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Article

A Novel Multispecies Toxicokinetic Modeling Approach in Support of Chemical Risk Assessment

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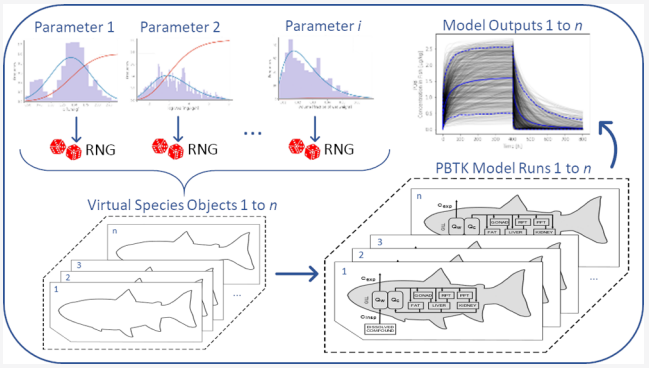
ABSTRACT: Standardized laboratory tests with a limited number of model species are a key component of chemical risk assessments. These surrogate species cannot represent the entire diversity of native species, but there are practical and ethical objections against testing chemicals in a large variety of species. In previous research, we have developed a multispecies toxicokinetic model to extrapolate chemical bioconcentration across species by combining single-species physiologically based toxicokinetic (PBTK) models. This "top-down" approach was limited, however, by the availability of fully parameterized single-species models. Here, we present a "bottom-up" multispecies PBTK model based on available data from 69 freshwater fishes found in Canada. Monte Carlo-like simulations were performed using statistical distributions of model parameters derived from these data to predict steady-state bioconcentration factors (BCFs) for a set of well-studied chemicals. The distributions of predicted BCFs for 1,4-dichlorobenzene and dichlorodiphenyltrichloroethane largely overlapped those of empirical data, although a tendency existed toward overestimation of measured values. When expressed as means, predicted BCFs for 26 of 34 chemicals (82%) deviated by less than 10-fold from measured data, indicating an accuracy similar to that of previously published single-species models. This new model potentially enables more environmentally relevant predictions of bioconcentration in support of chemical risk assessments.



KEYWORDS: *physiologically based toxicokinetic model, PBTK model, cross-species extrapolation, bioaccumulation, database development*

1. INTRODUCTION

Ecotoxicological studies conducted to characterize the toxicological effects and bioaccumulation potential of chemicals provide critical data needed to perform environmental risk assessments (ERA).[1] Fish are a vital element of such studies, as they are of ecological, commercial, recreational, and cultural importance and are regarded as sentinels of aquatic ecosystem quality.[2−4] Accordingly, many standard test guidelines that support chemical risk assessment have been developed using fish.[5−8]

Most ecotoxicological test protocols rely on a few surrogate species to predict toxicity and bioaccumulation in ecological receptors of concern.[9] Results from experiments conducted using these test protocols are used to derive metrics that may be used to inform ERA, such as half-maximal effect concentrations (EC_{50}) and bioconcentration factors (BCFs). The derived values are then used with safety factors and assumed to be protective of all species within a group, for example, all fishes.[1] Given the vast diversity of fishes, however, with over 34,000 described species globally,[10] risk assessments based on data from a few tested model species will inescapably lead to over- or underestimation of potential risk to other, untested species.[11,12] Time, cost, and ethical considerations make it impossible to address this issue by simply performing more whole-animal tests. Therefore, there is an increasing demand for alternative methods to predict chemical risks across the diversity of species.

These predictive methods can be applied to explain differences in the chemical concentration time-course at the target site (also referred to as toxicokinetics) or differences in the interaction of a chemical with molecular structures of the organism (also referred to as toxicodynamics).[13,14] Toxicokinetics and dynamics are mutually dependent, and a detailed understanding of chemical concentration at the target site is often considered a prerequisite to quantitatively study toxicodynamics. Thus, various toxicokinetic models, including physiologically based toxicokinetic (PBTK) models, have

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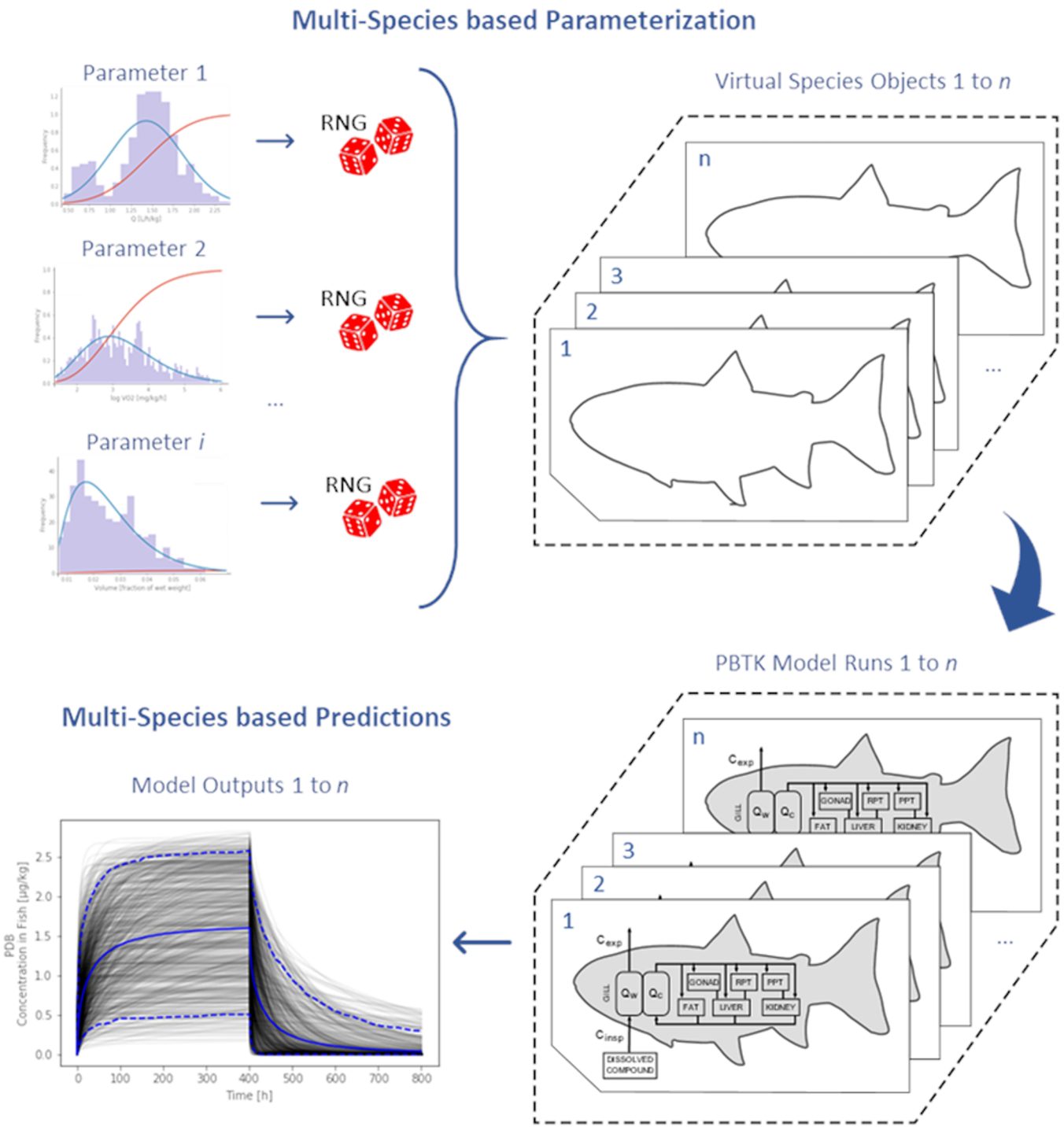

Figure 1. Conceptional representation of the multispecies PBTK model framework. Statistical distributions of model input parameters are resampled using random number generators (RNGs) to obtain a set of ecologically plausible "virtual species objects." Simulations performed using these "virtual species objects" are then complied to produce multispecies model outputs, including the distribution of predicted BCFs.

received much attention in recent years.[15−18] By predicting toxicokinetic processes mechanistically over time and specific to various tissues and organs, PBTK models facilitate extrapolation between levels of biological organization, exposure conditions, and among species.[19] The first PBTK models for fish were used to predict the uptake and accumulation of neutral organic chemicals in rainbow trout (*Oncorhynchus mykiss*).[20,21] Several other single-species PBTK models were since developed, encompassing species such as channel catfish (*Ictalurus punctatus*),[22] lake trout (*Salvelinus namaycush*),[23] fathead minnow (*Pimephales promelas*),[17] roach (*Rutilus rutilus*),[24] European eel (*Anguilla anguilla*),[25] and zebrafish (*Danio rerio*).[24,26] All these previous models require full sets of model parameters to describe the specific physiology of individual species. Some of these parameters, such as tissue volumes, are easily measured. However, information for other parameters, such as blood flow to different tissues, only exists for a limited number of species.[16] Consequently, the number of fully parameterized PBTK models has been restricted to the few species for which

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complete sets of model parameters were available, limiting the overall applicability of the approach and the scope of previous "top-down" multispecies models that are basically combinations of complete PBTK models.[24]

For some of the model parameters commonly used in PBTK models, scientific literature contains abundant information for numerous fish species. This information is often reported for purposes other than toxicokinetic modeling, for example, the gonadosomatic index which is a measurement of the gonad volume relative to the whole-body volume as a phenotypical endpoint. By using this information as a resource to provide additional information in support of PBTK modeling efforts, it may be possible to describe a broader range of toxicokinetic behaviors relevant to chemical risk assessments.

The goal of the present study was to improve current strategies for bioconcentration assessments by implementing a novel multispecies PBTK modeling approach that includes available inter- and intraspecies parameter values in a "bottom-up" model framework in which intra- and interspecies variability flows from the entirety of available parameter values. To achieve this, a database of existing literature values and newly measured physiological and anatomical data from Canadian freshwater fishes was established and used to derive underlying statistical distributions of model parameters. Parameters sampled from these distributions were used in Monte Carlo-like simulations to make probabilistic predictions of BCFs for a set of data-rich chemicals and compare the resulting distributions. Here, the BCF (L kg^{-1}) is defined as the steady-state chemical concentration in a fish resulting from a water-only exposure, divided by the aqueous chemical concentration. Predicted BCFs for a range of chemicals were then compared to measure values to evaluate model sensitivity to changes in selected parameters and provide a quantitative assessment of model accuracy, with the aim to establish the applicability domain of the model. Our new approach enables researchers and decision-makers to predict the expected distributions of BCF across a diversity of fishes, rather than individual values, and thereby facilitates more realistic assessments.

2. MATERIALS AND METHODS

2.1. Experimental Design and Model Conceptualization. A multispecies PBTK model for the prediction of whole-body and tissue-specific concentrations of neutral organic compounds in fish was developed based on a previously published model structure.[24] This structure was amended with modules to enable Monte Carlo-like simulations of chemical bioconcentration based on the inherent interspecies variability of model parameters (Figure 1). A structured literature search was conducted to gather available model parameter values for freshwater fishes in Canada (Supporting Information, Section 1). The resulting database (Supporting Information, Section 3; available for download with the model code) was then used to generate "virtual species objects," each of which represents a set of PBTK model parameters that are biologically plausible but do not necessarily correspond to the existing species of fishes. Here, instead of using single-species parameter sets, each parameter of a virtual species was drawn from a statistical distribution deduced from the database. Steps to ensure conservation of total tissue volume and total arterial blood flow to tissues were introduced in an initialization process to ensure the biological plausibility of these virtual species objects.

2.2. Literature Search. A literature search for model parameters was conducted in 2018 using the Web of Science database (Clarivate Analytics, Boston, USA). Search terms for each input parameter were specified (Supporting Information, Tables S1−S4), except for the model parameter oxygen consumption rate (VO_2; mg h^{-1} kg^{-1}), which was retrieved from the FishBase database (www.fishbase.org, accessed October 18, 2018). The search results were then exported and manually processed in three additional steps outlined in the Supporting Information (Section 1).

2.3. Model Structure and Implementation. The model was implemented in the open-source software Jupyter Notebooks (www.jupyter.org, version 5.5.0) and written in the programming language Python (Python Software Foundation, Delaware, USA, version 3.6.5). The model consists of seven compartments representing blood, adipose fat, gonads, kidney, liver, poorly perfused tissues (muscle, skin, and carcass), and richly perfused tissues (viscera and brain) (Supporting Information, Figure S4). A set of mass balance differential equations was used to describe the rate of change of the mass of chemical in each compartment. These equations were then simulated for the time course to calculate the chemical concentration in each compartment as a function of time. Uptake and elimination of water-borne compounds was assumed to occur *via* the gills. In the absence of reliable cross-species biotransformation data across the diversity of fishes included here, hepatic biotransformation was set to zero. The fish growth rate was also set to zero based on the assumption that empirical BCFs used to evaluate model performance were corrected for growth.[8] Chemical flux across the gills was described using the flow-limited model given by Erickson and McKim[27] (Supporting Information, eq S25). This model predicts that the rate of branchial exchange is limited by the fish's effective respiratory volume (Q_w; L h^{-1} kg^{-1}) or the product of its cardiac output (Q_C; L h^{-1} kg^{-1}) and an equilibrium blood:water partition coefficient (P_{bw}; dimensionless), whichever is smaller. For all but relatively hydrophilic chemicals, Q_w tends to be less than $Q_c \cdot P_{bw}$, resulting in a water flow limitation on uptake and elimination. Chemical flux between blood and tissues was assumed to be blood flow-limited, resulting in an equilibrium distribution between each tissue and venous blood exiting the tissue. A detailed description of the model structure and equations can be found in Supporting Information, Section 5. The model code is available as a Jupyter Notebook on GitHub (https://github.com/NikaGoldring/multi-species-PBTK).

Input parameter values collected in the structured literature search were stored in a MySQL (Oracle Corporation, Redwood Shores, USA, version 5.6) database using the administration tool phpMyAdmin (Free Software Foundation, Inc., Boston, USA, version 4.8.0). MySQL Connector/Python software (Oracle Corporation, Redwood Shores, USA, version 8.0) was used to access the database and enable reading, filtering, and writing into the database *via* Python code (Supporting Information, Section 3).

Each input parameter value follows a specific underlying mathematical probability distribution. If a sample from this distribution is representative, that is, enough data points are available, it is possible to derive the unknown distribution by fitting a probability density function (PDF) to the data using nonlinear regression methods. Using the Python module SciPy (www.scipy.org, version: 1.1.0), the input parameters of the multispecies PBTK model were fitted to normal, gamma, and

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uniform distributions in order to find the best fit to the data (Supporting Information, Section 4). The resulting PDF for each input parameter, based on values for all species represented in the database, then informed the parameterization of the model. To this end, instead of using single-species values, each input parameter was drawn from its statistical distribution and assigned to a virtual species object using a random number generator (RNG) of the Python module NumPy (www.numpy.org, version: 1.14.3).

Initial simulations were performed under the assumption that parameter values can be drawn independently from their respective statistical distributions. It is possible, however, that two or more input parameters are correlated with one another, invalidating this assumption. Therefore, a correlation analysis was performed to investigate the effect of correlation among model inputs. For each parameter, the arithmetic means were calculated for each species if more than one value was available. Pearson correlation coefficients and *p*-values among these mean values were then calculated using SciPy. For those parameters exhibiting significant correlations, ordinary least square regression analysis was performed in SciPy. These regressions were used in the initialization function of the model to account for the identified linear correlation of model parameters. The input parameter with the most reliable underlying distribution, that is, most available data to derive the distribution, was drawn from its PDF, and the correlated value was calculated from the respective linear regression function (Table 2). Model results derived with this alternative setting for model parameterization were then compared to those obtained by simple resampling of parameter distributions.

2.4. Model Verification and Sensitivity Analysis. The model functions were verified by comparing outputs of the new model implementation with previously published outputs of existing models. The primary difference between this and previous PBTK modeling efforts with fish relates to changes in the initialization step which are required to perform the Monte Carlo-like simulation. Tissue volumes and blood flows were checked after the value assignment in the resampling function to verify that biologically plausible virtual species objects were generated. More specifically, it was ensured that the sum of tissue fractions did not exceed 1 and that tissue volumes and blood flows summed up to the respective wet weight and cardiac output of the virtual species object (for further details, see Supporting Information, Tables S11, and eqs S14−S17).

A sensitivity analysis was performed on the three most important model parameters determined in previous PBTK modeling efforts:[17,20,24,28] whole-body lipid content, cardiac output, and oxygen consumption rate (Supporting Information, Section 7). Model runs with two representative chemicals with high and intermediate *n*-octanol−water partitioning coefficients (log K_{ow}; dimensionless), specifically dichlorodiphenyltrichloroethane (DDT, log K_{ow} = 6.91) and 1,4-dichlorobenzene (PDB, log K_{ow} = 3.44), were conducted. For each chemical, whole-body lipid content, cardiac output, and oxygen consumption rate were independently set to one of five different values (Supporting Information, Table S13). All parameters except that which was fixed for a particular model run were resampled in the manner described in the text to generate a distribution of modeled outputs. The impact of a change in each parameter on predicted BCFs was evaluated under steady-state conditions (*i.e.*, when the internal concentration changed by less or equal to a factor of 0.0005 over the time step [1 h] of the simulation). Body wet weight and exposure temperature were set to 10 g and 15 °C, respectively, for this exercise, recognizing that most fish used for bioconcentration experiments are in this size range.

2.5. Model Validation. The model validation was performed by comparing BCFs predicted by the multispecies model to published BCFs found in the Ecotoxicology Knowledgebase (ECOTOX, US EPA, accessed April 6, 2020). For these simulations, the model was run with repeated sampling of parameter values for 1000 iterations, that is, virtual species objects. The first validation step was a comparison of modeled and measured BCFs for PDB and DDT. The second validation step consisted of a correlation analysis of mean modeled and measured BCFs for 34 neutral organic chemicals (Supporting Information, Table S12). This list was based on a similar list of chemicals used for validation of a single-species PBTK model published by Stadnicka *et al.*[17] Modeled and measured BCFs were averaged and plotted on a log scale. Model accuracy was then quantified as the root mean squared error (RMSE).

3. RESULTS AND DISCUSSION

3.1. Literature Search and Database. The majority of the values in the constructed physiological database were obtained from peer-reviewed literature studies (Table 1). For Q_C, a total number of 343 values were found for freshwater fishes occurring in Canada. Lipid content (alpha$_i$; fraction of tissue wet weight) for all tissues represented by the PBTK model resulted in a total of 366 values. All 1,017 VO_2 values were obtained from the FishBase database,[10] and no additional literature search was conducted. The data set for tissue volumes (V_i; L, calculated from fraction of whole-body wet weight) comprised 1016 values across all tissues. Of the 73 total blood flow (Q_i; L h^{-1} kg^{-1}) values, 39 were taken from the literature, mostly from previously published PBTK modeling studies. Additional estimates of Q_c, alpha$_i$, Q_i, and V_i were obtained from unpublished studies conducted at the Toxicology Centre (University of Saskatchewan, Canada). These studies are described in Supporting Information (Section 2). Detailed results of the literature search for each

Table 1. Total Number of Values for the Model Input Parameters (for Reference, see Supporting Information, Section 2): Cardiac Output (Q_C; L h^{-1} kg^{-1}), Oxygen Consumption Rate (VO_2; mg h^{-1} kg^{-1}), Lipid Content of the Compartment (Alpha$_i$; Fraction of Tissue Wet Weight), Blood Flow to Compartment (Q_i; L h^{-1} kg^{-1}), and Volume of Compartment (V_i; L, Calculated From Fraction of Whole-Body Wet Weight), Contained in the Physiological Database

source	Q_C	VO_2	alpha$_i$	Q_i	V_i
literature	339	1017	292	39	840
unpublished data	4		74	34	176
total number of values in the database	343	1017	366	73	1016

[a]Literature values were obtained from a structured review of peer-reviewed literature. Unpublished data were obtained from data sets generated at the Toxicology Centre (University of Saskatchewan, Canada). All values from both sources are contained in an Excel file, which is provided in Supporting Information for this article.

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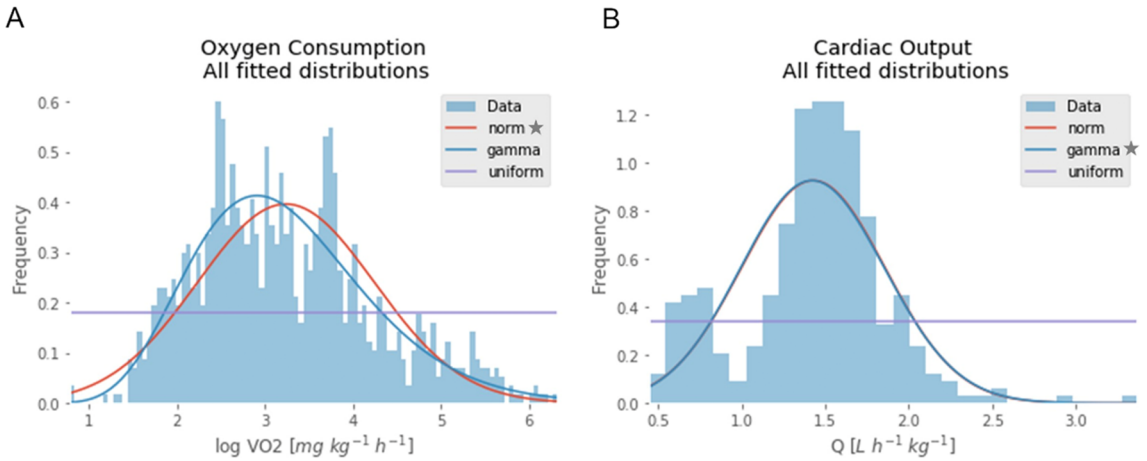

Figure 2. Histograms of (A) oxygen consumption rate (VO_2; mg h^{-1} kg^{-1}) and (B) cardiac output (Q_C; L h^{-1} kg^{-1}) with fitted statistical PDFs. The red line represents the fit to a normal distribution, the blue line is the fit to a gamma distribution, and the purple line is the fit to a uniform distribution. The best-fit distributions are highlighted with an asterisk: that for VO_2 is a normal distribution, while that for Q_c is a gamma distribution.

input parameter are listed in Supporting Information, Tables S1−S4.

A total of 225 freshwater fish species that are members of 32 families have been described in Canada.[10] The families with the highest number of species (in parentheses) within this list are Cyprinidae (57), Salmonidae (37), Catostomidae (18), Percidae (16), Centrarchidae (13), Petromyzontidae (11), Ictaluridae (10), and Cottidae (10). The final physiological database contains 2815 parameter values from 69 different fish species (31% of total) representing 23 families (72% of total).

The purpose of collecting data from multiple species was to derive an underlying statistical distribution for each physiological model parameter. These distributions were ultimately used to represent the inter- and intraspecies variability of model input parameters and served as the basis of multispecies PBTK modeling. Several ecologically important families are not represented in the database at all (*e.g.*, Osmeridae), while others are underrepresented (*e.g.*, Petromyzontidae). Conversely, several families are overrepresented (*e.g.*, Salmonidae), reflecting the use of individual species (*e.g.*, rainbow trout) as laboratory test models. This is particularly apparent for more complex to determine cardiovascular parameters, such as tissue blood flow distribution. There should be a dedicated effort to fill in these data gaps going forward. In such cases, this overrepresentation has the potential to bias modeled findings. To date, however, published PBTK models have been parameterized for only a few fish species.[17,24] Despite its limitations, therefore, the multispecies PBTK model represents a significant increase in the extent of species coverage.

Statistical distributions for Q_C and VO_2, which are of high importance to model predictions,[28] were based on a substantial amount of data. A large amount of information pertaining to tissue volumes was also available. In contrast, the amount of information for blood flows to individual tissues was highly limited (5 total studies; Table S2). For the gonads and adipose fat, this information was insufficient to fit statistical distributions to measured values. These parameters were therefore represented by truncated uniform distributions.

In addition to parameter values obtained through the literature search, the database was augmented with experimentally determined values from this study, as well as previously unpublished values for cardiac output, blood flows, tissue volumes, and lipid contents from studies conducted with white sturgeon (*Acipenser transmontanus*), northern pike (*Esox lucius*), white sucker (*Catostomus commersonii*), lake whitefish (*Coregonus clupeaformis*), and walleye (*Sander vitreus*) at the Toxicology Centre, University of Saskatchewan, Canada (L. Weber, N. Hogan and C. Grimard, unpublished data; please refer to the Supporting Information for experimental details).

3.2. Parameter Distributions and Correlation Analysis. The distributions of all model input parameters were developed from the collected multispecies parameter values. Descriptive parameters for the best fit PDFs were then estimated (Supporting Information, Section 4) and served as inputs for parameterization of virtual species objects. Uniform and gamma distributions were truncated between the minimum and maximum values found in the literature. Normal distributions were only truncated at the origin.

Cardiac output data were best fit to a gamma distribution, while data for VO_2 were best described by a normal distribution (Figure 2). In a gamma distribution, the probability density is skewed toward either side of the mean value, in this case toward lower values. Across species, most model parameters followed a gamma distribution, where the most frequent value was smaller than the mean value. Thus, the highest probability of a parameter value occurring in freshwater fishes is typically not well represented by the mean. This result supports the use of distributions rather than mean values. While the mode of each derived parameter distribution represents the value with the highest probability of occurring in nature, these distributions also include values at both extreme ends. Including these less frequent but possible values supports more realistic PBTK model predictions.[15,29]

The correlation analysis revealed 17 significant parameter correlations (Pearson correlation coefficient > |0.70|, p-value < 0.05) within the database. In seven of these cases, input parameters were correlated with more than one other input parameter. The 10 variable pairs that showed the most reliable correlations (number of available values that formed the bases for the correlation analysis, highest correlation coefficient) were used to calculate linear regression functions. During a subsequent analysis of model performance, three of these regression functions caused disproportionate and unrealistic

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values in the generation of virtual species objects and were therefore excluded. For the remaining seven regressions, the input parameter with the most reliable underlying distribution, that is, most available data to derive the distribution, was drawn from its PDF, and the correlated value was calculated from the respective linear regression function (Table 2).

Table 2. Regression Summary for Correlated Model Input Parameters

parameter (x)	parameter (y)	slope	SE slope	R^2	n
$alpha_{blood}$	$alpha_{fat}$	61.9868	6.466	0.968	4
$alpha_{fat}$	V_{kidney}	0.0084	0.001	0.931	4
$alpha_{fat}$	$alpha_{kidney}$	0.0926	0.04	0.73	3
$alpha_{fat}$	Q_{liver}	0.0292	0.003	0.981	3
$alpha_{blood}$	V_{liver}	1.175	0.058	0.993	4
V_{liver}	$alpha_{liver}$	4.040	0.478	0.922	7
Q_{rpt}	Q_{ppt}	1.4762	1.077	0.32	5

[a]Correlated parameters are listed along with the slope of each fitted regression function, the standard error of the slope (SE), the coefficient of determination (R^2), and the number of values from which the regression was developed (n). Regressions were forced to intercept the origin. Abbreviations: $alpha_i$ = lipid content of a compartment (i), Vi = volume of a compartment (i), Q_i = blood flow to a compartment (i), where i=blood, fat, kidney, or liver; rpt=richly perfused tissue, ppt=poorly perfused tissue.

RMSE and R^2 values calculated by regressing predicted BCFs against measured values for 34 chemicals (see 3.3.2 Validation) did not differ substantially for the simple model parameterization (*i.e.*, with values drawn independently from each PDF; RMSE = 0.766, R^2 = 0.641) and the correlation-based parameterization (RMSE = 0.755; R^2 = 0.639). Thus, including correlations among model parameters during the parameterization of virtual species objects had little effect on model accuracy or the strength of the linear correlation between modeled and measured BCFs. However, this result reflects the current state of the physiological database and could change when more data become available in the future.

3.3. Multispecies PBTK Model. *3.3.1. Sensitivity Analysis.* The sensitivity analysis showed that predicted BCFs tend to increase in proportion to whole-body lipid content, although the BCF predicted for any given simulation may be substantially different from the mode of the distribution because of variability in other model parameters (Figure S5). This finding is similar to that reported in other PBTK modeling efforts[16] and reflects the fact that bioconcentration of neutral organic compounds is largely driven by passive partitioning of chemicals out of the water column and into fish tissue lipid.[30] In accordance with previous findings,[28] BCF predictions were unaffected by variation of cardiac output for both chemicals.

The VO_2 rates obtained from the FishBase database included data that resulted in weight-normalized values ranging from 0.76 to 6.33 log mg h^{-1} kg^{-1}. Modeling this range of VO_2 values highlighted performance limitations of the model. When the VO_2 is very low, the simulation time required to achieve steady state for a high log K_{ow} chemical (*e.g.*, DDT) increases dramatically. High levels of whole-body lipid content increase this simulation time even more. Based on these results, we suggest that simulations of VO_2 rates <1.0 log mg h^{-1} kg^{-1} should not be performed for chemicals with log K_{ow}s > 6.5. Based on the FishBase database, however, such low VO_2 rates represent the lower bound of what is physiologically possible, and this limitation does not significantly affect the applicability domain of the model. Even for PBD, however, most of the simulations obtained at the two lowest levels of VO_2 failed to arrive at steady state within the imposed limits of computation time (max. of 20,000 h of simulated exposure time, which took just over 10 h to run; Figure S5). Increasing the computation time for each iteration, that is, virtual species objects, would have resulted in a higher percentage of simulations achieving steady state, but this was infeasible, given the need to run the model repeatedly to generate a distribution of BCFs. Parallel computing might be a solution to this problem; however, we prioritized the ability of the model to run on a personal computer.

Reduced respiration results in less water and, by extension, less chemical passing over the gills in a given time period. This will reduce the water flow limitation on chemical flux across the gills described earlier. Low VO_2 values may be associated with metabolic rate depression, occurring at low temperatures or when fasting.[31] Other species have naturally low VO_2 rates as an adaptation to their habitat and/or lifestyle.[32] To our knowledge, measurements of chemical uptake in fish that possess very low VO_2 values have not been performed. Thus, it remains unclear how rapidly high log K_{ow} chemicals are taken up under these circumstances. Potentially, dermal uptake could assume a more prominent role in fish with low metabolic activity, for example, European eels.[25] To describe this uptake, it would be necessary to develop a PBTK model that represents the skin as a discrete compartment.[33]

3.3.2. Validation. In the first validation step with single compounds, a comparison of modeled and measured multispecies BCF values for PDB showed a partial overlap of the two distributions (Figure 3). The modeled values ranged from 36 to 565, whereas the measured data contained smaller values, for example, BCF = 0.25 for western mosquitofish (*Gambusia affinis*), and higher values, for example, BCF = 1400 for rainbow trout (*Oncorhynchus mykiss*). The modeled BCFs for DDT ranged from 3402 to 185,284. Again, modeled and measured BCF distributions were largely overlapping, with some smaller values and one extreme outlier (largemouth bass, *Micropterus salmoides*: BCF = 317,000) in the measured data set.

Observed differences in the quality of agreement between measured and modeled BCFs for PDB and DDT may be due in part to the varying availability of measured BCFs for different chemicals and species, depending on their presence in the ECOTOX database. While the model predictions were based on all species within the database, the literature values were limited to the available studies. For PDB, literature values for BCF were mainly recorded for rainbow trout (56 out of 60 total values).

Because the multispecies model incorporates physiological variability within and among many fish species, a broad range of predicted BCFs was expected. The measured data were similarly broad, suggesting that physiological variation may explain much of the variability in reported BCFs within and among species. Despite similarities in the breadth of measured and modeled distributions, their modes were substantially different (Figure 3). There are at least two possible explanations for these findings. First, many of the physiological and anatomical parameters from which PDFs were derived were obtained from sub-adult and adult animals and/or relatively large fish species. In contrast, most reported BCFs

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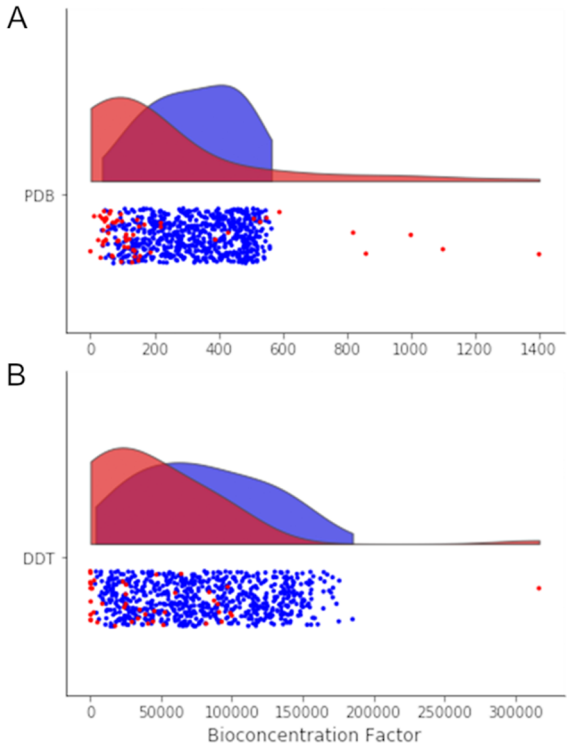

Figure 3. Comparison of modeled and measured multispecies BCF values for (A) PDB and (B) DDT. For each chemical, two data sets (modeled: blue and measured: red) were plotted as a scatterplot and half violin plot. Scatterplot results were randomly displaced along the *Y* axis to permit discrimination of values. Half violin plots were created using the Python package Seaborn.

have been obtained using small fish species (*e.g.*, sheepshead minnows) or early life stages of larger species,[34,35] both of which generally contain low levels of whole-body lipid. Second, not all values in the ECOTOX database were obtained using standardized *in vivo* testing procedures (*e.g.*, OECD TG 305[8]). It is possible, therefore, that some low values reflect failure of fish to achieve steady state (for steady-state BCF determinations), use of an inappropriately high test chemical concentration (leading to incomplete dissolution), or some other methodological issue.[36] PBD and DDT are both predicted to undergo little if any biotransformation in fish (Supporting Information Table S12). Biotransformation is unlikely, therefore, to be responsible for observed differences in measured and modeled BCFs.

In the second validation step, mean BCF values calculated from empirical data were regressed against mean values of modeled data on a logarithmic scale for 34 chemicals (Figure 4). The RMSE for this relationship was 0.76 log units. Model predictions for most chemicals (82%) were within a factor of 10 of measured data. Only six chemicals were outside this range: 1,2-difluorobenzene, 1,2-dibromobenzene, hexachloro-1,3-butadiene, fenvalerate, permethrin, and 2,3,7,8-tetrachlorodibenzofuran. For each of these chemicals, the model tended to overpredict measured values.

In a previous model validation exercise performed using single-species PBTK models,[17] 95% (rainbow trout) and 88% (fathead minnow) of the predicted internal concentrations were less than 10-fold different from measured concentrations. Similar, modeling studies with zebrafish showed that 88%[26] and 84%[24] of predicted internal concentrations were within 5-fold of measured data. Thus, the accuracy of the multispecies model appears to be comparable or only marginally lower compared to that of previous single-species PBTK models, while the species applicability domain is significantly higher.

In the absence of reliable cross-species biotransformation data, the biotransformation rate was set to zero. Although this approach is consistent with previous PBTK modeling efforts, it is important to note the potential impacts of this assumption on model predictions. For chemicals that undergo significant biotransformation in fish, failure to account for this activity can result in underestimation of chemical elimination and could lead to overestimation of true BCFs, depending on the relative rates of other elimination pathways (*e.g.*, branchial efflux). As such, failure to account for this activity could have contributed to the observed trend toward overestimation of empirical BCF

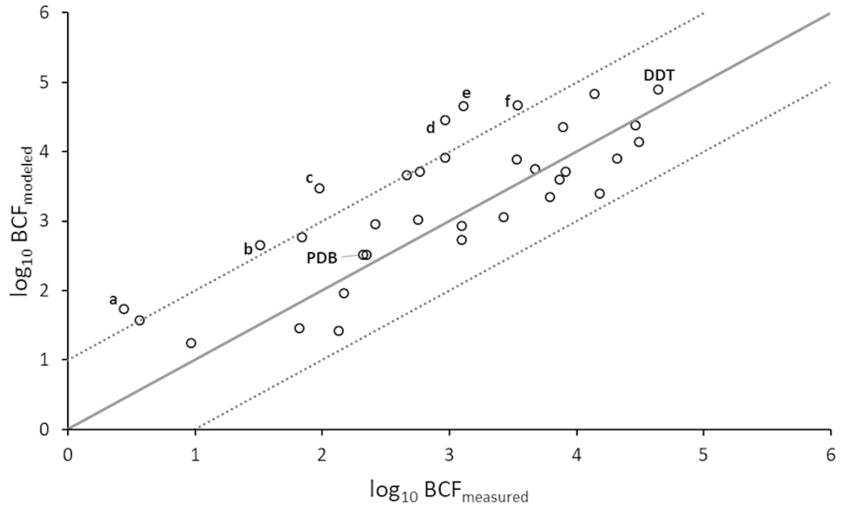

Figure 4. Relationship between modeled and measured multispecies BCF values for various chemicals. Mean values of multispecies BCF from modeled predictions and measured literature data for 34 chemicals (Supporting Information, Table S12) were plotted on a logarithmic scale. The solid line represents the line of equality; dashed lines represent a 10-fold deviation from equality. Letters and abbreviations: a = 1,2-difluorobenzene, b = 1,2-dibromobenzene, c = hexachloro-1,3-butadiene, d = fenvalerate, e = permethrin, f = 2,3,7,8-tetrachlorodibenzofuran, PDB = 1,4-dichlorobenzene, DDT = dichloro-diphenyltrichloroethane.

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values. To improve model accuracy, biotransformation of chemicals should be included.[15] One way to achieve this goal is to measure biotransformation *in vitro* and then use various scaling factors to extrapolate this information to the intact tissue where this activity occurs.[37] A more generic approach to account for biotransformation was demonstrated by Arnot, *et al.*,[38] who developed a Quantitative Structure−Activity Relationship (QSAR) to predict apparent whole-body rates of biotransformation (k_B, given as k_M by the authors; d^{-1}) from the chemical structure. This QSAR is provided as part of the BCFBAF module in US EPA's EPI Suite software package.

To assess the possible impact of biotransformation on the model validation exercise, k_B values were obtained for all 34 chemicals in the validation data set (Supporting Information Table S12). This information was then used to evaluate measured and modeled BCFs shown in Figure 4. Fifteen chemicals in the data set have estimated log K_{ow} values > 5. For nine of these, there was good agreement between observed and modeled BCFs (well below a 10-fold difference; data points not labeled with a letter). All nine of these chemicals have a predicted k_B < 0.1 d^{-1}. Of the six remaining chemicals, four (pentachlorophenol, fenvalerate [d], permethrin [e], and 2,3,7,8-tetrachlorodibenzofuran [f]; letters in brackets refer to the annotation in Figure 4) exhibited a large difference between the measured and modeled BCFs (approaching 10-fold or greater). Each of these has a predicted k_B value > 0.1 d^{-1}. Thus, the existing data for several high log K_{ow} chemicals suggest that measured BCFs lower than those predicted by the current model may be due to biotransformation. The rate of biotransformation required to impact chemical bioaccumulation increases with decreasing log K_{ow} due to the dominant influence of chemical flux across the gills. Indeed, modeled simulations indicate that even very high rates of biotransformation are unlikely to have a discernible impact on predicted BCFs for chemicals with log K_{ow} values between 1 and 3.[39] It is unlikely, therefore, that moderate rates of biotransformation (k_B < 1 d^{-1}) predicted for 1,2-difuorobenzene, 1,4-difluorobenzene, 1,2-dichlorobenzene, and PDB (a−d in Figure 4; log K_{ow} values from 2.13 to 3.44) can explain large differences between modeled and measure BCFs.

Because the fish biotransformation QSAR provides biotransformation rates expressed on a whole-body basis, this information cannot be input directly to the current PBTK model. It would be possible, however, to use these predictions to estimate corresponding rates of hepatic intrinsic clearance (CL_{int}; L h^{-1} kg^{-1}) by running the existing *in vitro−in vivo* extrapolation models[34] in reverse. A CL_{int} value determined in this manner could be input directly to a mass−balance equation for liver tissue as a first-order clearance constant.[40]

3.4. Model Use for Ecological Risk Assessment. Multiple reviews of PBTK modeling have emphasized the lack of data needed to derive model parameters,[16,41] which has limited these efforts to a few selected surrogate species. A generic PBTK modeling approach for fish provided by Grech *et al.*[42] addressed the issue of intraspecies variability in model parameters as well as the life-cycle and temperature dependencies of these inputs. Predictions of this model were generally accurate: for six out of the nine chemicals investigated in this study, 50% of predictions were within a factor of three of measured values. For three more lipophilic chemicals, 75% of predictions were within a factor of three of measured values. However, this approach employed uniform distributions to describe parameter variability, and diversity was limited to four species: rainbow trout, zebrafish, fathead minnow, and three-spined stickleback. In contrast, the presented multispecies PBTK model employs PDFs, which are based on a statistical evaluation of the existing parameter data, to represent known variation in model inputs. Not surprisingly, this model yields a relatively broad range of predicted BCFs for fish. In doing so, however, the model provides a mechanistic explanation for much of the observed variability in empirical BCFs, which is comparably large.

The accuracy of the multispecies PBTK model, as characterized by its ability to predict measured BCFs for fish, is like that of previously published single-species models. Unlike previous models, however, the multispecies model could be used to tailor bioaccumulation assessment efforts to conditions of special interest, including specific taxonomic groups or geographic locations. This approach could be particularly useful in assessing the risk of site-specific chemical exposure.[11] For this application, chemical fate models[43] could be used to determine likely sites of potential exposure. The database of physiological values could then be filtered for species most likely to occur in these types of environments. Thus, the multispecies approach presented in this study supports the inclusion of nonmodel species with potentially greater relevance to local ecosystems.[44] Apart from the prediction of steady-state BCFs, the presented model might also be very valuable for researchers working under non-steady-state conditions, for example, when conducting toxicity tests. In this context, the model could be used to explore how species differences in anatomy and physiology, combined with study-specific parameters such as fish size and temperature, could impact the time to steady state. For example, a small fish at warm temperatures containing little whole-body lipid might be shown to achieve steady state in a few hours or days, while for a large fish at cold temperatures containing a large amount of whole-body lipid, exposure to the same chemical may not result in the steady state for weeks or months.

The multispecies PBTK model also supports other concepts central to ERA efforts such as the adverse outcome pathway (AOP) framework. While most AOPs are initially developed based on qualitative evidence, researchers developing quantitative AOPs need to account for toxicokinetics when describing key event relationships.[45] In the future, the generated database of physiological information of freshwater fishes occurring in Canada should be expanded to other geographic regions. With the collection of more physiological data, it may also become possible to simulate chemical accumulation occurring at different life stages, facilitating the assessment of risk throughout a fish's lifetime. Additional refinements to the model can be considered, including chemical uptake from the diet (yielding a steady-state bioaccumulation factor, or BAF), chemical elimination to feces, hepatic biotransformation, and diffusion limitations on chemical flux across the gills. With respect to dietary uptake and fecal egestion, however, there would be a need to specify model parameters (*e.g.*, feeding rate and dietary composition) that tend to be study-specific, while at the same time accounting for species in differences gastrointestinal anatomy and physiology (*e.g.*, presence/absence of a stomach, gut transit time) that impact dietary uptake efficiency. Similarly, in the case of gill diffusion limitations, it would be necessary to account for species differences in gill morphometry (*e.g.*, number and dimensions of secondary gill lamellae). Presently, therefore, use of this model as a screening-level tool for

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bioaccumulation assessment argues for the current, simpler description.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acs.est.1c02055.

Additional information on the conducted literature search, experimental generation of parameters, database construction, parameter distributions, model inputs, parameters and equations of the PBTK model, and sensitivity analysis (PDF)

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Notes

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