Supporting Information:

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

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

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

$$
K_{\text{plasma/water}} = \text{protein}_{\text{plasma}} \times K_{\text{protein/water}} + \text{albumin}_{\text{plasma}} \times K_{\text{albumin/water}} + \text{storage lipid}_{\text{plasma}} \times K_{\text{storage lipid/water}} + \text{membrane lipid}_{\text{plasma}} \times K_{\text{membrane lipid/water}} + \text{water}_{\text{plasma}}
$$

(1)

In this equation, $\text{protein}_{\text{plasma}}$ is the non-albumin protein content of plasma (as volume fraction mL/mL), $\text{albumin}_{\text{plasma}}$ is the albumin content of plasma (as volume fraction) etc. The composition data used for the estimation of $K_{\text{plasma/water}}$ are provided in Table S1 (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.

<table>
<thead>
<tr>
<th></th>
<th>water $w_{\text{plasma}}$</th>
<th>albumin $\text{albumin}_{\text{plasma}}$</th>
<th>protein $\text{protein}_{\text{plasma}}$</th>
<th>storage lipid $\text{storage lipid}_{\text{plasma}}$</th>
<th>membrane lipid $\text{membrane lipid}_{\text{plasma}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.965</td>
<td>0.006</td>
<td>0.009</td>
<td>0.010</td>
<td>0.010</td>
</tr>
</tbody>
</table>

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

$$
K_{\text{sorbing plasma components/water}} = \frac{K_{\text{plasma/water}} - \text{water}_{\text{plasma}}}{(1 - \text{water}_{\text{plasma}})}
$$

(2)

Table S2 lists the used test chemicals with CAS numbers, octanol-water partition coefficients log $K_{\text{OW}}$ (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 $K_{\text{sorbing comp/w}}$. 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 $K_{\text{sorbing comp/w}}$ is estimated using eq. (1) and (2) above).

Table S2: Details on the used test chemicals.

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

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

Here, \(Q_R\) is the respiration rate (LW/d), \(C_{W,in}\) and \(C_{W,out}\) the chemical concentrations in inflowing and outflowing ventilated water (mol/LW), \(P_{gills} * A_{gills}\) (LW/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.

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

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

\[
\frac{dM_{blood \ in \ periphery}}{dt} = Q (C_{blood,gills} - C_{blood,periphery}) - k_2 V_{periphery} C_{blood,periphery} K_{periphery/blood} \tag{5}
\]
Here, \( k_2 \) is the elimination rate constant in the periphery (1/d), \( V_{\text{periphery}} \) is the volume of the periphery and \( K_{\text{periphery/blood}} \) 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 (\( Q_{\text{free}} \) and \( Q_{\text{bound}} \)). 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.](image)

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

\[
\frac{dM_{\text{water}}}{dt} = Q_R \left( C_{W,\text{in}} - C_{W,\text{out}} \right) + P_{\text{gills}} * A_{\text{gills}} \left( C_{\text{free,blood,gills}} - C_{W,\text{out}} \right) \quad (6)
\]

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

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

Here, \( Q_{\text{free}} \) is the flow rate of the aqueous portion of blood (L/W/d), \( k_{\text{sorb}} \) (L/W/L_{\text{sorb comp}}/d) and \( k_{\text{des}} \) (1/d) are sorption or desorption rate constant for binding to non-aqueous blood constituents, \( V_{\text{free,gills}} \) is the aqueous volume of gill blood, \( V_{\text{sorb,gills}} \) is the non-aqueous volume of gill blood, \( C_{\text{free,blood,periphery}} \) and \( C_{\text{free,blood,gills}} \) are the freely dissolved chemical concentrations (mol/L_W) in blood flowing into the gills and out of the gills and \( C_{\text{bound,blood,gills}} \) is the bound chemical concentration (mol/L_{\text{sorb component}}) in blood flowing out of the gills.
\[
dM_\text{non-aqueous blood in gills} \quad \frac{dt}{dt} = Q_{\text{bound}} (C_{\text{blood,periphery}} - C_{\text{blood,gills}}) + k_{\text{sorb}} \cdot C_{\text{blood,gills}} \cdot V_{\text{sorb,gills}} = k_{\text{des}} \cdot C_{\text{bc}} \tag{8}
\]

\[
dM_\text{non-aqueous blood in periphery} \quad \frac{dt}{dt} = Q_{\text{bound}} (C_{\text{blood,gills}} - C_{\text{blood,periphery}}) + k_{\text{sorb}} \cdot C_{\text{blood,periphery}} \cdot V_{\text{sorb,periphery}} \tag{9}
\]

\[
dM_\text{aqueous blood in periphery} \quad \frac{dt}{dt} = Q_{\text{free}} (C_{\text{blood,gills}} - C_{\text{blood,periphery}}) - k_{\text{sorb}} \cdot C_{\text{free}} \cdot V_{\text{sorb,periphery}} - k_{2} \cdot V_{\text{periphery}} \cdot C_{\text{blood,periphery}} \tag{10}
\]

\[
Q_{\text{bound}} \text{ is the flow rate of the non-aqueous portion of blood (L/sorb component/d) and } C_{\text{bound}} \text{ is the bound chemical concentration in blood flowing into the gills.}
\]

\[
dM_\text{non-aqueous blood in periphery} \quad \frac{dt}{dt} = Q_{\text{bound}} \cdot C_{\text{blood,gills}} \cdot V_{\text{sorb,gills}} + k_{\text{sorb}} \cdot C_{\text{blood,periphery}} \cdot V_{\text{sorb,periphery}} \tag{9}
\]

\[
C_{\text{blood,gills}} = \frac{C_{\text{blood}} \cdot V_{\text{blood}} - C_{\text{blood,periphery}} \cdot V_{\text{periphery}}}{V_{\text{blood,gills}}} \tag{11}
\]

\[
C_{\text{blood,periphery}} = \frac{C_{\text{blood}} \cdot V_{\text{blood}} - C_{\text{free,periphery}} \cdot V_{\text{periphery}}}{V_{\text{blood,periphery}}} \tag{12}
\]

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 \(m_{\text{body}}\) (g):

\[
cardiac \text{ output (L/h/kg}_{\text{fish}} = (0.23 \cdot T - 0.78) \cdot \left(\frac{m_{\text{body}}}{500}\right)^{-0.1} \tag{13}
\]

The volume of gill cells is estimated from the fractional gill weight \(G_{\text{FW}}\) (0.0247 g_{\text{gills}}/g_{\text{fish}}), gill cell content \(G_{\text{cells}}\) (556 * 10^6 cells/g_{\text{gills}}), and the gill cell diameter \(d_{\text{gill}}\) (0.00151 cm):
\[ V_{\text{gills}} = G_{\text{cells}} \times G_{\text{FBW}} \times m_{\text{body}} \times \frac{1}{6} \pi d_{\text{gill}}^3 \] (14)

The blood volume is derived from the fractional blood volume \( B_{\text{FBW}} \) (0.034 mL\text{blood/gbody}, [7]):
\[ V_{\text{blood}} = B_{\text{FBW}} \times m_{\text{body}} \] (15)

The organism-water partition coefficient is calculated as
\[ K_{\text{organism/water}} = \text{lipid content}_{\text{organism}} \times K_{\text{octanol/water}} \] (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]
  
  \[
  \begin{array}{c|c|c}
  \text{water}_{\text{blood}} & \text{protein}_{\text{blood}} & \text{lipid}_{\text{blood}} \\
  0.89 & 0.096 & 0.014 \\
  \end{array}
  \]

- **gills** [9]
  
  \[
  \begin{array}{c|c|c}
  \text{water}_{\text{gills}} & \text{protein}_{\text{gills}} & \text{lipid}_{\text{gills}} \\
  0.73 & 0.205 & 0.065 \\
  \end{array}
  \]

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 \( P_{\text{through layer}} \times A \) in cm³/s are calculated for each of the individual layers of this total barrier using the diffusion coefficient of the chemical within the layer \( D_{\text{chemical in layer}} \) (cm²/s), the partition coefficient between layer and water \( K_{\text{layer/water}} \) (L_water/L_layer), the exchange surface area between the layers \( A_{\text{gills}} \) (cm²) and the layer thickness \( d_{\text{layer}} \) (cm).
\[ P_{\text{through layer}} \times A = D_{\text{chemical in layer}} \times K_{\text{layer/water}} \times A_{\text{gills}} \times \frac{1}{d_{\text{layer}}} \] (17)

These individual permeability surface area products are then used to estimate the total permeability surface area product \( P_{\text{gills}} \times A \) in cm³/s) in the gills.
\[ P_{\text{gills}} \times A = \frac{1}{P_{\text{mucus}} \times A} + \frac{1}{P_{\text{membrane}} \times A} + \frac{1}{P_{\text{cytosol}} \times A} + \frac{1}{P_{\text{ABL}} \times A} \] (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 ($D_{\text{chemical in water}} = 7.5 \times 10^{-6} \text{ cm}^2/\text{s}$ 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. ($A_{\text{gills}} = 29.4 \text{ cm}^2$). 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 $d_{\text{mucus}} = 6 \times 10^{-5} \text{ cm}$, $d_{\text{cytosol}} = 1.4 \times 10^{-3} \text{ cm}$, $d_{\text{membrane}} = 3.68 \times 10^{-5} \text{ cm}$ and $d_{\text{ABL}} = 3 \times 10^{-4} \text{ cm}$.

For sorption kinetics an arbitrary range of $k_{\text{des}}$ 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 ($k_{\text{sorb}}$ in L_water/L_sorbing component/d) can be calculated using the partition coefficient between sorbing blood components and water ($K_{\text{sorbing components/water}}$) (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

$$k_{\text{sorb}} = k_{\text{des}} \times K_{\text{sorbing components/water}}$$

The concentration-time profiles for all test chemicals are shown below:
Figure S3: Concentration-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 $0.4 \text{ 1/d}$ (i.e. tenfold slower elimination kinetics).

Figure S4: Change in uptake efficiency ($E_{\text{uptake}}$), elimination efficiency ($E_{\text{elimination}}$) and bioconcentration factor (BCF) for a scenario of a chemical with a log $K_{OW} = 6$ and a whole-body elimination rate constant of $0.4 \text{ 1/d}$ 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 $E_{\text{elimination}}$. 
Figure S5: Calculated elimination efficiencies $E_{\text{elim}}$ for varying sorption rate constants $k_{\text{sorb}}$ and varying partition coefficients $K_{\text{sorbing plasma components/water}}$.

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.

References


