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1 Introduction

FROM:
Walker, J., M.C. Newman, and M. Enache. *Fundamental QSARs for Metal Ions.* Taylor & Francis, Boca Raton, FL., 213, p. 288

1.1 THE CONCEPT OF STRUCTURE–ACTIVITY RELATIONSHIPS (SARS)

SAR resides at the intersection of biology, chemistry, and statistics.

McKinney et al. (2000, p. 9)

McKinney et al. (2000) identified the essential steps in generating SARs. It is critical at the beginning to identify the mechanism underpinning the bioactivity of interest. Once the underpinnings are defined, the relevant toxicants and their relevant qualities can be identified with the intention of using them to understand and predict trends within the toxicant class. Next, a qualitative or quantitative approach that relates toxicant qualities to bioactivity is formulated. Approaches range from simple dichotomous categorizations to complex quantitative models generated with a variety of statistical techniques. Such SARs or quantitative SARs (QSARs) are relevant to the specified toxicant class and bioactivity. Additional SARs might be needed to address other classes or activities.

The QSAR approach for organic compounds is well established in contrast to the nascent approach for inorganic chemicals such as metal ions. As a late nineteenth-century QSAR example, the Meyer-Overton rule related anesthetic potency to its oil–water or oil–air partition coefficient. This theme of relating organic compound bioactivity or accumulation to lipophilicity still dominates much of QSAR literature about nonpolar organic contaminants. There are numerous cases where additional qualities based on other molecular structures or properties of organic compounds are included to develop QSARs for different organic contaminant classes. These qualities are often quantified in metrics of nucleophilicity, electrophilicity, molecular topology, and steric qualities (Newman and Clements 2008). QSARs based on lipophilicity, nucleophilicity, electrophilicity, molecular topology, or steric qualities have been developed for pollution prevention, toxicity screening, risk assessment, and web applications (Walker 2003).

In contrast, qualitative rules such as the d-orbital electron-based Irving-Williams series (Brezonik et al. 1991) are well established for ordering the relative bioactivities of subsets of metals, but quantitative relationships for metal ions have remained inexplicably underdeveloped in toxicology and risk assessment (Newman et al. 1998; Walker and Hickey 2000). Fortunately, this underdevelopment is now recognized as such and is steadily being resolved, as illustrated by the studies described in Chapter 5.

1.2 METALS IN THE MOLECULAR ENVIRONMENT

Metals can be classified based on an array of qualities. Some are more useful than others for quantitatively predicting intermetal differences in bioactivity. Following the lead of numerous authors, most notably Nieboer and Richardson (1980), this

treatment will focus on classification schemes that link biological mode of action to coordination chemistry.

There are several candidate metal classification schemes to employ for SAR and QSAR generation (Duffus 2002). The easiest to eliminate at the onset is classification based on whether the metal is an unstable or stable nuclide. This classification is irrelevant because our intent is not prediction of effects arising from different types of ionizing radiations. It is prediction of adverse effects from chemical interaction between metal and organism. Classification based on natural abundances such as *bulk*, *abundant*, or *trace* elements is unhelpful because we wish to make predictions for toxicological effects at unnatural, as well as natural, concentrations. However, there are cases in which natural abundance or natural occurrence information can provide valuable insight, as exemplified by the studies of Fisher (1986) and Walker et al. (2007), respectively. Another general classification of metals is the dichotomous division of metals as either being heavy or light metals. The general cutoff between these two groupings (circa 4 g cm^{-3}) has been applied loosely to highlight the toxicity of many heavier metals. Obviously, a dichotomous schema has minimal utility here, especially for creating QSARs. At a slightly finer scale, Blake (1884) did note more than a century ago a correlation between atomic number and metal toxicity. Conforming to the Irving-Williams series, toxicity to mice increased progressively with atomic numbers from manganese (atomic number 25, density 7.43) to copper (atomic number 29, density 8.96) (Jones and Vaughn 1978), but this increase also corresponded with the progressive addition of d-orbital electrons from $[Ar]3d^5 4s^2$ to $[Ar]3d^{10}4s^1$. Such a scheme based on density or atomic number does not incorporate important periodicities influencing metal toxicity. A schema framed around the periodic table seems more amenable because metal binding to critical biochemicals can easily be related to the classic periodicities therein. Certainly, trends in the nature and occupation of the outer valence shell can be discussed starting from this classic vantage, e.g., qualities of d- versus s- and p-block elements (Barrett 2002; Walker et al. 2003). However, this approach requires extension to generate related quantitative metrics of binding tendencies. For example, zinc ($[Ar]3d^{10}4s^2$) was less toxic in the above progression (Jones and Vaughn 1978) than might have been anticipated based on atomic number, density, or the number of d-orbital elections alone. With the maturation of coordination chemistry as a predictive science, relevant quantitative metrics have emerged that combine several metal ion properties into directly useful metrics. Continuing the example, the empirical softness index (σ_p) described later conveniently resolves the inconsistency just noted for zinc toxicity. These schemes framed on classic periodicity-related binding tendencies are favored here.

> The primary purpose of classifying [metal ions] in (a), or hard, and (b) or soft, is to correlate a large mass of experimental facts. All the criteria used for the classification are thus purely empirical; they simply express the very different chemical behavior of various [metal ions].
>
> **Ahrland (1968, p. 118)**

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Ahrland (1968, p. 118)

TABLE 1.1
Classification of Metal Ions According to Nieboer and Richardson (1980)

Metal Ion Class	Metal Ions
b	Au^+, Ag^+, Cu^+, Tl^+
	Hg^{2+}, Pd^{2+}, Pt^{2+}
	Bi^{3+}, Tl^{3+}
Intermediate or borderline	Cd^{2+}, Co^{2+}, Cr^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Ni^{2+}, Pb^{2+}, Sn^{2+}, Ti^{2+}, V^{2+}, Zn^{2+}
	Fe^{3+}, Ga^{3+}, In^{3+}
a	Cs^+, K^+, Li^+, Na^+
	Ba^{2+}, Be^{2+}, Ca^{2+}, Mg^{2+}, Sr^{2+}
	Al^{3+}, Gd^{3+}, La^{3+}, Lu^{3+}, Sc^{3+}, Y^{3+}

Note: The actinides and lanthanides are class (a) metals. Although placed in this table as an intermediate metal ion, Pb^{2+} tends toward class (b) more than most intermediate metal divalent ions in that part of this table. Cd^{2+} also is classified as being along the line between class (b) and intermediate metal ions.

Pearson (1963) and Ahrland (1968) developed the hard and soft acids and bases (HSAB) concept that fulfills many of the practical requirements for metal ion SARs and QSARs. Their approach was to quantify differences in metal ion bond stability during complexation with different ligand atoms. The electrophilic metal ion was envisioned as a Lewis acid and the nucleophilic donor atom of the ligand as a Lewis base.* Class (a) and (b) metals were designated hard and soft Lewis acids, respectively. The soft/hard facet of HSAB theory refers to how readily the outer valence shell deforms during interaction between the metal ion and ligand donor atom. This quality of metal ions generally corresponds to nonpolarizable (hard, class [a]) and polarizable† (soft, class [b]) during interaction with donor atoms of ligands.

The class (a) and (b) metals are clustered predictably in the periodic table, with intermediate (borderline) metals being found between these clusters. The exact borders for these classes of metals vary in the published literature because the tendencies used to separate the metals are continuous and a discrete classification is partially arbitrary.

The widely applied Nieboer and Richardson (1980) tabulation of these metal ions classes is summarized in Table 1.1. The general trend in bond stability of the class (a) metal ions with various ligand donor atoms is O>N>S and that for class (b) metal ions is S>N>O (Nieboer and Richardson 1980). Borderline metal ions are more complex, having binding tendencies intermediate between class (a) and (b) metals. Interactions between the hard class (a) metal ions and ligands tend to be ionic in

* Recollect that a Lewis acid is a species that can accept an electron pair and a Lewis base is one that can donate an electron pair.

† A polar bond is one in which a dipole is formed along the bond axis. Polarizability in this treatment generally corresponds with the readiness of the valance shell to deform during metal-ligand interaction.

nature and those for class (b) tend to be covalent. Those of intermediate metal ions vary in degrees in the covalent nature to their bonds with ligands.

The coordination chemistry-based approach for qualitatively predicting trends in metal ion bioactivities has been applied successfully for several decades. In the early 1960s, Shaw (1961) drew from the field of coordination chemistry to relate metal toxicity to metal–ligand bond stabilities. Using the then-maturing HSAB theory, Jones and Vaughn (1978) related toxicity directly to a continuous metric of metal ion softness, σ_p. Williams and Turner (1981) extended this approach by adding more toxicity data and considering mono-, di- and trivalent metal ions. This general approach continues to be expanded and refined to generate metal ion QSARs.

1.3 METALS IN AND EFFECT ON WHOLE ORGANISMS

Coordination chemistry directly influences metal–biological interactions, although metal essentiality can introduce additional features (Fraústo de Silva and Williams 1993). Relevant interactions include adsorption to biological surfaces, bioaccumulation, and toxicological effect. This chapter broadly describes these biological phenomena and, through examples, relates them to metal coordination chemistry. Such relationships between metal ion characteristics and bioactivity were referred to as *ion character-activity relationships* (ICARs) by Newman and coworkers (e.g., Ownby and Newman 2003). The quantitative rendering of these relationships has been called, alternatively, *quantitative ICARs* (QICARS) by Newman et al. (1998) and *quantitative cationic-activity relationships* (QCARs) by Walker et al. (2003). ICARs will be discussed in the remainder of this chapter, while detailed discussions of QCARs and QICARs are presented in later chapters.

> Many attempts have been made to correlate the physiological action of the elements with their physical or chemical properties, but with only partial success.
>
> **Mathews (1904, p. 290)**

> [T]he degree of toxicity of ions is largely determined by their affinity for their electrical charges, this affinity determining the readiness with which they tend to abandon the ionic state to enter into chemical combination with protoplasmic compounds.
>
> **Erichsen Jones (1940, p. 435)**

> [T]he fungostatic action of metal cations is related to the strength of covalent binding to surface ionogenic groups on the cell ...
>
> **Somers (1961, p. 246)**

> The results of this investigation ... establish a toxicity sequence ... that is of very general significance in aquatic biology and one that is also firmly based on the principles of co-ordination chemistry.
>
> **Shaw (1961, p. 755)**

> Quantitative ion character-activity relationships can be developed for a range of effects based on metal–ligand binding theory.
>
> **Newman et al. (1998, p. 1423)**

> Developing and validating Quantitative Cationic Activity Relationships or (Q)CARs to predict the toxicity [of] metals is challenging because of issues associated with metal speciation, complexation and interactions within biological systems and the media used to study these interactions.
>
> **Walker et al. (2003, p. 1916)**

As reflected in these quotes, the idea that metal ion biological activity is relatable to coordination chemistry is more than a century old. What is new is our emerging capability to quantitatively predict metal–biological activity with coordination chemistry-based metrics. Our understanding of coordination chemistry has advanced substantially, bringing along with it an assortment of convenient metrics for quantifying differences in metal chemistries. Although the pioneering work of Alfred Werner that began the field of coordination chemistry took place a century ago, the HSAB concepts that permeate discussions here and in other chapters came together only in the last half of the twentieth century (e.g., Pearson 1963, 1966). Hard and soft acids and bases theory now has evolved to such an extent that it is applied to develop both organic and inorganic QSARs (Carlson 1990). An array of potential physicochemical metrics has emerged with more refinements made every year. They are actively being assessed for their relative advantages in facilitating quantitative prediction of metal bioactivity (e.g., Kaiser 1980). Reviews by Newman et al. (1998), Ownby and Newman (2003), and Walker et al. (2003) reconfirm the viability of predicting metal activity with metal ion coordination chemistry metrics. Studies such as those of Wolterbeek and Verberg (2001), Kinraide and Yermiyahu (2007), and Kinraide (2009) enhance their potential each year by comparing and refining metrics. Complementing this growth in physicochemical metrics is the increasingly comprehensive and sound effects database available for use in quantitative models. Enough progress had been made as we enter the new millennium that general metal selection approaches for developing these relationships are beginning to emerge (e.g., Wolterbeek and Verburg 2001). It is the explicit goal of this book to synthesize this recent work, and in so doing, facilitate further advancement toward establishing powerful QSARs for metals.

1.3.1 Accumulation in the Organism

A metal ion must interact with a biological surface before being taken up and having an effect. Such interactions can be conveniently modeled with the Langmuir model.

$$n = \frac{KCM}{1 + KC} \tag{1.1}$$

where n is the measured amount of metal adsorbed per unit of adsorbent mass, C is the measured equilibrium dissolved metal concentration, M is the estimated

adsorption maximum for the adsorbent, and K is the estimated affinity parameter for the adsorbent that reflects the bond strength involved in adsorption (Newman 1995). Numerous examples show that adsorption differences can be related to coordination chemistry metrics. The K values for a series of class (a) metals adsorbing to *Spirogyra* sp. (Crist et al. 1988) were correlated with polarizing power, Z^2r^{-1}, of the metals where Z is the ion charge and r is the ion radius (Figure 1.1 top panel). The Z^2r^{-1} metric quantifies the energy of the ion during electrostatic interaction with a ligand. The general conclusion from Crist et al.'s (1988) metal–algal sorption study was that the affinity parameter (K) was dictated by charge density if adsorption involved strong metal–ligand bonds, but by energy of hydration if weak metal–ligand bonds were involved. Similarly, maxima for adsorption of a series of class (a), intermediate, and class (b) metals to *Saccharomyces cerevisiae* (Chen and Wang 2007) increased as the χ^2r of the metal ion increased (Figure 1.1 bottom panel). (χ is electronegativity and r is the ion radius.) The χ^2r metric reflects the relative degree of covalent versus electrostatic nature of bonds during metal interaction with a ligand (Newman et al. 1998). Similar studies include those exploring simple or competitive metal adsorption to other microalgae (Crist et al. 1992; Xue et al. 1988), brown macroalgae (Raize et al. 2004), and bacteria (Can and Jianlong 2007; Zamil et al. 2009).

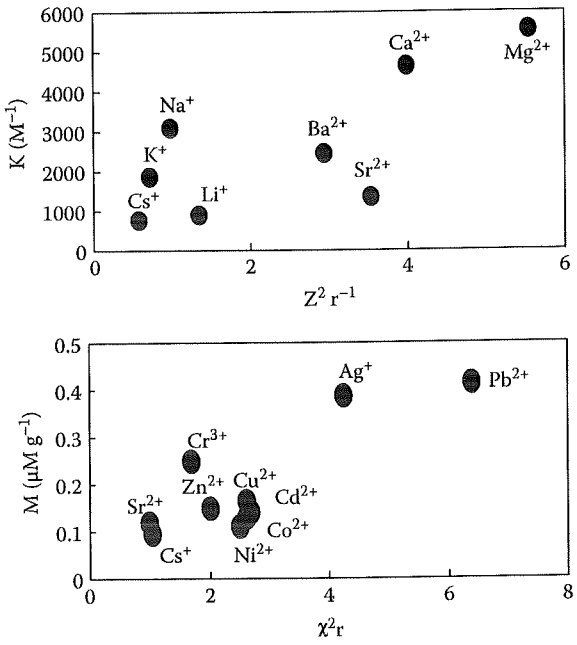

FIGURE 1.1 Trends in adsorption metrics as a function of coordination chemistry for alkali and alkali earth (class [a]) metals and the alga, *Spirogyra* sp. (top panel), and a series of class (a), intermediate, and class (b) metals and the bacterium, *Saccharomyces cerevisiae* (bottom panel). The units of K in the top panel are M^{-1} from an algal suspension with 0.5×10^{-3} to 0.1×10^{-1} M of dissolved metal.

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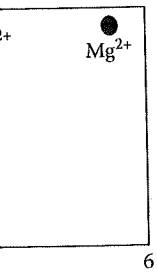

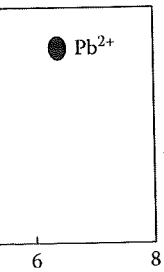

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Adsorption models such as that of Crist et al. (1992) can be linked to the biotic ligand model (BLM) with which aquatic toxicologists infer dissolved metal bioactivity (Di Toro et al. 2001). The BLM estimates equilibrium concentrations or activities of dissolved metals species that can compete for and adsorb to ligands on biological surfaces. The dissolved metal bioavailability and potential effect are assumed to result from its interaction with a ligand such as those of a gill sodium or calcium channel protein (Di Toro et al. 2001). For instance, cadmium diffusion through crustacean gill is facilitated by its interaction with a calcium-binding membrane protein (Rainbow and Black 2005). The interaction might simply lead to metal entry into the cell or production of an adverse effect such as ionoregulatory disruption. The relative amounts and binding qualities of calcium and cadmium competing for passage through the membrane via this calcium-binding protein would influence the rate of cadmium introduction into gill cells. QSARs and BLMs are discussed in Chapter 6.

Adsorption involved in other exposure routes, such as gut uptake, is also related to coordination chemistry. Movement across the gut can involve straightforward mechanisms such as those just described or more involved mechanisms. Two class (b) metals provide examples in which crucial complexation processes began in solution before any direct interactions occurred with the cell membrane. Methylmercury forms a stable bond with the sulfur atom of cysteine ($HO_2CCH(NH_2)CH_2SH$) and can enter cells as a complex via an essential amino acid uptake mechanism (Hudson and Shade 2004). Cadmium also strongly binds to compounds with thiol groups such as those of the tripeptide, glutathione (glutamic acid-cysteine-glycine), and cysteine. These cadmium complexes can gain entry to the gut epithelial cells by a specific organic anion transport mechanism (Pigman et al. 1997).

At the other extreme from this thiol-linked transport of class (b) metals, membrane transport of class (a) metals such as potassium or sodium does not involve covalent bonding. Large electronegativity differences between the metal ion and biochemical ligand donor atoms result in bonds of a predominantly ionic nature:* class (a) metals are present primarily as the free aquated ion and bind only weakly to biochemical groups (Fraústo de Silva and Williams 1993). They move into cells by passive diffusion or active pumping. Passive diffusion involves carrier (ionophores) or channel proteins (Ovchinnikov 1979). Cation permeability and ionophore/channel selectivity are determined by the qualities of associated ionic bonding, Van der Waals forces, and hydrogen bonds. Features such as charge density (that is, cation radius and charge) and related hydration sphere characteristics influence ionic interactions. As an example, the electrostatic interactions in the K^+ channel cavity strongly influence selectivity, greatly favoring K^+ over the smaller Na^+ (Bichet et al. 2006). Cation interactions during Na^+/K^+ ATPase pumping also involve noncovalent interactions with RCO_2^- groups that cause conformational shifts in the protein helices to facilitate transmembrane movement (Fraústo de Silva and Williams 1993).

* Fajan's rules dictate that the partial covalent nature of a metal's ionic bond with an anion will increase with high charge of either of the interacting ions, small cation radius, or large anion radius. The covalent nature of the bond also is higher with a noninert gas electron configuration of the cation, e.g., the more covalent nature of Cu^{2+} ($\{Ar\}3d^9$) bonds, in contrast to the ionic bonds formed by Na^{+1} ($\{Ne\}$) (Barrett 2002).

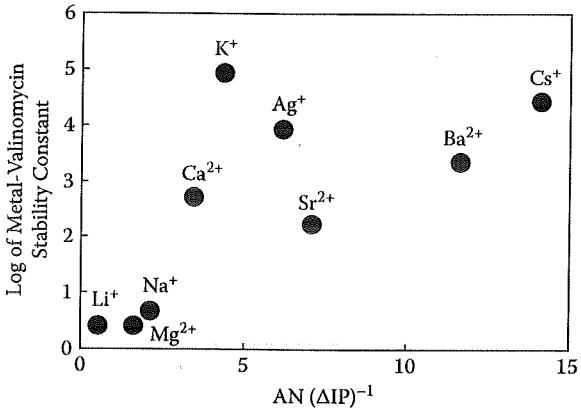
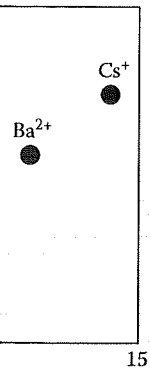


FIGURE 1.2 Ovchinnikov's (1979) estimated stability constants for valinomycin complexes (in methanol solvent) with a series of class (a) metals and one class (b) metal (Ag^+) were plotted against the $AN(\Delta IP)^{-1}$ metric. The $AN(\Delta IP)^{-1}$ metric combines the influences of ion size or inertia and atomic ionization potential (Newman et al. 1998). As is clear in this figure, valinomycin is very selective for K^+ and bonding with the one class (b) metal ion is more stable than might have been expected from the class (a) metal ion pattern.

Trends in metal–ligand stability constants for the ionophore, valinomycin, (Ovchinnikov 1979) illustrate several points about class (a) cation movement across membranes (Figure 1.2). The $AN(\Delta IP)^{-1}$ metric (Kaiser 1980) in Figure 1.2 combines the atomic number (AN) and the difference in ionization potential (ΔIP) between the ion oxidation number OX and OX-1. Clearly, coordination chemistry trends influence ionophore binding. Another point illustrated in this figure is that extreme specificity might be designed into transport processes that fill an essential biological role: the K^+ stability constant is much higher than expected from the general trend. Favored transport can be designed into such structures through a combination of features such as ion size and charge, involved ligand donor atoms, and the configuration of ligand groups (Fraústo de Silva and Williams 1993). As an illustration of the exacting design associated with selectivity, a mutation changing only two amino acid clusters in the K^+ channel materially decreases selectivity (Heinemann et al. 1992). Such features are layered onto the general trends predictable from general metal coordination chemistry.

Once a metal enters the cell, a wide range of processes occur that, again, are best understood based on general binding tendencies. By design, the metallothionein proteins and phytochelatin oligopeptides bind intermediate and class (b), but not class (a), metals. Because metallothioneins are synthesized and distributed unevenly among tissues, there will be an associated high accumulation of some metals such as cadmium in organs like the mammalian kidney. This binding to metal-cysteinyl thiolate clusters is crucial to essential element (copper and zinc) and nonessential element (cadmium, mercury, and silver) regulation, sequestration, and elimination in all phyla. More generally, methylmercury forms stable covalent S–Hg bonds with diverse proteins in various tissues (Harris et al. 2003) and will become associated



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with tissues to a degree related to its delivery rates to potential binding sites and the number of binding sites in the tissue. As another general example, differences in metal binding tendencies produced differential metal accumulation in the various tissues of crayfish (Lyon et al. 1984).

Biomineralization processes play a role in bioaccumulation and are strongly influenced by physicochemical features of metals. Jeffree (1988) found a strong correlation between alkaline earth metal accumulation with age in freshwater mussels (*Velesunio angasi*) and the corresponding metal hydrogen phosphate solubility. Cellular compartmentalization in *Helix aspersa* resulted in intermediate metals (cobalt, iron, manganese, and zinc) being incorporated into sparingly soluble pyrophosphate granules, and class (b) metals (cadmium, lead, and mercury) become associated with sulfur donor atoms of cytosolic proteins (Hopkin and Nott 1979; Simkiss 1981a). Some zinc was also associated with the cytoplasmic proteins. Simkiss (1981b) indicated that another intermediate metal (cobalt) could be found in the calcium/magnesium pyrophosphate granules that also contained protein-rich layers. Some class (a) (barium, strontium) and (b) (silver) metals were also present in the distinct layers of these granules.

Coordination chemistry influences bioavailability, internal transformations, and distribution of metals among internal pools. Fisher (1986) related elemental bioaccumulation in unicellular marine phytoplankton to binding tendencies. He used the logarithm of the corresponding metal hydroxide's solubility product to reflect metal affinity for intermediate ligands such as those with oxygen donor atoms. The Log K_{sp}-MOH was correlated with metal bioaccumulation in phytoplankton, although as illustrated in Figure 1.3, any of a series of other such metrics might have been applied as effectively. In a second excellent paper (Reinfelder and Fisher 1991), the distribution of metals in diatom cells and consequent bioavailability to zooplankton grazers was found to reflect fundamental binding chemistries. The fraction of cellular metal associated with soluble cytoplasmic proteins was higher for class (b) and intermediate metals than for class (a) metals. Moreover, bioavailability to copepods of class (b) and intermediate metals was higher than that for class (a) metals. Similar phenomena occur in metazoans as can be illustrated with the work of Howard and Simkiss (1981), who quantified class (a), intermediate, and class (b) metal binding to hemolymph proteins of the snail, *Helix aspersa*. Based on our previous discussions, a clear trend can be produced to explain intermetal differences in hemolymph protein binding (Figure 1.4). Similar to trends in Figure 1.3, the softer metals (those with outer valence orbitals that readily deform during bonding with ligands) are bound more stably to the plasma proteins than are the harder metals.

As suggested by the above exploration of Howard and Simkiss's data, a metal might be eliminated from the organism at a rate predictable from its binding chemistry. Lyon et al. (1984) explored this theme by introducing a series of class (a), intermediate, and class (b) metals into the open circulatory system of crayfish (*Austropotamobius pallipes*) and measuring elimination from the hemolymph (Figure 1.5). They fit the observed decreases in hemolymph metal concentrations to a first-order elimination model with two mathematical compartments. The percentage of the introduced metal remaining in the hemolymph (C_m) fit the straightforward model

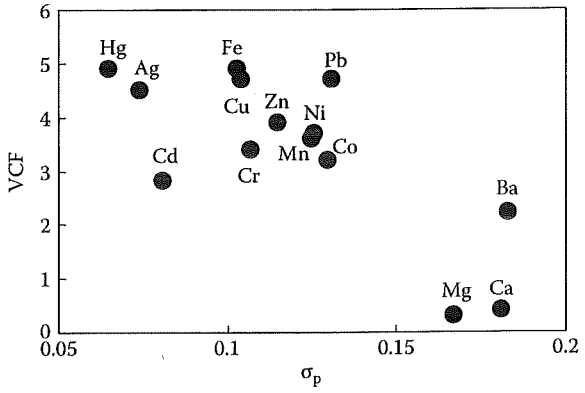

FIGURE 1.3 Phytoplankton volume concentration factor (VCF) (Fisher 1986) is correlated with metal softness index, σ_p. The units for VCF are (Moles of metal $\times \mu m^{-3}$ of cell)/(Moles of dissolved metal $\times \mu m^{-3}$ of ambient water). The softness index reflects the ion's tendency to share electrons during interaction with ligand donor atoms. The three class (a) metals shown are clustered on the bottom right, indicating low bioaccumulation factors compared to the intermediate and class (b) metals in the upper left of the figure. This illustration displays only those metals for which softness parameter values were available in Table 2 of McCloskey et al. (1996). (Data from McCloskey, J.T., M.C. Newman and S.B. Clark. 1996. Predicting the relative toxicity of metal ions using ion characteristics: Microtox® bioluminescence assay. *Environ. Toxicol. Chem.* 15:1730–1737.)

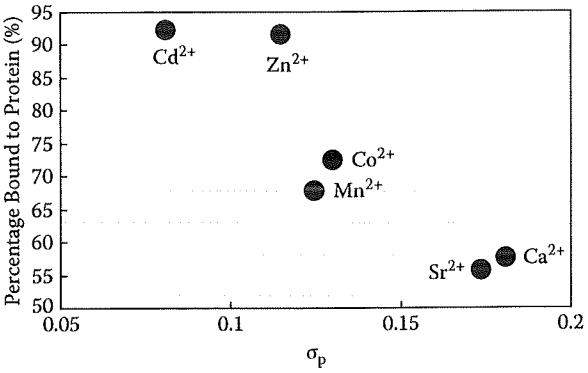

FIGURE 1.4 The percentage of injected metal bound to gastropod hemolymph protein as related to the metal ion softness.

$$C_m = Ae^{-k_{Fast}t} + Be^{-k_{Slow}t} \tag{1.2}$$

where t is the time elapsed since introduction into the hemolymph, A and B are estimated constants, and k_{Fast} and k_{Slow} are estimated rate constants for elimination from the fast and slow compartments. A general trend emerges if the half-life of a metal in the slow compartment ($T_{1/2} = \ln 2\ (k_{Slow})^{-1}$ is plotted against $\chi^2 r$. Half-life increased

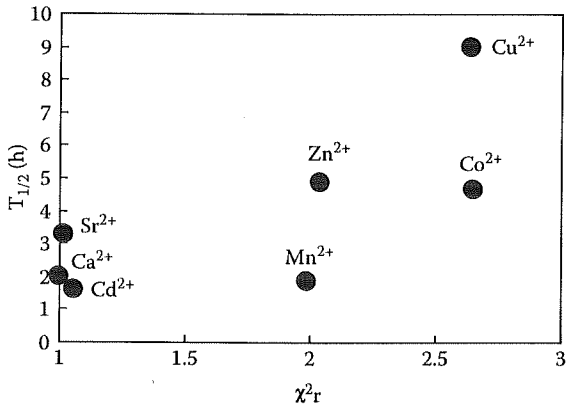

FIGURE 1.5 Elimination of metal ions from crayfish hemolymph is related to metal ion softness. Strong covalent bonding slows elimination. In this illustration, the iron datum was omitted because it was derived from the citrate salt, whereas the other metals were prepared from chloride salts. Based on trends shown here and in Figure 1.3, one could incorrectly assume that metal softness might always be the best descriptor for predicting trends. However, as Ahrland explains, "soft and polarizable are not synonymous; a soft acceptor is certainly always polarizable, but a highly polarizable acceptor need not necessarily be soft, i.e., have (b) properties. For metal ion acceptors, the outer d-electrons are as essential as the polarizability" (Ahrland, S. 1968. Thermodynamics of complex formation between hard and soft acceptors and donors. *Struct. Bond.* 5:118–149).

as the covalent nature of the metal bond with biochemical ligands increased: strong covalent bonding slowed elimination.

1.3.2 Biomolecule-to-Organism Manifestations of Metal Toxicity

It should be no surprise to the reader at this point that metal binding differences have also been used to explain intermetal differences in toxicity. The simplest of such effects, in vitro inhibition of enzyme catalysis, can be related to metal affinity to intermediate ligands such as those with oxygen donor atoms (Newman et al. 1998) (Figure 1.6). Examining several enzyme inhibition data sets, Newman et al. (1998) suggested that they could best be modeled with the absolute value of the logarithm of the first hydrolysis constant (i.e., K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$), although clear trends also emerge if plotted against the softness index (σ_p). The |Log K_{OH}| reflected metal ion binding affinity for intermediate ligands.

Expanding outward on the biological hierarchy scale from biomolecules to cells, additional mechanisms emerge that could produce differences in metal ion toxicity. Mechanisms include intermetal differences in transport, disruption of ion regulation, binding to and altering protein or nucleic acid functioning, and oxyradical generation. Newman et al. (1998) found coordination chemistry–based trends in metal lethality to cultured cells from fish (Babich et al. 1986; Babich and Borenfreund 1991; Magwood and George 1996) and hamster (Hsie et al. 1984), bacterial bioluminescence suppression (McCloskey et al. 1996; Newman and

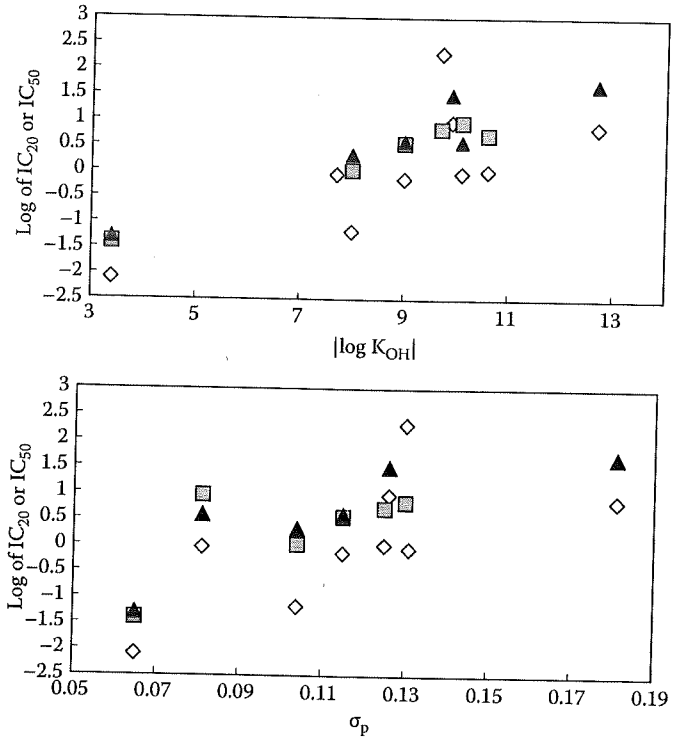

FIGURE 1.6 In vitro inhibition of enzyme activity by metal ions is correlated with the absolute value of the logarithm of the first hydrolysis constant (i.e., K_{OH} for $M^{n+} + H_2O \rightarrow MOH^{n-1} + H^+$) and the softness index (σ_p). Inhibition data for these three enzymes were produced by Christensen (1971/1972) and Christensen and Tucker (1976). White diamonds = catfish carbonic anhydrase IC_{50}, grey squares = white sucker lactic dehydrase IC_{20} and black triangles = white sucker glutamic oxaloacetic transaminase IC_{20}.

McCloskey 1996), and fungal germination (Somers 1961) (Figure 1.7 top panel). Weltje (2002) found similar trends for bacterial bioluminescence inhibition by a series of lanthanides.

Some central themes can be underscored by comparing the McCloskey et al. (1996) and Weltje (2002) data. Modeling trends for bacterial bioluminescence inactivation by divalent metal ions, Newman and McCloskey (1996) found that the |log K_{OH}| metric produced the best fitting model of a series of candidate models. This suggested that intermetal differences in inactivation were related to differences in affinity for intermediate ligands. This research was expanded (McCloskey et al. 1996) by exploring trends for twenty mono-, di-, and trivalent cations that included class (a), intermediate, and class (b) metals. Although an adequate model was produced by using σ_p alone, the best model for describing the intermetal inactivation trends incorporated both $\chi^2 r$ and |log K_{OH}|. Together, the degree of covalency in metal–ligand bonds and metal affinity for intermediate ligands influenced inactivation.

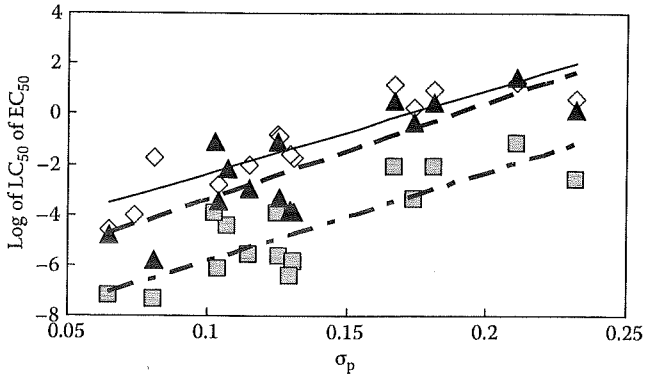

FIGURE 1.7 Lethal and sublethal effects of metal ions on *Daphnia magna* are predictable with the softness index (σ_p). White diamonds = reproductive impairment with chronic exposure (Log EC_{50} after 3 weeks), grey squares = mortality with chronic exposure (Log LC_{50} after 3 weeks), and black triangles = mortality with acute exposure (Log LC_{50} after 48 hours). Effects data were taken from Khangarot and Ray (1989) and Biesinger and Christensen (1972). The regression r^2 values for the linear models fit through the acute and chronic lethality, and chronic reproductive impairment data were 0.83, 0.84, and 0.78, respectively.

Complementing this work, Weltje (2002) took the same approach to study the trivalent, class (a) lanthanide ions. This study was especially interesting for two reasons. The rapidly growing, worldwide use of the rare earth elements (Haxel et al. 2002) makes understanding and predicting lanthanide ion toxicities important (Zhang et al. 1999). But more germane here, how electrons are added to the 4*f* shell of this series of elements permits clear demonstration of how electrostatic interactions influence class (a) metal ion toxicity. The electron occupancy of the seven 4*f* orbitals increases from 0 for lanthanum to 14 for lutetium (see Table 1 of Moeller [1975] for exact configurations). Because the 4*f* electrons are not effective at shielding the nuclear charge, a progressive decrease of ionic radii manifests from lanthanum to lutetium, i.e., the lanthanide contraction (Barrett 2002). Consequently, the charge density (reflected by $Z^2\, r^{-1}$) for these trivalent ions increases steadily from lanthanum to lutetium. Weltje (2002) demonstrated a corresponding increase in toxicity: bioluminescence inhibition increased as the energy of the metal ion increased relative to electrostatic interactions with biochemical ligands.

Expanding the biological scale of organization still further outward to the whole metazoan level, it is easy to find published relationships between metal ion qualities and adverse effects. Newman et al. (1998) tabulated a series of such studies. Included effects were acute and chronic lethality, and developmental and reproductive effects. Test species ranged widely from *Planaria* to mice. Walker et al. (2003) also examined the literature addressing this theme, clarifying several important themes. And more such relationships are published each year. As examples, Lewis et al. (1999) produced QSAR models for mice and rats exposed to more than twenty metal ions and Van Kolck et al. (2008) produced models for bioconcentration and lethality for two mollusks.

Barium toxicity by disruption of normal K^+ and Na^+/K^+ channel functioning (Das et al. 1988; Delfino et al. 1988; Taglialatela et al. 1993; Bradberry and Vale 1995) is a particularly informative example of the role played by binding tendencies on effects in metazoans. In this case, it is the class (a) metal ion binding characteristics that emerge as important. Tatara et al. (1998) determined LC_{50} values for nematodes (*Caenorhabditis elegans*) exposed to a series of mono-, di-, and trivalent metal ions including Ba^{2+}. They produced a QSAR with $\chi^2 r$ and |Log of K_{OH}|, but found that Ba^{2+} was much more toxic than predicted from the general QSAR model.* Tatara et al. explained this difference using the relative charge densities of K^+ and Ba^{2+}. The atomic radii of K^+ and Ba^{2+} are 1.38 and 1.36 Å, respectively, but these very similar radii are associated with ions of different charge. The result is very different ion charge densities as reflected in the metric, $Z^2 r^{-1}$. The bonding to the K^+ channel is much more stable for Ba^{2+} than K^+, resulting in a blocking of the essential passage of K^+ through membrane ionophores of excitable tissues. Nervous and muscle tissue could not function properly. This explained the atypical toxicity of Ba^{2+} to the nematode.

In summary, differences in metal coordination chemistries produce differences in the bioaccumulation and effects of single metals. Associated trends can be predicted with basic metrics of metal–ligand interactions.

1.3.3 Metal Interactions in Mixtures

Quantitative means of coping with joint action of metals in mixture have lagged behind those used to quantify effects of single metals. This has produced a body of mixture publications that are more descriptive or graphical than those for single metals. In some extreme cases, they are insufficient for quantifying joint effects despite common use. Typical studies include the toxic unit approach in research such as that implemented by Sprague and Ramsay (1965) and Brown (1968), and also the isobole graphical approach taken by Nash (1981), Broderius (1991), or Christensen and Chen (1991). Some approaches, such as the rudimentary concentration additivity context of toxic units, can be misinformative, tending to confuse as much as advance understanding. The classic quantitative models based on independent and similar joint action provide the best chance of exploring metal ion mixture effects as correlated with coordination chemistry.

Joint action of mixtures is quantified differently depending on whether the mixed toxicants are thought to be acting independently or by a similar mode of action (Finney 1947). In practice, independently acting chemicals are notionally those acting by different modes. In other instances, similar joint action is assumed: the mixed toxicants share a dominant mode of action and display similar toxicokinetics. Mixed toxicants can result in potentiation in which the presence of one chemical at nonlethal levels makes another toxic or more toxic. Mixed toxicants can also be synergistic. In that case, the two or more toxicants together at the specified levels are more toxic than would be predicted by simply summing the effect expected for each alone at those concentrations. The opposite (antagonism) can also occur if the

* Lewis et al. (1999) later found similar outlier behavior for barium mouse and rat toxicities.

mixed toxicants produce an effect less than predicted from simply summing the expected effect of each one alone. Functional, dispositional, or receptor-based modes for antagonism exist. Functional antagonism occurs if the toxicants change the process leading to adverse effect in opposite directions, neutralizing each other's effect. Dispositional antagonism occurs if the toxicant influences the uptake, movement, deposition, or elimination of the other(s). Receptor antagonism occurs if the toxicants block or compete in a material way with the other from a receptor. A relevant example might be the competition of metal ions for movement through ion channels, such as that described by Vijverberg et al. (1994). A quick review of the discussions above should reveal the capacity of metals to interact in these manners and for these interactions to be related to intermetal binding trends.

Again, modeling joint action of mixed toxicants is based on whether the mixed chemicals are thought to be predominately independent or similar in action for an adverse effect. The joint effect (P_{M1+M2}) of two independently acting metals ($M1$ and $M2$) combined at concentrations C_{M1} and C_{M2} that alone would produce P_{M1} and P_{M2} proportions (or probabilities) of effect would be predicted with the model,

$$P_{M1+M2}=P_{M1}+P_{M2}\,(1-P_{M1}) \quad \text{or} \quad P_{M1}+P_{M2}-P_{M1}\,P_{M2} \tag{1.3}$$

As discussed in Chapter 8, any deviation from ideal independence might be detected by inserting a parameter (ρ) to be estimated into Equation (1.3) in place of the implied 1 and then testing for significant deviation from 1 for the parameter estimate (Newman and Clements 2008).

$$P_{M1+M2}=P_{M1}+P_{M2}-\rho P_{M1}\,P_{M2} \tag{1.4}$$

Depending on study goals, a general linear modeling approach might be applied to these kinds of data. If there are more metals in the mixture, the independent joint action model can be expanded to Equation (1.5).

$$P_{M1+M2+M3\ldots}=1-(1-P_{M1})(1-P_{M2})(1-P_{M3})\ldots \tag{1.5}$$

The approach is different for mixed metals that are assumed to have a material degree of interaction due to a similar mode of action. Such situations produce toxicant effect–concentration relationships for each of the toxicants alone that have the same slopes ($Slope_{Common}$) (Finney 1947). Extending the notation above to the case of similar joint action,

$$\mathrm{Pr}obit(P_{M1})=Intercept_{M1}+Slope_{Common}(Log\,C_{M1}) \tag{1.6}$$

$$\mathrm{Pr}obit(P_{M2})=Intercept_{M2}+Slope_{Common}(Log\,C_{M2}) \tag{1.7}$$

The logarithm of the relative potency of these mixed toxicants (e.g., ρ_{M2}) can then be estimated (Equation [1.8]) and used to predict the combined effects of these mixed toxicants (Equation [1.9]).

$$Log\ \rho_{M2} = \frac{Intercept_{M2} - Intercept_{M2}}{Slope_{Common}} \tag{1.8}$$

$$\Pr obit(P_{M1+M2}) = Intercept_{M1} + Slope_{Common} Log\ (C_{M1} + \rho_{M2} C_{M2}) \tag{1.9}$$

The relative potency, ρ_{M2}, functions here much like a currency exchange rate functions. It converts concentration of one metal into the equivalent concentration of the other. If one were interested in detecting trends away from perfect similar joint action and toward independent action, the absolute difference between estimated slopes from Equations (1.6) and (1.7), i.e., $|Slope_{M1} - Slope_{M2}|$ could be used as a metric of deviation from similar mode of action.

Newman and Clements (2008) reanalyzed the binary metal mixture effect on bacterial bioluminescence data of Ownby and Newman (2003) using these models (Figure 1.8). A common slope was anticipated if two mixed metals were

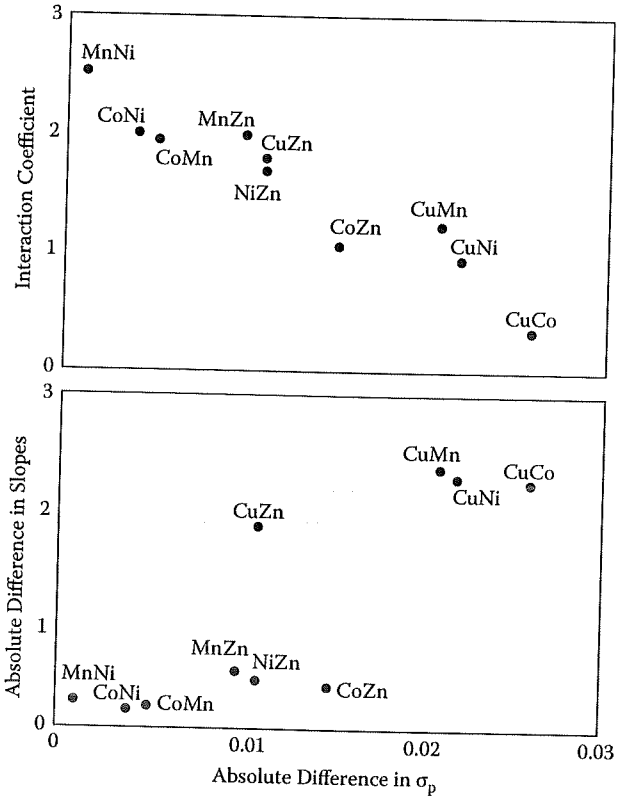

FIGURE 1.8 Deviations from perfect independent (top panel) and perfect similar (bottom panel) action for binary mixtures of metals are predictable from differences in paired metal softnesses. Specific paired metals in mixtures are indicated next to each data point, e.g., MnNi = a binary mixture of Mn^{2+} and Ni^{2+}. This figure is Figure 9.8 from Newman, M.C., and W.H. Clements. 2008. *Ecotoxicology: A Comprehensive Treatment.* Boca Raton, FL: CRC Press.

$$\frac{}{M2} \quad (1.8)$$

$$(C_{M1}+\rho_{M2}C_{M2}) \quad (1.9)$$

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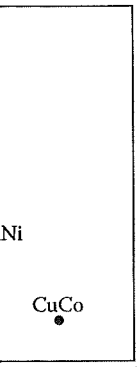

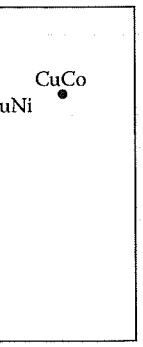

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similar in action. The absolute value of the difference in the probit model slopes for each metal (Equations [1.6] and [1.7]) reflected the degree of deviation from the similar action assumption. As anticipated, a clear trend emerged if this absolute difference were plotted for ten binary mixtures against the differences in the softness indices of the mixed metals (Figure 1.8 bottom panel). The more similar the paired metals' softness indices, the more they tended toward similar action. It is interesting to note that this approach does not require that a complex series of binary metal mixture experiments be conducted. It requires only probit model slopes from single-metal tests. However, mixture experiments are required to explore this approach from the vantage of independent action. Based on metal independent action modeling, the magnitude of deviation of the interaction coefficient (ρ in Equation [1.4]) from 1 suggests the degree of deviation from perfect independent action (Figure 1.8 top panel). The more dissimilar the softness indices of the paired metals, the more the estimated ρ deviated from 1. Independence increased the more dissimilar the coordination chemistry of the paired metals. Clearly, joint action of metals was influenced by coordination chemistry of the combined metals.

1.4 CONCLUSION

> [S]everal resolvable issues require attention before the QICAR approach has the same general usefulness as the QSAR approach. These issues include exploration of more explanatory variables, careful evaluation of ionic qualities used to calculate explanatory variables, examination of models capable of predicting effects for widely differing metals (e.g., metals of different valence states), effective inclusion of chemical speciation, examination of more effects, and assessment of the applicability of QICARs to phases such as sediment, soils, and foods.
>
> **Newman et al. (1998, p. 1424)**

It is now generally accepted that QSAR-like models can be generated for metal ions based on fundamental coordination chemistry trends. Examples ranging from adsorption to biological surfaces, to metal interactions in mixtures, to accumulation and effects in metazoans were used in this short chapter to demonstrate this point. Class (a) metal toxicity was easily related to electrostatic interactions with biological ligands. Trends in effects of class (b) and intermediate metals were related to qualities more closely linked to covalent bonding. Models involving a wider range of class (a), intermediate, and class (b) metals might require more than one explanatory variable based on different binding tendencies.

What is currently needed is a sustained exploration of the approach and further refinement of metrics and methodologies. Progress toward filling the information gaps highlighted in the above quote is evident in the literature, e.g., Can and Jianlong (2007), Kinraide and Yermiyahu (2007), Newman and Clements (2008), Ownby and Newman (2003), Walker et al. (2003), Wolterbeek and Verburg (2001), and Zamil et al. (2009) and Zhou et al. (2011). These models will very likely emerge in the next two decades to the level enjoyed now by QSAR models for organic compounds.

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