SUPPORTING INFORMATION

Models used to predict chemical bioaccumulation in fish from *in vitro* biotransformation rates require accurate estimates of blood-water partitioning and chemical volume of distribution.

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Text S1. Polyparameter linear free energy relationships

Storage lipid-water (K_{SLW}), membrane lipid-water (K_{MLW}), serum albumin-water (K_{APW}), and non-albumin protein-water (K_{NPW}) partition ratios were predicted using polyparameter linear free energy relationships (ppLFER) according to Endo et al. [1]. Polyparameter linear free energy relationships are multiple regression models that use several descriptors as independent variables to characterize the free energy of partitioning. The logarithm of the partition ratio (log K) is calculated as the sum of the contributions of the various inter-molecular interactions:

$$\log K = eE + sS + aA + bB + vV + c \qquad \text{(S1)}$$

where E, S, A, B, and V are properties of the solute, and e, s, a, b, v, and c are properties of the two solvent phases. Solvent descriptors (e, s, a, b, v) are given by Endo et al. [1] and quantify the magnitude of the difference in the corresponding solute–solvent interactions between the two solvent phases. The solute descriptors are excess molar refraction (E), dipolarity/polarizability (S), solute H-bond acidity (A), solute H-bond basicity (B), and molar volume (V). Solute descriptors for the chemicals in Table 3 of the main text were obtained from the UFZ-LSER Database [2]. Chemicals were identified within the UFZ website using either the CAS number or, if unavailable, the universal SMILES format. Solute descriptors for each chemical are given in Table S3.

Text S2. *Estimation of chemical volume of distribution*

Apparent chemical volumes of distribution (V_D; L blood kg fish^{-1}) for chemicals ranging in log K_{OW} between 2 and 9 are summarized for several fish species in Table 3 of the main text. The V_D may be obtained directly using a traditional pharmacokinetic test design wherein a bolus dose of chemical is administered by intravascular (IV) injection and the chemical concentration in blood is measured over time [3] (Figure 6C of the main text). For chemicals that exhibit simple 1-

compartment elimination kinetics, the V_D can be determined as the administered dose divided by the chemical concentration at t=0 (C_0) or the dose divided by the product of the area under the curve (AUC) and the estimated elimination rate constant ($k_{T,B}$).

Alternatively, V_D may be determined as the ratio of fish-water and blood-water partitioning values (V_D=K_{FW}/K_{BW}; Equation 4 of main text). The K_{BW} may be determined *in vivo* from measured chemical concentrations in blood and expired water [4] or *in vitro* using different partitioning systems [5,6]. The K_{BW} may also be estimated from *in vitro* binding data reported in trout blood plasma [7−9] as:

$$K_{BW} = v_{WB}/\phi_B \quad \text{(S2)}$$

where ϕ_B is the unbound chemical fraction in blood plasma (unitless) and v_{WB} is the water content of blood (0.89 L water L blood^{-1} [5]).

For V_D values derived as K_{FW}/K_{BW}, the standard deviation of V_D for each test chemical (SD_{V_D}) can be propagated from the standard deviations of K_{FW} ($SD_{K_{FW}}$) and K_{FW} ($SD_{K_{BW}}$) using the equation:

$$SD_{V_D} = V_D\sqrt{(SD_{K_{FW}}/K_{FW})^2 + (SD_{K_{BW}}/K_{BW})^2} \quad \text{(S3)}$$

Ideally, for the derivation of V_D, the K_{FW} would be measured in the same study as K_{BW} using the same measurement approach. However, for many studies the K_{FW} was not reported and in these cases, we used Equation 11 (Table 1 of main text) to estimate fish-water partitioning. In such cases, the SD_{KFW} was unavailable and was therefore not included in the calculation of SD_{VD} (Equation S3).

TABLES

Table S1. Independent variable inputs to the *in vitro-in vivo* extrapolation (IVIVE; [10]) and bioaccumulation (B; [11]) sub models.

Parameter	Value	Description[a]
Octanol-water partition ratio (K_{OW}; L water L octanol^{-1})		Varied between 2 and 9
Body weight of modelled fish (W_B; kg fish)	0.01	Assumed
Liver S9 protein concentration (C_{S9}; mg protein mL S9^{-1})	1.0	Assumed
Substrate depletion rate constant (k_{DEP}; h^{-1})		Varied between 0.01 h^{-1} and 10 h^{-1}
Fish acclimatation temperature (T; °C)	15	Assumed
Liver S9 protein content (L_{S9}; mg protein g liver^{-1})	163	[10]
Fractional liver weight (L_{FBW}; g liver g fish^{-1})	0.015	[12]
Fractional liver blood flow (Q_{FRAC}; unitless)	0.259	[13]
Fractional water content of blood (v_{WB}; L water L blood^{-1})	0.889	[5]
Fractional total lipid content of blood (v_{LB}; L lipid L blood^{-1})	0.014	[5]
Fractional total protein content of blood (v_{PB}; L protein L blood^{-1})	0.096	$1-(v_{LB}+v_{WB})$
Fractional total protein content of S9 (v_{PS9}; L protein L S9^{-1})	0.0010	[14]
Fractional total lipid content of S9 (v_{LS9}; L lipid L S9^{-1})	0.0003	[14]
Fractional water content of S9 (v_{WS9}; L water L S9^{-1})	0.9987	$1-(v_{LS9}+v_{PS9})$
Fractional whole-body total lipid content (v_{LF}; L lipid L fish^{-1})	0.050	Assumed
Fractional whole-body total protein content (v_{LF}; L lipid L fish^{-1})	0.161	[2]
Fractional whole-body water content (v_{PF}; L lipid L fish^{-1})	0.789	$1-(v_{LF}+v_{PF})$
Density of fish (d_F; kg fish L water^{-1})	1.00	Assumed
Particulate organic carbon content (C_{POC}; kg POC L water^{-1})	4.6×10^{-6}	[15]
POC binding constant (α_{POC}; unitless)	0.35	[16]
Dissolved organic carbon content (C_{DOC}; kg DOC L water^{-1})	1.0×10^{-6}	[15]
DOC binding constant (α_{DOC}; unitless)	0.08	[17]
Total aqueous chemical concentration (C_W; mg chemical L water^{-1})	1.0	Assumed

[a]Describes the parameter, its reference, or the equation used to estimate it.

Table S2. Dependent variable inputs to the *in vitro-in vivo* extrapolation [10] and bioaccumulation [11] sub models.

***In vitro-in vivo* extrapolation model**		
Parameter	**Equation**	**Reference**
In vitro intrinsic clearance rate ($CL_{INT,S9}$; mL S9 h^{-1} mg^{-1})	k_{DEP}/C_{S9}	
In vivo intrinsic clearance rate ($CL_{INT,LIV}$; L S9 d^{-1} kg^{-1})	$CL_{INT,LIVS9}\times L_{S9}\times L_{FBW}\times 24$	
Blood-water partition ratio (K_{BW}; L water L $blood^{-1}$)	Varied	Table 1 of main text
S9 system-water partition ratio (K_{S9W}; L water L $S9^{-1}$)	Varied	Table 1 of main text
Unbound fraction in S9 system (ϕ_{S9}; unitless)	v_{WS9}/K_{S9W}	
Unbound fraction in blood plasma (ϕ_P; unitless)	v_{WBL}/K_{BW}	
Clearance binding term (f_U; unitless)	ϕ_P/ϕ_{S9}	
Cardiac output (Q_C; L blood d^{-1} kg^{-1})	$([(0.23\times T)-0.78]\times[W_B/500]^{-0.1})\times 24\times 10^3$	[18]
Tissue blood flow (Q_H; L blood d^{-1} kg^{-1})	$Q_C\times Q_{FRAC}$	[13]
Hepatic clearance (CL_H; L blood d^{-1} kg^{-1}	$([Q_H\times f_U\times CL_{INT,LIV}]\times[v_{WS9}/v_{WBL}])$ / $(Q_H+[f_U\times CL_{INT,LIV}]\times[v_{WS9}/v_{WBL}])$	[19,20]
Fish-water partition ratio (K_{FW}; L water kg $fish^{-1}$)	Varied	Table 1 of main text
Apparent volume of distribution (V_D; L blood kg $fish^{-1}$)	K_{FW}/K_{BW}	
Whole-body biotransformation rate constant (k_B; d^{-1})	CL_H/V_D	
Bioaccumulation model		
Parameter	**Equation**	**Reference**
Gill uptake rate constant (k_1; L water kg $fish^{-1}$ d^{-1})	$1 / ((0.01 + K_{OW}^{-1}) \times W_B^{0.4}$	[11]
Gill elimination rate constant (k_2; d^{-1})	$k_1/(v_{LF} \times K_{OW})$	[11]
Fecal egestion rate constant (k_E; d^{-1})	$0.125 \times (0.02 \times W_B^{-0.15} \times e^{(0.06 - T)})$ / $(5.1\times10^{-8} \times K_{OW} + 2)$	[11]
Growth rate constant (k_G; d^{-1})	Assumed negligible (0 d^{-1})	[11]
Freely dissolved chemical fraction in water (Φ; unitless)	$1/(1+C_{DOC}\times\alpha_{DOC}\times K_{OW}+C_{POC}\times\alpha_{POC}\times K_{OW})$	[11]
Bioconcentration Factor (BCF; L water kg $fish^{-1}$)	$((k_1\times C_W\times\Phi]/[k_2+k_B+k_G+k_E])/C_W$	[11]

Table S3. Chemical solute descriptors (*E*, *S*, *A*, *B*, and *V*; Ulrich et al. [2]) and polyparameter linear free energy relationship (ppLFER)-estimated partition ratios for storage lipid-water (K_{SLW}); membrane lipid-water (K_{MLW}), albumin protein-water (K_{APW}), and non-albumin protein-water (K_{NPW}) partitioning (Equation S1).

Chemical	***E***	***S***	***A***	***B***	***V***	**log K_{MLW}**	**log K_{SLW}**	**log K_{APW}**	**log K_{NPW}**
Paraoxon	0.81	1.71	0.00	1.18	1.89	1.62	1.55	1.65	1.07
Benzene	0.61	0.52	0.00	0.14	0.72	2.22	2.16	1.92	1.14
Tetrachloroethane	0.60	0.76	0.16	0.12	0.88	2.67	2.37	2.44	1.60
Pentachloroethane	0.65	0.66	0.17	0.06	1.00	3.40	3.25	3.03	2.23
Methyltestosterone	1.54	2.51	0.21	1.27	2.52	3.36	3.05	3.26	2.72
Carprofen	2.07	2.18	1.03	0.89	1.94	3.53	1.54	3.42	2.75
Parathion	1.20	1.49	0.00	0.88	2.00	3.51	3.73	3.11	2.59
Hexachloroethane	0.68	0.68	0.00	0.00	1.12	4.02	4.29	3.51	2.74
Cyclo salicylate	1.20	1.29	0.02	0.47	1.73	4.25	4.50	3.73	3.11
Pyrene	2.81	1.71	0.00	0.28	1.58	5.35	5.40	4.40	3.85
Chlorpyrifos	1.37	1.36	0.00	0.61	2.15	5.21	5.73	4.50	4.01
Polysantol	0.65	0.78	0.31	0.58	2.09	5.02	5.18	4.43	3.92
Methoxychlor	1.85	2.08	0.00	0.82	2.37	5.01	5.33	4.44	3.93
Ambrofix	0.63	0.64	0.00	0.50	2.10	5.40	6.22	4.63	4.16
Galaxolide	1.09	1.15	0.00	0.63	2.25	5.40	6.08	4.67	4.21
Trifluralin	1.01	1.20	0.00	1.08	2.20	3.53	3.93	3.05	2.67
Karanal	0.64	1.04	0.00	0.74	2.36	5.12	5.90	4.50	4.05
Nonylphenol	0.78	0.89	0.53	0.33	2.04	5.83	5.63	5.21	4.60
PCB 52	1.90	1.48	0.00	0.15	1.81	6.07	6.50	5.20	4.58
PCB 153	2.18	1.74	0.00	0.11	2.06	7.04	7.58	6.05	5.44
Diethylhexylphthalate	0.66	1.06	0.00	0.90	3.40	7.97	9.50	6.92	6.70
PCB 202	2.44	2.00	0.00	0.06	2.30	8.04	8.70	6.93	6.33
PCB 209	2.72	2.26	0.00	0.02	2.55	9.01	9.78	7.78	7.20

Table S4. Log-transformed trout liver S9-water (K_{S9W}), blood-water (K_{BW}), and whole fish-water (K_{FW}) chemical partition ratios estimated using empirically-based (Emp), composition-based (Comp), and polyparameter linear free energy relationship (ppLFER) prediction methods.

Chemical	**log K_{OW}**	**log K_{S9W}**			**log K_{BW}**			**log K_{FW}**		
		Emp	**Comp**	**ppLFER**	**Emp**	**Comp**	**ppLFER**	**Emp**	**Comp**	**ppLFER**
Paraoxon	2.00	0.07	0.01	0.01	0.74	0.45	0.51	0.68	0.82	0.79
Benzene	2.13	0.08	0.02	0.02	0.82	0.54	0.69	0.81	0.94	1.08
Tetrachloroethane	2.39	0.12	0.04	0.05	0.99	0.75	1.05	1.07	1.18	1.40
Pentachloroethane	3.22	0.34	0.21	0.24	1.57	1.51	1.73	1.90	1.99	2.16
Methyltestosterone	3.36	0.40	0.27	0.27	1.67	1.65	1.93	2.04	2.13	2.23
Carprofen	3.79	0.60	0.52	0.19	1.97	2.08	1.96	2.47	2.55	2.16
Parathion	3.83	0.62	0.55	0.50	2.00	2.12	2.03	2.51	2.59	2.55
Hexachloroethane	4.14	0.79	0.79	0.90	2.23	2.42	2.47	2.82	2.90	3.06
Cyclo salicylate	4.70	1.14	1.30	1.10	2.64	2.98	2.71	3.38	3.46	3.28
Pyrene	4.90	1.27	1.49	1.96	2.78	3.18	3.63	3.58	3.66	4.20
Chlorpyrifos	4.96	1.31	1.55	2.27	2.83	3.24	3.81	3.64	3.72	4.46
Polysantol	5.00	1.33	1.59	1.77	2.85	3.28	3.45	3.68	3.76	3.99
Methoxychlor	5.08	1.39	1.66	1.90	2.91	3.36	3.52	3.76	3.84	4.10
Ambrofix	5.10	1.40	1.68	2.75	2.93	3.38	4.22	3.78	3.86	4.92
Galaxolide	5.30	1.53	1.88	2.62	3.07	3.58	4.12	3.98	4.06	4.79
Trifluralin	5.34	1.56	1.92	0.63	3.10	3.62	2.14	4.02	4.10	2.71
Karanal	5.60	1.74	2.18	2.43	3.29	3.88	3.92	4.28	4.36	4.60
Nonylphenol	5.76	1.85	2.34	2.26	3.41	4.04	4.09	4.44	4.52	4.55
PCB 52	6.10	2.08	2.68	3.03	3.66	4.38	4.57	4.78	4.86	5.22
PCB 153	6.34	2.24	2.92	4.11	3.83	4.62	5.61	5.02	5.10	6.29
Diethylhexylphthalate	7.60	3.12	4.18	6.02	4.75	5.88	7.42	6.28	6.36	8.17
PCB 202	7.73	3.21	4.31	5.22	4.85	6.01	6.69	6.41	6.49	7.39
PCB 209	8.27	3.58	4.85	6.30	5.24	6.55	7.75	6.95	7.03	8.47

Table S5. Linear regression equations obtained by regressing log transformed chemical partition ratios against log K_{OW}. The partition ratios were generated for trout liver S9 (K_{S9W}), blood (K_{BW}), and whole fish (K_{FW}) for test chemicals presented in Table 3 of the main text.

Phase	**Partitioning approach**	**Entire log K_{OW} range**	**log K_{OW} > 4**
Liver S9 system	Empirical	$\log K_{S9W} = 0.58 \times \log K_{OW} - 1.46$; $R^2 = 0.97$	$\log K_{S9W} = 0.68 \times \log K_{OW} - 2.08$; $R^2 = 0.99$
	Composition-based	$\log K_{S9W} = 0.77 \times \log K_{OW} - 2.24$; $R^2 = 0.94$	$\log K_{S9W} = 0.99 \times \log K_{OW} - 3.54$; $R^2 = 0.99$
	ppLFER	$\log K_{S9W} = 0.91 \times \log K_{OW} - 2.77$; $R^2 = 0.85$	$\log K_{S9W} = 1.26 \times \log K_{OW} - 4.90$; $R^2 = 0.90$
Blood	Empirical	$\log K_{BW} = 0.72 \times \log K_{OW} - 0.74$; $R^2 = 0.99$	$\log K_{BW} = 0.73 \times \log K_{OW} - 0.79$; $R^2 = 1.00$
	Composition-based	$\log K_{BW} = 0.98 \times \log K_{OW} - 1.61$; $R^2 = 0.99$	$\log K_{BW} = 1.00 \times \log K_{OW} - 1.72$; $R^2 = 1.00$
	ppLFER	$\log K_{BW} = 1.13 \times \log K_{OW} - 2.10$; $R^2 = 0.93$	$\log K_{BW} = 1.35 \times \log K_{OW} - 3.24$; $R^2 = 0.88$
Whole fish	Empirical	$\log K_{FW} = 1.00 \times \log K_{OW} - 1.32$; $R^2 = 1.00$	$\log K_{FW} = 1.00 \times \log K_{OW} - 1.32$; $R^2 = 1.00$
	Composition-based	$\log K_{FW} = 0.99 \times \log K_{OW} - 1.20$; $R^2 = 1.00$	$\log K_{FW} = 1.00 \times \log K_{OW} - 1.24$; $R^2 = 1.00$
	ppLFER	$\log K_{FW} = 1.22 \times \log K_{OW} - 1.99$; $R^2 = 0.93$	$\log K_{FW} = 1.37 \times \log K_{OW} - 2.91$; $R^2 = 0.88$

FIGURES

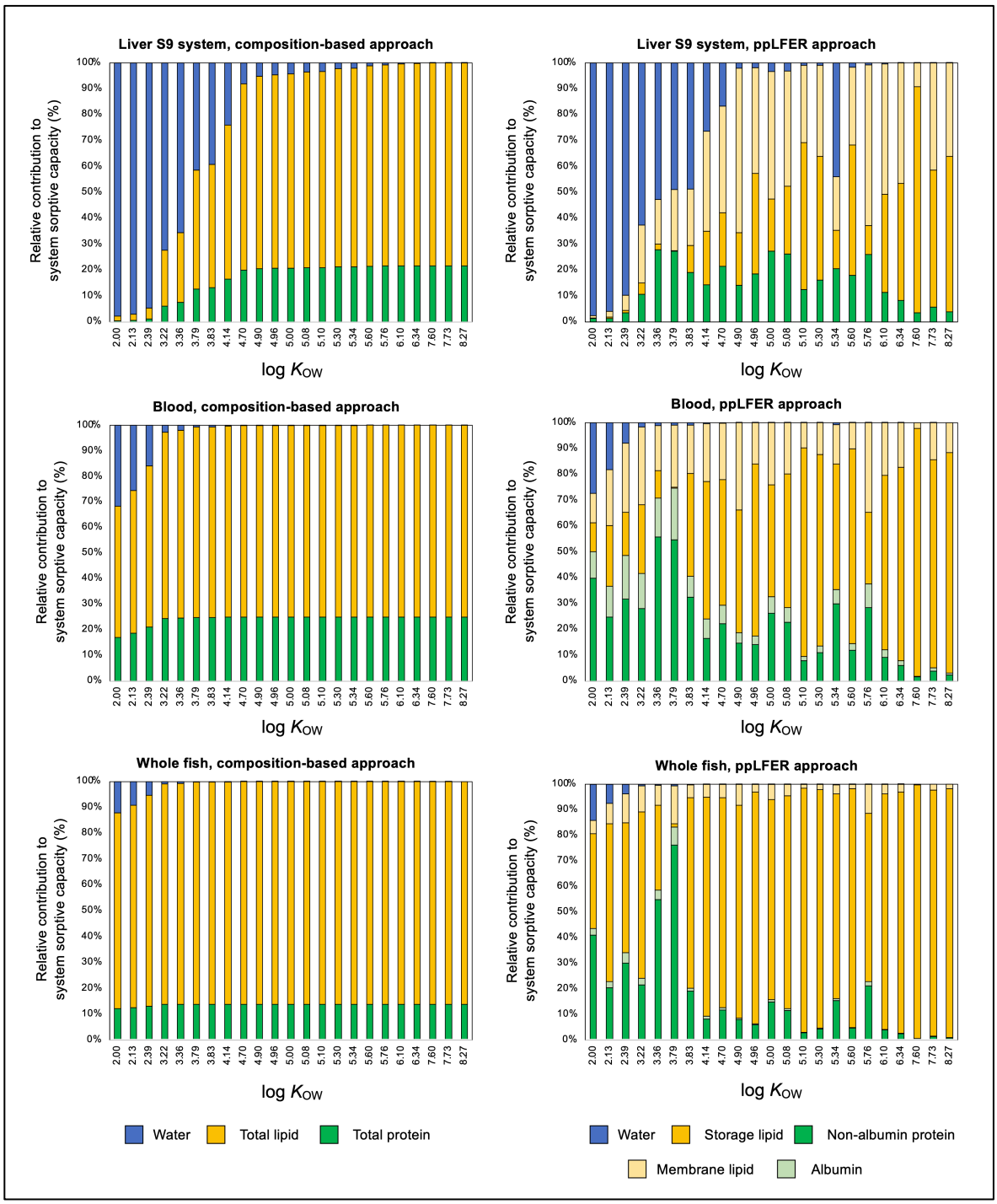

Figure S1. Relative contribution of lipid, protein, and water components (x) to the system (S) sorptive capacity for liver S9 incubation media, blood, and whole fish, estimated using composition-based and ppLFER prediction approaches. Volume fractions (v) for each component used are presented in Table 2 of the main text and the calculated system partition ratios (K_{SW}) for trout liver S9, blood, and whole fish are in Table S4. The relative contribution for each component was calculated as $v_X \times K_{XW}/K_{SW}$, where K_{XW} is the partition ratio for x-water partitioning [1].

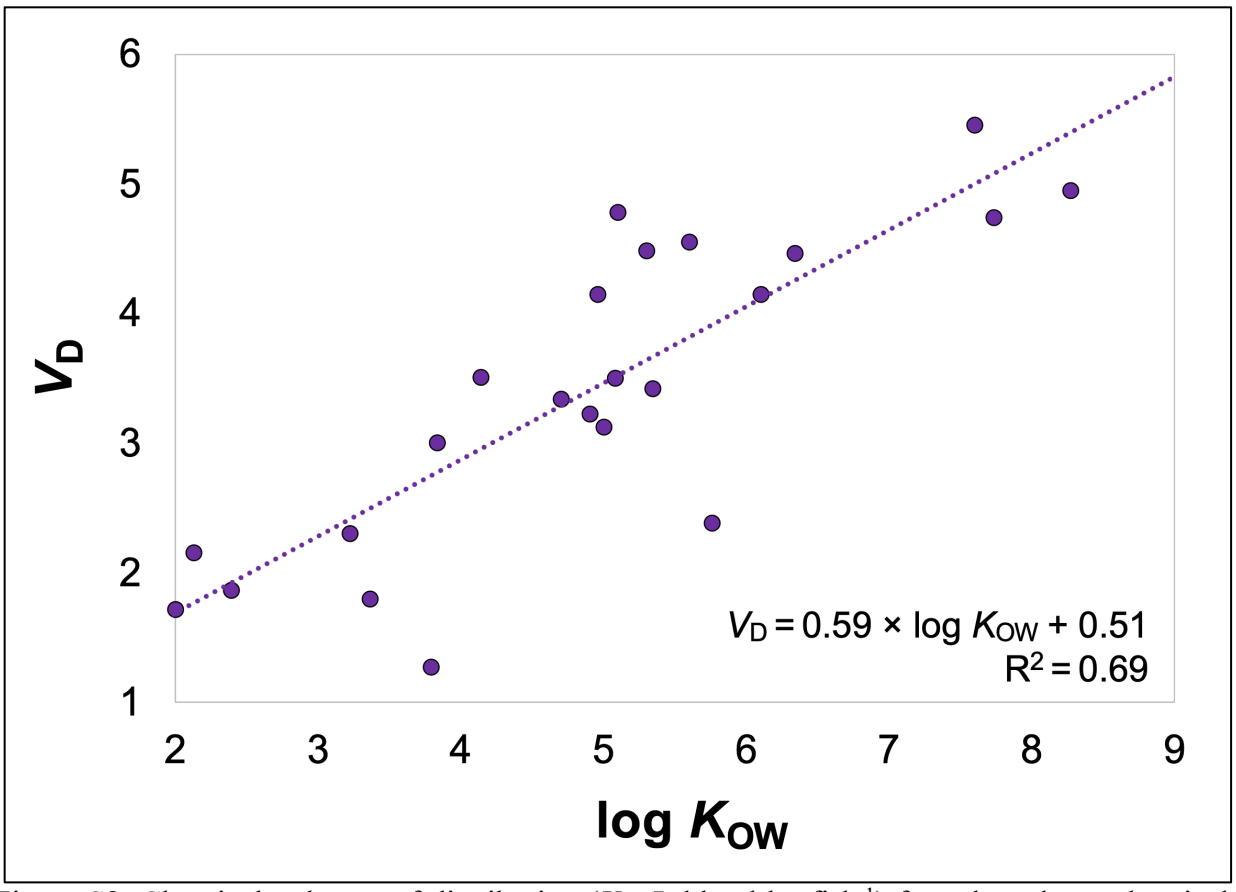

Figure S2. Chemical volumes of distribution (V_D; L blood kg fish^{-1}) for selected test chemicals calculated using K_{BW} (Equation 13 of main text) and K_{FW} (Equation 14 of main text) values estimated from ppLFERs. The equation for the linear model (purple dotted line) describing the relationship between V_D and log K_{OW} is provided in the figure.

REFERENCES

[1] Endo S, Brown TN, Goss KU. 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.

[2] Ulrich N, Endo S, Brown TN, Watanabe N, Bronner G, Abraham MH, Goss, KU. 2017. UFZ-LSER database v 3.2, Helmholtz Centre for Environmental Research- UFZ, Leipzig, Germany.

[3] Kleinow KM, Nichols JW, Hayton WL, McKim JM, Barron MG. 2008. Toxicokinetics in fishes. In: Di Giulio, R.T., Hinton, D. E. (Eds.), The Toxicology of Fishes. CRC Press, Boca Raton, FL, pp. 55–152.

[4] Fitzsimmons PN, Fernandez JD, Hoffman AD, Butterworth BC, Nichols JW. 2001. Branchial elimination of superhydrophobic organic compounds by rainbow trout (*Oncorhynchus mykiss*). *Aquat Toxicol.* 55:23–34.

[5] Bertelsen SL, Hoffman AD, Gallinat CA, Elonen CM, Nichols JW. 1998. Evaluation of logK_{OW} and tissue lipid content as predictors of chemical partitioning to fish tissues. *Environ. Toxicol. Chem*. 17:1447–1455.

[6] Nichols, J.W., McKim, J.M., Lien, G.J., Hoffman, A.D., Bertelson, S.L. 1991. Physiologically based toxicokinetic modeling of three waterborne chloroethanes in rainbow trout (*Oncorhynchus mykiss*). *Toxicol. Appl. Pharmacol.* 110, 374–389.

[7] Schultz IR, Hayton WL. 1999. Interspecies scaling of the bioaccumulation of lipophilic xenobiotics in fish: An example using trifluralin. *Environ Toxicol Chem* 18:1440−1449

[8] Escher BI, Cowan-Ellsberry CE, Dyer S, Embry MR, Erhardt S, Halder M, Kwon J-H, Johanning K, Oosterwijk MTT, Rutishauser S, Segner H, Nichols JW. 2011. Protein and lipid binding parameters in rainbow trout (*Oncorhynchus mykiss*) blood and liver fractions to extrapolate from an in vitro metabolic degradation assay to in vivo bioaccumulation potential of hydrophobic organic chemicals. *Chem Res Toxicol* 24:1134–1143.

[9] Laue H, Hostettler L, Badertscher RP, Jenner KJ, Sanders G, Arnot JA, Natsch A. 2020. Examining uncertainty in in vitro-in vivo extrapolation applied in fish bioconcentration models. *Environ. Sci. Technol.* 54:9483−9494

[10] Nichols JW, Huggett DB, Arnot JA, Fitzsimmons PN, Cowan-Ellsberry CE. 2013. Towards improved models for predicting bioconcentration of well-metabolized compounds by rainbow trout using measured rates of in vitro intrinsic clearance. *Environ Toxicol Chem.* 32:1611−1612.

[11] Arnot JA, Gobas F. 2003. A generic QSAR for assessing the bioaccumulation potential of organic chemicals in aquatic food webs. *QSAR Comb Sci.* 22:337−345.

[12] Schultz IR, Hayton WL. 1999. Interspecies scaling of the bioaccumulation of lipophilic xenobiotics in fish: An example using trifluralin. *Environ Toxicol Chem* 18:1440−1449

[13] Nichols JW, McKim JM, Andersen ME, Gargas ML, Clewell HJ, Erickson RJ. 1990. A physiologically based toxicokinetic model for the uptake and disposition of waterborne organic chemicals in fish. *Toxicol Appl Pharmacol* 106:433–447.

[14] Saunders LJ, Diaz-Blanco G, Lee Y-S, Otton SV, Gobas FAPC. 2020a. Hepatic clearance binding terms of in rainbow trout: Application of a streamlined sorbent-phase dosing method. *Environ Sci Technol Let.* 7, 672–676.

[15] US Environmental Protection Agency. 2003. Methodology for deriving ambient water quality criteria for the protection of human health (2000) Technical support document, Vol 2: Development of national bioaccumulation factors. EPA 822/R-03/030. Washington, DC.

[16] Seth R, Mackay D, Muncke J. 1999. Estimating the Organic Carbon Partition Coefficient and Its Variability for Hydrophobic Chemicals. *Environ Sci Technol.* 33:2390−2394.

[17] Burkhard LP. 2000. Estimating Dissolved Organic Carbon Partition Coefficients for Nonionic Organic Chemicals. *Environ Sci Technol.* 22:4663−4668.

[18] Erickson RJ, McKim JM. 1990. A model for exchange of organic chemicals at fish gills: flow and diffusion limitations. *Aquat Toxicol.* 18:175−198.

[19] Wilkinson GR, Shand DG. 1975. Commentary: A physiological approach to hepatic drug clearance. *Clin Pharmacol Ther.* 18:377−390.

[20] Krause, S., Goss, K.-U. 2018. In vitro-in vivo extrapolation of hepatic metabolism for different scenarios - A toolbox. *Chem. Res. Toxicol.* 31. 1195–1202.