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INVERSE RELATIONSHIP BETWEEN BIOCONCENTRATION FACTOR AND EXPOSURE CONCENTRATION FOR METALS: IMPLICATIONS FOR HAZARD ASSESSMENT OF METALS IN THE AQUATIC ENVIRONMENT

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Abstract—The bioconcentration factor (BCF) and bioaccumulation factor (BAF) are used as the criteria for bioaccumulation in the context of identifying and classifying substances that are hazardous to the aquatic environment. The BCF/BAF criteria, while developed as surrogates for chronic toxicity and/or biomagnification of anthropogenic organic substances, are applied to all substances including metals. This work examines the theoretical and experimental basis for the use of BCF/BAF in the hazard assessment of Zn, Cd, Cu, Pb, Ni, and Ag. As well, BCF/BAFs for Hg (methyl and inorganic forms) and hexachlorobenzene (HCB) were evaluated. The BCF/BAF data for Zn, Cd, Cu, Pb, Ni, and Ag were characterized by extreme variability in mean BCF/BAF values and a clear inverse relationship between BCF/BAF and aqueous exposure. The high variability persisted when even when data were limited to an exposure range where chronic toxicity would be expected. Mean BCF/BAF values for Hg were also variable, but the inverse relationship was equivocal, in contrast with HCB, which conformed to the BCF model. This study illustrates that the BCF/BAF criteria, as currently applied, are inappropriate for the hazard identification and classification of metals. Furthermore, using BCF and BAF data leads to conclusions that are inconsistent with the toxicological data, as values are highest (indicating hazard) at low exposure concentrations and are lowest (indicating no hazard) at high exposure concentrations, where impacts are likely. Bioconcentration and bioaccumulation factors do not distinguish between essential mineral nutrient, normal background metal bioaccumulation, the adaptive capabilities of animals to vary uptake and elimination within the spectrum of exposure regimes, nor the specific ability to sequester, detoxify, and store internalized metal from metal uptake that results in adverse effect. An alternative to BCF, the accumulation factor (ACF), for metals was assessed and, while providing an improvement, it did not provide a complete solution. A bioaccumulation criterion for the hazard identification of metals is required, and work directed at linking chronic toxicity and bioaccumulation may provide some solutions.

Keywords—Metals Bioconcentration factor Hazard assessment Bioaccumulation Toxicity

INTRODUCTION

Bioaccumulation, along with persistence and acute toxicity, is used for aquatic environmental hazard identification to determine the potential for adverse effects to biota. Hazard identification is the determination of the adverse effects that a substance has an inherent capacity to cause and is based on its intrinsic properties [1]. Because it is based on a substance's fundamental and inherent properties, hazard identification criteria should be independent of exposure conditions. Specific issues and such as those that may be encountered locally and regionally are not considered in hazard identification but rather are dealt with in risk assessment, which integrates hazard identification, dose–response assessment, and exposure assessment.

In addition to its use as a criterion for hazard identification, bioaccumulation can also be a component of other regulatory toolboxes and is used in many jurisdictions for prioritization and risk assessment [2–4]. For example, aquatic toxicity, bioaccumulation, and persistence are applied in the internationally harmonized system for hazard classification of chemical substances, based on hazard identification, that has been developed within the framework of the Organization for Economic Cooperation and Development (Paris, France) [1]. Bioaccumulation is also used for categorization of substances in Canada, in life-cycle impact assessment models [5], and in screening-level risk assessment evaluations [6]. The criteria used to evaluate bioaccumulation in these contexts are the bioconcentration factor (BCF) and the bioaccumulation factor (BAF). For hazard classification, the BCF/BAF criterion is usually applied as a threshold, above which a substance is deemed bioaccumulative and therefore possessing the potential for long-term environmental impacts. The threshold BCF/BAF values used to classify substances typically range between 500 and 5,000, depending on the jurisdiction [1,7]. The criterion is generally applied to all substances including metals and metal compounds.

The BAF and BCF represent one of the most simplified models for bioaccumulation [8,9]. It is a single-compartment model that predicts partitioning between exposure medium (water in this study, but also soil or sediment) and biota. Both BCF and BAF are generally calculated as the ratio, at equi-

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librium, of internal biota concentration to exposure concentration. Although the calculation of BAF and BCF are usually the same (e.g., see [7]), the interpretations are slightly different, with accumulation in organisms arising from water only for BCF and from water and dietary sources for BAF. Therefore, in general, BAF is derived from measurements in natural environments and BCF is more readily measured under laboratory conditions.

In general, bioaccumulation of substances is widely accepted as one of the key factors in understanding and identifying their potential environmental hazard. To produce adverse effects, metals must bioaccumulate (where uptake exceeds elimination) in excess of a threshold concentration at the specific site of action. The BCF/BAF criterion is the only bioaccumulation model considered for hazard identification in spite of the fact that there are other models available. For example, some recent metal-specific bioaccumulation models include McGeer et al. [10], DiToro et al. [11], and Santore et al. [12], although it must be recognized that these are metal specific and not designed or easily adaptable for hazard identification. An important feature of these metal-specific models is validation, specifically, linking bioaccumulation to adverse impact. In terms of environmental protection, the issue of validation is important because, as noted by Beyer [13], an overemphasis on bioaccumulation potential as an independent endpoint often diverts attention and resources from the more important concern of whether metal concentrations in the environment result in impacts.

A number of recent studies have questioned whether use of the BCF/BAF model is appropriate for describing the relationship between bioaccumulation and the potential effects for naturally occurring inorganic substances such as metals [2,3,8,14–17]. These criticisms were based on the argument that the BCF/BAF model was originally developed and validated for a fairly limited number of neutral, lipophilic, synthetic organic substances with narcosis as the mode of toxic action. Additionally, those works argue that the simple ratio of internal concentration to external exposure (i.e., the BCF or the BAF) does not recognize the complex internal metal dynamics of uptake, internal sequestration, storage, active elimination, and nutrient essentiality or the potential for adverse effects.

The purpose of our study was to provide a detailed examination of the bioaccumulation of some metals in relation to the BCF, BAF, and hazard identification principles. We considered inorganic metal substances and compounds and excluded organometallic compounds with the exception of methylmercury. We focused efforts on two aspects, one being the theoretical underpinnings of the BCF/BAF model in relation to the state of the science on bioaccumulation of metals in aquatic organisms. The other aspect was an assessment of some of the bioaccumulation data available in terms of the practical implications of using BCF and BAF as criteria for aquatic hazard identification. While the bioaccumulation of metals from environmental media other than the aquatic medium can also lead to impacts, we chose to focus on the aquatic because of the volume of data available and the fact that metal BCFs and BAFs derived from soil and sediments studies tend to be orders of magnitude lower that those from aquatic (data not shown). In addition, much of the regulatory concern for metals in the context of environmental hazard classification is in relation to the aquatic medium [1,7].

THEORETICAL BASIS OF THE BCF/BAF MODEL

The use of BCF and BAF, subsequently referred to as BCF for simplicity, as quantitative measures that indicate the potential toxicological impact of substances [4,18] was developed and validated for neutral hydrophobic organic substances [4,19–27]. This development as an indicator of the long-term hazard potential was due to the limited data on the chronic toxicity of these substances. As such, BCF is of most value when little or no long-term toxicological data are available [1].

One of the most important theoretical conditions of the BCF model in terms of its applicability to hazard identification of chemical substances is that BCFs should be independent of exposure. In other words, hazard identification is based on the intrinsic properties of substances [1], and for BCF to be considered as an intrinsic property, it should remain constant over a range of conditions. Meeting this condition means that differences in BCFs among substances are related to variations in bioaccumulation, which includes uptake and elimination as well as metabolic and natural degradation/depuration [21,23,28].

Diffusion is the mechanism of uptake and accumulation for neutral organic substances in biota and is the key aspect of their bioaccumulation that ensures that BCFs are independent of exposure. Neutral organic substances, because they are intrinsically lipophilic and hydrophobic, accumulate in biota via simple passive diffusion across the lipid bilayer of biological membranes as predicted by Fick's Law [29]. Because lipid solubility is directly related to biological membrane permeability [9,30], uptake of neutral organic substances is driven by the thermochemical partitioning between the water phase of the environmental medium and the lipid phase of the animal. Uptake into biota by passive diffusion satisfies the assumption that BCF be independent of exposure. Therefore, the validation of the BCF model as an indicator of the bioaccumulative nature of neutral organic substances is related to their intrinsic hydrophobic and lipophilic chemical properties.

The fact that the BCF model is essentially a hydrophobicity model [26] has been exploited to derive even more simplified estimates of bioaccumulation potential. Studies have illustrated a direct relationship between the octanol-to-water partition coefficient (K_{ow}) of a substance and its BCF [20,23,25,27,31,32]. This BCF to K_{ow} relationship results from the link between K_{ow} and cell membrane permeability [33]. In addition, studies have shown an inverse relationship between BCF and water solubility [25,31,34,35]. Furthermore, the theoretical physiochemical basis of the experimental associations between BCF, K_{ow}, and water solubility for lipid-soluble organic compounds is based on fugacity, and this has been derived and discussed by Mackay [28], McCarty and Mackay [36], and Newman [9].

APPLICATION OF THE BCF MODEL TO METALS

The BCF model has been derived and validated, both experimentally and theoretically, but only for a limited number of lipophilic, nonionic synthetic organic substances that undergo minimal metabolism within an organism [4]. The fundamental differences that exist between the physical, chemical, and toxicological properties of organic and inorganic substances would indicate that this model might not apply to the latter and has been reviewed [2,26,36]. These fundamental physicochemical differences between organic and inorganic substances are carried over to their mechanisms of uptake by biota. Lipophilicity, K_{ow}, and fugacity, while key correlates for bioaccumulation of

organic substances, are generally considered irrelevant and unrelated to accumulation of metals [1,9,29,37,38]. One of the important assumptions of the BCF model is that it reflects equilibrium conditions between exposure and tissue concentrations. While this equilibrium can be verified for lab-based exposures (BCFs), organisms sampled from natural conditions (BAFs) may not fulfill this assumption. Often there is not enough information given to assess whether or not equilibrium has been achieved, and if it has not, BAF values would be underestimates of real values.

Bioaccumulation of naturally occurring substances occurs along a continuum of exposure, and trace amounts of metals, both essential and nonessential, can be found in all biota [39,40]. These two studies demonstrate that, while it is possible to calculate BCFs from accumulations that occur under natural conditions, these values can be as high as 300,000 and are generally meaningless in terms of evaluating the potential for toxicity or environmental hazard [14,41]. In addition to background accumulation, aquatic biota are also able to regulate internal concentrations of metals through active regulation, storage, or a combination of these two [42–49]. Furthermore, the degree of uptake and ultimate internal fate of metals in aquatic biota is strongly influenced by availability and/or transfer processes such as ligand binding and receptor site competitive interactions. These bioaccumulation-controlling processes act at the level of the aquatic medium (e.g., geochemical speciation), the biological membranes (e.g., cationic competition), the vascular and intercellular transfer mechanisms, and the intracellular matrix [14,37,38,50–58]. While diffusional uptake of neutral inorganic complexes can occur [59,60], uptake of ions via physiological mechanisms that exhibit saturable kinetics is much more common and toxicologically relevant [9,29,33,61–63]. Similarly, physiological processes, usually renal, biliary, or branchial, generally control elimination. Additionally, sequestering, detoxification, and storage occurs [55,61] (also see below). As a result of these physiological processes, biota often actively regulate metal bioaccumulation via dynamic feedback systems that respond to environmental loading and maintain homeostasis [51,57,63]. These physiological processes have evolved over time because of the natural occurrence of metals, allowing biota to adapt with excesses and to accumulate because of nutritional dependency. As a result of the host of factors that influence metal uptake and accumulation, BCF values for metals are likely not to be independent of exposure. The independence between BCF values and exposure is a central feature of the BCF model and its use in hazard classification.

Because of active regulation and homeostatic control of metal bioaccumulation, the BCF model is problematic in terms of how it can be applied to metals and inorganic metal substances. Physiological control over accumulation suggests that bioaccumulation will vary with exposure, thus potentially invalidating BCF as a hazard criterion. Additionally, with respect to bioaccumulation from natural background levels and nutritionally essential elements, some degree of accumulation is normal and/or essential and completely unrelated to potential impact of anthropogenic releases to the environment (the focus of hazard identification of substances). The BCF is an aggregate measure of all these sources and does not distinguish between different forms of bioaccumulated metal. These issues do not arise in the hazard identification of the purely anthropogenic neutral organic substances that form the basis of the BCF model.

A further complicating factor in the application of BCF to metals is the fact that many aquatic organisms store metals in detoxified forms, such as in inorganic granules or bound to metallothionein-like proteins [64–67]. The use of granules as a storage mechanism is of particular note in the context of BCFs because extremely high tissue concentrations are often associated with this storage mechanism but unrelated to adverse impact. For example, two types of granules are known in mollusks. One of these is calcium phosphate based, capable of storing Cd, Cu, Co, Fe, Mn, Ni, and Zn [55,68] and rendering these metals nonbioavailable to both the mollusk and organisms that consume them [69–71]. Another granule type is derived from Cu-S complexes that appear to be products of normal lysosomal breakdown of metallo-sulfur proteins such as metallothioneins [72]. These granules have been shown to not only complex Cu but also Cd and Ag [68], with the end result that the metal is either excreted, recycled, or permanently stored. While sequestered and stored metal may not result in direct impacts on the organism itself, there exists the potential for impacts in predators through dietary uptake.

In summary, based on the assumptions underlying the BCF model and on the naturally occurring background concentrations of the elements in biota, it would appear that the theoretical basis for applying BCF to the hazard identification of metals is problematic. Complicating factors include the fundamental physicochemical differences between organic and inorganic substances and how these relate to the complexity and diversity of mechanisms for metal uptake, accumulation of essential and nonessential elements from natural background, homeostatic control of accumulation, and internal detoxification and storage. However, none of these issues diminishes the importance of bioaccumulation as a factor in assessing the environmental hazard associated with metals. To correctly assess potential hazards, it would be necessary to distinguish between essential nutritional accumulation, that which is sequestering and stored, and accumulation that causes adverse effects. Because BCFs are based on the whole-body concentration, the BCF model does not distinguish between these different forms of bioaccumulation and therefore it would seem unlikely that the criterion would be correlated to adverse effects such as chronic toxicity.

APPLICATION OF BCF TO METAL BIOACCUMULATION DATA

Methods

In this work, we have reviewed literature data to evaluate the relationships between chronic exposure and metal bioaccumulation in aquatic biota in terms of both whole-body metal concentration and BCF. The goal was to assess practical aspects of using BCFs as a criterion for hazard identification. Although BCFs are sometimes provided in the literature, many metal-exposure studies reporting whole-body concentrations do not include calculated BCF values. However, it is often possible to calculate BCF from whole-body concentration data and exposure or body concentration from BCF and exposure data. The data we reviewed were available from experimental exposures (BCF) and reports of samplings from natural environments (BAF). Because BCFs and BAFs are calculated in an identical manner [7] and are considered similarly in the regulatory context, we did not distinguish between these two types of data sources except in a few specific cases where an in-depth attempt was made to explain anomalous and/or variable data.

We collected and evaluated waterborne-exposure data on Zn, Cd, Cu, Pb, Ni, Ag, Hg, and hexachlorobenzene (HCB; $C_6H_4Cl_2O$). These substances were chosen for practical as well as theoretical reasons with the availability of reasonable amounts of suitable information as the primary consideration. Data were collected from the primary literature with the help of common sources such as the AQUIRE database [73] as well as Jarvinen and Ankley [74] being used to identify additional studies. Zn, Cd, Cu, Pb, Ni, and Ag were included in this study because these represent metals of general concern in terms of environmental protection and they span the continuum from nutritionally essential, such as Zn, Cu, and Ni, to nonessential, such as Pb and Ag. We included Hg for comparative purposes, as it can occur in the organic methylmercury form, with potentially different bioaccumulation trends from the other metals. Data for Hg were subdivided into studies where biota were exposed to methylmercury and those where they were exposed to inorganic Hg, usually as a chloride or nitrate salt. Hexachlorobenzene was also added to the comparative list as a synthetic organic pollutant. As such, HCB was chosen as an ideal substance, one for which BCF values could be assumed to accurately describe its bioaccumulative nature.

As prerequisites for data suitability, we required exposure and whole-body metal levels measured by accepted analytical techniques and an assessment of exposure in the context of guidelines associated with standard BCF test methodologies [75,76]. In this respect, we considered experimental exposure data to be acceptable only when whole-body concentration data were available and when the exposure duration was at least 28 d for fish and 14 d for invertebrates and plants or shorter periods if equilibrium had been demonstrated. The metal concentrations from biota sampled from natural environments (BAF) were assumed to be at equilibrium, although it must be recognized that often not enough information is given to assess whether or not this has been achieved. When data were available on a dry-weight basis, it was converted to wet weight for BCF calculations with dry-to-wet conversion ratios of 0.1 for algae, plants, and mollusks and 0.2 for arthropods, annelids, and fish [74]. When a range of exposure concentrations was given, the average was used. In a few cases, control exposure levels were reported as below a quantified detection limit, and exposure value at or slightly less than the reported limit of detection was used. A full listing of the data used in this study is presented in tabular format in the Appendix (See SETAC Supplementary Data Archive, Item ETC-22-05-001; http://etc.allenpress.com).

We have analyzed the data to show the relationships between exposure concentration and bioaccumulation in terms of whole-body metal concentration and BCF. To enable comparisons and because of the volume of information, data were sorted into 11 different species groups following the approach used by Jarvinen and Ankley [74], which were aquatic microphytes and algae (designated as algae), annelids, arthropods (other than insects), insects, mollusks, salmons, centrarchids, cyprinids, sticklebacks, killifish, and other fish species. For each metal, the species represented in these groups varied with the information available. For example, when one of the fish species groups, e.g., cyprinids, consisted of a small number of observations, the cyprinids were included in the other fish grouping. Additionally, in a few cases, data for specific metals and species groups were further subdivided to better understand patterns of bioaccumulation. Linear regression of log-transformed data was used to determine the slope and intercept of the best-fit line and to test the slope for significance from zero. For each metal, regression analysis was done within each species group and for all data combined.

The mean BCF value for each metal and associated coefficient of variations (CV) was calculated using all available data. To illustrate the effect of outliers, mean BCF and CV values were also recalculated for some metals after removal of extremely high or low data points. Data for potential exclusion in recalculations were identified as values greater or less than 3 SD from the mean as well as by visual assessment and these data points were clearly identified in the appropriate figure and discussed in the text. Mean BCF values were also calculated over a narrowed exposure range. The narrowed range was chosen to limit the amount of data by approximately 50 to 75% and to bracket the chronic water-quality guidelines and criteria [77–85]. The restricted-range mean BCF values were calculated to evaluate the possibility of linking BCF to an exposure range over which chronic effects would be expected to occur (i.e., environmentally relevant exposure conditions). These restricted exposure conditions were selected to include a concentration range spanning from just below the water-quality or guideline values to just above, and for each metal, the actual range depended on the amount of data available.

As an alternative to BCF, we derived accumulation factors as a parallel measure but calculated as the increase in concentration that results from an increase in exposure [86]. The only data available to calculate these values were studies with two or more exposure concentrations. Hence, the lowest exposure concentration (usually controls) and its associated whole-body concentration were subtracted from higher exposure and concentration values (respectively). The ACF is analogous to a BCF but represents the additional accumulation that results from incremental exposure. As with BCF values, it was assumed that tissue concentrations had reached equilibrium. There were sufficient data to calculate a database of ACF values for Zn, Cd, Cu, and Pb.

HCB accumulation and BCF

The mean BCF of 69,796 for HCB was significantly higher and the CV of 36% was significantly less than for the metals examined (see Table 1). The mean derived from all the data was heavily influence by six data points from the study of Baturo and Lagadic [87], where BCF values were as high as 1,533,000 and all but one of the values was higher than 3 SD above the mean. The exclusion of these six data points reduced the mean BCF by 74% to 18,391 and the CV to only 14%. Limiting the data to the range of 0.1 to 1 μg/L resulted in a 29% increase in the mean BCF to 23,667, but CV remained unchanged at 15% (Table 1).

Bioaccumulation data for HCB clearly showed that, as exposure concentrations increased, body concentrations increased (Fig. 1A, C, and E). Data for mollusks were split into two subgroups, namely those from field studies and those from laboratory studies, the former exhibiting HCB concentrations that were between 2 and 3.5 orders of magnitude greater (Fig. 1A, open squares) than those from laboratory studies (Fig. 1A, filled squares). The concentration-to-exposure relationship for mollusk field studies yielded a slope of 1.71 ± 0.29 ($p < 0.05$, $n = 6$; Fig. 1A), while that of lab studies was 0.68 ± 0.15 ($p < 0.05$, $n = 9$, Fig. 1A). Therefore, both within species groups and overall, the whole-body concentration to exposure relationship was significant (Table 2).

Table 1. Mean bioconcentration factor and bioaccumulation factor (BCF) values and associated standard deviation for seven metals and hexachlorobenzene (HCB). The data for mercury were separated into exposures using methylmercury and those where the exposure substance was an inorganic salt of mercury. Values are shown using all the available data and, for some substances, the effect of outlier data is illustrated by recalculating means with data from specific studies removed. Additionally, the BCF mean values were calculated over a limited range of exposure concentrations, and for each substance, this limited range was chosen to encompass concentrations where chronic toxicity might be expected to occur (based on water-quality guidelines/criteria). The accumulation factor (ACF) is also shown for those metals with sufficient data; standard deviation (SD)

Substance/metal	Variable	Mean	SD	*n*
HCB	BCF: all exposures	69,796	24,888	82
	BCF: all exposures[a]	18,391	2,595	76
	BCF: 0.1–1 μg/L[a]	23,667	3,660	42
Zinc	BCF: all data	3,957	8,771	143
	BCF: all data[b]	3,394	8,216	133
	BCF: 10–110 μg/L	2,941	6,006	45
	BCF: 10–110 μg/L[b]	1,852	3,237	43
	ACF: all data[b]	326	1,462	68
	ACF: all data[bc]	158	233	67
Cadmium	BCF: all data	1,866	4,844	226
	BCF: 0.1–3 μg/L	2,623	6,009	52
	ACF	600	2,510	97
	ACF[d]	352	615	96
Copper	BCF: all data	1,854	4,465	128
	BCF: all data[e]	1,144	1,720	122
	BCF: 1–10 μg/L	1,224	1,835	50
	ACF[e]	660	865	52
	ACF[ef]	456	659	46
Lead	BCF: all data	598	1,102	66
	BCF: 1–15 μg/L	410	647	14
	ACF	350	431	33
Nickel	BCF: all data	1,613	8,411	52
	BCF: all data[g]	157	135	49
	BCF: 5–50 μg/L[g]	106	53	27
	ACF[g]	39	112	6
Silver	BCF: all data	1,233	2,338	29
	BCF: 0.4–5 μg/L	884	484	17
Mercury, inorganic	BCF: all data	4,955	10,109	60
	BCF: 0.1–1 μg/L	14,550	15,859	15
Mercury, methyl	BCF: all data	8,952	24,675	53
	BCF: 0.1–1 μg/L	9,023	25,929	39

[a] Data from Baturo and Lagadic [87] removed.
[b] Outliers from Shuster and Pringle [89] as well as Mirenda [90] removed; see Figure 2 and text.
[c] Extreme value of Burbidge [160] removed.
[d] Extreme value of Pesch and Stewart [161] removed.
[e] Outliers from Shuster and Pringle [89] as well as Winner [117] removed; see Figure 4 and text.
[f] Data from McLusky and Phillips [119] removed.
[g] Data from Wilson [140] removed.

The BCF data revealed a relationship to exposure that was generally invariant (Fig. 1, Table 2). Arthropods were the only species group to show a significant BCF versus exposure slope, and this was a positive relationship. Overall, when all the data were pooled, the regression analysis revealed significant positive slope to the relationship between BCF and exposure concentration (Table 2).

Hexachlorobenzene was included in our assessment as typical of the neutral and lipid-soluble organic substances that fulfill the theoretical context of the BCF model. The organochlorine, which was used as a fungicide and chemical feedstock in manufacturing, is recognized as a persistent and bioaccumulative substance with the potential to biomagnify [88]. Based on these properties, the relatively low variability in the mean BCF, the elevated values of that mean, and the general lack of correlation between exposure concentration and BCF, it is reasonable to suggest that the BCFs illustrate the inherent bioaccumulative nature of HCB and are indicative of the hazard associated with HCB. This illustrates that the BCF model does apply to the substances for which it was designed.

Zinc accumulation and BCF

One of the most notable features of the mean for Zn BCF was the variability (Table 1) as typified by a CV of 223%. Recalculation of the mean with the values of Shuster and Pringle [89] and Mirenda [90] (Fig. 2A and C, open squares) not included resulted in a 14% reduction in BCF for Zn but little change in data variability (CV 242%; Table 1). The data of Shuster and Pringle [89] were not included as they was characterized by extremely high tissue concentrations relative to the exposure concentration. The data of Mirenda [90] had very low tissue concentrations (and consequently low BCF values, ranging from 0.5 to 1.3) for exposure concentrations up to 130,000 μg/L, and some of these data points are off the axis scale of Figure 2A. When the range of exposure values was limited to 10 to 110 μg Zn/L, the range where chronic effects are predicted to begin, the mean BCF value was slightly reduced, to a value of 2,941. Within the reduced exposure range, removal of the outlier data as described above (two data points from Shuster and Pringle [89]) decreased the mean BCF by

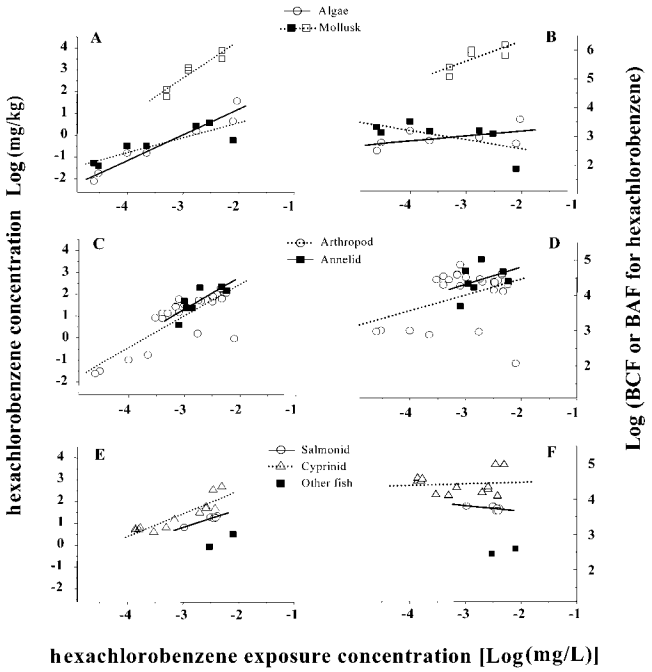

Fig. 1. Effect of chronic hexachlorobenzene (HCB) exposure on HCB content in aquatic biota (**A**, **C**, **E**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for HCB (**B**, **D**, **F**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 2. Note that, due to distinct differences in tissue concentration, data for the mollusk species group were separated into two groups, and these are shown in **A** and **B** as open and filled squares (see text for details). As well, regression lines are not provided for the group designated as other fish.

37%, but the CV nonetheless remained elevated at 175% (Table 1).

The accumulation of Zn by eight species groups is shown in Figure 2A, C, E, and G, and the associated regression variables are given in Table 3. The data clearly illustrate that internal Zn content is well regulated. All eight species groups exhibited very slight increases in whole-body concentration over a dramatic increase in exposure concentration. Only arthropods and cyprinid species showed significant increases in whole-body Zn concentration with increasing exposure level (Table 3 and Fig. 2A, C, E, and G). When all data were pooled across the eight species groups, the overall concentration-to-exposure relationship showed a slight accumulation (Table 3) over the range of exposures, although the coefficient of determination (r^2) was low. From data for all species and all exposures except Shuster [89], the mean Zn content was 46.2 $\pm$ 50.7 μg Zn/g tissue (mean $\pm$ SD, n = 137, CV of 110%). Therefore, the carcass concentration data illustrate clearly that Zn does bioaccumulate in aquatic biota, but there is an inconsistent relationship between exposure concentration and whole-body concentration of Zn. In fact, most species did not show significant increases in Zn accumulation when exposure levels increased, even when exposure concentrations reached those that would be predicted to cause chronic effects. This suggests that adverse effects related to Zn exposure are independent of whole-body accumulation, as recently discussed by Alsop et al. [91].

Due to the general lack of increased whole-body and tissue concentrations at higher Zn exposure levels, the Zn BCF data showed an inverse relationship to exposure concentration (Fig. 2B, D, F, and H and Table 3). The highest BCF values for Zn were at low and naturally occurring exposure concentrations, while the lowest BCF values were at elevated Zn exposure

Table 2. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne hexachlorobenzene (HCB) exposure concentration to HCB content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for HCB ($\log_{10}:\log_{10}$ basis). Data are grouped by species as shown in Figure 1, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data are pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	n
BCF/BAF versus exposure							
By species	Algae	0.17	0.10	3.53*	0.36	0.31	9
	Arthropods	0.44*	0.18	5.35*	0.57	0.18	29
	Annelids	0.49	0.52	5.77*	1.43	0.15	7
	Mollusk[a]	0.19	0.22	4.84	0.69		15
	Salmonids	−0.16	0.10	3.33*	0.26	0.34	7
	Cyprinids	0.04	0.15	4.56*	0.46	0.01	13
Overall	Species mean[a]	0.20	0.14	4.60	0.72		6
	All data	0.35*	0.13	5.03*	0.41	0.08	82
Content versus exposure							
By species	Algae	1.17	0.10	3.53	0.35	0.96	9
	Arthropods	1.44*	0.18	5.35*	0.57	0.71	29
	Annelids	1.49*	0.52	5.77*	1.43	0.62	7
	Mollusk[a]	1.19	0.22	4.84	0.69		15
	Salmonids	0.84*	0.10	3.33*	0.26	0.92	7
	Cyprinids	1.04*	0.15	4.56*	0.46	0.83	13
Overall	Species mean[a]	1.20	0.14	7.60	0.71		6
	All data	1.35*	0.13	5.03*	0.41	0.58	82

[a] Statistical significance of the mean of species was not assessed.

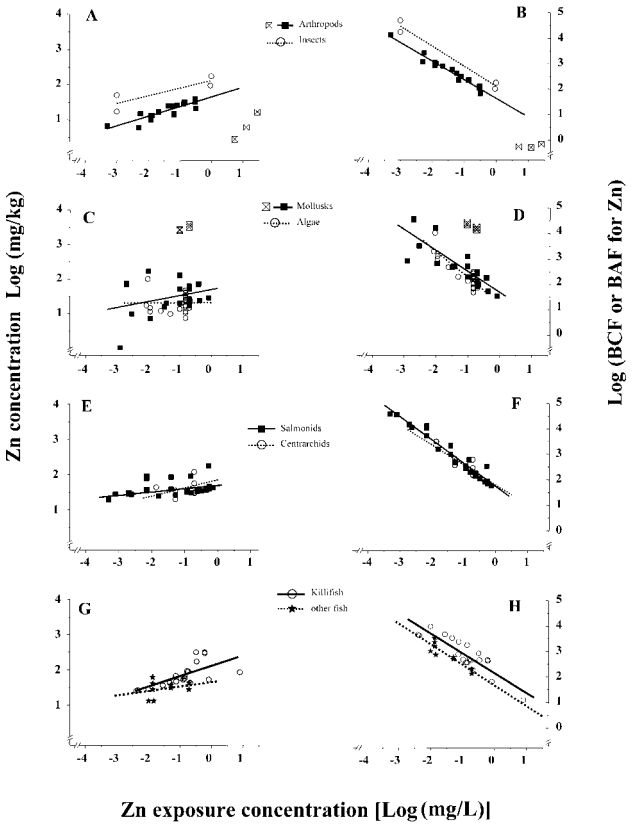

Fig. 2. Effect of chronic Zn exposure on Zn content in aquatic biota (**A**, **C**, **E**, **G**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Zn (**B**, **D**, **F**, **H**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 3. Note that regression lines are not provided for the group designated as other fish and open symbols filled with an X are outliers that were not included in the regression analysis (see text for details).

levels. In all cases, the relationship of BCF to exposure was significant and negative. Therefore, while bioaccumulation of Zn occurs, the BCF for Zn should not be considered as a measure that describes this Zn bioaccumulation. In fact, Zn BCF values are much more closely correlated to Zn exposure concentration than they are with bioaccumulation (see coefficients of determination; Table 3). In terms of aquatic hazard classification, neither BCF nor body concentration seem to be reliable indicators of the potential for adverse effects. This is in agreement with both Alsop et al. [91] and Galvez et al. [92], who concluded that regulatory strategies based on total tissue Zn concentrations would not be successful. Taken together, the variability in mean Zn BCF values and the inverse relationship indicate that Zn BCF is not an intrinsic property of Zn.

The Zn data illustrate that, when BCF criteria are applied as threshold values for hazard identification, it may lead to conclusions that are inconsistent with toxicological data. In this context, the inverse relationship of BCF to exposure erroneously suggests that hazard is less at elevated exposure concentrations. However, water-quality guidelines and criteria [78,85] provide for waterborne Zn concentrations above which adverse impacts on aquatic biota can be expected and therefore increasing exposure results in increased hazard. This is supported by data showing tissue accumulation as exposure concentration increases. Thus, the conclusions drawn from the measured toxicological data as well as tissue concentrations and those derived from the application of BCF threshold criteria values are inconsistent.

The physiological basis for the inverse relationship of BCF to Zn exposure concentration arises from Zn uptake and control mechanisms. At low environmental Zn levels, animals are able to sequester and retain Zn in tissues for essential functions [93]. When Zn exposure levels are chronically elevated, aquatic animals are able to control bioaccumulation. There is clear evidence that many species actively regulate their body Zn concentrations, including Crustacea, such as *Homarus gammarus*, *Carcinus maenas*, *Maia squinado*, *Crangon crangon*, *Palaemon elegans*, *P. serratus* [94], and *Austropotamobius pallipes* [95,96]; the oligochaetes *Lumbriculus variegatus* and *Neries diversicolor* [96]; mussels such as *Mytilus edulis*, *Dreissena polymorpha*, *Unio pictorum*, and *Velesunio ambiguus* [96–98]; the gastropod *Nucella reticulatus* [99]; as well as *Oncorhynchus mykiss* [91,100]. As it does with Cu, the amphipod *Echinogammarus pirloti* does not actively excrete excess Zn but takes it up at a low net rate relative to its body growth rate [94], thus illustrating another burden control strategy. Detoxification both through binding to proteins such as metallothionein [44,69] and storing as Zn phosphate granules [94,101,102] has also been discussed. While the chironomids *Chironomus riparius* and *Stictochironomus histrio* do not appear to actively regulate their zinc body concentrations, Zn is lost with each cast exuvium [103]. This process may effectively reduce body concentrations but possibly only on an intermittent basis under an ongoing exposure and may also occur for other biota that molt.

Although total Zn carcass concentration is not well correlated with Zn exposure, radiotracer studies in rainbow trout have shown that chronic waterborne Zn^{2+} exposure results in dramatic and complex alterations in gill uptake kinetics [104] and that these are linked to Ca^{2+} dynamics [105]. Included in the changes are a decreased affinity and an increase in the total number of binding sites [104]. While these changes appear to be reliable indicators of exposure, it is unclear how they can be exploited for environmental hazard classification and therefore further development is required.

There is little evidence to suggest that metals such as Zn biomagnify in aquatic food webs. For example, Leland and Kuwabara [106] state that the classic idea of biomagnification, developed from studies of DDT, does not hold for most metals. Absorption of metals from food is highly variable because of the variety of free and bound forms of the ions that are possible in food [107]. In addition, competition between related elements for active transport sites is also variable. Although there is no evidence that zinc biomagnifies in aquatic systems, it is an essential element that many organisms accumulate to high levels and elevated accumulation rates may sometimes be mistaken as trophic transfer [108].

Cadmium accumulation and BCF

At 1,866, the mean BCF for Cd was lower than that of Zn (at 3,957), although it was similar with respect to variability (CV of 265%). Limiting the range of exposure values to 0.1 to 3 μg Cd/L increased the mean BCF to about 2,600 and the SD to about 6,000 (CV = 230%; Table 1). Whether the full data set or the limited-exposure range data was used, high variability was a key feature of the mean BCF values for Cd.

An increase in whole-body Cd concentration was apparent in most of the species examined (Fig. 3A, C, E, and G). Anal-

Table 3. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Zn exposure concentration to Zn content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Zn ($\log_{10}:\log_{10}$ basis). Data are grouped by species as shown in Figure 2, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
By species	Algae	−1.00*	0.12	1.31*	0.14	0.74	24
	Insects	−0.79*	0.09	2.11*	0.19	0.98	4
	Arthropods[a]	−0.73*	0.04	1.64*	0.07	0.96	17
	Mollusks[a]	−0.83*	0.13	1.70*	0.19	0.71	20
	Salmonids	−0.92*	0.04	1.73*	0.06	0.96	29
	Centrarchids	−0.80*	0.20	1.78*	0.24	0.69	9
	Killifish	−0.84*	0.10	2.14*	0.12	0.78	20
	Other fish	−0.87*	0.16	1.63*	0.26	0.80	9
Overall	Species mean[b]	−0.85	0.03	1.76	0.09		8
	All data[a]	−0.84*	0.03	1.74*	0.06	0.77	132
Content versus exposure							
By species	Algae	0.002	0.12	1.31*	0.14	0.00001	24
	Insects	0.21	0.09	2.11*	0.19	0.76	4
	Arthropods[a]	0.27*	0.04	1.64*	0.07	0.74	17
	Mollusks[a]	0.17	0.13	1.70*	0.19	0.10	20
	Salmonids	0.07	0.04	1.71*	0.07	0.12	29
	Centrarchids	0.20	0.20	1.78*	0.24	0.12	9
	Killifish	0.30*	0.08	2.10*	0.09	0.45	20
	Other fish	0.13	0.16	1.63*	0.26	0.08	9
Overall	Species mean[b]	0.17	0.04	1.75	0.09		8
	All data[a]	0.17*	0.04	1.72*	0.05	0.14	132

[a] Outlier data points were not included in the regression analysis; see Figure 2 and text.
[b] Statistical significance of the mean of species was not assessed.

ysis of the trends was complicated by the variation in the data, particularly for fish groups such as salmonids (Fig. 3E) and sticklebacks (Fig. 3G), where a significant accumulation was absent and coefficients of determination were low (Table 4). Killifish and aquatic insects showed the highest accumulation as exposure concentration increased (Fig. 3G and A, respectively).

In spite of significant increases in body concentration over the range of exposure concentrations for a number of species groups, the relationship of Cd BCF to Cd exposure concentration was negatively correlated (Fig. 3B, D, F, and H and Table 4) except for killifish. This negative relationship between BCF and exposure was generally lower than for Zn but was nonetheless significant. The highest BCF values for Cd were at low and naturally occurring exposure concentrations. The inverse BCF to exposure relationship illustrates that, although Cd concentration increases with exposure, internal accumulation does not rise as quickly as exposure levels and therefore indicates a significant degree of control over Cd accumulation. Therefore, bioaccumulation of Cd does occur, but as with Zn, the high variability of the mean BCF values and the negative correlation between exposure and BCF indicates that BCF for Cd is neither an intrinsic property nor an optimum descriptor of Cd bioaccumulation.

It is generally agreed that the bioaccumulation of Cd does not serve a nutritional purpose, although recently this notion has been challenged for some marine organisms [109]. As seen from the relative differences in the scales of Zn and Cd body concentration axes in Figures 2 and 3, accumulations of Cd tend to be much lower than those of nutritionally essential elements such as Zn. Although there is little evidence of active regulation of internal Cd concentrations, it is clear from the inverse BCF-to-exposure relationship that some physiological control over Cd accumulation can be achieved. For example, reduced branchial uptake in response to exposure has been demonstrated in rainbow trout [110–112]. As well, growth dilution of Cd stores in decapods shows that a form of regulation is possible [102]. Detoxification of accumulated Cd is also common. For example, binding of Cd to low molecular weight proteins such as metallothionein occurs in many animals [55], including the rainbow trout [111], the barnacle *Semibalanus balanoides* [102], the scallop *Mizuhopecten yessoensis* [113], the marine gastropod *Nassarius reticulatus* [99], and possibly *Daphnia magna* [114]. An example of an animal with tissue-specific granule storage of Cd is the marine isopod *Idotea baltica*, in which granules are stored in the hepatopancreas [115]. Storage of Cd as granules in the kidney is also common in vertebrates. These studies illustrate that carcass concentrations of Cd significantly above normal levels can be tolerated and physiological processes adapted to result in acclimation. A mechanistic understanding of chronic bioaccumulation control mechanisms for Cd is incomplete and, as such, how this information might be included in a model for hazard classification is not clear. However, it is deserving of further efforts.

As discussed in the review by Suedel et al. [108], there is little evidence to suggest that cadmium biomagnifies in aquatic systems. For example, Ferard et al. [116] examined the transfer

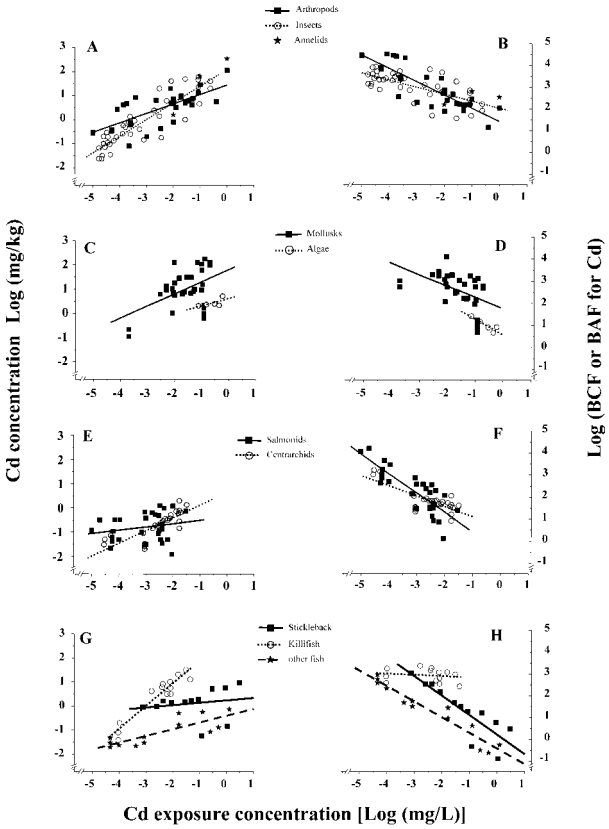

Fig. 3. Effect of chronic Cd exposure on Cd content in aquatic biota (**A**, **C**, **E**, **G**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Cd (**B**, **D**, **F**, **H**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 4.

of cadmium in an experimental food chain consisting of algae (*Chlorella vulgaris*), zooplankton (*Daphnia magna*), and fish (*Leucaspias delineatus*) and illustrated that Cd concentrations decreased with increasing trophic level.

Copper accumulation and BCF

The mean BCF value for Cu was similar to that of Cd (Table 1), and again, the variability of the BCF data was very high (CV = 241%). Within the data set, the study of Shuster and Pringle [89] provided four relatively high BCF values (Fig. 4C and D, open triangle), while two very low BCF values came from the study of Winner [117] (both values <1.0). Note that the body burden data from this latter study are shown in Figure 4A (open circles marked with an x), but the corresponding BCF values were off the scale of the axis in Figure 4B. The removal of these six data points reduced the mean BCF by 39% and associated variability by 62% (Table 1, CV = 152%). Selecting BCFs from Cu exposures over the limited concentration range of 1 to 10 μg/L did not result in a change in either the mean or the variability (CV = 150%).

The accumulation data (Fig. 4A, C, E, and G and Table 5) for Cu illustrate that, except for the algae and other fish groups, all species groups experienced a generalized increase in carcass concentration as exposure levels of Cu increased. The accumulation trend for Cu in algae was not significant, but the range of exposure concentrations was limited and data were edited for an outlier (Fig. 4G). The data point not included in the analysis for algae (labeled as algae but including diatoms, macrophytes, and other plants) was for the aquatic moss *Rhychostegium riparioides* from the study of Mersch et al. [118]. The data on Cu tissue concentrations in mollusks required an in-depth examination primarily due to the values for the oyster *Crassostrea virginica* reported by Shuster and Pringle [89]. These values (Fig. 4C, open triangles) were omitted from the regression analysis (Table 5) because they were ninefold higher than the next highest value and almost 50 times above the mean body concentration for other mollusks of 14.5 ± 17.8 mg/kg ($n = 30$). As this was the only datum we had from *C. virginica* in our database, it is not known if the Cu hyperaccumulation observed in the Shuster and Pringle [89] study is a species-specific trait. A detailed examination of Cu accumulation with supplemental data from, e.g., estuarine monitoring programs may shed light on differences between this species and other mollusks.

To explain the variability in the remaining mollusk data for Cu concentration, it was further subdivided to show mussels (*Mytilus edulis* and *Dreissena polymorpha*; Fig. 4C, filled squares) as distinct from other mollusk species (Fig. 4C, open circles). The log-log concentration to exposure relationship for the mussel subgroup had a slope and intercept of 0.79 ± 0.24 and 2.60 ± 0.40 (for both, $p < 0.05$, $n = 13$; Fig. 4C, solid line), respectively, while that of the remaining mollusks was 0.76 ± 0.20 and 1.95 ± 0.46 (for both, $p < 0.05$, $n = 17$; Fig. 4C, dotted line). Although data are limited, mussel species may have a relatively lower Cu body concentration compared with other mollusk species. However, in terms of bioaccumulation during exposure, the similarity of the slope values indicates that uptake patterns are similar. For this reason, the regression data reported in Table 5 are for all mollusk data except for Shuster and Pringle [89].

As with the mollusk data, the annelid species group had two distinct data clusters for Cu body concentrations that were further subdivided into a high accumulation group and a lower accumulation group (Fig. 4A). The high accumulation group was dominated by the study of McLusky and Phillips [119] (Fig. 4A, filled squares). Data for a lower accumulating group were from three studies, Young et al. [120] (Fig. 4A, filled triangles), Pesch and Morgan [121], and Milanovich et al. [122] (these latter two studies shown as filled stars in Fig. 4A). After examining the available information, we conducted regression analyses on individual studies as opposed to grouped data. The McLusky and Phillips [119] study provided data on *Phyllodoce maculata* and yielded a concentration-to-exposure relationship with a slope of 0.62 ± 0.10 ($p < 0.05$, $n = 7$). The data of Young et al. [120] for *Eudistylia vancouveri* provided a slope of 0.36 ± 0.12 (not significant, $n = 4$). Note that the analysis of the arthropod data did not include the Winner [117] data, which were characterized by very low Cu concentrations in *Daphnia magna* in spite of relatively high exposure levels (Fig. 4A, open symbols marked with an X). The study of Winner [117] demonstrated the ability of dissolved organic carbon to reduce the bioavailability of Cu, perhaps explaining the low body concentrations.

The BCF values for Cu are shown in Figure 4B, D, F, and H and the associated regression analysis variables are given in Table 5. Algae (Fig. 4H), arthropods (Fig. 4B), and salmonids (Fig. 4F) each showed a significant and negative slope for the BCF-to-exposure relationship (Table 5). The mollusk and the other fish species groups (Fig. 4D and H, respectively) had negative BCF versus concentration slopes but, due to the variability in the data, these were not significant. For the an-

Table 4. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Cd exposure concentration to Cd content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Cd ($\log_{10}:\log_{10}$ basis). Data are grouped by species as shown in Figure 3, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
	Algae	−0.72*	0.20	0.59*	0.14	0.81	5
	Insects	−0.32*	0.06	2.06*	0.19	0.46	40
	Arthropods	−0.61*	0.07	1.43*	0.19	0.74	31
	Mollusks	−0.50*	0.17	1.79*	0.29	0.21	36
	Salmonids	−0.87*	0.11	−0.40	0.36	0.69	29
	Centrarchids	−0.47*	0.08	0.64*	0.22	0.59	26
	Sticklebacks	−0.90*	0.17	0.23	0.28	0.73	12
	Killifish	−0.05	0.10	2.81*	0.26	0.03	13
By species	Other fish	−0.72*	0.06	−0.40*	0.17	0.93	13
	Species mean[a]	−0.57	0.09	0.97	0.37		9
Overall	All data	−0.49*	0.04	1.25*	0.12	0.38	209
Content versus exposure							
	Algae	0.28	0.20	0.59*	0.14	0.41	5
	Insects	0.68*	0.06	2.06*	0.19	0.79	40
	Arthropods	0.39*	0.07	1.43*	0.19	0.53	31
	Mollusks	0.50*	0.17	1.79*	0.29	0.21	36
	Salmonids	0.13	0.11	−0.40	0.36	0.05	29
	Centrarchids	0.53*	0.08	0.64*	0.22	0.64	26
	Sticklebacks	0.10	0.17	0.23	0.28	0.03	12
	Killifish	0.94*	0.10	2.81*	0.26	0.90	13
By species	Other fish	0.28*	0.06	−0.40*	0.17	0.66	13
	Species mean[a]	0.43	0.09	0.97	0.37		9
Overall	All data	0.51*	0.04	1.25*	0.12	0.41	209

[a] Statistical significance of the mean of species was not assessed.

nelid species group, data were split (described above; see Fig. 4B) into that for *P. maculata* and *E. vancouveri* and the slopes of the BCF versus exposure concentration relationship for both species was significant at -0.38 ± 0.10 ($p < 0.05$, $n = 7$) and -0.64 ± 0.12 ($p < 0.05$, $n = 4$), respectively. The mean of these two values is presented in Table 5.

The Cu accumulation and BCF data clearly show that aquatic animals are able to modulate Cu bioaccumulation, as would be expected from a nutritionally required element. Although Cu accumulated as exposure concentration increased, for most species groups, the increase in concentration was proportionally less than that of exposure, thus illustrating an ability to regulate and producing a negative relationship between exposure and BCF. The ability to regulate internal Cu concentrations has been demonstrated in a wide variety of aquatic organisms, including marine species such as *Palaemon elegans*, *Crangon crangon*, *Homarus gammarus*, *Carcinus maenas*, and *Echinogammarus pirloti*, as reviewed by Rainbow [94], as well as *Neanthes arenaceodentata* [121] and *Eudistylia vancouveri* [120]. It has also been shown that freshwater fish such as the rainbow trout actively regulate Cu via sequestering into the liver and elimination via the bile, a process that involves Cu-specific transport mechanisms [123,124]. Detoxification of Cu through binding to metallothionein-like proteins has also been shown to be of significance in both marine and freshwater organisms [44,55,61,102]. In addition, detoxification and storage of Cu in granules has been shown [102,125], and this may explain the relatively shallow slope of BCF versus exposure relationship for mussels and other mollusks (Fig. 4D). As noted earlier, the BCF measure does not distinguish among physiologically essential Cu, internally stored and detoxified Cu, and excess accumulation that can produce adverse effects. As with Zn and Cd, the variability of mean BCF values and the negative correlation between BCF and exposure concentration indicates that BCF is not an intrinsic property of Cu.

There is no evidence that copper biomagnifies in aquatic systems, although it does appear to be transferred through food chains [108]. As reviewed by Lewis and Cave [126], copper accumulation in aquatic organisms at different trophic levels varies considerably and depends on several factors, including the physiological requirements of the organism, the source of copper, exposure duration, migration patterns, and chemical speciation.

Lead accumulation and BCF

The mean BCF for Pb was the lowest of all the metals on which we collected data (Table 1). As with the other metals, there was considerable variation around the mean with a CV of 184%. Narrowing the range of exposure values to from 1 to 15 μg Pb/L decreased the mean BCF value by about 30%, and variation was approximately the same (CV of 158%; Table 1).

All species groups displayed increases in body concentrations for Pb as exposure levels increased (Fig. 5). The regression analysis (Table 6) showed a distinct variation in ac-

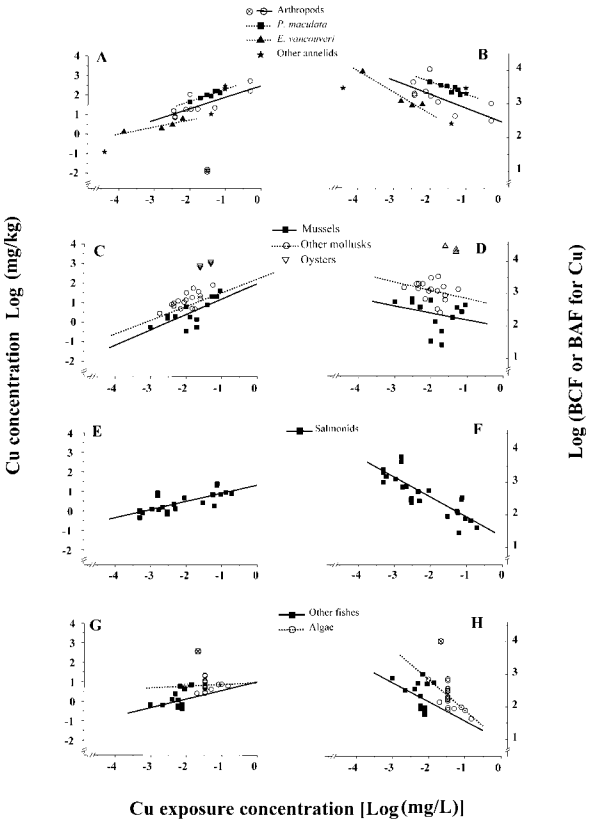

Fig. 4. Effect of chronic Cu exposure on Cu content in aquatic biota (**A**, **C**, **E**, **G**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Cu (**B**, **D**, **F**, **H**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 5. Note that regression lines are not provided for the group designated as oysters and open symbols filled with an X are outliers that were not included in the regression analysis (see text for details).

cumulation rates, being low for fish, high for algae, and intermediate for arthropods (Fig. 5A; Table 6). The analysis of the arthropod data was done excluding the single elevated value of Brown [127], where tissue concentrations were 175-fold higher than any of the other arthropod values (Fig. 5A, marked open square). As with Cu, the data available for mollusks showed different accumulation patterns, and therefore this group was separated. A relatively high accumulation group, from the study by Schulz-Baldes [128] with *Mytilus edulis* (Fig. 5C, filled squares), was characterized by a relatively low rate of accumulation, with a slope of 0.47 ± 0.07, $n = 3$ (Fig. 5C, filled squares and dashed line). Another mussel study, by Kraak et al. [129], measured Pb accumulation in *Dreissena polymorpha* (Fig. 5C, open squares) and yielded an exposure-to-body concentration relationship with a slope of 0.92 ± 0.09 (significant, $n = 5$). The average of the two mussel studies is presented in Table 6.

When all the data were pooled, the overall body concentration-to-exposure relationship from all data produced a slope of 1.0 (Table 6). While this suggests that each unit increase in exposure was matched by an equal increase in concentration, this was clearly an artifact of pooling the data. An examination of data on a species and individual study basis reveals that there is some degree of control over accumulation (Table 6, overall concentration by species). The disparity between results of the regression analysis for the overall data set and that of the species groups would appear to be due to the range of body concentrations that occur over the exposure range. When all of the data were pooled together, the subtleties of the actual exposure to accumulation relationships are not illustrated.

The BCF versus exposure values showed a significant and inverse relationship for all species groups (Fig. 5B and D and Table 6), with algae and fish exhibiting the lowest and highest BCF rates of change with exposure, respectively. For subgroupings within the mollusk data set (as above), the individual studies with *M. edulis* [128] (Fig. 5D, filled squares) and *D. polymorpha* [129] (Fig. 5D, open squares) produced BCF versus exposure slopes of −0.52 ($n = 3$) and −0.08 ($n = 5$), respectively, and the average of these two is presented in Table 6. As with the body-concentration data, the overall pooling of all of the Pb BCF data did not provide an accurate reflection of the data (Table 6).

No studies were identified in the scientific literature demonstrating that Pb tissue concentrations can be actively regulated by aquatic biota. However, Pb will bind to metallothionein and also probably has a higher affinity for other metabolic ligands, as it is often associated with deposited inorganic granules with high concentrations of calcium [47]. Hopkin and Nott [130] demonstrated that the shore crab (*Carcinus maenas*) detoxifies lead in calciferous granules in the midgut gland. The detoxification and storage of Pb in shellfish has been suggested for the zebra mussel *Dreissena polymorpha* [129,131], the blue mussel *Mytilus edulis* [132,133], the Eastern oyster *Crassostrea virginica* [89,134,135], and the softshell clam *Mya arenaria* [134]. Ideally, a bioaccumulation measure linked to the potential impact of Pb should be able to distinguish between accumulated Pb that is detoxified and stored and that which is available to cause toxic impacts. Therefore, Pb bioaccumulation is characterized by the storage of detoxified forms, an inverse relationship between BCF and exposure concentrations, and considerable variability associated with the mean BCF values.

According to reviews by Eisler [136] and Suedel et al. [108], there is no evidence that lead biomagnifies in higher trophic levels of either freshwater or marine food webs. As reviewed by Demayo et al. [137], dietary lead may be virtually unavailable to fish such as rainbow trout. This is supported by lab and field studies [138,139] that show decreasing Pb concentrations with increasing trophic level. While dietary Pb may be unavailable to some species, it must be recognized that, for others, dietary Pb can be taken up; however, the very low efficiency of uptake [138] ensures that it does not biomagnify.

The data for Ni were somewhat limited compared with those for Zn, Cd, Cu, and Pb. Nonetheless, there were sufficient data to calculate a mean BCF, which was similar to Cu and Cd but exceptionally variable, with a CV of 521% (Table 1). The exceptional variation for the Ni BCF was associated with the study of Wilson [140], using the bivalve *Cerastoderme edule*, where BCF values were as high as 59,600 (Fig. 6D, see marked open squares). The removal of these three exceptional BCF values resulted in a 10-fold decrease in the mean BCF value and in a SD that was approximately the same as the mean, to yield a CV of 86%. When the exposure concentrations were limited to the range 5 to 50 μg/L, the mean BCF value was further reduced by 33% (Table 1).

Nickel accumulation and BCF

The Ni accumulation data are shown in Figures 6A and C. Although the data are somewhat limited, there is an overall trend of increased body concentrations as exposure concentrations increased. Both overall and within each of the indi-

Table 5. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Cu exposure concentration to Cu content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Cu ($\log_{10}:\log_{10}$ basis). Data are grouped by species as shown in Figure 4, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
	Algae[a]	−0.92*	0.24	0.93*	0.35	0.44	21
	Annelids[b]	−0.51	0.11	2.17	0.26	0.86	11
	Arthropods	−0.41*	0.12	2.46*	0.21	0.55	12
	Mollusks[a]	−0.30	0.21	2.19*	0.41	0.07	30
	Salmonids	−0.58*	0.08	1.30*	0.18	0.72	25
By species	Other fish	−0.56	0.38	0.97	0.87	0.12	17
	Species mean[c]	−0.55	0.09	1.67	0.38		6
Overall	All data[a]	−0.30*	0.07	2.10*	0.15	0.13	121
Content versus exposure							
	Algae[a]	0.08	0.24	0.93*	0.35	0.01	21
	Annelids[b]	0.49	0.11	2.17	0.26	0.85	11
	Arthropods	0.59*	0.12	2.46*	0.21	0.71	12
	Mollusks[a]	0.70*	21	2.19*	0.41	0.29	30
	Salmonids	0.42*	0.08	1.30*	0.18	0.56	25
By species	Other fish	0.44	0.38	0.97	0.87	0.08	17
	Species mean[c]	0.45	0.09	1.67	0.38		6
Overall	All data[a]	0.70*	0.07	2.10*	0.15	0.45	124

[a] Outlier data points were not included in the regression analysis; see Figure 4 and text.
[b] Mean of two studies used; see text and Figure 4 for details.
[c] Statistical significance of the mean of species was not assessed.

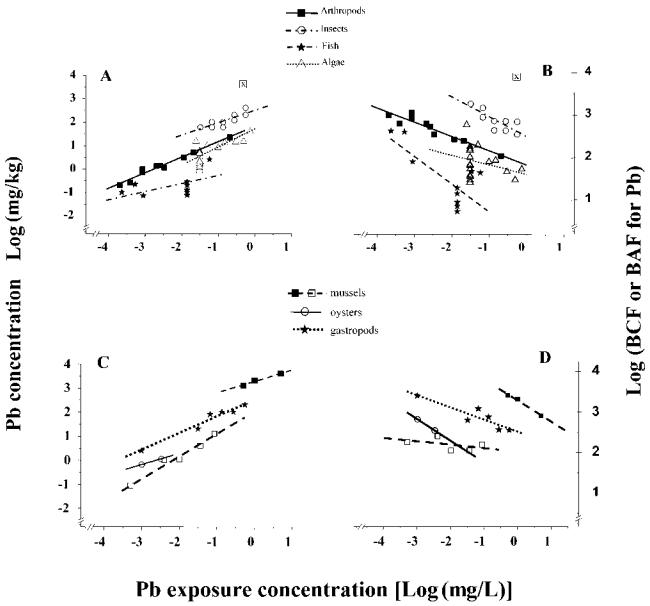

Fig. 5. Effect of chronic Pb exposure on Pb content in aquatic biota (**A**, **C**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Pb (**B**, **D**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 6. Note that, due to distinct differences in tissue concentrations, data for the mussel species group was separated into two groups, and these are shown in **C** and **D** as open and filled squares (see text for details).

vidual species groups, there was a significant slope associated with the accumulation versus exposure relationship (Table 7). However, as with the other metals, these accumulation slopes were less than unity and therefore BCF was inversely correlated with exposure. The negative relationship between Ni BCF and Ni exposure concentration was significant in all cases tested (Table 7). The data illustrate that, although Ni bioaccumulates, the resulting BCF values are inversely correlated with exposure and therefore BCF cannot be considered as an inherent property of Ni, and this undermines it use in hazard identification.

As a nutritionally essential element, the fact that the bioaccumulation of Ni occurs is unequivocal. No studies were identified that illustrated active regulation of Ni tissue concentrations in aquatic biota, although Ni has not been studied to the same extent as some of the other essential metals. An improved understanding of the mechanisms and physiology of Ni bioaccumulation and relationships to chronic impact are clearly required.

There is no evidence that nickel biomagnifies in aquatic food webs [108]. Watras et al. [141] studied nickel accumulation in *Daphnia magna* fed nickel-enriched algae and demonstrated that nickel is not transferred significantly between trophic levels. In a field study reported by Mathis and Cummings [142], nickel concentrations were also found to decrease with increasing trophic level in a food web characterized by clams, oligochaetes, omnivorous fish, and carnivorous fish, again demonstrating that food chain transfer of nickel is minimal.

Silver accumulation and BCF

Data on Ag BCFs were relatively limited; however, as with the other metals, the BCF values were highly variable, with a

Table 6. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Pb exposure concentration to Pb content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Pb ($\log_{10}$: $\log_{10}$ basis). Data are grouped by species as shown in Figure 5, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
By species	Algae	−0.23	0.18	1.60*	0.24	0.09	18
	Insects	−0.47*	0.11	2.50*	0.11	0.69	10
	Arthropods[a]	−0.34*	0.04	1.83*	0.11	0.88	10
	Gastropods	−0.30*	0.07	2.51*	0.11	0.81	6
	Mussels[bc]	−0.30	0.08	2.65	0.11	0.55	8
	Fish	−0.65*	0.19	0.07	0.44	0.56	11
Overall	Species mean[b]	−0.38	0.06	1.86	0.40		6
	All data	0.01	0.09	2.33*	0.17	0.0001	66
Content versus exposure							
By species	Algae	0.77*	0.18	1.60*	0.24	0.53	18
	Insects	0.53*	0.11	2.50*	0.11	0.74	10
	Arthropods[a]	0.66*	0.04	1.83*	0.11	0.96	10
	Gastropods	0.70*	0.07	2.51*	0.11	0.92	6
	Mussels[bc]	0.70	0.08	2.65	0.11	0.98	8
	Fish	0.35	0.19	0.07	0.44	0.26	11
Overall	Species mean[b]	0.62	0.06	1.86	0.04		6
	All data	1.01*	0.09	2.33*	0.17	0.66	66

[a] Outlier data point of Brown [127] was not included in the regression analysis; see Figure 5 and text.
[b] Statistical significance of the mean of species was not assessed.
[c] Values for mollusks represent an average of individual species and studies; see Figure 5 and text.

CV of 190% (Table 1). Reducing the Ag exposure range to 0.4 to 5 μg/L resulted in only a 16% decrease in the mean BCF, although the variation was reduced considerably to a CV of 43%. Data on bioaccumulation clearly show that Ag tissue and body concentrations rise with increasing exposure concentration. However, body concentration does not increase in equal proportion to exposure concentration and therefore an inverse relationship between exposure and BCF occurs (Table 8 and Fig. 7).

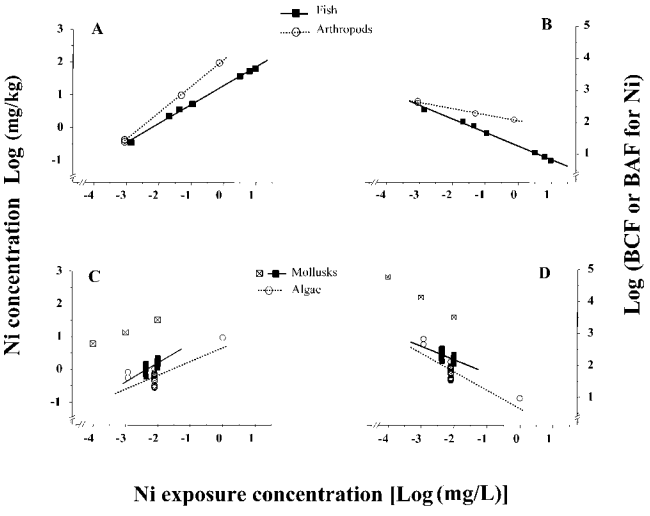

Fig. 6. Effect of chronic Ni exposure on Ni content in aquatic biota (**A**, **C**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Ni (**B**, **D**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for each species group. The regression variables are given in Table 7. Note that, within the mollusk species group, there were outlier data points that were excluded from the regression analysis, and these are shown as open squares filled with an X in **C** and **D** (see text for details).

No studies were identified in the scientific literature demonstrating that Ag tissue concentrations can be actively regulated by aquatic biota. Studies such as those of Bryan [143] suggest that Ag is not actively regulated but rather stored. This premise is supported by studies with rainbow trout and European eel, which illustrate the time course of tissue-specific internal Ag accumulation [144,145]. However, the recent study by N. Bury (Kings College London, UK, personal communication) suggests that Ag-specific transporters may exist in the gill of rainbow trout and opens the possibility of some degree of control over uptake and accumulation.

Mercury accumulation and BCF

The mean BCFs for methylmercury and inorganic Hg (Table 1) were greater than those for other elements assessed but were also highly variable (CVs of 276 and 204% respectively). When all the BCF data were used to calculate these means, the bioaccumulative nature of methylmercury was apparent and its mean BCF was considerably higher (Table 1). Limiting the data to the range of 0.1 to 1 μg/L resulted in no substantive change in mean and CV for methylmercury and a large, nearly threefold, increase in mean BCF for inorganic Hg (CV of 109%). The dramatic increase in mean BCF inorganic Hg was associated with limiting the data to the lower 25% of the exposure range. In other words, 75% of the exposures for inorganic Hg were at concentrations above 1 μg/L.

The bioaccumulation data for Hg show that, in general,

Table 7. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Ni exposure concentration to Ni content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Ni ($\log_{10}:\log_{10}$ basis). Data are grouped by species as shown in Figure 6, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
By species	Algae	−0.58*	0.11	0.65*	0.23	0.66	17
	Arthropods	−0.19*	0.02	2.05*	0.03	0.98	4
	Mollusks[a]	−0.42*	0.14	1.35*	0.31	0.32	20
	Fish	−0.42*	0.02	1.26*	0.02	1.00	7
Overall	Species mean[b]	−0.40	0.08	1.33 ± 0.15			4
	All data	−0.53*	0.07	1.11*	0.16	0.53	51
Content versus exposure							
By species	Algae	0.42*	0.11	0.65*	0.23	0.50	17
	Arthropods	0.81*	0.02	2.05*	0.03	1.00	4
	Mollusks[a]	0.58*	0.14	1.35*	0.31	0.49	20
	Fish	0.58*	0.02	1.26*	0.02	1.00	7
Overall	Species mean[b]	0.60	0.08	1.33 ± 0.15			4
	All data	0.47*	0.07	1.11*	0.16	0.48	51

[a] Outlier data points from Wilson [140] were not included in the analysis; see Figure 6 and text.
[b] Statistical significance of the mean of species was not assessed.

tissue concentrations of Hg increased with increased exposure, although the trends were not always clear (Table 9 and Fig. 8, C, E, and G). For inorganic Hg exposures, the cyprinid species showed a significant positive relationship between exposure and concentration (Fig. 8E, open squares and solid line, and Table 9). Although not tested due to the paucity of data, salmonids would appear to have a similar relationship [146] between whole-body concentrations and inorganic Hg exposure (Fig. 8E, open circles). In contrast, mollusks showed a significant reduction in tissue concentration as inorganic Hg exposure increased (Fig. 8C, open circles and solid line, and Table 9). Bioaccumulation data for methylmercury were also equivocal, showing a significant and positive relationship between exposure and accumulation for some fish species groups but not for other species groups (Fig. 8A, C, E, and G, filled symbols). There was a relative lack of data on Hg exposures in invertebrates and therefore the insect, annelid, and arthropod groups were pooled for regression analysis. The relationship between BCF and exposure concentration was generally negative, although there was considerable variability in some species groups; a few others, such as cyprinids exposed to inorganic Hg and centrarchids exposed to methylmercury, did not have an inverse relationship (Table 9, Fig. 8B, D, F, H).

As with the other metals, water chemistry influences Hg bioavailability, but additionally, accumulation is strongly affected by methylation/demethylation reactions, which are microbially mediated [147–149]. It is generally agreed that methylmercury is the most bioaccumulative form of Hg and it is known to biomagnify in aquatic food webs [150–155], and this was seen in the mean BCFs when all the data were used. Overall, the high BCF values are a function of high assimilation efficiency, particularly of the neutral and lipid-soluble methylmercury, and the fact that, for fish at least, the eliminated very slowly relative to uptake [156].

Given the neutral and lipophilic nature as well as the biomagnification potential of methylmercury, our hypothesis was that Hg bioaccumulation would be consistent with the theoretical BCF model (i.e., relatively constant over the exposure range, as HCB was). While this occurred for a few species groups, there was considerable variability and also an unexpected negative relationship between BCF and exposure for a number of groups. The data available are not extensive, but it

Table 8. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Ag exposure concentration to Ag content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Ag ($\log_{10}:\log_{10}$ basis). Data are grouped to show the overall relationship when all data are pooled together as shown in Figure 7

		Regression variables					
Variable	Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure							
Overall	All data	−0.54	0.07	1.22	0.23	0.64	23
Content versus exposure							
Overall	All data	0.46	0.07	1.22	0.23	0.72	23

Table 9. Regression coefficients (slope and intercept given with standard error of means [SEM]) for the linear relationship of waterborne Hg exposure, either as methylmercury (CH_3-Hg) or inorganic Hg salt, to the Hg content in aquatic biota as well as bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Hg ($\log_{10}$: $\log_{10}$ basis). Data are grouped by species as shown in Figure 8, where the associated best fit lines are shown. Overall relationships for slope and intercept are given as either the mean of species groups or the linear regression when all data were pooled. The number of observations is shown for each relationship, and * indicates that the slope or intercept are significantly different ($p < 0.05$) from zero as determined by the regression analysis

	Regression variables							
Variable		Species group	Slope	SEM	Intercept	SEM	Coefficient of determination	*n*
BCF/BAF versus exposure								
By species	Hg salt	Insects, annelids, and arthropods	−0.39	0.42	2.04	0.42	0.09	11
	Hg salt	Mollusks	−1.45*	0.11	−0.32	0.20	0.37	30
	Hg salt	Cyprinids	−0.20	0.14	2.83*	0.43	0.71	15
	CH_3-Hg	Salmonids	−0.84*	0.26	1.02	0.79	0.46	14
	CH_3-Hg	Centrarchids	0.07	0.30	3.23*	0.95	0.01	7
	CH_3-Hg	Other fish	−0.38	0.25	1.93*	0.71	0.10	22
Overall	Hg salt	All data	−1.00*	0.12	0.43	0.24	0.61	60
	CH_3-Hg	All data	−0.17	0.11	2.83*	0.35	0.05	49
Content versus exposure								
By species	Hg salt	Insects, annelids, and arthropods	−0.61	0.42	2.04	0.42	0.19	11
	Hg salt	Mollusks	−0.45*	0.11	0.32	0.20	0.37	30
	Hg salt	Cyprinids	0.80*	0.14	2.83*	0.43	0.71	15
	CH_3-Hg	Salmonids	0.16	0.26	1.02	0.79	0.03	14
	CH_3-Hg	Centrarchids	1.07*	0.30	3.23*	0.95	0.72	7
	CH_3-Hg	Other fish	0.62*	0.25	1.93*	0.71	0.24	22
Overall	Hg salt	All data	−0.002	0.11	0.43	0.24	0.0000	60
	CH_3-Hg	All data	0.83*	0.11	2.83*	0.35	0.53	49

would appear that the BCF model may not fully capture the complexities of Hg uptake and accumulation in some species. Clearly, further investigation would be beneficial in this regard, and discriminating among field and laboratory exposures as well as accounting for the influence of water chemistry are examples of avenues to pursue. Additionally, a better understanding of the relative exposure to methylmercury and inorganic Hg forms during chronic exposures to either methylmercury or inorganic Hg would be useful. It is likely that, whether the exposure regime involves dosing with methylmercury or with an inorganic salt of Hg, there will be at least some exposure to both methylated and inorganic forms of Hg.

Accumulation factors for Zn, Cd, Cu, and Pb

The concept of the ACF variable was to provide a ratio of accumulation that was similar to BCF but that accounted for only the increased accumulation that arises from an increase in exposure concentration (see *Methods*). By removing the preexisting concentrations, which (presumably) cause no adverse effect, from the calculation, the focus is on accumulation that results from elevated metal exposure. Sufficient data were available for ACF calculations for Zn, Cu, Cd, and Pb, with only a few data points available for Ni. Among Zn, Cu, Cd, and Pb, the mean ACF values were lower than BCF values by a factor in the range 1.7 to 21.5 defined by Pb and Zn, respectively. The difference between BCF and ACF values was most dramatic for Zn, for which the mean ACF value was 90% lower (Table 1). In the cases of Zn and Cd, the mean ACF values were severely skewed by extremely high single ACF values, and removal of these single values dramatically reduced both the mean and its associated variability (Table 1). The variability of the mean ACF values was in general a bit lower compared with the mean BCF values but was nonetheless elevated, with CVs ranging from 123 to 175%.

The differences between mean ACF and BCF values provide some insight into the bioaccumulation of naturally oc-

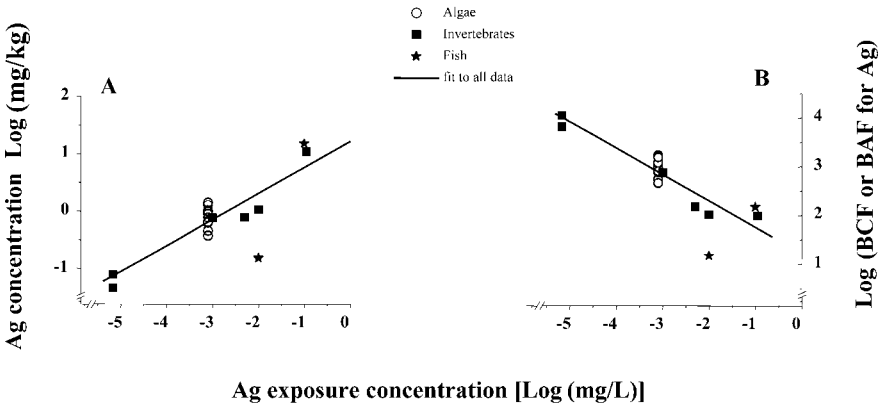

Fig. 7. Effect of chronic Ag exposure on Ag content in aquatic biota (**A**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Ag (**B**). Data are on a log-log basis and the best fit line from the linear regression analysis is shown for all species grouped together. The regression variables are shown in Table 8.

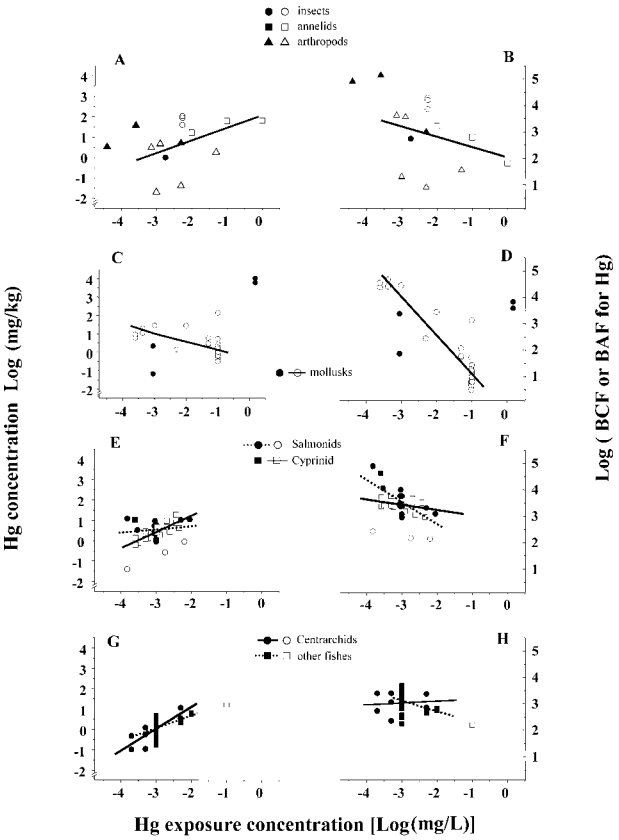

Fig. 8. Effect of chronic Hg exposure on Hg content in aquatic biota (**A**, **C**, **E**, **G**) and associated bioconcentration factor and bioaccumulation factor (BCF/BAF) values for Hg (**B**, **D**, **F**, **H**). Data are on a log-log basis and, for each species group, filled symbols represent exposures where methylmercury was administered, while open symbols represent those for an inorganic Hg salt such as the chloride or nitrate. The best fit line from the linear regression analysis is shown for species groups with seven or more data points expect for insects, arthropods, and annelids, where data for inorganic exposure (open symbols in **A** and **B**) were pooled for analysis. The regression variables are shown in Table 9.

curring substances. Both ACFs and BCFs are measures of accumulation, but ACF values are limited to the additional whole-body concentration of metal that results from an additional exposure to that metal. The BCF values not only include the incremental amounts but also include all accumulations, including those that result from normal exposure concentrations. An example is Zn, with a mean ACF value that is a mere 9.6% of the mean BCF value (Table 1). This 90% reduction illustrates not only the nutritional accumulation of Zn for essential physiological functions but also the ability of animals to regulate Zn concentrations when faced with elevated exposure levels. The difference between AFC and BCF values is not only a feature of essential minerals, as mean ACF values for Cd and Pb were 81 and 41% less than mean BCF values (Table 1). Therefore, even for nonessential elements, the preexisting low-level exposure concentrations prior to experimental exposures, which are assumed to result in no toxic effects, play a significant role in BCF values. However, the ACF approach could have a significant impact on hazard classification when one considers the threshold values range from 500 to 5,000, and therefore the use of ACFs may preclude classification of some metals under some hazard classification schemes.

The ACF data were also evaluated to determine if there

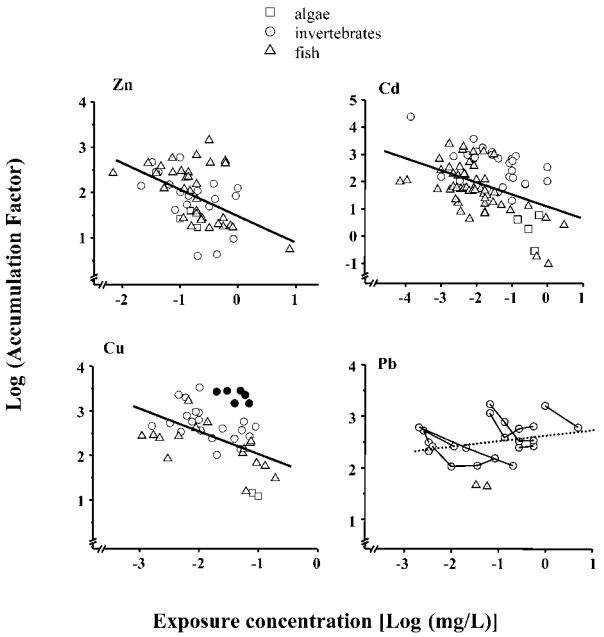

Fig. 9. Accumulation factor (ACF) as a function of aquatic exposure concentration for Zn (top left panel), Cd (top right panel), Cu (bottom left panel), and Pb (bottom right panel) for algae (squares), invertebrates (circles), and fish (triangles). The ACF values were calculated as the ratio of concentration to exposure after removing preexisting background (see text for details). The best fit lines derived from linear regression analysis with all data pooled are shown as solid lines for Zn, Cd, and Cu and a dotted line for Pb. Note that, in the case of Cu, data from one study were omitted from the analysis, and this has been indicated by filled symbols. For Pb, solid lines connecting points show data from exposure series within individual studies.

were relationships to exposure concentrations. With all the data pooled by metal, there was a significant and negative relationship between ACF values for Zn, Cd, and Cu and exposure concentration (Fig. 9). The log-log linear regressions of ACF to exposure concentration (Fig. 9) slope for Zn was -0.58 ± 0.14 ($n = 58$, $r^2 = 0.23$, $p < 0.05$), for Cd was -0.46 ± 0.10 ($n = 88$, $r^2 = 0.18$, $p < 0.05$), and for Cu was -0.51 ± 0.13 ($n = 40$, $r^2 = 0.30$, $p < 0.05$). Note that, for Cu, the mollusk data of McLusky and Phillips [119] were omitted from the analysis, as previously discussed. As revealed by the dotted line in the Pb panel of Figure 9, only the ACF values for Pb showed a nonsignificant regression slope when all the data were pooled. However, when trends within studies as per the solid lines in the Pb panel are examined, it is clear that there was a negative correlation between exposure and ACF within each of the individual studies. Therefore, with respect to the ACF values for Zn, Cd, Cu, and Pb, the ACF values are dependent on exposure concentration. As previously discussed, this negative relationship results from an ability to acclimate to elevated exposures and thereby control metal accumulation. In addition to active regulation, the negative correlation is also a function of uptake being a rate-limited process.

CONCLUSIONS

The accumulation of Zn, Cd, Cu, Pb, Ni, and Ag in aquatic biota were, in general, remarkably consistent, particularly for Zn, where total body/tissue concentration varied little over a wide range of exposure concentrations, exposure conditions, and species. However, mean BCF values for the six metals were characterized by high variability, and there was an inverse

relationship between BCF and exposure concentration. Therefore, using the weight of evidence available, it is virtually impossible to derive a meaningful BCF value that one could say is representative of the BCF for each of the metals. Even when BCFs are limited to the exposure range where chronic toxicity might be expected (based on water-quality guidelines), it is not possible to derive a precise and accurate BCF value. In addition, the inverse correlation between BCF and exposure for these metals produces the possibility of inadequate and faulty predictions for hazard. For example, hazard identification based on BCFs indicates that hazard is reduced as concentration increases, a conclusion that is inconsistent with the toxicological literature. Because the hazard identification of chemical substances is intended to be based on their intrinsic properties, which by definition are independent of exposure, the variability in the values of BCF versus exposure concentration for metals should preclude the use of BCFs for hazard identification of inorganic metal substances.

Clearly, bioaccumulation is a characteristic of the metals examined, but the BCF parameter does not characterize this bioaccumulation nor is it related to the potential for toxic impacts. This conclusion has a theoretical, chemical, physiological, and pragmatic basis. The BCF model was designed, developed, and adapted to describe neutral and lipid-soluble organic substances of anthropogenic origin, and its application to metals for the purposes of hazard identification is not supported by the scientific data.

This is not to say that bioaccumulation of metals is unimportant. Understanding and predicting bioaccumulation of metals is one of the key requirements in understanding their fate and toxicity in aquatic environments and for environmental protection measures. However, the BCF criterion does not reflect the current understanding of metal bioaccumulation and cannot predict it. Bioaccumulation of metals follows a different paradigm relative to neutral organics. For example, metal uptake occurs via specific mechanisms that can often be modified as a result of exposure. Additionally, low-level accumulation at background concentrations is a natural phenomenon, detoxification and elimination of accumulated metals is part of acclimation, and toxicity (acute) is predominantly associated only with charged cations. The ACF values, while offering the potential to deal with at least one of the shortcomings of the BCF model (bioaccumulation at low exposure levels), are insufficient as a replacement for BCF. Moreover, due to the storage of metals in granules and as metallothioneins, metal bioaccumulation is not necessarily indicative of secondary poisoning or chronic effects.

Recommendations for alternative approaches

Accumulation models specific to metals have been published for Cu, Cd, Zn, Cr, and Ni [86,157–159], but they have not been adapted for use in the context of hazard identification. Other metal-specific bioaccumulation models such as the biotic ligand model [10–12] or the free-ion activity model [54] might be developed to elaborate a criterion that could be used as the basis for hazard identification. However, currently these models only predict acute toxicity. The origin of bioaccumulation as a criterion in hazard identification for organic substances was based, along with persistence, on their link to potential chronic impacts (toxicity and biomagnification). Linking bioaccumulation to chronic impacts is exceptionally difficult, in part because chronic indicators and effects are not as clearly delineated as they are for acute toxicity. It is important that bioaccumulation not be an independent end point in and of itself. Instead, directed and validated links between a bioaccumulation criterion and chronic impacts in the environment are needed.

Mechanistically based chronic toxicity and bioavailability models for metals are currently under development and may provide a validated bioaccumulation criterion for use in hazard identification. The focus of these developing models is primarily chronic toxicity predictions for the purpose of improved water and effluents quality criteria and guidelines. This interdisciplinary research applies aquatic geochemistry, physiology, and toxicology to link waterborne and dietary exposure, bioaccumulation, and adverse effects. It therefore seems possible that the results may provide a scientifically validated bioaccumulation criterion for aquatic hazard identification and thereby fill the regulatory gap that exists due to the lack of validity of the BCF model as applied to metals.

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