**Literature Review Report:
Interactions Between Trace
Metals and Aquatic Organisms**

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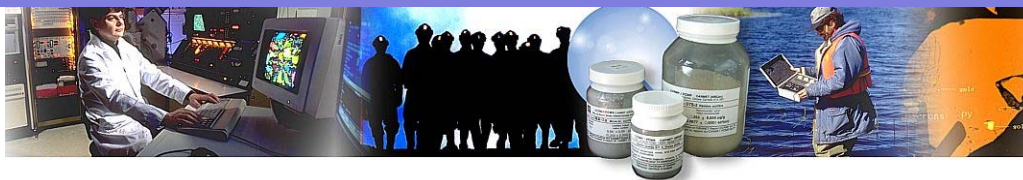
Literature Review Report: Interactions Between Trace Metals and Aquatic Organisms

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EXECUTIVE SUMMARY

This report is an update of a literature review conducted for MEND in 1993 on the development and application of tools to predict the effects of transition metals in sediment, water and aquatic food. Several of the tools discussed in 1993 have now been validated, and in some cases adopted by regulatory agencies. These tools can be used for mine effluent receiving waters to assess the potential for metal toxicity in sediment, to derive site specific water quality guidelines or to assess the risk for secondary exposure. These approaches include the acid volatile sulphide – simultaneous extracted metals (AVS – SEM) approach to predict the absence of toxicity in anoxic sediments, the biotic ligand model (BLM) to predict waterborne metal toxicity and the dynamic multipathway bioaccumulation model (DYMBAM) that predicts metal bioaccumulation in aquatic organisms.

Predicting metal bioavailability and toxicity in sediments remains a challenge. For sediments lacking oxygen, an approach based on the assumption that metals bound to sulphides are not bioavailable was successfully developed and applied to predict absence of toxicity for several metals (cadmium, copper, lead, nickel and zinc). The models presented in 1993 that predict free metal ion concentrations based on adsorption equilibrium with iron hydroxides have been used in several studies. However, this approach has not been fully validated or adopted by regulatory agencies. The AVS – SEM approach was further developed to use the difference between AVS and SEM rather than the ratio of the two as originally proposed, and to include normalization for the organic matter content of the sediment. The approach is based on the assumption that sedimentary metal in the presence of excess sulphides and in the presence of organic matter will have low bioavailability. The AVS – SEM approach has been used successfully by regulatory agencies, e.g. United States Environmental Protection Agency. However, this approach is restricted to a relatively small number of metals and only predicts the absence of toxicity.

For metals in the water column, the BLM approach allows prediction of acute toxicity of several metals within a factor of 2 to 3, as well as chronic toxicity for invertebrates. However, the capacity to predict metal speciation in natural waters and the extension of the BLM from acute to chronic toxicity still requires research. The BLM approach is based on the free ion activity model (FIAM) that was discussed in the 1993 MEND literature review. The approach uses geochemical equilibrium models to estimate the amount of metal bound to a biotic ligand, e.g., fish gills, for a specified water chemistry. Metal toxicity is then estimated based on the given water chemistry. Significant progress has been made since 1993 in the capacity to predict metal speciation in natural waters using geochemical models. However, available studies suggest that these models do not always provide accurate estimates. Therefore, efforts should be made to use, if available, corrected default parameters and to validate results with actual measurements. Nevertheless, the BLM approach normally allows the prediction of metal toxicity within a factor of 3 or less. This is a major improvement on the current approach since metal toxicity based on total concentrations can vary by several orders of magnitude as a function of different water chemistries. Application of the BLM to acute metal toxicity to fish (rainbow trout and fathead minnows) has been extremely successful and is based on a solid mechanistic understanding of

the effects of metals on fish regulation of ion concentrations in internal fluids. Extension of the BLM to other organisms and to chronic toxicity has also been moderately successful. However, the underlying mechanisms of toxicity are not fully understood and the acute BLM framework does not always directly apply. Nevertheless, the BLM approach has been adopted to different extents by regulatory agencies such as the United States and the European Union, and accounts for metal bioavailability in acute and chronic water quality guidelines and ecological risk assessments.

Food has been shown to be an important route of metal exposure and data from laboratory feeding experiments are currently integrated in the DYMBAM modelling approach that considers metal uptake from water and diet. There is no consensus in the literature on the relative importance of waterborne *versus* dietary metals for biological uptake and toxicity. Toxicity has most often been associated with waterborne exposure. For metal uptake however, dietary exposure can be significant and in some cases can be the major route of exposure for freshwater invertebrates (for example *Daphnia magna*, *Sialis velata* or *Chaoborus punctipennis*). The DYMBAM approach explicitly considers both routes of exposure and has been used successfully to predict metal bioaccumulation in laboratory and field exposures. Generally, the approach cannot predict metal toxicity, as metal toxicity is not directly related to total body metal accumulation.

Based on the above, future research efforts should focus on the chronic toxicity and particulate metal. Major progress have been achieved regarding our capacity to predict the acute effects of waterborne metals but additional research is required to expand the approach for chronic effects, especially for fish and plants. In addition, our ability to predict the effects of metal in sediment and metal in suspended particulate matter is still limited. Research should aim towards an improved assessment of the metal speciation in the exposure media, and a better understanding of the physiological effects and adaptation to trace metals.

SOMMAIRE EXÉCUTIF

Ce rapport est une mise à jour de la revue de la littérature effectuée pour le NEDEM en 1993, au sujet du développement et de l'application de méthodes de prédiction pour les effets des métaux de transition présents dans les sédiments, l'eau et la nourriture dans le milieu aquatique. Plusieurs des méthodes examinées en 1993 ont maintenant été validées et certaines d'entre elles ont été adoptées par des organismes de réglementation. Ces approches peuvent être utilisées pour les milieux aquatiques qui reçoivent des effluents miniers pour évaluer le potentiel de toxicité des sédiments, pour déterminer des critères de qualité de l'eau propre à un site et pour évaluer le risque d'exposition secondaire. Ces méthodes comprennent l'utilisation des sulfures volatils à l'acide et des métaux extractibles simultanément (AVS - SEM) pour prédire l'absence de toxicité dans les sédiments anoxiques, le modèle du ligand biotique (BLM) pour prévoir la toxicité des métaux présents dans l'eau et le modèle dynamique de bioaccumulation à voies de d'exposition multiples (DYMBAM) pour prédire la bioaccumulation des métaux dans les organismes aquatiques.

Prédire la biodisponibilité et la toxicité des métaux présents dans les sédiments demeure un défi. Dans le cas des sédiments peu oxygénés, une méthode a été élaborée en se basant sur l'hypothèse selon laquelle les métaux liés aux sulfures ne sont pas biodisponibles et cette méthode a été utilisée pour prédire l'absence de toxicité de plusieurs métaux (cadmium, cuivre, plomb, nickel et zinc). Les modèles présentés en 1993 qui prédisent les concentrations d'ions métalliques libres d'après l'équilibre d'adsorption avec les hydroxydes de fer ont été utilisés dans plusieurs études. Toutefois, cette méthode n'a pas été pleinement validée ou adoptée par des organismes de réglementation. L'élaboration de l'approche des AVS -SEM a été poursuivie et inclut l'utilisation de la différence entre les AVS et SEM plutôt que le rapport entre les deux tel qu'initialement prévu et inclut également une normalisation pour le contenu en matière organique du sédiment. La méthode est basée sur l'hypothèse selon laquelle les métaux présents dans les sédiments ont une biodisponibilité peu élevée lorsqu'il y a excès de sulfures et présence de matière organique. La méthode AVS - SEM a été utilisée avec succès par des organismes de réglementation, notamment l'Environmental Protection Agency des États-Unis. Toutefois, cette méthode n'est utilisable que pour un nombre relativement restreint de métaux et elle ne peut prédire que l'absence de toxicité.

Pour les métaux dans la colonne d'eau, le BLM permet la prédiction de la toxicité aiguë de plusieurs métaux à l'intérieur d'un facteur de 2 à 3 et de la toxicité chronique pour les invertébrés. Cependant, il faut poursuivre la recherche pour pouvoir utiliser un jour le BLM pour prédire la spéciation des métaux dans les eaux naturelles et pour prédire autant la toxicité chronique que la toxicité aiguë. Le BLM est basé sur le modèle de l'activité des ions libres (FIAM) qui a été analysé dans la revue de la littérature effectuée pour le NEDEM en 1993. La méthode utilise des modèles de l'équilibre géochimique afin d'évaluer pour une composition chimique de l'eau donnée la quantité de métal liée à un ligand biotique, par exemple les branchies des poissons. La toxicité des métaux est ensuite évaluée d'après la composition chimique de l'eau. Depuis 1993, d'importants progrès ont été réalisés à l'égard de la capacité de

prévoir la spéciation des métaux dans les eaux naturelles au moyen des modèles géochimiques. Mais les études disponibles suggèrent que ces modèles ne fournissent pas toujours des évaluations précises. Par conséquent, il est souhaitable d'utiliser les paramètres par défaut corrigés s'ils sont disponibles et de valider les résultats en les comparant avec les mesures réelles. Néanmoins, le BLM permet normalement de prédire la toxicité des métaux à l'intérieur d'un facteur de 3 ou moins, ce qui constitue une importante amélioration par rapport à la méthode actuelle étant donné que la toxicité des métaux basée sur les concentrations totales peut varier de plusieurs ordres de grandeur en fonction des diverses compositions chimiques de l'eau. L'utilisation du BLM pour prédire la toxicité aiguë des métaux pour les poissons (truites arc-en-ciel et têtes-de-boules) a été extrêmement concluante et est basée sur une solide connaissance mécanistique des effets des métaux sur les concentrations ioniques dans les fluides internes des poissons. L'application du BLM à d'autres organismes et à la toxicité chronique a produit des résultats modérément prometteurs. Toutefois, les mécanismes sous-jacents à la toxicité ne sont pas encore parfaitement bien connus et l'approche conceptuelle du BLM pour la toxicité aiguë n'est pas toujours directement applicable. Le BLM a tout de même été adopté à différents degrés par des organismes de réglementation, notamment aux États-Unis et dans l'Union européenne, et il sert à prédire la biodisponibilité des métaux dans les critères pour la qualité de l'eau pour la vie aquatique (toxicité aiguë et chronique) et dans les évaluations du risque écologique.

La nourriture est une importante voie d'exposition aux métaux et les données des expériences d'alimentation en laboratoire sont actuellement intégrées à l'approche de modélisation du DYMBAM qui tient compte de la bioaccumulation des métaux provenant de l'eau et de la nourriture. Les divers auteurs ne s'entendent pas sur l'importance relative des métaux présents dans l'eau *par rapport* aux métaux présents dans la nourriture pour ce qui est de la bioaccumulation et de la toxicité. La toxicité des métaux a le plus souvent été reliée à une exposition aux métaux présents dans l'eau. Toutefois, pour ce qui est de la bioaccumulation des métaux, l'exposition aux métaux présents dans la nourriture peut jouer un rôle important et, dans certains cas, elle peut être la principale voie d'exposition pour les invertébrés d'eau douce (par exemple *Daphnia magna*, *Sialis velata* ou *Chaoborus punctipennis*). Le DYMBAM tient compte explicitement des deux voies d'exposition, et il a été utilisé avec succès pour prédire la bioaccumulation de métaux lors d'expositions en laboratoire et sur le terrain. En règle générale, la méthode ne permet pas de prédire la toxicité des métaux parce que celle-ci n'est pas directement liée à l'accumulation totale de métaux dans l'organisme.

Par conséquent, les efforts de recherche devraient mettre l'emphase sur la toxicité chronique et les métaux particulaires. Des progrès importants ont été faits en ce qui concerne notre capacité à prédire les effets aigus des métaux dissous, mais des travaux de recherche supplémentaires sont requis pour adapter cette approche aux effets chroniques, en particulier pour les poissons et les plantes. De plus, notre capacité de prédiction des effets des métaux dans les sédiments et dans la matière en suspension est encore limitée. La recherche devrait viser à améliorer l'évaluation de la spéciation de métaux dans les milieux d'exposition et à développer une meilleure compréhension des effets et de l'adaptation physiologiques aux métaux traces.

1 INTRODUCTION

This report provides an updated review of advances in the development, validation and regulatory applications of the tools proposed in 1993 to predict the impacts of transition metals in aquatic ecosystems. A literature review report was completed in 1993 for the "Mine Environment Neutral

> **Current report is an update of a literature review conducted for MEND in 1993 on the development and application of tools to predict the effects of metals in sediment, in water and in food.**

Drainage" (MEND) program on possible means to evaluate the biological effects of sub-aqueous disposal of mine tailings. The document provided selective literature reviews on the biological uptake of metals, their bioaccumulation and their possible toxic effects on aquatic organisms in sub-aqueous disposal of mine tailings. Specific tools to predict the effects of metals in the aquatic environment were suggested in the report. Since 1993, research has advanced considerably, and some of the possible methods to predict impacts of metals have been adopted as part of a revised approach to manage biological impacts. The major advances regarding metals risk assessment are related to exposures through contaminated sediments, through the water column and through contaminated food. It should be noted that both the 1993 and the current report focus on transition metals (Cu, Ni, Zn, Cd, Pb, etc.) and do not address other important elements associated with mine tailings (e.g. As, Se, Mo and Sb).

It is recognized that total metal concentrations in sediment are not a good predictor of metal impacts, and geochemical approaches such as equilibrium partitioning models are now used as alternatives in sediment quality guidelines. While the long-term fate of metals in aquatic systems is the eventual deposition into sediments, it is often very difficult to link metal contamination in sediments with sediment toxicity. Comparisons of porewater metal concentrations and water quality guidelines are commonly used to assess the risk associated with sedimentary metals. However, the present understanding and capacity to model the geochemistry of metals in the solid and dissolved phases of sediments offers an alternative approach to predicting impacts that is based on more representative and relevant sediment characteristics. The 1993 review presented adsorption models and the acid volatile sulphides (AVS) approach. The adsorption models have been used in several studies since 1993. The AVS approach has been widely used since 1993 and was adopted in 2005 by the United States Environmental Protection Agency (US EPA 2005). The AVS approach could be used for example to assess if metals that have accumulated in sediment downstream of a mine discharge point have the potential to be toxic to benthic organisms.

Bioavailability is also an important concept in ecological risk assessment of metals in surface waters. The Free Ion Activity model (FIAM), which was discussed in the 1993 report, has led to the development of several bioavailability models to predict the toxicity of metals to aquatic species. These models are now commonly referred to as the "biotic ligand models" (BLMs), based on the software that was adopted by the US EPA (2007). Extensive research efforts have

been dedicated to the development and validation of BLMs and have led to the adoption of this approach by several jurisdictions for the development of water quality guidelines or ecological risk assessments (Niyogi and Wood 2004). While BLMs were originally derived for predicting acute toxicity of metal to fish (rainbow trout and fathead minnows) the approach has been successfully extended to chronic metal toxicity and other test species including invertebrates and some aquatic plant species. Extending the BLM to predict chronic toxicity still requires more research and development to address the challenges associated with longer exposure. Primarily, the mechanisms of chronic toxicity differ from those of acute toxicity. In addition, the longer exposure time for chronic toxicity requires the consideration of dietary effects and addition of food to the test media. Feedback between dietary exposure to metals and waterborne (gill) uptake also need to be considered. Finally, fish (and perhaps other higher organisms) tend to acclimate to chronic metal exposure. The current BLM approach can be used to derive site-specific water quality guidelines for surface waters that are receiving mine discharges.

Better understanding of metal uptake and mechanisms of metal detoxification have allowed the derivation of the Dynamic Multipathway Bioaccumulation Model (DYMBAM) approach, which can predict the relative contribution of water versus dietary exposure of metals. The current water quality guidelines are largely based on laboratory-derived acute and chronic toxicity measurements for which water was the primary route of exposure to metal. To predict metal impacts *in situ*, the question of the importance of contaminated food versus water arises. For some benthic species, it has been demonstrated that metal uptake is dominated by dietary exposure. Laboratory feeding experiments, such as those described in the 1993 literature review, have allowed the determination of physiological parameters required for the DYMBAM and therefore it can effectively predict metal uptake in several species of aquatic organisms (Luoma and Rainbow 2005). However, whole body metal uptake in most cases is not directly correlated to metal toxicity. Nevertheless, the DYMBAM approach is a promising tool to address issues related to metal bioavailability for the ecological risk assessment of metals in the aquatic environment. This approach could be used to predict metal accumulation in aquatic species downstream of a mine effluent discharge point and assess the ecological risk for organisms feeding on these species.

2 APPROACHES FOR MEASURING BIOAVAILABILITY OF METALS IN SEDIMENTS

Several physical, chemical and biological factors can influence metal bioavailability in sediments. Thus, sediment quality guidelines based on total metal concentrations cannot be used to predict metal toxicity (Simpson and Batley 2007). Aside from sediment quality guidelines, impacts can be assessed through laboratory based sediment toxicity tests, but with some limitations. Specifically, toxicity is difficult to link to levels of metal

> **It is still a challenge to predict metal bioavailability and toxicity in sediments. For sediment without oxygen, an approach based on the assumption that metals bound to sulphides are not bioavailable has been successfully developed and applied to predict absence of toxicity for several metals (cadmium, copper, lead, nickel and zinc).**

concentrations in sediments (Chapman *et al*. 1998) and test conditions might not be relevant for the *in situ* environment. It is also recognized that sampling and spiking of sediments with metals can lead to substantial overestimation of actual metal bioavailability. Accordingly, evaluating the risks associated with contaminated sediments is difficult, and the most popular method is a weight of evidence approach that combines chemical, toxicological and biological lines of evidences.

Similar to the aqueous environment, metal geochemistry plays a key role in determining the bioavailability and impacts of metals in sediments. Sedimentary solid phases that can bind metals and therefore affect their bioavailability include organic carbon, sulphides, and iron and manganese oxy-hydroxides. Although, no physical-chemical fractions actually represent the true bioavailable fraction in sediments, a clear distinction can be made between anoxic sediments and oxic sediments. In the presence of oxygen, dissolved sulphides may be present in sediment to some extent (e.g. Widerlund and Davison 2007) but in general, metal speciation is expected to be largely controlled by oxides and organic fractions. In absence of molecular oxygen however, the partitioning of many of the environmentally important metals will be dominated by sulphides.

2.1   Oxic sediments

The approach proposed in the 1993 literature review to predict free metal concentrations based on sorption equilibrium with oxy-hydroxides has been applied in several subsequent studies but has not been validated or implemented as a regulatory tool. For example, studies on aquatic plants (St-Cyr and Campbell 2000) and freshwater bivalves (e.g. Flessas *et al*. 2000, Wang *et al*. 1999, Giguère *et al*. 2003, Perceval *et al*. 2002 2004 and 2006) have used the sorption equilibrium approach to estimate free metal concentrations.   However few studies have compared the estimation of free ion concentrations, based on sequential extractions of the superficial sediment, and direct measurements. Vigneault *et al*. (2005) reported that the equations developed by Tessier (1992) can provide good estimates of free zinc concentrations if applied on suspended solids collected from receiving waters, and by neglecting organic matter as

a binding phase. However, this approach did not provide accurate estimates of free cadmium and free copper concentrations. To date, the only equation that accounts for sorption to organic matter was derived for cadmium (Tessier *et al.* 1993). Adsorption equilibrium with oxyhydroxides is a recognized approach to estimate metal distribution in sediment (Fairbrother *et al.* 2007), however it has not been adopted as a regulatory tool to manage contamination of sediments with metals.

2.2   Anoxic sediments

Since the 1993 literature review the method of calculating the ratio of acid volatile sulphides and simultaneously extracted metals to predict absence of metal toxicity in anoxic sediment has been further developed. The modified method that is based on the acid volatile sulphides and simultaneously extracted metals difference (AVS-SEM) is widely used and has been adopted by the US EPA and in the European Union for risk assessment for metals.

The AVS-SEM method was adopted by the US EPA as a procedure to develop sediment quality benchmarks for mixtures of cadmium, copper, lead, nickel and zinc (US EPA 2000). As proposed by Ankley *et al.* (1996), the US EPA combines the approaches for interstitial water concentrations and AVS-SEM. The latter approach is still based on the assumption that in the presence of excess sulphides, many metals will predominately bind as sulphides (and be non-bioavailable), and thus no toxicity will be observed (Di Toro *et al.* 1990). The improved approach is to determine the difference in AVS-SEM (Hansen *et al.* 1996). This provides the amount of SEM_{metal} that is not bound to sulphides and hence is potentially bioavailable. The approach has been verified by several short-term, life cycle, and long-term laboratory and field studies (Chapman *et al.* 1998). The current approach is to normalize the AVS-SEM difference for the organic carbon content of the sediment (US EPA 2000). For example, Burton *et al.* (2005) showed that zinc was not chronically toxic in sediments with excess SEM normalized for organic carbon of less than 147 µmol per g of organic carbon. The AVS-SEM approach has been commonly used as a line of evidence to exclude metals as potential toxicants in contaminated sediments (e.g. Ferraro and Cole 2002). In addition, Di Toro (2005) proposed to extend the approach for the development of a sediment biotic ligand model (see section 3.4 for details) which would allow the prediction of toxicity rather than the prediction of the lack of toxicity. Finally, the AVS-SEM approach was recommended by the US EPA for exposure estimates provided underlying hypotheses and related uncertainties are taken into account in the risk characterization phase (Fairbrother *et al.* 2007). Recently, the AVS-SEM approach was applied to characterize sediments from European ecoregions (Burton *et al.* 2007). Interestingly, Vandegehuchte *et al.* (2007) found that the free nickel activity and AVS-SEM (normalized for organic carbon) methods were equally good at predicting nickel toxicity to the benthic oligochaete *Lumbriculus variegatus*.

The AVS-SEM approach has some constraints; specifically that it is limited to anoxic sediments and to only five metals (cadmium, copper, lead, nickel and zinc). Also, the approach was developed mainly with spiked sediment data, for which porewater metal concentrations are likely

to be much higher than for actual contaminated samples. Sediments are very heterogeneous; for example, the depth of the oxic layer can vary both spatially and temporally. Therefore, measuring AVS-SEM on sediment samples may not be representative of actual exposure conditions for a given benthic organism. Also, the approach does not consider differences in interspecies sensitivities and modes of exposure (Hare *et al.* 1994). Finally, the insolubility of some copper and nickel sulphides in AVS extraction and experimental artefacts, such as the oxidation of copper sulphides during the SEM extraction have been reported (Simpson *et al.* 1998).

The AVS-SEM analysis could be applied to sediment collected in waters that receive or will receive mine effluent to assess the potential for metal toxicity. These analyses could be conducted for example on sediments that are collected as part of baseline or environmental effects. These data would be used to estimate the potential for discharged metals to contribute to effects, if any, on the benthic community downstream of a discharge point.

3 INTERACTIONS BETWEEN DISSOLVED TRACE METALS AND AQUATIC ORGANISMS

3.1 Background information

Over the last 15 years, environmental scientists have dedicated research into improving the understanding of metal speciation and its relationship to ecotoxicity, and in developing models that accurately predict trace metal toxicity. Much effort was focused on the concept that the free metal ion was directly related to bioavailability, thus forming the basis of the free ion activity model (FIAM). The core assumption of the FIAM is that metal bioavailability is under thermodynamic control. This equilibrium assumes that the rate of metal uptake across a cell surface is slow compared to the rate of diffusion to the cell surface, so that the surface-bound metal rapidly comes into pseudo-equilibrium with the metal in the bulk solution (Batley *et al.* 2004). Campbell (1995) reported exceptions to the underlying hypothesis, but found that most cases conformed to the FIAM. The current conclusion is that free ion is an important form, and possibly the only bio-reactive form (Paquin *et al.* 2002).

> **The biotic ligand model approach allows prediction of the acute and chronic toxicity of several metals within a factor of 2 to 3. Nevertheless, the capacity to predict metal speciation in natural waters and extend the BLM approach from acute to chronic toxicity still requires research.**

The FIAM was formulated in the early 1980's based on experiments conducted in the 1970's and more recently evolved into the biotic ligand model (BLM). Progress towards the free ion concept began in the 1970's, when scientists demonstrated that the free metal ion activity, specifically the cupric ion, was a better predictor of toxicity than the total dissolved metal concentration (e.g. Sunda and Lewis 1978). Reduction in toxicity as a result of competition by hardness cations (Ca^{2+} and Mg^{2+}) with metal ions for binding sites on the organism was also identified (Zitko *et al.* 1973; Zitko 1976). These studies, along with Pagenkopf *et al.*'s (1974) formulation of a chemical equilibrium model to describe the effect of water chemistry on metal speciation and toxicity, marked the advent of the metal bioavailability era. A significant advancement came in 1983, when Morel devised a theoretical scheme for the FIAM. It illustrated the interaction between the metal and organism based on a general chemical equilibrium principle. The model also incorporated the binding of free metal ions and other metal complexes to cellular binding sites at the site of toxic action while considering competition by other cations such as protons and hardness cations.

The major feature missing in Morel's depiction of FIAM was a description of how it may be applied in practice. This was satisfied by Pagenkopf (1983) who proposed the gill surface interaction model (GSIM) at about the same time. The GSIM allowed for an interpretation of toxicity test results for both individual metals (e.g. Cd, Cu, Pb, and Zn) and metal mixtures in fish. Approximately 10 years later, Playle *et al.* (1992, 1993a and 1993b) provided additional

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information on the effect of anionic complexation and competition with other cations on trace metals binding to fish gills. These data were later integrated with toxicity data by Di Toro *et al.* (2001), who developed a version of the BLM to predict the acute toxicity of copper and silver to several freshwater species.

The BLM's use has now been extended to regulatory systems. For example, the US EPA has incorporated the BLM into their water quality criteria development for copper (US EPA 2007). Prior to the BLM's integration, the freshwater copper criterion recommendations involved a hardness-dependent value. The use of the BLM improved the relevance of the criterion by including other critical water chemistry factors that are known to affect Cu toxicity. Most importantly, the BLM includes metal complexation by dissolved organic carbon (DOC) and inorganic ligands, the competitive interactions at the site of toxicity (i.e. biotic ligand), and the effect of pH, none of which were accounted for by the hardness correction method. At a low DOC concentration of ~2 mg/L, the BLM adjusted Water Quality Criteria (WQC) for copper is comparable to the previous hardness corrected criteria. However at a higher DOC concentration of 10 mg/L, the BLM adjusted WQC is about five times higher (Reiley 2007). The use of the BLM means that a single WQC will not exist for comparison purposes and implementation guidance is currently under preparation by the US EPA (Reiley 2007). Other countries are following suit. For example, regulatory agencies in Australia, New Zealand and the European Union (EU) are reviewing the BLM. In the EU, the BLM approach was used for the environmental risk assessments for copper, nickel and zinc. In Canada, Environment Canada and the Canadian Council of Ministers of the Environment (CCME) have decided to integrate the BLM approach into the development of Canada's water quality guidelines for metals (Niyogi and Wood 2003). CCME water quality guidelines currently in revision are using the BLM to normalize the effect of water chemistry on toxicity for some metals, for example silver.

For new or existing mines, the BLM approach can be used to assess the potential for metal toxicity in a specific receiving water. One limitation however is that the list of parameters measured for water quality monitoring does not always include all the required parameters for the BLM models. Both dissolved inorganic and organic carbon concentrations are key ligands for metal and need to be measured. Also, major cations (calcium, magnesium, sodium and potassium) and major anions (chlorides and sulphates) also need to be measured separately. For some metals, such as silver, dissolved sulphide is also a key parameter. Nevertheless, the BLM has been used to derive site-specific water quality guidelines. For example, the BLM was used in several cases to derive site-specific discharge objectives in Ontario (P.G. Welsh, Ontario Ministry of the Environment, personal communication). Another potential application of the BLM approach for mining would be to assess the potential effect of water borne metals on aquatic communities downstream of a discharge point taking in to account site-specific bioavailability.

3.2 Development of the FIAM/BLM approach

The BLM provides direct, quantitative estimates of metal toxicity, and was developed as a regulatory tool for predicting the site-specific toxicity of trace metals across a wide range of water quality variables, such as pH, hardness and dissolved organic matter concentration. The BLM is a mechanistic approach based on estimating metal binding to a biologically active uptake site for different aqueous chemistries (Figure 1). The binding of cations to the biotic ligand is assumed to be at equilibrium as is the chemical speciation in the exposure medium. Chemical speciation calculations compute the free metal, metal hydroxide, and competing cation activities to which the biotic ligand is exposed and these are used to estimate the amount of metal bound to the biotic ligand.
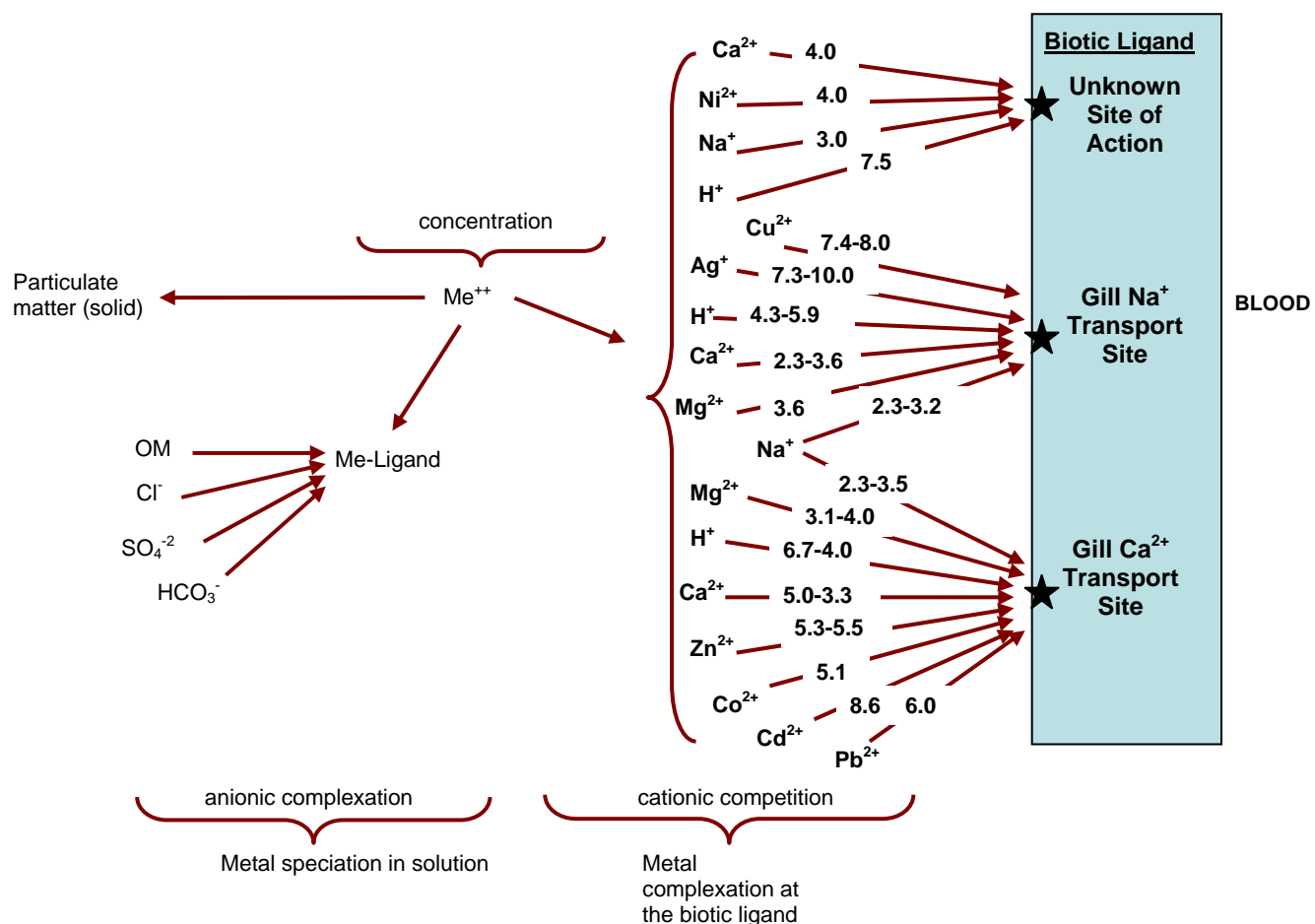
Figure 1. The BLM framework for metal speciation in solution and metal binding at the site of toxicity (or at the biotic ligand). Ranges of binding affinities (Log K values) for the various cations are included in the diagram. Adapted from Niyogi and Wood (2004).

Several models based on a similar framework have been proposed in the literature, such as the model developed by Di Toro *et al.* (2001) and the development and subsequent validation of the approach is still the focus of intense research efforts worldwide. While the original design of the BLM was as an acute toxicity prediction model for individual metals and for a limited number of aquatic species, efforts are now focusing on increasing the number of species and metals that can be included in the BLM approach. Research is also underway to extend the model to chronic toxicity in the context of water quality guidelines/criteria, risk assessment, and environmental effects monitoring, as well as the acute and chronic toxicity of complex effluents.

The BLM approach is a very active field of research and several studies were published recently on different aspects of the BLM. This report will focus on studies that tested the actual capacity of models to accurately predict metal toxicity. The underlying mechanisms and the research work related to the development of the BLM framework were reviewed elsewhere (e.g. Niyogi and Wood 2004) and will not be discussed in detail. However, a general description of the BLM framework is provided below and describes how metal speciation in solution and metal binding at the biological surface are estimated and linked to predict toxicity to an organism.

3.2.1   Metal speciation estimation in solution

A key component of the BLM approach is the estimation of trace metal complexation by inorganic and organic ligands in solution. While the use of chemical equilibrium models for complexation by inorganic and synthetic ligands is relatively straightforward, predicting complexation by naturally occurring dissolved organic matter is more challenging, given its heterogeneous character. In general, humic substances are the major component of DOC, ~ 50 to 80 % of DOC (e.g. Buffle 1988). Humic substances play a significant role in metal speciation by complexing many metals, preventing precipitation and controlling their speciation and transport (Morel 1993). Binding of metals by humic substances is affected by factors such as charge, binding site distribution, variable reaction stoichiometry and competitive nature of the ion binding (Unsworth *et al.* 2006). Sutton and Sposito (2005) recently proposed that humic substances are collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and hydrogen bonds. These associations are capable of organizing into micellar structures in suitable aqueous environments. Therefore, the metal, the nature of the DOC, and the competition between other metals are among the factors that need to be considered when predicting metal complexation by DOC.

Basically, two different modelling approaches have been used in the BLM. The first approach (e.g. Di Toro *et al.* 2001), uses a modified CHESS speciation code (Santore 1995) that includes the WHAM V (Tipping 1994) approach to model complexation by natural organic matter. WHAM V is based on an extensive dataset for natural organic matter and considers mono- and bi-dentate complexation and the influence of electrostatic interactions on binding. Proton binding and competitive metal binding are described for two types of acid groups. Each group is assigned a range of intrinsic pKs that are given median and range

values. The updated WHAM VI model includes improved modelling for trivalent metals present in low concentrations (Tipping 1998). Also, the proton and metal affinity are not completely correlated in the updated model to allow for the generation of wider range of metal binding strengths. The MINEQL$^+$ (Schecher 1994) speciation code has also been used (e.g. McGeer *et al.* 2000). However, this approach is not recommended since it employs a single binding constant measured for a natural organic matter sample and therefore ignores the polyelectrolytic and multidentate nature of natural organic matter.

3.2.2   Metal complexation at the biotic ligand

The other key component of metal speciation estimated using the BLM is complexation by the biotic ligand itself, for example, fish gills. Two approaches are currently used to estimate the amount of metal associated with a biological surface. The first, which is more mechanistically based, relies on measured conditional stability constants of the metals to the biological surface (Macrae *et al.* 1999). The second BLM approach is more empirical and uses complexation by a hypothetical biologically active surface ligand as a fitting parameter to relate metal speciation to the observed metal toxicity (De Schamphelaere and Janssen 2002). In the latter approach binding of metal complexes such as CuOH$^+$ (De Schamphelaere and Janssen 2002) and AgCl (Paquin *et al.* 2002) may also be considered in addition to the free metal ion, though without any physico-chemical evidence for their actual existence (see Campbell (1995) and Campbell *et al.* (2002) for a discussion of the possible role of such ternary complexes). The formation of a ternary complex between lead, humic substances and the algal surface of *Chlorella kesslerii* has also been suggested for consideration in future BLM frameworks (Slaveylova *et al.* 2003; Lamelas and Slaveykova 2007; Slaveykova 2007).

The measured or estimated binding constants are often roughly constant for different biological ligands in toxicity testing at relatively high metal concentrations. This is presumably because surface complexation is associated with similar low-affinity chemical moieties in each case. For example, Heijerick *et al.* (2002) noted the similarity of published binding constants for zinc with experimentally derived log K_{ZnBL} values of; 5.3 to 5.6 (Alsop and Wood 2000) and 5.1 (Galvez *et al.* 1998) for rainbow trout, and estimated log K_{ZnBL} values of 5.4 for steelhead trout (Cusimano *et al.* 1986) and 5.3 for *Daphnia magna* (Heijerick *et al.* 2002). Conditional stability constants for copper are also similar for fish gills, with experimentally derived log K_{CuBL} values of 7.5 for rainbow trout, 7.3 for brook trout (Macrae *et al.* 1999), and 7.4 for fathead minnows (Playle *et al.* 1993b). The estimated binding constants are slightly higher for the invertebrate *Daphnia magna*, with an estimated log K_{CuBL} of 8.0 (De Schamphelaere *et al.* 2002). Differences are also expected for non-chitinous invertebrates such as *Lumbriculus variegatus* (Meyer *et al.* 2002). Some of these differences may also be due to differences in experimental and model design, as there are no standardized methodologies and procedures for deriving data for conditional stability constants.

In the BLM framework, major cations are assumed to compete with the metal of interest for binding sites of the aqueous ligands and at the biotic ligand (Figure 1). For acute BLMs, sodium,

calcium and magnesium are typically included as competing cations that are capable of binding to the biotic ligand. However, for chronic Cu toxicity to invertebrates, the competitive effect of major cations is of much less importance than for acute toxicity to fish. For example, the chronic Cu BLM developed for *Daphnia magna* by De Schamphelaere and Janssen (2003) did not include calcium or magnesium as competing cations. Furthermore, work by Schwartz and Vigneault (2007) also found the effect of major cations to be negligible when developing a chronic Cu toxicity BLM for *Ceriodaphnia dubia*. Potassium does not affect most metal toxicity and is generally not included for modelling purposes (e.g. De Schamphelaere and Janssen 2002). An exception would be for the development of a thallium BLM, where the competitive interaction of potassium would be of importance. In fact, Hassler *et al.* (2007) recently developed a BLM to predict thallium toxicity to algae that incorporates potassium as the only competing major ion.

The effect of pH on metal toxicity conceptualized in the BLM is similar to the effect of major cations (Figure 1), and is based on aqueous metal speciation and competition of H^+ with metals for binding sites at the biological surface. Depending on the pH, H^+ concentration changes metal speciation for all metals. In addition, for some metals, H^+ also competes for binding sites on the biological surface, possibly reducing toxicity. However, pH can have direct physiological effects on the uptake of metals by aquatic organisms, which are not usually directly considered in the BLM framework. For example, Heijerick *et al.* (2002) suggested that the intrinsic binding affinity of the biotic ligand for metals could be affected by pH for zinc and the freshwater alga *Pseudokirchneriella subcapitata*. Similarly, François *et al.* (2007) found that pH has a non-competitive effect on manganese and cadmium uptake by *Chlamydomonas reinhardtii* that needs to be considered in the BLM framework.

To illustrate the competitive effect of H^+ using the BLM developed by Cowgill and Milazzo (1991), we estimated copper and silver binding to the gills of fathead minnows from pH 5 to pH 9 in Lake Superior water (Figure 2). In this exercise, any observed effect was related only to competition for binding to the gill surface and not to changes in copper or silver speciation in solution as total dissolved metal concentrations were increased with pH to keep free metal concentrations constant. In a toxicologically relevant metal concentration range, the direct competition of protons for binding to the gill surface is weak due to the higher affinity of Cu^{2+} and Ag^+ ions. In fact, no experimental data supports competition of H^+ with silver; the small reduction of Ag^+ bound to the gill at pH below 6 is presumably an artefact related to the use of an empirically derived lower proton binding constant for the silver BLM (Paquin *et al.* 2002). Similarly, no competitive effect of H^+ was found for the acute toxicity of zinc to *Daphnia magna* (Heijerick *et al.* 2002). In contrast, a significant competition of H^+ was found for the chronic toxicity of zinc to *Daphnia magna* (Heijerick *et al.* 2005) which highlights the significant differences between the mechanisms of chronic and acute toxicity of zinc to *Daphnia magna*.
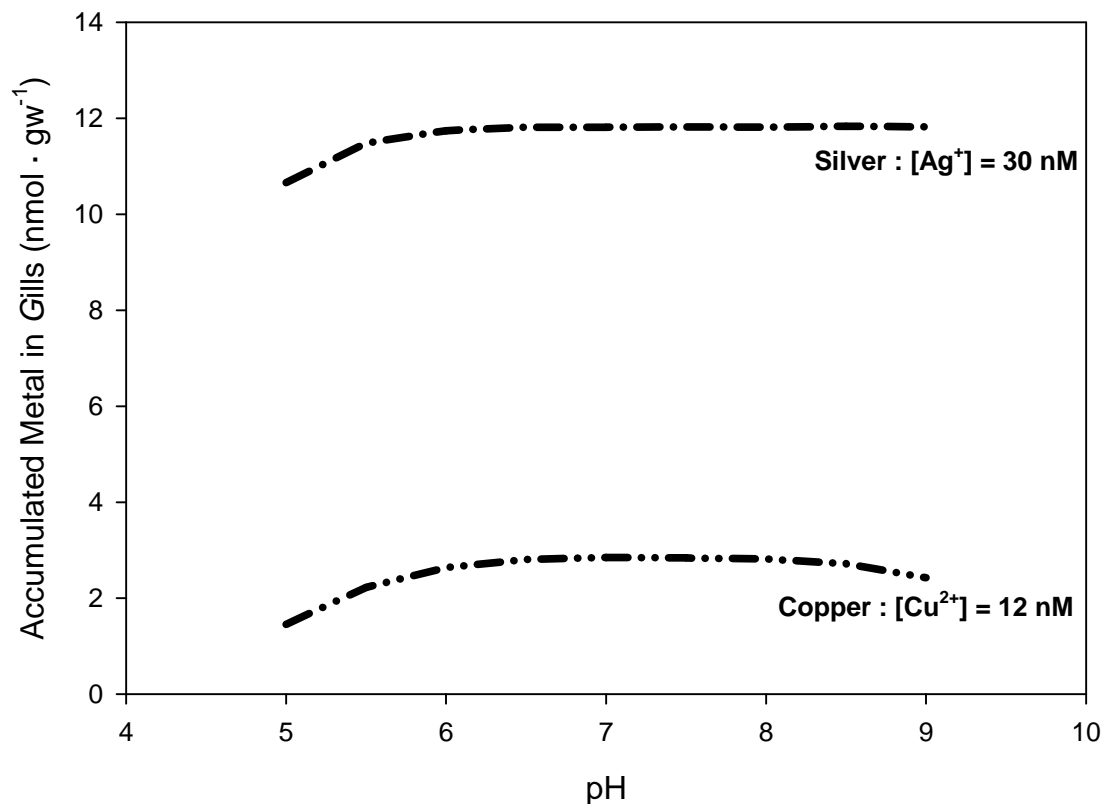
Figure 2. Effect of pH on BLM (2002) predicted Cu^{2+} and Ag^+ accumulation in the gills of *Pimephales promelas*. Average Lake Superior water chemical composition taken from Erickson *et al.* (1996). Freshwater criteria maximum concentrations (US EPA 2002) used as total dissolved concentrations, 205 nM Cu and 32 nM Ag, at pH 5. Total dissolved concentrations were increased with increasing pH so that free copper and free silver concentrations remained constant. Silver BLM : Log $K_{H+-gills}$ = 4.3 and Log $K_{Ag+-gills}$ = 7.3. Copper BLM : Log $K_{H+-gills}$ = 5.4 and Log $K_{Cu2+-gills}$ = 7.4.

3.3   Validation of the FIAM/BLM approach

The toxicity of trace metals can vary by a few orders of magnitude when based on total metal concentrations. However, with a better understanding of the mitigating effects of dilution water chemistry, the seemingly variable nature of trace metal toxicity can be more accurately predicted using the BLM. Metal toxicity based on total metal concentrations can vary by an order of magnitude (or more) in different natural waters. By comparison, studies that validated the BLM approach with independent datasets indicate that BLM-predicted toxicity is generally within a factor of two of measured toxicity. This is a reasonable improvement in our capacity to predict metal toxicity, especially considering that toxicity endpoints measurements alone, especially in natural waters, are associated with a relatively large variability. Furthermore, metal speciation

predictions are based on several individual chemical measurements and are also associated with a large variability. The approach has the value of being mechanistically based, at least for the prediction of trace metal acute toxicity to certain indicator species. Therefore, the BLM provides a more relevant and more precise approach to predict metal toxicity than using total metal concentrations or hardness-adjusted total concentrations. Validation studies have addressed both the metal speciation and the metal toxicity components of the BLM approach. Studies that focussed on validating predictions of metal speciation using mostly the Windermere humic aqueous model (WHAM) are briefly presented below. Studies that compared acute and chronic BLM toxicity predictions with measured toxicity are also presented below and summarized in Table 2.

3.3.1   Validation studies – Geochemistry

Along with research efforts to develop the BLM approach to predict metal toxicity, many studies over the past 15 years have focussed on measuring metal speciation in aquatic systems. Metal speciation is defined as the quantitative distribution of an element (e.g. a metal in its free and complexed forms) (Templeton *et al.* 2000) and can profoundly affect metal bioavailability and toxicity to aquatic organisms. Publications on measured metal speciation mainly focused on the measurement of labile metals using methods such as diffusive gradients in thin films technique (DGT) (e.g. Zhang and Davison 2000; Unsworth *et al.* 2005; Unsworth *et al.* 2006; Sigg *et al.* 2006) and competing ligand exchange methods (Mandal *et al.* 2002; Apte *et al.* 2005; Chakraborty *et al.* 2006). Although labile metal species may be indicators of metal bioavailability (Apte *et al.* 2005), the free metal ion concentration is believed to be a better predictor of bioavailability as discussed previously (Morel 1983). However, there is a limited availability of methods for reliably measuring free metal ion concentrations directly at environmentally relevant levels. These methods include the Wageningen Donnan membrane technique (e.g. Temminghoff *et al.* 2000), equilibrium ion-exchange technique (e.g. Fortin and Campbell 1998), and some electrochemical techniques such as Square Wave Anodic Stripping Voltammetry with a Rotating Disk Electrode (e.g. Pickering 2002). The most common difficulty with metal speciation techniques is that detection limits are not sufficient for natural waters where concentrations are generally very low (Sigg *et al.* 2006). Therefore, there is still no routine method available to directly measure metal speciation at environmentally relevant concentrations, even though the ability to estimate metal speciation is critical to predict impacts related to metals. Nevertheless, a relatively small number of studies have directly compared metal speciation model predictions with measured speciation.

Fair agreement between measured and predicted metal species concentrations was reported for several metals, including copper, zinc, cadmium and nickel. In 1999, Bryan *et al.* (1999) tested the predictive ability of WHAM model V in natural waters containing dissolved organic matter (DOM). The purpose of the study was to determine whether DOM behaved as isolated humic substances, on which the model is based. This was undertaken by studying copper binding to DOM using a Cu ion selected electrode (ISE) and comparing the results to their optimized WHAM predictions (Bryan *et al.* 1999). A reasonably good fit was found. In 2002, Bryan *et al.*

(2002) undertook a more comprehensive validation study. Fifteen freshwater samples containing large amounts of DOC were measured for free copper by ISE and results were comparable to predictions by Model V and the recently improved Model VI. Guthrie *et al.* (2005) undertook a similar study for a variety of trace metals (Ni^{2+}, Cu^{2+}, Zn^{2+} and Cd^{2+}) in freshwater samples from lakes in Rouyn-Noranda, Québec, which were affected by discharge from a nearby copper smelter. Free metal ion and/or labile metal complex concentrations measured by cathodic and anodic stripping voltammetry were compared to Model V and VI predictions. Both versions were found to predict the speciation of Ni, Zn and Cd reasonably well, but in general, WHAM VI performed better than WHAM V. Meylan *et al.* (2004) also found that free or labile zinc concentrations predicted using geochemical models including WHAM VI were similar to measured concentrations using DGT, competitive ligand exchange and voltammetry. Similarly, Mandal *et al.* (2002) reported good agreement between measured labile nickel and WHAM V estimates for a series of lakes and rivers in the Sudbury area. Aung *et al.* (2008) recently reported a good agreement between zinc and nickel bound to DOC measured by DGT and estimated using WHAM VI for an urban river in Japan. For strongly acidic conditions, WHAM VI provided fair estimates of labile aluminum, manganese and copper in acid mine drainage from a high arctic coal mine compared to DGT measurements (Søndergaard *et al.* 2008). Similarly, WHAM VI was able to predict free nickel concentrations within a factor of 1.2 for mine effluent receiving water with low DOC and a slightly acidic pH (Vigneault and Schwartz, unpublished results). However, a European study involving several speciation techniques noted that metal speciation models were reasonably good at predicting the dominant species of trace metals in freshwaters but that the free copper and lead concentrations can be under predicted by several orders of magnitude (Unsworth *et al.* 2006). It should be emphasized however, that the WHAM and Nica-Donnan models tested in this study worked reasonably well at relatively high metal concentrations, i.e. in the range of concentrations associated with metal acute toxicity and to some extent chronic toxicity. However, performance of these models at lower metal concentrations is much less satisfactory, especially for metals with strong affinities for natural organic matter such as copper and lead.

The current approach used in BLM estimations is generally based on standard assumptions regarding the nature of the DOC and a limited number of input parameters. Bryan *et al.* (2002) used the percent of active DOC as a fitting parameter between measured and predicted free copper concentrations for several freshwaters. They found that, on average, 65% of DOC in the samples should be assumed to be active humic substances in the model. In addition, the competition by other dissolved metals such as Mg, Al, Ca, Fe(II), Fe(III), and Zn were taken into account. Model VI has improved representations of the minimal high affinity binding sites in humic substances, which are sensitive for Fe(III) competition. Hence, a better fit to the observed data was found and the predictions were within a factor of 3.6 in 95% of the cases (Bryan *et al.* 2002) when competition was included. Iron competition is not included in most currently used acute BLM models, e.g. (US EPA 2007).

Other recent studies have also highlighted some limitations on the capacity to predict free metal concentrations in natural and receiving waters. In contrast to nickel, zinc and cadmium, Guthrie *et al.* (2005) found that both WHAM V and VI underestimated free copper concentrations more

than 2-fold compared to their voltammetry measurements. Gimpel *et al.* (2003) also found large differences between measured copper speciation and predicted speciation using WHAM. They obtained better prediction using a modified stability constant for copper binding to fulvic acid instead of the WHAM default constant. For zinc and nickel, Zhang (2004) obtained good prediction of measured free zinc and nickel concentrations using WHAM VI when competition with iron was considered and when the stability constants for some inorganic complexes were corrected. For nickel, Doig and Liber (2007) found that WHAM VI provided adequate prediction of free nickel concentrations in presence of fulvic acid but tended to overpredict free nickel in presence of humic acid when compared to ion-exchange measurements. Gopalapillai *et al.* (2008) reported a similar overestimation of free nickel by WHAM VI compared to ion-exchange measurements. Van Laer *et al.* (2006) corrected a similar overestimation of the free nickel concentrations by modifying the binding parameters of fulvic acid and carbonates. Similarly, Cheng *et al.* (2005) proposed an alternative binding constant for zinc and organic matter, as the default value did not provide a good estimate of competitive interactions with aluminum and iron. Later, Cheng and Allen (2006) noted that WHAM VI provided good predictions for free zinc concentrations in spiked surface waters, but complexation was overestimated at pH 8.0 and at zinc concentrations lower than 65 $\mu g \cdot L^{-1}$. Major differences between ultrafiltration measurements and WHAM VI predictions were also noted for chromium, copper, iron and nickel in shallow groundwaters (Pourret *et al.* 2007c). Lastly, in marked contrast to the results described above (Guthrie *et al.* 2005), Meylan *et al.* (2004) found that WHAM VI and other models overestimated free copper concentrations compared to actual measurements. Therefore, depending on the physico-chemical conditions, free metal concentrations may not be accurately predicted by speciation models, especially for low ambient metal concentrations.

Research efforts are currently devoted to developing and applying WHAM for a larger set of trace metals and to complex effluents or receiving waters. Tipping (2007) has recently modified the WHAM VI database to model the interactions of inorganic and methyl-mercury with humic substances. Using ultrafiltration measurements, Pourret *et al.* (2007a; 2007b) demonstrated that WHAM VI can be used to predict the speciation of rare earth elements in natural waters with a range of characteristics. For aluminum, Cory *et al.* (2007) reported good predictions of inorganic aluminum in Swedish lakes but poor predictions in Swedish rivers. The use of metal speciation models in receiving waters requires validation because of the potential presence of synthetic organic ligands in industrial or municipal waste waters (Tipping 1998). For example, the poor agreement that was found between WHAM VI predicted free nickel concentrations and ion-exchange measurements for a series of mine effluents (Gopalapillai *et al.* 2008) could be related to the presence of mining reagents that can chelate metals. Accordingly, the results of metal speciation models used to predict impacts of metals in waters affected by mine effluent discharges should be used with caution.

3.3.2   Validation studies – Acute toxicity

3.3.2.1   Fish

The acute BLM for fish is based on detailed studies of the effects of metals on gill iono-regulation. Validation studies were completed to assess the predictive capacity of the models on copper, silver, zinc and more recently on nickel.

For copper, Santore *et al.* (2001) designed a BLM to predict the effects of water chemistry on the acute toxicity of metals to aquatic biota. They tested their BLM for acute copper toxicity on fathead minnows by comparing their predicted of LC_{50} (concentration that resulted in 50% mortality) to the toxicity test results for two effluent-impacted streams in the United States and found the correlation to be generally within a factor of two.

For silver, the model developed by McGeer *et al.* (2000) to predict acute toxicity to rainbow trout was in agreement, within a factor of 2, with the toxicity measured in 31 toxicity tests from 10 independent studies. Di Toro *et al.* (2001) also developed a BLM to predict acute silver toxicity using the gill binding parameters reported by Bury *et al.* (1999) for fathead minnows and rainbow trout. However, Bielmyer *et al.* (2007) recently found that this model did not accurately predict the acute toxicity of silver to fathead minnows, especially in soft waters.

Acute BLMs for zinc toxicity to fathead minnows and rainbow trout were developed by Santore *et al.* (2002) but to date the validation of their predictive capacity has not been completed in natural waters. While the same datasets were used for the model development and the model testing, their model generally predicted the acute zinc toxicity within a factor of 2 for rainbow trout. For fathead minnows however, the model was less accurate in predicting the acute toxicity of zinc in part because the datasets for this fish included different age classes. De Schamphelaere *et al.* (2004) developed a model to predict the lethality of zinc to rainbow trout in laboratory water as well as in natural surface waters (De Schamphelaere *et al.* 2005) and in all of the cases the model fit measured toxicity values within a factor of 2.

Deleebeeck *et al.* (2007b) recently developed a BLM to predict the acute toxicity of Ni BLM to rainbow trout and fathead minnows. Their BLM was tested against 96h LC50 data for both larval and juvenile fathead minnow studies found in the literature and the results were in good agreement, i.e. within a factor of 2.2.

3.3.2.2   Invertebrates

Two models have been developed and validated for the prediction of the acute toxicity of Cu to daphnids. Santore *et al.* (2001) developed a BLM for *Daphnia pulex* by calibrating the lethal accumulation concentration to published toxicity data using the stability constants obtained for the acute toxicity to fathead minnows. They found the predictions of their *D. pulex* model to be generally within a factor of 2 of the measured toxicity. Based on more detailed experiments on the effect of competing cations, De Schamphelaere *et al.* (2002) developed a similar model to

predict the acute toxicity of copper to *Daphnia magna.* They later refined and tested their *Daphnia magna* Cu BLM using independent toxicity test data conducted with 25 reconstituted media representative of European natural waters and 19 spiked European natural waters (De Schamphelaere *et al.* 2002). Using their refined models, 50% percent effect concentrations (EC_{50}) were predicted within a factor of 2 both in the reconstituted and spiked waters. Further improvement of the predictive capacity was later achieved by accounting for the source of DOC, based on ultraviolet absorbance measurements (De Schamphelaere *et al.* 2004). Bossuyt *et al.* (2004) demonstrated that the model could be successfully applied to several cladoceran species, predicting EC_{50} within a factor of 2 for 27 out of 28 cladocerans in four European waters. An extensive validation study has also been conducted for *D. magna*, *D. pulex* and *D. obtusa* in 37 freshwater samples from Chile (Villavicencio *et al.* 2005) using the models described above. It was found that all models predicted toxicity within a factor of 2 most of the time and that the model originally developed by De Schamphelaere *et al.* (2002) was the best model for their dataset. BLMs developed for acute metal toxicity to invertebrates are semi-empirical since they do not rely on actual measurements of metal binding to the biotic ligand and the mechanisms of toxicity are not fully understood. De Schamphelaere *et al.* (2007a) recently demonstrated that while the protective effect of Na for acute copper toxicity is observed for most species of cladocerans, the current BLM framework is most likely oversimplified since there is evidence for an upper limit to the protective effect of Na. This is illustrated by the binding constant for Na to the biotic ligand being in the same order of magnitude as the constant for Ca and Mg. Based on principles of chemical equilibrium, the binding constant of monovalent Na to an organic ligand should be much less than binding constant of divalent Ca and Mg.

Two models were developed to predict zinc toxicity to daphnids. Santore *et al.* (2002) developed a BLM for the acute toxicity of zinc to *Daphnia magna.* When tested using the same data used to develop the model, 11 out of the 12 predicted endpoints were within a factor of 2 of the measured values. Heijerick *et al.* (2002) developed a model and conducted a validation study using 17 reconstituted media to predict zinc acute toxicity to *Daphnia magna*. Acute toxicity was accurately predicted within a factor of 2. De Schamphelaere *et al.* (2005) also obtained similar accuracy in a subsequent validation study of this model.

Development and validation of a BLM for other metals, such as nickel, are still in the preliminary stages. For example, Keithly *et al.* (2004) conducted toxicity studies with *Ceriodaphnia dubia* and *Hyalella azteca* with the intention of developing a reliable BLM for acute nickel toxicity. The LC_{50} results for the initial calibration were within a factor of 2, except for one *C. dubia* result with a sample pH of 8.6 (Schubauer-Berigan *et al.* 1993). Nickel carbonate ($NiCO_3$) is expected to be the dominant Ni species present at the noted pH and it is presumed by the BLM to be non-bioavailable. Additional studies are needed to understand Ni accumulation and toxicity in high pH waters. Nevertheless, this study showed that the BLM approach should be applicable for nickel. A more recent study of Ni toxicity to *Daphnia magna,* and applied to *Ceriodaphnia dubia*, was conducted by Deleebeeck *et al.* (2007a). They developed an improved model that considered the protective effect of Mg, which was not included in previous models. The measured acute toxicity (as 48-h EC_{50}) of 15 out of 16 samples fell within a factor of 2 of the predictions by the improved BLM. A model to predict the acute

toxicity of nickel to cladocerans is under development.   Deleebeeck *et al.* (2007c) recently demonstrated that the intrinsic sensitivity of cladocerans to nickel in soft water versus hard water was not significantly different. They have also found that the accuracy of BLM prediction was improved if different binding constants for Ca and Mg were used for hard and soft waters.

3.3.3   Validation studies – Chronic toxicity

3.3.3.1   Fish

The BLM approach for chronic toxicity is still in the early stages of development. A chronic BLM for zinc was developed (De Schamphelaere and Janssen 2004) and, in the case of rainbow trout, it appears that calcium is the most important cation to consider for chronic zinc toxicity. It was also found that 30-d mortality was a more sensitive endpoint than growth. De Schamphelaere *et al.* (2005) tested this model using eight natural waters spiked with zinc and under a wide range of water chemistry variables that affect bioavailability (e.g. pH, DOC, Ca, Mg, Na). The trout model predicted toxicity within a factor of 2.1.

Only one study has been published to date on the development and validation of a chronic nickel BLM for rainbow trout. Deleebeeck *et al.* (2007b) observed reduction of 17-d mortality with increased concentrations of Ca^{2+}, Mg^{2+} and H^+. However, Ni toxicity is not fully understood and therefore the protective mechanisms of these cations are still under investigation. In contrast to standard BLM assumptions, the LC_{50} expressed in free nickel concentrations decreased with pH increase and therefore the effect of pH cannot be modelled using the default BLM framework. Nevertheless, they were able to develop a model that predicted nickel toxicity within a factor of two in natural surface waters.

Applying the BLM framework to chronic toxicity is associated with several challenges. A complication is that chronic exposures require feeding which may directly affect metal toxicity. Work by Schwartz and Vigneault (2007) and Kolts *et al.* (2006) also found that the DOC derived from feeding the test organisms for chronic exposures can affect copper speciation and needs to be accounted for in BLM estimations. Diet influences metal binding to fish gills. For example, Kamunde *et al.* (2003) reported that dietary sodium reduced gill Cu uptake in rainbow trout during chronic exposure. The BLM implicitly assumes that the properties of the epithelial binding site or "biotic ligand" are constant, unaffected by general water chemistry (pH, hardness) or by pre-exposure to metals. In fact, for fish there is now convincing evidence that the metal-binding properties of the biotic ligand (e.g., the apparent surface density of binding sites, their affinity for the metal of interest) are affected by these variables, and that the resulting changes significantly alter the metal sensitivity of the target organism (Kamunde *et al.* 2002; Klinck *et al.* 2007; Niyogi and Wood 2003). These findings imply that a range of metal-binding properties for the biotic ligand will have to be incorporated into models for chronic toxicity (Niyogi and Wood 2004). Another complication is that the toxicity mechanisms that were identified for acute metal toxicity are not necessarily the same for chronic exposures. For acute toxicity to fish, the site of action for metal toxicity has been identified as the gills. For chronic toxicity, the actual site of

action can vary depending on the nature of the toxicity responses and on the actual route of exposure. Metals have been shown to impair chemosensory function in fish and the fish olfactory system has been suggested as an alternative biotic ligand for development of a chronic BLM for fish (Pyle and Mirza 2007; McIntyre *et al.* 2008a and b).

3.3.3.2   Invertebrates

Similar to fish, there is limited work on the development of chronic BLMs for invertebrates. Heijerick *et al.* (2005) developed and validated a chronic Zn BLM for *D. magna*. They developed BLMs to predict no observed effects concentrations (NOEC) and 50% effect concentrations (EC_{50}) using the linear relationship between the zinc activity and the competing cation activity. De Schamphelaere *et al.* (2005) later tested the EC_{50} model in eight natural surface waters. In waters with pH 6 to 8, the model predicted chronic zinc toxicity to *D. magna* within a factor of two, but above pH 8 toxicity was underestimated by a factor of 3 to 4.

Recently, a model was developed and validated for chronic Cu toxicity to *C. dubia* (Schwartz and Vigneault 2007). It was found that the effect of major cations on reproductive inhibition of Cu was negligible so only the effect of pH (competitive interactions of H^+) was included in their model. Food-derived DOC was also included as a ligand for copper in solution. The developed BLM tended to slightly overestimate toxicity, but was able to predict toxicity within a factor of two for 5 out of 6 natural waters.

3.3.3.3   Algae

Heijerick *et al.* (2002) have developed and validated a chronic zinc BLM for algae. They investigated the individual effects of pH and major cations (Ca, Mg, K, and Na) on zinc toxicity to the green alga *Pseudokirchneriella subcapitata* and compared the results to predictions of the BLM initially developed by De Schamphelaere and Janssen (2002). Their results showed that proton competition reduced the toxicity of zinc but the effect of pH was non-linear. The absence of linearity indicates that the original BLM hypothesis (the binding characteristics of the biotic ligand are independent of the test medium) does not hold true for this species of algae. This study led to the conclusion that further investigation was required to improve the BLM for chronic predictions, which initiated numerous studies on the prediction of chronic metal toxicity in algae. Meanwhile, De Schamphelaere *et al.* (2005) developed a bioavailability model for the chronic toxicity of zinc to *P. subcapitata,* based on an empirical relationship between toxicity expressed in free zinc activity and pH..

Similar to zinc, the relationships between free Cu and pH were used to predict chronic copper toxicity to algae. De Schamphelaere *et al.* (2003) completed a comprehensive study on the effects of pH, water hardness, and the source and concentrations of organic carbon on chronic copper toxicity to *P. subcapitata* using a 72-h growth inhibition study as the endpoint. The concentrations resulting in 10% and 50% growth inhibition fell within a range of 14 to 176 µg Cu/L (12-fold range) and 27 and 507 µg Cu/L (20-fold range), respectively. Results of this study indicate that when toxicity endpoints are calculated based on total dissolved Cu concentrations,

water chemistry has a significant effect on copper toxicity, with the exception of water hardness. However, when toxicity endpoints are calculated based on free copper activities, only pH significantly affects toxicity. Therefore, an empirical relationship between pH and free copper was developed. This model predicted toxicity within a factor of 2 for 81% of the data obtained using copper-spiked European surface waters. The importance of pH and DOC (and therefore free copper activity) for the toxicity of copper to *P. subcapitata* was also highlighted by Heijerick *et al.* (2005) based on tests using laboratory and natural waters. More recently, De Schamphelaere and Janssen (2006) suggested using the relationship between free copper and pH to predict chronic copper toxicity to algae for regulatory purposes. They found that the effect of pH on copper toxicity to algal growth was statistically different for the algal species tested. Nevertheless, a single regression based on the combined data for all the algal species provided estimation of chronic toxicity within a factor of two for 94 - 100% of the cases. This pH-based linear model is proposed as an approximation since a mechanistically based BLM is not yet available for algae. To achieve this, De Schamphelaere *et al.* (2005) suggested using surface-bound or internalized copper as predictors of toxicity instead of free copper activity.

Other studies looked at extending the BLM approach to predict algal growth inhibition for additional metals such as thallium. Although in the early stage, a study by Hassler *et al.* (2007) showed that the BLM for *Chlorella sp.* could successfully predict the competitive interaction between thallium and potassium. Chronic toxicity testing found that due to the greater affinity of thallium for the biotic ligand in comparison to potassium, a 40-160 fold excess potassium is needed to suppress the toxic effect of thallium. The competitive effect of potassium on thallium uptake fluxes could be modeled using BLM-derived stability constants. In addition, it is evident that measuring ambient potassium concentrations is critical for establishing water quality guidelines for thallium.

3.4   Applicability of the FIAM/BLM approach to sediments

Recently, Di Toro *et al.* (2005) suggested extending the AVS-SEM technique to the prediction of toxicity using geochemical modelling and the BLM approach. As mentioned in the previous section, AVS-SEM alone has been used to predict the absence of toxicity. They suggested the use of WHAM to predict partitioning of metals to sediment particulate organic carbon, and then to use the acute BLM to predict toxicity of metals to benthic organisms. This work is one of the initial steps in developing a sediment BLM and thus, only partitioning to sediment particulate organic carbon is considered in addition to the AVS. Initial calculations showed that based on mass balance, detailed pore water chemistry can be ignored for the calculations, except for pH. The measured and predicted toxicity were consistent, which suggests that further development of the sediment BLM approach is warranted. It should be noted however that extrapolation of the BLM framework to benthic invertebrates is not straightforward. Borgmann *et al.* (2004) evaluated an extensive dataset on metal bioaccumulation by the benthic invertebrate *Hyalella azteca* and provided evidence that understanding the potential effects of major cations on uptake is required before the BLM approach can be applied to chronic toxicity.

Table 1.   Summary of some of the available BLM validation studies in acute and chronic toxicity prediction for various trace metals. Studies were selected that used an independent dataset to validate the developed models. This is only a subset of the available publications related to the BLM.

Metal	Toxicity/ endpoint	Organism(s)	Predictions	Reference
Cu(II)	Acute, LC_{50}	*Pimephales promelas* (fathead minnows), *Daphnia pulex*	Generally ≤ a factor of 2	Di Toro *et al.* 2001 and Santore *et al.* 2001
	Acute, EC_{50}	*Daphnia magna*	≤ a factor of 2	Janssen *et al.* 2003
	Acute, EC_{50}	*Daphnia magna*	≤ a factor of 1.3	De Schamphelaere *et al.* 2004
	Acute, EC_{50}	*Daphnia magna, Daphnia pulex* and *Daphnia obtusa*	Mostly ≤ a factor of 2	Villavicencio *et al.* 2005
	Chronic, $NOEC/EC_{10}$	*Daphnia magna*	≤ a factor of 2	Janssen *et al.* 2003
	Chronic, IC_{25}	*Ceriodaphnia dubia*	5 out of 6 natural waters. ≤ a factor of 2	Schwartz and Vigneault 2007
	Chronic, $EC_{10}/EC_{50}/NOEC$	*Pseudokirchneriella subcapitata*	Generally ≤ a factor of 2	De Schamphelaere *et al.* 2003
	Chronic, EC_{10}/EC_{50}	*Pseudokirchneriella subcapitata* *Chlorella vulgaris* *Chlamydomonas reinhardtii,*	Generally ≤ a factor of 2	De Schamphelaere and Janssen 2006
Ag(I)	Acute, LC_{50}	*Ceriodaphnia dubia*	For *C. dubia* 6 out of 9 within a factor of 2	Bielmyer *et al.* 2007
		Pimephales promelas (fathead minnows)	For *P. promelas* 5 out of 10 within a factor of 2	
	Acute, LC_{50}	Rainbow trout	Generally ≤ a factor of 2	McGeer *et al.* 2000
Zn(II)	Acute, EC_{50}	*Daphnia magna*	≤ a factor of 1.9	Heijerick *et al.* 2002
	Acute/chronic, $EC_{50}/NOEC$	*Daphnia magna*	≤ a factor of 2	De Schamphelaere *et al.* 2005
	Chronic, LC_{10}/LC_{50}	Rainbow trout	Generally ≤ a factor of 2	De Schamphelaere *et al.* 2005
	Chronic, LC_{10}/LC_{50}	Rainbow trout	≤ a factor of 2	De Schamphelaere and Janssen 2004
	Chronic, $EC_{50}/NOEC$	*Pseudokirchneriella subcapitata*	Generally ≤ a factor of 1.5	Heijerick *et al.* 2002
	Chronic, $EC_{50}/NOEC$	*Daphnia magna*	≤ a factor of 1.5	Heijerick *et al.* 2005
	Chronic, $NOEC/EC_{10}$	*Daphnia magna*	≤ a factor of 2	Janssen *et al.* 2003
Ni(II)	Acute, EC_{50}	*Daphnia magna* *Ceriodaphnia dubia*	≤ a factor of 2	Keithly *et al.* 2004
		Daphnia magna *Ceriodaphnia dubia*	≤ a factor of 2	Deleebeeck *et al.* 2007a
	Acute/chronic, LC_{50}	Rainbow trout, fathead minnow	≤ a factor of 2	Deleebeeck *et al.* 2007b
Tl(I)	Chronic, EC_{50}	*Chlorella* sp.	Successfully predicted competitive interaction between Tl(I) and K	Hassler *et al.* 2007

4   INTERACTIONS BETWEEN PARTICULATE TRACE METALS AND AQUATIC ORGANISMS

4.1   Background information

In general, and particularly for short-term acute exposure, metal toxicity is related to waterborne exposure only. Accordingly, current water quality guidelines are generally based on "dissolved" metals. The BLM approach discussed earlier considers only waterborne exposure. However, to predict chronic metal toxicity, the importance of

> **Data from laboratory feeding experiments are currently integrated in a modelling approach that considers metal uptake from the water and from the diet. In general, the approach can not predict metal toxicity, as it is not directly related to total body metal accumulation.**

metal exposure through the particulate phase is recognized, and is gaining attention (Meyer *et al.* 2005). In particular, ecological risk assessment requires information about long-term effects of metal exposure. During chronic exposures, metal uptake by the organisms can occur not only through water but also through food, which could contribute significantly to observed metal uptake (e.g. Goulet *et al.* 2007). As mentioned previously, the development of the BLM approach has been possible through studies of the mechanisms of metal toxicity in fish, e.g., impairment of the gill respiratory system by certain waterborne metals. However, the gill is the main route of entry for waterborne metals, while uptake and accumulation of dietary metal have been shown to occur largely in the internal tissues of the fish such as the gut and the hepatopancreas (Handy *et al.* 2005). Furthermore, the relative importance of food versus water for metal uptake tends to be metal specific and depends on several biological factors such as animal behaviour, e.g., benthic versus pelagic. Consequently, a review of sources of metal to freshwater invertebrates by Hare *et al.* (2003) concluded that for some species dietary uptake cannot be ignored.

4.2   Dietary metals

There is conflicting evidence in the literature on the relevance of dietary exposure to predict metal toxicity. The mechanisms of diet-borne metal toxicity are complex and have led to varying results from different research groups. A study by Hook and Fisher (2001) showed that reproduction of cladoceran crustaceans are inhibited by chronic exposure to Ag in algal food sources. Similarly, a study by De Schamphelaere *et al.* (2004) found that reproduction of *Daphnia magna* was reduced by 40% after chronic dietary Zn exposure. In addition, a recent study found that dietary Cd and Cu significantly affected the survival, reproduction and feeding rate of *Ceriodaphnia dubia* (Sofyan *et al.* 2006). Finally, reproduction was inhibited in marine copepods fed with diatoms contaminated with Ag, Zn, Cu and Ni (Bielmyer *et al.* 2006). In contrast, Canli (2005) found that dietary Zn had a stimulating effect on *D. magna* reproduction and also enhanced its tolerance to Zn. Furthermore, De Schamphelaere *et al.* (2004) found a stimulating effect on *D. magna* growth and reproduction at the highest Cu dietary exposure level that was tested, 1400 µg Cu·g dry wt^{-1}. At an exposure level of 3000 µg Cu·g dry wt^{-1}, however,

they found significant reductions in growth and reproduction (De Schamphelaere *et al.* 2007b). Of interest, Rickwood and Dubé (2006) found toxicity to fathead minnow larvae when exposed to mine effluent (e.g., reduction in hatching success and increases in deformities) was reduced when exposure included the effluent and contaminated food. Thus, it is evident that the relative importance of water versus diet exposure will vary depending on the metals and organisms considered. Furthermore, it will likely depend on other factors such as the actual level of metals in exposure media.

4.3   Dynamic multipathway bioaccumalation model (DYMBAM)

The best approach to predict the relative importance of waterborne versus dietary exposure for the uptake of metals is the recently developed model "dynamic multipathway bioaccumulation model" (DYMBAM). An earlier version of this kinetic model was applied by Reinfelder *et al.* (1998) to determine trace metal biomagnification. The results of the study suggested that small differences in assimilation efficiency or the elimination rate constant may determine whether a trace metal will be biomagnified with trophic levels. DYMBAM can account for metal accumulation by a broad range of aquatic organisms that are chronically exposed to metals. The model is mechanistically based, but also considers empirical knowledge of geochemical influences, biological differences, and the chemical differences between metals.

DYMBAM has been shown to reliably predict metal accumulation for both laboratory (Yu and Wang 2002b and 2002a; Guan and Wang 2004b and 2004a) and field samples (Luoma and Rainbow 2005). A field study compared bioaccumulation data from 15 literature sources (7 metals, 14 species of aquatic organisms, and metal concentrations varying up to 7 orders of magnitude) to the predictions by DYMBAM. In general, the model estimates agreed within two-fold of the experimental data (Luoma and Rainbow 2005). The reason for DYMBAM's success is its incorporation of influx and efflux rates of diet-borne metals from the biota, in addition to the dilution of internal metal concentrations as a result of organism growth. In fact, several studies have shown its ability to relate toxicity to metal exposure (Simpson 2005; Simpson and King 2005; King *et al.* 2005). DYMBAM has also been utilized for marine sediment samples to determine Cd uptake and accumulation in the Pacific blue mussel (*Mytilus trossulus*) (Widmeyer and Bendell-Young 2007). The study by Goulet *et al.* (2007) examined accumulation of Cd in *D. magna* when exposed via dietary and waterborne sources, and the predictive capability of DYMBAM in relating Cd bioaccumulation to chronic toxicity in the organism. The DYMBAM predictions were within a factor of 3 of the measured Cd in *D. magna* and demonstrated that at low Cd concentrations in the water, diet was the main route of exposure while the inverse was true at elevated aqueous Cd concentrations. However, in that study, no relationship between diet-borne exposure and chronic toxicity to the invertebrate was observed.

DYMBAM remains a promising tool for ecological risk assessment of metals in the aquatic environment. A study by Simpson (2005) on copper effect concentrations for benthic organisms in sediments using DYMBAM concluded that a single value for sediment quality guideline (SQG) may not be sufficient. It was proposed that a better approach would be to have a guideline range that can be applied to different types of sediments. The DYMBAM approach is also useful

to predict metal bioaccumulation in natural waters, and the availability of reliable prediction models is increasing. In fact, DYMBAM has been recommended as an alternative to bioaccumulation/bioconcentration factors under the US EPA framework for the metals risk assessment (Fairbrother *et al.* 2007). However, one should note that metal bioaccumulation is not necessarily directly related to metal toxicity, partly due to an organism's ability to regulate and detoxify metals, especially essential trace metals. At present, the DYMBAM approach does not account for metal detoxification within the organism, which can lead to difficulties in predicting toxicity (Rainbow 2002).

In the context of the mine effluent discharge, the DYMBAM approach could be used to predict metal accumulation in aquatic species and assess the ecological risk for organisms feeding on these species. Water quality parameters needed would be the same as for the BLM approach. However, the DYMBAM requires uptake and depuration constants that are specific to each aquatic species and therefore the approach would be applicable to species that have been studied in laboratory.

5 ACKNOWLEDGEMENTS

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