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MATHEMATICAL RELATIONSHIPS BETWEEN METRICS OF CHEMICAL BIOACCUMULATION IN FISH

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Abstract: Five widely used metrics of bioaccumulation in fish are defined and discussed, namely the octanol–water partition coefficient (K_{OW}), bioconcentration factor (BCF), bioaccumulation factor (BAF), biomagnification factor (BMF), and trophic magnification factor (TMF). Algebraic relationships between these metrics are developed and discussed using conventional expressions for chemical uptake from water and food and first-order losses by respiration, egestion, biotransformation, and growth dilution. Two BCFs may be defined, namely as an equilibrium partition coefficient K_{FW} or as a nonequilibrium BCF_K in which egestion losses are included. Bioaccumulation factors are shown to be the product of the BCF_K and a novel equilibrium multiplier M containing 2 ratios, namely, the diet-to-water concentration ratio and the ratio of uptake rate constants for respiration and dietary uptake. Biomagnification factors are shown to be proportional to the lipid-normalized ratio of the predator/prey values of BCF_K and the ratio of the equilibrium multipliers. Relationships with TMFs are also discussed. The effects of chemical hydrophobicity, biotransformation, and growth are evaluated by applying the relationships to a range of illustrative chemicals of varying K_{OW} in a linear 4-trophic-level food web with typical values for uptake and loss rate constants. The roles of respiratory and dietary intakes are demonstrated, and even slow rates of biotransformation and growth can significantly affect bioaccumulation. The BCF_Ks and the values of M can be regarded as the fundamental determinants of bioaccumulation and biomagnification in aquatic food webs. Analyzing data from food webs can be enhanced by plotting logarithmic lipid-normalized concentrations or fugacities as a linear function of trophic level to deduce TMFs. Implications for determining bioaccumulation by laboratory tests for regulatory purposes are discussed. *Environ Toxicol Chem* 2013;32:1459–1466. © 2013 SETAC

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INTRODUCTION

Bioaccumulation of organic chemicals in fish and other organisms that may constitute food chains is a concern because of both possible adverse effects on the organisms themselves and the potential for exposure to predators, including humans, that may consume these organisms. The focus here is on bioaccumulation in fish, but similar principles apply to bioaccumulation in other aquatic water-breathing organisms, and they also may apply to air-breathing organisms such as birds and mammals. As a result, a global initiative has been launched to evaluate commercial chemicals for their capacity to bioaccumulate [1,2]. As part of this initiative, various kinds of bioaccumulation data and metrics are used to determine whether and to what extent chemicals are bioaccumulative. Extensive literature exists on bioaccumulation from scientific and regulatory perspectives, examples being the reviews by Barber [3,4], Mackay and Fraser [5], Arnot and Gobas [6], Ehrlich et al. [7], Burkhard et al. [8], and Gobas et al. [9], the latter summarizing the conclusions of a SETAC-sponsored workshop held in 2008. These and other reviews have pointed out the existence of several metrics of bioaccumulation that differ in definition, in regulatory application, and in adoption by the scientific community.

Our objective here is to define and discuss the relationships between 5 common bioaccumulation metrics for aquatic organisms with a view to clarifying their relative merits and applicability for bioaccumulation assessments. We first briefly define and discuss the bioaccumulation metrics, then apply a mass balance model to examine and quantify the relationships between them. We seek to provide novel insights into the underlying processes resulting in bioaccumulation and provide guidance for improving and selecting data for bioaccumulation assessments.

BIOACCUMULATION METRICS

For the current analysis, we define and describe 5 common metrics for assessing bioaccumulation. Differences exist in the definitions and usage of these terms; however, the definitions given here are used to develop mathematical relationships in the next section. The octanol–water partition coefficient (K_{OW}) is widely used as an indicator of hydrophobicity and thus the partitioning of a chemical from water into lipids and other organic phases such as protein [10]. The K_{OW} is primarily controlled by the solubility of the substance in water, because the solubility of neutral, liquid nonpolar organic chemicals in octanol is relatively constant. A log K_{OW} value of 5 is often used as a bioaccumulation assessment criterion; however, depending on the regulatory program, lower values are also used to categorize bioaccumulation potential. Whereas K_{OW} gives a reasonable and conservative estimate of lipid–water partitioning for nonpolar hydrophobic substances [11], it may not accurately simulate partitioning for more polar and ionogenic organic chemicals and other chemical classes such as organofluorines and silicones. Direct empirical measurement is essential in such cases.

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The bioconcentration factor (BCF) is defined here as the nondimensional ratio of the volumetric concentrations in fish C_F (mol/m^3) and in water C_W (mol/m^3). The BCF is then deduced as C_F/C_W after prolonged exposure when a steady state is reached. Chemical uptake is by exposure to chemicals in the water only. In a regulatory context, a BCF of 5000 (i.e., 5% of K_{OW} of 10^5) is often used as a screening tool for evidence of high bioconcentration; however, depending on the regulatory program, lower values are also used to categorize bioaccumulation potential [7]. Empirical determinations of BCFs are preferred. For a nonmetabolizing substance in a fish with a zero or negligible growth rate and with no exposure to chemical in the diet or loss of chemical through fecal egestion, the BCF can be regarded as a fish–water thermodynamic partition coefficient K_{FW}. When the rate of approach toward equilibrium is relatively slow, as applies to very hydrophobic, persistent substances, one must feed the fish a diet containing no chemical; thus, loss by fecal egestion will occur and there may be an apparent loss of chemical by increasing body weight. The BCFs of untransformed chemicals measured in unfed fish (i.e., no chemical uptake from the diet and no fecal egestion) are thus expected to be greater than those in fish that are fed uncontaminated food (i.e., no chemical uptake but fecal egestion occurs). Ambiguity can occur between the truly dissolved and total concentration in water [12,13], but here we assume the former to apply. Reaching a true steady state or equilibrium may not always be feasible experimentally, but this can be circumvented by measuring the uptake rate constant from water (k_1) and the clearance rate constant from the organism (k_2) and calculating the BCF as k_1/k_2. For screening purposes, an approximate BCF may be estimated as the product of K_{OW} and the volume fraction lipid content (L_C) of the whole organism. This is the basis of the simple correlation for neutral, nonpolar organic chemicals that the BCF is approximately 0.05 K_{OW} [14], where 0.05 corresponds to the median lipid content in small fish in laboratory BCF tests [6].

Standard methods such as the Organization of Economic Co-operation and Development (OECD) 305 bioconcentration: flow-through test provides testing guidance [15]; unfortunately, key principles outlined in these guidelines cannot always be followed in practice [6]. Bioconcentration factors may be reported on a wet-weight or lipid-normalized basis. Growth dilution and biotransformation during the test results in a reduction in BCF, but a growth-corrected concentration and BCF can be estimated and reported.

The bioaccumulation factor (BAF) is defined here in a similar fashion as the nondimensional BCF; in other words, BAF is C_F/C_W at steady state, except that in this case the fish is exposed to both water and food; thus, an additional input of chemical from dietary assimilation takes place. The concentrations used here are mol/m^3 wet weight in the fish and mol/m^3 in water. The fish concentration also can be expressed on a mol/m^3 lipid basis. A necessary additional loss of chemical results from fecal egestion, and apparent loss by growth dilution may occur. If biotransformation occurs, it also affects the BAF. Bioaccumulation factors, which are a function of trophic level position occupied by the organism, are usually determined from field data and rarely from laboratory tests with exposure from both water and diet. Ambiguity about whether truly dissolved or total concentrations in water are used to calculate BAFs may occur. Additional uncertainty occurs because water and diet concentrations may vary during exposure in the field; thus, use of a single concentration in the water or fish obtained by sampling may contribute error. Temporal variability in the chemical concentration in the water and diet also can complicate the interpretation of BAF data. Determining the approach to steady state is usually not feasible in the field. For regulatory purposes, a BAF exceeding 5000 has often been applied when defining a chemical as bioaccumulative, although other criteria have also been suggested [6,7].

The biomagnification factor (BMF) is preferably defined as the lipid-normalized ratio of the concentration in the predator (C_2mol/m^3 lipid) to that of the diet (C_1 mol/m^3 lipid); thus, BMFs exceeding 1.0 indicate an increase in lipid concentration and thus also an increase in thermodynamic potential or fugacity with ascending position in the food chain or food web. The BMFs also can be defined on a wet-weight basis, but this definition lacks thermodynamic significance. In a regulatory context, BMFs exceeding 1.0 are a cause for concern because these substances tend to exhibit their highest concentrations in upper-trophic-level organisms, including humans. Biomagnification factors are influenced by the approach to steady state, by growth dilution, and by biotransformation rates in both prey and predator, making simple interpretation difficult. The review by Gobas et al. [9] favored the use of BMF for characterizing the bioaccumulative nature of substances because biomagnifying substances differ fundamentally from non-biomagnifying substances in that their fugacity and chemical potential increase from prey to predator in a functioning ecosystem. Non-biomagnifying chemicals cannot achieve such an increase. Biomagnification factors can be determined from laboratory or field studies; however, defining a chemical concentration in the prey can be difficult if the predator has a varied diet in the natural environment. A significant advantage of the BMF is the lack of a need to measure or estimate the concentration in water. Standard test protocols for determination of the BMF have been developed as outlined in the recently updated OECD 305 bioaccumulation test guidelines [16].

When reporting BMFs, BCFs, and BAFs, it is essential to define clearly the units of concentration that can be on a whole-body basis, for a specific tissue such as muscle, or on a lipid-normalized basis.

The trophic magnification factor (TMF) is similar to a BMF that expresses a simple predator–prey relationship, but the TMF describes the increase or decrease in concentrations of the chemical in multiple organisms that occupy successively higher trophic levels or positions within a food web, often graphically; thus, TMFs can be fractional as well as integral numbers [17,18]. A favored method is to obtain ^{15}N (stable isotope) data for each species sampled, use an enrichment factor of 3.4 parts per thousand in ^{15}N, and assume similar nitrogen sources to indicate a unit trophic level increase [19]. Trophic magnification factors have the considerable advantage that they provide a basis for analyzing data from a diversity of species in real-world aquatic ecosystems; however, reliable TMF determination requires comprehensive and insightful sampling of the ecosystem [17].

A 6th bioaccumulation metric is the biota–sediment accumulation factor, discussion of which is beyond the scope of the present study. It is the ratio of concentration in an organism (that may or may not dwell in the sediment) to that in the sediment. Chemical exposure is from all sources, including diet, sediment, and water. A frequently used concentration in sediment is the organic-carbon (OC)-normalized value, the implication being that most of the hydrophobic organic chemical present is associated with the OC fraction of sediment solids. Biota–sediment accumulation factors, expressed as ratios of lipid-normalized concentration in the organism to the OC-normalized concentration in sediment, are particularly enlightening because they contain

information on the equilibrium status of the organism and sediment phases [8,20].

Mathematical relationships among bioaccumulation metrics in single organisms

Our aim is to develop and exploit algebraic relationships between the various metrics of bioaccumulation. In the interest of simplicity, we first assume that rates of growth and biotransformation are negligible. We use volumetric units such as mol/m^3 rather than conventional mass (mol/kg) units to simplify the equations and render the metrics dimensionless. Conversions to units of mol/kg or g/kg are readily accomplished by multiplying by the fish density (kg/L or kg/m^3). Furthermore, we first assume steady-state conditions. The algebraic relationships derived below are summarized in Table 1.

The conventional steady-state bioaccumulation equation for aquatic organisms [21] is

$$\begin{aligned} C_F &= (k_R C_W + k_D C_D)/(k_V + k_E + k_M + k_G) \\ &= (k_R C_W + k_D C_D)/k_T \end{aligned} \quad (1)$$

Where k_T is

$$k_V + k_E + k_M + k_G \quad (2)$$

It follows that

$$C_F = (k_R/k_T)C_W + (k_D/k_T)C_D \quad (3)$$

Here, k_R (m^{3}water/d) is the rate constant for chemical uptake from the water by gill respiration, k_D (m^3 food/d) is the rate constant for dietary uptake, k_T is the total elimination rate constant (d^{-1}) comprising the rate constants for loss by respiratory ventilation (k_V), such as $k_R/(L_C \times K_{OW})$, by egestion (k_E), by biotransformation or metabolism (k_M), and by growth (k_G), all with units of d^{-1}. Both k_R and k_D contain an uptake or assimilation efficiency. The C_D, C_W, and C_F are chemical concentrations in diet, water, and fish, respectively, all with units of mol/m^3 or g/m^3. When scaling between organisms, normalizing the rate constants with respect to organism mass is often done to account for the effect of size, but this convention is not employed here.

For simple bioconcentration in fish, the uptake equation suggests 2 fish–water concentration ratios or BCFs, depending on whether the fish is fed uncontaminated food. If k_M, k_E, and k_G are insignificant compared with k_V, thermodynamic equilibrium is approached between the water and the fish, and the corresponding BCF_E can be designated as a partition coefficient K_{FW}. If the fish is fed a clean diet that does not contain the test chemical, as may be necessary if the exposure duration is long, C_D is zero; only the first term in Equation 3 applies. We designate this kinetic BCF as BCF_K, as given by

$$BCF_K = C_F/C_W = k_R/k_T \quad (4)$$

If k_M and k_G are zero, this kinetic BCF_K reduces to $k_R/(k_V + k_E)$, in which egestion of feces can provide an additional loss route. In short, BCF_K includes k_E in the denominator and will be smaller than the partition coefficient K_{FW}, especially for hydrophobic chemicals for which k_R and k_V are relatively small compared with k_E. In the subsequent discussion, we focus on BCF_K as being the more relevant and useful BCF metric. With growth, the BCF is reduced, but a growth correction can be applied to estimate the BCF for no growth. Growth can reduce the BCF, and growth rates between tests can be variable; hence, correcting for growth rates provides BCFs that are more appropriately comparable.

For bioaccumulation, both chemical uptake terms in Equation 1 apply, and

$$\begin{aligned} \text{BAF} &= C_F/C_W = k_R/k_T + (k_D/k_T)(C_D/C_W) \\ &= k_R/k_T + (k_R/k_T)(k_D/k_R)(C_D/C_W) \\ &= BCF_K[1 + (k_D/k_R)(C_D/C_W)] = BCF_K M \end{aligned} \quad (5)$$

The BAF can be regarded as the product of BCF_K and the multiple M; namely $[1 + (k_D/k_R)\ (C_D/C_W)]$. This multiple proves to be a key quantity when characterizing bioaccumulation, because it is the factor by which the concentration in the fish exceeds its steady state or near-equilibrium value as a result of food uptake and digestion. We refer to it as an "equilibrium multiplier" and later show that it is closely related to the ratio of the fugacity in the organism to that of its diet. Now k_D/k_R is the ratio of the rate constants for dietary uptake and respiration and includes differences in chemical uptake efficiencies from the water and the diet. It has units of m^3 food/m^3 water. This ratio is fairly constant for most chemicals, but it can be smaller for hydrophobic chemicals of log K_{OW} exceeding 7 that have lower dietary assimilation efficiencies [21–23]. The food–water concentration ratio, C_D/C_W, is the BAF of the prey, and it can vary greatly in magnitude and has units of m^3 water/m^3 food. The product $(k_D/k_R)(C_D/C_W)$ is thus the ratio of absolute rates (e.g., mol/d) of chemical uptake from diet and uptake from water and is a property of both the fish and its diet.

These equations can be used to undertake some illustrative calculations of the role of K_{OW} as a determinant of BCF and BAF. For a small fish, k_D may be typically 0.01/d, and k_R may be 200/d, so the magnitude of k_D/k_R is approximately 5×10^{-5}m^3 food/m^3 water [21]. Using this k_D/k_R ratio of approximately 5×10^{-5}, the uptake rates by the 2 routes

Table 1. Bioaccumulation metrics and algebraic relationships

Metric	Description	Algebraic relationships
K_{OW}	Octanol–water partition coefficient	C_{OCT}/C_W
BCF_E	Equilibrium BCF with only respiration, no dietary input of chemical and no egestion losses	$C_O/C_W = k_R/k_V$
BCF_K	Kinetic, non-equilibrium BCF with only respiration, no dietary input of chemical but with egestion losses and possible metabolic and growth dilution losses	$C_O/C_W = k_R/k_T$
BAF	Dietary and respiratory inputs of chemical and all losses included	$C_O/C_W = BDF_K \times M$ where $M = [1 + (k_D/k_R)\ (C_D/C_W)]$
BMF	Ratio of concentrations or fugacities of a predator to a unique prey	$C_2/C_1 = (BCF_{K2}/BCF_{K1})(M_2/M_1)$
TMF	Ratio of organism concentrations or fugacities per unit increase in trophic level	Obtained from the slope of a plot of concentration vs trophic level or from weighted BMFs
BSAF	Ratio of concentration in organism to concentration in sediment	C_O/C_S

become equal when C_D/C_W is approximately $1/(5 \times 10^{-5})$ or 2×10^4. For food of lipid content 0.05 and assuming partitioning to be controlled by K_{OW}, this corresponds to 0.05 $K_{OW} = 2 \times 10^4$ or $K_{OW} = 4 \times 10^5$. The implication is that when $K_{OW} >> 4 \times 10^5$, uptake is primarily from the diet. When $K_{OW} << 4 \times 10^5$, uptake is primarily from water, and the BCF and BAF are approximately equal. For these lower K_{OW} chemicals, equilibrium between water and fish is approached because of the fast rates of transport between water and fish, and dietary uptake then becomes relatively inconsequential.

When describing BMFs, we first use whole organism concentrations. The BMF_{21} for species 2 (predator) consuming only species 1 (prey) is given by

$$BMF_{21} = C_{F2}/C_{F1} = BAF_2/BAF_1 = BCF_{K2}[1 + (k_{D2}/k_{R2})(C_{D2}/C_W)]/(C_{F1}/C_W) \quad (6)$$

and because C_{D2} is C_{F1}

$$BMF_{21} = BCF_{K2}(C_W/C_{F1} + k_{D2}/k_{R2}) \quad (7)$$

Alternatively, Equation 6 can be expressed as

$$BMF_{21} = (BCF_{K2}/BCF_{K1})(M_2/M_1) \quad (8)$$

This relationship between BCF and BMF was first derived in a presentation by Gobas [24]. Both ratios in parentheses in Equation 7 are small; thus, BMF_{21} is much smaller than BCF_{K2}. The ratios are approximately equal when K_{OW} is 4×10^5. For less hydrophobic chemicals, the ratio C_W/C_{F1} is more significant and depends inversely on K_{OW}. As noted earlier, the ratio k_D/k_R is relatively independent of K_{OW}, but when log K_{OW} exceeds a value of 7, this ratio decreases because of the decrease in the food assimilation efficiency caused by the increasing water phase resistance (i.e., the low internal water phase concentration), which retards transport from the gastrointestinal tract to the blood. An additional separate issue is the increased partitioning to particulate matter that reduces the dissolved concentration in the water [12,13].

Equation 8 shows that BMF_{21} is controlled by the ratio of BCFs, which is mainly determined by the relative lipid contents of predator and prey, and more importantly by the ratio of the equilibrium multipliers M_2/M_1 that characterize the respective departures from equilibrium. Biomagnification can thus be viewed as being primarily caused by an increase in these equilibrium multipliers from prey to predator, or M_2/M_1.

For more hydrophobic chemicals, the ratio C_W/C_{F1} becomes negligible, so BMF_{21} approaches $BCF_{K2}(k_{D2}/k_{R2})$. For very hydrophobic chemicals, BCF_{K2} is approximately k_{R2}/k_{T2}; thus, BMF_{21} approaches k_{D2}/k_{T2}, and because k_{T2} approaches k_{E2} when growth and biotransformation are relatively slow, BMF_{21} approaches k_{D2}/k_{E2}. This ratio has been termed Q, and it represents a maximum achievable BMF [25]. It is controlled by the loss in sorptive capacity and mass of the food as it is converted to fecal matter such that k_{D2}/k_{E2} may be approximately 4 for small fish. An implication is that organisms with high food intake and lipid absorption efficiencies approaching 100% will experience high BMFs. Homeotherms such as mammals and birds are obvious examples [23].

The TMF can be regarded as an average BMF in which dietary uptake of several different organisms occurs, possibly at different trophic levels (TLs) or positions [8]. The TMF is then a weighted average of the individual BMFs with the weighting being done on the dietary preference fractions. Rather than regard the TMF as a ratio of concentrations, it is perhaps better viewed as the multiple by which the concentration increases per unit increase in TL. It is also similar in magnitude to the ratio of the BAF to BCF or M. It can be determined from the slope of a plot of concentration in organisms versus their assigned TL, preferably for illustrative purposes on a logarithmic scale [17,18], namely

$$logTMF = (logC_n - logC_1)/(n - 1) \quad (9)$$

where n refers to the number of the TL extending from 1 at the base of the food chain to n at higher levels. One may use wet weight or lipid concentrations, or, as is discussed later, fugacities; however, standard practice is to use lipid concentrations for lipid-soluble substances.

For all of these bioaccumulation metrics, biotic concentrations may be defined, as above, on a whole-body or lipid-normalized basis. Lipid normalization increases all concentrations, the BCFs and BAFs by the factor $1/L_C$, and better conveys the equilibrium status between organisms. If biotic concentrations are expressed in units of mass/mass, for example, mg/kg, the previous equations must be modified to include the organism density. If 1.0 kg/L is assumed, numerical values are unchanged.

Derivation in terms of fugacity

Equations 1 to 9 have been derived using conventional concentration units. A similar derivation can be done using fugacity, yielding identical results. Essentially, C is replaced by Z × f where Z (mol/m^3 × Pa) is the fugacity capacity of the chemical in the phase of interest which may be water, whole fish, fish lipids, or whole sediment, or sediment OC, and f is fugacity (Pa). Partition coefficients such as K_{OW} are then ratios of corresponding Z values. Degradation rate processes can be expressed as D values, such as V.Z.k., where V is the volume of phase and k is the rate constant. For advective or flow processes, D is GZ, where G is a flow rate. The review by Mackay and Fraser [5] demonstrates these conversions. Burkhard and colleagues [8] have highlighted the utility of expressing the bioaccumulation metrics as fugacity rather than concentration ratios to compare different data in terms of a common currency, that is, relative equilibrium status and to provide a weight of evidence for bioaccumulation assessment.

Rather than present these alternative equations here, we confine our discussion to some insights revealed by them. The equilibrium BCF or K_{FW} when expressed as a ratio of fish and water fugacities is simply 1.0. If growth, biotransformation, or fecal egestion is significant, the BCF_K is less than K_{FW}, implying that although steady state is reached, true equilibrium is not achieved. Second, a BAF may correspond to an increase in fugacity because the fish absorbs chemical from the food, which experiences a decrease in lipid content during digestion and hence a decreasing Z value and a corresponding increase in fugacity [23]. If monitored aquatic species show an increase in fugacity or lipid-normalized concentration with trophic level, this indicates significant biomagnification as distinct from simple bioconcentration. Third, a wet-weight BMF that is C_2/C_1 is also $(Z_2/Z_1)(f_2/f_1)$ and is then determined by 2 principal factors: the ratio of lipid contents, or Z values, and the ratio of fugacities. In contrast, a BMF expressed as a ratio of lipid-normalized concentrations is simply f_2/f_1 provided that most partitioning is to lipids. For this reason, lipid-normalized BMFs are preferred. Possibly the nature of the lipids and thus their affinity for the chemical, or Z values, differ as discussed by Jonker [26]. For

organisms of low lipid content, and for ions, other phases such as proteins can contribute to the Z value [10,27]. When interpreting BMF data from the field, one must assess the individual contributions of these 2 ratios. A wet-weight BMF can exceed 1.0 as a result of lipid content differences even with no fugacity increase. Fourth, the characteristic time for uptake is $1/(k_T)$ or $V,Z/D_T$ days, namely, the time required to approach 63% of steady state. The total D value for all losses is D_T. This time can be quite long for hydrophobic chemicals, so having an estimate available when designing and interpreting laboratory tests is useful.

ILLUSTRATIVE FOOD WEB RELATIONSHIPS

To illustrate these food web relationships, we construct a simple food chain consisting of 4 organisms, all with equal lipid contents of 5% and identical rate constants for uptake and loss processes. The chemicals are assumed to be conventional nonpolar substances with a range of K_{OW} values. In the interest of simplicity, no dependence of rate constants on organism size is included, and because conditions are steady state, no organism sizes need be defined. The dissolved concentration in water is 1 mg/m^3. Organism 1 is phytoplankton and achieves bioconcentration equilibrium with the water, there being no dietary uptake. We set k_M and k_G to have negligible values of 5×10^{-5} d^{-1} for all other organisms. Organisms 2, 3, and 4 are fish comprising linear trophic positions in a linear food chain. Their egestion rate constants k_E are assumed to be an illustrative factor of 4 less than k_D, reflecting the reduction of the sorbing capacity of the ingested food as it is depleted in mass and lipid content. Table 2 gives the results of simulating this food web for 6 chemicals (A–F) varying in hydrophobicity. Chemical F is included to demonstrate the effect of increasing k_M and k_G for chemical E by a factor of 10 to 5×10^{-4} d^{-1}.

Concentrations in organism 1 respond linearly to increasing K_{OW}. The BCF_K values in all other organisms are calculated assuming fecal egestion; thus, these BCFs are dependent on K_{OW}, because k_V and hence k_T depend on K_{OW}. Because hydrophobicity increases, all concentrations and BAFs increase as expected.

For the less hydrophobic substances A and B of log K_{OW} 4 and 5, uptake and loss is primarily by respiration, and near equilibrium exists between water and the fish. All BAFs are close to the BCF_K; thus, BMFs are close to 1.0, and half-times for uptake and loss are relatively short. The effects of diet and food chains are negligible.

For chemical C with log K_{OW} of 5.6 (K_{OW} of 4×10^5), the rates of uptake by respiration and dietary intake are equal and the equilibrium multiplier M is 2; thus, for fish 2, the BAF is twice the BCF_K. The BMF is 1.59, and this is lower than M because the concentration in the diet is the higher equilibrium value corresponding to BCF_E. With increasing trophic level, M increases from 2 to 2.6 and 3.08, indicating an increasing departure from equilibrium, that is, biomagnification. The factors by which M increases, 2.6/2 and 3.08/2.6, equal the respective BMFs. Interestingly, in the current simulated foodweb, the BMF decreases with increasing trophic level because, as Equation 7 shows, the term C_W/C_D decreases; however, this may not be generally applicable. For fish 2, 50% of the intake is from food, whereas for fish 3, this increases to 67% because of higher food concentrations. The uptake and clearance half times for fish 4 are 55 d; thus, a steady state cannot be reached in a standard bioconcentration test (~28 d).

For chemicals D and E with higher values of K_{OW}, dietary intake and fecal egestion increasingly dominate, and concentrations and BAFs increase. Biomagnification factors also increase, approaching the maximum assumed value of 4, that is, the ratio k_D/k_E. The food web shows clear biomagnification as M increases. The half-times to steady state become very long at 106 d and 238 d; thus, steady state may not occur in the environment as concentrations fluctuate and organisms grow and diets change. Assuming a calculated steady-state condition to apply may be a reasonable approximation for regulatory purposes because it circumvents problems of estimating growth rates and dietary changes.

Chemical F is identical to chemical E, but the rate constants for growth and biotransformation (metabolic conversion) are increased by a factor of 10. The effect is a considerable reduction in bioaccumulation, especially for very hydrophobic substances such as chemical E. Biotransformation and growth at higher trophic levels can result in trophic dilution, that is, BMFs are <1.0. Figure 1 illustrates this approach using data from Table 2, in which it is assumed that all lipid contents are 5% and fugacities are thus proportional to concentrations. Interestingly, the lines are closest to linear for the most hydrophobic substances in which the term C_W/C_F in Equation 7 is small relative to k_D/k_R.

Table 2. Concentrations (mg/m^3) and bioaccumulation parameters for illustrative chemicals of varying K_{OW} in a linear food web in water of dissolved concentration 1 mg/m^3[a]

Chemical	A	B	C	D	E	F
K_{OW}	10^4	10^5	$10^{5.6}$	10^6	10^7	10^7
C_1, K_{FW1}, C_{D2}	500	5000	20 000	50 000	50×10^4	50×10^4
BCF_K fish 2, 3 & 4	497	4695	15900	30300	6.7×10^4	5.1×10^4
C_2, BAF_2	509	5869	31700	106100	173×10^4	133×10^4
$BMF_{2\text{-}1}$	1.02	1.17	1.59	2.12	3.47	2.67
M_2	1.02	1.25	2.00	2.50	26	26
C_3, BAF_3	509	6072	41100	191 000	584×10^4	347×10^4
$BMF_{3\text{-}2}$	1.00	1.03	1.29	1.80	3.37	2.60
M_3	1.03	1.29	2.59	6.30	87.7	67.7
C_4, BAF_4	509	6120	48500	320 000	1955×10^4	895×10^4
$BMF_{4\text{-}3}$	1.00	1.01	1.18	1.67	3.34	2.58
M_4	1.03	1.30	3.05	10.6	293	175
% from diet, fish 4	2.5	23.3	67.2	90.5	99.7	99.4
Half time (d)	1.72	16.3	55.0	105	231	178

[a]All organisms have lipid contents of 5% (L_C) and have rate constants (/d), k_R of 200, k_V of $200/(L_C \times K_{OW})$. Organisms 2 to 4 (fish) have k_D of 0.01, k_E of $k_D/4$, negligible values of k_M of 0.00005 and k_G of 0.00005, except for chemical F, which has higher rate constants for metabolic conversion and growth k_M of 0.0005 and k_G of 0.0005, (half-lives 3.8 yr).

K_{OW} = octanol–water partition coefficient; BCF = bioconcentration factor; BAF = bioaccumulation factor; BMF = biomagnification factor.

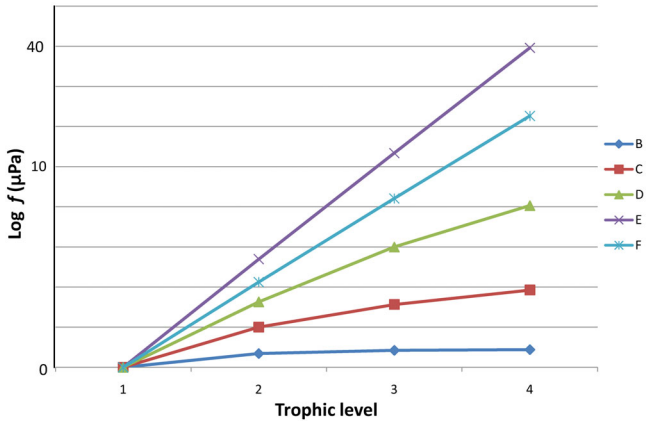

Figure 1. Plot of fish fugacities for chemicals B to F as a function of trophic level for the data in Table 1, assuming all chemicals have a molar mass of 250 g/mol. The Z value of water (Z_W) is 4 mol/m^3 × Pa, and the Z value of each fish is 0.05 K_{OW} Z_W. The fugacity of the water is 1.0 μPa in all cases.

Typically feeding relationships in food webs are complex; thus, the equation for BAF of the predator must be modified to include all food sources. For example, if fish 4 consumes equal quantities of fish 2 and 3, the BAF equation becomes

$$BAF_4 = BCF_{K4}[1 + 0.5(k_D/k_R)(C_2/C_W) + 0.5(k_D/k_R)(C_3/C_W)] \quad (10)$$

The result is a value intermediate between BMFs assuming 100% diets of fish 2 or 3. Under these conditions, a TMF is more appropriate, but a BMF based on average prey compositions can be valuable for explaining the observed exposure of indicator species of regulatory concern.

Table 3 gives the concentrations in fish 4 of chemical D assuming various proportions of fish 2 and 3 as diet. The TMFs and BMFs based on average food composition are also given. In this case a simple linear dependence of concentration on diet proportions occurs, but this assumes that dietary uptake rates (feeding) are independent of food type (energy density) and that the loss rate constant is independent of diet composition, digestion efficiency, and proportion. These assumptions will likely not apply in practice in a real aquatic ecosystem with complex bioenergetics. The equilibrium multiplier M in this case is the average of the 2 contributing M values. An implication is that monitoring data for concentrations at different trophic levels could be used to estimate effective Ms and shed light on dietary preferences, especially for model-based screening or risk assessments. An ideal example is the extensive monitoring data for 127 PCB congeners in 23 species in Lake Hartwell by Walters et al. [19].

Powell and colleagues [28,29] have demonstrated that when analyzing bioaccumulation data in food webs, converting all biotic concentrations to fugacities and then plotting log fugacities as a function of trophic level obtained from stable N isotope

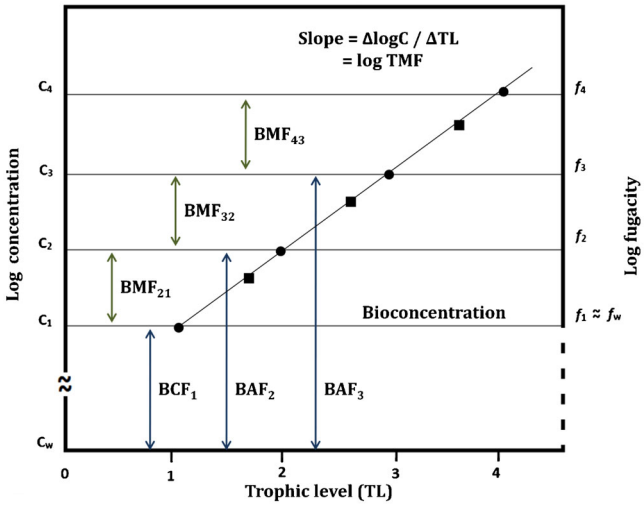

Figure 2. Relationships between bioconcentration, biomagnification, bioaccumulation, and trophic magnification expressed as chemical concentrations and fugacities. The round symbols are for a linear food web, and the square symbols represent a nonlinear food web with multiple dietary items. BCF = bioconcentration factor; BMF = biomagnification factor; BAF = bioaccumulation factor; TMF = trophic magnification factor.

measurements is illuminating. Figure 2 is a modification of figures from these presentations. Each interval in log f corresponds to a BMF, but this factor is not influenced by differences in lipid content as is the case with BMFs expressed as concentration ratios. If fugacity BMFs are constant, the log f data will lie on a straight line and extrapolation to a trophic level of 1 (probably a planktonic organism) will yield their fugacity and approximately that of the water. This extrapolation may be impossible if there is exposure from the sediments, and any assertions must be treated with caution, recognizing that BMFs will likely vary with organism physiology and size as well as biotransformation and growth rates and departures from steady state. In addition, the assumption that lipid–water and K_{OW} are equal may be invalid. This method can be used to estimate the prevailing fugacity in water or sediment and circumvents the issue of estimating bioavailability. This is noteworthy because the fish concentration data are likely more accurate than those in water. In such cases, using the biotic data to deduce a likely or effective dissolved concentration in water may be preferable.

Figure 2 further illustrates the relationships between concentration, fugacities, and bioaccumulation metrics in a linear food web. In this case the BCF establishes the condition at the base of the food web corresponding to the assumption of equi-fugacity between water and planktonic organisms. At higher trophic levels each BAF is increased by the multiple M; thus, BMFs are essentially ratios of M. At high trophic levels, which are of primary regulatory concern, the concentrations are controlled by the species-specific BCF and the appropriate value of M as discussed previously.

Table 3. Effect of increasing the trophic position of fish 4 for the concentration of chemical D by varying the dietary proportions of fish 2 (trophic level 2) and fish 3 (trophic level 3)[a]

Diet % fish 3	0	25	50	75	100
Diet % fish 2	100	75	50	25	0
Diet concentration	106 000	127 000	149 000	170 000	191 000
C_4	191 000	223 000	255 000	288 000	320 000
BMF	1.80	1.75	1.72	1.69	1.67
TMF	2	2.25	2.5	2.75	3

[a]Organism 1 is assigned a trophic position of 1.0.
BMF = biomagnification factor; TMF = trophic magnification factor.

For example, for chemical D in Table 3, the base concentration of organism 1 is 50 000 mg/m^3; in other words, the BCF_{E1} is also 50 000, as is BCF_E for all other organisms with the same lipid contents. Because organism 2 to organism 4 have egestion losses, their BCF_K values are lower; in other words, 30 300. For fish 2, M_2 is 2.5; thus its BAF is 106 100, M_3 is 6.3 and BAF_3 is 191 000, M_4 is 10.6 and BAF_4 is 320 000. The BMFs are ratios of values of M. On a logarithmic scale, which is more thermodynamically appropriate, log C_W is zero, and the log concentrations in organism 1 to organism 4 are 4.7, 5.03, 5.28, and 5.5, respectively. A 4.7-log-unit concentration increase from water to organism 1 was caused by bioconcentration, then only a subsequent 0.8 log unit increase in concentration in fish 4 occurs because of both bioaccumulation and biomagnification. On the contrary, viewing bioaccumulation from a linear concentration perspective suggests that for chemical D bioconcentration causes only an increase from 1 in water to 50 000 g/m^3 in organism 1 at the base of the food web, whereas the increase from organism 1 to organism 4 is 270 000 mg/m^3. Depending on which perspective is adopted, bioconcentration or biomagnification may be viewed as the more important process contributing to the overall concentrations, fugacities, and resulting exposures throughout the food web.

DISCUSSION

The metrics of K_{OW}, BCF, BAF, and BMF are often regarded as independent quantities, each reflecting different exposure and uptake conditions. Although this is correct, the fact that they are closely related mathematically with the high degree of interdependence demonstrated here may not be appreciated. The relationships assume that the standard model (Equation 1) is correct and that K_{OW} is a reasonable surrogate for lipid water partitioning, which is not the case for substances with fundamentally different partitioning properties such as ionogenic substances. However, as illustrated elsewhere, the standard model may be applicable for ionogenic organic chemicals if the appropriate surrogates for chemical partitioning are included [27]. The relationships apply to the truly dissolved concentration in water. The use of a total (dissolved and sorbed) concentration in water introduces a bioavailability complication external to the organism that can contribute to apparently lower BAFs beyond a log K_{OW} of approximately 6 or 7 when BAFs are calculated based on the total (bulk) water concentrations.

The structures of the derived uptake equations suggest the importance of establishing BCFs, preferably BCF_K. The BAFs are simple multiples M of BCF_K (Equation 5). Biomagnification factors are also multiples of BCF_K (Equation 7) and are ratios of the corresponding values of M. The TMFs can be viewed as essentially weighted averages of BMFs or preferably as the slopes of plots of log fugacity versus TL.

The simulations indicate that the key chemical and physiological properties of the fish are as follows. First, the lipid contents play an important role in determining K_{FW} and BCF_K and hence the other bioaccumulation metrics. Defining an equivalent lipid content, including nonlipid organic matter such as protein, may be preferable, particularly when lipid contents in organisms or tissues are relatively low (i.e., $<$2% v/v) [10,21,30]. Second, the equilibrium multiple M by which the BAF exceeds the BCF depends on the ratio k_D/k_R, which, in concert with C_D/C_W, controls the relative uptake rates from food and water. The M thus reflects the organism's bioenergetics, which dictates the rate of food intake relative to the corresponding oxygen demand and respiration rate. Third, the egestion rate constant k_E probably depends largely on the absorption efficiency of lipids and other dietary components and controls the maximum BMF for very hydrophobic substances as k_D/k_E [23,25]. Forth, dietary preferences determine the M values for each food item consumed and thus the overall M from all sources and hence the TMF. However, different dietary items may have different digestion characteristics. Fifth, the rate constants for growth dilution and biotransformation can play a significant role by decreasing BCFs, BAFs, BMFs, and TMFs [31,32]. These rates are most important for hydrophobic chemicals for which $k_R/(L_C \times K_{OW})$ and hence k_V, the respiration loss rate constant, is small.

In principle, from a BCF measurement and knowledge of these parameters, all of the bioaccumulation quantities can be estimated and if necessary confirmed by laboratory tests or from monitoring data. This enables the relative importance of respiratory and dietary intakes and the contributions of the 4 loss processes to be determined. The response kinetics also can be estimated, which are important for designing bioaccumulation and toxicity tests.

Concerns have been expressed that BCFs lack ecological relevance, are technically challenging to measure for higher K_{OW} chemicals, and are variable because of changing lipid contents. However, a strong case can be made for measuring BCF_K reproducibly and accurately under defined laboratory test conditions in which fish are fed a chemical-free maintenance diet so that egestion losses are included, thus facilitating the estimation of BAFs and BMFs from BCF_K.

For substances of log K_{OW} 4 and less, a steady-state BCF can be achieved experimentally in a relatively short test. Bioaccumulation factors are likely to be close to this BCF, BMFs are likely to be 1.0 or less, and near equi-fugacity applies. A useful check on the magnitude of an estimated BCF_E is that it is approximately $L_C \times K_{OW}$.

For more hydrophobic substances for which dietary uptake is critical, BAFs and BMFs are potentially predictable from BCFs using values of M deduced from knowledge of the fish physiology and the chemical concentrations in the diet. To calculate concentrations and BAFs in a food web, the concentration of BCF in organism 1 is first estimated, then M_2 is deduced followed by BAF_2, then M_3 and BAF_3, and so on. A major disadvantage of BCF and BAF is that they require a concentration in water that may be uncertain and difficult to measure accurately. The use of BMFs avoids this difficulty. Ultimately, however, the absolute concentrations, not their ratios, are of concern from an exposure and risk assessment perspective.

An attractive but demanding ecological modeling approach is to measure or estimate the water and sediment concentrations, the various rate constants, and dietary preferences for organisms in the food web, deduce the biotic concentrations, then iterate toward an optimal simulation by varying the exposure concentrations and other adjustable parameters such as dietary preferences and rate constants inherent in values of M. This could provide a mechanistic as well as empirical confirmation of the presence of biomagnification.

All bioaccumulation metrics are valuable. The BCF is a principal determinant of concentrations in food webs, because its magnitude reflects a thermodynamic quantity and it can be determined reproducibly in laboratory tests by reaching steady state or by using a k_1/k_2 approach. In conjunction with the equilibrium multiple M, it drives BAFs and BMFs and can be used to estimate or confirm measured BAFs and BMFs, provided that appropriate physiological data are available, as is usually the

case for well-characterized test organisms. The present study illustrates the relationships between the various bioaccumulation metrics and provides a possible methodology for converting between measures.

An attractive option is to modify the OECD 305 test as described in the proposal to include dietary uptake [16]. If fish are fed a maintenance diet of known chemical concentration in a defined food matrix, one may estimate k_D, the dietary assimilation efficiency, and k_T. These test data are also particularly valuable for obtaining estimates of k_M [31]. A BAF and BMF can thus be estimated from experimental data. In some cases, maintaining the fish until steady state is reached may be possible. Another option is to use advance information on the uptake and loss rate constants to design a test in which the fish are rapidly brought to a predetermined relatively high concentration well below effect levels; then their diet is adjusted to that corresponding to the predetermined concentration, to maintain that fish concentration at near-steady state for a prolonged period. Such a test would provide unequivocal quantitative empirical evidence of the extent of bioaccumulation.

A general conclusion from this analysis is that a combination of laboratory tests, monitoring data, and mass-balance modeling can provide a convincing and consistent quantitative picture of the bioaccumulation phenomena. A demonstrated lack of consistency is also valuable as an indication of unusual chemical or physiological properties or assumptions that deserve further investigation.

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