters for these BLM versions are summarized in Table 1. Neither model has the capability to fully model the metal complexation in terms of the full heterogeneity of natural waters, which include colloids and particulates, as well as dissolved ligands.

A number of previously published applications of the BLM allow for more than just the free ion to interact at the biotic ligand and to exert a toxic effect. These include the development of a silver BLM that was proposed by Paquin et al. (1999), the current version of the copper BLM, which was previously proposed by Santore et al. (2001), and a copper BLM for Daphnia magna that has been proposed by De Schamphelaere and Janssen (2002). In each of these, metal species other than M\(^{n+}\) are able to exert a toxic effect at a BL. In the silver application, AgCl is considered to be bioreactive, while, in the versions for Cu, [Cu(OH)]\(^{+}\) is implicated as being a bioreactive form, in addition to [M\(^{n+}\)]. In each of these sets of computations, WHAM V reactions in the BLM were used to calculate the degree of organic complexation of dissolved metal.

The metal–biotic ligand interactions follow the approach of Playle and co-workers (Playle et al., 1992, 1993a,b; Janes and Playle, 1995), who characterized Cu–, Cd– and Ag–gill interactions over a range of water chemistry conditions by assuming that gill sites behaved in the same way as any other dissolved ligand. The gill is composed of a suite of negatively charged proteins to which cations can bind, and the biotic ligand represents a physiologically active subset of these sites. The BLM assumes that the biotic ligand occurs on a biological surface, such as a gill membrane, and metal binding can be characterized as a surface adsorption process. Competition is assumed to occur between the toxic metal and Cu\(^{2+}\), Mg\(^{2+}\), Na\(^+\) and H\(^+\) (McGeer et al., 2000; Santore et al., 2001; Schwartz and Playle, 2001; De Schamphelaere and Janssen, 2002) for binding sites on the biotic ligand. K\(^+\) has also been tested as a competing ligand, but was not found to influence Cu or Zn toxicity to daphnids (De Schamphelaere and Janssen, 2002; Heijerick et al., 2002a) or Zn toxicity to the algae Raphidocelis subcapitata (Heijerick et al., 2002b).

Different published versions of the BLM have used different means to estimate the binding of the metal to the BL. Binding constants for the interaction between metals and protons and the biotic ligand have been derived from gill-loading experiments conducted by Playle and co-workers using juvenile rainbow trout (described above) (Playle et al., 1992, 1993a,b; Janes and Playle, 1995). The version of the BLM that was originally developed to predict the acute toxicity of silver (Paquin et al., 1999) was concurrently calibrated to the gill silver accumulation data of Janes and Playle (1995) and silver toxicity data for rainbow trout, fathead minnow and Daphnia magna (Bury et al., 1999b; Bills et al., 1997). McGeer et al. (2000) focused on the underlying mechanism of toxicity that results from binding of silver to the gills of rainbow trout, the inhibition of branchial sodium- and potassium-activated adenosine triphosphatase activity (Na\(^+\)/K\(^+\)-ATPase activity, or NKA activity as used hereafter). The inhibition of this enzymatic activity by silver and by some other metals disrupts the ability of aquatic organisms to actively regulate certain internal ion pools and may cause death if conditions are extreme enough (Wood et al., 1996, 1999; Morgan et al., 1997; Webb and Wood, 1998; Bury et al., 1999a,b). Recognizing this, they used a Ag–NKA ‘gill binding constant’ that had been previously determined by Wood et al. (1999). They also specified gill binding constants for other competing cations using data from Bury et al. (1999a,b) and from Galvez and Wood (1997). They showed that use of these mechanically based estimates of binding constants led to very good predictions of rainbow trout LC\(_{50}\) values over a wide range of silver concentrations and water quality characteristics. The fact that the mechanically based Ag–NKA binding constant evaluated by McGeer et al. (2000) was comparable to the accumulation/toxicity based constant evaluated by Paquin et al. (1999) (log \(K_{\text{Ag–Gill}}\) = 7.3 vs. 7.6) was a fortuitous result. It suggests that the general approach of calibrating the model to toxicity data is of practical utility. In particular, it may be useful if the mechanism of toxicity of a metal is less well understood than it is for both copper and silver or, for more practical reasons, as in cases where it may not be
Some of the conditional constants that have been derived to characterize metal binding to biotic ligands, for different versions of the BLM, are summarized in Table 2. The similarity among constants (given the experimental errors involved in deriving these constants) for the same metal but different organisms suggests that the mechanism of binding to the ligand is similar for different organisms. The fact that protons do not mitigate toxicity in the same manner for all of these organisms may suggest that the mechanism of toxicity varies across organisms, or that other differences in related physiological processes exist. Alternatively, it may also reflect the possibility that the test conditions were not adequate to arrive at a definitive evaluation of the proton binding constant.

MacRae (1994), MacRae et al. (1999) demonstrated a clear link between copper accumulation at the fish gill and copper toxicity. Hence, pursuant to the approach of Di Toro and co-workers (USEPA, 1999a; Di Toro et al., 2001), the link to toxicity is made by assuming that the dissolved metal LC50, which varies with water chemistry, is always associated with a fixed critical level of metal accumulation at the biotic ligand. This fixed lethal accumulation at 50% mortality, referred to as the LA50, is the concentration of the metal–biotic ligand complex, Me:BL, that is associated with 50% mortality. It is assumed to be constant, regardless of the chemical characteristics of the water (Meyer et al., 1999).

There are several important features of the BLM of acute toxicity that distinguish it from earlier models upon which it is based. First, it synthesizes within the context of a single model framework many of the important interactive factors (e.g. inorganic and organic complexation and cation competition) that affect metal bioavailability, while at the same time providing a way to predict a toxic effect concentration of a metal to an organism over a range of water quality conditions. In addition, while it recognizes the importance of the free ion activity of the metal, the free ion is not necessarily the only available metal form and, furthermore, it places emphasis on the prediction of the dissolved metal concentration that is associated with the effect of interest at a given water quality. Thus, the 96-h LC50 value is predicted to be the dissolved metal concentration that is required for metal accumulation at the biotic ligand to equal the LA50. The LA50 is evaluated by calibration of the BLM to metal toxicity data in studies where the chemistry is reasonably well known. Once evaluated, the BLM may be used to
predict the total dissolved metal concentration, the predicted dissolved LC50, that is associated with the LA50 for a given set of water quality characteristics. It has been shown that, when suitably calibrated, the BLM can be used to successfully predict LC50 values over a wide range of water quality characteristics. Fig. 7 illustrates how a version of the BLM that was developed for copper is able to predict fathead minnow LC50 values to within a factor of approximately two over a range of effect levels that spans approximately two orders of magnitude.

To date, there have been a number of applications of various aspects of the generalized BLM framework to the acute toxicity of metals, for a variety of both organisms and metals (e.g. Paquin et al., 1999; McGeer et al., 2000; Santore et al., 2001; De Schamphelaere and Janssen, 2002). More recently, as described in the remainder of this special BLM issue of CBP, Bell et al. (2002), Heijerick et al. (2002b) and Campbell et al. (2002) explore the potential for application of the BLM framework at the cellular level, such as would be appropriate in its application to algae. Similarly, Tao et al. (2002) and Macdonald et al. (2002) present results for the binding of Cu and Pb, respectively, to fish gills, and Meyer et al. (2002) consider the potential for relating whole body accumulation of Cu to its toxicity to Lumbriculus variegatus. De Schamphelaere et al. (2002) and Bury et al. (2002) describe recent applications of the BLM to the analysis of the acute toxicity of Cu and Ag, respectively, to invertebrates, while Santore et al. (2002) present results for Zn toxicity to fish and invertebrates. Although the details of how these various applications of the BLM framework were performed may differ to some degree, the underlying conceptual approach, described above, is generally the same in each case. Initial efforts to extend the application of the BLM to marine waters and to chronic effect levels are also in progress (refer to Ward and Kramer, 2002; Hogstrand et al., 2002 for marine studies using Ag).

4. Ongoing developments: the BLM, the future

With regard to chemistry, future BLM development efforts will undoubtedly benefit from improvements in the ability of chemists to reliably measure a wide range of free metal ion concentrations at lower concentrations than is currently possible. This will allow the development of more detailed models of metal binding to organic matter, gill surfaces and other sites where binding of metals leads to the manifestation of toxic effects. It will also allow easier and more complete validation of the chemistry component of the models developed.

At the metal concentrations used in acute toxicity applications of the BLM, it can be argued that strong ligands have been saturated and do not exert a controlling influence over metal speciation. At the lower metal concentrations that are relevant to chronic effects, however, the strong binding sites may well become more important (see Bryan et al., 2002; Bianchini and Bowles, 2002; Smith et al., 2002; Ward and Kramer, 2002 for further discussion of metal complexation in natural waters). This may be particularly problematic for BLM applications that model metal–DOM interactions as a single discrete ligand with a single binding constant. The use of multiple binding site models that take into account competition from other cations are more likely to be able to account for lower-concentration, higher-affinity binding sites, but such applications will need to be properly tested over the relevant concentration ranges. In addition to strong binding sites in DOM, there are...
other, high-affinity ligands in natural waters that need to be accounted for. Bianchini and Bowles (in press) discuss the need to include reduced sulfur ligands, including inorganic sulfide species and thiols.

No doubt, more sophisticated models of metal binding to biotic ligands (e.g. gills) will be developed in the same way that models such as WHAM (V) have advanced the prediction of metal speciation in solution (Bryan et al., 2002 describe recent WHAM results). Modeling metal binding to algal and bacterial cell surfaces, as well as uptake into the cell, would seem to be a logical progression in this regard (see Bell et al., 2002; Campbell et al., 2002). As part of these developments, it may also be desirable to further investigate the degree to which metal species other than the free ion (M\textsuperscript{2+}), for example M(OH)	extsuperscript{+}, can bind at the biotic ligand and exert toxicity. Studying such interactions at a fundamental level will prove difficult without the improved measurement techniques mentioned above.

Another area where chemistry may shed further light on the prediction of metal–organism interactions is related to the consideration of kinetic vs. thermodynamic control (Campbell et al., 2002). One of the principal assumptions of the BLM is that metal uptake is under thermodynamic control (i.e. that internalization is slow relative to the transport of the metal from the bulk solution to the biological surface and its reaction at this epithelial interface). Comparisons of calculated metal diffusion rates with metal uptake rates measured, on a common (membrane) areal basis, suggest that under certain conditions, e.g. low ambient metal concentrations, the diffusive supply of the metal from the bulk solution may prove to be the rate-limiting step. Under such conditions, all labile diffusive species contribute to metal uptake, and metal uptake is insensitive to changes in the metal distribution amongst the different labile forms. This result is inconsistent with the standard view, that is, that metal bioavailability should be sensitive to changes in metal speciation. It will clearly be important to determine for which metals and under what conditions diffusive supply of the metal may be the rate-limiting step.

The current formulation of the BLM assumes that metal uptake across the gill is slow relative to metal influx with the inspired water, and that the gill surface achieves equilibrium with the inspired water. Thus, the BLM does not consider the possible influence of gill ventilation rates on metal uptake or toxicity. Recent studies on Cd uptake by filter-feeding molluscs suggest that this assumption may not stand up to scrutiny (Tran et al., 2001, 2002). The fact that the organism may add carbon dioxide and ammonia to the water as it passes through the gill chamber may also influence water chemistry and, hence, metal speciation in the gill micro-environment, in comparison to the chemistry of the ambient water (e.g. Playle, 1998). Hence, this may be another area where more detailed consideration could improve the predictive capability of the BLM.

It has long been recognized that the reaction kinetics vary by metal, and some metals are known to exhibit relatively slow complexation kinetics (e.g. Ni\textsuperscript{2+}, Al\textsuperscript{3+}; Co\textsuperscript{3+} Cr\textsuperscript{3+}); for such metals, the assumption of thermodynamic control should be carefully examined. Ma et al. (1999) showed that a 24-h period was required to approach equilibrium conditions for copper. Having similar information for these other metals would help to ensure that experimental design and equilibrium model simulation results are internally consistent. Another possibility is to begin to consider the modeling of non-equilibrium conditions.

With regard to the involvement of anions in metal transport across epithelial membranes, another frequent assumption is that metal complexes shed their ligands before the metal traverses the biological membrane, i.e. it is the metal cation that enters the cell, independently of the (anionic) ligand. While this is not a strict requirement of the BLM, it is typically an initial assumption that is invoked in using such models to interpret toxicity data. A review of the animal physiology literature reveals several suggestions that Cd, Cu, and Zn carbonate/chloride complexes may be taken up by red blood cells (see Campbell et al., 2002 for further discussion). The postulated transported species (e.g. [Cd(OH)(HCO\textsubscript{3})][Cl\textsuperscript{-1}] and [Zn(OH)(HCO\textsubscript{3})][Cl\textsuperscript{-1}]) are entirely speculative; no thermodynamic data exist to suggest that such mixed-ligand complexes actually exist in solution, let alone at the transport sites. However, it might be possible to explain these recent experimental data not in terms of the transport of intact ternary complexes, but rather as the co-transport of the carbonate anion and one or more metal species. If such co-transport were widespread for metals and anions, then the whole BLM construct would crumble! The fact that, on the contrary, the BLM
does a good job of predicting (acute) toxicity suggests that co-transport cannot be a widespread phenomenon at external epithelial membranes (respiratory structures and the integument). The case with internal epithelial membranes (intestine, erythrocytes, hepatocytes, lymphocytes, etc.) may differ, however; ligand concentrations are much higher in the internal environment, and as a consequence it is perhaps more likely to encounter co-transport phenomena.

With regard to physiology and toxicology, it is envisioned that a variation of the BLM of acute toxicity will be needed for additional types of organisms, with perhaps an improved mechanistic understanding of the causes of differences in species sensitivity forming the basis of this effort. Acute toxicity data for fish are readily available for many metals, but far fewer data with measured metal burden and influence of water chemistry have been collected for invertebrates. More studies are needed, such as that reported by Gensemer et al. (2002), who evaluated the role of water hardness on copper toxicity using the cladoceran Ceriodaphnia dubia.

It will also be necessary to extend the time scale of application of the BLM, such that it is applicable to chronic exposures and effects. This will in turn place greater demands on the chemical sub-models of the BLM in order to reliably predict metal speciation over lower metal concentration ranges than was required in previous applications to acute toxicity. Chronic toxicity studies using metals are starting to consider the influences of water characteristics on toxicity, and the mechanisms of toxicity. Four recent chronic studies are included in this issue: Bianchini and Wood (2002) examine the physiological effects of chronic silver exposure in Daphnia magna; McGeer et al. (2002) describe the role of DOC on bioavailability and toxicity of Cu to rainbow trout during chronic exposure; Brauner and Wood (2002) review the influence of DOC on ionic silver in the survival and development of embryos and larval trout; and Ward and Kramer (2002) report on the influence of salinity on the chronic toxicity of silver to mysid shrimp.

The application of the BLM to other organisms will clearly need to include additional marine organisms, in which case it may be necessary to include waterborne metal exposure via the gastrointestinal tract, in addition to the gill. One such study explored the movement and binding of silver in the gut of the European flounder (Hogstrand et al., 2002), and similar lines of investigation will likely add to our understanding of the importance and mechanisms associated with this route of exposure.

Finally, it is envisioned that the BLM could be used as an aid in the interpretation of results from in vitro cell-culture tests and tests with excised gill arches. One example of this type of in vitro study is described by Taylor et al. (2002), who modeled branchial copper binding to excised rainbow trout gill arches. Together, model and data could be used to extrapolate such test results to alternative exposure conditions.

It is worth noting that, for metals such as Ag and Cu, which tend to interfere with Na\(^+\) balance, the ameliorating influence of Ca\(^{2+}\) on metal toxicity is not simply due to the mitigating effect of competing cations, as is commonly represented in applications of the BLM. Benefits to the organism are also likely to occur as a result of the decrease in the Na\(^+\) efflux component that occurs at elevated Ca\(^{2+}\) levels (Fig. 3, point 2). In effect, Ca\(^{2+}\) acts as a stabilizer of tight junctions in epithelial membranes, reducing diffusive loss of ions such as Na\(^+\) and Cl\(^-\) via paracellular pathways. This effect helps to minimize reduction in plasma ion pools associated with metal exposure. Experiments should be designed to test more rigorously this hypothesis, that Ca\(^{2+}\) has only a limited influence on the tendency for copper and silver to accumulate to the gill surface (and perhaps to the biotic ligand itself), but will, nonetheless, offer protection against toxicity through the indirect ‘tightening’ of the gill epithelium.

The extent to which Ca\(^{2+}\) protects against metal toxicity, and perhaps the mechanism by which Cu\(^{2+}\) is protective, may also be species-dependent. For instance, acute waterborne Ag\(^+\) exposure was recently found to significantly reduce plasma Na\(^+\) levels in crayfish as a result of the combined inhibition of Na\(^+\) uptake (J\(_{\text{in}}\)) and an increase in Na\(^+\) efflux (J\(_{\text{ex}}\)) (see right side of Fig. 1; Fig. 3, points 2–4) (Grosell et al., 2002a). In comparison, Ag\(^+\) disrupts the plasma Na\(^+\) balance in rainbow trout by inhibiting the unidirectional Na\(^+\) uptake component alone without an effect on efflux. It may be expected that Ag\(^+\) partially interferes with Ca\(^{2+}\) sites in the paracellular pathway in crayfish (Fig. 3, point 2). Therefore, changes in water Ca\(^{2+}\) concentrations would be predicted to ameliorate the toxicity of Ag\(^+\) in crayfish more so than...
would be predicted in fish. These differences highlight the necessity to incorporate differences in species sensitivities to acute metal toxicity in the BLM. Di Toro et al. (2001) and Santore et al. (2001) found that reducing the critical concentration of metal binding to the biotic ligand was suitable for predicting acute toxicity in species with different sensitivities to copper. It is possible that species differences may also be predicted by manipulating the conditional stability constants for that species differences may also be predicted by metal–biotic ligand interactions. Alternative, physiologically based approaches may also be viable (see Grosell et al., 2002b; Paquin et al., 2002), but these will require modification to the current chemical equilibrium-based BLM framework. The key point is that future BLM development efforts should profit from the consideration of new physiological information as it becomes available, in addition to toxicity information, as efforts proceed to extend the BLM to other aquatic species.

It is also assumed in the BLM framework that the stability of the metal–biotic ligand and of the various cation–biotic ligand complexes do not change across the different water chemistries tested. We must remember, though, that the biotic ligand is part of a living organism which is under tight regulatory control and very likely to change in response to environmental perturbations. The nature and dynamic properties of the biotic ligand need to be considered in the future development of biotic ligand models. From a biochemical perspective, the biotic ligand probably represents proteins involved in metal uptake at the critical site of toxicity. For many invertebrates, and certainly for fish, such proteins are found at the surface of ion-transporting cells of the gills (Olsson et al., 1998; Wood, 2001). Ion-transporting cells take up abundant ions and trace elements from the surrounding water to maintain osmolarity and mineral homeostasis. Metals enter the epithelium through transport proteins that are there to enable uptake of type A metals, such as Na\(^+\) (Bury et al., 1999a; Grosell et al., 2002a) and Ca\(^{2+}\) (Verbost et al., 1989; Hogstrand et al., 1996). There are also a number of transporters that are dedicated to essential transition and type B metals, such as Fe\(^{2+}\), Cu\(^+\) and Zn\(^{2+}\), but may also be permeable to non-essential elements (Bury et al., submitted). Typical for metal transporters is that they have been highly conserved through evolution, and because they define bioavailability, this is probably one reason why water chemistry has a similar, general effect on bioavailability of a given metal to a daphnid as it has to a fish. It is thought that the abundance of these transport proteins on the apical surface of ion-transporting cells results in preferential uptake of toxic metals in these cells and, thus, in a very high effective dose at the site of toxicity (Wood et al., 2002; Luoma et al., in press).

It is now well known that metal uptake by the gills changes over time by factors such as growth phase (Hogstrand et al., 1995, 1998) and metal exposure (Hogstrand et al., 1995, 1998; Grosell et al., 1997, 2002a). Specifically, metal uptake by the fish gill is typically reduced in response to exposure to the same metal. For zinc, it has been shown that this decrease in uptake rate is brought about by a reduction in gill affinity (decreased log \(K\)) for zinc (Hogstrand et al., 1995). Yeasts respond in a similar fashion to zinc exposure and in these organisms the mechanism involved is a removal of high-affinity zinc transporters, while those with lower affinity are still expressed (Gitan et al., 1998). Likewise, exposure of yeast to copper results in reduced expression of the high-affinity copper importer, CTR1 (Pena et al., 1998). The significance of these molecular studies, in terms of refinement of biotic ligand models, is that they indicate that there may not be a single log \(K\) value between a metal and the biotic ligand, but several, and that the relative abundance of each binding moiety varies according to conditions, such as previous metal exposure. Thus, better understanding of the molecular physiology of metal uptake and regulation in aquatic species should improve BLM predictions by providing the affinities of actual metal transporters and the conditions that determine the abundance of each binding site.

Significant progress has been made in the development and application of the BLM of acute metal toxicity in fresh water. An important challenge in the foreseeable future will be to extend the BLM approach to chronic metal toxicity and to dietary metal exposure. In the case of establishing a chronic BLM, there are several issues that could be important. For example, although exposure to low concentrations of copper and silver both initially elicit ionoregulatory disturbances (i.e. plasma Na\(^+\) reduction) over the first 7–14 days of exposure, prolonged exposure is usually followed by a recovery in plasma Na\(^+\) levels back to control levels (Lauren and Wood, 1987; Galvez et al., 1998; Galvez and Wood, 2002). Furthermore,
although plasma Na$^+$ levels increase back to normal, other ionoregulatory endpoints, such as Na/K-ATPase or unidirectional Na$^+$ uptake, may remain perturbed. Interestingly, Na/K-ATPase activity values, although initially inhibited by the metal, are increased to the point that levels are actually significantly elevated above control levels after 1 month of exposure. Often, acclimation to metals during chronic exposure makes organisms more tolerant to acute lethal challenges to the metal (Galvez et al., 1998; Galvez and Wood, 2002 for Ag; Dixon and Sprague, 1980 for Cu; Alsop et al., 1999 for Zn; Hollis et al., 2000 for Cd). These phenomena obviously complicate the use of these endpoints to model chronic metal toxicity. It has also been shown that the BLM characteristics of the gill, such as the binding site density and the conditional stability constants, change in response to long-term metal exposure (Szebedinzsky et al., 2001). There is also the confounding factor imposed by exposing aquatic organisms to metals via the diet. Recently, studies have shown that dietary Cd distributes to various tissues in fish, including the gills, resulting in a reduction in the binding affinity for waterborne cadmium and an increase in the binding site density of waterborne cadmium (Szebedinzsky et al., 2001). Once again, the physiology of aquatic organisms is complex and should be considered within the BLM framework.

These advances in understanding how aquatic organisms regulate the levels of fluids and ions in internal compartments have provided a physiological basis for the BLM interactions that occur with respect to the organism (McGeer et al., 2000). They should continue to facilitate the interpretation of the results of experimental investigations that are being performed as part of ongoing BLM development efforts. Further investigations of the mechanisms of toxicity of other metals, including Cd, Zn, Ni, and Pb, will also likely lead to improved understanding of metal–organism interactions and improved representations of these interactions in the context of the acute BLM framework. At the same time, it is envisioned that they will also serve as a basis for future development of the BLM, as its future applicability is extended into other areas of toxicological interest, such as species sensitivity and time-variable exposure (e.g. Grosell et al., 2002b; Paquin et al., 2002).

Application of the BLM to multi-metal predictions based on representative metal discharges to ambient waters is another complex area that may need to be addressed in the future. This approach would consider both multi-metal interactions with regard to inorganic and organic complexation reactions, and multi-metal interactions as they affect the organism. In their simplest form, these might involve interactions at the same site of action with the same biotic ligand, as might be considered for copper and silver, which both inhibit Na/K-ATPase activity. It is expected that the development of a BLM for metal mixtures will be more difficult when the mode of action of toxicity varies for the different stressor metals (e.g. in the case of copper and silver, which primarily have an ionoregulatory effect, and nickel, which is understood to primarily affect the respiratory system).

Much effort has clearly been directed at the development of the biotic ligand model for aquatic species that reside primarily in the water column of aquatic systems. However, the potential utility of the BLM approach is also being considered for other media, including aquatic sediments and terrestrial settings. A sediment BLM would be applicable to sediment-dwelling organisms, including both animals and aquatic macrophytes. The equilibrium partitioning approach that has been developed for metals offers, in essence, a first approximation to a sediment BLM, in that it considers the primary binding phase in sediments to be acid-volatile sulfide (Ankley et al., 1996). It also considers the importance of metal mixtures (i.e. Cu, Cd, Ni, Pb, Zn and Ag) on the metal complexation capacity of the sediments, which in this case is controlled by acid-volatile sulfide. A recent refinement to this approach, one that includes carbon normalization (Di Toro et al., 1999; USEPA, 2000), is also conceptually consistent with the representation of NOM complexation in the BLM, although such NOM interactions are represented in a much simpler fashion than in applications of the BLM that have been explored to date. It is clear that the range of chemical concentrations to be considered in sediments will differ markedly from the water column, but the general conceptual approach is likely to be quite similar to the water column BLM. The important question that remains to be answered is whether or not a more detailed representation of the chemistry, in situations where the simultaneously extracted metal exceeds the AVS, will improve the
explanatory power of the carbon-normalized approach to predicting effects of metals on benthic organisms.

An initiative to develop a BLM for European soils is just getting started for both plants and animals. As in sediments, the chemical environment and concentration ranges of interest will be much different than in the water column, and soils will have unique characteristics to address as well. For example, the wetting and drying of soils over time and aging of soil–metal mixtures may have important effects on metal availability and toxicity. Even so, the notion that bioavailability is important has been demonstrated to be of great significance in the evaluation of potential effects in the water column and sediments of aquatic systems, and it is envisioned that this will be true in soils as well.

As the chemical and physiological mechanisms of toxicity become better understood, it is envisioned that the BLM may become an increasingly mechanistic framework, such that the current uncertainty in predictions will eventually be reduced and/or viewed with increased understanding. As it stands today, much of what is now known about the chemistry, physiology and toxicology of metals will be of use in the near term in efforts to extend the applicability of the BLM approach to other areas of interest, including chronic toxicity, marine organisms and species sensitivity. It is expected that the regulatory agencies will continue to draw upon these advances in an ongoing effort to develop regulations that reflect the current version of ‘good science’ as it continues to evolve.

With regard to the regulatory implementation of the BLM, there are considerably more variables to consider than in previous approaches, such as application of a hardness-based WQC. This is considered to be a positive development, in light of what is known about the importance of water quality characteristics other than hardness on metal availability and toxicity to aquatic organisms. However, specification of a narrowly defined design condition, based on some critical freshwater flow rate and a single set of water chemistry characteristics, would tend to be so restrictive that it would be difficult to estimate what level of protection was being provided to aquatic life. An alternative that is being considered by the US EPA is to adopt a Monte Carlo-based approach to varying the water quality parameter inputs based on a statistical characterization of site water conditions. Such probabilistic techniques will need to be further developed in order to enhance the practical utility of the BLM in the regulatory arena.

A workshop on WQC was recently held at the University of Ghent, in Ghent, Belgium (Universiteit Ghent, 2002). The purpose of this workshop was to explore the state of science related to the bioavailability of metals in aquatic environments and to discuss possible integration of the recent scientific findings into the new regulatory frameworks in Europe and elsewhere. It was attended by 65 participants from academic, national regulatory agencies and industry organizations. The workshop participants concluded that the science and knowledge on the bioavailability of metals in surface waters are rapidly evolving and need to be, where possible, integrated into regulatory frameworks. It was further recognized that integrating bioavailability aspects for metals, within the dissolved phase, decreases uncertainty and increases ecological relevance. It was concluded that the bioavailable metal fraction should hence be used as far as possible for the setting of WQC and for compliance checking. It was stated that, in addition to (1) chemical measurement of metal bioavailability of metals (through specialized techniques such as complexation capacity and free ion activity) and (2) ecotoxicological tools (e.g. water effect ratios), the application of (3) toxicity–bioavailability models in regulatory frameworks should be further explored. The latter models may vary from simple equations to detailed models, with the most advanced so far being the BLM approach, as described in this journal issue.

5. Concluding remarks

It is clearly not possible to forecast accurately what lies ahead in future development of the BLM. What can be said, however, is that the research described in the papers of this journal issue represents a distinct milestone in the ongoing evolution of the BLM approach and, more generally, of approaches to performing ecological assessments for metals in aquatic systems. They also establish a benchmark to which future developments in the scientific and regulatory arenas can be compared. Finally, the papers demonstrate the importance and usefulness of the concept of bioavailability and of evaluative tools such as the BLM.
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