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Can poly-parameter linear-free energy relationships (pp-LFERs) improve modelling bioaccumulation in fish?

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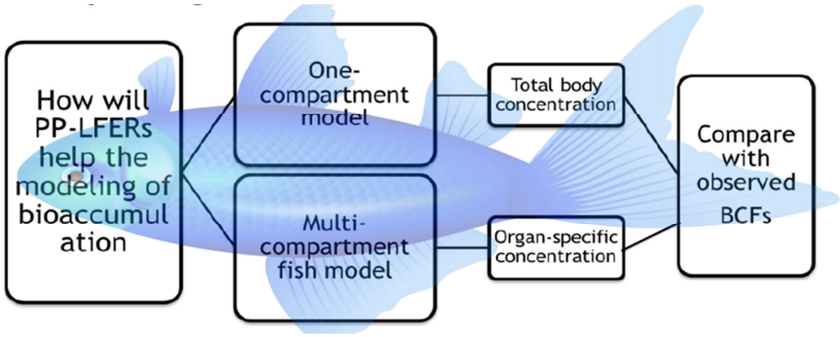
Abstract

A wide range of studies have characterized different types of biosorbent, with regards to their interactions with chemicals. This has resulted in the development of poly-parameter linear free energy relationships (pp-LFER) for the estimation of partitioning of neutral organic compounds to biological phases (e.g., storage lipids, phospholipids and serum albumins). The aims of this study were to explore and evaluate the influence of implementing pp-LFERs both into a one-compartment fish model and a multi-compartment physiologically based toxicokinetic (PBTK) fish model and the associated implications for chemical risk assessment. For this purpose, fish was used as reference biota, due to their important role in aquatic food chains and dietary exposure to humans. The bioconcentration factor (BCF) was utilized as the evaluation metric. Overall, our results indicated that models incorporating pp-LFERs (R^2=0.75) slightly outperformed the single parameter (sp) LFERs approach in the one-compartmental fish model (R^2=0.72). A pronounced enhancement was achieved for compounds with log K_{OW} between 4 and 5 with increased R^2 from 0.52 to 0.71. The little improvement was caused by the overestimation of lipid contribution and underestimation of protein contribution by sp-approach, which cancel each other out. Meanwhile, a greater improvement was observed for multi-compartmental PBTK models with consideration of metabolism, making all predictions fall within a factor of 10 compared with measured data. For screening purposes, the K_{OW}-based (sp-LFERs) approach should be sufficient to quantify the main partitioning characteristics. Further developments are required for the consideration of ionization and more accurate quantification of biotransformation in biota.

Graphical abstract



Highlights

- Incorporating pp-LFERs approach into fish model resulted in greater improvement in the PBTK fish model than that in one-compartment fish model.
- sp-LFERs approach overestimated the lipid contribution and underestimated protein contribution to the total partition between fish and water, which cancelled out each other.
- Large uncertainties are caused by quantification of biotransformation.
- Uncertainties in screening assessments are larger than differences between the pp-LFER and sp-LFER models.

Keywords

Partition coefficients, pp- LFER, bioaccumulation, biotransformation

1 Introduction

Bioaccumulation in aquatic species is a critical endpoint in the regulatory assessment required by authorities, such as the European Chemical Agency (ECHA) and the United States Environmental Protection Agency (Gobas et al., 2009). One widely used assessment metric is the bioconcentration factor (BCF), which assesses the bioaccumulative potential of a chemical to biota through constant aqueous exposure under well-controlled laboratory conditions (Mackay et al., 2013). One principle of the Registration, Evaluation, Authorization and Restriction of Chemicals (REACH) regulation is that testing of chemicals on animals should be a last choice (Van der Jagt et al., 2004; Parliament and Union, 2006; Laue et al., 2014). Much effort has been devoted to developing predictive models to estimate BCFs, where no *in vivo* data are available. Typically, chemical is preliminary screened and assessed based on physicochemical properties, like octanol-water partitioning coefficient (K_{OW}). It's widely used as an indicator of hydrophobicity and thus the partitioning of a chemical from water to lipids and other organic phases (e.g., protein) (Debruyn and Gobas, 2007).

Equilibrium partition coefficients for organic chemicals from environmental compartments to a tissue/organism are normally estimated by the total lipid content in combination with the K_{OW} (Mackay, 2001). So chemical concentrations in an organism/tissue are often normalized to the total lipid content, assuming that all lipids have identical sorption properties and the non-lipid fraction has a negligible sorption capacity (Endo et al., 2013). However, the suitability of this simplified approach has been questioned (Hermens et al., 2013; Endo and Goss, 2014a). It has been reported that the sorption capacity varies among different types of lipids (e.g., storage and membrane lipids) (Endo et al., 2011). Furthermore, the non-lipid components (e.g., proteins and serum) could also be a significant accumulation phase for organic compounds, especially for the H-bond donor compounds (Endo et al., 2012). More importantly, correlations with K_{OW} are expected to be valid only for restricted chemical domains (Hermens et al., 2013). As attention on contaminants in the environment with more complex structures, like hormones,

pharmaceuticals and surfactants grows, the task to go beyond K_{OW} and explore more refined approaches to mechanistically modelling bioaccumulation is urgently needed.

Much effort has been made for the exploration and development of poly-parameter linear free energy relationships (pp-LFER), which could account for the contribution of different specific and non-specific inter-molecular interactions (Abraham et al., 1994; Abraham et al., 2015). Undeman et al. (2011) estimated the total sorption capacity of the human body directly using the pp-LFERs calibrated for composite tissues/organs, showing limited benefit over the traditional sp-LFERs approach (Undeman et al., 2011). This could be attributed to the unavailability of different pp-LFERs equations in individual biological phase (e.g., neutral lipid, phospholipid and protein) at that time. A single pp-LFER for partitioning to composite tissue/organ (e.g., blood, liver and brain) they used, which may only work well for the calibrated chemicals. If a very diverse set of study chemicals out of the calibration domain was applied to pp-LFERs of composite tissue/organ, large errors may occur. For instance, models calibrated by data set from very polar compounds, which predominately partition into the aqueous phase, may not work well in a biological phase calibrated by compounds mainly partitioning to lipid (Geisler et al., 2011). Thus, if different chemicals have different preferred phases within a composite material (e.g., fat tissue is a composite material mainly made up by water, neutral lipid, phospholipid and protein), a pp-LFERs need to be established for individual biological phase instead of the whole bulk compartment. However, the individual pp-LFER for a separate biological phase was not available previously.

Recently, a number of studies have characterized different types of lipids, with regards to their chemical interactions (Endo et al., 2011; Geisler et al., 2012). Meanwhile, pp-LFERs for estimation of partitioning of neutral organic compounds to biological phases have also been calibrated, e.g., storage lipids (Geisler et al., 2012), phospholipids (Endo et al., 2011), serum albumins (Endo and Goss, 2011a) and muscle protein (Endo et al., 2012). In addition, preliminary evaluation has been carried out to directly compare partition coefficients to tissues calculated by pp-LFER models and K_{OW}-based models, indicating an order-of-magnitude

approximation (Endo et al., 2013). Furthermore, another initial evaluation was conducted to examine the effect of pp-LFERs approaches on pharmacokinetic (PBPK) models (Salmina et al., 2016). But they did not incorporate metabolic transformation, which would be a critical issue for rapidly metabolized compounds. Consequently, a comprehensive study to explore their benefit for the prediction of bioaccumulation potential and interpretation of biomonitoring results is desirable.

The main objective of this study was to explore the influence of implementing pp-LFERs on the estimation of bioconcentration factors in different types of fish model. Fish were used as a reference biota due to their important role in human daily diet and the fact that they act as an essential biosorbent for organic chemicals. Additionally, enough data availability exists for model evaluation compared to other species. In this study, two types of fish model: a one-compartment fish model (Arnot and Gobas, 2004) and a multi-compartment physiologically based toxicokinetic (PBTK) model (Nichols et al., 1990) were set up with incorporated sp or pp-approaches. Differences between model outputs were evaluated, and predicted BCFs were used to compare with measured BCFs. The implications for research and regulatory practices with regard to chemical risk assessment are also discussed.

2 Methods

2.1 General approach

Two types of mechanistic fish models were selected in this study, the one-compartment fish model (Arnot and Gobas, 2004), which assumes the chemical concentration is the same throughout the organism, and the multi-compartment PBTK model (Nichols et al., 1990), which considers chemical concentration may differ between various organs and tissues. Their selection in the chemical risk assessment depends on the question being addressed and the ease of data collection under different scenarios (Landrum et al., 1992). The one-compartment model is suitable for preliminary risk assessment with simple inputs, while the multi-compartment model is preferred in higher-tier assessments to quantify organ-specific concentration. These two

representative models were implemented under both traditional sp-LFER (traditional K_{OW} - driven) and newly-developed pp-LFERs to explore their performance in term of BCF prediction.

To eliminate difference caused by input parameters, the only distinction between these two approaches of pp-LFERs and sp-LFERs models, is the way of calculating partition coefficients to tissues/organs. All other equations and parameterizations were not modified in these two modelling approaches. Firstly, both models were run using a set of chemicals with the same measured descriptors. Thus, the potential errors in the measurement of chemical descriptors will be eliminated by using the same chemical descriptors for both approaches. Then the compiled dataset with measured BCFs was used as the endpoint to compare with the model predictions. Only chemicals present in neutral form in natural water were considered in this evaluation process.

2.2 General fish model

2.2.1 One-compartment model

For the one-compartment model, fish was described as a well-mixed compartment and thus the target chemical was assumed to be homogeneous in the whole fish body. In this type model, K_{OW} was regarded as a surrogate of lipid to quantify partition process. Chemical concentration in fish (C_b, kg kg^{-1}) could be modelled using following first-order equation:

$$dC_b/dt = k_u C_w - k_e C_b \quad (1)$$

where k_u is the uptake rate constant via gill ventilation (L kg^{-1} d^{-1}), C_w is the truly dissolved chemical concentration in the water column (kg L^{-1}). k_e is the total elimination rate constant (d^{-1}), including respiratory exchange back to water (k_w), fecal egestion (k_f), biotransformation (k_m) and growth dilution (k_g). These four elimination rate constants were calculated following the same treatment of Arnot fish model (Arnot and Gobas, 2004). In this study, the organism was assumed to be fed completely “clean” food during the entire exposure period. Though the dietary uptake could be omitted from a BCF model, fecal egestion should be included to

account for the redistribution of the target compound between the organism and its gut (Armitage et al., 2013). The detailed parameterization is contained in Table S1. The steady-state condition was assumed. So BCFs were used to compare the difference between predicted and observed values. Under steady state (dC_b/dt=0), chemical concentrations in the organism and BCF could be calculated by:

$C_b=k_uC_w/k_e$ (2) and $BCF=C_b/C_w=k_u/k_e$ (3)

In all calculations, the diet was assumed to be 1.5% total lipid (1.2% neutral lipid, 0.3% phospholipid for pp-LFER calculation), 15% non-lipid organic matter (NLOM) and 83.5% water (Armitage et al., 2013). Mass-based tissue fractions were converted to volume-based tissue fractions assuming densities of 0.9, 0.9, 1.0 and 1.0 kg L^{-1} for neutral lipid, phospholipid, NLOM and water, respectively.

2.2.2 Multi-compartment PBTK model

Chemical accumulation by fish can also be simulated by the physiologically based toxicokinetic (PBTK) fish model developed by Nichols and co-workers, which treats whole fish with individual compartments, like adipose, liver and kidney separately (Nichols et al., 1990). It is particularly useful to predict chemical concentration when a specific tissue/organ is the dominant site of action. The rainbow trout was used as a reference fish, due to being used as a standard fish in many studies and has relatively abundant data. Detailed parameterizations were presented in Table S5 but are also presented elsewhere (Nichols et al., 2007). The amount of the chemical in each compartment is calculated using the following relationship:

$$dA_i/dt=Q_i\times(C_{art}-C_{vi}) \quad (4)$$

where A_i is the chemical amount in compartment i (µg), Q_i is the arterial blood flow to compartment i (L h^{-1}), C_{art} is the chemical concentration in arterial blood (µg L^{-1}), C_{vi} is the chemical concentration in venous blood after compartment i (µg L^{-1}).

$$C_b=\Sigma A_i/BW \quad (5)$$

where C_b is the average chemical concentration in the whole fish body (μg kg^{-1}), ΣA_i is the chemical amount in all compartments (μg), BW is the body weight of fish (kg).

In order to facilitate the comparison, the PBTK model employed several empirical relationships provided by (Arnot and Gobas, 2004), including the calculation of C_{wd} (dissolved chemical concentration in water), C_d (chemical concentration C_d=0 in diet, assuming only ingesting completely "clean" food), G_v (total ventilation volume) and partition coefficient between fish and water ($K_{fish/water}$). The considered compartment includes the liver, fat, kidney, richly perfused compartment and poorly perfused compartment for rainbow trout.

2.3 Biotransformation

In general, models require information on metabolic biotransformation to improve estimation for chemicals that are subject to biotransformation (Arnot et al., 2008). Even slow rates of biotransformation may significantly affect bioaccumulation in fish (Mackay et al., 2013). So the treatment of biotransformation was considered and described in detail as below for the two types of fish model. However, the measured data and available models for estimating biotransformation rates (both whole body and tissue-specific) were extremely limited (Nichols et al., 2006). The extrapolation approach described below is a first approximation and should be used with caution due to the high uncertainty.

2.3.1 One – compartment model

For the one-compartment model, the experimental biotransformation rate constants (k_m) were selected preferentially to predicted values from BCFBAF submodel in EPISuite (US EPA, 2012), which was normalized to a 10 g fish at 15 ℃. These were converted to mass and temperature specific $k_{m,x}$ value as (US EPA, 2012):

$$k_{M,\,X} = k_M (W_X / W_N)^{-0.25} \times exp\,(0.01 \times (T_X - T_N)) \quad (6)$$

where W_x is the study-specific mass of the organism (kg), W_N is the normalized mass of the organism (0.01 kg), T_x is the study-specific temperature, T_N is the normalized water temperature (15 ℃).

2.3.2 Multi-compartmental model

For the PBTK model, the whole-body metabolism rate k_m taken from the EPISuite database (US EPA, 2012) was used to back-calculate the metabolism rate in liver. Experimental values were also preferred and used where possible. Thus, the hepatic clearance (CL_H, L h^{-1} kg^{-1}) was expressed as below and was normalized to the weight of fish:

$$CL_H = k_m \times V_{d,blood} \quad (7)$$

where the $V_{d,blood}$ (L kg^{-1}) is the apparent volume of distribution, referenced to the chemical concentration in mixed blood. This could be regarded as the sorption capacity of the fish relative to that of blood, and can be approximated by dividing the $K_{fish\text{-}water}$ by $K_{blood\text{-}water}$ (Nichols et al., 2006). If the rate of biotransformation is very high, then the CL_H is rate-limited by the total blood flow to the liver (Nichols et al., 1990). This is just a first approximation of extrapolation of biotransformation rates, since it will be affected by many factors, e.g., the extra hepatic metabolism and protein binding (Nichols et al., 2007).

2.4 General pp-LFERs

Poly-parameter linear free energy relationship (pp-LFERs) are multiple linear regression models that use several solute- or sorbate-specific descriptors as independent variables (Endo and Goss, 2014a). There are three widely used forms of pp-LFERs expressed as:

$$\log K = c + sS + aA + bB + vV + eE \quad (8)$$

$$\log K = c + eE + sS + aA + bB + lL \quad (9)$$

$$\log K = c + sS + aA + bB + vV + lL \quad (10)$$

where K is the partition coefficient between two phases. Equation (8) is used for partitioning between a condensed phase and a gas phase, and Equation (9) is used for partitioning between two condensed phases. The capital letters stand for the chemical descriptors: S refers to dipolarity/polarizability, A and B are the hydrogen bond acidity and basicity, L is the logarithm of the partition coefficient between hexadecane and air, E is the excess molar refraction (cm^3 $mol^{-1}/10$), and V refers to the McGowan volume (cm^3 $mol^{-1}/100$). The lower cases letters s, a, b,

227 *v*, and *l* are regression coefficients and c is the regression constant, which indicate the
228 complementary properties of the partitioning system. The Equation (10) uses *V* and *L* and has
229 the advantage of wider application to organosilicons and highly fluorinated compounds (Endo
230 and Goss, 2014b). It is therefore preferred to be used. The selected pp-LFERs in this study are
231 summarized in Table S3. It is generally expected that the extrapolation of a model beyond its
232 calibrated domain may cause larger prediction errors than that would be expected for
233 interpolation. Special caution should be taken for the serum albumin, whose fitting to data was
234 not as good as other biological systems (Endo and Goss, 2011b). The ranges of individual
235 descriptors used in each equation are summarized in Table S6 for each biological system in this
236 study.

237 2.5 Implementation of pp-LFERs

238 2.5.1 Incorporating pp-LFERs in the one-compartment model

239 In the one-compartment model, the partition coefficient between fish and water is quantified as
240 (Arnot and Gobas, 2004):

$$K_{fish/water} = (f_{lipid}/D_{water} + f_{NLOM} \times \beta/D_{NOLM})\, Kow + f_{water} \quad (11)$$

241 where f_{lipid}, f_{NLOM} and f_{water} are the volume fractions of lipid, non-lipid organic matter (NLOM)
242 and water, as quantified in Table S2; β is the proportionally constant of *NLOM* to octanol, D_{water}
243 and D_{NOLM} are the densities of lipid and non-lipid organic matter.

244 Replacing the sp-LFERs by pp-LFERs, the partition coefficients are modified as:

$$K_{fish/water} = (K_{storage\ lipid/water} \times f_{storage\ lipid}/D_{lipid}) + (K_{phospholipid/water} \times f_{phospholipid}/D_{phospolipid}) + (K_{protein/water} \times f_{protein}/D_{protein} + f_{water}/D_{water}) \quad (12)$$

245 where $f_{storage\ lipid}$, $f_{phospholipid}$ and $f_{protein}$ are the volume fractions of storage lipid, phospholipid and
246 protein of fish defined in Table S4, *K* values indicate the individual partition coefficients
247 between target biological medium and water, and *D* is the corresponding density of each tissue.
248 The densities of storage (neutral) lipid, phospholipid, protein and water are assumed to be 0.93,

1, 1.4 and 1 kg L^{-1} (Endo et al., 2013). A similar treatment was performed for the partition coefficient between gut and fish ($K_{gut\text{-}fish}$).

2.5.2 Incorporating pp-LFERs into PBTK model

In the PBTK model, the $K_{blood\text{-}water}$ was derived as (Bertelsen et al., 1998):

$$K_{blood/water}=10^{0.72\times \log Kow +1.04 \log(\alpha_b)}+\gamma_b \quad (13)$$

where the α_b is the lipid content of blood tissue, γ_b is the water content of blood tissue and other partition coefficients $K_{organ/blood}$, including $K_{liver/blood}$, $K_{fat/blood}$, $K_{muscle/blood}$ and $K_{kidney/blood}$, are calculated from $K_{blood/water}$ as:

$$K_{organ/blood}=(10^{0.72\times Log\ Kow +1.04 \log(\alpha_i)+0.86}+\gamma_i)/K_{blood/water} \quad (14)$$

Where the α_i and γ_i are the lipid and water contents in the individual organ. The composition of each organ was as assumed to the defaults for rainbow trout in the original PBTK model. But in pp-LFER PBTK model, the $K_{organ/water}$ was calculated based on the biological composition of each organ, mainly containing neutral lipid, phospholipid, protein and water. The specific composition of each biological compartment (e.g., blood, kidney and liver) is presented in Table S5. It was assumed here that total lipid only contains neutral lipid and phospholipid. The fraction of bovine serum albumin (BSA) was selected from a study based on mammals (Endo et al., 2013). The treatment of fat content in lean tissues (all compartments exclude the fat) and the temperature dependence of partitioning is detailed in the supporting information. The bovine serum albumin was only considered to be present in the blood tissue, since its existence is fairly minimal and its variation may increase the model uncertainty. The $K_{organ/blood}$ was calculated in the pp-LFERs approach as:

$$K_{organ/blood}=K_{organ/water}/K_{blood/water} \quad (15)$$

2.6 Solute descriptors

Experimentally measured solute descriptors are available for thousands of chemicals and have been compiled as a free-of-charge database (http://www.ufz.de/index.php?en=31698). The initial chemical dataset including 235 compounds (Brown and Wania, 2009), were selected

from 1460 individual chemicals which were considered to fall within the range environmentally relevant compounds. Several updated experimental values of descriptors were also added from the recently published literature to cover more polar and complex chemicals, including organosilicon compounds, highly polyfluorinated chemicals, flame retardants (e.g., polybrominated diphenyl ethers, hexabromocyclododecane, bromobenzenes, trialkyl phosphates), pesticides, polychlorinated biphenyls (PCBs) and heterocyclic aromatic as well as nitroaromatics compounds (Geisler et al., 2011; Stenzel et al., 2013a, b). Ionization was not taken into account in this study, as the pp-LFER approaches to ionic chemicals are still a subject of on-going research. No successful application to environmental and biological processes have been reported so far (Endo and Goss, 2014a). Selected chemicals were categorized into different polarities according to A and B values defined here: nonpolar (both A and B $\leq$ 0.2), monopolar (including H-bond acceptor (A >0.2 but B <0.2) or H-bond donor (A <0.2 but B>0.2), and bipolar (both A and B >0.2) compounds. Their individual impact on pp-LFERs is characterized.

Two subsets of compounds were added to the whole dataset. One represented chemicals with strong H-donor function (A>0.3), because substantial differences in the "aA" term have been observed for the pp-LFER equations for octanol and storage lipids for this type of chemical. Thus, partitioning to octanol and storage lipid are expected to be different, which contrasts with most typical assumptions that the octanol is a good surrogate for lipids. The other subset contained complex compounds with more than one polar functional group per molecule. The selected compounds cover hormones and hormone active compounds (e.g., estrone, bisphenol A, phthalate esters), fungicides, herbicides and mycotoxins. The representative functional groups included alcohol, amide, carbonyl, nitrite, ester, epoxide and phenyl groups. Ignorance of ionization could potentially generate uncertainty, since the partitioning behaviour of ionic species is different from neutral species (Abraham and Acree, 2010).

2.7 Compilation of measured BCFs dataset

The main source of observed BCF data was extracted from Arnot and Gobas (2006). It contains multiple BCF measurements for chemicals in different fish species with varying physiological

conditions, which reflect realistic variations in BCFs across different fish species and system conditions. The dataset mainly contained nonpolar compounds and was firstly used to test the model performance for the one-compartment model (Arnot and Gobas, 2004). The majority of data points are from studies using common carp (*Cyprinus carpio*), fathead minnow (*Pimephales promelas*), zebrafish (*Danio rerio*) and rainbow trout (*Oncorhynchus mykiss*).The chemicals with observed BCFs from studies in rainbow trout were extracted to produce a subset of 41 distinct compounds and 355 data points, which was used to evaluate the PBTK model under sp and pp approaches requiring specific physical fish information. In addition, other publicly available data were also compiled to cover additionally observed BCFs for complex polar chemicals, such as the Pesticide Property Database (http://sitem.herts.ac.uk/aeru/ppdb/en/index.htm). It is ideal to have study-specific experimental information water temperature, fish weight, and lipid content to predict individual BCF values. However, many studies did not record such information. Consequently, a value of 5% was used as a first approximation of whole body lipid content (Arnot and Gobas, 2006). All selected experimental BCF values were lipid normalized.

2.8 Inter-comparison of models

A difficult task is to systematically compare the results from pp-LFER and sp-LFER models. One approach is to compare the predicted results directly (Gotz et al., 2007). Here, we used space maps to present the variations in models outputs as a function of partition coefficients, like K_{AW}, K_{OA} and K_{OW} (Brown and Wania, 2009). Firstly, the entire dataset was used to compare the predicted values of partition coefficients calculated by sp/pp-approach and the predicted concentration in fish. Individual contributions of different forms of intermolecular interaction to partitioning from organs/tissues to water can be compared to explore the dominant interactions. The statistical analysis was conducted using average model bias (MB) and average absolute model bias (AMB) to assess model performance as calculated below:

$$MB = \frac{\sum_{i=1}^{n} log(\frac{BCF_M}{BCF_E})}{n} \quad (16)$$

$$AMB = \frac{\sum_{i=1}^{n} ABS\left[log(\frac{BCF_M}{BCF_E})\right]}{n} \quad (17)$$

where BCF_M is the modelled bioconcentration factor, BCF_E is the measured bioconcentration factor, n is the number of observations, *ABS* means the absolute deviation. *MB* represents the average factor by which the model output deviates from the observation. It is useful to indicate the direction of any systematic bias. The root mean square error (RMSE) and the square of correlation coefficient (R^2) were also used to characterize model performance.

In this study, the only difference between model inputs is the replacement of octanol-based sp-LFER with pp-LFERs. Therefore, any observed differences will be attributable to this difference. The experimental errors in measuring the partitioning coefficients were not considered in this study. In order to keep the inputs same and reduce the uncertainty from the measurement of K_{OW} (Linkov et al., 2005), K_{OW} used in sp-LFERs was also derived from pp-LFERs instead of using measured K_{OW} values.

3 Results & Discussion

3.1 Comparison of outputs by the sp/pp-approaches

In order to identify the types of chemicals for which the implementation of pp-LFERs would make a significant difference, the predicted concentration of fish and partition coefficients were compared for chemicals possessing a wide range of partitioning properties using the solute descriptors. The results are presented in chemical partitioning plots as a function of the chemicals' octanol-air-water partitioning properties, described by K_{AW} and K_{OA} (Figure 1). In addition, the influence of the polarity is also illustrated in Figure 1 (a, b). The different categories of nonpolar, monopolar and bipolar compounds were defined based on the descriptor values of A and B in Section 2.6. A quantitative assessment of the relative contribution of the

different solute descriptors in the pp-LFERs for the partition coefficients was presented in Figure S2.

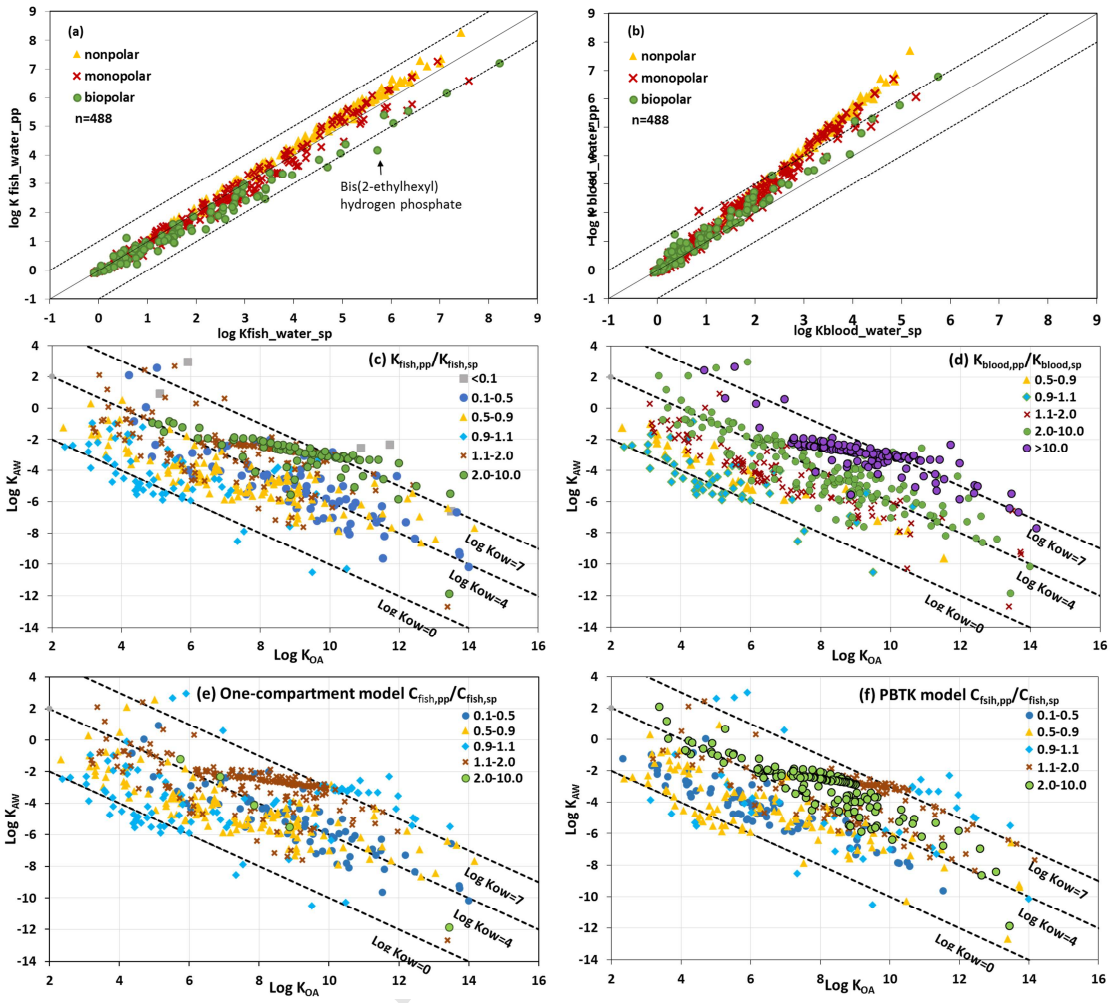


Figure 1**.** Comparison of calculated logarithmic fish-water partition coefficients (a) and blood-water partition coefficients (b) by pp-LFERs and sp-LFERs values with different defined polarities. The multiple colours and symbols represented different polarities defined by A and B. For nonpolar compounds, both A and B ≤0.2 (N=156); for monopolar compounds, either A or B is >0.2 (N=224); for bipolar compounds, both A and B >0.2 (N=108). Chemical partitioning space plots indicated the ratios of partition coefficient between water and whole fish (c) also blood (d); concentrations in fish calculated using sp and pp approach in one-compartment model (e) and in multi-compartment PBTK models (f). Different colours indicated the magnitude of the quotient. The diagonal lines indicate the log K_{OW} equal to 0, 4 and 7.

3.1.1 Comparison of $K_{fish\text{-}water}$ by the sp/pp-approaches

In general, the log $K_{fish/water}$ was estimated consistently via both approaches. 99% of selected substances the observed differences was less than one log unit. Compounds with different polarities indicated slightly different deviations as in Fig 1. For all nonpolar compounds in the dataset, the $K_{fish/water}$ value calculated by pp-LFERs was larger than that calculated by sp-LFERs. However, the compounds with bipolar functional groups tended to show a larger difference between $K_{fish/water}$ calculated by these two approaches. The largest difference of log $K_{fish\text{-}water}$ was observed for bis(2-ethylhexyl) hydrogen phosphate, up to 1.5 log unit, with a strong H-bond donor/acceptor (A=0.96, B=1.12). Its log $K_{lipid/water}$ was less than log K_{OW} by 2 log units, leading to the large deviation of calculated $K_{fish\text{-}water}$. The overestimation of BCFs may be expected for such type of compounds by directly using Kow.

When looking at the dependency of deviation with the different range of log K_{OW} values (Figure 1-c), the discrepancy also gradually raised with increased hydrophobicity. For hydrophilic compounds (log K_{OW} <0), both approaches agreed well with each other within approximately a factor of 10. For chemicals with log Kow>4 and log K_{OA} >8, the pp-approach generally predicted $K_{fish\text{-}water}$ on average two times higher than that predicted by sp-approach. But the deviation did not consistently propagate to the predictions of concentration in fish. Both approaches agreed reasonably well for hydrophilic (log Kow<0) and highly hydrophobic compounds (log Kow>7) with the quotient between 0.9-1.1, while the deviation occurred on the calculation of $K_{fish\text{-}water}$ was up to 35 times. The underlying explanation could be that $K_{fish\text{-}water}$ has different extent of impact on the determination of BCFs, which is dependent on chemical hydrophobicity. For instance, $K_{fish\text{-}water}$ was observed to contribute a greater degree to the bioaccumulative potential for hydrophobic chemicals with a high tendency of bioaccumulation (Kuo and Di Toro, 2013b). While, partitioning to organic carbon (bioavailable portion) contributed more to BCF values for super-hydrophobic compounds (Kuo and Di Toro, 2013b).

3.1.2 Comparison of $K_{blood\text{-}water}$ by sp/pp-approaches

Greater differences were observed for the log $K_{blood/water}$ calculated by sp-LFERs and pp-LFERs approaches, indicating increased divergence with higher partition coefficients between blood and water for compounds with different polarities. 72% of selected substances fell within a difference of less than a log unit. In the total data set, the largest difference up to 2.5 log units was found for 1, 2, 3, 4, 5, 6, 7, 8-octachloronaphthalene in the category of nonpolar compounds. This compound has an extremely high L=12.88, leading to higher partition coefficients between biological tissue and water than that between octanol and water.

A different trend was observed for the relationship between hydrophobicity and the deviations of the predictions by sp-LFERs and pp-LFERs models than that for the $K_{fish/water}$. For 86% of the selected substances, the pp-LFERs model estimated higher blood-water partition coefficients than the sp-LFERs model. Larger discrepancies were observed with increasing hydrophobicity for all three types of compounds. Especially for nonpolar compounds, the deviation between the sp-LFERs and pp-LFERs models indicated a positive relationship between the log K_{OW} and a high correlation coefficient of R^2=0.96 was observed (Figure S1-a). A higher deviation resulting from incorporating pp-LFERs was expected for polar compounds than for nonpolar compounds. The underlying reason for this unexpected results could be caused by the inclusion of protein in the pp-approach. The predicted partitioning coefficients of protein have larger deviation (1-2 log units) than $K_{storage\ lipid\text{-}water}$ for nonpolar compounds, which increased with hydrophobicity (Endo et al., 2012). The absolute values of Ll+Vv terms, describing van der Waals interactions, was plotted against hydrophobicity (illustrated in Figure S1). The sum of Ll and Vv consistently increased in all biological systems as in Figure S1. The divergences grew between different biological compositions and octanol with increased hydrophobicity. Therefore, the greater deviation probably occurs as a consequence of not properly capturing the behaviour of van der Waals' forces for chemicals with high values of L. The difference between predicted concentrations between sp-LFERs and pp-LFERs from the PBTK model is similar to that from

the one-compartment model, since both models employed several identical empirical relationships (Arnot and Gobas, 2004).

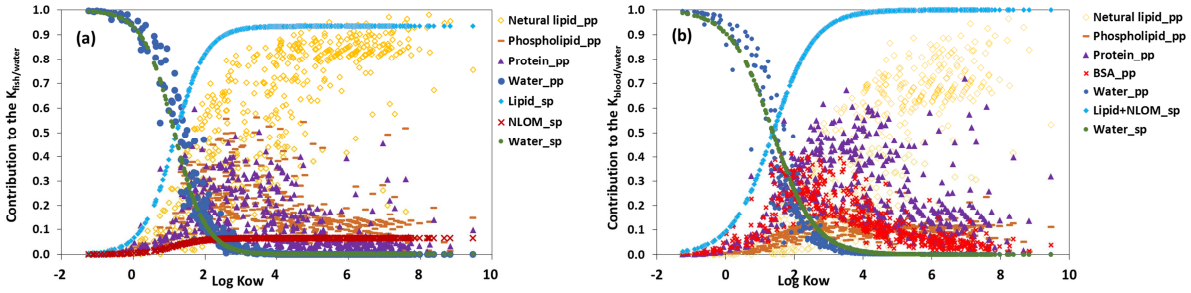


Figure 2. Contribution to total partition capacity by different biological tissues with the full range of K_{OW}: (a) individual contribution to the total $K_{fish/water}$; (b) individual contribution to the total $K_{blood/water}$.

3.2 Contribution to the total sorption capacity

In order to explore the importance of neutral lipids, phospholipids (membrane), proteins, serum albumin (BSA) and water as sorptive matrixes, the contribution of each biological phase calculated via sp-LFERs and pp-LFERs was plotted as a function of log K_{OW} in Figure 2. The greatest disparity is the dominant tissue contributing to the total partitioning capacity. For the one-compartment fish model, the sp-LFERs model only considered neutral lipid, water and NLOM (a relative sorptive capacity proportional to lipid). Therefore, the contribution of each biological sorbent to the total partitioning capacity presented a continuous trend the change of chemical hydrophobicity (illustrated in Figure 2-a). However, the shifting trend was more complex for the pp-LFERs model, with additional consideration of protein and phospholipid without directly relating to octanol. It is obvious that the contributions of water and lipid were consistent for hydrophobic and hydrophilic chemicals for both models, since the water and lipid are the absolute predominant sorptive matrixes. For the chemical with moderate to high K_{OW} values ($2<\log K_{OW}<6$), the phospholipid and protein made important contributions, up to 39% for protein and 61% for phospholipid, respectively. This also explains that the large deviation in calculated partition coefficients between fish/blood and water for a chemical with moderate hydrophobicity (Figure 1-c).

For the PBTK models employing pp-LFERs, the individual contribution was also calculated between blood and water for the whole range of K_{OW} in Figure 2-b. A similar trend was observed for predicted blood-water partitioning as the comparison for the $K_{fish/water,}$ which continuously changed with the varied K_{OW} values. However, the pp-LFERs model predicted more dispersed values in the individual biological compartments. The protein and BSA also contributed to the total blood-water partitioning up to 72% and 41%, respectively. Their individual contribution did not indicate a consistent shift with the increased log K_{OW}, especially for protein, whose points were scattered on a wide range of log K_{OW} between 2 and 9. This reflects the fact that hydrophobicity is not a perfect indicator for absorption to protein. For example, eicosanoic acid is the most hydrophobic compound in the database with log Kow=9.47. However, protein contributes 32% to the total blood-water partition coefficients. BSA contributed most in the moderate range of log K_{OW}=1 ~ 5, based on the currently used chemical set. Phospholipids also contributed between 10~20% for compounds with Log K_{OW} >1 peaking at about log K_{OW}=4~5. It is noteworthy that the regression relationship used for calculating blood-water partition coefficients, was originally derived from compounds with a limited log K_{OW} range from 1.46 to 4.04. Thus, any compounds outside this range may cause potential errors and should be used with caution (Bertelsen et al., 1998; Nichols, 2002). This relationship is still commonly used in PBTK modelling (Hendriks et al., 2005; Han et al., 2007; Stadnicka et al., 2012). Evaluation of the regression equation to describe tissue/water partitioning is out of the scope of this study.

From the comparison of the contribution to the total fish/blood-water partition coefficients above, it also could help to explain how the difference occurs. In the range of log K_{OW} from 2 to 6, protein provides an important contribution to both partition coefficients. Using octanol as equivalent to lipid could overestimate the contribution of lipid, but the sp-LFER approach could also underestimate the contribution of protein. As a result, the total partition coefficient calculated by the sp and pp-approaches could be expected to be different within a reasonable range, since the underestimation and overestimation could proportionally cancel out with each

other. The similar result was also observed in comparing the lipid-octanol model and pp-LFERs model to predicting partition coefficients of tissue-water (Endo et al., 2013).

3.3 Comparison with experimental data

3.3.1 One-compartment model

There are 835 data points from fish species chosen from the experimental database for 110 distinct compounds (Arnot and Gobas, 2006). The chemicals covered the K_{OW} range from -0.15 to 8.67. However, most data points fell in the log K_{OW} range between 3~5 as illustrated in Figure S3. In order to examine the magnitude of the deviation correlated by the hydrophobicity between predictions and measurements, the impact of applying pp-LFER equations to the individual ranges of log K_{OW} and the whole dataset was explored and presented in Table S7. In general, the pp-LFER model performed slightly better in terms of predicting BCF, with increased coefficient of determination (R^2) and absolute model bias for the whole dataset. The deviations between the sp and pp-LFERs model predictions, did not show a pronounced K_{OW} dependency. The pp-LFERs model did not generally improve the coefficient of determination, for compounds with log K_{OW} <3. The underestimation is most severe for log K_{OW} <1 with an average 2.9 log units for both approaches. This is because the calculation of $K_{fish/water}$ is predominantly determined by water (illustrated in Figure 2). Thus the effect of replacing sp with pp-LFERs is minimal. Therefore, there is no clear advantage observed for using pp-LFERs model instead of sp-LFERs for compounds with log K_{OW} < 2. For the middle range of log K_{OW} from 4 to 5, the BCFs predicted by pp-LFERs were found to better fit observed values compared the sp-LFERs model. This is due to a better quantification of partitioning behaviour of polar compounds such as phenols in this range, by adding separate consideration of protein and phospholipid, which makes signification contribution in such case.

For very hydrophobic compounds (7<log K_{OW} <9), both models predicted the selected BCFs reasonably well (R^2=0.80-0.96). This is because lipids are the main sorbing matrix in this K_{OW} range. In addition, it has been demonstrated that depuration kinetics are more important for hydrophobic chemicals with higher bioaccumulation potentials. While, partitioning to dissolved/

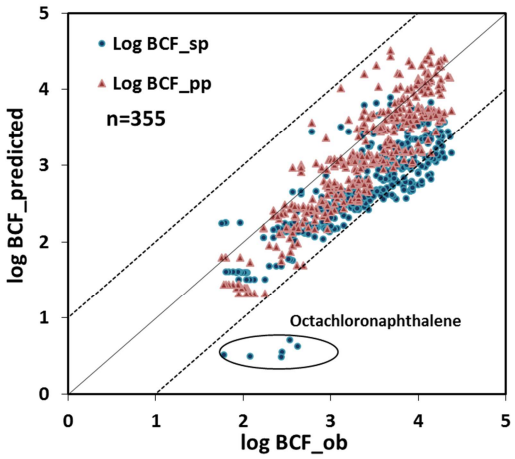
485 particulate organic carbon (the bioavailable part) plays an important role for highly hydrophobic
486 chemicals (Kuo and Di Toro, 2013b). Therefore, improved partition coefficients may not greatly
487 influence the model performance using the pp-LFERs model in the high log K_{OW} range (7~9).
488 On the other hand, chemicals with low bioaccumulative potential (log BCF≤ 2) are generally
489 mainly determined by fish-water partitioning coefficients (K_{bw}) and thus more pronounced
490 improvement would be expected (Kuo and Di Toro, 2013b). Consequently, the comparison
491 should be made with caution for the very hydrophilic and super-hydrophobic compounds, due
492 the limited data points.

493 **3.3.2 PBTK model**

494 In total, 41 distinct compounds with 355 data points with log K_{OW} from 2.4 to 8.7 for rainbow
495 trout were selected. Results of statistical analysis are presented in Table S7 and S8. Most
496 compounds have low polarity, with relative small Aa and Bb values. Greater improvement was
497 observed when pp-LFERs models were used in the PBTK model compared that in the one-
498 compartment model. This could be attributable to more pp-LFERs equations incorporated in the
499 model, not only for the blood-water system, but also covering kidney, liver, and fat and water
500 partitioning composed by varied biological composition. In the one-compartment model, sp-
501 LFERs were only replaced with the partition coefficients between whole body and water. The
502 K_{OW}-driven sp-LFERs PBTK model tended to underestimate BCFs for 96% of the selected
503 measurements. One underlying explanation could be that the partitioning behaviour could not be
504 well characterized by means of octanol-water partitioning. Particularly for highly hydrophobic
505 nonpolar compounds, the divergence increased with the increasing hydrophobicity as discussed
506 in Section 3.1.2.

507 When metabolism was included, the pp-LFER model also performed better in all the statistical
508 analysis. All deviations fell within a factor of 10. A paired t-test was conducted to indicate
509 whether there is a statistical difference ($p<0.05$). All the compounds fell within 1 log unit via
510 incorporation of pp-LFERs equations. The correlation of determination was improved from 0.67
511 to 0.80 while the absolute model bias (AMB) decreased by half from 0.68 to 0.34. The largest

512 deviation occurred for octachloronaphthalene predicted by the sp-LFERs model, which also had
513 the largest divergence when comparing the blood-water partition in the whole dataset discussed
514 previously. This further supports the fact that sp-LFERs underestimated the blood water
515 partitioning and potentially also partitioning to other biological compartments (kidney, liver and
516 fat). However, both models tended to underestimate the BCFs for the whole dataset. This could
517 be due the parameterization uncertainty, mainly from hepatic biotransformation extrapolated
518 from the whole-body metabolism rate. It has been demonstrated that biotransformation may
519 have a greater impact on the PBTK model than that in the one-compartment model, which
520 results from the different structure of both models (Nichols et al., 2007; Stadnicka et al., 2012).



521

522 Figure 3. Comparison between measured log BCF_ob with predicted log BCF using sp/pp-
523 approaches in the multi-compartment PBTK model. The dashed lines represent a factor of 10
524 between the predicted and measured BCFs.

525 3.4 Practical implications

526 pp-LFERs model can potentially provide improved insights about the prediction of potential
527 bioaccumulation. The impacts of using pp-LFERs were different in the one-compartment fish
528 model and PBTK fish model. For the one-compartment model, pp-LFERs improved model
529 performance for chemicals with log K_{OW} 4-5, via better quantifying the protein/phospholipid-
530 water partition coefficients. However, the differences between predictions via sp-LFERs and
531 pp-LFERs model are relatively small for the whole range of K_{OW}. This is because better

quantification of individual partitioning processes does not guarantee significant improvement overall. Besides, elimination kinetics could be the most important parameters in the determination of BCFs for highly bioaccumulative substances (Kuo and Di Toro, 2013a). As a consequence, such simplified models are generally incorporated in multimedia fate models and are used for the chemical screening and risk assessment. The sp-LFERs incorporated in one-compartment fish models is, therefore, good enough for these purposes.

This situation could be different for the PBTK fish model, which offers more detailed information on organ-specific concentrations and which is potentially more insightful for understanding potential exposure routes for target fish organs. It is important to understand specific pathways to target sites and bioaccumulation along food chains, if predators preferentially consume certain body parts (Ankley et al., 2010; Stadnicka et al., 2012). Therefore, the pp-LFERs model would clearly benefit from a better description and characterization of biological composition and water partition coefficients. Although the flawed regression equations used in this study are limited in terms of their applicable domains, lipid was still not suggested as a good indicator to predict partition coefficients in this case as discussed above, particularly for very hydrophobic and polar compounds. In addition, the pp-LFERs model also could help with the extrapolation of partition coefficients in PBTK model to another fish species, if the biological composition in individual organ/tissues could be accurately quantified.

3.5 Limitations

In this study, all the values for solute descriptors were based on experiments, which have been reported in the literature for more than 2000 compounds and freely at http://www.ufz.de/index.php?en=31698. However, this could hamper its wide application if the solute descriptor values are not available for target compounds (Stenzel et al., 2013b). For the purpose of fast chemical screening, predictive methods that only require molecular structure are desirable. Prediction models, such as ABSOLV, a commercial QSAR model that predicts the pp-LFER solute descriptors for compounds with SMILES notations (Stenzel et al., 2014), may

559 be useful. This works well for chemicals with relatively simple molecular structures, but further
560 development is needed for H-donor compounds and chemical with complex structures (Geisler
561 et al., 2015).

562 Ionization was not taken into account in this study, as pp-LFERs approach for ionic chemicals is
563 still a subject of ongoing research. No successful applications to environmental and biological
564 processes have been reported so far (Endo and Goss, 2014a). However, since many complex/
565 multifunction chemicals may ionize in biota, there is a strong need for the investigation of ionic
566 chemicals (Endo and Goss, 2014a; Bittermann et al., 2016). Meanwhile, the development of
567 one-compartment models for ionic compounds indicated improved performance via
568 consideration of partitioning processes to phospholipids (Armitage et al., 2013). In our study,
569 phospholipids also appeared to play an important role in distribution.

570 ## 3.6 Conclusions

571 Overall, pp-LFERs models slightly outperformed sp-LFERs models for the whole dataset in a
572 one-compartment model, especially for compounds in the log Kow range 4~5. Greater
573 improvement was found when pp-LFERs were incorporated into a multi-compartment PBTK
574 model. The impact of pp-LFERs incorporation could be further evaluated by the organ-specific
575 concentrations/bioaccumulative potential. Therefore, for screening purposes conducted by
576 simplified one-compartment model, the sp-LFERs approach is probably good enough to
577 quantify the main partition characteristics in most cases. For more detailed study aimed to
578 understand exposure pathways to target sites, or dietary exposure for predators preferentially
579 consuming certain organs/tissues, it is suggested the pp-LFERs should be incorporated in the
580 PBTK model to improve the accuracy of the description of partition processes.

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5 References

Abraham, M.H., Acree, W.E., Jr., 2010. Equations for the transfer of neutral molecules and ionic species from water to organic phases. J Org Chem 75, 1006-1015.

Abraham, M.H., Chadha, H.S., Whiting, G.S., Mitchell, R.C., 1994. Hydrogen-Bonding .32. An Analysis of Water-Octanol and Water-Alkane Partitioning and the Delta-Log-P Parameter of Seiler. J Pharm Sci-Us 83, 1085-1100.

Abraham, M.H., Gola, J.M.R., Ibrahim, A., Acree Jr, W.E., Liu, X., 2015. A simple method for estimating in vitro air-tissue and in vivo blood-tissue partition coefficients. Chemosphere 120, 188-191.

Ankley, G.T., Bennett, R.S., Erickson, R.J., Hoff, D.J., Hornung, M.W., Johnson, R.D., Mount, D.R., Nichols, J.W., Russom, C.L., Schmieder, P.K., Serrrano, J.A., Tietge, J.E., Villeneuve, D.L., 2010. Adverse outcome pathways: A conceptual framework to support ecotoxicology research and risk assessment. Environmental Toxicology and Chemistry 29, 730-741.

Armitage, J.M., Arnot, J.A., Wania, F., Mackay, D., 2013. Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish. Environmental Toxicology and Chemistry 32, 115-128.

Arnot, J.A., Gobas, F., 2004. A food web bioaccumulation model for organic chemicals in aquatic ecosystems. Environmental Toxicology and Chemistry 23, 2343-2355.

Arnot, J.A., Gobas, F.A.P.C., 2006. A review of bioconcentration factor (BCF) and bioaccumulation factor (BAF) assessments for organic chemicals in aquatic organisms. Environ Rev 14, 257-297.

Arnot, J.A., Mackay, D., Bonnell, M., 2008. Estimating metabolic biotransformation rates in fish from laboratory data. Environmental Toxicology and Chemistry 27, 341-351.

Bertelsen, S.L., Hoffman, A.D., Gallinat, C.A., Elonen, C.M., Nichols, J.W., 1998. Evaluation of log K_{OW} and tissue lipid content as predictors of chemical partitioning to fish tissues. Environmental Toxicology and Chemistry 17, 1447-1455.

Bittermann, K., Spycher, S., Goss, K.-U., 2016. Comparison of different models predicting the phospholipid-membrane water partition coefficients of charged compounds. Chemosphere 144, 382-391.

Brown, T.N., Wania, F., 2009. Development and exploration of an organic contaminant fate model using poly-parameter linear free energy relationships. Environmental Science & Technology 43, 6676-6683.

Debruyn, A.M.H., Gobas, F.A.P., 2007. The sorptive capacity of animal protein. Environmental Toxicology and Chemistry 26, 1803-1808.

Endo, S., Bauerfeind, J., Goss, K.U., 2012. Partitioning of neutral organic compounds to structural proteins. Environ. Sci. Technol. 46, 12697-12703.

Endo, S., Brown, T.N., Goss, K.U., 2013. General model for estimating partition coefficients to organisms and their tissues using the biological compositions and polyparameter linear free energy relationships. Environ. Sci. Technol. 47, 6630-6639.

Endo, S., Escher, B.I., Goss, K.U., 2011. Capacities of membrane lipids to accumulate neutral organic chemicals. Environ. Sci. Technol. 45, 5912-5921.

626 Endo, S., Goss, K.-U., 2011a. Serum Albumin Binding of Structurally Diverse Neutral Organic
627 Compounds: Data and Models. Chemical Research in Toxicology 24, 2293-2301.
628 Endo, S., Goss, K.U., 2011b. Serum albumin binding of structurally diverse neutral organic
629 compounds: data and models. Chemical Research in Toxicology 24, 2293-2301.
630 Endo, S., Goss, K.U., 2014a. Applications of polyparameter linear free energy relationships in
631 environmental chemistry. Environ. Sci. Technol. 48, 12477-12491.
632 Endo, S., Goss, K.U., 2014b. Predicting partition coefficients of Polyfluorinated and
633 organosilicon compounds using polyparameter linear free energy relationships (PP-LFERs).
634 Environ. Sci. Technol. 48, 2776-2784.
635 Geisler, A., Endo, S., Goss, K.-U., 2011. Partitioning of polar and non-polar neutral organic
636 chemicals into human and cow milk. Environment International 37, 1253-1258.
637 Geisler, A., Endo, S., Goss, K.U., 2012. Partitioning of organic chemicals to storage lipids:
638 elucidating the dependence on fatty acid composition and temperature. Environ. Sci. Technol.
639 46, 9519-9524.
640 Geisler, A., Oemisch, L., Endo, S., Goss, K.-U., 2015. Predicting storage-lipid water
641 partitioning of organic solutes from molecular structure. Environmental Science & Technology.
642 Gobas, F.A.P.C., de Wolf, W., Burkhard, L.P., Verbruggen, E., Plotzke, K., 2009. Revisiting
643 Bioaccumulation Criteria for POPs and PBT Assessments. Integrated Environmental
644 Assessment and Management 5, 624-637.
645 Gotz, C.W., Scheringer, M., Macleod, M., Roth, C.M., Hungerbuhler, K., 2007. Alternative
646 approaches for modeling gas-particle partitioning of semivolatile organic chemicals: Model
647 development and comparison. Environmental Science & Technology 41, 1272-1278.
648 Han, X., Nabb, D.L., Mingoia, R.T., Yang, C.-H., 2007. Determination of Xenobiotic Intrinsic
649 Clearance in Freshly Isolated Hepatocytes from Rainbow Trout (Oncorhynchus mykiss) and Rat
650 and Its Application in Bioaccumulation Assessment. Environmental Science & Technology 41,
651 3269-3276.
652 Hendriks, A.J., Traas, T.P., Huijbregts, M.A.J., 2005. Critical Body Residues Linked to
653 Octanol−Water Partitioning, Organism Composition, and LC50 QSARs: Meta-analysis and
654 Model. Environmental Science & Technology 39, 3226-3236.
655 Hermens, J.L.M., de Bruijn, J.H.M., Brooke, D.N., 2013. The octanol–water partition
656 coefficient: Strengths and limitations. Environmental Toxicology and Chemistry 32, 732-733.
657 Kuo, D.T.F., Di Toro, D.M., 2013a. Biotransformation model of neutral and weakly polar
658 organic compounds in fish incorporating internal partitioning. Environmental Toxicology and
659 Chemistry 32, 1873-1881.
660 Kuo, D.T.F., Di Toro, D.M., 2013b. A reductionist mechanistic model for bioconcentration of
661 neutral and weakly polar organic compounds in fish. Environmental Toxicology and Chemistry
662 32, 2089-2099.
663 Landrum, P.F., Lee, H., Lydy, M.J., 1992. Toxicokinetics in Aquatic Systems - Model
664 Comparisons and Use in Hazard Assessment. Environmental Toxicology and Chemistry 11,
665 1709-1725.
666 Laue, H., Gfeller, H., Jenner, K.J., Nichols, J.W., Kern, S., Natsch, A., 2014. Predicting the
667 bioconcentration of fragrance ingredients by rainbow trout using measured rates of in vitro
668 intrinsic clearance. Environ. Sci. Technol. 48, 9486-9495.
669 Linkov, I., Ames, M.R., Crouch, E.A.C., Satterstrom, F.K., 2005. Uncertainty in Octanol−Water
670 Partition Coefficient: Implications for Risk Assessment and Remedial Costs. Environmental
671 Science & Technology 39, 6917-6922.
672 Mackay, D., 2001. Multimedia Environmental Models: The Fugacity Approach, Second Edition.
673 Taylor & Francis.
674 Mackay, D., Arnot, J.A., Gobas, F., Powell, D.E., 2013. Mathematical relationships between
675 metrics of chemical bioaccumulation in fish. Environmental Toxicology and Chemistry 32,
676 1459-1466.
677 Nichols, J., 2002. Modeling the Uptake and Disposition of Hydrophobic Organic Chemicals in
678 Fish Using a Physiologically Based Approach. in: Krüse, J., Verhaar, H.M., de Raat, W.K.
679 (Eds.). The Practical Applicability of Toxicokinetic Models in the Risk Assessment of
680 Chemicals. Springer Netherlands, pp. 109-133.

Nichols, J.W., Fitzsimmons, P.N., Burkhard, L.P., 2007. In vitro-in vivo extrapolation of quantitative hepatic biotransformation data for fish. II. Modeled effects on chemical bioaccumulation. Environmental Toxicology and Chemistry 26, 1304-1319.

Nichols, J.W., McKim, J.M., Andersen, M.E., Gargas, M.L., Clewell, H.J., 3rd, Erickson, R.J., 1990. A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. Toxicol Appl Pharmacol 106, 433-447.

Nichols, J.W., Schultz, I.R., Fitzsimmons, P.N., 2006. In vitro–in vivo extrapolation of quantitative hepatic biotransformation data for fish: I. A review of methods, and strategies for incorporating intrinsic clearance estimates into chemical kinetic models. Aquat Toxicol 78, 74-90.

Parliament, E., Union, t.C.o.t.E., 2006. Regulation (EC) no. 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the registration, evaluation, authorization and restriction of chemicals (REACH), establishing a European Chemicals Agency, amending Directive 1999/45/EC and repealing Council Regulation (EEC) no. 793/93 and Commission Regulation (EC) no. 1488/94, as well as Council Directive 76/769/EEC and commission Directives 91/155/EEC, 93/67/EEC, 93/105/CE and 2000/21/EC. Official Journal of the European Union 396.

Salmina, E.S., Wondrousch, D., Kühne, R., Potemkin, V.A., Schüürmann, G., 2016. Variation in predicted internal concentrations in relation to PBPK model complexity for rainbow trout. Science of The Total Environment 550, 586-597.

Stadnicka, J., Schirmer, K., Ashauer, R., 2012. Predicting concentrations of organic chemicals in fish by using toxicokinetic models. Environ. Sci. Technol. 46, 3273-3280.

Stenzel, A., Goss, K.U., Endo, S., 2013a. Determination of polyparameter linear free energy relationship (pp-LFER) substance descriptors for established and alternative flame retardants. Environ. Sci. Technol. 47, 1399-1406.

Stenzel, A., Goss, K.U., Endo, S., 2013b. Experimental determination of polyparameter linear free energy relationship (pp-LFER) substance descriptors for pesticides and other contaminants: new measurements and recommendations. Environ. Sci. Technol. 47, 14204-14214.

Stenzel, A., Goss, K.U., Endo, S., 2014. Prediction of partition coefficients for complex environmental contaminants: Validation of COSMOtherm, ABSOLV, and SPARC. Environmental toxicology and chemistry 33, 1537-1543.

Undeman, E., Czub, G., McLachlan, M.S., 2011. Modeling bioaccumulation in humans using poly-parameter linear free energy relationships (PPLFERS). Science of The Total Environment 409, 1726-1731.

US EPA, 2012. Estimation Programs Interface Suite™ for Microsoft® Windows, v 4.11. United States Environmental Protection Agency, Washington, DC, USA.

Van der Jagt, K., Munn, S., Tørsløv, J., de Bruijn, J., 2004. Alternative approaches can reduce the use of test animals under REACH. Report Eur 21405, 1-25.