Equilibrium Biopartitioning of Organic Anions – a Case Study for Humans and Fish

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Abstract

In this work we combine partition coefficients between water and membrane lipid, storage lipids, the plasma protein albumin as well as structural protein with the tissue dependent fraction of the respective phases in order to obtain a clearer picture on the relevance of various biological tissues for the bioaccumulation of 31 organic anions. Most of the partition coefficients are based on experimental data, supplemented by some predicted ones. The data suggest that the plasma protein, albumin, will be the major sorption matrix in mammals. Only small fractions of the studied chemicals will occur freely dissolved in an organism. For the investigated acids with pKa < 5, partitioning is dominated by the ionic species rather than the corresponding neutral species. Bioconcentration in fish is not expected to occur for many of these acids unless pH in the aqueous environment is low or specific sorption mechanisms are relevant. In contrast, biomagnification in terrestrial mammals would be expected for most organic anions if they are not sufficiently metabolized. We conclude that sorption is important for the toxicokinetics of ionizable organic chemicals and the dominating sorbing matrices are quite different from those for neutral species.

Keywords: bioaccumulation, organic anions, partitioning, bioconcentration, risk assessement, chemical regulation

1. Introduction

Among the many organic chemicals that have to be regulated there are many that are ionizable (i.e., have at least one ionic species depending on the pH). In the regulation procedure these ionizable chemicals need to be assessed –among others– for their bioaccumulation potential and their toxicity. For these tasks it is fundamental that we understand the biopartitioning of ionic species. For neutral organic chemicals our understanding of biopartitioning has reached a rather high level(Endo et al., 2013) but it is still in its infancy for ionic organic species. In recent papers we have investigated the partitioning of organic ionic species to distinct biological matrices such as phospholipids, structural proteins and albumin.(Bittermann et al., 2014; Henneberger et al., 2016a, b) Here, we combine this information in order to shed some light on the partition behavior of some typical organic acid anions in organisms. Cations were excluded from this study due to limited data availability. Armitage et al. (Armitage et al., 2013) have presented a toxicokinetic model for the bioaccumulation of ionizable chemicals in fish. Their model had a focus on kinetic processes and was built on the assumption that partition coefficients of ionic species can be estimated by substracting an empirically found constant from the logarithmic partition coefficients of the corresponding neutral species. Similar approaches have also been used by others in models for the bioconcentration of ionizable chemicals in fish (Erickson et al., 2006; Fu et al., 2009). This approach goes back to a work of Escher and Schwarzenbach on membrane partitioning of phenolic acids (Escher and Schwarzenbach, 1996). However, in a later work Escher and Sigg presented data revealing that correction by a single constant does not really work when different compound classes need to be covered (Escher and Sigg, 2004). Our own work also does not support this approach (Bittermann et al., 2014). In the pharmaceutical literature there is also the attempt to relate anionic partition coefficicents to the Kow of the neutral species based on an empirical relationship calibrated with a small number of experimental data (Rodgers and Rowland, 2006). For our work here we concluded that it was important to use reliable, mostly experimental partition coefficients rather than less reliable estimated values in order to analyse the equilibrium partition preferences of ions in organisms. The specific goal of this work was to answer three questions: 1) What is the relative importance of different sorption matrices (proteins, phospholipids, triglycerides) for the partitioning of acidic chemicals in an organism (here a typical human is used as an example)? 2) Can we infer a distinct organ-specific

partitioning of acidic chemicals from the available data using composition and size of organs that are typical for humans? 3) What is the expected bioaccumulative behavior of non-metabolized, acidic chemicals in fish (here a rainbow trout) at a physiological pH of 7.4 and at various external pH values. For all questions, we start with considering the ionic species alone and then discuss the partitioning of the total compound (i.e., neutral and ionic species).

2. Methods

2.1 General Approach

In our recent work we were able to generate equilibrium sorption data for about 40 organic anions in structural proteins, albumin and phospholipids.(Bittermann et al., 2014; Henneberger et al., 2016a, b) These data were supplemented with data from the literature and will serve as the basis for the work presented here. It was not possible though to base the work solely on experimental data because there is only limited overlap between the investigated ionic chemicals from the different datasets. We therefore had to resort to estimation methods for ionic partition coefficients in order to fill data gaps. Available estimation methods for ionic partitioning to albumin and structural proteins have a rather narrow application domain (Henneberger et al., 2016b; Linden et al., 2017) and were avoided. Thus, we decided to use only those ions for which we had experimental values for their partitioning from water to albumin and to structural protein. For four of these chemicals we also had experimental phospholipid-water partition coefficients (4-n-octylbenzenesulfonate, bromoxynil, ibuprofen and pentachlorophenol). For the remaining ions, the phospholipid-water partition coefficients were derived from a quantum chemically based approach, namely COSMO*mic*, which we expect to possess a very wide application range (Bittermann et al., 2014). This procedure left us with 31 organic acid anions representing pharmaceuticals, pesticides and other chemicals. Most of these chemicals are carboxylic acids (n=23), particularly benzoic acids (n=8) and naphthoic acids (n=7).

For the partitioning of the neutral species we largely rely on pp-LFER predictions as outlined in earlier work (Endo and Goss, 2011; Endo et al., 2012; Geisler et al., 2012). Both, experimental partition coefficients as well as those from the pp-LFER equations are for 37°C.

This work is limited to the equilibrium partitioning of ions although kinetic uptake and elimination processes may strongly affect the actual concentrations in organisms. In fact, kinetics may be more important for ionic species than for neutral ones because ions are known to have a much lower membrane permeability (Saparov et al., 2006) and are expected to be more susceptible to active transport processes across membranes (Roth et al., 2012). Nevertheless, equilibrium partitioning must be understood first before more complex kinetically controlled scenarios can be investigated.

All calculations here assume that partitioning within an organism can be treated as additive with respect to the different sorption matrices of an organ or organism.. It is also assumed that partition coefficients that have been measured with isolated proteins and lipids are representative of *in vivo* partitioning. These are standard assumptions in pharmaco- and toxicokinetic modeling and at least for neutral organic chemicals they are supported by some experimental evidence (Endo et al., 2013) For plasma-muscle partitioning of ions we have some qualitative indication that this approach should work as well (see SI Figure S7 in (Henneberger et al., 2016b)). Again following pharmacokinetic practice we assume that water, storage lipids, phospholipids, structural proteins and albumin are the relevant sorbing matrices for neutral organic species. For ionic species we follow the same approach but ignore storage lipids (triglycerides). This is supported by experimental evidence showing that partitioning of ions to organic solvents is orders of magnitude smaller than partitioning to phospholipids (Escher and Sigg, 2004). Neglect of storage lipids as sorbing compartment for ions is further supported by energetic considerations: a sorbing organic matrix likely needs ionic (or zwitterionic) functional groups in order to efficiently compete with the strong water dipole for organic ions because of the rather attractive ion-dipole interactions that occur in water. While proteins and phospholipids possess such ionic functions, storage lipids do not. A comprehensive investigation of ionic partitioning in organisms also needs to include ion-trap effects that arise from pH differences within the body and/or between an organism and surrounding water (Fu et al., 2009; Neuwoehner and Escher, 2011).

2.2 Specific Calculations

Table 1 shows the fractional composition for a total human and for the major organs that we have used for our calculations.

Table 1

Composition of a woman (BW = 60 kg, H = 163 cm, BMI = 22.6 kg/m^2). Blood volumes within the organs were estimated with Krogh´s cylinder model (Krogh, 1922) and subtracted from the organ volumes reported in (Willmann et al., 2007). Composition of the organs was taken from (Schmitt, 2008a; Schmitt, 2008b) Interstitial volume in the organs and albumin concentration therein was taken from (Schmitt, 2008b) and (Ellmerer et al., 2000) respectively. All values are given in mL.

	total volume of organs (without capillaries)	**volume of phospholipids**	**volume of storage lipids**	**volume of structural protein**	**volume of water**	**volume of albumin in interstitial space of organs**
Adipose	22076	42.5	15177	990	5845	20.66
Brain	1311	89.1	56.9	106	1059	0.04
Gut	1223	21.9	48.7	151	1000	0.83
Heart	343	14.8	13.7	49.1	265	0.32
Kidneys	427	12.1	4.3	57.3	352	0.59
Liver	1843	73.3	30.4	275	1463	2.21
Lung	1034	7	7.3	39.3	977	2.75
Muscle	19114	79.1	76	2946	15997	16.26
Skin	3516	25.7	231	751.4	2501	6.33
Spleen	231	2.1	0.9	34.6	193	0.31
Gonads	12	0.3	0	1.3	10	0.01
Blood	4800	16.3	15.8	624	3986	158
total	**55929**	**384**	**15662**	**6025**	**33649**	**208.32**

This total composition from Table 1 together with the corresponding partition coefficients listed in the Supporting information (SI Table S1) allows the calculation of the relative sorption capacity for each ionic species, with the following formulas:

(1)

(2)

(3)

(4)

where $f_{i\ matrix}$ is the fraction of the total amount of a chemical species i that resides in ´matrix´ at equilibrium, V is the volume of the respective biological matrix (see Table 1) and $K_{i\ xy}$ is the equilibrium partition coefficient between matrix x and y (in L/L). When needed these were calculated from weight based partition coefficients in SI Table S1 using density of 1.36 kg/L for all proteins. Subscripts are used as follows: w – water, alb –albumin, prot – structural proteins, p.lip - phospholipids. Partition coefficients between two matrices are calculated via the thermodynamic cycle using the known matrix-water partition coefficients. The relative concentrations in the matrices at equilibrium are directly given by these partition coefficients.

The partitioning of the total chemical (ionic plus neutral species) is obtained by applying above formulas to the neutral species as well (including storage lipids as additional sorbing matrix) and then weighing the results for both species according to their fractionation in water that is calculated from the Henderson and Hasselbalch equation:

(5)

with α_{acid} being the fraction of the neutral acid and (1- $\alpha_{i\ acid}$) as the fraction of the acid anion. The pK_a values used for this calculation are listed in SI Table S2.

3. Results & discussion

3.1 Matrix specific partitioning

For a typical human adult with a composition as shown in Table 1, the expected relative sorption capacity of different matrices for each anion is shown in Figure 1.

Fig. 1. Relative sorption capacity (calculated as product of partition coefficients and relative volume of the matrix) of different matrices for the investigated anions, sorted according to chemical classes (all data refer to the anionic species although the names of the neutral chemicals are shown).

Almost all anions are predominately sorbed to albumin, with structural proteins and phospholipids as the next relevant sorbing matrices. Despite its dominating contribution to the volume of a human, water plays only a minor role for hosting these chemicals. Besides the relative distribution of the total internal amount of a chemical it is also interesting to look at the relative concentrations in various matrices (see SI Table S3). In this case, albumin is even more dominating than for the mass distribution because albumin has the highest loadings while contributing little to the total volume of humans.

Figure 2 shows the expected relative sorption capacity for the neutral species of the tested organic acids. Storage lipids are typically expected to dominate the partitioning of neutral organic chemicals. It is therefore interesting to see that the picture is somewhat different for these rather polar neutral molecules. For all of them, storage lipids, phospholipids and structural proteins contribute in the same order of magnitude to the overall accumulation in the organism.

Fig. 2. Relative sorption capacity (calculated as product of partition coefficients and relative volume of the matrix) for the neutral species of the tested organic acids based on estimated partition coefficients (listed in SI Table S4) and matrix volumes (Table 1).

The distribution of the sum of both molecular species of each chemical at pH 7.4 is given in SI Table S5. It differs little from the situation shown for the ionic species alone in Figure 1 because the anionic fraction in water of all chemicals studied here amounts to more than 99% of the total chemical at the physiological pH of 7.4. Sorption of the neutral species is not sufficiently extreme to compensate for this fractionation.

3.2 Organ specific partitioning

Among all matrices investigated above, albumin has shown the highest affinity for organic anions. Most of the albumin (ca. 75%) is located in the blood and so blood is estimated to carry the highest load of organic anions as compared to the organs (see Figure 3 and SI Table S6). Differences in the calculated mass loadings of different organs are mostly due to the different volume of these organs. Hence, muscles are the next important compartment for storing organic anions followed by the skin and adipose. This picture does

not change much if both species of each chemical are considered together (i.e., anionic and neutral species), as already discussed above: at pH 7.4 the neutral species make up less than 1 % of the total aqueous concentration and their respective partition coefficients are not high enough to let neutral species dominate the overall partitioning of the investigated chemicals. We have to note here that in reality there might be organ specific sorption processes for ions that are not considered in our modeling approach because too little systematic knowledge about such processes is available. A prominent example for such a specific binding is the binding of long chain perfluorinated acids to fatty acid binding proteins in the liver (Luebker et al., 2002; Woodcroft et al., 2010; Zhang et al., 2013).

Fig. 3. Sorption capacity (calculated as product of partition coefficients and relative volume of the organs) of different organs of a human (see Table 1 for characteristics) for various organic anions (although the names of the neutral species were used in the graphic all data apply to the anionic species).

3.3 Bioconcentration in fish

So far, our work was only concerned with partitioning within organisms. For the bioaccumulation perspective, partitioning between the environment and the organism is important. The relevant metric for regulation is currently the bioconcentration factor (BCF) which is defined as the steady-state concentration in fish over the concentration in the water it is exposed to. This BCF should not exceed the value of 2000 for a fish with 5 % lipids. While standardization of total lipid content is useful for neutral hydrophobic chemicals it is irrelevant for ionic chemicals because their sorption is not dominated by storage lipids but by proteins and to some extent by phospholipids as shown above. For our calculations here, we looked at a 1 kg rainbow trout with the following composition in volume %: storage lipids 11%, phospholipids 1.0 %, structural proteins 15.8 %, blood proteins 0.27 %, water 69.8 % (adapted from (Nichols et al., 1990)). For our calculations we assumed that the partition coefficient for bovine serum albumin can also be used for fish

blood transport protein although we have no further evidence for this assumption. We note that this is a major uncertainty in our model approach. Partition equilibrium can be seen as a worst case estimation for bioconcentration because it does not account for any metabolism. Here, we use the organism-water partition coefficients of the anionic and neutral species as an indicator of bioaccumulation trends of different ionizable organics that could be used in a lower tier screening of chemicals in the regulatory process. Figure 4 shows how the different sorption matrices contribute to the overall distribution between rainbow trout and water for the neutral and the respective anionic species (the indicated relative contributions refers to the non-logarithmic *K*-value).

Fig. 4. Logarithmic partition coefficients for the neutral and the anionic species between water and whole fish (rainbow trout). The color coding indicates how different sorption matrices contribute to this overall partitioning (note that the indicated relative contributions refer to the non-logarithmic *K*-value).

For most chemicals (74 %) the neutral species exhibit stronger sorption from water into fish than their corresponding ionic species. In those cases where we find the ionic species to possess a higher affinity for fish than the neutral species this is always due to an exceptionally high partitioning to albumin. The latter is likely due to a combination of a favorable three dimensional structure and favorable ionic/and or H-bond interactions (Linden et al., 2017). The neutral species of these chemicals will have almost the same 3D structure but not the same ionic/and or H-bond interactions. Our partition coefficient to albumin for neutral species are all predicted with a model that does not account for 3D effects. We can thus not be sure whether these values are really correct or whether –for these chemicals- the neutral form would have actually have a much higher affinity to albumin so that the neutral species would still dominate in the total affinity to fish.

The overall sorption of the investigated chemicals at physiological pH is always dominated by the ionic species, simply because all studied chemicals are > 99% ionized at pH 7.4, while the equilibrium partition coefficients of the ions are less than 100 times smaller than that of their corresponding neutral species. Thus, for the chemicals studied here the organism/water partition coefficients of the ionic species can also be used

as an approximation of the total partition coefficients for the sum of both species (anionic and neutral) for each chemical as long as the pH value in the exposure medium is identical to the physiological pH.

If ionizable chemicals partition between two aqueous compartments that are separated by a membrane, which is only permeable to the neutral species, then partition equilibrium is reached when the concentration of the neutral species is the same on both sides of the membrane. If, however, the pH value on both sides of the membrane differs then the equilibrium concentration of the ionized species is different on both sides of the membrane and hence also the total concentration of the ionizable chemical (Neuwoehner and Escher, 2011) This so-called ion-trap effect will increase the bioaccumulation of acids if the pH in the exposure water is smaller than the internal pH in the organism. The ion-trap effect can easily be calculated for the worst-case assumption that the ions have no membrane permeability at all:

(6)

Hence, at pH 6.4 in the exposure medium there would be an increase in the sorption coefficients of most acids by one log unit for an organism that keeps up an internal pH of 7.4. In this case two of the investigated chemicals would slightly exceed the BCF threshold of 2000 (assuming that no metabolism takes place): pentachlorophenol and 4-n-octylbenzenesulfonate. At pH 5.4 which is the lower end of what one would typically expect for natural waters more than 50% of the discussed anions would exceed the BCF of 2000 if no metabolism occurs.

For biomagnification in terrestrial organisms the acceptable thresholds for equilibrium partitioning are much lower than for bioconcentration. For chemicals that are not volatile (log K_{oa} > 5) and that do not metabolize, log $K_{organism/water}$ would have to be smaller than 1 in order to be assessed as non-bioaccumulative based on purely physico-chemical reasoning (Czub and McLachlan, 2004; Armitage and Gobas, 2007; Kelly et al., 2007; Goss et al., 2013). The partition coefficients shown in Figure 4 for fish can also be taken as an approximation for terrestrial vertebrates because the overall composition does not vary greatly between fish and other vertebrates (compare data from Table 1 and (Nichols et al., 1990)). One can see that most chemicals exceed this threshold of log $K_{organism/water}$ >1. Thus, bioaccumulation for acidic chemicals might be more of an issue for terrestrial organisms than for aquatic species. Another effect that might contribute to the

bioaccumulation of ionizable chemicals, which is not relevant for hydrophobic neutral molecules, is active transport across membranes with carrier proteins (Ng and Hungerbühler, 2014).

The discussion in this work gives a first idea of certain trends when it comes to the biopartitioning of organic acids. For a complete picture, more processes will have to be considered in a quantitative way (e.g., active transport, ion-trap effects within organisms in different organelles, metabolism, electric fields across membranes). In addition to the organic acids investigated here, organic bases, zwitterions and permanent ions also have to be considered.

The bioaccumulation metrics BCF and BMF are defined for steady state situations in which the sum of all kinetic uptake processes matches the sum of all kinetic clearance processes. For chemicals with low or no metabolism these accumulation metrics are close to what one can expect from equilibrium partitioning, which is what we discussed here. However, the more important metabolism becomes the more these metrics become kinetically controlled which means that also uptake kinetics and thus membrane permeability become an issue (Armitage et al., 2013).

We are just at the beginning of a sound mechanistic understanding of the toxicokinetics of organic ions in organisms and there is a long way ahead. We hope that this work helps to raise awareness for the existing knowledge gap and that more work in this direction can be triggered.

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