Environmental Toxicology

A Toxicokinetic Framework and Analysis Tool for Interpreting Organisation for Economic Co-operation and Development Guideline 305 Dietary Bioaccumulation Tests

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Abstract: The Organisation for Economic Co-operation and Development guideline 305 for bioaccumulation testing in fish includes the option to conduct a dietary test for assessing a chemical's bioaccumulation behavior. However, the one-compartment toxicokinetic model that is used in the guidelines to analyze the results from dietary bioaccumulation tests is not consistent with the current state of the science, experimental practices, and information needs for bioaccumulation and risk assessment. The present study presents 1) a 2-compartment toxicokinetic modeling framework for describing the bioaccumulation of neutral hydrophobic organic chemicals in fish and 2) an associated toxicokinetic analysis tool (absorption, distribution, metabolism, and excretion [ADME] B calculator) for the analysis and interpretation of dietary bioaccumulation test data from OECD-305 dietary tests. The model framework and ADME-B calculator are illustrated by analysis of fish dietary bioaccumulation test data for 238 substances representing different structural classes and susceptibilities to biotransformation. The ADME of the chemicals is determined from dietary bioaccumulation tests and bioconcentration factors, biomagnification factors, and somatic and intestinal biotransformation rates. The 2-compartment model cannot. This insight is important for applying a weight-of-evidence approach to bioaccumulation assessment where information from aqueous and dietary test endpoints can be integrated to improve the evaluation of a chemical's bioaccumulation potential. *Environ Toxicol Chem* 2020;39:171–188. © 2019 SETAC

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INTRODUCTION

The assessment of the potential of chemical substances to bioaccumulate in the biosphere has become an important component of global environmental stewardship of chemicals in commerce. Bioaccumulation, which involves the absorption, internal distribution, biotransformation, and excretion of chemical substances by biological organisms, has the potential to cause concentrations of chemicals in organisms that are sufficiently high to cause adverse biological responses. Various countries have developed national legislation that requires the assessment of the bioaccumulation behavior of chemicals as

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part of efforts aimed at controlling substances that can cause human health and/or environmental effects (US Environmental Protection Agency 1976; Government of Canada 1999; European Commission 2006; Japanese Ministry of the Environment 2018). Also, international programs, such as the United Nations Stockholm Convention (United Nations Environment Programme 2001), include bioaccumulation assessment of priority chemicals with the goal of protecting human and environmental health at the global level.

For many years, the Organisation for Economic Cooperation and Development (OECD) has developed test guidelines for evaluating the bioaccumulation potential of chemicals. Before 2012, the OECD bioaccumulation guidelines included methods for the measurement of the bioaccumulation of chemicals from water into fish (Organisation for Economic Co-operation and Development 1996). Bioconcentration factors (BCFs) determined in laboratory studies using aqueous

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exposure of a test substance that follow the OECD-305 guidelines have become important metrics for priority setting and the assessment and regulation of chemicals. However, several developments led the OECD to revise its guidelines. First, it was found that bioconcentration tests are not always technically feasible. In particular, chemicals that are very hydrophobic are difficult to test in aqueous bioconcentration tests. Second, dietary exposure is often the dominant route of chemical bioaccumulation for very hydrophobic substances (Qiao et al. 2000) in fish and many other animals but is not considered in bioconcentration tests. Third, bioconcentration tests are expensive and involve substantial animal use. Cheaper and less animal-intensive bioaccumulation testing strategies are needed to meet regulatory objectives involving the evaluation of large numbers of chemicals. Fourth, government regulations evolved in response to new scientific information recognizing bioaccumulation metrics other than the BCF including the dietary bioaccumulation or biomagnification factor (BMF) and the trophic magnification factor (European Chemicals Agency 2017).

The revised OECD guidelines (Organisation for Economic Co-operation and Development 2012) for bioaccumulation testing now provide recommendations for conducting dietary bioaccumulation tests in addition to aqueous bioconcentration tests. A guidance document includes a variety of suggestions on how to analyze the results from dietary bioaccumulation tests (Organisation for Economic Co-operation and Development 2017). However, the basic fish-food kinetic modeling framework used in the OECD-305 guideline has not kept up with recent developments in bioaccumulation science, dietary bioaccumulation testing methods, and information needs and limits the interpretation of dietary bioaccumulation test results. For example, the current OECD-305 bioaccumulation model views a fish as a single homogeneous compartment, whereas most experimental tests recognize both the gut contents (which are often removed prior to chemical analysis) and the fish body and, in some cases, specific organs of the fish (e.g., liver). Also, the OECD-305 fish bioaccumulation model includes terms for uptake and overall depuration but provides no further description on how or to what extent concentrations are affected by biotransformation, gill elimination, fecal excretion, and growth dilution. In addition, the OECD-305 bioaccumulation model only provides a mass balance of the absorbed chemical and not of the administered chemical. This practice ignores the fate of large amounts (and often the majority) of the administered chemical in the test. Furthermore, the OECD-305 model framework only recognizes lipids as the phase in which bioaccumulation occurs and ignores the contribution of proteins and other media which can play an important role in the bioaccumulation of perfluorinated substances (Martin et al. 2003) and organisms with low lipid content (Arnot and Gobas 2004). Also, the OECD-305 model framework includes methods for the growth correction of the BCF and BMF that violate mass balance (Gobas and Lee 2019). Finally, the current OECD-305 toxicokinetic framework limits the ability of dietary bioaccumulation tests to provide valuable information such as the BCF, biotransformation rates, and other

information on the absorption, distribution, metabolism, and excretion (ADME) of chemicals in fish.

It is the objective of the present study to develop and investigate a refined toxicokinetic modeling framework and an associated toxicokinetic analysis tool for the analysis and interpretation of the dietary bioaccumulation test data that is in sync with current empirical methods of bioaccumulation testing and information needs. This framework for analysis provides more information than is obtained from the current toxicokinetic framework described in the OECD-305 guidance document. The main objectives of the refined toxicokinetic framework for the OECD-305 dietary bioaccumulation are to 1) derive the BCF and other bioaccumulation metrics from the results of dietary bioaccumulation test; 2) provide a full accounting of the mass balance of chemical administered to fish; 3) characterize the ADME profile of chemicals in fish relevant to bioaccumulation assessments; 4) quantify somatic and intestinal biotransformation rates, which are useful for the development of in vitro and quantitative structure-activity relationship-based methods for assessing biotransformation rates of chemicals in fish; 5) provide error estimates for bioaccumulation metrics derived from the results of dietary bioaccumulation tests; 6) provide a statistical framework for testing bioaccumulation metrics against regulatory criteria values; and 7) identify methods for delineating the relative importance of dietary and respiratory uptake routes of chemical bioaccumulation in fish under field conditions.

An additional objective of this model framework is to avoid or minimize adding steps to the OECD-305 dietary test protocol that increase effort and cost. The ultimate goal of the present study was to advance the use of OECD-305 dietary bioaccumulation testing in regulatory decision-making.

THEORY

The refined toxicokinetic framework for dietary bioaccumulation tests includes the following main structural differences from the kinetic framework included in the OECD-305 guidance document: 1) The fish is represented by a 2compartment model, which includes both the gastrointestinal cavity (lumen) and the body (soma). The current OECD-305 toxicokinetic framework assumes a one-compartment fish model. 2) A full mass balance of the chemical administered to the fish in the test is provided. The current OECD-305 toxicokinetic framework accounts for only the mass of absorbed chemical, not ingested chemical. 3) The distribution of the chemical between the intestinal tract and the body of the fish, including enterohepatic cycling of the parent compound, is taken into account. The OECD-305 toxicokinetic framework does not include this process. 4) Recognition that hydrophobic organic chemicals accumulate not only in lipids but also in proteins and that the sorptive capacities of chemicals for lipids and proteins can vary among chemicals. The OECD-305 toxicokinetic framework only recognizes lipids as the medium in which bioaccumulation occurs. 5) Recognition of error in measurements and calculations and incorporation of

uncertainty in the determination of bioaccumulation metrics. 6) Inclusion of statistical methods for testing whether the BCF derived from a dietary bioaccumulation test exceeds regulatory criteria.

The 2-compartment toxicokinetic framework is presented in the ADME-B(ioaccumulation) calculator (included in the Supplemental Data), which is a freely accessible Excel spreadsheet program that can be used to interpret the results from OECD-305-style dietary bioaccumulation tests. The equations used are summarized in Table 1 (Equations 1–46) and a description and explanation of the methods, including a discussion of assumptions and limitations, is presented.

The 2-compartment fish toxicokinetic model

The 2-compartment fish toxicokinetic model includes the gastrointestinal contents and the body (soma) of the fish (Figure 1). In dietary bioaccumulation tests where gut contents are removed prior to chemical analysis of the fish tissue, the gastrointestinal contents (or digesta) include the intestinal flora and gastric enzymes and represent an important site where biotransformation can occur. The body of the fish includes all of the tissues that are part of the fish sample. If tissues of the gastrointestinal tract (e.g., gut wall but not gut lumen) are included in the fish sample analysis, then any somatic biotransformation also reflects biotransformation in tissues of the gastrointestinal tract.

A chemical enters the lumen as a result of food ingestion, and chemical transfer occurs from the body of the fish via diffusive transfer and bile excretion. The chemical is removed from the lumen through chemical transfer into the fish body, fecal egestion, and biotransformation in the lumen. The chemical in the fish body is the result of uptake from the intestinal lumen and from water via the gills and skin. It is removed from the fish body via chemical transfer from the fish into the gastrointestinal contents via diffusion and bile excretion, respiratory elimination via the gills and skin, and biotransformation in the fish body (somatic biotransformation). Also, pseudoremoval of the chemical occurs as a result of growth dilution. Growth dilution does not actually remove the chemical from the fish but lowers the concentration of the chemical in the fish because of an increase in the biomass of the fish. The mass-balance equations for the fish body (B) and the gastrointestinal contents (G) are presented in Equations 1 to 4 (Table 1). This model was first described in Lo et al. (2015) and Gobas and Lo (2016). The 2-compartment toxicokinetic model differs from the one-compartment model in that 1) the concentration of the chemical in the fish only applies to chemical in the fish body and excludes chemical in the lumen of the gastrointestinal tract and 2) the model provides insights into the dynamics of the chemical in the fish that a onecompartment model cannot provide. Specifically, the model describes chemical exchange between the body of the fish and the water, fecal excretion, biotransformation in the body of the fish and in the lumen of the gastrointestinal tract, and growth dilution. The merit of the 2-compartment model is that it

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provides more mechanistic information than the onecompartment formulation while not requiring additional experimental effort in determining concentrations of the chemical in the gastrointestinal tract. This means that the model can be implemented in accordance with the current OECD-305 guideline for conducting dietary bioaccumulation tests. It is important to stress that, as long as fish gut contents are removed before the analysis of the fish body in dietary bioaccumulation tests, the current OECD-305 kinetic bioaccumulation model provides identical results for the depuration rate constant and the dietary uptake efficiency as the 2compartment fish toxicokinetic model but cannot determine the BCF, biotransformation rate constants in the fish body and intestines, and fecal excretion or include bioaccumulation in nonlipid tissues because these processes are not included in the model. The domain of applicability of the 2-compartment fish toxicokinetic model includes nonionic organic chemicals (log K_{OW} between approximately 3 and 9.2) with affinities for lipids and proteins.

Determination of bioaccumulation metrics from dietary bioaccumulation test data

The application of the 2-compartment modeling framework to the dietary bioaccumulation tests involves the derivation of the uptake clearance rate (k_{B1} ; L water kg fish wet wt⁻¹ d⁻¹) and 7 rate constants for respiratory elimination (k_{B2} ; per day), chemical transfer from fish body to gastrointestinal contents (k_{BG} ; per day), chemical transfer from gastrointestinal contents to fish body (k_{GB} ; per day), somatic biotransformation (k_{BM} ; per day), biotransformation in the gastrointestinal contents (k_{GM} ; per day), fecal egestion from the gastrointestinal tract (k_{GE} ; per day), and growth dilution (k_{GD} ; per day). If these rate constants can be determined from the test, then it is possible to derive various bioaccumulation metrics and assess the relative importance of the diet and the water as sources for the bioaccumulation of chemicals in fish under various environmental conditions. As described in more detail in Lo et al. (2015, 2016), the use of nonmetabolizable reference chemicals in the dietary bioaccumulation test can facilitate the derivation of the biotransformation rate constants. Methods for the derivation of the uptake clearance rate and rate constants as well as the total depuration rate constant and the dietary uptake efficiency are described, with corresponding equations presented in Table 1. Equations 1 and 2 present the mass-balance equations for the body of the fish and the intestinal contents, respectively, whereas Equations 3 and 4 present the same mass-balance equations in the more familiar concentration format.

Depuration rate constant

The depuration rate constant of the test (k_{BT}) and nonmetabolizable reference ($k_{BT,R}$) chemicals is the sum of the rate constants for respiratory elimination (k_{B2}), fecal excretion (k_{BE}), somatic biotransformation (k_{BM} , with $k_{BM} = 0$ for $k_{BT,R}$) and growth dilution (k_{GD} ; Equation 5 in Table 1). It can be derived

TABLE 1: Equations for the 2-compartment mass balance bioaccumulation model in fish

Equation	Equation no.
$\frac{\mathrm{d}M_{\mathrm{B}}}{\mathrm{d}t} = k_{\mathrm{B1}}^* \times M_{\mathrm{WD}} + k_{\mathrm{GB}} \times M_{\mathrm{G}} - (k_{\mathrm{B2}} + k_{\mathrm{BG}} + k_{\mathrm{BM}}) \times M_{\mathrm{B}}$	(1)
$\frac{\mathrm{d}M_{\mathrm{G}}}{\mathrm{d}t} = \mathrm{G}_{\mathrm{I}} \times \mathrm{C}_{\mathrm{D}} + k_{\mathrm{BG}} \times \mathrm{M}_{\mathrm{B}} - (k_{\mathrm{GB}} + k_{\mathrm{GE}} + k_{\mathrm{GM}}) \times \mathrm{M}_{\mathrm{G}} = 0$	(2)
$\frac{\mathrm{dC}_{\mathrm{B}}}{\mathrm{dt}} = \left(\frac{k_{\mathrm{B1}} \times V_{\mathrm{W}}}{W_{\mathrm{B}}}\right) \times C_{\mathrm{WD}} + \left(\frac{k_{\mathrm{GB}}}{k_{\mathrm{GB}} + k_{\mathrm{GE}} + k_{\mathrm{GM}}}\right) \times \frac{G_{\mathrm{I}}}{W_{\mathrm{B}}} \times C_{\mathrm{D}} - \left(k_{\mathrm{B2}} + \left(\frac{k_{\mathrm{BG}} \times (k_{\mathrm{GE}} + k_{\mathrm{GM}})}{k_{\mathrm{GB}} + k_{\mathrm{GE}} + k_{\mathrm{GM}}}\right) + k_{\mathrm{BM}} + k_{\mathrm{GD}}\right) \times C_{\mathrm{B}}$	(3)
$\frac{dC_B}{dt} = k_{B1} \times C_{WD} + E_D \times F_D \times C_D - k_{BT} \times C_B$	(4)
$k_{\text{BT}} = k_{\text{B2}} + k_{\text{BE}} + k_{\text{GD}}$ where	(5a)
$k_{\text{BE}} = \left(\frac{k_{\text{BG}} \times (k_{\text{GE}} + k_{\text{GM}})}{k_{\text{GB}} + k_{\text{GE}} + k_{\text{GM}}}\right) = k_{\text{BG}} \times (1 - \text{E}_{\text{D}})$	(5b)
$\ln C_{\rm B} = b_0 + b_1 \times t$	(6)
$k_{\rm BT} = -b_1$	(7)
$ln W_B = b_2 + b_3 \times t$	(8)
$k_{\rm GD} = b_3$	(9)
$E_{\rm D} = \frac{k_{\rm GB}}{k_{\rm GR} + k_{\rm GR} + k_{\rm GM}}$	(10)
$E_{D,R} = \frac{k_{GB}}{k_{CD} + k_{CR}}$	(11)
$E_{\rm D} = \frac{C_{\rm B,t=0} \times k_{\rm BT}}{2} \times \frac{1}{1} = -\frac{e^{\rm b_0} \times b_1}{1}$	(12)
$C_{\rm D} \times F_{\rm D} = 1 - e^{-k_{\rm BT} \times t_{\rm u}} \qquad C_{\rm D} \times F_{\rm D} \times (1 - e^{b_{\rm I} \times t_{\rm u}})$ $k_{\rm BM} = k_{\rm BT} - k_{\rm RT,R}$	(13)
$k_{\text{BT,R}} = k_{\text{B2}} + k_{\text{BE}} + k_{\text{GD}} \approx \frac{1}{\omega} \times \frac{1}{K_{\text{OW}}} + \beta$	(14)
$k_{\text{BT,R}} = k_{\text{B2}} + k_{\text{BE}} + k_{\text{GD}} = k_{\text{B2}} + k_{\text{BG}} \times (1 - E_{\text{D}}) + k_{\text{GD}}$	(15)
$k_{\rm GM} = \left(\frac{1}{\rm F_{\rm D}} - \frac{1}{\rm F_{\rm D,R}}\right) \times k_{\rm GB}$	(16)
$\frac{1}{E_{D,R}} = m \times K_{OW} + n$	(17)
$\Phi_{\rm BM} = \frac{k_{\rm BM} \times M_{\rm B}}{k_{\rm BM} \times M_{\rm B} + k_{\rm GM} \times M_{\rm G}}$	(18)
$\Phi_{\rm GM} = \frac{k_{\rm GM} \times M_{\rm G}}{k_{\rm BM} \times M_{\rm B} + k_{\rm GM} \times M_{\rm G}} = 1 - \Phi_{\rm BM}$	(19)
$k_{\rm GE} = \frac{G_{\rm GE}}{W_{\rm C}}$	(20)
$G_{GE} = [(1 - \varepsilon_L) \times \phi_{DL} + (1 - \varepsilon_P) \times \phi_{DP} + (1 - \varepsilon_N) \times \phi_{DN} + (1 - \varepsilon_W) \times \phi_{DW}] \times G_I$	(21)
$\frac{dW_G}{dt} = G_I - \delta \times W_G$	(22)

(Continued)

TABLE 1: (Continued)

Equation	Equation no.
$W_{G} = \frac{G_{I}}{\delta} = \frac{F_{D} \times W_{B}}{\delta}$	(23)
$k_{\rm GB} = \frac{E_{\rm D,R}}{1 - E_{\rm D,R}} \times k_{\rm GE}$	(24)
$k_{\rm BG} = K_{\rm GB} \times k_{\rm GB} \times \frac{W_{\rm G}}{W_{\rm R}} \times \frac{d_{\rm B}}{d_{\rm G}}$	(25)
$K_{GB} = \frac{C_G}{C} = \frac{\left[\phi_{GL}/d_L + \phi_{GP} \times \chi/d_P + \phi_{GN} \times \theta/d_N + \phi_{GW}/(K_{OW} \times d_W)\right] \times d_G}{\left[1 + \frac{1}{2}\left(\frac{1}{2} + \frac{1}{2}\right) + \frac{1}{2}\left(\frac{1}{2} + \frac{1}{2}\right)\right] \times d_G}$	(26)
$\phi_{GL} = \frac{(1 - \varepsilon_L) \times \phi_{DL}}{(1 - \varepsilon_L) \times \phi_{DL}}$	(27)
$\phi_{LL} = \frac{(1 - \varepsilon_L) \times \phi_{DL} + (1 - \varepsilon_P) \times \phi_{DP} + (1 - \varepsilon_N) \times \phi_{DN} + (1 - \varepsilon_W) \times \phi_{DW}}{(1 - \varepsilon_P) \times \phi_{DP}}$	(28)
$\begin{array}{l} \varphi_{GP} \stackrel{-}{=} (1 - \epsilon_{L}) \times \varphi_{DL} + (1 - \epsilon_{P}) \times \varphi_{DP} + (1 - \epsilon_{N}) \times \varphi_{DN} + (1 - \epsilon_{W}) \times \varphi_{DW} \\ (1 - \epsilon_{N}) \times \varphi_{DN} \end{array}$	(29)
$\begin{split} \phi_{GN} &= \frac{1}{(1 - \epsilon_L) \times \varphi_{DL} + (1 - \epsilon_P) \times \varphi_{DP} + (1 - \epsilon_N) \times \varphi_{DN} + (1 - \epsilon_W) \times \varphi_{DW}} \\ &\qquad \qquad $	(30)
$\phi_{GW} = \frac{1}{(1 - \varepsilon_L) \times \phi_{DL} + (1 - \varepsilon_P) \times \phi_{DP} + (1 - \varepsilon_N) \times \phi_{DN} + (1 - \varepsilon_W) \times \phi_{DW}}$ $\frac{1}{(1 - \varepsilon_L) \times K_{DW} + \lambda}$	(31)
$\frac{1}{k_{B2}} = \frac{1}{2} \times \frac{1}{k_{B2}}$	(32)
$k_{B2} = \frac{1}{W_B \times K_{BW}}$	(33)
$\frac{k_{\rm B1}}{k_{\rm B2}} = \frac{k_{\rm BW}}{k_{\rm B2}} = K_{\rm BW}$	(34)
$K_{BW} = \frac{\phi_{BL} \times K_{OW}}{d_L} + \frac{\phi_{BP} \times \chi \times K_{OW}}{d_P} + \frac{\phi_{BW}}{d_W}$	(25)
$k_{\rm B1} = K_{\rm BW} \times k_{\rm B2}$	(35)
$BMF = \frac{C_{B}}{C_{D}} = \frac{F_{D} \times E_{D}}{k_{BT}}$	(36)
$BMF_L = BMF \times \frac{L_D}{L_B}$	(37)
$L_{D} = \phi_{DL} + \phi_{DP} \times \chi + \phi_{DN} \times \theta + \frac{\phi_{DW}}{K_{OW}}$	(38)
$L_{B} = \phi_{BL} + \phi_{BP} \times \chi + \frac{\phi_{BW}}{K_{OW}}$	(39)
$BCF_{ww,t} = \frac{C_B}{C_{WT}} = \frac{k_{B1}}{k_{BT} \times (1 + C_{OC} \times K_{OC})}$	(40)
$BCF_{L,t} = \frac{BCF_{ww,t}}{L_{B}} = \frac{k_{B1}}{k_{BT} \times (1 + C_{OC} \times K_{OC}) \times L_{B}}$	(41)
$BCF_{5\%,t} = 0.05 \times BCF_{L,t} = \frac{0.05 \times k_{B1}}{k_{BT} \times (1 + C_{OC} \times K_{OC}) \times L_{P}}$	(42)
$BAF_{ww,t} = \frac{k_{B1} + k_{D} \times C_{D,field}/C_{WD,field}}{k_{D} \times (1 + C_{D} \times K_{D})}$	(43)
$BMF_{L} = \frac{e^{b_{0}}}{C_{D} \times (1 - e^{b_{1} \times t_{0}})} \times \frac{L_{D}}{L_{B}}$	(44)

(Continued)

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TABLE 1: (Continued)

Equation	Equation no.
$BCE_{ray} = -$ 0.05 × $W_B^{C_3}$ × 10 ^{C_4}	(45)
$\sum_{SW,t}\frac{C_2}{\left(C_1 + \frac{C_2}{K_{OW}}\right)} \times C_{OX} \times b_1 \times (1 + C_{OC} \times K_{OC}) \times L_B$	
$0.05 \times \left(\frac{1}{\omega}\right) \times K_{BW}$	(46)
$bCr_{5\%,t} = -\frac{1}{b_1 \times K_{OW} \times (1 + C_{OC} \times K_{OC}) \times L_B}$	

b₀ = estimated regression coefficient; b₁ = estimated regression coefficient; b₂ = estimated regression coefficient; b₃ = estimated regression coefficient; b₄ = bioaccumulation factor based on the total concentration of the chemical in the water expressed on a wet weight basis (L water/kg fish wet wt); BCF5%t = bioconcentration factor based on the total concentration of the chemical in the water normalized to a fish with a 5% lipid content (L water/kg fish wet wt); BCF_{Lt} = bioconcentration factor based on the total concentration of the chemical in the water expressed on a lipid equivalent basis (L water/kg lipid); BCF_{ww,t} = bioconcentration factor based on the total concentration of the chemical in the water expressed on a wet weight basis (L water/kg fish wet wt); BMF = biomagnification factor (kg food/kg fish wet wt); BMF_L = biomagnification factor expressed on a lipid equivalent basis (kg lipid/kg lipid/kg lipid); C_1 = estimated regression coefficient; C_2 = estimated regression coefficient; C_3 = estimated regression coefficient; $C_B = concentration$ of the chemical in the body of the fish (g chemical/kg fish wet wt); $C_{B,t=0} = concentration$ of the chemical in the body of the fish at the beginning of the depuration phase (g chemical/kg fish wet wt); C_D = concentration of the chemical in ingested food (g chemical/kg food); C_{D,field} = concentration of the chemical in ingested food under a field condition (g chemical/kg food); C_G = concentration of the chemical in the gastrointestinal contents (g chemical/kg digesta); C_{OC} = concentration of organic carbon in the water (kg/L); C_{0X} = dissolved oxygen concentration in the water (mg O₂/L); C_{WD} = dissolved concentration of the chemical in the water (g chemical/L); C_{WD}, field = dissolved concentration of the chemical in the water under a field condition (g chemical/L); C_{WT} = total concentration of the chemical in the water (g chemical/L); d_B = density of the body of the fish (g/mL); d_G = density of the digesta (g/mL); d_L = density of lipid (g/mL); d_N = density of nondigestible organic matter (g/mL); d_P = Density of protein (g/mL); d_W = density of water (g/mL); E_D = dietary uptake efficiency of the test chemical (unitless); E_{DR} = dietary uptake efficiency of the non-metabolizable reference chemical (unitless); E_W = gill chemical uptake efficiency (unitless); F_D = proportional feeding rate (kg food/kg fish ww/d); G_{GE} = fecal egestion rate (kg digesta/d); G_I = ingestion rate of food (kg food/kg); G_V = gill ventilation rate (L/d); k_{B1}* = rate constant for respiratory uptake (d⁻¹); k_{B1} = clearance rate for respiratory uptake (L water/kg fish wet wt/d); k_{B2} = rate constant for respiratory elimination (d⁻¹); k_{BE} = rate constant for fecal excretion (d⁻¹); k_{BC} = rate constant for chemical transfer from the fish body to the gastro-intestinal contents (d⁻¹); k_{BT} = rate constant for biotransformation of the chemical in the body of the fish (d⁻¹); k_{BT} = depuration rate constant of the test chemical (d⁻¹); $k_{BT,R}$ = depuration rate constant of the non-metabolizable reference chemical (d⁻¹); k_D = dietary uptake clearance rate (kg food/kg fish wet wt/d); k_{GB} = rate constant for chemical transfer from the gastrointestinal contents to the fish body (d⁻¹); k_{GD} = rate constant for growth dilution (d⁻¹); k_{BW} = rate constant for fecal excretion of the chemical from the gastro-intestinal tract (d⁻¹); k_{BW} = rate constant for biotransformation of the chemical in the gastro-intestinal tract (d⁻¹); k_{BW} = rate constant for biotransformation of the chemical in the gastro-intestinal tract (d⁻¹); k_{BW} = a dimensionless fish-water partition coefficient (unitless); K_{BW} = fish-water partition coefficient (L/kg fish wet wt); K_{GB} = gastro-intestinal content-fish body partition coefficient (unitless); K_{OC} = dissolved organic carbon-water partition coefficient (unitless); K_{OC} = octanol-water partition coefficient (unitless); L_B = fraction of lipid-equivalent content in the fish body (kg lipid/kg fish wet wt); L_D = fraction of lipid-equivalent content in the diet (kg lipid/kg food); M_B = mass of the chemical in the body of the fish (g); M_G = mass of the chemical in the digesta (g); M_{VVD} = mass of the dissolved chemical in the water that the fish is exposed to (g); m = estimated regression coefficient; n = estimated regression coefficient; t = time (d); t_u = duration of the exposure (uptake) period (d); W_B = fish body weight (kg wet wt); W_G = amount of the digesta (kg); β = estimated regression coefficient; δ = digesta evacuation rate constant (d⁻¹); ϵ_L = dietary assimilation efficiency of lipid (unitless); ϵ_{N} = dietary assimilation efficiency of nondigestible organic matter (unitless); ϵ_{P} = dietary assimilation efficiency of protein (unitless); ϵ_{W} = dietary assimilation efficiency of water (unitless); θ = sorptive capacity of test chemical to nondigestible organic matter relative to octanol (unitless); λ = estimated regression coefficient; ϕ_{BL} = lipid content of the fish body (kg lipid/kg fish); ϕ_{BM} = proportional contribution of somatic biotransformation (unitless); ϕ_{BP} = protein content of the fish body (kg protein/kg fish); ϕ_{BW} = water content of the fish body (kg water/kg fish); ϕ_{DL} = lipid content of the diet (kg lipid/kg food); ϕ_{DP} = protein content of the diet (kg protein/kg food); ϕ_{DN} = nondigestible matter content of the diet (kg nondigestible matter/kg food); ϕ_{DW} = water content of the diet (kg water/kg food); ϕ_{GM} = proportional contribution of gastro-intestinal biotransformation (unitless); χ = sorptive capacity of test chemical to protein relative to octanol (unitless); $\omega =$ estimated regression coefficient.

from the slope of a simple linear regression of the natural logarithm-transformed concentrations of the test chemical in the body of the fish against time in the depuration phase (Equations 6 and 7 in Table 1).



FIGURE 1: Conceptual diagram of the 2-compartment fish bioaccumulation model illustrating compartment 1, the fish body (gray), and compartment 2, the intestinal content (taupe). Also shown are the transport (black arrow) and transformation (red) processes considered in the model framework for the interpretation of Organisation for Economic Co-operation and Development guideline 305 dietary bioaccumulation tests including the uptake clearance rates (k_{B1}) for respiratory (L water.kg fish body⁻¹.d⁻¹) and (k_D) for dietary (kg food.kg fish body⁻¹.d⁻¹) uptake, rate constants (d⁻¹) of chemical transfer 1) from the intestines to the body of the fish (k_{GB}); 2) from the body of the fish to the intestines (fecal egestion) to the receiving environment (k_{G2}); rate constants (d⁻¹) for chemical transformation in the body of the fish (somatic transformation; k_{BM}) and in the intestinal contents (intestinal transformation; k_{GM}) and for growth dilution (k_{GD}).

Growth dilution rate constant

The growth dilution rate constant (k_{GD}) can be determined from a linear regression of the natural logarithm of body weight of the fish versus time as described in the OECD-305 guidelines and Equations 8 and 9 (Table 1).

Dietary uptake efficiency of the test and reference chemicals

The dietary uptake efficiencies of the test (E_D) and reference ($E_{D,R}$) chemicals are defined in Equations 10 and 11, respectively (Table 1), and can be derived from the results of dietary bioaccumulation tests in a fashion similar to that described in the OECD-305 guideline from the fractional feeding rate (F_D), the concentration of the chemical in administered diet (C_D), the depuration rate constant (k_{BT}), and the concentration of the chemical in fish at the beginning of the depuration phase ($C_{B,t=0}$) according to Equation 12 (Table 1). Values for k_{BT} , $k_{BT,R}$, and $C_{B,t=0}$ can be obtained from the linear regression of the natural logarithm of the concentrations of the chemical in the fish body against time (Equations 6 and 7).

Somatic biotransformation rate constant

The somatic biotransformation rate constant ($k_{\rm BM}$) represents the biotransformation that occurs in the body of the fish. It can be derived from the depuration rate constant ($k_{\rm BT}$) measured in the dietary bioaccumulation test when nonbiotransformable reference chemicals are used in the test. The underlying principle is that nonmetabolizable reference chemicals can reveal the combined depuration rate of the test chemical through all depuration processes (i.e., gill elimination, net fecal egestion, and growth dilution) other than biotransformation. Consequently, the somatic biotransformation rate constant can be determined as the difference between the depuration rate constant of the test chemical $(k_{\rm BT})$ and the depuration rate constant of the nonmetabolizable reference chemical ($k_{BT,R}$) according to Equation 13 (Table 1). Given that elimination and excretion rates are known to be related to the K_{OW} of the chemical, it is important to use a reference chemical with the same log K_{OW} value as the test chemical. Such a reference chemical may be difficult to find. However, a range of nonbiotransformable reference chemicals with varying K_{OW} , encompassing the K_{OW} of the test chemical, can be used to develop a quantitative relationship between $k_{\text{BT,R}}$ and K_{OW} for nonbiotransformable chemicals that can then be used to derive the $k_{\text{BT,R}}$ for the test chemical. For example, a simple linear relationship between $k_{BT,R}$ and $1/K_{OW}$ (Equation 14 in Table 1) may be adequate (Gobas and Lo 2016). Other relationships, such as log $k_{BT,R}$ versus log K_{OW} , may also be useful. If no reference chemicals are used in the test, then $k_{\rm BT,R}$ can be estimated as the sum of $k_{\rm B2}, k_{\rm BE}$ (i.e., product of $k_{\rm BG}$ and the fraction of the chemical not absorbed in the gut, i.e., $1 - E_D$), and k_{GD} of the test chemical (Equation 15 in Table 1), while $k_{\rm BM} = 0$ when deriving $k_{\rm BT,R}$. The derivation of $k_{\rm B2}$ and $k_{\rm BG}$ is discussed below in sections Rate constant for chemical elimination from the fish body to the water via the respiratory tract and Rate constant for chemical transfer from the gastrointestinal contents to the fish body. If the depuration rate constant of the reference chemicals ($k_{BT,R}$) is greater than the depuration rate constant of the test chemicals ($k_{\rm BT}$), then $k_{\rm BM}$ cannot be determined and is referred to as indeterminable in Supplemental Data, Spreadsheet S1.

Intestinal biotransformation rate constant

The intestinal biotransformation rate constant (k_{GM}) represents the biotransformation that occurs in the contents of the gastrointestinal tract, which is the compartment that is not normally analyzed as part of a bioaccumulation test. This is typically the gut lumen. The intestinal biotransformation rate constant can be derived from the measured dietary absorption efficiency of the test chemical (E_D) if nonbiotransformable reference chemicals are used in the test. The essence of this method is that the dietary uptake efficiency of nonmetabolizable reference chemicals ($E_{D,R}$) can represent the extent of dietary absorption of the test chemical in the absence of intestinal biotransformation of the test chemical, as defined in Equation 11 (Table 1). The intestinal biotransformation rate

constant can then be determined from the difference in absorption efficiencies of the test and the nonmetabolizable reference chemical according to Equation 16 (Table 1). Because dietary absorption efficiencies may also be related to the K_{OW} of the chemical, it is important to use a range of nonbiotransformable reference chemicals with varying K_{OW} , encompassing the K_{OW} of the test chemical so that an empirical relationship between E_{D,R} and K_{OW} can be developed for nonbiotransformable chemicals that can be used to estimate this parameter for the test chemical (Lo et al. 2015). It has been shown that E_{D.R} for nonbiotransforming chemicals in fish follows a nonlinear relationship with K_{OW} for neutral hydrophobic chemicals with a log $K_{OW} > 3$ (i.e., Equation 17 in Table 1). A nonlinear regression can therefore be applied to observations of $E_{D,R}$ for reference chemicals of different K_{OW} , from which an appropriate $E_{D,R}$ for the test chemical can be determined. The derivation of k_{GM} also requires the determination of k_{GB} , which is discussed below in section Rate constant for chemical transfer from the gastrointestinal contents to the fish body. It is important to note that k_{GM} can only be determined if errors in $E_{D,R}$ and E_D are sufficiently small. If E_D is greater than $E_{D,R},$ then $k_{\rm GM}$ is referred to as indeterminable in Supplemental Data, Spreadsheet S1. It should also be emphasized that k_{GM} applies to the mass of chemical in the gastrointestinal tract (M_G), whereas $k_{\rm BM}$ applies to the chemical mass in the fish's body (M_B). To compare the fractional contribution of somatic (Φ_{BM}) and gastrointestinal (Φ_{GM}) biotransformation, the rate constants need to be multiplied by the corresponding masses of the parent substance in the fish's body (M_B) and the intestinal tract (M_G), as described by Equations 18 and 19 (Table 1).

Fecal excretion rate constant

The fecal excretion rate constant ($k_{\rm GE}$) represents the fraction of the chemical mass in the gastrointestinal contents (i.e., lumen) that is egested in fecal matter per unit of time. Test chemical is removed from the intestinal contents via fecal egestion, intestinal biotransformation, and uptake into the fish body. The fecal excretion rate constant (k_{GE}) is the ratio of the fecal egestion rate (G_{GE}, kg digesta dry wt per day) and the amount of digesta (W_G, kg dry wt) in the gastrointestinal tract (Equation 20 in Table 1). The value of G_{GF} can be determined experimentally from fecal collection measurements. However, this is often difficult to do experimentally. An alternative and likely more accurate empirical method for determining G_{GE} is by adding a nonabsorbable tracer such as chromic oxide to the diet and measuring the increase in concentrations in the fecal matter over that in administered diet attributable to food absorption by the fish (Fenton and Fenton 1979; Gobas et al. 1999). Also, G_{GE} can be estimated from the dietary ingestion rate, the composition of the diet, and the assimilation efficiencies of the diet constituents (Arnot and Gobas 2004) using values for the assimilation efficiencies of the various food constituents (i.e., Equation 21 in Table 1). A reasonable estimate of the amount of digesta (W_G) in the intestinal tract can be derived by assuming that feeding of fish in a bioaccumulation test is reasonably well described by a

continuous process and that food absorption by the fish removes a constant fraction of the contents of the intestines per unit of time (i.e., Equation 22 in Table 1). This approach is consistent with observations indicating that the decrease in gastrointestinal contents follows an exponential relationship with time, suggesting that the rate of emptying the gastrointestinal tract in units of kg dry weight/d is proportional to the amount of food in the intestinal tract (Fange and Grove 1979). A mean steady-state amount of digesta can then be estimated from the feeding rate (G₁) and the digesta evacuation rate (δ ; Equation 23 in Table 1). For small fish such as juvenile rainbow trout, the fish's 95% gastrointestinal evacuation time (t_{E 95}) for a meal is approximately 1.45 d (Fange and Grove 1979) and δ is 3/1.45 or approximately 2 d⁻¹. Hence, for a 1-g fish, which is fed 2% of its body weight per day, a W_G of 0.02 × 1/2 or 0.01 g dry weight can be derived.

Rate constant for chemical transfer from the gastrointestinal contents to the fish body

The rate constant for chemical transfer from the gastrointestinal contents to the fish body ($k_{\rm GB}$) represents the fraction of the chemical mass in the gastrointestinal contents (i.e., lumen) that is absorbed by the body of the fish per unit of time. The rate constant $k_{\rm GB}$ can be derived from the dietary absorption efficiency of the reference chemical $E_{\rm D,R}$. The underlying principle is that nonmetabolizable reference chemicals in the intestinal tract can either be egested as fecal matter or absorbed by the fish. Hence, if both $E_{\rm D,R}$ and $k_{\rm GE}$ can be determined from the results of the test (as described above), then $k_{\rm GB}$ can be derived by rearranging Equation 11 into Equation 24 (Table 1).

Rate constant for chemical transfer from the fish body to the gastrointestinal contents

The rate constant for chemical transfer from the fish body to the gastrointestinal contents (k_{BG}) represents the fraction of the mass of parent (i.e., untransformed) test chemical in the body of the fish that is transferred to the intestinal tract per unit of time. This chemical transfer normally includes passive diffusion and biliary excretion of the parent chemical. Transformation of the chemical in the liver and other tissues of the fish body is included in k_{BM} and not in k_{BG} . It is possible that a metabolite(s) of the test chemical in the intestinal tract and then reabsorbed (Gaillard et al. 2017). This particular contribution to enterohepatic recirculation of chemicals is not captured in the model.

The rate constant for chemical transfer from the fish body to the gastrointestinal contents (k_{BG}) can be estimated from k_{GB} according to Equation 25 (Table 1). This method assumes that the partition coefficient of the untransformed test chemical between the fish body and the gastrointestinal contents (K_{GB}) can be estimated. The ADME-B calculator estimates this partition coefficient from the composition of the digesta and the body of the fish and the K_{OW} of the substance according to Equation 26 (Table 1). The composition of the fish's body and fish food is often known or measurable. The composition of the digesta can also be measured, but this can be difficult in small fish. In the absence of measurements, it can be approximated from the dietary composition using estimates of the dietary assimilation efficiencies of lipids (ϵ_L), protein (ϵ_P), nondigestible organic matter (ϵ_N), and water (ϵ_W) using Equations 27 to 30 (Table 1), following Arnot and Gobas (2004). The dietary lipid assimilation efficiency is well characterized at approximately 0.92 in rainbow trout (Gobas et al. 1999), and protein and water assimilation efficiencies in fish are approximately 0.75 and 0.5, respectively (Lo et al. 2015). The dietary assimilation efficiency of nondigestible organic matter is assumed to be 0. If available, alternative values can be entered into the ADME-B calculator. For substances within the domain of applicability (log K_{OW} between approximately 3 and 9.2), the water assimilation efficiency has no significant effect on the derivation of k_{BG} of organic substances because water has a negligible capacity to solubilize very hydrophobic chemicals compared to lipids, proteins, and other organic materials. Hence, its exact value is inconsequential in bioaccumulation calculations for these chemicals.

Rate constant for chemical elimination from the fish body to the water via the respiratory tract

The rate constant for chemical elimination from the fish body to the water via the respiratory tract $(k_{\rm B2})$ involves the transfer of chemicals from the fish to the water via the gills and skin. For hydrophobic organic substances, this process involves mostly passive diffusion and gill ventilation (Gobas and Mackay 1987). For substances with high $K_{\rm OW}$, $k_{\rm B2}$ is often small and of little relevance in dietary bioaccumulation tests except for its relationship with the respiratory uptake clearance rate $k_{\rm B1}$ and the BCF. If the dietary bioaccumulation test involves multiple nonbiotransformable reference chemicals, it is possible to determine k_{B2} from the slope of the relationship of the depuration rate constants of the reference chemicals ($k_{BT,R}$) and K_{OW} following Gobas and Lo (2016; Equation 31 in Table 1). If the dietary bioaccumulation test did not include reference chemicals, then the ADME-B calculator uses the model in Arnot and Gobas (2004) for deriving the uptake clearance rate (k_{B1}) and the elimination rate constant (k_{B2} ; Equation 32 in Table 1). This model estimates gill ventilation rates and gill uptake efficiencies of the test chemicals in fish. The main disadvantage of this approach is that $k_{\rm B2}$ is not determined from the results of the dietary bioaccumulation test. Discrepancies between the model and the test can cause error in the determination of k_{B1} .

Derivation of the uptake clearance rate for chemical uptake from the water via the respiratory route (gills and skin)

The uptake clearance rate of chemicals in fish (k_{B1}) is the volume of water cleared (of chemical) by the body of the fish over time and has units of liters of water per kg of fish body per day. It involves the transfer of chemicals from water via the

gills and skin into the fish. Because a dietary bioaccumulation test does not include respiratory uptake of the chemical in the fish, it appears that $k_{\rm B1}$ cannot be determined in dietary bioaccumulation tests. However, dietary bioaccumulation tests do involve respiratory elimination, which is the reverse process of respiratory uptake. Because respiratory exchange of hydrophobic organic chemicals in fish is passive in nature, k_{B1} and k_{B2} are related according to Equation 33 (Table 1), providing a method to determine k_{B1} from k_{B2} (Equations 34 and 35 in Table 1). If reference chemicals are used in dietary bioaccumulation tests, BCFs can be determined from the results of the test. If no reference chemicals are used in the dietary bioaccumulation tests, $k_{\rm B1}$ cannot be derived directly from the results of the dietary bioaccumulation tests but can be estimated using an external model for the uptake clearance rate (k_{B1}) and the elimination rate constant (k_{B2}) .

Derivation of the BMF of the test chemical in fish

The BMF represents the ratio of the steady-state concentrations of the chemical in the fish and the diet that the test organisms were exposed to. Because concentrations of hydrophobic organic chemicals in fish do not always achieve a steady state in the test, the BMF is best derived according to Equation 36 (Table 1), using the kinetic approach. This BMF has units of kg food dry weight per kilogram of fish wet weight and is dependent on the preparation and application of the food in the test. In most dietary bioaccumulation studies, the food consists of dry pellets. In such studies, the BMF compares dry weight- and wet weight-based concentrations, which is tantamount to comparing "apples" and "oranges." This BMF is difficult to interpret and relate to regulatory bioaccumulation criteria. However, this BMF can be expressed on a lipid equivalent basis as BMF_L (Equation 37 in Table 1) using Equations 38 and 39 (Table 1). This equation is similar to that described in the OECD-305 guideline but considers not only the lipids as the medium in which chemicals bioaccumulate but also protein, nondigestive matter (e.g., carbohydrates), and (although unimportant) water. Tissues with a high protein content are known to preferentially accumulate certain polyfluorinated hydrocarbons (Martin et al. 2003) and can have a significant effect on bioaccumulation behavior if the lipid content of fish or fish food is low. Measurements of the protein content of the fish and food are sometimes useful additions to the OECD-305 dietary bioaccumulation test. The BMF_L is a useful metric to assess the biomagnification of a range of hydrophobic chemicals with affinities for lipids, proteins, and carbohydrates. A useful value for the BMF₁ that can be used as criterion value to indicate chemicals with biomagnification potential is 1. Substances with a BMF_L <1 can be expected to lack biomagnification potential as long as the test species is representative of the biomagnification potential in other species. Substances with a BMF_L significantly >1 indicate a potential to biomagnify. Biomagnification is of toxicological concern because it amplifies the chemical activity and fugacity in the food web and can cause high concentrations in organisms at the top of the food chain.

Derivation of the BCF of the test chemical in fish

The BCF is a frequently used metric in regulatory evaluations. Although the BCF is not a primary metric generated by a dietary bioaccumulation test, it can be derived from the results of a dietary bioaccumulation test for chemicals according to Equation 40 (Table 1). Because the BCF used for regulatory evaluations is based on the total concentration of the chemical in the water, Equation 40 includes an estimation of the bioavailable fraction of the chemical in the water, which is a function of the amount of organic carbon in the water and the organic carbon–water partition coefficient. To account for differences in the body composition (e.g., lipid and protein content) among fish, the BCF can be also expressed on a lipid equivalent basis according to Equation 41 (Table 1). The BCF normalized to a fish with a 5% lipid content (BCF_{5%,t}) is calculated according to Equation 42 (Table 1).

Derivation of the bioaccumulation factor of the test chemical in fish

The bioaccumulation factor (BAF) is different from the BCF in that it includes uptake of chemicals in fish from both water and diet. It is used as a metric of bioaccumulation in regulatory evaluations in Canada and the United States. The BAF is a function of the relative concentrations of the chemical in water and food sources, which can vary substantially among aquatic environments or test systems. The ADME-B calculator uses Equation 43 (Table 1) to assess the BAF in fish as a function of user-defined concentrations of the chemical in water and diet.

Error analysis

In the ADME-B calculator, the error in the determination of each rate constant is either calculated or estimated through the rule of uncertainty propagation (Farrance and Frenkel 2012). This method is simpler and less time- and computing-intensive than methods based on Monte Carlo simulations (Papadopoulos and Yeung 2001). Following the rule of uncertainty propagation for a quantity of interest q which is a function of variables $x_1, x_2 \dots x_n$ (i.e., $q = f[x_1, x_2 \dots x_n]$), the uncertainty (estimated as standard error) of q (s_q) is approximated as

$$s_{q} = \sqrt{\left(\frac{\partial q}{\partial x_{1}} \times s_{x1}\right)^{2} + \left(\frac{\partial q}{\partial x_{2}} \times s_{x2}\right)^{2} + \dots + \left(\frac{\partial q}{\partial x_{n}} \times s_{xn}\right)^{2}}$$
(47)

where $\partial q/\partial x_1$, $\partial q/\partial x_2$, and $\partial q/\partial x_n$ are the partial derivatives of q with respect to x_1 , x_2 , and x_n , respectively; and s_{x1} , s_{x2} , and s_{xn} are the standard errors of x_1 , x_2 , and x_n , respectively. This method assumes that errors in the variables are uncorrelated with one another. In cases where errors in variables are correlated (e.g., regression coefficients), the standard error s_q is approximated as (Farrance and Frenkel 2012)

$$s_{q} = \sqrt{\sum_{i=1}^{n} \left(\frac{\partial q}{\partial x_{i}} \times s_{xi}\right)^{2} + 2\sum_{i=1}^{n} \sum_{j=1}^{n} \left(\frac{\partial q}{\partial x_{i}}\right) \times \left(\frac{\partial q}{\partial x_{j}}\right) \times Cov(x_{i}, x_{j})}$$
(48)

where $\partial q/\partial x_i$ and $\partial q/\partial x_j$ are the partial derivatives of q with respect to x_i and x_j , respectively, and $Cov(x_i, x_j)$ is the covariance of x_i and x_j . The estimated covariance of regression coefficients, such as those used in Equation 6 in Table 1, can be obtained directly from the estimated variance–covariance matrix of the regression coefficients calculated by most statistical computer programs and is also included in the ADME-B calculator. The equations for deriving the estimated uncertainties of the parameters in the 2-compartment model are provided in the Supplemental Data (Section S1) and are included in the ADME-B spreadsheet model.

Statistical testing

To provide a statistically based method for testing whether the bioaccumulation metrics derived from the results of bioaccumulation tests are greater than regulatory limits (e.g., BMF_L of 1 and regulatory BCF criteria values of 2000 or 5000), the ADME-B calculator includes a one-sided Student t test based on the following assumptions: 1) the sampling distribution of the test statistic is assumed to follow a Student tdistribution; 2) for testing the BMF_L, the degrees of freedom are determined from the number of observations (n) in the depuration phase of the test that are used to determine the depuration rate constant k_{BT} as n-2; 3) for testing the BCF derived from tests that included reference chemicals, the degrees of freedom are determined as n-2 from the smallest number of observations (n) of the 2 regressions used in the determination of k_{BT} (i.e., Equation 6 in Table 1) and $k_{BT,R}$ (i.e., Equation 14 in Table 1); 4) for testing the BCF derived from tests that did not include reference chemicals, the degrees of freedom are determined as n-2 from the smallest number of observations (n) of the 3 regressions used in the derivation of the BCF, namely regressions used to determine a) the depuration rate constant k_{BT} , b) the gill uptake efficiency (E_W; n = 12), and c) the oxygen consumption rate (n = 4967). The test applies a significance level of 0.05 or any other userspecified value relevant to the evaluation. To test whether these assumptions are reasonable, p values calculated from the t test were compared to results from Monte Carlo simulations. To obtain meaningful results from Monte Carlo simulations, the models for calculating BMFL and BCF were rewritten in terms of the independent empirical observations required for determining BMF_L and BCF (i.e., Equations 44-46 in Table 1).

MATERIALS AND METHODS

ADME-B calculator

The equations summarized in Table 1 were used to create a spreadsheet model for deriving bioaccumulation metrics from OECD-305 dietary bioaccumulation tests using the 2compartment model. The current version of the ADME-B calculator is included in the Supplemental Data, and updates can be freely downloaded from the Simon Fraser University website (Environmental Toxicology Research Group 2019) or requested from the corresponding author.

ADME-B calculator application and evaluation

To evaluate and test the refined toxicokinetic framework for the OECD-305 dietary bioaccumulation test, the spreadsheet model was applied to the results of 27 OECD-305 dietary bioaccumulation tests performed at Exxon Mobil Biomedical Sciences, one test performed at Simon Fraser University (Lo et al. 2015), and 4 dietary bioaccumulation tests reported in the literature (Sijm et al. 1992; Fisk et al. 1998; Stapleton et al. 2004; Inoue et al. 2012). Descriptions of the dietary bioaccumulation tests conducted at Exxon Mobil Biomedical Sciences and Simon Fraser University are included in the Supplemental Data (Section S2) and Lo et al. (2015, 2016).

Tests involved juvenile rainbow trout (Oncorhynchus mykiss; 27 tests), carp (3 tests), guppies (one test), and fathead minnow (one test). Results for 238 organic chemicals (166 unique chemicals) were evaluated, including aliphatic and cyclic hydrocarbons, parent and alkylated aromatic hydrocarbons, and halogenated aliphatic and aromatic hydrocarbons and representatives from several oxygen-, nitrogen-, or sulfur-containing compound classes. The only chemical property required for the interpretation of dietary bioaccumulation tests for bioaccumulation assessment in fish is $K_{\rm OW}$. The $K_{\rm OW}$ values of all the test chemicals were obtained from EPI Suite (Ver. 4.1; US Environmental Protection Agency 2012), except for the K_{OW} of PCBs, which were obtained from Mackay et al. (2006) and Hawker and Connell (1988); Kow values are reported in the Supplemental Data (Spreadsheet S1). The log K_{OW} values of the test chemicals obtained from EPI Suite varied between 3.3 and 13.2. Dietary bioaccumulation test results for 106 chemicals (87 unique chemicals) of the 238 chemicals were included in previous analyses (Lo et al. 2015, 2016; Gobas and Lo 2016), which took advantage of available data not required by the OECD-305 guideline and addressed uncertainty in the feeding rate in some of the experiments. The analysis in the present study is tailored to OECD-305 dietary bioaccumulation tests and differs from the analysis in Lo et al. (2015, 2016) in that 1) the present study only considered information from the depuration period, whereas Lo et al. (2015, 2016) used data from both the uptake and depuration periods and 2) the present study used reported feeding rates, whereas Lo et al. (2015, 2016) estimated feeding rates from dietary uptake efficiencies of the reference chemicals. Because the majority of studies analyzed did not use multiple reference chemicals, we calculated k_{B2} according to Equation 32 (Table 1) for all studies except that of Lo et al. (2015), for which we used Equation 31 (Table 1), which is specific to tests that use reference chemicals. Evaluation of the analysis tool involved the calculation of all rate constants and associated bioaccumulation metrics for the full set of 238 test chemicals, including uncertainty estimates. The bioaccumulation metrics evaluated in the present study are the lipid-normalized BMFs (BMF₁) and the wet weight-based BCFs for fish with a 5% lipid content (BCF_{5%,t}).

Information on the test conditions required and used in the evaluation and testing of the ADME-B calculator includes fish

body weight (W_B ; kg wet wt), the concentration of the chemical in the diet (C_D; grams of chemical per kilogram of food), the proportional feeding rate (F_D; kilograms of food per kilogram of fish wet wt per day), the duration of the feeding period (t_u; days), the lipid content of the fish body (ϕ_{BL} ; kilograms of lipid per kilogram of fish wet wt), the protein content of the fish body (ϕ_{BP} ; kilograms of protein per kilogram of fish wet wt), the lipid content of the diet (ϕ_{DL} ; kilograms of lipid per kilogram of food), the protein content of the diet (ϕ_{DP} ; kilograms of protein per kilogram of food), the water content of the diet (ϕ_{DW} ; kilograms of water per kilogram of food), the water temperature (T; °C), the dissolved oxygen concentration in water (C_{OX} ; milligrams of O₂ per liter), the rate constant for growth dilution $(k_{GD}; per day)$, and the time-course concentrations of the test chemical in the depuration phase (t, C_B; days, grams of chemical per kilogram of fish wet wt). This information was obtained from the dietary bioaccumulation tests and used as input to the ADME-B calculator (Supplemental Data, Spreadsheet S1). The assimilation efficiencies of lipid, protein, nondigestible organic matter, and water ($\epsilon_L,~\epsilon_P,~\epsilon_N,$ and $\epsilon_W;$ unitless) were assumed based on the values reported by Lo et al. (2015) as discussed. Because many dietary bioaccumulation tests did not include reference chemicals, the dietary absorption efficiency of the nonmetabolizable reference chemicals (E_{D.R}; unitless; standard error [SE]) was estimated using the findings for the reference chemicals reported in Lo et al. (2016):

$$E_{D,R} = \frac{1}{5.6 \times 10^{-9} (\text{SE1.8} \times 10^{-9}) \times K_{OW} + 1.9 (\text{SE0.1})}$$
(49)

This approach is reasonable because in 13 independent dietary tests that involved use of hexachlorobenzene as a single reference chemical, the measured dietary uptake efficiency of hexachlorobenzene was in agreement with the estimate determined from Equation 49 for 12 of these tests, with the exception of one test which likely assumed an erroneous feeding rate. The depuration rate constant of the reference chemical $(k_{\text{BT,R}})$ was estimated according to Equation 15 (Table 1). The rate constant for respiratory elimination ($k_{\rm B2}$) was calculated according to Equation 32 (Table 1). The gill chemical uptake efficiency (E_W) in Equation 32 was estimated using findings reported in Gobas and Mackay (1987). The gill ventilation rate (G_V) in Equation 32 was estimated based on oxygen requirement data for fish (US Environmental Protection Agency 1998) with details provided in the Supplemental Data (Section S1). Other kinetic parameters and bioaccumulation metrics were calculated using equations presented in Table 1.

Statistical testing

To evaluate whether the Student *t* test included in the ADME-B calculator provides reasonable estimates of the statistical significance of tests that involve comparison of the BCF or BMF_L to regulatory criteria, Monte Carlo simulations (n = 1000) of equations 44 to 46 were conducted using the @Risk 7.6 software (Palisade), assuming that input variables (specified in Table 1) were normally distributed and independent (except for regression coefficients, for which correlation was considered).

RESULTS AND DISCUSSION

ADME-B calculator

The ADME-B calculator consists of 4 modules: 1) a worksheet in which experimental data and test conditions are entered and bioaccumulation metrics are derived, 2) an ADME profiler that illustrates the internal distribution and transformation of the test chemical in the test fish and in field-exposed fish, 3) an error analysis module that includes the equations used to calculate error, and 4) a statistical analysis tool to test bioaccumulation metrics against regulatory criteria values. The application of the ADME-B calculator to one of the test chemicals (i.e., 1,2,3,4,5,6,7,8-octahydrophenanthrene) in the present study is detailed in Supplemental Data, Spreadsheet S1, with the corresponding ADME-B profile illustrated in Figure 2. Figure 2A illustrates that in the dietary bioaccumulation test of 1,2,3,4,5,6,7,8octahydrophenanthrene E_{D} was 0.186 (±0.033 SE) and k_{BT} was 0.244 (\pm 0.018 SE) d⁻¹, 18.6% of the ingested dose was directly absorbed into the fish body, whereas a total of 65.7% of the ingested dose was biotransformed in the intestines and 17.1% was egested unabsorbed in fecal matter. Within the body of the fish, 12.7% of the ingested dose (or 12.7/18.9 = 67% of the absorbed dose) was biotransformed in the fish, 1.66% was excreted into the intestines, 1.58% was eliminated via the gills and skin back to the water, whereas growth dilution accounted for a 2.92% pseudoloss of the ingested dose. In the intestines, 0.31% of the ingested dose is reabsorbed after excretion, whereas 1.08% of the ingested dose is biotransformed in the intestines after excretion from the body of the fish and 0.28% of the ingested dose is egested in fecal matter after excretion. The BCF_{ww,t} of 1,2,3,4,5,6,7,8-octahydrophenanthrene derived from the dietary bioaccumulation test results is 2702 (±233 SE) L/kg wet weight and is not significantly greater than 5000 (p > 0.05). The BMF_L is 0.117 (±0.014 SE) kg lipid/kg lipid. The somatic biotransformation rate constant (k_{BM}) is 0.167 (±0.019 SE) d⁻¹, and the intestinal biotransformation rate constant (k_{GM}) is 3.05 (±0.93 SE) d⁻¹.

Figure 2B shows that in a field scenario where the concentrations of 1,2,3,4,5,6,7,8-octahydrophenanthrene in water and the prey of the fish are, respectively, 0.01 μ g/L and 100 μ g/kg food, approximately 69% of the chemical that the fish is exposed to is from the water and 31% is from the diet. In this scenario the majority of the chemical in the fish body is from the water, and biotransformation in the body of the fish is the main route of depuration. The field BAF under these conditions is 2930 (±219 SE) L/kg wet weight and is greater than the corresponding BCF_{ww,t} of 2702 (±233 SE) L/kg wet weight because it includes dietary exposure. It is important to note that concentrations of 1,2,3,4,5,6,7,8-octahydrophenanthrene in water and food that are different from those selected in this example will alter the relative roles of the water and food exposure pathways and the value of the BAF. This can be investigated by using the ADME-B calculator.

Application and evaluation of the ADME-B calculator

The results of the application of the ADME-B calculator to the dietary bioaccumulation test data of 238 test chemicals in

(**A**)







FIGURE 2: The profile of absorption, internal distribution, biotransformation, and excretion of 1,2,3,4,5,6,7,8-octahydrophenanthrene in fish expressed in terms of the fraction of the administered chemical intake rate in units of grams per day in a dietary bioaccumulation test (A) and in a field exposure environment (B). Yellow boxes indicate routes of chemical uptake, and red boxes indicate routes of chemical elimination. BAF = bioaccumulation factor; BCF = bioconcentration factor; $C_D =$ concentration of the chemical in the diet; C_W = concentration of the chemical in water; k_{BG} = rate constant of chemical transfer from the body to the intestines; k_{BM} = rate constant for chemical transformation in the body; k_{GB} = rate constant of chemical transfer from the intestines to the body; k_{GD} = rate constant for growth dilution; k_{GM} = rate constant for biotransformation in the gastrointestinal contents; k_{GE} = rate constant for fecal egestion from the gastrointestinal tract; M_B = chemical mass in the fish's body; M_G = mass of chemical in the gastrointestinal tract; W_B = fish body weight; G_1 = food ingestion rate.

32 OECD-305 dietary bioaccumulation tests are presented in the Supplemental Data (Spreadsheet S1). Ten additional substances for which dietary bioaccumulation tests were completed were excluded from analysis because tissue concentrations after the end of the uptake period were either not detected or in error (because of contamination) and likely exhibit a very low bioaccumulation potential. The measured total depuration rate constants for all test chemicals ($k_{\rm BT}$) ranged from 0.00069 (±0.0021 SE) to 3.8 d⁻¹ (±0.25 SE; Figure 3A). For 5 substances (i.e., 2-isopropyldecalin, PCB 52, PCB 153, PCB 155, and PCB 209), $k_{\rm BT}$ was not statistically different from 0 (p > 0.05) because of slow depuration, producing large lower error bars that cannot be displayed in Figure 3A.

The calculated dietary chemical uptake efficiency (E_D) ranged from 0.0085 (±0.0027 SE) to 1.7 (±0.19 SE; Figure 3B). Dietary uptake efficiencies (E_D) of 200 of the 238 test chemicals fell below 53%, which is considered the maximum mean dietary uptake efficiency for nonmetabolizable reference chemicals in OECD-305-style dietary bioaccumulation studies that use standard fish diets similar to those used by Lo et al. (2015) and reflects the absorption efficiency of the food in the test, which was previously measured with chromic oxide tracers to be approximately 50% (Gobas et al. 1999). Dietary uptake efficiencies >1 were calculated for 5 substances and can be attributed to errors in the determination of the feeding rate that were corrected for in the analysis of Lo et al. (2016) but not in the present analysis. It is important to stress that alternative dietary dosing methods involving single-bolus treatments, gavage, and oils as the delivery method can produce dietary uptake efficiencies that are different from those determined in OECD-305 tests that administer the chemical through daily feeding of fish food over an extended period of time. Also, the composition of the diet can have an effect on the dietary uptake efficiency (Gobas et al. 1993). The use of reference chemicals in dietary bioaccumulation tests is recommended to improve interstudy comparison of dietary uptake efficiencies.

Somatic biotransformation rate constants were obtained from the dietary bioaccumulation tests for 172 of the 238 chemicals and ranged from essentially 0 to 3.7 (\pm 0.25 SE) d⁻¹ (Figure 3C). For 66 substances, depuration rate constants of the test chemicals were not distinguishable from those of the corresponding reference chemicals, and the somatic biotransformation rate constant was therefore considered to be 0 d⁻¹. Intestinal biotransformation rate constants were obtained from the dietary bioaccumulation tests for 190 of the 238 chemicals and ranged from essentially 0 to 101 (\pm 33.8 SE) d⁻¹ (Figure 3D). For 48 substances, dietary uptake efficiencies of the test chemicals were not distinguishable from those of the corresponding reference chemicals, and the intestinal biotransformation rate constants were therefore considered to be 0 d⁻¹. Lower error bars of the somatic and intestinal biotransformation rate constants equal to 0 d⁻¹ could not be displayed in Figure 3C,D. For the 137 substances for which both intestinal and somatic biotransformation rate constants could be determined, there was no apparent correlation between intestinal and somatic biotransformation rate constants (Supplemental Data, Figure S1), suggesting that intestinal and somatic biotransformation are subject to different transformation pathways. Figure 4A shows that when fish are exposed via the diet, biotransformation of 120 out of 137 chemicals occurs predominantly in the lumen of



FIGURE 3: Distributions of the calculated total depuration rate constant, k_{BT} (**A**); dietary chemical uptake efficiency, E_D (**B**); somatic biotransformation rate constant, k_{BM} (**C**); intestinal biotransformation rate constant, k_{GM} (**D**); lipid-equivalent biomagnification factors, BMF_L (**E**); and bioconcentration factors, BCF_{5%,t}, normalized to a fish with a 5% lipid content based on total chemical concentration in water (**F**) using the fish absorption, distribution, metabolism, and excretion (ADME)-B calculator for 238 (**A**, **B**, **E**, **F**), 172 (**C**), and 190 (**D**) test chemicals in 32 dietary bioaccumulation tests. Data are presented in order of increasing values. Error bars represent the estimated standard error of the mean. Solid red lines represent the BMF_L of 1 kg lipid/kg lipid (**E**) and the regulatory BCF criterion of 5000 L/kg wet weight (**F**); solid blue line represents the regulatory BCF criterion of 2000 L/kg wet weight.



FIGURE 4: Proportional contribution of somatic biotransformation (ϕ_{BM} , solid blue squares, left axis) and intestinal biotransformation (ϕ_{GM} , open red dots, right axis) to the overall mass of chemical biotransformed for 137 test chemicals under dietary exposure only (**A**) and aqueous exposure only (**B**) conditions. Data are presented in order of increasing values of ϕ_{BM} .

the fish (i.e., $\phi_{GM} > 0.5$). However, when fish are exposed via the water, the majority of the test chemicals (133 out of 137) are predominantly biotransformed in the body of the fish (i.e., $\phi_{BM} > 0.5$). Similar results were reported by Lo et al. (2016). This means that the role of somatic or intestinal biotransformation processes in mitigating the bioaccumulation of chemicals in fish is dependent on the exposure pathway.

Lipid-equivalent biomagnification factors (BMF_L) and bioconcentration factors normalized to fish with a 5% lipid content (BCF_{5%,t}) of the test chemicals are tabulated in the Supplemental Data (Spreadsheet S1). The BMF_L varied from 0.0025 (\pm 0.0009 SE) to 24 (\pm 72 SE) kg lipid/kg lipid (Figure 3E), with BMF_L values >1 being observed in 35 cases for 19 unique chemicals with a log K_{OW} ranging between 5.7 and 8.3. The great majority of the calculated BCF_{5%,t} values fell between 100 and 400 000 L/kg fish wet weight (Figure 3F). Figure 5 illustrates that the calculated BMF_L and BCF_{5%,t} values were positively correlated for chemicals with log K_{OW} values ranging between 3.3 and 9.2 (3 chemicals with log K_{OW} between 11.8 and 13.2



FIGURE 5: The relationship between calculated lipid-equivalent biomagnification factors (BMF_L) and bioconcentration factors normalized to a fish with a 5% lipid content based on total chemical concentration in water (BCF_{5%,t}) using the absorption, distribution, metabolism, and excretion (ADME)-B calculator for 238 test chemicals (log K_{OW} ranged 3.3–9.2) in 32 dietary bioaccumulation tests. The dotted black line represents the fitted log BMF_L – log BCF_{5%,t} linear regression of the test chemicals. Solid red lines represent the BMF_L criterion of 1 kg lipid/kg lipid and the regulatory BCF criterion of 5000 L/kg wet weight. Error bars represent the estimated standard error of the mean. Also shown is a possible classification scheme for bioaccumulative, bioaccumulative, and not bioaccumulative in a regulatory sense. B = bioaccumulative; not B = not bioaccumulative; vB = very bioaccumulative.

were excluded because they are out of the domain of applicability for conducting reliable dietary bioaccumulation tests following OECD-305 guidelines):

L

$$\log BCF_{5\%,t} = 0.65 \ (\pm 0.036 \ SE) \times \log BMF_{L} + 4.02 \ (\pm 0.044 \ SE) n = 235; r^{2} = 0.58; p < 0.001$$
(50)

The main underlying factor contributing to this correlation is that BMF_L and $BCF_{5\%,t}$ share the same depuration rate constant $(k_{\rm BT})$. The underlying factor (other than measurement error) contributing substantial variation in the relationship between the BMF_L and $\mathsf{BCF}_{5\%,t}$ is the biotransformation rate of the chemical in the lumen of the fish, which affects the BMF to a much greater degree than the BCF. The lack of correlation between intestinal and somatic biotransformation rates further contributes to the variation in the relationship between the BMF_L and BCF_{5%,t} (Supplemental Data, Figure S1). The correlation between the BCF and BMF is therefore highly dependent on the selection of chemicals used in constructing the correlation. Including substances that are biotransformed in the lumen of the fish confounds the development of simple guantitative relationships between BCFs and BMFs. Figure 5 confirms the lack of a simple quantitative relationship between BCFs and BMFs that can be used to derive the BCF from the BMF or the BMF from the BCF. Equation 50 and similar equations derived in other studies may therefore be of limited use for assessing BCFs from BMFs measured in dietary bioaccumulation tests. A useful predictive relationship between the BMF_L and $BCF_{5\%,t}$ can only be expected for recalcitrant substances that do not biotransform in the intestines (Lo et al. 2016).

Statistical testing

Supplemental Data, Figure S2, shows that p values for testing the hypotheses that $BMF_L > 1$ and $BCF_{5\%,t} > 5000$ determined by error propagation in ADME-B calculator and Monte Carlo simulations are in good agreement. Error propagation identified 35 cases where BMF_L was >1, of which 24 cases (for 16 unique chemicals) exhibited a p < 0.05 and in 11 cases (for 7 unique chemicals) $p \ge 0.05$. Monte Carlo simulations showed 33 cases where BMF_L was >1, of which 26 cases (for 16 unique chemicals) exhibited a p < 0.05 and in 7 cases (for 5 unique chemicals) $p \ge 0.05$. Error propagation identified 67 cases where the calculated $\mathsf{BCF}_{5\%,t}$ was >5000, with 50 cases exhibiting a p < 0.05 and 17 cases a $p \ge 0.05$. Monte Carlo simulations identified 66 cases where the calculated BCF_{5%,t} was >5000, with 53 cases exhibiting a p < 0.05 and 13 cases a $p \ge 0.05$. The main difference between error propagation and Monte Carlo simulations as methods for testing exceedance of criteria values occurred for chemicals with measured depuration rate constants that exhibited a large error compared to the mean. In those cases, Monte Carlo simulations produced very wide frequency distributions for the BMF_L and $BCF_{5\%,t}$ that were unable to provide realistic estimates of the means and standard errors, whereas error propagation did provide reasonable estimates of the BMF₁ and BCF_{5%,t}. Comparison of the error propagation method and the Monte Carlo simulation method indicates that the ADME-B calculator's method for testing BCFs and BMFs to criteria values is adequate and produces confidence values and p values that are similar to or more conservative when compared to evaluating statistical significance determined by Monte Carlo simulations. The advantage of error propagation over Monte Carlo simulations is that it is less computingintensive than Monte Carlo simulations and less sensitive to the selection of frequency distributions that correctly describe the experimental metrics.

Both the BMF_L and BCF_{5%,t} exceeded their corresponding criteria values of 1 and 5000 in a statistically significant (p < 0.05) fashion in 23 cases for 15 unique chemicals (i.e., selected polychlorinated biphenyl and polybrominated diphenyl ether congeners, hexachlorobenzene, mirex and tris[4-chlorophenyl] methane). All of these chemicals can be regarded as very bio-accumulative substances (Figure 5). Figure 6, which presents BMF and BCF relationships for several chemical classes, shows that all of these very bioaccumulative substances are halogenated organic compounds with log K_{OW} values of 5.7 and 8.3 (Figure 6A). In 27 cases for 16 unique chemicals (i.e., selected polychlorinated biphenyl congeners and polynaphthenic hydrocarbons, hexachlorobenzene and tris[4-chlorophenyl]methanol), the BCF_{5%,t} exceeded 5000 in a statistically significant (p < 0.05) fashion, whereas the BMF_L did not statistically exceed 1 (Figure 6A–C).

These substances are less bioaccumulative than substances for which both the BMF_L and $BCF_{5\%,t}$ exceed the criteria values of 1 and 5000, respectively. These substances should be regarded as bioaccumulative rather than very bioaccumulative because of their high degree of bioaccumulation from water but lack of biomagnification from food sources because of limited uptake from food resulting from intestinal biotransformation, low bioavailability, and/or other processes (Figure 5). We believe that this approach provides a better technical basis for ranking chemicals for their bioaccumulation potential than the current approach in the European Union (European Chemicals Agency 2017) that uses different bioconcentration-based criteria for bioaccumulative (BCF \ge 2000) and very bioaccumulative (BCF \ge 5000) substances. The great majority of the test chemicals (i.e., 186 cases for 139 unique chemicals) exhibited a BMF_L that was not significantly $(p \ge 0.05) > 1$ and a calculated BCF_{5%,t} that was not significantly $(p \ge 0.05)$ greater than the BCF criterion value of 5000. These chemicals may be considered nonbioaccumulative in a regulatory context (Figure 5), although they possess significant capacity for bioaccumulation from water that may cause toxic effects if environmental concentrations reach sufficiently high levels.

All substances with a BMF_L statistically significantly >1 exhibited a BCF > 5000. This indicates that an empirical BMF_L that is significantly (p < 0.05) > 1 is adequate proof that the $BCF_{5\%,t}$ can be expected to be >5000. However, only 23 of 50 chemicals with a BCF_{5%,t} significantly (p < 0.05) >5000 exhibited a BMF_L significantly (p < 0.05) >1. This illustrates that substances with a $BCF_{5\%,t} > 5000$ do not necessarily biomagnify in fish and hence are less bioaccumulative in the environment than chemicals that do biomagnify. The main reason for the lack of biomagnification of chemicals with a BCF > 5000 is the limited uptake of chemical from the gastrointestinal contents because of intestinal biotransformation and/or poor dietary bioavailability. One of the limitations of statistical testing for bioaccumulation assessment is for bioaccumulation tests in which the test substance exhibits a depuration rate constant (k_{BT}) that is not statistically different from 0 d⁻¹. This may occur in tests of insufficient duration or test with very slowly depurating substances. In such cases, the BCFs and BMFs derived from the results of the tests contain large uncertainty and are therefore not significantly greater than or less than the criteria values. In these cases, a better test design is required to obtain meaningful information. One of the strengths of statistical testing is that the regulatory criteria for bioaccumulation can be tested in a scientifically valid fashion.

Merits and limitations

The advantage of the dietary bioaccumulation test over the aqueous bioconcentration test is that somatic and intestinal biotransformation rates can be simultaneously determined in a single test. The ADME-B calculator can use dietary bioaccumulation test results to assess somatic and intestinal biotransformation rates under a variety of potential environmental exposure conditions. In most cases, bioconcentration tests can only reveal the somatic biotransformation rates because



FIGURE 6: The relationship between calculated lipid-equivalent biomagnification factors (BMF_L) and bioconcentration factors normalized to a fish with a 5% lipid content based on total chemical concentration in water (BCF_{5%,1}) using the absorption, distribution, metabolism, and excretion (ADME)-B calculator for 58 halogenated organic compounds (**A**), 79 aliphatic hydrocarbons (**B**), 87 aromatic hydrocarbons (**C**), and 14 oxygen-containing compounds (**D**). PCB = polychlorinated biphenyl; PBDE = Polybrominated diphenyl ethers.

intestinal biotransformation only has a small (and therefore difficult to determine) effect on the mass balance of the chemical in the fish. One of the limitations of the dietary bio-accumulation test is that it cannot account for biotransformation of the chemical in the gill compartment, which can affect the uptake clearance rate (k_{B1}) and hence the BCF (Camenzuli et al. 2019). However, neglecting gill metabolism ensures that conservative BCF estimates are derived from dietary test data using the ADME-B calculator described in the present study. If more definitive BCF values are required, aqueous tests can be performed as part of a tiered testing strategy.

The advantage of the 2-compartment fish toxicokinetic model over the one-compartment fish model is that it can account for the effect of the exposure pathway on the bio-accumulation of biotransforming chemicals. For example, it can explain why a chemical can exhibit a low BMF_L because

of susceptibility to biotransformation in the lumen but a high $\mathsf{BCF}_{5\%,t}$ if the chemical is not or is slowly transformed in the body of the fish. We therefore recommend that the 2compartment fish toxicokinetic modeling framework is applied in a weight-of-evidence approach for regulatory bioaccumulation assessment so that information on both BCF and BMF test endpoints can be integrated to provide an improved evaluation of a chemical's bioaccumulation potential, as illustrated in the present study. The advantage of the ADME-B calculator over currently used methods (e.g., OECD-305 technical guidance manual) is that it better informs interpretation and provides greater insights into the bioaccumulation process by using the results of dietary bioaccumulation tests more effectively. The effectiveness of the ADME-B calculator can be enhanced by making minor modifications to the dietary bioaccumulation test protocol, such as including the removal of the intestinal contents from fish and

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the use of nonbiotransforming reference chemicals in the test. The removal of the intestinal contents in a dietary bioaccumulation test is most important for chemicals that are biotransformed rapidly in the body of the fish. For such substances the mass of chemical in the fish body is low, and the mass of chemical in the intestinal contents following dietary ingestion of administered contaminated food can make up a substantial fraction of the mass in the fish sample during the uptake phase if the intestinal contents are not removed. The ratio of the chemical mass in the intestinal contents (M_{G}) relative to that in the body of the fish at the end of the uptake period (and beginning of the depuration phase; M_B) for the 238 tests investigated varied between 0.015 and 10⁵ (Supplemental Data, Figure S3). For 57 of the 238 substances, the chemical mass in the intestinal contents at the end of the uptake period was greater than that in the body of the fish (i.e., $M_G/M_B > 1$; Supplemental Data, Figure S3). For these substances, removal of the gut contents can have a substantial effect on the determination of the BCF and BMF. Also, for 57 of the 238 chemicals the chemical mass in the intestinal contents at the end of the uptake period was <10% of that in the body of the fish (i.e., $M_G/M_B < 0.1$; Supplemental Data, Figure S3). In these tests, the removal of intestinal content is not expected to have a significant effect on the determination of the BCF and BMF. The use of nonbiotransforming reference chemicals in the dietary bioaccumulation test can enhance the determination of biotransformation rates (Lo et al. 2015) and BCFs (Gobas and Lo 2016). Another modification that is useful when administering diets is the inclusion of chromic oxide in the diet. The increase in the concentration of chromic oxide in fecal matter over that in the food (i.e., the dietary assimilation efficiency term $[\epsilon_{f}]$ in the ADME-B calculator) allows empirical determination of the fecal egestion rate, which plays an important role in the excretion and biomagnification of chemicals in fish and other organisms. In future work, we plan to test the reliability of the 2-compartment fish model and the associated ADME-B calculator to determine BCFs of hydrophobic organic chemicals by comparing BCFs derived from dietary bioaccumulation tests to those derived from aqueous bioconcentration tests conducted for the same chemicals, fish species, and experimental conditions. We recommend that future updates of the OECD 305 test guideline incorporate the findings of the present and future studies for improving the conduct, analysis, and interpretation of laboratory bioaccumulation tests.

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4599.

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Data Availability Statement—The ADME-B model and the data used in the present study are included in data files 1 and 2

of the Supplemental Data. Data pertaining to this article are located at figshare (https://wiley.figshare.com/etc).

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