Supporting Information:

Could chemical exposure and bioconcentration in fish be affected by slow binding kinetics in blood?

Sophia Krause[a,*], Kai-Uwe Goss[a,b]

[a] Helmholtz Centre for Environmental Research, Department of Analytical Environmental Chemistry, Permoserstr. 15, 04318 Leipzig, Germany

[b] University of Halle-Wittenberg, Institute of Chemistry, Kurt-Mothes-Str. 2, 06120 Halle, Germany

*Address correspondence to sophia.krause@ufz.de

Content

Section 1: Details on the test chemicals used for the desorption experiments

The partition coefficients of the test chemicals for plasma $K_{plasma/water}$ are estimated using poly-parameter free energy relationships (ppLFERs). The general equation for this approach is the following [1]:

$$K_{plasma/water} = protein_{plasma} * K_{protein/water} + albumin_{plasma} * K_{albumin/water} + storage\ lipid_{plasn} * K_{storage\ lipid/water} + membrane\ lipid_{plasma} * K_{membrane\ lipid/water} + water_{plasma} \quad (1)$$

In this equation, $protein_{plasma}$ is the non-albumin protein content of plasma (as volume fraction mL/mL), $albumin_{plasma}$ is the albumin content of plasma (as volume fraction) etc. The composition data used for the estimation of $K_{plasma/water}$ are provided in Table S**1** (as volume fractions mL/mL). The data for albumin and non-albumin protein is derived from the given protein content of plasma (assuming a density of 1.38 g/mL for unit conversion [2]), the data for lipid is taken from the literature [3].

Table S1: Composition data used for estimation of the plasma-water partition coefficients.

water w_{plasma}	$albumin_{plasma}$	$protein_{plasma}$	storage $lipid_{plasma}$	membrane $lipid_{plasma}$
0.965	0.006	0.009	0.010	0.010

The chemical specific protein-water, albumin-water, storage lipid-water and membrane lipid-water partition coefficients ($K_{protein/water}$, $K_{albumin/water}$, $K_{storage\ lipid/water}$, $K_{membrane\ lipid/water}$) can be retrieved from the UFZ LSER database [4]. From the calculated plasma-water partition coefficient $K_{plasma/water}$, the partition coefficient between the sorbing plasma components only and water is derived:

$$K_{sorbing\ plasma\ components/water} = \frac{K_{plasma/water} - water_{plasma}}{(1 - water_{plasma})} \quad (2)$$

Table S2 lists the used test chemicals with CAS numbers, octanol-water partition coefficients log K_{OW} (retrieved from the UFZ LSER database), the used plasma dilutions and used chemical concentrations as well as the calculated partition coefficients between sorbing plasma components and water log $K_{sorbcomp/w}$. Note that the octanol-water partition coefficients are only included here to provide insight on the chemical's hydrophobicity; the octanol-water partition coefficients are not used for the estimation of the partition coefficient between sorbing plasma components and water (instead log $K_{sorbcomp/w}$ is estimated using eq. (1) and (2) above).

Table S2: Details on the used test chemicals.

test chemical	CAS	log K_{OW} [L/L]	used plasma dilutions	used chemical concentration [mg/L]	log $K_{sorbcomp/w}$ estimated [L/L]
phenanthrene	85-01-8	4.4	20x, 50x	0.25	4.32
n-propylbenzene	103-65-1	3.7	2x, 5x	0.5	3.48
1,8-dibromooctane	4549-32-0	4.8	25x, 100x	0.1	4.57
1,2,3,4-tetrachlorobenzene	634-66-2	4.6	20x, 50x	0.1	4.70
di-n-pentylether	693-65-2	4.3	5x, 25x	0.5	3.83
n-hexylbenzene	1077-16-3	5.3	25x, 100x	0.05	5.11
chlorpyrifos	2921-88-2	5.2	20x, 62.5x	0.1	4.31

1,4-dibromobenzene	106-37-6	3.8	2x, 5x	0.5	3.90
pyrene	129-00-0	4.6	50x, 100x	0.025	4.71
1,2,4-trichlorobenzene	120-82-1	4.1	5x, 25x	0.5	4.12

Section 2: Models for quantitative evaluation of the impact of sorption kinetics

To investigate the influence of sorption kinetics in gill blood on chemical uptake, two steady-state models are compared. Both models represent the uptake of the substance via ventilation, the transport into the periphery of the organism with the blood flow and the elimination of the chemical in the periphery. One model represents a scenario with sorption kinetics in the blood, the other model represents a scenario with instantaneous equilibrium in the blood. Both models are expressed in the form of linear systems of equations and solved in Excel for steady state condition.

a) Model with instantaneous binding equilibrium in blood

The model assuming instantaneous binding equilibrium in blood is depicted in Figure S1. Chemical uptake, elimination via biotransformation and exchange via blood flow are modelled as kinetic processes and instantaneous equilibrium between gills and gill blood and between periphery and peripheral blood is assumed.

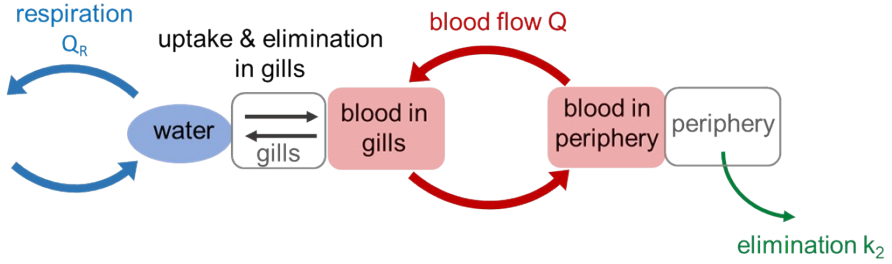


Figure S1: Schematic representation of the model assuming instantaneous binding equilibrium in blood. Kinetic exchange between respired water, blood in gills and blood in periphery is modelled.

The following mass balances can be formulated for the different compartments:

$$\frac{dM_{water}}{dt} = Q_R\left(C_{W,in} - C_{W,out}\right) + P_{gills} * A_{gills}\left(\frac{C_{blood,gills}}{K_{blood/water}} - C_{W,out}\right) \quad (3)$$

Here, Q_R is the respiration rate (L_W/d), $C_{W,in}$ and $C_{W,out}$ the chemical concentrations in inflowing and outflowing ventilated water (mol/L_W), $P_{gills} * A_{gills}$ (L_W/d) the permeability surface area product for permeation through the gills, $C_{blood,gills}$ is the chemical concentration in blood flowing out of the gills and $K_{blood/water}$ is the blood-water partition coefficient of the chemical.

$$\frac{dM_{blood\ in\ gills}}{dt} = Q\left(C_{blood,periphery} - C_{blood,gills}\right) + P_{gills} * A_{gills}\left(C_{W,out} - \frac{C_{blood,gills}}{K_{blood/water}}\right) \quad (4)$$

Here, Q is the blood flow rate through the gills (L_{blood}/d), $C_{blood,periphery}$ is the chemical concentration in blood flowing from periphery into gills.

$$\frac{dM_{blood\ in\ periphery}}{dt} = Q\left(C_{blood,gills} - C_{blood,periphery}\right) - k_2 V_{periphery} C_{blood,periphery} K_{periphery/blood} \quad (5)$$

Here, k_2 is the elimination rate constant in the periphery (1/d), $V_{periphery}$ is the volume of the periphery and $K_{periphery/blood}$ is the periphery-blood partition coefficient. To represent steady state condition, all mass balances are set to $\frac{dM}{dt} = 0$.

b) Model with sorption kinetics in blood

The model representing sorption kinetics in blood is illustrated in Figure S2. Again chemical uptake, elimination via biotransformation and exchange via blood flow are modelled as kinetic processes. Additionally, transitioning of the chemical between bound and freely dissolved state, i.e. transitioning between aqueous and non-aqueous blood, is also represented as kinetic process. Because of the discrimination between aqueous and non-aqueous blood, a discrimination must also be made here between a flow rate of aqueous and non-aqueous blood (Q_{free} and Q_{bound}). Between gills and aqueous gill blood and between periphery and aqueous peripheral blood instantaneous equilibrium is assumed.

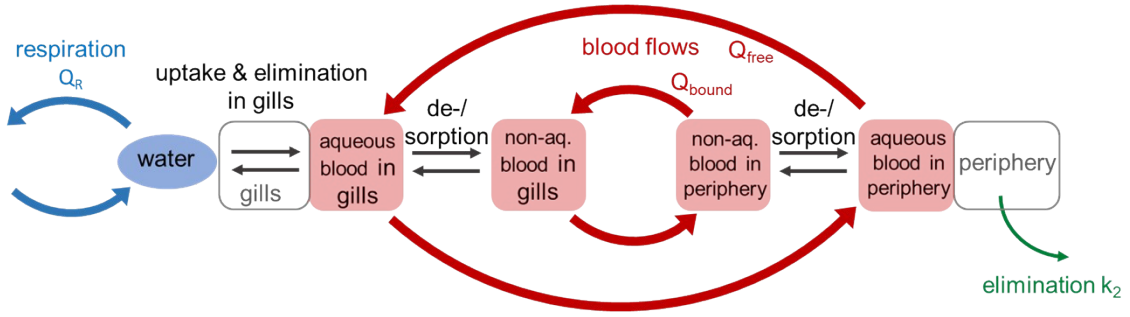


Figure S2: Schematic representation of the model representing sorption kinetics in blood. In this model, kinetic exchange between respired water, aqueous blood in the gills, non-aqueous blood in the gills and aqueous and non-aqueous blood in the periphery is modelled.

The following mass balances can be formulated for the different compartments:

$$\frac{dM_{water}}{dt} = Q_R\,(C_{W,in} - C_{W,out}) + P_{gills} * A_{gills}\,(C_{blood,gills}^{free} - C_{W,out}) \quad (6)$$

Here, the variables Q_R, $C_{W,in}$, $C_{W,out}$ and $P_{gills} * A_{gills}$ are identical to the ones used in eq. (3). $C_{blood,gills}^{free}$ is the freely dissolved chemical concentration in gill blood.

$$\frac{dM_{aqueous\ blood\ in\ gills}}{dt} = Q_{free}\left(C_{blood,periphery}^{free} - C_{blood,gills}^{free}\right) + P_{gills} * A_{gills}\,(C_{W,out} - C_{blood,gills}^{free}) - k_{sorb} * C_b * V_{sorb,gills} + k_{des} * C_{blood,gills}^{bound} * V_{sorb,gills} \quad (7)$$

Here, Q_{free} is the flow rate of the aqueous portion of blood (L_W/d), k_{sorb} ($L_W/L_{sorb\ comp}$/d) and k_{des} (1/d) are sorption or desorption rate constant for binding to non-aqueous blood constituents, $V_{free,gills}$ is the aqueous volume of gill blood, $V_{sorb,gills}$ is the non-aqueous volume of gill blood, $C_{blood,periphery}^{free}$ and $C_{blood,gills}^{free}$ are the freely dissolved chemical concentrations (mol/L_W) in blood flowing into the gills and out of the gills and $C_{blood,gills}^{bound}$ is the bound chemical concentration (mol/$L_{sorb\ component}$) in blood flowing out of the gills.

$$\frac{dM_{non-aqueous\ blood\ in\ gills}}{dt} = Q_{bound}\left(C^{bound}_{blood,periphery} - C^{bound}_{blood,gills}\right) + k_{sorb} * C^{free}_{blood,gills} * V_{sorb,gills} - k_{des} * C^{bo} \quad (8)$$

Q_{bound} is the flow rate of the non-aqueous portion of blood ($L_{sorb\ component}$/d) and $C^{bound}_{blood,periphery}$ is the bound chemical concentration in blood flowing into the gills.

$$\frac{dM_{non-aqueous\ blood\ in\ periphery}}{dt} = Q_{bound}\left(C^{bound}_{blood,gills} - C^{bound}_{blood,periphery}\right) + k_{sorb} * C^{free}_{blood,periphery} * V_{sorb,peripher} * C^{bound}_{blood,periphery} * V_{sorb,periphery} \quad (9)$$

$$\frac{dM_{aqueous\ blood\ in\ periphery}}{dt} = Q_{free}\left(C^{free}_{blood,gills} - C^{free}_{blood,periphery}\right) - k_{sorb} * C * V_{sorb,periphery} - k_2 * V_{periphery} * C^{free}_{blood,periphery} \quad (10)$$

k_2 is the elimination rate constant in the periphery (1/d), $V_{periphery}$ is the volume of the periphery and $K_{periphery/blood}$ is the periphery-blood partition coefficient. To represent steady state condition, all mass balances are set to $\frac{dM}{dt} = 0$.

For calculation of the BCF, the total concentrations in blood flowing into and out of the gills are needed. These total blood concentrations are derived from the provided freely dissolved and bound steady-state blood concentrations ($C_{blood\text{-}free}$ and $C_{blood\text{-}bound}$) according to

$$C_{blood,gills} = \frac{C_{blood-free,gills} * V_{blood-free,gills} + C_{blood-bound,gills} * V_{blood-bound,gills}}{V_{blood,gills}} \quad (11)$$

$$C_{blood,periphery} = \frac{C_{blood-free,periphery} * V_{blood-free,periphery} + C_{blood-bound,periphery} * V_{blood-bound,periphery}}{V_{blood,periphery}} \quad (12)$$

Section 3: Input Parameters for model application

As written in the main text, physiological data for a 10 g fish with 5 % body fat at 15 °C is used. The gill blood flow is assumed to be 100 % of the cardiac output. Cardiac output is calculated using the allometric formula given by Erickson and McKim (Erickson and McKim 1990) from temperature T (° C) and bodyweight m_{body} (g):

$$cardiac\ output\ (L/h/kg_{fish}) = (0.23 * T - 0.78) * \left(\frac{m_{body}}{500}\right)^{-0.1} \quad (13)$$

The volume of gill cells is estimated from the fractional gill weight G_{FBW} (0.0247 g_{gills}/g_{fish}, [5]), gill cell content G_{cells} (556 * 10^6 cells/g_{gills}, [6]) and the gill cell diameter d_{gill} (0.00151 cm, [6]):

$$V_{gills} = G_{cells} * G_{FBW} * m_{body} * \frac{1}{6}\pi {d_{gill}}^3 \quad (14)$$

The blood volume is derived from the fractional blood volume B_{FBW} (0.034 mL_{blood}/g_{body}, [7]):

$$V_{blood} = B_{FBW} * m_{body} \quad (15)$$

The organism-water partition coefficient is calculated as

$$K_{organism/water} = lipid\ content_{organism} * K_{octanol/water} \quad (16)$$

The partition coefficients for the different tissues (e.g. gills and blood) are calculated using eq. (6) presented in the main text.

The following composition information for blood and gills is used (as volume fractions in mL/mL):

- blood [3, 8]

$water_{blood}$	$protein_{blood}$	$lipid_{blood}$
0.89	0.096	0.014

- gills [9]

$water_{gills}$	$protein_{gills}$	$lipid_{gills}$
0.73	0.205	0.065

The ventilation rate is calculated according to the algorithm from Arnot et al. [10]. Based on the assumption that only 70 % of the ventilated volume is actually available for respiration, the respiration rate is calculated as 0.7 * ventilation rate [11].

The uptake of chemicals from the respired water into the blood is estimated via permeability. For doing so, the approach from Larisch et al. [12] is used and it is assumed that a barrier consisting of aqueous boundary layers (ABL), mucus, cell membrane and cytosol must be overcome for uptake into the blood. Separate permeability surface area products ($P_{through\ layer} * A$ in cm^3/s) are calculated for each of the individual layers of this total barrier using the diffusion coefficient of the chemical within the layer $D_{chemical\ in\ layer}$ (cm^2/s), the partition coefficient between layer and water $K_{layer/water}$ (L_{water}/L_{layer}), the exchange surface area between the layers A_{gills} (cm^2) and the layer thickness d_{layer} (cm).

$$P_{through\ layer} * A = D_{chemical\ in\ layer} * K_{layer/water} * A_{gills} * \frac{1}{d_{layer}} \quad (17)$$

These individual permeability surface area products are then used to estimate the total permeability surface area product ($P_{gills} * A$ in cm^3/s) in the gills.

$$P_{gills} * A = \frac{1}{\frac{1}{P_{mucus} * A} + \frac{1}{P_{membrane} * A} + \frac{1}{P_{cytosol} * A} + \frac{1}{P_{ABL} * A}} \quad (18)$$

For the partition coefficients between water and mucus, ABL and cytosol a value of 1 is assumed (i.e. the layers were assumed to have the same sorption capacity as pure water), for the partition coefficient between membrane and water the hexadecane-water partition coefficient serves as a surrogate. We note that assuming an identical sorption capacity of mucus and cytosol as compared to pure water is a simplification leading to a certain parameter uncertainty. However, more precise estimation of the partition coefficients for mucus and water would require precise knowledge of the composition of these two phases. However, since precise compositional data

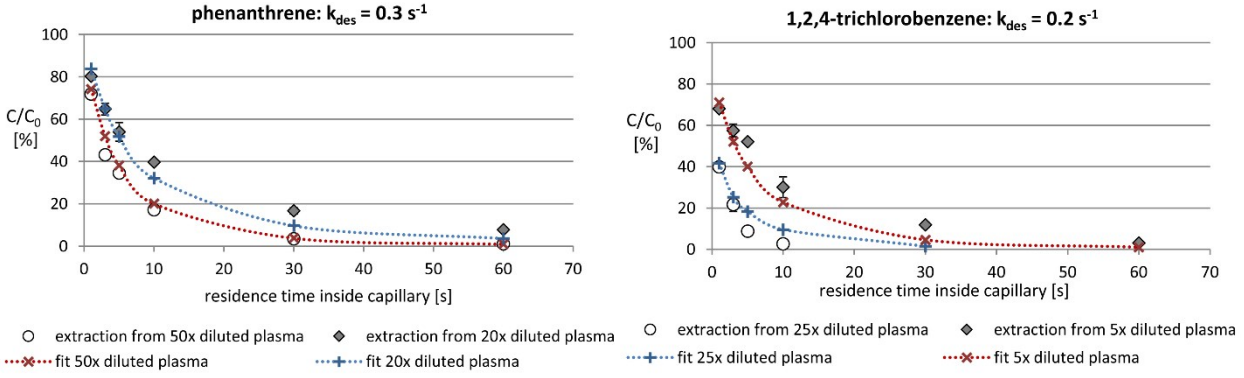
are not available, this procedure would lead to uncertainties in a similar way, except that the estimation would be far more complicated than the assumption we have made. For simplicity, we also assume the value of the hexadecane-water partition coefficient being equal to the octanol-water partition coefficient. For the diffusion coefficients in cytosol and ABL, the diffusion coefficient in pure water is assumed ($D_{chemical\ in\ water}$ = 7.5 * 10^{-6} cm²/s as average value for > 900 chemicals). For the diffusion coefficient in mucus, the higher viscosity of the mucus is taken into account by assuming the diffusion coefficient in pure water divided by 1.7 ('mucus factor' by Larisch et al. [12]). The exchange surface area of the gills is also obtained from Larisch et al. (A_{gills} = 29.4 cm²). The diffusion coefficient in the membrane is estimated as 0.32 $*$ diffusion coefficient in pure water [13]. For the individual layer thicknesses, the 'physiological data sheet' of Larisch et al. [12] is used, where the layer thickness in the cytosol is multiplied by a factor of 2 to take into account the tortuosity (i.e. the intertwined diffusion path of the molecule through the cytosol) yielding d_{mucus} = 6 * 10^{-5} cm, $d_{cytosol}$ = 1.4 * 10^{-3} cm, $d_{membrane}$ = 3.68 * 10^{-5} cm and d_{ABL} = 3 * 10^{-4} cm
For sorption kinetics an arbitrary range of k_{des} from 0.4 1/s to 4 $*$ 10^{-7} 1/s was chosen to evaluate the impact of sorption kinetics. The relationship between desorption rate constant and sorption rate constant is described in SI section 4. For elimination, a whole-body elimination rate constant k_2 of 4 1/d was chosen. This rate constant was estimated using the 'B-compass fish' tool [9] assuming a rather fast hepatic *in vitro* rate constant of 10 1/h [14].

Section 4: Relationship between desorption rate constant and sorption rate constant and concentration-time profiles for all test chemicals

From a known (or assumed) desorption rate constant, the sorption rate constant (k_{sorb} in $L_{water}/L_{sorbing\ component}$/d) can be calculated using the partition coefficient between sorbing blood components and water $K_{sorbing\ components/water}$ (a detailed description on how the required partition coefficient was calculated for the test chemicals is provided in section 1 of this SI) according to the following equation

$$k_{sorb} = k_{des} * K_{sorbing\ components/water} \quad (19)$$

The concentration-time profiles for all test chemicals are shown below:



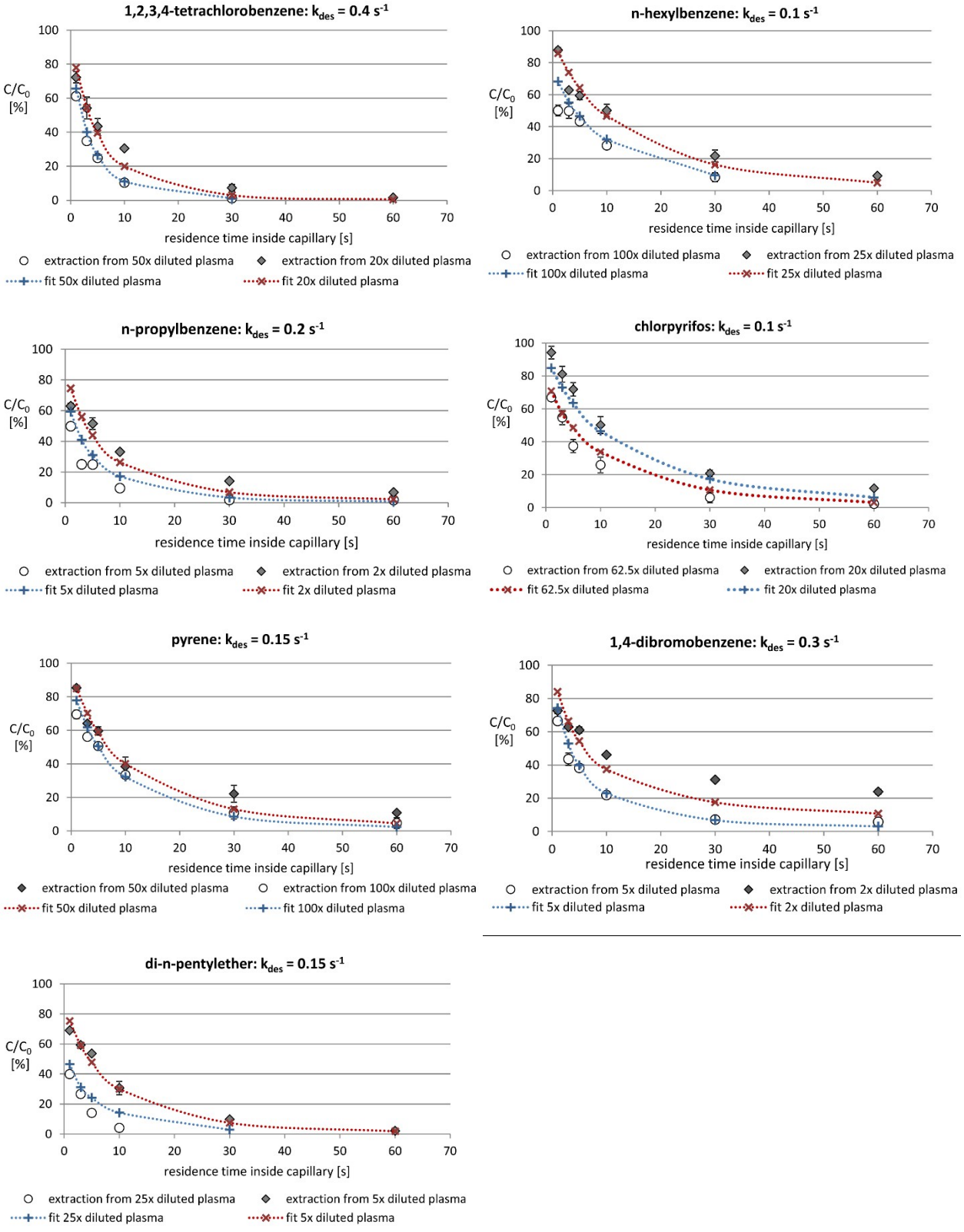


Figure S3: Cocentration-time profiles with corresponding fits for all test chemicals.

Section 5: Modeled impacts of sorption kinetics in blood on uptake, elimination and BCF for a slower whole-body elimination rate constant

As an addition to the calculations presented in the main text, we here present implications of slow sorption kinetics for a scenario of a chemical with a log K_{OW} = 6 and a whole-body elimination rate constant k_2 of 0.4 1/d (i.e. tenfold slower elimination kinetics).

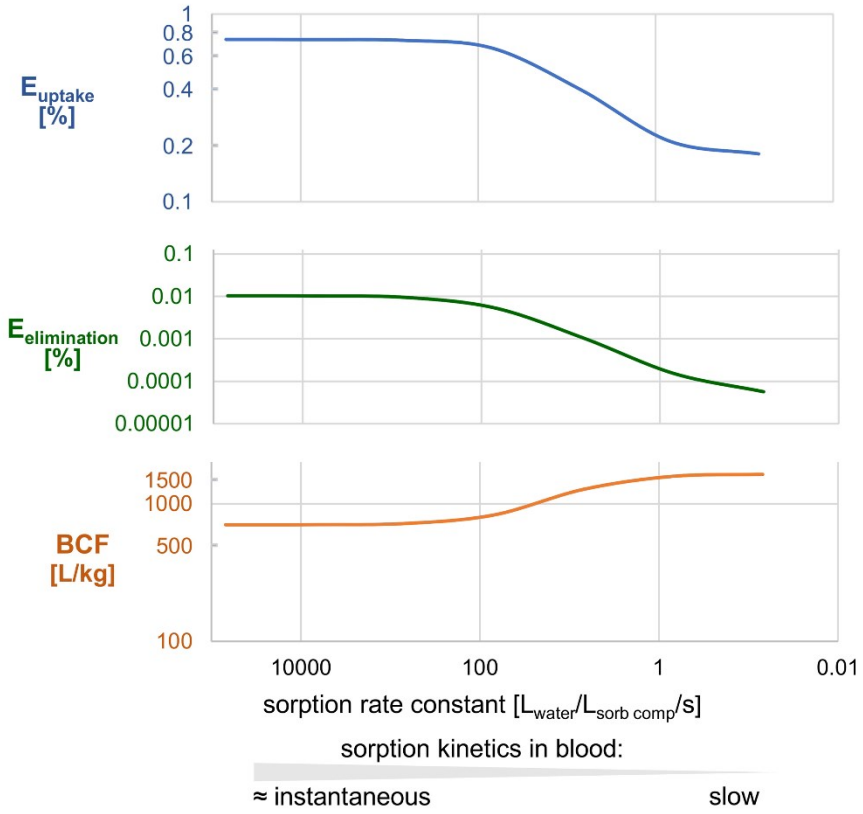


Figure S4: Change in uptake efficiency (E_{uptake}), elimination efficiency ($E_{elimination}$) and bioconcentration factor (BCF) for a scenario of a chemical with a log K_{OW} = 6 and a whole-body elimination rate constant of 0.4 1/d depending on the sorption kinetics in blood.

The figure shows that slower sorption kinetics in blood still lead to decreasing uptake and elimination efficiency and increasing BCF values. However, the magnitude of the effect that occurs is now smaller for elimination efficiency and BCF as compared to the example in the main text.

Section 6: Calculation of the elimination efficiency in dependency of partition coefficient between sorbing plasma components and water and sorption rate constant

As mentioned in the main text, the impact of sorption kinetics decreases with decreasing log K_{OW}. Figure S5 shows this for the elimination efficiency $E_{elimination}$.

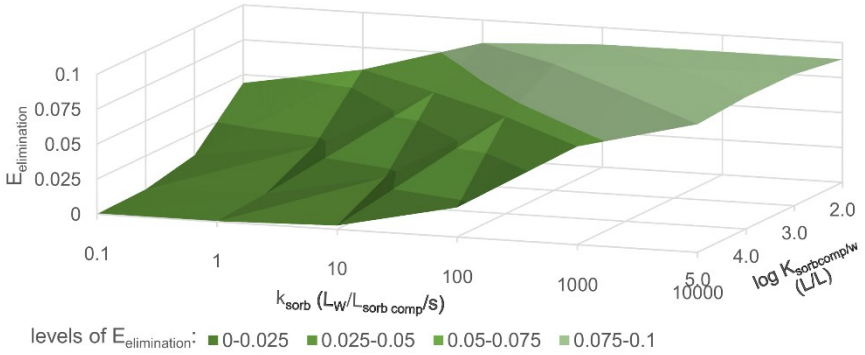


Figure S5: Calculated elimination efficiencies $E_{elimination}$ for varying sorption rate constants k_{sorb} and varying partition coefficients $K_{sorbing\ plasma\ components/water}$.

Analogous to the findings presented in the main text, the elimination efficiency also becomes less sensitive to a potential limitation due to slow sorption kinetics for less hydrophobic chemicals.

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