INCORPORATING METAL SPECIATION AND BIOAVAILABILITY INTO WATER QUALITY GUIDELINES FOR PROTECTING AQUATIC ECOSYSTEMS

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ABSTRACT

Guidelines for metals in aquatic ecosystems are typically based on total concentrations. However, it is well established that metal bioavailability (ie. the ability of a metal to bind or traverse the cell membrane) is critically dependent on the physicochemical form, or speciation, of a metal. As such, guidelines based on total concentrations may be overprotective, since only a fraction of the total concentration may be bioavailable. Following a risk-based approach, a decision tree was developed to account for metal speciation and bioavailability in aquatic ecosystems. The decision tree includes three increasingly complex levels of analysis: (i) total acid-soluble (unfiltered) metal concentration; (ii) dissolved (filtered) acid-soluble metal concentration, and (iii) the concentration of specific metal species (eg. geochemical modelling) or groups of metal species (eg. chemical measurement) and/or biological testing. The complexity of analysis for a water sample depends on guideline conformity at each level of the decision tree. As part of this approach, algorithms for hardness-dependency were derived for Cd, Cr(III), Cu, Pb, Ni and Zn in freshwaters ($\leq 2.5\%$ salinity), because of its established effect on the bioavailability of these metals. The measurement of metal speciation and bioavailability permits guidelines for protecting aquatic ecosystems to be potentially relaxed on a site-specific basis. This signifies a move to develop chemical indicators further so that they better reflect biological effects.

Key words: metal speciation, water quality guidelines, aquatic ecosystems, hardness, geochemical modelling.

INTRODUCTION

Guidelines for metals in aquatic ecosystems are typically based on total (ie. dissolved and particulate) concentrations (Gardiner and Zabel 1989; CCREM 1991; US EPA 1995a, b; Roux et al. 1996). However, it is well established that metal bioavailability (ie. the ability of a metal to bind or traverse the cell membrane) is critically dependent on the physicochemical form, or speciation, of a metal. Although the relationship between metal speciation and bioavailability is complex, metals present as the free metal ion, or as weak complexes that are able to dissociate at a cell or gill membrane, are generally considered more bioavailable than metals in strong complexes or adsorbed to colloidal and/or particulate matter (Hamelink et al. 1994). Guidelines based on total metal concentrations will generally be overprotective, since only a fraction of the total metal concentration in natural waters will be generally bioavailable, especially in samples containing appreciable concentrations of suspended particulate matter and/or dissolved organic matter (DOM).

From a regulatory standpoint, it makes sense to test for guideline conformity, in the first instance, on the basis of total metal concentration, because it is both simpler and less expensive to undertake, with less opportunity for sample contamination. However, guideline values are usually derived from laboratory toxicity tests with sensitive organisms exposed to metals present as simple inorganic complexes, ie. metals that occur in a potentially bioavailable form (Warne 1998). Where guideline values are exceeded on the basis of total metal concentration, a hierarchy of measurements of increasing complexity may be prescribed, which

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provide increasingly refined methods to identify specific metal species, or groups of metal species, that exert toxic effects. As is the practice in the USA, the onus is placed on the 'polluter' to demonstrate guideline conformity at the necessary level of complexity, viz. concentration of total, dissolved or bioavailable metal. A decision tree for determining guideline values, which accounts for metal speciation and bioavailability, is shown in Figure 1.

The general aim of this study was to incorporate metal speciation into the Australian water quality guidelines to better indicate the biological effects of metals in aquatic ecosystems on a site-specific basis (ie. a potential relaxation of national guideline values).

INCORPORATION OF METAL SPECIATION INTO THE GUIDELINES (i) Total metal analysis

The first step in using the decision tree approach is to measure the total metal concentration of a water sample. The measurement of total metal concentration, however, is not without controversy. The total metal concentration can be operationally separated into a particulate (> 0.45 μ m) and dissolved (< 0.45 μ m) phase by filtration through a membrane filter (eg. 0.45 μ m cellulose acetate membrane). The dissolved phase will invariably include colloidal material (ie. 0.01 - 0.45 µm) (Horowitz et al. 1996). From an analytical standpoint, the recovery of metals from the particulate phase will depend upon the ability of the dissolution technique to dissolve all of the metal. It has been reasonably argued (Batley 1989; Martin et al. 1991) that metals not solubilised by acid potentially have a low bioavailability. Thus, although methods have been described which measure total metal concentration, the standard methods adopted by agencies, such as the United States Environmental Protection Agency (Martin et al. 1986, 1991), have been for acid-soluble metals. These methods initially require sample preservation by acidification to pH < 2, and then use different levels of acid treatment to measure either total recoverable metals or acid-soluble metals. Total recoverable metals are typically determined after digestion of the sample in a mixture of 0.15 M HNO_{3} and 0.10 M HCl, while acid-soluble metals are those released by acidification of the sample to pH < 2 at room temperature. The requirement for the stringency of the former treatment and the potential for sample contamination have been challenged (Martin et al. 1986). Recent results for acidsoluble metals have been found to be almost identical to those for total recoverable metals (Martin et al. 1991).



Figure 1. Risk-based decision tree, which incorporates metal speciation and bioavailability, to modify measured total metal concentrations. DTA = Direct Toxicity Assessment

(ii) Factors influencing metal speciation and bioavailability

Metal speciation and bioavailability in fresh surface waters (defined here as having a salinity $\leq 2.5\%$) may be strongly influenced by a variety of physicochemical parameters, including hardness (ie. primarily Ca and/ or Mg concentration), alkalinity, pH, natural DOM and redox potential (Stumm and Morgan 1996). The latter two parameters, in addition to salinity, are also relevant to estuarine and marine systems. A summary of the major physicochemical parameters controlling the speciation of a variety of environmentally-relevant metals is given in Table 1. Australasian Journal of Ecotoxicology

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Metal ^a	Major controls on metal speciation affecting guideline values
Aluminium	pH ^b , inorganic complexation, organic complexation ^c
Arsenic	Valency state [As(III), As(V)]
Cadmium	pH ^b , inorganic complexation, organic complexation ^c
Chromium	Valency state [Cr(III), Cr(VI)]
Copper	pH ^b , inorganic complexation, organic complexation
Lead	pH ^b , inorganic complexation, organic complexation ^c
Mercury	CH ₃ Hg content, inorganic complexation, organic complexation
Nickel	pH ^b , inorganic complexation
Selenium	Valency state [Se(IV), Se(VI)]
Uranium	pH ^b , inorganic complexation, organic complexation
Vanadium	Valency state [V(IV), V(V)], inorganic complexation
Zinc	pH ^b , inorganic complexation, organic complexation

Table 1. Major factors controlling metal speciation.

^a The term metal also includes the metalloids, As and Se.

^b Important in freshwaters only.

^c Requires experimental confirmation.

Hardness

The bioavailability of Cd, Cr(III), Cu, Ni, Pb and Zn in freshwater typically decreases with increasing hardness (CCREM 1991; US EPA 1995a, 1995b), with Ca and/or Mg competing with metals for surface binding sites on cell membranes (Markich and Jeffree 1994). As such, hardness-dependent algorithms have been used to determine guideline values for these metals overseas, including Canada (CCREM 1991; Porter *et al.* 1995), South Africa (Roux *et al.* 1996), the UK (Gardiner and Zabel 1989) and the USA (US EPA 1995a, 1995b). For example, the current Canadian water quality guideline for Cd (Porter *et al.* 1995) is hardness-dependent; guideline Cd concentrations are 0.01, 0.03, 0.05, 0.06 μ g L⁻¹ at a hardness of 30, 90, 150 and 210 mg L⁻¹ as CaCO₃, respectively.

Although the previous Australian water quality guidelines (ANZECC 1992) for protecting freshwater ecosystems from Cd, Cu, Pb, Ni or Zn, acknowledged the importance of water hardness, they provided no quantitative method for calculating metal guideline values, given a particular water hardness. For example, the 1992 guideline for Ni ranged from 15-150 μ g L¹ 'depending on hardness'. Such a guideline was of limited value to water resource managers for determining the concentration of Ni that may have been safely released into a particular receiving water. This issue has been addressed in the current guidelines (ANZECC & ARMCANZ 2000) by defining specific algorithms for Cd, Cr(III), Cu, Ni, Pb and Zn (Table 2), to permit calculation of a hardness-modified guideline value.

Table 2. General form of the hardness-dependent algorithms describing guideline values for selected metals in freshwaters.

Metal	Hardness-dependent algorithm ^a		
Cadmium	$HMGV = GV(H/30)^{0.89}$		
Chromium(III)	$HMGV = GV(H/30)^{0.82}$		
Copper	$HMGV = GV(H/30)^{0.85}$		
Lead	$HMGV = GV(H/30)^{1.27}$		
Nickel	$HMGV = GV(H/30)^{0.85}$		
Zinc	$HMGV = GV(H/30)^{0.85}$		

^a HMGV, hardness-modified guideline value (μ g L⁻¹); GV, guideline value (μ g L⁻¹) at a hardness of 30 mg L⁻¹ as CaCO₃; H, measured hardness (mg L⁻¹ as CaCO₃) of a fresh surface water ($\leq 2.5\%$). Slope values range from 0.82 to 1.27 (see text).

Insufficient data are available to derive hardnessdependent algorithms specifically for Australian and New Zealand freshwater organisms (Markich *et al.* 2002). Consequently, algorithms developed as part of the North American guidelines (Porter *et al.* 1995; US EPA 1995a, 1995b) for the protection of freshwater organisms were adopted. Moreover, these hardnessdependent algorithms have been expressed in a mathematically correct form for the revised Australian guidelines (equation 1).

HMGV =
$$GV\left(\frac{H}{30}\right)^a$$
 [Eq. 1]

where HMGV is the hardness modified guideline value (μ g L⁻¹), GV is the guideline value (μ g L⁻¹) at a hardness of 30 mg L⁻¹ as CaCO₃, H is the measured hardness (mg L⁻¹ as CaCO₃) of a fresh surface water and *a* is a constant (ie. slope factor).

The slope factors, *a*, of the hardness-dependent algorithms for Cd, Cr(III), Cu, Ni, Pb and Zn, which range from 0.82 to 1.27 (Table 2), were taken directly from

North American data (US EPA 1995a, 1995b). They were derived empirically by pooling values of the slopes of fitted regressions for toxicity (log, LC50) versus hardness (\log_{a} mg L⁻¹ as CaCO_a) for individual test organisms, particularly species of fish and crustaceans. The slope factors have been justified within a mechanistic framework (Meyer 1999), based on the concept of competitive binding of cations to fish gills (Playle 1998). Meyer (1999) derived a general equation relating toxicity (LC50) with hardness, alkalinity and pH. It was rationalised that the regression slopes of log, LC50 versus log, hardness for metals such as Cd, Cu, Ni, Pb and Zn should range from 0.8 to 1.3, for hardness and alkalinity values between *ca*. 20 and *ca*. 400 mg L⁻¹ as CaCO₂. For extremely low values (*ca.* 1 mg L¹ as CaCO₂) of hardness and alkalinity the slope is ca. 0, whilst for extremely high (ca. 1000 mg L^{1} as CaCO₂) values of both variables the slope is *ca*. 2.

The hardness-dependent algorithms should be used in freshwaters (or estuarine waters) with a salinity $\leq 2.5\%$. The majority of data used to develop the hardness-dependent algorithms occurred within the hardness range 25 - 400 mg L⁻¹ as CaCO₃, and therefore, the algorithms are most accurate in this range (Porter *et al.* 1995; US EPA 1995a, 1995b; Meyer 1999). Most surface freshwaters in Australia and New Zealand have a hardness between 25 and 400 mg L⁻¹ as CaCO₃. Estuarine waters with a salinity of 2.5‰ typically have a water hardness of about 450 mg L⁻¹ as CaCO₃.

Nevertheless, for surface freshwaters with a hardness $< 25 \text{ mg } \text{L}^1 \text{ or } > 400 \text{ mg } \text{L}^1 \text{ as } \text{CaCO}_3$, guideline values should be calculated using the measured ambient hardness of the surface water. Limiting the use of algorithms to hardness values $> 25 \text{ mg } \text{L}^1$ as CaCO₃ could potentially result in underprotective guideline values in extremely soft waters (eg. 3 - 4 mg L^1 as CaCO₃ in Magela Creek, Northern Territory, Australia). Similarly, limiting the use of algorithms to hardness values $< 400 \text{ mg} \text{L}^{-1}$ as CaCO₃ could potentially result in overprotective guideline values in extremely and bardness values in extremely hard waters (eg. 540 mg L^{-1} as CaCO₃ in Lake Edward, South Australia). Common sense should be exercised when extrapolating outside the usual range of hardness.

An increase in water hardness is typically associated with an increase in alkalinity (as Ca and/or Mg carbonate). Consequently, the hardness-dependent algorithms developed for each metal may also incorporate the effects of alkalinity, depending on the pH of the waters. For acidic waters (pH < 6), hardness and alkalinity are typically uncoupled, whereas in neutral and alkaline waters (pH 6 - 9) both variables may be closely coupled. For some metals (eg. Cu), alkalinity has been shown to have a greater influence on reducing toxicity than hardness, due to the formation of non-toxic metal-carbonate (eg. CuCO₃) complexes (Hunt 1987; Markich and Jeffree 1994).

pН

Apart from water hardness, pH is an important parameter that may influence metal bioavailability in freshwaters. A decrease in the pH may increase the free metal ion concentration, resulting in metal desorption from colloidal and particulate matter, and dissociate some inorganic and organic metal complexes. For example, a decrease in pH from 6.0 to 5.0 increased the bioavailability of U to a freshwater bivalve by an order of magnitude, due to an increase in the calculated concentrations of UO₂²⁺ and UO₂OH⁺ (Markich et al. 2000). In contrast, a decrease in pH from 7.5 to 6.0 reduced the bioavailability of Cd to three freshwater organisms (water flea, amphipod and fish) by two- to nine-fold (Schubauer-Berigan et al. 1993). Free Cd (Cd²⁺) was calculated to be the dominant (ca. 90%) species in the test waters over the entire pH range studied. It is widely considered that an increase in proton (H⁺) concentration competitively inhibits the binding of the free metal ion (eg. Cd²⁺) at the cell membrane surface (Campbell and Stokes 1985), and hence, reduces metal (Cd) bioavailability.

Dissolved organic matter

Natural DOM (primarily as humic and fulvic acids) is an important parameter that may reduce metal bioavailability in surface waters (Daly et al. 1990; Erickson et al. 1996; Playle 1998), by binding metals in metal-organic complexes. For example, Daly et al. (1990) showed the that toxicity (96 h LC50) of Cu to the Australian freshwater shrimp, Paratya australiensis, decreased by a factor of about two as the concentration of natural DOM increased two-fold. Copper typically forms strong complexes with natural DOM (Moore and Ramamoorthy 1984). This is due to the borderline, hard/ soft acid nature of the Cu2+ ion, which makes it a versatile electron acceptor, able to form strong bonds with ligands such as fulvic or humic acids, containing O, N and S functional groups. As a consequence, Cu-DOM complexes form a major proportion of total Cu in many surface waters. For example, Breault et al. (1996) concluded that Cu-DOM complexes ranged from 84 to 99% of total Cu (13 μ g L⁻¹) in seven freshwater streams of varying water chemistry.

In contrast to Cu, Cd forms relatively weak complexes with natural DOM (Moore and Ramamoorthy 1984). Sufficiently high concentrations of natural DOM are needed to form appreciable proportions of Cd-DOM Metal speciation and water quality guidelines

complexes. For example, Stackhouse and Benson (1988) found no significant (P > 0.05) differences in the toxicity of Cd to the water flea, *Daphnia magna*, at natural DOM (as humic acid) concentrations ranging from 0 to 5.0 mg L⁻¹. However, the authors found that Cd toxicity was substantially reduced (by 75%) at 50 mg L⁻¹.

Synthetic organic ligands such as oxines or xanthates, present in some industrial and/or agricultural effluents, may complex metals to form lipid-soluble metal complexes. These complexes can diffuse directly through the hydrophobic cell membrane, transporting both the ligand and metal into the cell, where both may exert separate toxicity (Ahsanullah and Florence 1984; Stauber and Florence 1987). However, the concentrations of these lipid-soluble metal complexes usually represent less than 1% of the total metal concentration in natural waters, and typically are of minor environmental significance.

Redox potential

A change in redox potential (eg. from oxic to reducing conditions) can lead to changes in the valency state of several elements, including As, Cr, Fe, Mn and Se, and hence in their bioavailability. This may also involve precipitation and/or dissolution reactions of metal sulfides or hydroxide species. Florence and Stauber (1991) reported that the toxicity (72 h EC50) of the Australian diatom, *Nitzschia closterium*, to As(III) (7 μ g L¹) was > 250 times higher than As(V) (> 2000 μ g L¹).

Salinity

Salinity has been reported to be an important parameter affecting metal speciation and bioavailability in estuarine or marine waters. It has been generally established that metal bioavailability decreases with increasing salinity [see review by Hall and Anderson (1995)]. For example, Bryant et al. (1985) found that the toxicity of Ni to the amphipod, Corophium volutator, declined six-fold (ie. from an LC50 of 5.6 to 34 mg L^{-1}) with an increase in salinity from 5 to 35‰. Such effects have been attributed to a decline in the free metal ion concentration with the concomitant formation of metal-chloride complexes, which are considered to have a relatively low bioavailability. Other potential mechanisms for the effects of salinity include osmotic stress, changes in activity coefficients and alteration of the sorption characteristics of the cell membrane (eg.gill) surface.

SUMMARY

Conflicting results have been reported on the effect of pH, natural DOM and salinity on metal bioavailability for a given metal, precluding the development of empirical algorithms like those given for water hardness. For example, Cu bioavailability has been found both to increase (eg. Erickson *et al.* 1996) and decrease (eg. Horne and Dunson 1995) with decreasing pH over the range 3.0 to 7.0. Similarly, Cd bioavailability has been reported to both increase (Laegreid *et al.* 1983) and decrease (Stackhouse and Benson 1988) due to the formation of Cd-DOM complexes.

(iii) Dissolved metal analysis

The next qualification of the total (acid-soluble) metal guideline trigger value is to measure the dissolved metal concentration (Figure 1). If the total (acid-soluble) metal concentration trigger value is exceeded, it is recommended that the original, unacidified, water sample be filtered, in the first instance, through a 0.45 µm (or similar) membrane filter and then acidified (pH < 2) at room temperature, prior to being re-analysed [ie. dissolved (acid soluble) metal]. Filtration through a 0.45 µm membrane is a traditional, yet practical, choice, but will invariably include colloidal matter, which is capable of sorbing metals. Although ultrafiltration, using a 0.015 µm membrane filter, provides a better separation of the dissolved and particulate metal fraction than the 0.45 µm membrane, it has practical problems (Batley 1989). Some of the problems associated with using filtration to define dissolved trace metal concentrations in natural water samples are discussed by Horowitz et al. (1996).

Filtration is particularly relevant for freshwater samples with high levels of suspended solids, where a high proportion of a metal may be bound to particulate matter (eg. Cu, Hg, Pb or U). In many estuarine or marine waters, the contribution of suspended particulate matter to the total metal concentration is usually insignificant (Batley 1989). However, for some metals such as Al, Fe or Mn, the particulate concentration may substantially exceed the dissolved concentration. The decision tree approach (Figure 1) assumes that metals bound to suspended particulate matter have a negligible or low bioavailability. While this assumption is generally true for some organisms (eg. several species of unicellular algae or bacteria), other organisms (eg. selected species of molluscs or crustaceans) may derive metals from both the dissolved and particulate phase (Reinfelder et al. 1998). Therefore, the bioavailability of suspended, particulatebound metals may need to be considered.

(iv) Analysis of specific metal species and biological testing

If guideline values are exceeded on the basis of dissolved metal concentrations, the risk to aquatic organisms may still be minimal, if metal speciation is considered. Metal speciation may be evaluated using direct chemical measurement, geochemical modelling and/or biological testing (Figure 1). Ultimately, biological measurement will provide absolute confirmation of chemical measurements and/or predictions.

Chemical measurement

The many approaches to the direct measurement of metal speciation have been well-reviewed (Batley 1989; Tessier and Turner 1995; Buffle and Horvai 2000). Chemical measurement techniques, such as anodic stripping voltammetry, ion selective electrodes or ligand competition methods, have been used successfully to detect either labile (ie. inorganic and weakly-bound organic) metal species or free metal ions. Given the importance of organic complexation, the measurement of the complexation capacity of water for a particular metal is particularly important. Only when the complexation capacity is exceeded, will labile and potentially bioavailable metal species be present at biologically significant concentrations. Complexation capacity can be determined by titrating a water sample with ionic metal (eg. Cu²⁺) and measuring the increase in uncomplexed metal, either chemically (eg. Cu²⁺ selective electrode) or biologically (eg. using unicellular algae or bacteria).

It is clearly advantageous if the results of simple chemical measurements can be used to predict metal bioavailability, or failing this, if more complex chemical measurements can be used as a surrogate for biological testing. Copper speciation has been extensively studied in natural waters. Measurements of labile Cu may be highly related to bioavailable Cu (eg. from tests with unicellular algae or bacteria) in both marine and freshwaters (pH 5-9), except where natural DOM levels are high (Stauber et al. 1996). Because Cu forms strong complexes with natural DOM, complexation capacity titrations yield reliable measures of labile metal and of the potential of the DOM to bind added Cu. An example outlining the use of the decision tree for interpreting the concentration of Cu in a freshwater sample, is given in Box 1.

Chemical measurement techniques that might be used to determine the speciation of environmentally-relevant metals are summarised in Table 3. Provided these techniques do not underestimate the bioavailable metal concentration, their use can be justified in providing something better than simply a dissolved metal analysis. A number of studies have successfully related measured groups of metal species (eg. labile Zn) to bioavailable metal concentrations in natural waters, using a range of aquatic organisms and metals (Table 3).

A problem confronting speciation studies is the lack of analytical methods that can determine the concentrations of specific inorganic species, such as the free metal ion. Ion-selective electrodes determine free metal ion concentrations, but are generally insensitive, and prone to interferences, in most natural waters (Batley 1989). Their application has largely been limited to studies of Cu speciation (Xue and Sunda 1997). Electrochemical procedures, such as anodic and cathodic stripping voltammetry, have been used to elucidate the extent of complexation by DOM for a range of metals (Batley 1989; Tessier and Turner 1995). Size fractionation techniques, such as dialysis, have also been used (Apte et al. 1989; Buffle et al. 1992), although these methods, like electrochemical procedures, give an operationally-defined group of metal species which is often difficult to relate to actual metal species.

For covalently-bonded molecules (organometallic species), such as methylmercury and organoarsenic compounds, chromatographic separation (GC or HPLC) followed by element specific detection using atomic absorption, emission or fluorescence spectrometry is the favoured approach (Marshall and Momplasir 1995).

The determination of oxidation states is important for elements such as As, Se or Cr. This may be achieved by selective extraction (Blaylock and James 1993), coprecipitation (Cranston and Murray 1978), electrochemical determination (Florence 1986) or volatilisation following species-selective derivatisation (Marshall and Momplasir 1995).

Sample storage is a critical issue confronting metal analysis. Metal species may not actually be in thermodynamic equilibrium at the time of collection, and biological processes may alter metal speciation during storage. Refrigeration and storage of the water sample in the dark is considered a minimum requirement (Batley 1989). An alternate approach is the fixing of certain metal species by immobilisation onto selective adsorbents in the field (Boussemart and van den Berg 1994). This is probably the best approach for unstable metal species, although sample contamination must be strictly controlled.

Geochemical speciation modelling

Geochemical speciation modelling has evolved considerably over the last 25 years. A number of geochemical speciation models are currently available,

Box 1. *Example of using the decision tree for Cu speciation in ambient waters*

A sample of unfiltered water collected from a freshwater stream (pH 7.2, dissolved oxygen 7.9 mg L⁻¹, conductivity 186 μ S cm⁻¹, turbidity 5.1 NTU, dissolved organic carbon 4.5 mg L⁻¹) was acidified (pH < 2) at room temperature and analysed for total Cu by inductively coupled plasma mass spectrometry (ICPMS). Water hardness was also measured (ie. 80 mg L⁻¹ as CaCO₃), since the bioavailability of Cu is known to be influenced by water hardness in freshwaters. This value was used to calculate a hardness-modified guideline value for total Cu (Table 2) in the stream of 3.2 μ g L⁻¹. The measured total (acid-soluble) Cu concentration of 14.5 μ g L⁻¹ exceeded the hardness-modified guideline value.

Using the decision tree, the next step is to filter a sub-sample of the original stream water (unacidified) using a 0.45 μ m or similar membrane filter, followed by acidification (pH <2) of the sample at room temperature. The dissolved (acid-soluble) Cu concentration was found to be 6.4 μ g L¹, a value which still exceeded the hardness-modified Cu guideline (ie. 3.2 μ g L¹). As a further option, another sub-sample of the original stream water (unacidified) was filtered through a 0.015 μ m membrane filter and analysed for Cu. The measured value of 5.0 μ g L¹ still exceeded the hardness-modified Cu guideline value.

Following the decision tree approach, metal speciation was considered. Chemical measurements and geochemical speciation modelling were used to determine the speciation of Cu in the unacidified, filtered (0.015 μ m) water sample. For the latter technique, a detailed measure of the major and minor cations and anions is required, including the dissolved organic carbon concentration. Such data are used as input into a speciation model, such as PHREEQE96, which has a carefully reviewed equilibrium constant database. Anodic stripping voltammetry (ASV) measurements of the water sample revealed a labile Cu concentration (of inorganic metal species and weakly bound organic complexes) of 2.0 μ g L⁻¹. This concentration is below the hardness-modified Cu guideline value (ie. 3.2 μ g L⁻¹). It is generally believed that the labile metal fraction is a good indicator of metal bioavailability. Complementary modelling calculations of dissolved inorganic Cu concentration (1.8 μ g L⁻¹) were consistent with the ASV results. These estimates of Cu bioavailability could be confirmed using a toxicity test with a sensitive organism, such as a unicellular green alga.

In summary, although the total (acid-soluble) Cu concentration of the water sample initially exceeded the hardness-modified Cu guideline value, a very small proportion (ca. 14%) was considered to be bioavailable. Thus, a total (acid-soluble) Cu concentration of 14.5 μ g L¹ is considered low risk to organisms inhabiting the freshwater stream used in this example.

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Metal	Technique ^a	Species measured	Related to metal bioavailability ^b	
			Tested	Organism
Aluminium	CSV, cation exchange resin, ligand competition, spectrophotometry or solvent extraction AAS	Reactive Al, inorganic, non-complexed monomeric	Yes	Algae, fish
Arsenic	HPLC- or GC-Hydride-AAS (AFS), ICPMS	As(III), As(V), organoarsenic acids	Yes	Algae, bacteria
Cadmium	ASV, ligand competition	Labile Cd or Cd ²⁺	Yes	Algae, annelids, crustaceans, fish, molluscs
Chromium	IC, resin separations, spectrophotometry	Cr(III), Cr(VI)	Yes	Algae, bacteria
Copper	ASV, CSV, ISE, ligand competition	Labile Cu or Cu ²⁺	Yes	Algae, amphibians, bacteria, crustaceans, fish, molluscs
Lead	ASV, CSV, ligand competition	Labile Pb or Pb ²⁺	Yes	Algae
Mercury	AFS, ASV	Hg(II), methyl Hg	Yes	Algae
Nickel	CSV, ligand competition	Labile Ni	Yes	Algae
Selenium	AFS, IC	Se(IV), Se(VI), organoSe	Yes	Algae, bacteria, crustaceans, fish, insects
Uranium	CSV, TRLFS, UV-Vis	$U(VI), UO_2^{2+}, UO_2OH^+$	Yes	Molluscs
Vanadium	Capillary electrophoresis, IC	V(IV), V(V)	No	
Zinc	ASV, CSV, ligand competition	Labile Zn	Yes	Algae, crustaceans, fish, insects

Table 3. Summary of metal speciation techniques in natural waters.

^a CSV, cathodic stripping voltammetry, AAS, atomic absorption spectrometry, HPLC, high performance liquid chromatography, GC, gas chromatography, AFS, atomic fluorescence spectrometry, ICPMS, inductively coupled plasma mass spectrometry, ASV, anodic stripping voltammetry, ISE, ion selective electrode, IC, ion chromatography, TRLFS, time-resolved laser-induced fluorescence spectroscopy, UV-Vis, ultraviolet-visible spectrophotometry.

^b Apte and Batley (1995) and Markich (1998).

including PHREEQE (Parkhurst *et al.* 1980), MINTEQ (Allison *et al.* 1991), HARPHRQ (Brown *et al.* 1991) and MINEQL (Schecher and McAvoy 1994). These are used to ascertain the speciation of a particular element(s) in water under specified conditions (eg. measured ion concentrations, pH, pE and temperature). Speciation may involve one or more of the gaseous, aqueous or solid (colloidal and/or particulate) phases.

Most geochemical speciation models employ the concept of chemical equilibrium. This concept assumes that the system being modelled is in thermodynamic equilibrium, and calculates speciation using either a Gibbs free-energy minimisation technique or an equilibrium constant method (Markich and Brown 1999). Given a similar thermodynamic database, these models should produce similar speciation results. However, several of the most commonly-used geochemical speciation models, including MINTEQ, have some equilibrium constants that are inappropriate, or in error (Serkiz *et al.* 1996). The output from geochemical speciation models is only as reliable as the input data. Given the potential difficulties, speciation modelling should only be undertaken by those expert in this area, otherwise the speciation results cannot be used to justify why a guideline value may be exceeded for a particular metal.

Geochemical speciation models should be validated for their ability to predict metal bioavailability in natural waters. Models such as MINTEQ come close to handling adsorption, although they consider only single solid phases and not the heterogeneous surfaces of natural systems. In the case of organic complexation, it is necessary to assume binding constants for natural DOM which, depending on the water, may vary naturally by several orders of magnitude. The use of geochemical speciation models as an unambiguous predictive tool is currently limited.

Biological testing

While chemical measurement techniques and geochemical speciation modelling may detect and predict the forms of metals in aquatic systems, respectively, they do not directly demonstrate adverse biological effects. Biological testing is one experimental approach that directly measures the bioavailable fraction of a metal. Toxicity, for example, is simply a generic measure of a particular biological response or endpoint. A toxicity test may measure lethal (eg. death) or sub-lethal (eg. growth inhibition, reproduction or enzyme activity) responses of organisms. Acute toxicity tests, based on invertebrate and fish mortality, have been traditionally used. However, over the past decade there has been a major research thrust towards the development of chronic toxicity tests (Adams 1995). Chronic tests determine the response of an organism over a number of generations, or a significant portion of the organism's life span. Such tests may be of longterm duration (weeks or months), or short-term in the case of single-celled algae that divide daily. Toxicity testing is discussed in more detail by Warne (1998).

In acute studies, toxicity invariably results from metal bioaccumulation. However, the relationship between chronic toxicity and metal bioaccumulation is still unclear, mainly because mechanisms of chronic toxicity remain poorly understood (McDonald and Wood 1993). In chronic metal exposures, organisms have the ability to sequester metals as phytochelatins, metallothioneins or in granular deposits (Roesijadi 1992; Ahner and Morel 1995). Such processes essentially decouple toxicity and bioaccumulation.

The use of water-effect ratios by the US EPA (1994) is a related approach to metal speciation that addresses, through toxicity testing, the effect of natural complexing agents in reducing metal bioavailability. Toxicity measurements are performed in both metal-spiked natural and laboratory (reconstituted) water, and the ratio of LC50 values (the concentration of metal causing a 50% mortality) for the test species is termed the water-effect ratio. It leads to a site-specific but organism-specific relaxation of national guideline values (ie. water effect ratio x national guideline value). If using test species that respond at or near guideline concentrations, the result is similar to that proposed in the revised Australian water quality guidelines (ANZECC & ARMCANZ 2000).

(v) Models of metal-organism interaction

An important development in understanding the mechanisms of metal-organism interaction is the development of the free ion activity model (FIAM) (Morel 1983; Campbell 1995). This model postulates that the biological effects of metals are best predicted

by the activity of the free metal ion, rather than total metal concentration. Broadly, the FIAM identifies surface complexation at cell membranes (such as fish gills) as a major determinant in metal bioavailability. The model requires the determination of the metal speciation of the aqueous medium to which the organism is exposed, and subsequently, relates the activity of the free metal ion to biological effect. The model assumes that the aqueous medium is in equilibrium with the cell membrane surface.

The FIAM has been largely successful in predicting the biological effects of several metals (eg. Cd, Cu, Mn and Zn) to a variety of aquatic organisms (eg. algae, bacteria, crustaceans, molluscs and fish); however, several exceptions (eg. lipid-soluble metal complexes) have been reported (Campbell 1995). Most studies have used algae, fish or crustaceans. The FIAM has been extended by Brown and Markich (2000) by integrating biological receptor theory to obtain a more rigorous conceptual model; one that more precisely quantifies the interaction of chemical species at biological receptor sites.

Another extension of the FIAM is the fish gill surface interaction model [see review by Playle (1998)]. This model attempts to predict metal bioavailability by incorporating measured (conditional) metal-gill surface binding constants into a geochemical speciation model. This approach explicitly considers competitive effects (eg. by Ca²⁺) and complexation effects (eg. by DOM) on metal binding to freshwater fish gills in a manner which is more predictive and mechanistically-based than descriptive empirical equations. The models developed to date have only considered the acute toxic effects of a few metals (eg. Ag, Cd, Cu and Co) at the gills of rainbow trout or fathead minnows (Playle 1998) and more recently to daphnids De Schamphelaere and Janssen (2002). Further work needs to test the applicability of the model to other freshwater fish species, in addition to other freshwater organisms (eg. molluscs or crustaceans).

PRACTICAL GUIDE TO USING THE DECISION TREE

Total (acid-soluble) metal concentration

A typical application of the decision tree approach (Figure 1) for a water sample, would be to first measure the total (acid-soluble) metal concentration and compare this with the guideline trigger value. If the water sample is from a freshwater system (salinity \leq 2.5‰), and if the metal is one whose bioavailability is known to be hardness-dependent (ie. Cd, Cr(III), Cu, Pb, Ni or Zn), the guideline value should be modified to account for the hardness of the ambient waters using

metal-specific algorithms (Table 2). Applying the general form of the algorithms given in Table 2, guideline values for Cd, Cr(III), Cu, Ni and Zn will increase by about a factor of ten as the water hardness increases from 30 (soft) to 400 (extremely hard) mg L^1 as CaCO₃, whereas guideline values for Pb will increase by about a factor of 27 over the same range of hardness. The range of guideline values for each metal reflect the slope factor, *a*, of each metal-specific hardness algorithm (Table 2).

The use of 'clean' sampling and handling techniques is strongly recommended to minimise trace metal contamination at minimally-polluted sites, and hence, provide reliable measures of trace metals, such as Cd, Cu, Cr, Hg, Ni, Pb and Zn (Apte *et al.* 1998; Markich and Brown 1998). For some metals, such as Cd or Pb, 'conventional' sampling procedures may result in concentrations 10-100 times higher than those measured using 'clean' sampling techniques. Rigorous quality control procedures are also essential for both accurate (metal spikes or standard reference materials) and precise (field and procedural blanks or replicates) trace metal analysis.

Dissolved (acid-soluble) metal concentration

If the total (acid-soluble) metal concentration exceeds the guideline value (modified for water hardness with Cd, Cr(III), Cu, Pb, Ni or Zn), then a measurement of the dissolved (acid-soluble) metal concentration should be made, and compared with the same guideline value (Figure 1). From a practical standpoint, it is crucial that the membrane filter and membrane housing used is adequately cleaned (acid-washed and rinsed in deionised water) prior to filtration (Batley 1989). Otherwise, metal contamination of the water sample may occur as a result of metals leaching from the membrane. Filtration should ideally be carried out at the time of sample collection to minimise changes in speciation with time (Batley 1989).

From a practical standpoint, several water samples may be collected simultaneously from a given site. One should be immediately acidified (pH < 2) and analysed for total metal concentration. A second sample should be filtered, acidified (pH < 2) and retained for dissolved metal analysis, if required. A third sample may also be collected for metal speciation work, if required. This procedure minimises the effects of potential changes to the general chemistry and speciation of the surface water that may occur over time, in the event that dissolved metal analysis and speciation analysis are required.

Metal speciation/bioavailability

If the dissolved (acid-soluble) metal concentration exceeds the guideline value, a more complex suite of techniques are available to chemically measure and/or predict metal speciation or directly evaluate metal bioavailability, through toxicity testing (Figure 1). These approaches, however, all require a high level of expertise.

Geochemical speciation modelling, including the use of algorithms (eg. water hardness), is the ultimate aim of most regulators. Presently, however, the most common application of geochemical speciation modelling is the prediction of the total metal fraction which occurs as inorganic metal species. This is relevant given that guideline values are largely derived from laboratory studies with metals added to the test waters (ie. reconstituted waters) as inorganic salts. Such systems are typically devoid of natural DOM (or suspended particulates), and thus, metal-organic complexation is negligible. Depending on the chemical characteristics of the test water, the free metal ion may comprise a varying fraction of the total inorganic metal concentration. For example, Holm et al. (1995) showed that Cd^{2+} typically comprised > 90% of total Cd in fresh surface water at pH 7 and 23 mg L¹ humic acid. Conversely, Breault et al. (1996) showed that Cu²⁺ typically comprised < 5% of total Cu in fresh surface water at pH 7 and 8.3 mg L¹ fulvic acid.

From a practical perspective, toxicity testing in minimally-polluted aquatic ecosystems has limited use. That is, metal guideline values (typically expressed as the inorganic metal concentration) may be up to an order of magnitude lower than levels that are toxic to sensitive test organisms. Polluted aquatic ecosystems offer more scope for evaluating guideline values through toxicity testing. In situations where biological testing can be used to evaluate metal toxicity, a general consensus needs to be reached with respect to the types of test organisms used and the likely ranges of their sensitivity to specific metal exposures. Other issues that need to be considered include whether acute and/or chronic toxicity endpoints are measured and whether local (site-specific) or standard test species should be used. Such issues will more than likely need to be agreed upon by both the 'polluter' and the regulators or catchment managers on a site-specific basis.

SHORTCOMINGS OF THE REVISED GUIDELINES

The hardness-dependent algorithms were largely derived using metal toxicity data for fish and crustaceans. It is assumed that hardness-dependence is the same for all freshwater organisms. Perhaps of greater importance, is the need to extend toxicity studies to incorporate a greater variety of freshwater organisms, such as algae and molluscs, to test the assumption that the hardness-dependent algorithms describe all freshwater species. One shortcoming of this approach is that, to produce the final algorithm, increased errors result from the pooling of the individual slopes of the regressions (US EPA 1985). As a result, the reasonably good statistical fits ($r^2 = 0.60 - 0.75$) of individual regressions for the selected metals are reduced ($r^2 = 0.40 - 0.60$) in the pooled regressions. Therefore, the generic hardness-dependent algorithms can be used to predict a guideline value for a particular metal with a confidence limit of about \pm 50%.

The revised Australian water quality guidelines for protecting aquatic ecosystems account only for the effects of water hardness (which usually incorporates alkalinity) with six metals (ie. Cd, Cr(III), Cu, Pb, Ni and Zn). There is some evidence which indicates that the uptake and toxicity of Al, Cr(VI), U and V in freshwater organisms is also reduced with increasing water hardness, but insufficient data are currently available to develop hardness-dependent algorithms. One potential shortcoming with the use of generic hardnessdependent algorithms is that hardness and alkalinity, although frequently related, may be uncoupled in certain circumstances. For example, a surface freshwater with a high natural hardness could possibly exhibit low alkalinity as a result of acid drainage. This scenario has the potential to be underprotective to freshwater organisms, for a particular water hardness, given that the ameliorative capacity of the alkalinity component has been reduced.

Apart from water hardness, metal bioavailability can be strongly affected by a range of other important water quality parameters, including pH, natural DOM concentration and salinity. No algorithms are presently available to describe water quality guidelines for different metals whose bioavailability will vary as a function of these water quality parameters. A multiple regression algorithm, that includes water hardness (alkalinity), pH and natural DOM to describe a water quality guideline for a given metal in a freshwater system, would be potentially more beneficial for environmental protection than the use of water hardness alone.

At present, no universally applicable technique exists for determining metal bioavailability. Although toxicity testing provides a direct determination of metal bioavailability, it is labour-intensive and costly. Chemical measurements that are specific for particular metal species (eg. ion-selective electrode - free metal ion concentration) or operationally-defined groups of metal species (eg. anodic stripping voltammetry - labile metal concentration) are more cost-effective, but provide a more equivocal indication of metal bioavailability than direct toxicity testing. Geochemical speciation modelling, using routine packages (eg. MINTEQ), is perhaps the most cost-effective technique available, with the added advantage of providing a predictive capability, but suffers from being the most equivocal in determining metal bioavailability. Chemical measurements and geochemical speciation modelling may serve as valuable tools if performed in conjunction with biological tests, where particular metal species (eg.free metal ion or labile metal species) can be related to a biological effect (eg. toxic response). These techniques are not trivial and should be only undertaken by appropriately experienced personnel.

FUTURE DIRECTIONS

Future revisions of the Australian water quality guidelines may consider using the free metal ion concentration as the basic guideline value. This value could then be 'scaled-up' to reflect a reduction in the bioavailable (ie. free) metal concentration due to the site-specific chemical characteristics (eg. hardness, DOM concentration or salinity). The implementation of this concept would require that original toxicity data be converted from total to free metal ion concentrations. This assumes that the water chemistry provided in the original data source is sufficient to accurately predict the free metal ion concentration using geochemical speciation modelling. The free metal ion concentrations, rather than the total concentrations, would then be fitted using an appropriate distributionbased extrapolation model (Warne, 1998) to obtain a final guideline value. Such an approach has been used by Batley et al. (1999) for freshwater organisms exposed to Cu.

Markich and Jeffree (1994) proposed that Ca concentration may be a better parameter than total hardness (Ca + Mg) for the protection of freshwater organisms, since Ca is far more effective at ameliorating metal toxicity than Mg. Indeed, in most freshwaters, Ca is the prevalent (70%) hardness cation. The current German water quality guidelines actually use Ca concentration instead of total hardness for Cu, Zn and Cd to protect freshwater fisheries (Rump and Krist 1992). Markich and Jeffree (1994) recommended that total hardness (Ca + Mg) will be more useful only when the concentration of Mg considerably exceeds that of Ca in fresh surface waters.

The latest research has identified the surface complexation of metals to cell membranes as a major determinant of metal bioavailability to aquatic organisms, and is attempting to predict metal bioavailability by incorporating the binding constants of metals to cell membranes (eg. fish gills) into speciation models (Playle 1998). This approach aims to bridge the gap between speciation modelling and toxicity testing, but at present there are insufficient data for metals other than Cd and Cu. Further work extending this concept to other organisms. (De Schamphelaere and Janssen 2002).

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