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Bioconcentration, bioaccumulation, biomagnification and trophic magnification: a modelling perspective

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We present a modelling perspective on quantifying metrics of bio-uptake of organic chemicals in fish. The models can be in concentration, partition ratio, rate constant (CKk) format or fugacity, Z and D value (fZD) format that are shown to be exactly equivalent, each having its merits. For most purposes a simple, parameter-parsimonious one compartment steady-state model containing some 13 parameters is adequate for obtaining an appreciation of the uptake equilibria and kinetics for scientific and regulatory purposes. Such a model is first applied to the bioaccumulation of a series of hypothetical, non-biotransforming chemicals with $\log K_{OW}$ (octanol–water partition ratio) values of 4 to 8 in 10 g fish ranging in lipid contents to deduce wet-weight and lipid normalized concentrations, bioaccumulation and biomagnification factors. The sensitivity of biomagnification factors to relative lipid contents is discussed. Second, a hypothetical 5 species linear food chain is simulated to evaluate trophic magnification factors (TMFs) showing the critical roles of K_{OW} and biotransformation rate. It is shown that lipid normalization of concentrations is most insightful for less hydrophobic chemicals ($\log K_{OW} < 5$) when bio-uptake is largely controlled by respiratory intake and equilibrium (equi-fugacity) is approached. For more hydrophobic chemicals when dietary uptake kinetics dominate, wet weight concentrations and BMFs are more insightful. Finally, a preferred strategy is proposed to advance the science of bioaccumulation using a combination of well-designed ecosystem monitoring, laboratory determinations and modelling to confirm that the perceived state of the science contained in the models is consistent with observations.

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Environmental significance

It is internationally accepted that the three principal criteria for evaluating the adverse environmental effects of chemicals are: P persistence, B bioaccumulation and T toxicity. Here we address B and show that the use of simple and commonly accepted criteria for B can be flawed, especially for hydrophobic substances. We argue that mass balance models can contribute significantly to B assessments, especially when combined with laboratory tests under controlled conditions and well-designed monitoring programs. As specific examples, we present a modeling perspective on bioaccumulation factors of hypothetical relatively hydrophobic chemicals in a series of small fish ranging in lipid contents. We deduce wet-weight and lipid-normalized concentrations, bioaccumulation factors and biomagnification factors. We discuss the implications of lipid contents, which have unappreciated effects on both wet-weight and lipid-weight concentrations and their ratios. It is shown that lipid normalization of concentrations is more insightful for less hydrophobic chemicals (*i.e.* $\log K_{OW} < 5$) because bio-uptake is largely controlled by respiratory intake and equilibrium is approached, implying that lipid normalized concentration ratios are more insightful and less variable. On the contrary, for very hydrophobic chemicals dietary uptake kinetics dominate, and wet weight concentrations are more insightful, especially when estimating trophic magnification factors that are increasingly applied for regulatory purposes, often without a full appreciation of their inherent variability and uncertainties. We suggest strategies to advance the science of bioaccumulation, including more data on partitioning ratios for key biological tissues and the acquisition of more data on sediment/water concentration and fugacity ratios, spatial and temporal variabilities, reproductive losses and changing diets as a function of growth.

1 Introduction

Bioaccumulation of organic substances is an important component of chemical risk assessment for both scientific and

regulatory purposes. Chemical concentrations in biota that are orders of magnitude larger than those in water and air are important for several reasons. Such large concentrations may adversely affect organisms across food webs, especially if internal concentrations reach toxic levels. Rather than measure the usually low concentrations in water or air it may be preferable to measure the relatively higher concentrations in biota resulting from bioaccumulation, but this requires information on the magnitude and determinants of these relative

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concentrations. Studies of bioaccumulation fall generally into one of the following categories: ecosystem monitoring, laboratory tests under controlled conditions, mass balance modelling, and *in vivo* and *in vitro* ADME studies. In this contribution we address insights that can be gained from modelling focusing primarily on aquatic organisms that respire in water and sediments but recognizing that similar principles apply to air-breathing mammals, birds, and reptiles. In Table 1 we define the bio-uptake factors and terminology employed herein and widely used in modelling and monitoring studies.^{1–5} The bio-concentration factor (BCF) expresses the increase in concentration, but with little or no increase in fugacity as measured in tests such as OECD 305.⁶ The bioaccumulation factor (BAF) includes a further concentration increase as well as a fugacity increase. It can be viewed as the product of the BCF and a ‘multiplier’ dependent on the BAF of the diet and the ratio of the rates of dietary uptake and respiratory uptake.⁷ The biomagnification factor BMF is essentially the ratio of the BAFs of the predator and the prey and may involve an increase in both concentration and fugacity. The TMF as the slope of the log concentration *vs.* trophic position is related to the mean BMF of the species comprising the food web. Considerable literature exists on these factors and especially BMFs and TMFs that may yield the highest concentrations and exposures.^{8–12} In the terminology of MacDonald *et al.*,¹³ a BCF represents solvent switching from water to lipid at a constant fugacity, while BAF, BMF and TMF represent additional solvent depletion as the ingested lipid solvent is hydrolysed causing an increase in fugacity.

In Table 1 the water concentration may be of whole water or (as in this study) only truly dissolved chemical. Biotic concentrations may be expressed as wet weight or lipid normalized quantities or they may be specific to defined tissues. Trophic magnification factors are generally obtained from the slope of a plot of log lipid normalised concentrations *vs.* trophic position or level, the latter being deduced from ¹⁵N measurements.^{8,14,15}

It is obviously critical to define the concentration units derived from the mass balance equations for comparison with monitoring data. The most commonly used units are whole body (wet weight) C_{FW} and lipid-normalized C_{FL} concentrations where C_{FL} equals C_{FW}/L , and L is the lipid content. Here, subscript F refers to the organism (fish), W to wet weight and L to lipid normalised.

Homogenizing the whole fish, and using a standard method of lipid extraction may be used to determine these concentrations. It can be experimentally demanding to homogenize large fish, thus it may be convenient to analyse only part of the

carcass; for example, a fillet that is largely muscle and is preferred for human consumption. The concentration in a fillet can be significantly different from that of the whole fish and this difference also applies to lipid-normalized concentrations because the lipid content of the fillet is usually much lower than that of the whole fish. For example, Niimi and Oliver¹⁶ obtained such data for PCBs in rainbow trout and showed that for the more recalcitrant congeners (penta- to deca-chloro) the muscle concentrations C_{FM} (ng g^{-1} ww) averaged a factor of 3.84 lower than that of the whole fish concentrations C_{FW} (ng g^{-1} ww). This is largely attributable to differences in the reported lipid content of 9.8% in the whole fish and 1.7% in muscle, a factor of 5.76. For example, if C_{FW} is 100 ng g^{-1} ww then C_{FM} may be approximately $100/3.84$ or 26 ng g^{-1} ww. The corresponding lipid-normalized wet-weight and muscle concentration will be respectively $26/0.098$ or 1020 ng g^{-1} lw and $26/0.017$ or 1532 ng g^{-1} lw, a factor of $5.76/3.84$ or 1.5 difference. Lipid-normalized muscle or fillet concentrations are thus likely to be significantly larger than lipid-normalized wet-weight concentrations for substances such as PCBs.

Another complication is the simplifying assumption that hydrophobic chemicals partition only to lipids. It is generally accepted that there is appreciable partitioning into other non-lipid phases such as protein. For example, the ratio of protein to lipid partition coefficients with respect to water being of the order of 0.03¹ implying that less chemical partitions into protein tissue compared with lipid. It follows that if the fillet protein content is much higher than that of the lipid, much of the solute will reside in the protein phase. The lipid-normalized concentration would then be over-estimated. Finally, there can be concerns about the accuracy of lipid content measurements, especially at low lipid levels as may occur in planktonic organisms of low lipid content for which the lipid-normalized concentrations are much larger than wet weight concentrations and are very sensitive to errors in lipid measurement. Endo, Goss and Brown^{17–19} have demonstrated that partitioning to different biotic phases is more accurately evaluated using Abraham or poly-parameter LFER methods rather than simple octanol–water partitioning (K_{OW}). We assume, however, that for the present screening-level purposes, the lipid–water partition coefficient is approximately equal to the octanol–water partition coefficient K_{OW} , recognizing that this is a significant simplification and does not apply to all chemicals, nor to all lipids.

Lipid-normalized concentrations prove to be very convenient when comparing concentrations between species in food webs, in part because they are proportional to fugacities, thus the equilibrium status of a chemical between water, sediment and organisms comprising food webs can be revealed by comparing

Table 1 Definitions for BCF, BAF, BMF and TMF

| Bio-uptake factors | Definition |
|------------------------------------|--|
| Bioconcentration factor (BCF) | Ratio of fish to water concentrations with no dietary intake |
| Bioaccumulation factor (BAF) | Ratio of fish to water concentrations with dietary intake |
| Biomagnification factor (BMF) | Ratio of fish to diet concentrations |
| Trophic magnification factor (TMF) | Averaged BMF over a food web of several trophic levels |



lipid normalized or organic carbon normalized concentrations. Obviously, C_{FW} for one fish should not be compared with C_{FL} for another. This issue can become critical when calculating BMFs and TMFs that necessarily involve comparison of concentrations between prey and predator fish. Common practice is to measure and report both wet-weight and lipid-normalized concentrations along with the identity of the tissues analysed and the measured lipid contents.

2 Background

2.1 Evolution of bio-uptake models

Models of bioaccumulation have evolved considerably from the early bioconcentration work of Neely *et al.*²⁰ More comprehensive dietary and respiratory uptake models such as that of Thomann²¹ address biomagnification in which the predators achieve higher concentrations than their prey.^{1,2} An issue common to all environmental models is the optimal number of compartments. To achieve greater fidelity to reality the number of compartments may be increased, but this is at the expense of requiring additional parameters and their associated uncertainties, especially those controlling inter-compartment transport and partitioning. A balance is needed between complexity and parsimony as dictated by the nature of the application as discussed by several authors.^{22,23} The currently preferred strategy is to use as parsimonious a model as possible consistent with satisfying the modelling objective.

It is common to refer to one-compartment models in which there is no attempt to describe differences in internal distributions. Rates of input by respiration and dietary uptake are defined using a gross input rate and uptake efficiencies. This implies the existence of an external compartment in which there is a split between absorbed and non-absorbed chemical. It can be argued that the simple one-compartment model actually contains three compartments in which only the splitting performance of the two external compartments is defined, thus simplifying the model. If the chemical is subject to biotransformation in the gut as described by Lo and Gobas¹⁰ or the chemical properties change in response to pH variation during

respiration as described by Erikson *et al.*,^{24–26} then it is essential to define the mass balances in these ‘external’ compartments.

For toxicokinetic and toxicodynamic or PBPK models in which uptake or effects in a target organ are evaluated it is essential to include that compartment explicitly.^{17,27} An extreme example is the recent model by Larisch *et al.*²⁸ that treats 10 internal organs and 3 external organs.

We believe a consensus has emerged that for many practical purposes a simple and parameter-parsimonious one compartment steady-state model with chemical uptake efficiencies is adequate to obtain an inherent appreciation of the dynamic uptake and loss processes as shown in Fig. 1. Exceptions to these processes are likely to occur when fish forage in regions that have particularly high or low contaminant concentrations, when spawning, or when losing large amounts of body mass in the winter.

The primary compartment of concern is the body, the organs, and the tissues, but it is essential to address chemical transport ‘splitting’ in the gut and the gill cavity as separate absorption efficiencies. The processes corresponding to the arrows in Fig. 1 can be expressed in conventional concentration-partition ratio, rate constant (CKk) or in fugacity-Z value, D value (fZD) format, but they are, or should be, algebraically equivalent.

The conventional differential and steady-state equations for uptake in CKk format are given in eqn (1)–(4), the various parameters being defined in Table 2.

Mass balance uptake differential equation in CKk format is:

$$\Delta C_{FW}/\Delta t = (k_R C_W + k_D C_D) - C_{FW}(k_V + k_E + k_M + k_G) \quad (1)$$

where C_{FW} is the wet-weight fish concentration, t is time, C_W is the water concentration, C_D is the diet concentration, k_R is the respiration uptake constant, k_D is the diet uptake constant, k_V is the respiration output constant, k_E is the egestion output constant, k_M is the biotransformation rate constant, and k_G is the growth rate constant. Integration from an initial fish concentration of zero and constant inputs yields eqn (2). At steady-state this reduces to eqn (3) and the resulting BMF is given by eqn (4).

$$C_{FW} = [(k_R C_W + k_D C_D)/k_T] \times [1 - e^{-k_T t}] \quad (2)$$

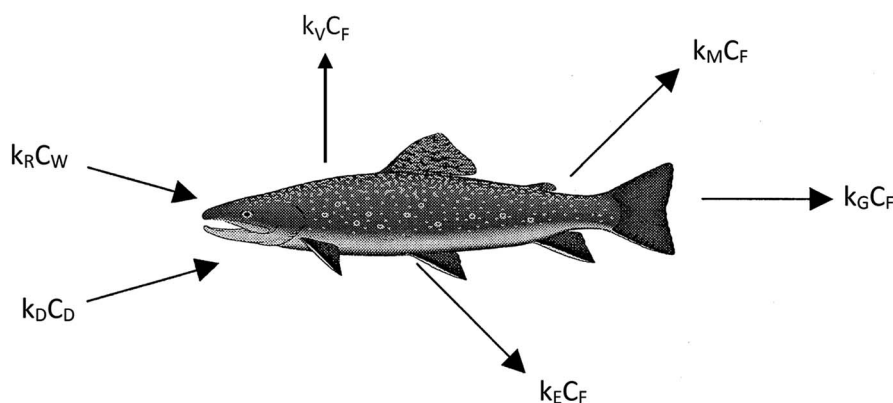


Fig. 1 Chemical uptake and clearance mechanisms modelled in this study.



Table 2 Definitions of Z and D values and relationships between the CKk and fDZ formats for a specimen calculation with a hypothetical relatively hydrophobic chemical of $\log K_{OW}$ of 6.0. Abbreviation for wet weight is ww and abbreviation for lipid weight is lw

| Quantity | CKk format (units) | Value | fDZ format | Value |
|------------------------------------|---|-----------|---|-----------------------|
| Chem. molar mass | MW (kg mol ⁻¹) | 0.1 | MW (kg mol ⁻¹) | 0.1 |
| Oct-water partition ratio | K_{OW} | 1 000 000 | $K_{OW} = Z_O/Z_W$ | 1×10^6 |
| Fish size | M_F (kg) | 0.01 | V_F (m ³) | 0.00001 |
| Lipid content of diet | L_D | 0.05 | L_D | 0.05 |
| Lipid content of fish | L_F | 0.10 | L_F | 0.10 |
| $Z_W = 1/H$ | | | | 0.1 |
| $Z_O = Z_W \cdot K_{OW}$ | | | | 100 000 |
| $Z_D = L_D \cdot Z_O$ | | | | 5000 |
| $Z_f = L_F \cdot Z_O$ | | | | 10 000 |
| Equilibrium BCF | $K_{OW} \cdot L_F$ | 100 000 | Z_F/Z_W | 100 000 |
| Net respiration rate | G_V (L per day) | 5 | G_V (m ³ per day) | 0.005 |
| Net feeding rate | G_f (kg per day) | 0.0006 | G_f (m ³ per day) | 0.6×10^{-6} |
| Transport parameters | | | | |
| Q values diet/egestion | $Q_C = k_D/k_E$ | 6 | $Q_f = D_D/D_E = Q_C \cdot Z_D/Z_F$ | 3 |
| Respiratory intake | k_R (L/kg per day) | 500 | $D_R = G_V \cdot Z_W$ | 0.0005 |
| Dietary intake | k_D (L/kg per day) | 0.06 | $D_D = G_f \cdot Z_D$ | 0.003 |
| Respiratory loss | $k_V = k_R/BCF$ (days) | 0.005 | $D_V = D_R$ | 0.0005 |
| Egestion | $k_E \sim k_D/Q_C$ (days) | 0.01 | $D_E = V_F \cdot Z_F \cdot k_E$ | 0.001 |
| Biotransformation | k_M , (days) | 0.01 | $D_M = V_F \cdot Z_F \cdot k_M$ | 0.001 |
| Growth dilution | k_G , (days) | 0.0025 | $D_G = V_F \cdot Z_F \cdot k_G$ | 0.00025 |
| Total loss rate | $k_T = k_V + k_E + k_M + k_G$ | 0.0275 | $D_T = D_V + D_E + D_M + D_G$ | 0.00275 |
| Exposure quantities | | | | |
| Water concentration | C_W (mg L ⁻¹ or g m ⁻³) | 0.001 | C_W (mol m ⁻³) | 0.00001 |
| Diet/water fugacity ratio | | | f_D/f_W | 1.5 |
| Diet concentration ww | C_{DW} (mg kg ⁻¹ , g m ⁻³) | 75 | C_{DW} (mol m ⁻³) | 1.125 |
| Diet concentration lw | C_{DL} (mg kg ⁻¹) | 1500 | C_{DL} (mol m ⁻³) | 22.5 |
| Water fugacity | f_W (Pa) | | $f_W = C_W/Z_W$ | 0.0001 |
| Diet fugacity | f_D (Pa) | | $f_D = C_{DW}/Z_D$ | 0.00015 |
| Calculated quantities | | | | |
| | Eqn (1)–(4) | | Eqn (5)–(8) | |
| Fish fugacity | f_F (Pa) | 0.00018 | $f_F = C_{FW}/Z_F$ | 0.000182 |
| Fish lipid fugacity | f_L (Pa) | | $f_L = C_{FL}/Z_L$ | 0.000182 |
| Fish concentration ww | C_{FW} (mg kg ⁻¹ , eqn (3)) | 181.82 | $C_{FW} = f_F \cdot Z_F$ (mol m ⁻³) | 1.8182 |
| Fish concentration lw | $C_{FL} = C_{FW}/L_F$ (mg kg ⁻¹) | 1818.2 | $C_{FL} = f_F \cdot Z_O$ (mol m ⁻³) | 18.182 |
| Bio-uptake factors | | | | |
| Bioacc. factor ww BAF _W | $BAF_W = C_{FW}/C_W$ | 181 818.2 | | |
| Bioacc. factor lw BAF _L | $BAF_L = C_{FL}/C_W$ | 181 8182 | | |
| Biomag. fact. ww BMF _W | $BMF_W = C_{FW}/C_{DW}$ | 2.4242 | | |
| Biomag. fact. lw BMF _L | $BMF_L = C_{FL}/C_{DL}$ | 1.2121 | | |
| Chem. quantity in fish | $C_{FW} \times M_F$ (mg) | 1.81 | $V \cdot Z_F \cdot f_F$ mol | 1.82×10^{-5} |
| Total uptake rate | (mg per day) | 0.05 | mol per day | 5×10^{-7} |
| Half-time | $\tau = \ln 2/k_T$, (days) | 25.2 | $\tau = \ln 2 \cdot V_F \cdot Z_F/D_T$ | 25.2 |

where k_T is the sum of the rate constants for all loss processes, namely ($k_V + k_E + k_M + k_G$). At steady-state when $k_T t \gg 1$, C_{FW} approaches $(k_R C_W + k_D C_D)/k_T$ and a bioaccumulation factor can be calculated as C_{FW}/C_W and a biomagnification factor as C_{FW}/C_D .

$$BAF_W = \frac{C_{FW}}{C_W} = \left(\frac{k_R C_W + k_D C_D}{C_W k_T} \right) \quad (3)$$

$$BMF_W = \frac{C_{FW}}{C_D} = \left(\frac{k_R C_W + k_D C_D}{C_D k_T} \right) \quad (4)$$

For hydrophobic chemicals when C_D is large and $k_D \gg k_R C_W/C_D$, BMF_W approaches k_D/k_T .

Further, for a slowly metabolized, hydrophobic chemical in a slow-growing fish $k_E \gg (k_V + k_M + k_G)$, BMF_W approaches k_D/k_E defined here as Q_C the ratio of dietary uptake and egestion rate constants.

The analogous uptake equations in fZD format are as follows, where D_T is the sum of the loss D values.

$$\frac{V_F Z_F \Delta f_F}{\Delta t} = (D_R f_W + D_D f_D) - f_F (D_R + D_E + D_M + D_G) \quad (5)$$



$$f_F = \frac{(D_R f_W + D_D f_D)}{(D_R + D_E + D_M + D_G)} \times (1 - \exp(-D_T t / V_F Z_F)) \quad (6)$$

When $D_T t / V_F Z_F \gg 1$ and steady state is approached, yielding eqn (7):

$$f_F \sim \frac{(D_R f_W + D_D f_D)}{(D_R + D_E + D_M + D_G)} = (D_R f_W + D_D f_D) / D_T \quad (7)$$

For less hydrophobic chemicals when $D_R \gg D_D$, f_F approaches f_W , equilibrium applies, BCF_W and BAF_W approach Z_F / Z_W and are approximately equal to $L \cdot K_{OW}$ where L is the fish lipid content. BMF_W becomes less relevant because dietary uptake is unimportant.

On the contrary, for a persistent, hydrophobic chemical in a slow-growing fish with dietary uptake, $D_E \gg (D_V + D_M + D_G)$, f_F approaches $f_D \cdot D_D / D_E$ or $f_D \cdot \tau$ where Q_f is defined as the ratio of dietary uptake and egestion D values. BAF_W , C_W and f_W become less relevant because respiratory uptake is relatively unimportant. The lipid normalized uptake metric BMF_L approaches Q_f and is the ratio of the fish and diet fugacities. As before, the wet weight BMF_W approaches Q_C and $Q_f \cdot (Z_F / Z_D)$ or approximately $Q_f \cdot (L_F / L_D)$. The two Q values are thus not equal and depend on the relative lipid contents of the fish (L_F) and diet (L_D). Q_f directly expresses the increase in fugacity corresponding to biomagnification, while Q_C expresses the corresponding increase in concentration.

Substitution of the various rate constants and D values in Table 2 into eqn (3) and (7) demonstrates the exact equivalence of the two formats for a chemical of moderate hydrophobicity. The steady-state eqn (3) and (7) are most readily interpreted, and are of most interest for both scientific and regulatory purposes. It is relatively straightforward to apply the basic equations to multiple organisms in food webs with defined dietary preferences and to organisms that respire in sediments and the water column. The principal challenge is to obtain accurate values for the various equilibrium and rate parameters and dietary preferences.

For hydrophobic substances, the egestion loss rate constant and D value are particularly important, since as discussed later egestion along with biotransformation play a critical role in determining the extent of biomagnification. The most rigorous approach is to define the input diet and output feces compositions and rates and as relative quantities of materials such as lipids and non-lipids including protein, carbohydrate, inert fibrous material, and water and assign partition ratios relative to water for each material. An example is the Arnot and Gobas¹ model that treats three materials, lipids, non-lipid organic matter (NLOM) and water in both diet and feces. Larisch *et al.*²⁸ treat five materials. The capacity of the feces to absorb and transport the chemical is inevitably lower than that of the ingested diet by a factor typically ranging from 3 to 10. This factor is primarily determined by the quantities of lipid transported in food and feces, thus a simple and very approximate approach is to suggest, as in Table 2, a multiple Q in the range 3 to 10 by which the egestion rate constant k_E or D value D_E is less

than that for the food. Inspection of the steady-state equation shows that for a persistent hydrophobic chemical in a fish that is not growing, the BMF will approach Q . It is noteworthy that Q_C in the CKk format is generally not equal to Q_f in the fZD format, thus the BMF expressed as a whole body or wet weight concentration ratio is generally unequal to the fugacity ratio. In principle, it is possible and potentially attractive to define a Q for each material and calculate a lumped Q_C or Q_f to deduce the egestion rate.

If there is no dietary uptake and the principal loss is by ventilation, a bioconcentration factor (BCF) can be calculated as k_R / k_V and equilibrium (equi-fugacity) is approached. If the very simplistic assumption is made that octanol and lipids have similar solvent properties for the chemical and lipids are the only absorbing phase, this BCF can also be estimated as the product of the fish lipid content and the octanol–water partition coefficient, namely $L_F \cdot K_{OW}$. If k_R is known, k_V can then be estimated as approximately k_R / BCF or $k_R / (L_F \cdot K_{OW})$. This BCF is effectively a thermodynamic partition ratio, however, it may be affected by weight gain (growth) or loss. For screening level purposes a lipid content L of 5% is often used thus a BCF of 5000 corresponds to a K_{OW} of 100 000 or $\log K_{OW}$ of 5. We accept the simplistic nature of this approach in that it applies only to a subset of chemicals. Other chemicals partition to other phases by electrostatic interactions, protein binding, and covalent bonding.

2.2 Relationships between the CKk and fZD mass balance equation formats

To illustrate the equivalence and relative merits of the two formats a simple spreadsheet was compiled to calculate the bio-uptake quantities from selected input parameters for a specimen chemical of $\log K_{OW}$ of 6.0, Henry's constant $10 \text{ Pa m}^3 \text{ mol}^{-1}$ and molar mass 100 g mol^{-1} in a 10 g fish of lipid content 10% with a growth rate constant of 0.0025 days. The fish is exposed to water at a concentration of 0.001 g m^{-3} and a diet of lipid content 0.05 g g^{-1} with a fugacity 1.5 times that of the water. The chemical is subject to biotransformation with a rate constant of 0.01 days^{-1} . Respiratory and dietary uptake parameters were taken from allometric relationships used by Arnot and Gobas¹ but quantities were rounded off to facilitate interpretation.

Calculations were done in both CKk and fZD formats independently and yield identical results as shown in Table 2, namely a fish wet weight concentration C_{FW} of 182 g m^{-3} or 1.82 mol m^{-3} . The corresponding lipid normalized concentrations are a factor of 10 greater. The equilibrium BCF of the fish is 100 000, the BAF_W is 181 800, and the BAF_L is 1 818 000. The calculated BMF_W is 2.42 and BMF_L is 1.21. The fugacity of the chemical in water is 0.1 mPa, the diet is 0.15 mPa and the fish is 0.18 mPa, thus the fish to diet fugacity ratio is 1.2, equal to the BMF_L , showing modest biomagnification.

The uptake processes are: respiration 10% and diet 90% with a total rate of 0.05 mg per day *i.e.* $0.5 \text{ } \mu\text{mol}$ per day. Loss processes are: respiration 17%, egestion 41%, biotransformation 35% and growth dilution 8%. At steady state the body burden is 1.81 mg or $18.1 \text{ } \mu\text{mol}$. The half-lives for uptake and



loss are both 25.2 days. The total input and loss rates are 0.05 mg per day, thus the residence time of the chemical in the fish is 36 days.

It is notable that the assumed ratio of dietary uptake to egestion rate parameters Q_C for the CKk format is 6 while Q_f for the fZD format is 3. This difference is attributable to the different lipid contents of the diet and fish since Q_f is $Q_C \cdot (L_D/L_F)$. Q_C and Q_f represent limiting maximum BMFs on a concentration and fugacity or lipid normalized basis respectively as is apparent from eqn (4) and (7). For example, increasing $\log K_{OW}$ to 8 and setting biotransformation and growth rates to zero result in a BMF_W of 5.98, approaching Q_C of 6 and a BMF_L of 2.99, approaching Q_f of 3. These Q values are critical determinants of BMFs for very hydrophobic chemicals. As K_{OW} increases, dietary uptake becomes the dominant input process and respiration becomes negligible. The fish is then unaffected by the concentration in water except that this water concentration controls concentrations at lower trophic levels.

Inspection of these results suggests that the CKk format is easier to understand and apply. Concentration ratios can, however, become very large and difficult to interpret and relative concentrations between fish and diet items can be misleading since both wet weight and lipid normalized concentrations can be used. This format proves to be most preferred for conditions under kinetic control as applies to hydrophobic chemicals.

The fZD format may be initially more difficult to apply, but it can provide additional insights into the bio-uptake process by revealing the relative equilibrium status between water, sediment and various aquatic species. Bio-uptake metrics expressed as fugacity ratios generally lie in the range 1 to 10 and are more easily interpreted. This format is most relevant when conditions are largely controlled by equilibrium processes as applies to less hydrophobic chemicals. Since BMF_L factors and fugacity ratios are equivalent, either can be used to characterize trophic magnification in food webs, however this implies that all partitioning is into lipids and in this simple case that lipids are equivalent to octanol.

Connolly and Pederson²⁹ first demonstrated this fugacity increase in monitoring data. This was followed by Gobas and colleagues^{30,31} who demonstrated experimentally that lipid digestion causes a fugacity increase in the digestive system and this elevated fugacity is transmitted into the body, causing

biomagnification. As noted earlier, this process of fugacity increase can be viewed as being caused by 'solvent depletion'.¹³ The fugacity format thus captures the fundamental cause of bioconcentration as being driven by differences in Z values (*i.e.* the capacity to absorb the chemical, dependent on solubility) between water and the fish, the ratio of which is a partition ratio. It also addresses the fundamental cause of bio-magnification reflecting a reduction in Z value in ingested food during lipid digestion and corresponding increase in fugacity.

Ultimately, since both formats yield identical results either or both can be used.

3 Results

3.1 Mass-balance equations applied to bioaccumulation in a simple predator-prey system

We now discuss several implications of the models comprising these equations, especially the sensitivity of desired outcomes to the parameter values of the selected chemical. A significant advantage of having available a simple and robust validated model is that the implications of changes in parameters can be explored mathematically rather than by actual, demanding, and expensive testing. To illustrate these implications we compile simple bioaccumulation models employing realistic ranges of properties of typical organisms and chemical parameters. The models are outlined in a recent study of TMFs to predict TMFs and BMFs.⁹ We suggest typical values for rate constants as a function of fish species and the chemical. Uptake and loss parameters are given in Table 3 for five species using allometric correlations suggested by Arnot and Gobas¹ as a function of fish mass and temperature. These rate constants (days^{-1}) are defined in Table 2. Typical numerical values are included and applied initially to a 10 g fish *i.e.* smelt, namely k_R (respiratory uptake) 470, k_D (dietary uptake) 0.063, k_G (growth dilution) 0.0025, and k_E (egestion) 0.0105, conveniently estimated for screening level purposes as a factor Q of 6 less than k_D . Dietary and respiratory assimilation efficiencies and the respiratory loss rate constant k_V are K_{OW} dependent and are estimated using parameters from the Arnot-Gobas model.¹ Values of k_M are later selected arbitrarily.

We first model the simple bioaccumulation of a series of hypothetical, non-biotransforming chemicals with $\log K_{OW}$ values of 4, 5, 6, and 8 in three smelt of different lipid contents

Table 3 Characteristics of selected food web species. Dietary and respiratory assimilation efficiencies and the respiratory loss rate constant k_V are K_{OW} dependent, k_V being estimated as $k_R/(L \cdot K_{OW})$

| Species | Phytoplankton | Zooplankton | Mysis | Smelt | Trout |
|--|---------------|----------------------|----------------------|--------|--------|
| Mass (g) | Not required | 0.0001 | 0.1 | 10 | 100 |
| Lipid content, L (g g^{-1}) | 0.05 | 0.03 | 0.2 | 0.1 | 0.25 |
| $Q_C = k_D/k_E$ | | 6 | 6 | 6 | 6 |
| Respiratory flow, G_W (L per day) | | 0.0049 | 0.44 | 8.77 | 39.2 |
| Dietary uptake (kg per day) | | 8.2×10^{-8} | 2.9×10^{-5} | 0.0015 | 0.010 |
| k_R (days^{-1}) | | 26 660 | 2376 | 474 | 212 |
| k_D (days^{-1}) | | 0.348 | 0.124 | 0.062 | 0.044 |
| k_G (days^{-1}) | | 0.025 | 0.0063 | 0.0025 | 0.0005 |
| k_E (days^{-1}) | | 0.0581 | 0.0206 | 0.0103 | 0.0073 |



exposed to contaminant in the same diet and the same respired water. These predator smelt occupy a functional trophic level (TL) of 2.0 and are designated either as lean (L) with a lipid content of 2%, mean (M) with 10%, and fat (F) with 20%. These rather extreme lipid contents are selected to facilitate interpretation of results. The water concentration is 1 mg m^{-3} , the diet has a lipid content of 10% and occupies a functional TL of 1.0 and is thus in equilibrium with the water as controlled by the lipid–water partition coefficient that is assumed to equal the octanol–water partition coefficient K_{OW} . These dietary concentrations are designated C_{DW} and C_{DL} on a wet-weight and lipid-weight basis respectively. An arbitrary fugacity Z -value Z_W of $0.01 \text{ mol m}^{-3} \text{ Pa}^{-1}$ in water is assumed corresponding to a Henry's law constant of $100 \text{ Pa m}^3 \text{ mol}^{-1}$ to enable fugacities to be calculated and compared for the water and fish. In each case the wet-weight fish concentrations C_{FW} and the lipid-normalized concentrations C_{FL} *i.e.* C_{FW}/L are deduced and compared. The bioaccumulation factors on a wet weight basis (BAF_W) and on a lipid-weight basis (BAF_L) are calculated as C_{FW}/C_W and C_{FL}/C_W respectively. The biomagnification factors on a wet-weight basis (BMF_W) and on a lipid-weight basis (BMF_L) are calculated as C_{FW}/C_{DW} and C_{FL}/C_{DL} respectively.

Also calculated are the percentages of uptake by diet and respiration and the percentages that each loss rate constant contributes to the total loss rate constant, thus identifying the dominant rate constant(s) and the half-times for uptake and clearance.

A selection of the results is given in Table 4 that gives calculated values of C_{FW} and C_{FL} for values of $\log K_{OW}$ of 4, 5, 6, and 8. In these simulations zero biotransformation is assumed *i.e.*, k_M is zero, but is varied later.

The columns on the left ($\log K_{OW}$ of 4.0) show that the lipid normalized concentration C_{FL} is fairly constant (10.8 to 11.3) as are values of BAF_L because the lipid is close to equilibrium with

the water, but the wet weight concentrations C_{FW} vary considerably (0.23 to 2.15) depending on the lipid content of the fish. The fish fugacities are directly proportional to values of BAF_L . This proportionality applies only if lipid is the only sorbing phase. The ratio of fish to diet fugacity equals BMF_L and varies from 1.08 to 1.13 indicating near-equilibrium and negligible biomagnification. The percentage chemical input from the diet is minimal (11.9%) because of the low concentration in the diet. The primary loss process is by ventilation, k_V , (95 to 99%) which depends inversely on lipid content, thus the rate constant for total loss is smallest for the fat fish, but the absolute rates of input and loss are equal in all three cases. Bioaccumulation factors on a wet-weight basis (BAF_W) range from 226 to 2150 reflecting the high variability in C_{FW} . Wet-weight biomagnification factors (BMF_W) vary similarly from 0.23 to 2.15. The total rate constant for loss ranges from 2.4 to 0.25 days^{-1} corresponding to short uptake and loss times of 0.3 to 2.8 days. The fat fish is slower to approach steady state because of its greater capacity for chemical. In this case it is clearly preferable to interpret the bioaccumulation phenomena on an equilibrium basis using C_{FL} or fugacity, as is normal practice recommended by Borga *et al.*⁸ and Burkhart *et al.*⁴ Use of wet-weight parameters can obscure the interpretation.

The columns in the centre-left with a higher value of $\log K_{OW}$ of 5.0 show that C_{FL} and C_{FW} now both vary considerably. Input is mainly or primarily from diet (57%) and remaining input is from respiration, reflecting the higher concentration in the diet. The primary loss processes are by ventilation (k_V) which depends inversely on lipid content, and egestion (k_E) which is independent of lipid content because the absolute rate is $C_{FW} \cdot k_E$. The rate constant for total loss is now less sensitive to fish lipid content and corresponds to uptake and loss half-times from 2.8 to 19 days. The ratio of fish to diet fugacities (BMF_L) now ranges from 2.21 to 1.51 indicating a higher fugacity in the

Table 4 Results of bioaccumulation estimations of a series of chemicals in 3 predator smelt differing in lipid contents, namely 'lean', 2%, 'mean' 10% and 'fat' 20% with properties given in Table 3. Note that BAF_L equals f_F/f_W and BMF_L equals f_F/f_D . The percentage contribution of each process to the overall loss rate constant k_T are included

| | $\log K_{OW} = 4.0, C_{DW} = 1.0, C_{DL} = 10.0$ | | | $\log K_{OW} = 5.0, C_{DW} = 10.0, C_{DL} = 100.0$ | | | $\log K_{OW} = 6.0, C_{DW} = 100.0, C_{DL} = 1000.0$ | | | $\log K_{OW} = 8.0, C_{DW} = 10\ 000, C_{DL} = 100\ 000$ | | |
|---------------|--|--------------------|--------------------|--|--------------------|--------------------|--|--------------------|--------------------|--|--------------------|--------------------|
| Predator | Lean | Mean | Fat | Lean | Mean | Fat | Lean | Mean | Fat | Lean | Mean | Fat |
| C_{FW} | 0.23 | 1.10 | 2.15 | 4.43 | 18.31 | 30.12 | 182.5 | 379.6 | 438.9 | 32 400 | 33 471 | 33 609 |
| C_{FL} | 11.3 | 11.0 | 10.8 | 221 | 183 | 151 | 9127 | 3796 | 2194 | 1.62×10^6 | 3.35×10^5 | 1.68×10^5 |
| Diet% | 11.9 | 11.87 | 11.87 | 57.17 | 57.17 | 57.17 | 92.89 | 92.89 | 92.89 | 99.75 | 99.75 | 99.75 |
| k_T | 2.36 | 0.48 | 0.25 | 0.25 | 0.06 | 0.04 | 0.036 | 0.018 | 0.015 | 0.0059 | 0.0057 | 0.0057 |
| $k_V\%$ | 99.5 | 97.30 | 94.74 | 94.78 | 78.42 | 64.50 | 64.89 | 26.99 | 15.60 | 4.00 | 0.83 | 0.41 |
| $k_E\%$ | 0.45 | 2.19 | 4.26 | 4.22 | 17.44 | 28.69 | 28.26 | 58.77 | 67.94 | 53.87 | 55.65 | 55.88 |
| $k_M\%$ | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| $k_G\%$ | 0.11 | 0.52 | 1.01 | 1.00 | 4.14 | 6.81 | 6.84 | 14.23 | 16.45 | 42.14 | 43.53 | 43.71 |
| BAF_W | 226 | 1104 | 2150 | 4426 | 18 307 | 30 116 | 1.83×10^5 | 3.80×10^5 | 4.39×10^5 | 3.24×10^7 | 3.35×10^7 | 3.36×10^7 |
| BAF_L | 1.13×10^4 | 1.10×10^4 | 1.08×10^4 | 2.21×10^5 | 1.83×10^5 | 1.51×10^5 | 9.13×10^6 | 3.80×10^6 | 2.19×10^6 | 1.62×10^9 | 3.35×10^8 | 1.68×10^8 |
| f_F/f_W | | | | | | | | | | | | |
| BMF_W | 0.23 | 1.10 | 2.15 | 0.44 | 1.83 | 3.01 | 1.83 | 3.80 | 4.39 | 3.24 | 3.35 | 3.36 |
| BMF_L | 1.13 | 1.10 | 1.08 | 2.21 | 1.83 | 1.51 | 9.13 | 3.80 | 2.19 | 16.20 | 3.35 | 1.68 |
| f_F/f_D | | | | | | | | | | | | |
| τ , days | 0.3 | 1.4 | 2.8 | 2.8 | 11.5 | 18.9 | 19.0 | 39.5 | 45.6 | 116.8 | 120.7 | 121.2 |



fish than that in the water in all cases and greater bio-magnification. In this case either C_{FW} or C_{FL} , or both, can be employed to interpret the bioaccumulation and trophic magnification.

The columns on the centre right ($\log K_{OW}$ of 6.0) show that C_{FW} now becomes more constant (182 to 440) and C_{FL} is now more variable (9127 to 2194). Diet is now responsible for 93% of the input because of its high concentration. There is appreciable biomagnification with BMF_W ranging from 1.8 to 4.4 while BMF_L ranges from 9.1 to 2.2 *i.e.* the effect of lipid content reverses the BMF trend. The primary loss processes are by ventilation and egestion. The uptake and loss half-times range from 19 to 46 days.

The columns on the right with $\log K_{OW}$ of 8 represent an extreme condition of super-hydrophobicity^{15,32} in which the diet is responsible for 99.8% of the inputs. The wet-weight concentrations are now nearly constant and the lipid-weight concentrations are highly variable. The primary loss processes are now by egestion (k_E) and growth (k_G) which are independent of lipid content, thus the rate constant for total loss ($\sim 0.006 \text{ days}^{-1}$) is also independent of lipid content and corresponds to a long half-time of 117 days. In this case it is clearly preferable to interpret the bioaccumulation phenomena using C_{FW} rather than C_{FL} because lipid normalization introduces an unnecessary variability. BMF_W ranges narrowly from 3.24 to 3.35 to 3.36 but the trend reverses for BMF_L with values of 16.2 to 3.35 to 1.68. C_{FL} is now highly variable while C_{FW} is fairly constant. For these conditions, it can be argued that it is preferable to use wet-weight concentrations and ratios.

These calculations illustrate several important features of BMFs and thus of TMFs. First, the wet-weight BMF_W is C_{FW}/C_{DW} , while the lipid-weight BMF_L is C_{FL}/C_{DL} , thus BMF_W is $BMF_L(L_F/L_D)$ where L_D and L_F are the lipid contents of the diet and fish respectively. When a fish consumes a lean diet and L_F/L_D is < 1.0 then BMF_L will exceed the BMF_W . On the contrary, when the fish is fatter than its diet the opposite occurs. Only when L_F and L_D are equal are the two BMFs equal as is apparent for the 'mean lipid' content fish. A BMF_L exceeding 1.0 in a predator may be attributable, not to biomagnification, but to a lean diet. At high values of K_{OW} lipid normalisation distorts the predator prey relationships if lipid contents are variable. These results suggest that when interpreting biomagnification data, both BMF_W and BMF_L should be inspected with the expectation that BMF_L will be more useful for less hydrophobic chemicals under equilibrium control and BMF_W more useful for highly hydrophobic chemicals under kinetic control.

Second, it may seem counter-intuitive that for highly hydrophobic substances lipid content is inconsequential because most of the chemical probably resides in the lipid phases. This insensitivity to lipid content arises because the fish concentration is controlled by the rate constant for loss k_T , and the principal contributing rate constants are independent of lipid content. Only k_V depends directly on lipid content, but it is small and insignificant, contributing less than 5% to the losses when $\log K_{OW}$ is 8.

Third, a common and correct justification for lipid normalization is that it reflects the fugacity of the fish relative to the

fugacity of the water and the diet. For the data in Table 2 BMF_L equals the ratio of the fish fugacity to that of the water and food. This ratio is close to 1.0 for the left-hand columns, but there is an increase in fugacity for the middle columns reflecting the expected biomagnification. For the columns on the right the fugacities in the fish are highly variable, specifically 16.2 to 1.68 times that of the water and food. Fugacities thus vary greatly depending on lipid levels of the predator. The biomagnification is not a result of thermodynamic partitioning, it is caused by the kinetic effect of a low and fairly constant value of k_T and the high and constant diet concentration that yield constant values of C_{FW} but highly variable values of C_{FL} . Fugacities then become less relevant indicators of bioaccumulation and biomagnification. In addition, varied lipid contents of the diet may impact various rate processes of the predator fish, including the diet ingestion rate, the growth rate, and ultimately the BMF. These types of impacts should be considered by modelers, however further discussion of this topic is beyond the scope of this study.

Simple bioaccumulation with biotransformation. To address biotransformation we again apply the simple bioaccumulation model to a series of chemicals with the same range of $\log K_{OW}$ values in the same 3 fish and again with the same water and diets, but biotransformation is introduced as an arbitrarily selected rate constant k_M of 0.01 days^{-1} corresponding to a half-life of approximately 70 days. The importance of this half-life has been reviewed by Arnot *et al.*^{33,34} and Goss *et al.*¹⁹ have suggested it as a direct metric of bioaccumulation potential.

The results in Table 5 are similar to those in Table 4 but biotransformation causes a reduction in all concentrations. The effect is greatest for the hydrophobic chemicals that have relatively slower non-biotransformation loss processes. Specifically, for $\log K_{OW}$ of 4 the fish concentrations are similar to those in Table 1. For $\log K_{OW}$ of 5 and 6 the concentrations in the fat fish are lower by a factor of up to 2 because k_M contributes up to 40% of the losses. For $\log K_{OW}$ of 8, k_M becomes the dominant (63%) loss rate constant and concentrations fall by nearly a factor of 3. These reductions in concentration are the direct result of k_M causing an increase in k_T .

For $\log K_{OW}$ of 8 the BMF_W values are all approximately 1.2 but the BMF_L values vary from 6.0 to 1.22 to 0.61 and are clearly being distorted by the varying lipid contents. The introduction of biotransformation as a significant loss process provides an additional incentive to avoid lipid normalization for very hydrophobic substances because the variation in C_{FW} values is considerably less than those of C_{FL} . Variation in lipid content for highly hydrophobic chemicals is inconsequential for steady state bioaccumulation and biomagnification, but it does affect the time required to reach steady state and this may be reflected in concentrations in ecosystems. It is thus interesting to explore how these assertions are reflected in food web simulations.

3.2 Linear food chain model to determine the TMF with and without biotransformation

A simple 5 species, 5 trophic level obligate food chain is defined in which the phytoplankton are assigned a TL of 1 while other species 2 to 5 have an exclusive diet of the species below. The



Table 5 Bioaccumulation simulations similar to Table 4 but including biotransformation with a rate constant of 0.01 days^{-1} corresponding to a half-life of approximately 70 days

| Predator | $\log K_{OW} = 4.0, C_{DW} = 1.0, C_{DL} = 10.0$ | | | $\log K_{OW} = 5.0, C_{DW} = 10.0, C_{DL} = 100.0$ | | | $\log K_{OW} = 6.0, C_{DW} = 100.0, C_{DL} = 1000.0$ | | | $\log K_{OW} = 8.0, C_{DW} = 10\,000.0, C_{DL} = 100\,000.0$ | | |
|---------------|--|-------------------|-------------------|--|-------------------|-------------------|--|-------------------|-------------------|--|-------------------|-------------------|
| | Lean | Mean | Fat | Lean | Mean | Fat | Lean | Mean | Fat | Lean | Mean | Fat |
| C_W | 0.22 | 1.08 | 2.07 | 4.26 | 15.71 | 23.67 | 143.31 | 241.91 | 264.67 | 1.2×10^4 | 1.2×10^4 | 1.2×10^4 |
| C_L | 11.24 | 10.82 | 10.33 | 212.76 | 157.07 | 118.35 | 7166 | 2419 | 1323 | 6.0×10^5 | 1.2×10^5 | 6.1×10^4 |
| Diet% | 11.87 | 11.87 | 11.87 | 57.17 | 57.17 | 57.17 | 92.89 | 92.89 | 92.89 | 99.75 | 99.75 | 99.75 |
| k_T | 2.37 | 0.49 | 0.26 | 0.26 | 0.07 | 0.05 | 0.05 | 0.03 | 0.03 | 0.02 | 0.02 | 0.02 |
| $k_V\%$ | 99.03 | 95.32 | 91.07 | 91.14 | 67.28 | 50.69 | 50.95 | 17.20 | 9.41 | 1.49 | 0.30 | 0.15 |
| $k_E\%$ | 0.44 | 2.14 | 4.09 | 4.05 | 14.97 | 22.55 | 22.19 | 37.45 | 40.98 | 20.06 | 20.30 | 20.33 |
| $k_M\%$ | 0.42 | 2.03 | 3.87 | 3.85 | 14.20 | 21.40 | 21.49 | 36.28 | 39.69 | 62.76 | 63.52 | 63.61 |
| $k_G\%$ | 0.11 | 0.51 | 0.97 | 0.96 | 3.55 | 5.35 | 5.37 | 9.07 | 9.92 | 15.69 | 15.88 | 15.90 |
| BAF_W | 2.2×10^2 | 1.1×10^3 | 2.1×10^3 | 4.3×10^3 | 1.6×10^4 | 2.4×10^4 | 1.4×10^5 | 2.4×10^5 | 2.6×10^5 | 1.2×10^7 | 1.2×10^7 | 1.2×10^7 |
| BAF_L | 1.1×10^4 | 1.1×10^4 | 1.0×10^4 | 2.1×10^5 | 1.6×10^5 | 1.2×10^5 | 7.2×10^6 | 2.4×10^6 | 1.3×10^6 | 6.0×10^8 | 1.2×10^8 | 6.1×10^7 |
| f_F/f_W | | | | | | | | | | | | |
| BMF_W | 0.22 | 1.08 | 2.07 | 0.43 | 1.57 | 2.37 | 1.43 | 2.42 | 2.65 | 1.21 | 1.22 | 1.22 |
| BMF_L | 1.12 | 1.08 | 1.03 | 2.13 | 1.57 | 1.18 | 7.17 | 2.42 | 1.32 | 6.03 | 1.22 | 0.61 |
| f_F/f_D | | | | | | | | | | | | |
| τ , days | 0.3 | 1.4 | 2.7 | 2.7 | 9.8 | 14.8 | 14.9 | 25.1 | 27.5 | 43.5 | 44.0 | 44.1 |

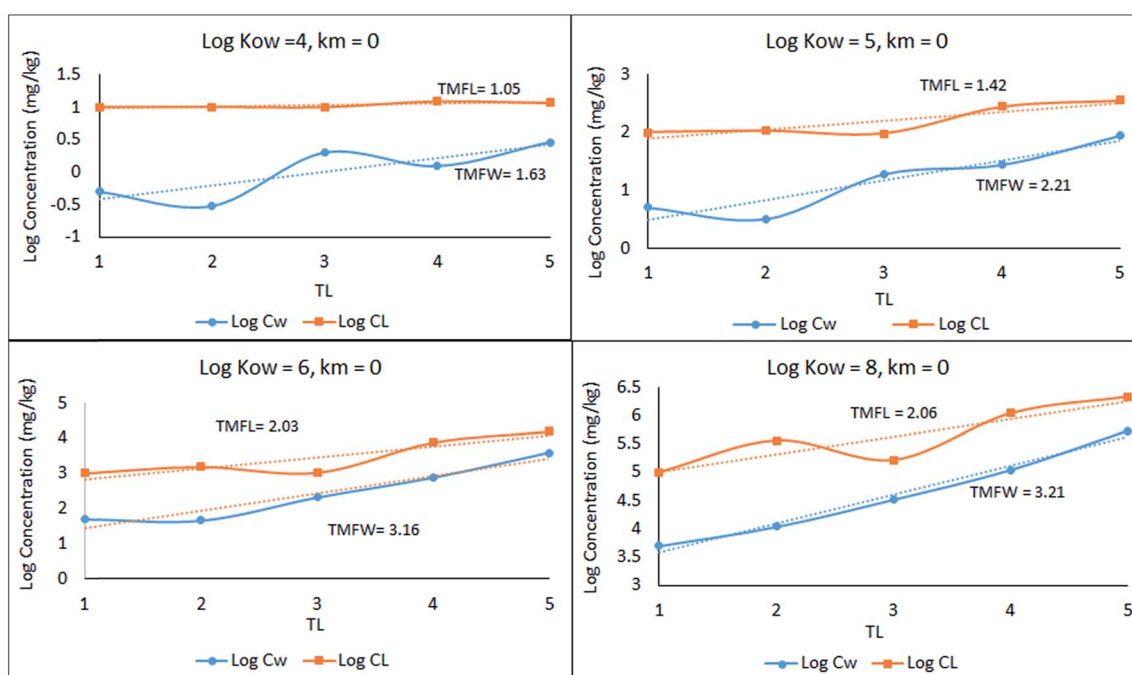


Fig. 2 Regression of log chemical concentrations (wet-weight and lipid-normalized) across a 5-species obligate food chain, where the species have various lipid contents.

fish properties listed in Table 3 are deduced from Arnot–Gobas⁴ correlations and are regarded as typical values for the masses of the specific species with a Q_C value *i.e.* k_D/k_E ratio of 6. The model was run as before for $\log K_{OW}$ of 4, 5, 6, and 8 and the concentrations C_{FW} and C_{FL} were calculated. Logarithmic values of both concentrations were plotted and regressed against trophic level to obtain a slope (β). The TMF is then calculated using the conventional method, namely the TMF is the antilog of the regression slope, β , of $\log C$ on TL.^{8,35,36} The results are presented in Fig. 2 as a series of TMF plots using

both $\log C_{FW}$ and $\log C_{FL}$ as a function of TL (lower and upper plots respectively) from which TMF_W and TMF_L can be obtained, expecting that since TMF is essentially an average BMF, the same general conclusions will apply as discussed earlier for the lean, mean, and fat fish.

There is a significant difference between wet-weight (C_{FW} and TMF_W) and lipid-normalized (C_{FL} and TMF_L) values. For less hydrophobic substances lipid normalization is clearly desirable because of the greater constancy of the C_{FL} values. As K_{OW} increases, the lipid-normalized lines have increased slopes



and become more variable. In contrast, the wet-weight lines become less variable and approach straight lines indicating constancy in BMF_W values. In contrast, for the more hydrophobic chemicals an improved regression is obtainable using TMF_W . In general, the two TMF values are unequal.

Part of the differences between the two TMFs arises because of systematic changes in lipid content (L) with TL. Generally, if L increases with TL the C_{FW} and C_{FL} lines tend to converge. Only if the lipid contents of all species are equal will the lines be parallel and TMF_W and TMF_L are equal. A simple numerical example illustrates this dependence of TMF on lipid variations.

For a 4-species linear food chain with a constant BMF_W of 3.0, the C_{FW} values for a hypothetical example could be 1, 3, 9 and 27. If the lipid contents are equal, both TMF_W and TMF_L will be 3.0. If the lipid contents increase from 0.05 at TL of 1 by multiples of 1.2 to 0.06, 0.072 and 0.0864, the TMF_W is unchanged at 3.0 but the TMF_L decreases to 2.5 which is $3.0/1.2$. Similarly, if the lipid contents decrease by a factor of 1.2, TMF_L increases to 3.6. These results suggest that lipid normalization is desirable for species that are approaching equilibrium with water with relatively insignificant dietary uptake, with fast respiratory exchange dominating. The opposite applies to hydrophobic chemicals that biomagnify appreciably. Lipid normalization can change the slope of the $\log C_F$ on TL regression line, causing TMF_W and TMF_L to diverge. This effect is most important for substances that biomagnify and are likely subjects of regulatory TMF evaluations.

We again suggest that both TMF_W and TMF_L be calculated from monitoring data. Their ratio is an indication of a systematic variation of L with TL. This ratio can be regarded as a trophic dependence on lipid content (TDL). If the ratio TMF_L/TMF_W and TDL is 1.0 there is no systematic dependence of TMF on L . A TDL < 1.0 indicates an increase in L with TL and a TDL > 1.0 indicates a decrease in L with increasing TL. An important implication is that if TMFs are to be used in a regulatory context it must be appreciated that TMF_L is a function of both biomagnification and systematic changes in species lipid contents. In practice, large values of both TMFs indicate appreciable biomagnification and either or both can be used.

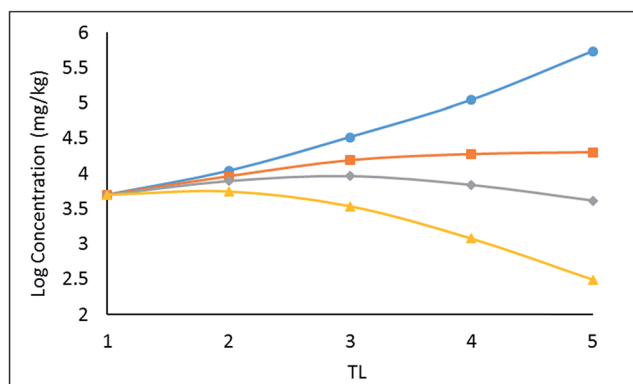


Fig. 3 Effect of biotransformation on $\log C_{FW}$ in a food chain for selected values of k_M , specifically reading down: 0 (blue), 0.01 (orange), 0.02 (grey) and 0.05 (yellow) days^{-1} . The corresponding TMF_W values are 3.21, 1.42, 0.95, and 0.49.

In Fig. 2 above, TMF_W exceeds TMF_L by a factor of approximately 1.6 indicating an increase in L with TL as is apparent from the lipid contents in Table 3.

Effect of biotransformation on TMFs. The simple food chain model was run for hydrophobic substances of varying biotransformation rate constants k_M which was applied to all species. Fig. 3 shows plots of $\log C_{FW}$ as a function of TL for k_M values ranging from 0 to 0.05 days^{-1} . The corresponding TMFs are given. As expected, an increase in k_M reduces concentrations, BMFs and TMFs, especially at higher trophic levels.

It is notable that the slopes of the $\log C_{FW}$ vs. TL lines are most variable at high trophic levels. This is because the rate constants for other losses are slower for large fish thus the introduction of a specific k_M has a greater effect on k_T and thus on the BMF_W . This raises an interesting possibility that if validated model is available then TMF_W data could be used to deduce biotransformation rate constants.

3.3 The key role of dietary and respiratory uptake rates

This analysis suggests that a major factor influencing bioaccumulation and biomagnification is the relative quantities of chemical taken up from water by respiration and from diet. Estimates of these rates require data on the volumetric or mass flows of water and food and the assimilation efficiencies in the gills and GI tract. Correlations and data³⁷ are available for these processes but improved estimates would be invaluable. A related issue is the location of biotransformation as being either somatic or intestinal as discussed by Gobas and Lo.^{10,31} In a simple one compartment model this may be of little importance but for more detailed multi-compartment models this can be critical, especially if QSARs are used to estimate biotransformation rate constants that differ in location and thus reactive environments within the fish. This can cause variations in predicted BCF and BMF values.

We believe that the three superhydrophobic permethylcyclsiloxanes D4, D5 and D6 serve as good examples because they cover a range in hydrophobicity from $\log K_{OW}$ of 6.98 for D4 to 8.09 for D5 and 8.87 for D6. These chemicals have been extensively studied in recent years. For D4, D5 and D6 respiratory uptake and loss are negligible thus it can be argued that the chemical observed in high trophic level species such as trout reached their destination entirely by dietary uptake from lower trophic level (planktonic) species, with water playing a negligible role as a source. Greater respiratory uptake is expected for D4, relative to D5 and D6, because of greater water solubility and lower to organic carbon partitioning coefficient (K_{OC}). This was tested using the model by setting the concentration in the plankton, then setting the water concentration as zero for all other species. As expected, this resulted in a negligible change in concentrations, BMFs and TMFs. A possible implication is that the source of these chemicals in lakes such as Lake Mjosa¹⁵ and marine ecosystems such as Tokyo Bay³⁶ is not the dissolved chemical in water, it is the contaminated suspended biomass discharged from waste water treatment plants. Clearly more efficient contaminant removal is desirable because the discharged biomass is a direct source of diet to the resident biota. In the case of D5 this explains why contamination in fish is apparently not fully mitigated by the



expected fast volatilization from the surfaces of these water bodies. Improved parameters for uptake from planktonic and other small organisms would be desirable because they play a critical role as the source of contamination.

4 Discussion

We first discuss the issue of whether or not to lipid normalize. In 1995 Hebert and Keenleyside³⁸ published a paper aptly titled "To normalize or not to normalize? Fat is the question." The authors acknowledged the value of lipid normalization when interpreting concentrations of hydrophobic contaminants in biota, but they pointed out that lipid normalization can lead to erroneous conclusions and direct interpretation of whole-body or wet-weight concentrations may be preferable. They cited three examples: concentrations of polychlorinated biphenyls (PCB) in herring gull (*Larus argentatus*) eggs in the Great Lakes, concentrations of hexachlorobenzene (HCB) in forage fish in the St. Clair and Detroit Rivers, and a hypothetical example of a hydrophobic contaminant in two species of fish. They concluded that both whole-body and lipid-normalized data should be examined and interpreted, as well as an alternative analysis of covariance (ANCOVA) approach. The paper has been cited over 100 times since 1995 testifying to its general acceptance. However, lipid normalization remains widely recommended and practiced, especially when assessing biomagnification and trophic magnification.^{8,39} The results from the present simulations support their assertion that both wet weight and lipid normalised concentrations should be used.

Second, the results presented here suggest that BMFs and TMFs of very hydrophobic chemicals subject to biotransformation are primarily dependent on K_{OW} and biotransformation half-lives. This is entirely consistent with the findings by Walters *et al.*⁴⁰ in their 'global synthesis' of over 1500 TMF measurements, most of which used lipid-normalized data. We suggest, however, that TMFs may be best determined using both lipid normalized concentrations and whole fish (wet-weight) concentrations. A benefit of the latter approach is that it avoids problems of lipid determination, especially when the organism has a very low lipid content or when fillets or muscle are the sampled media. It also avoids the dependence of BMFs on relative lipid contents of the predator and prey. Regressions of $\log C_{FW}$ vs. TL may be more robust than those using C_{FL} . An uncontentious conclusion is that it is desirable when processing monitoring data to obtain and evaluate both wet-weight and lipid normalised concentrations, and since there is no added cost, costs for lipid determination could be reduced.

Inclusion of benthic organisms in food webs is often essential because the subject chemicals may have partitioned into sediments and persisted there for a prolonged period of time. Estimation of pore water concentrations and hence sediment and pore water fugacities is fraught with uncertainties. It is likely that in many cases the prevailing sediment fugacity exceeds the water column fugacity because of organic carbon mineralization, thus fugacities at low trophic levels may be uncertain.⁴¹ Models can be useful for exploring the effect of

sediment/water fugacity ratios as discussed by Mackay *et al.*⁹ and Kim *et al.*³⁵

The deductions presented above assume that the basic uptake eqn (1) is correct and no other factors substantially influence the uptake. The assumption that K_{OW} equals the lipid-water partition coefficient is questionable but this should not affect the general conclusions concerning trends in hydrophobicity. Of course, real food webs are more complex and variable than those discussed here but we believe that the same principles may apply.

It must be appreciated that TMFs will differ when using wet-weight and lipid-normalised concentrations, especially if lipid contents vary throughout the food web. The differences in TMFs can be significant and in extreme cases one TMF may indicate trophic magnification whereas another TMF derived from the same data may indicate trophic dilution. This is most likely for hydrophobic chemicals that are appreciably biotransformed. In most cases, the two methods should yield similar, but unequal TMFs characterizing the extent of trophic magnification. For highly hydrophobic substances the calculation and evaluation of fugacities can be fraught with difficulties because concentrations are not influenced by lipid content. It is believed that these principles may apply to numerous hydrophobic substances including permethylcyclsiloxanes, phthalate esters, and halogenated hydrocarbons. Obviously when using BMFs and TMFs for regulatory purposes it is essential to appreciate the uncertainties introduced by these issues.

Finally, we comment on a semantic issue that it may be asserted that some chemicals biomagnify whereas others do not. Inspection of the uptake equation suggests that ALL chemicals biomagnify because k_D inevitably exceeds k_E and their ratio approaches Q . The reason that relatively hydrophilic chemicals do not apparently biomagnify is that the increase in fugacity caused by lipid digestion is mitigated by the relatively fast losses by ventilation or biotransformation. The experimental determinations of increases in fugacity in the GIT resulting from 'solvent depletion' by Gobas and colleagues⁴² present a compelling case for the fugacity increase in the GIT that is inevitably transmitted into the body of the fish, however, that increase may be subsequently dissipated by loss processes from the body that restore the body fugacity to a lower value similar to that of the food or water.

5 Conclusions: the continued evolution of bioaccumulation models; needs and priorities

In conclusion, we suggest that improvements in the science and modelling of bioaccumulation would benefit from advances in several subject areas.

Since bioconcentration is basically a partitioning phenomenon, there is a need for more data on partition ratios to all relevant tissues by experimental determinations, QSAR development and fundamental computational methods, including the influences of temperature and ionization especially for cations that can be highly toxic. Such developments would



enhance the use of tissue-specific parameters in PBPK models. Partitioning from sediment solids and pore water into benthic organisms can have a high degree of uncertainty, especially when water column concentrations are responding to changes in chemical emission rates. It would be useful to document passive sampling and other methods of determining sediment/water fugacity ratios.⁴¹

Chemical biotransformation or metabolism rates play a critical role in determining bioaccumulation, thus measurements and correlations need further improvement, including the differences between somatic (body) and gastrointestinal rates¹⁰ that occur in very different milieus.

Determination of trophic magnification in ecosystems must consider the effects of spatial and temporal variability, sediment-water fugacity ratios, reproductive losses and changing diets with growth.

There should be continuing consideration of the number and nature of fish compartments required for scientific and regulatory purposes, including consideration of the required accuracy and the degree of parsimony justified. The full implications of using steady state models as distinct from dynamic models also requires evaluation.

The various uptake and loss parameters are fundamentally linked to the organism's bioenergetics, thus consistency between these variables should be sought. There is a need for improvements in estimation methods for processes such as dietary and respiratory assimilation efficiencies and extents of digestion of various food items. The use of different *Q* values for each digested material should be explored.

As described here the model gives only point estimates of output quantities. Although incorporation of methods to evaluate probability and uncertainty would be useful it would be impractical for this paper because of the complexity and food web specificity. The probability and uncertainty associated with the model is a function of numerous variables that are dependent upon the sampled or defined food web under consideration. There is thus an obligation for the modeler to quantify the perceived accuracy of the results of each specific food web, especially as being best qualified to identify important parameters and covariances. There is increasing emphasis on presenting probabilistic estimates of model output using Monte Carlo and Bayesian approaches. A discussion of these methods, while beyond our scope here, deserves increased attention, especially when models are used for regulatory purposes. Nonetheless, Bayesian methods may readily be applied to a sampled food web using methods provided elsewhere (Powell *et al.*^{36,43}).

Overall, it seems likely that a preferred strategy to advance the science of bioaccumulation is to continue ecosystem monitoring, laboratory determinations of the bio-uptake processes and model development, preferably in concert and supported by regulatory incentives. It is obvious that quantitative estimations of bioconcentration, bioaccumulation, biomagnification and trophic magnification as used in regulatory programs are essential for exposure and risk assessment, especially for hydrophobic substances. Ultimately, regulatory decisions are best justified using data from well-designed and

carefully interpreted monitoring programs, preferably using benchmark chemicals. Models can play an invaluable complementary role to monitoring by confirming that the perceived state of the science contained in the models is consistent with the ecosystem observations.

Conflicts of interest

There are no conflicts to declare.

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References

- 1 J. A. Arnot and F. A. P. C. Gobas, A food web bioaccumulation model for organic chemicals in aquatic ecosystems, *Environ. Toxicol. Chem.*, 2004, **23**, 2343–2355.
- 2 D. Mackay and A. Fraser, Bioaccumulation of persistent organic chemicals: mechanisms and models, *Environ. Pollut.*, 2000, **110**, 375–381.
- 3 F. A. Gobas, W. de Wolf, L. P. Burkhard, E. Verbruggen and K. Plotzke, Revisiting bioaccumulation criteria for POPs and PBT assessments, *Integr. Environ. Assess. Manage.*, 2009, **5**, 624–637.
- 4 L. P. Burkhard, K. Borga, D. E. Powell, P. Leonards, D. C. Muir, T. F. Parkerton and K. B. Woodburn, Improving the quality and scientific understanding of trophic magnification factors (TMFs), *Environ. Sci. Technol.*, 2013, **47**(3), 1186–1187.
- 5 European Chemicals Agency (ECHA), *Guidance on Information Requirements and Chemical Safety Assessment*, ch. R.11: PBT/vPvB assessment, Helsinki, Finland, 2014.
- 6 OECD, Test No. 305: Bioaccumulation in Fish: Aqueous and Dietary Exposure, OECD Publishing, 2012.
- 7 D. Mackay, J. A. Arnot, F. A. Gobas and D. E. Powell, Mathematical relationships between metrics of chemical bioaccumulation in fish, *Environ. Toxicol. Chem.*, 2013, **32**(7), 1459–1466.
- 8 K. Borga, K. A. Kidd, D. C. G. Muir, O. Berglund, J. Conder, F. A. P. C. Gobas, J. R. Kucklick, O. Malm and D. E. Powell, Trophic magnification factors: consideration of ecology, ecosystems, and study design, *Integr. Environ. Assess. Manage.*, 2012, **8**, 64–84.
- 9 D. Mackay, A. K. D. Celsie, J. A. Arnot and D. E. Powell, Processes influencing chemical biomagnification and trophic magnification factors in aquatic ecosystems: implications for chemical hazard and risk assessment, *Chemosphere*, 2016, **154**, 99–108.
- 10 F. A. Gobas and J. C. Lo, Deriving bioconcentration factors and somatic biotransformation rates from dietary bioaccumulation and depuration tests, *Environ. Toxicol. Chem.*, 2016, **32**(12), 2968–2976.



- 11 L. P. Burkhard, J. A. Arnot, M. R. Embry, K. J. Farley, R. A. Hoke, M. Kitano, H. A. Leslie, G. R. Lotufo, T. F. Parkerton, K. G. Sappington, G. T. Tomy and K. B. Woodburn, Comparing laboratory and field measured bioaccumulation endpoints, *Integr. Environ. Assess. Manage.*, 2012, **8**(1), 17–31.
- 12 L. P. Burkhard, C. Cowan-Ellsberry, M. R. Embry, R. A. Hoke and K. A. Kidd, Bioaccumulation data from laboratory and field studies: are they comparable?, *Integr. Environ. Assess. Manage.*, 2012, **8**(1), 13–16.
- 13 R. Macdonald, D. Mackay and B. Hickie, Contaminant amplification in the environment, *Environ. Sci. Technol.*, 2002, **36**, 458A–462A.
- 14 D. E. Powell, *Trophic magnification factors (TMF): mathematical relationships with other measures used to interpret bioaccumulation in aquatic environments*, European Chemicals Agency (ECHA), Workshop on Scientific Advancements in Bioaccumulation Assessment, Helsinki, Finland, 2013.
- 15 K. Borga, E. Fjeld, A. Kierkegaard and M. S. McLachlan, Consistency in Trophic Magnification Factors of Cyclic Methyl Siloxanes in Pelagic Freshwater Food Webs Leading to Brown Trout, *Environ. Sci. Technol.*, 2013, **47**(24), 14394–14402.
- 16 A. J. Niimi and B. G. Oliver, Biological half-lives of polychlorinated biphenyl (PCB) congeners in whole-fish and muscle of rainbow trout (*Salmo gairdneri*), *J. Fish. Aquat. Sci.*, 1983, **40**, 1388–1394.
- 17 S. Endo, T. N. Brown and K. U. Goss, General model for estimating partition coefficients to organisms and their tissues using the biological compositions and polyparameter linear free energy relationships, *Environ. Sci. Technol.*, 2013, **47**(12), 6630–6639.
- 18 K. U. Goss, Predicting the equilibrium partitioning of organic compounds using just one linear solvation energy relationship (LSER), *Fluid Phase Equilib.*, 2005, **233**(1), 19–22.
- 19 K. U. Goss, T. N. Brown and S. Endo, Elimination half-life as a metric for the bioaccumulation potential of chemicals in aquatic and terrestrial food chains, *Environ. Toxicol. Chem.*, 2013, **32**(7), 1663–1671.
- 20 W. B. Neely, Estimating rate constants for the uptake and clearance of chemicals by fish, *Environ. Sci. Technol.*, 1979, **13**, 1506–1510.
- 21 R. V. Thomann, Bioaccumulation model of organic chemical distribution in aquatic food chains, *Environ. Sci. Technol.*, 1989, **23**, 699–707.
- 22 National Research Council (NRC), *Models in environmental regulatory decision making*, Committee on models in the regulatory decision process, Board on Environmental Studies and Toxicology, Washington, DC, 2007.
- 23 M. Z. Hauschild, M. A. Huijbregts, O. Jolliet, M. Macleod, M. Margni, D. Van de Meent, R. K. Rosenbaum and T. E. McKone, Building a model based on scientific consensus for life cycle impact assessment of chemicals: the search for harmony and parsimony, *Environ. Sci. Technol.*, 2008, **42**, 7032–7037.
- 24 R. J. Erickson, J. M. McKim, G. J. Lien, A. D. Hoffman and S. L. Batterman, Uptake and elimination of ionisable organic chemicals at fish gills: I. Model formulation, parameterization, and behaviour, *Environ. Toxicol. Chem.*, 2006, **25**, 1512–1521.
- 25 R. J. Erickson, J. M. McKim, G. J. Lien, A. D. Hoffman and S. L. Batterman, Uptake and elimination of ionisable chemicals at fish gills: II. Observed and predicted effects of pH, alkalinity, and chemical properties, *Environ. Toxicol. Chem.*, 2006, **25**, 1522–1532.
- 26 J. M. Armitage, J. A. Arnot, F. Wania and D. Mackay, Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish, *Environ. Toxicol. Chem.*, 2013, **32**(1), 115–128.
- 27 A. Celsie, D. Mackay, J. M. Parnis and J. A. Arnot, A fugacity-based toxicokinetic model for narcotic organic chemicals in fish, *Environ. Toxicol. Chem.*, 2016, **35**(5), 1257–1267.
- 28 W. Larisch, T. N. Brown and K. U. Goss, A toxicokinetic model for fish including multiphase sorption features, *Environ. Toxicol. Chem.*, 2017, **36**(6), 1538–1546.
- 29 J. P. Connolly and C. J. Pedersen, A thermodynamic-based evaluation of organic chemical accumulation in aquatic organisms, *Environ. Sci. Technol.*, 1988, **22**(1), 99–103.
- 30 F. A. P. C. Gobas, J. B. Wilcockson, R. W. Russel and G. D. Haffner, Mechanism of biomagnification in fish under laboratory and field conditions, *Environ. Sci. Technol.*, 1999, **33**, 133–141.
- 31 F. A. P. C. Gobas, X. Zhang and R. Wells, Gastrointestinal magnification: the mechanism of biomagnification and food chain accumulation of organic chemicals, *Environ. Sci. Technol.*, 1993, **27**, 2855–2863.
- 32 D. Mackay, D. E. Powell and K. B. Woodburn, Bioconcentration and Aquatic Toxicity of Superhydrophobic Chemicals: A Modeling Case Study of Cyclic Volatile Methyl Siloxanes, *Environ. Sci. Technol.*, 2015, **49**(19), 11913–11922.
- 33 J. A. Arnot, D. Mackay and M. Bonnell, Estimating metabolic biotransformation rates in fish from laboratory data, *Environ. Toxicol. Chem.*, 2008, **27**, 341–351.
- 34 J. A. Arnot, D. MacKay, T. F. Parkerton and M. Bonnell, A database of fish biotransformation rates for organic chemicals, *Environ. Toxicol. Chem.*, 2008, **27**, 2263–2270.
- 35 J. Kim, F. A. P. C. Gobas, J. A. Arnot, D. E. Powell, R. M. Seston and K. B. Woodburn, Evaluating roles of biotransformation, spatial concentration differences, organism home range, and field sampling design on trophic magnification factors, *Sci. Total Environ.*, 2016, **551–552**, 438–451.
- 36 D. E. Powell, N. Suganuma, K. Kobayashi, T. Nakamura, K. Ninomiya, K. Matsumura, N. Omura and S. Ushioka, Trophic dilution of cyclic volatile methylsiloxanes (cVMS) in the pelagic marine food web of Tokyo Bay, Japan, *Sci. Total Environ.*, 2017, **578**, 366–382.
- 37 R. Froese and D. Pauly, *FishBase: A global information system on fishes*, <http://www.fishbase.ca/home.htm>, 2015.
- 38 C. E. Hebert and K. A. Keenleyside, To normalize or not to normalize? Fat is the question, *Environ. Toxicol. Chem.*, 1995, **14**(5), 801–807.



- 39 L. P. Burkhard, K. Borga, D. E. Powell, P. Leonards, D. C. G. Muir, T. F. Parkerton and K. B. Woodburn, Improving the quality and scientific understanding of Trophic Magnification Factors (TMFs), *Environ. Sci. Technol.*, 2013, **47**, 1186–1187.
- 40 D. M. Walters, T. D. Jardine, B. S. Cade, K. A. Kidd, D. C. Muir and P. Leipzig-Scott, Trophic Magnification of Organic Chemicals: A Global Synthesis, *Environ. Sci. Technol.*, 2016, **50**(9), 4650.
- 41 F. A. Gobas and L. G. Maclean, Sediment-water distribution of organic contaminants in aquatic ecosystems: the role of organic carbon mineralization, *Environ. Sci. Technol.*, 2003, **37**, 735–741.
- 42 F. A. P. C. Gobas, J. B. Wilcockson, R. W. Russell and G. D. Haffner, Mechanism of biomagnification in fish under laboratory and field conditions, *Environ. Sci. Technol.*, 1999, **33**(1), 133–141.
- 43 D. E. Powell, M. Schøyen, S. Øxnevad, R. Gerhards, T. Böhmer, M. Koerner, J. Durham and D. W. Huff, Bioaccumulation and trophic transfer of cyclic volatile methylsiloxanes (cVMS) in the aquatic marine food webs of the Oslofjord, Norway, *Sci. Total Environ.*, 2017, **622–623**, 127–139.

