Equilibrium sorption of organic ions to selected proteins

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Eidesstattliche Erklärung

Hiermit versichere ich an Eides statt, dass ich die vorliegende Arbeit selbständig und ohne fremde Hilfe verfasst, andere als die von mir angegebenen Quellen und Hilfsmittel nicht benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht habe. Des Weiteren erkläre ich, dass ich die Dissertation weder in der gegenwärtigen noch in einer anderen Fassung bereits einer anderen Fakultät vorgelegt habe.

02. Januar 2017, Leipzig Luise Henneberger

Zusammenfassung

Viele umweltrelevante Chemikalien, wie zum Beispiel Pestizide, Pharmaka und zahlreiche Inhaltsstoffe von Kosmetika, sind ionische organische Chemikalien (IOCs). Allerdings sind die meisten Modelle für die Vorhersage der Gleichgewichtverteilungskoeffizienten, die im Bereich der Umweltrisikobewertung zum Einsatz kommen, nur für neutrale organische Chemikalien geeignet. Das Gleichgewichtsverteilungsverhalten von IOCs ist um ein Vielfaches komplexer als das von neutralen Chemikalien und es existieren kaum konsistente Datensätze für die Verteilung von IOCs. Eine Vielzahl von Studien hat gezeigt, dass die Verteilung von neutralen Chemikalien in biologische Systeme (Gewebe, Organe und ganze Organismen) zu meist durch die Lipidfraktion bestimmt wird. Für die in vivo-Verteilung von IOCs könnten vor allem Proteine als Sorptionsphasen eine Rolle spielen, aufgrund ihrer polaren Eigenschaften und da sie in biologischen Systemen in großer Menge vorkommen.

Das Ziel der vorliegenden Arbeit war es, zu untersuchen, wie die molekulare Struktur von organischen Ionen die Verteilung in ausgewählte Proteine beeinflusst. Dabei wurden Bovines Serumalbumin (BSA) und aus Hähnchenbrustfilet isoliertes Strukturprotein (Muskelprotein) als Modellproteine verwendet. Zunächst wurden zwei verschiedene Methoden für die Bestimmung der Proteinbindung getestet: Gleichgewichtsdialyse und ein Passive Sampling Ansatz. Die Gleichgewichtsdialyse wurde schließlich für die Versuche verwendet und Verteilungskoeffizienten für BSA ($K_{BSA/w}$) und Muskelprotein ($K_{MP/w}$) wurden für systematisch ausgewählte organische Anionen und Kationen, mit verschiedenen Grundstrukturen und funktionellen Gruppen, bestimmt. Die getesteten anionischen Chemikalien zeigten eine starke Verteilung in das BSA (log $K_{BSA/w}$ von bis zu 5,3), während die Kationen nur wenig an BSA sorbiert waren. Die Verteilung in das Muskelprotein war im Vergleich zu BSA weniger stark für die getesteten Anionen (bis zu 3,7 log Einheiten). Die gemessenen Kationen zeigten hingegen eine ähnlich starke Verteilung in beide Proteine. Des Weiteren stellte sich heraus, dass die Bindung an BSA stark durch die dreidimensionale Struktur der Testchemikalien beeinflusst wird und, dass ortho-substituierte organische Säuren eine deutlich schwächere Verteilung in das BSA aufweisen. So unterscheiden sich zum Beispiel die gemessenen Werte für log $K_{BSA/w}$ von

1- und 2-Naphthoesäure und von 3,4-Dichlorbenzoesäure und 2,6-Dichlorbenzoesäure jeweils um 1.5 Größenordnungen. Die beobachteten sterischen Effekte werden durch die im Rahmen dieser Studie ausgetesteten Vorhersagemodelle (z. Bsp. polyparameter linear free energy relationships (PP-LFERs)) nicht erfasst. Es werden daher für die korrekte Vorhersage der Bindung von IOCs an BSA alternative Modellierungsansätze benötigt. Für die Verteilung von neutralen, anionischen und kationischen Chemikalien in das Muskelprotein konnte erfolgreich eine PP-LFER Gleichung aufgestellt werden (R^2 = 0.89, RMSE = 0.29). Schließlich wurden die gemessenen Verteilungskoeffizienten für BSA und Muskelprotein verwendet, um Verteilungskoeffizienten zwischen Muskelgewebe und Blutplasma vorherzusagen ($K_{muscle/plasma}$). Die für $K_{muscle/plasma}$ berechneten Werte stimmen sowohl qualitativ, d.h. bezüglich der Unterschiede zwischen neutralen, anionischen und kationischen Chemikalien, als auch quantitativ, gut mit in der Literatur beschriebenen in vivo gemessenen Verteilungsverhältnissen überein. Diese Ergebnisse zeigen, dass die im Rahmen dieser Arbeit in vitro gemessen Verteilungskoeffizienten auch für die Beurteilung der in vivo-Verteilung von IOCs genutzt werden können.

Abstract

Many chemicals of environmental concern (e.g., pesticides, pharmaceuticals and ingredients of personal care products) are ionogenic organic chemicals (IOCs). However, partitioning models currently used for environmental risk assessment are only designed for neutral organic chemicals. Partitioning processes of IOCs are far more complex than for neutral chemical. Consistent datasets for the partitioning of IOCs are still widely missing. Numerous studies have shown that the partitioning of neutral chemicals into biological tissues and their constituents is mainly driven by the total lipid fraction. Proteins are expected to be a relevant partitioning phase for IOCs in vivo, because of their polar nature and their high prevalence in biological systems.

The aim of this study was to elucidate how the molecular structure of organic ions influences their partitioning to different proteins. Bovine serum albumin (BSA) and structural protein isolated from chicken breast fillet (muscle protein) were selected as model proteins. First, two different methods for the determination of protein binding, equilibrium dialysis and a passive sampling approach, were tested. The equilibrium dialysis method was selected and partition coefficients to BSA ($K_{BSA/w}$) and muscle protein ($K_{MP/w}$) were determined for systematically selected organic anions and cations with different basic structures and functional groups. The tested organic anions exhibited high affinities for BSA (log $K_{BSA/w}$ up to 5.3), while cationic test chemicals showed only weak binding to BSA. Binding to muscle protein was less strong compared to BSA for anionic chemicals (up to 3.7 log units). The tested organic cations showed similar sorption strength to both proteins. Interestingly, the results of this study also suggest that binding of IOCs to BSA is highly influenced by the three-dimensional structure of the chemicals and that ortho-substituted organic acids have substantially lower log $K_{BSA/w}$ values. For example log $K_{BSA/w}$ values of isomeric chemicals such as 3,4-dichlorobenzoic acid and 2,6-dichlorobenzoic acid, and 1-naphthoic acid and 2-naphthoic acid differed by more than 1.5 log units. The observed steric effects were not captured by the modeling approaches tested within this study (e.g., polyparameter linear free energy relationships (PP-LFERs)). Novel methods will be required for the accurate prediction of BSA binding of IOCs. For binding to muscle protein a PP-LFER model that is suitable for the

prediction of neutral, anionic and cationic chemicals was successfully developed (R^2 = 0.89, RMSE = 0.29). Finally, partition coefficients between muscle tissue and plasma were calculated ($K_{muscle/plasma}$) based on the determined partition coefficients to serum albumin and muscle protein. The calculated values for $K_{muscle/plasma}$ show a good qualitative (i.e., in terms of differences between neutral, anionic and cationic chemicals) and quantitative agreement with in vivo measured muscle tissue-plasma steady-state distribution ratios. These results indicate that the in vitro measured partition coefficients of this study can be used to gain valuable information on the in vivo partitioning behavior of IOCs.

Preface

The present work was performed between September 2012 and November 2015 at the Helmholtz Centre for Environmental Research, Leipzig at the Department of Analytical Environmental Chemistry. The thesis was written in cumulative form and is based on the following research articles:

Oemisch, L.; Goss, K.-U.; Endo, S., Ion exchange membranes as novel passive sampling material for organic ions: Application for the determination of freely dissolved concentrations. J. Chromatogr. A 2014, 1370, 17-24.

Henneberger, L.; Goss, K. U.; Endo, S., Equilibrium Sorption of Structurally Diverse Organic Ions to Bovine Serum Albumin. Environ. Sci. Technol. 2016, 50, (10), 5119-26.

Henneberger, L.; Goss, K. U.; Endo, S., Partitioning of Organic Ions to Muscle Protein: Experimental Data, Modeling, and Implications for in Vivo Distribution of Organic Ions. Environ. Sci. Technol. 2016, 50, (13), 7029-36.

Note that text passages in the summary are partly taken from the original publication without further indication. The abstracts of the original publications are included at the end of the thesis.

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1 Summary: Equilibrium sorption of organic ions to selected proteins

1.1 Introduction

Assessing the toxicity and the bioaccumulation potential of chemicals in the environment requires reliable information on their partitioning into biological compartments such as lipids and proteins. In the past, environmental scientists mainly focused on neutral organic chemicals and predictive tools with different levels of complexity have been developed for these chemicals. In simple models it is often assumed that the predominant sorption phase is the lipid fraction of an organism and that octanol is an appropriate surrogate for lipids. Models with more mechanistic insight explicitly consider different types of lipids and proteins as sorbing phases[1-3] and/or use more sophisticated approaches to predict relevant partition coefficients. For example polyparameter linear free energy relationships (PP-LFERs)[4, 5] have been established for partitioning to different biological phases including storage lipids,[6] phospholipids,[7] and proteins.[8, 9]

Although there is a need for predictive models that are capable of predicting the toxicity and the bioaccumulation potential of ionogenic organic chemicals (IOCs) as well, only a few attempts have been made so far to include IOCs in environmental partitioning models.[10] For a better understanding of bio-partitioning of IOCs, the relevant sorption phases for organic ions have to be identified. In contrast to neutral chemicals, IOCs are not expected to primarily partition to storage lipids, but rather to polar lipids (phospholipid membranes) and proteins.

At least two classes of protein may serve as significant sorption phases for IOCs in vivo. First, serum albumin and other serum proteins: Serum albumin is well-known for its ability to bind a broad range of chemicals, especially organic anions, reducing their freely dissolved fraction in plasma and increasing the sorption capacity of the plasma.[11-14] Additionally, for cationic and neutral chemicals a significant contribution of another plasma protein (α_1-acid glycoprotein) to the sorption capacity of the plasma has also been described.[15] Large amounts of data are available in the literature for binding of pharmaceuticals (including IOCs) to human serum albumin. However, reliability and

comparability of these data are difficult to evaluate. As reviewed by Nilsson et al.,[16] data for protein binding are often published without carefully controlling all the factors that can influence the partitioning process, e.g., temperature; the pH value; concentrations of the test chemical, salt, and the protein; non-specific adsorption to the used equipment; equilibrium disturbances; and leaking of dialysis membrane. Moreover, pharmaceuticals are often multifunctional molecules with complex structure. A collection of data for such diverse chemicals is difficult to interpret in terms of structural influences on the binding constant.

The second class of proteins that could be an important sorption phase for IOCs are structural proteins. Structural proteins make up the majority of the proteins in the human body, with a fraction of 10% by mass of the whole body. About half of the structural proteins (4% of the whole body mass) are muscle proteins (e.g., actin and myosin). The other half (6% of the whole body mass) include keratin and collagen.[17, 18] Generally, structural proteins are not expected to be a target site for toxic actions, but similar to serum albumin, sorption to structural protein can be substantial and affect the transport, distribution, and freely dissolved concentration of chemicals in the body. There are studies that focused on the sorption of organic chemicals to different structural proteins (i.e., gelatin, muscle protein, collagen, and keratin),[9, 19-21] but their targets were mainly neutral organic chemicals. Only a few studies have included binding data for IOCs as well.[20, 21]

Because for many chemicals partitioning data for proteins other than serum albumin are not readily available, partitioning models often consider the bulk protein fraction of an organism as one sorption phase, using serum albumin as a representative protein for all proteins.[2] Endo et al. have shown that for neutral chemicals, sorption to muscle protein and serum albumin is profoundly different.[9] This difference is reasonable, because sorption to serum albumin generally happens at specific binding sites,[9, 11, 22] while this is presumably not the case for structural proteins. A further study demonstrated that structural proteins can be a more important sorption phase than lipids for H-bond donor neutral chemicals in lean tissues.[1] Such general conclusions could not be made for IOCs so far, because consistent datasets for binding of IOCs to serum albumin and structural protein are missing.

1.2 Objective of this study

The aim of this study was to investigate how the molecular structure of IOCs (e.g., different basic structures, substitutions and charged functional groups) influences their sorption to selected proteins and to elucidate the sorption mechanisms. First, a method for the determination of protein binding of IOCs had to be established. Two different approaches were tested: a standard equilibrium dialysis method using customized dialysis cells and a passive sampling approach. Because convenient sorption materials for passive sampling of IOCs were not readily available, the applicability of ion exchange membranes (IEMs) for passive sampling of IOCs was tested. Although IEMs were found to be suitable for passive sampling of organic ions, equilibrium dialysis was finally chosen for the determination of protein-water partition coefficients in this study.

Two proteins were selected as model proteins: bovine serum albumin (BSA) and a mixture of different structural proteins isolated from chicken breast fillet (hereinafter referred to as "muscle protein"). Using the equilibrium dialysis method, partition coefficients between BSA and water ($K_{BSA/w}$) and muscle protein and water ($K_{MP/w}$) were determined for systematically selected IOCs. Particularly, many varyingly substituted benzenes and naphthalenes with an anionic functional group were included to study the influences of molecular structure on protein binding. More complex compounds (i.e., pesticides and pharmaceuticals) were included, because of their environmental relevance. All measurement conditions were thoroughly controlled, because partitioning processes of ions are potentially influenced by pH value and salt concentration. Additionally, influence of pH value, dependence on the concentration of inorganic and other organic ions, and reversibility of binding to BSA and muscle protein were determined experimentally.

The protein binding data obtained were correlated with various descriptors to gain an insight into the requirements of successful modeling for protein binding of IOCs and polyparameter linear free energy relationships (PP-LFERs) were tested for their suitability as a predictive tool. Finally, it was evaluated how far partitioning to serum albumin and muscle protein can explain muscle tissue-plasma partition coefficients that were measured in vivo.

1.3 Method development for protein binding

1.3.1 Equilibrium dialysis

Equilibrium dialysis is a well-established technique for the determination of protein binding. In this method a buffer solution with protein and a clean buffer solution are separated by a semi-permeable membrane (see also Figure 1). The test chemical is added to the system and the sample is incubated for a given time period. At equilibrium the freely dissolved concentration of the target chemical can be determined in the buffer-only solution.

For this study custom-made dialysis cells were used, consisting of two glass half-cells (volume approx. 5 mL each) separated by a dialysis membrane (Spectrum Laboratories, type Spectra/Por 4 RC with a molecular weight cutoff of 12-14 kD for BSA and type Spectra/Por 3 RC with a molecular weight cutoff of 3.5 kD for muscle protein). The dialysis cells were sealed with a PTFE ring and a plastic clamp and closed with plastic caps and PTFE septa (see Figure 1 for a photograph).

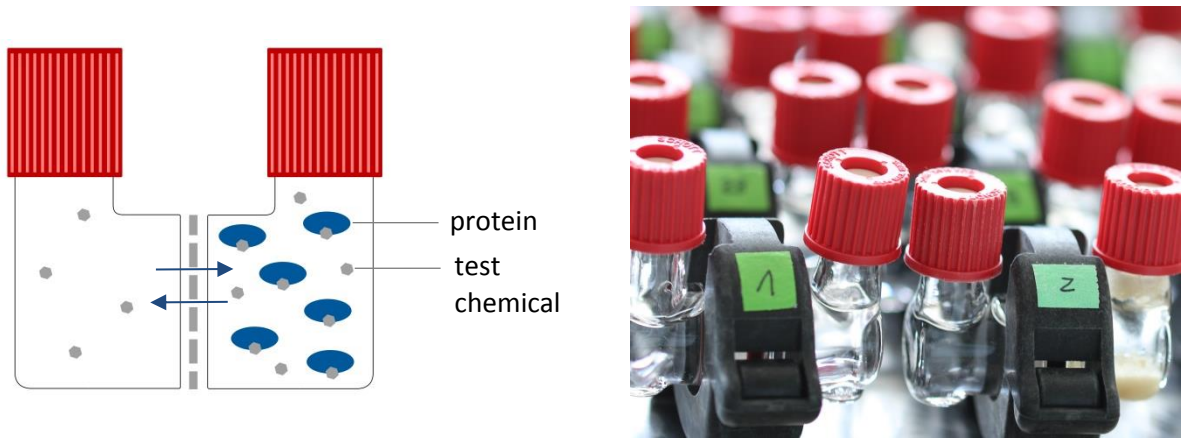

Figure 1 Experimental setup of dialysis method.

The dialysis cells with BSA were prepared as follows: one half-cell of the dialysis unit received 5 mL HBSS buffer and the other half-cell 4.9 mL BSA solution (1-50 g/L). All samples were spiked with 100 µL of a dilution of the test chemical in HBSS, which was prepared from a concentrated stock solution in methanol (final concentration of methanol in the system ≤0.5 vol%). The dialysis cells were magnetically stirred at 550 rpm at 37°C for equilibration. The experimental setup for the dialysis experiments with muscle protein was the same as with BSA, with minor modifications. The main difference

between BSA and muscle protein in the experiments was the solubility in water. Unlike serum albumin the muscle protein did not completely dissolve in water. Therefore, instead of pipetting a protein solution into the dialysis cells, a fixed amount of muscle protein was directly weighed into one half cell of the dialysis cells and 5 mL of a diluted solution of the test chemical in buffer were added. Because of the high amount of undissolved protein, stirring of the muscle protein suspension in the dialysis cells was not possible. Instead, the cells were equilibrated on an orbital shaker at 200 rpm at 37°C. The same amount of muscle protein (500 mg) was used for all experiments, because the final salt composition of the medium was found to be dependent on the amount of muscle protein. For both proteins three to four replicates of the dialysis cells were prepared. Aliquots of 100 µL were taken from the buffer-containing half-cell after two and three days (with no significant difference observed between the two time points). The concentration of the test chemicals was quantified in all samples by means of HPLC. More details on the instrumental analysis are provided in section 1.3.3. From the determined concentrations $K_{BSA/w}$ and $K_{MP/w}$ were calculated based on the mass balance calculation.

Control samples without protein were also prepared and measured in parallel. The average recovery of the test chemicals from the control samples was 91-117% for BSA and 87-105% for muscle protein. All sorption experiments were performed for individual chemicals and not with mixtures.

1.3.2 Passive sampling with ion exchange membranes (IEMs)

Equilibrium passive sampling with a polymeric sorbent such as solid phase microextraction (SPME) fibers is a useful tool for the determination of freely dissolved concentrations. In this method, the polymer is exposed to an aqueous solution containing the target chemical and the dissolved organic material such as protein. After equilibrium is established, the polymer phase is removed and the concentration in the polymer is measured. From the concentration in the polymer ($C_{polymer}$ [mmol/kg]) and the pre-determined polymer-water partition coefficient ($K_{polymer/w}$ [L/kg]), the freely dissolved concentration of the target chemical in the aqueous phase at the final equilibrium state (C_{free} [mmol/L]) can be calculated according to the following equation:

$$C_{\text{free}} = \frac{C_{polymer}}{K_{polymer/w}} \qquad (1)$$

The approach has been widely investigated and applied for neutral organic chemicals.[23-27] Several studies have also tried to adapt this technique to IOCs. For example polyacrylate (PA) coated SPME fibers have been used for passive sampling of weak acids and bases.[28-31] But these studies demonstrated that sorption by PA fibers is only significant for the neutral species of the studied weak acids and bases and this suggests that PA cannot effectively extract strong acids and bases that are fully ionized in water, unless the molecule contains a very large hydrophobic moiety in its structure. Very recently, "mixed-mode" SPME fibers that combine hydrophobic and cation exchange properties have been successfully applied for passive sampling of cationic pharmaceuticals and illicit drugs,[32-35] but the used material is not commercially available yet.

In this work the applicability of ion exchange membranes (IEMs) for passive sampling of IOCs was tested. Organic ions were expected to have a high affinity for IEMs of opposite charge due to ionic interactions. The IEMs applied for this study were obtained from FuMA-Tech GmbH (St. Ingbert, Germany). The anion exchange membrane "FAS" used for anionic test chemicals contains quaternary amines as ion exchange groups, Br^- as preloaded counterion and has an ion exchange capacity of 1.5 meq/g. The cation exchange membrane "FKS" for use with cationic analytes contains sulfonic acids as ion exchange groups, H^+ as preloaded counterion and has an ion exchange capacity of 1.2 meq/g. Both membranes were provided with a nominal thickness of 20 and 30 μm. The type of IEM used for the different test chemicals is shown in Table 1. For all experiments, discs (approximately 9 mm in diameter) were cut out of the IEMs with dissecting scissors, weighed on an analytical balance (1.5-3.5 mg, depending on membrane type and thickness) and conditioned in HBSS for at least 3 hours. To allow a complete extraction of the test chemicals from the IEMs after the passive sampling experiments, different extraction solutions were checked out in preliminary experiments. Either pure solvent or solvent-water mixtures, all containing inorganic acid, base or salt, were used.

The partitioning of 4-ethylbenzene-1-sulfonate, 2,4-dichlorophenoxyacetic acid and pentachlorophenol to the FAS membrane and of difenzoquat, nicotine and verapamil to

the FKS membrane was investigated. All test chemicals exhibited a sufficiently high affinity for the respective IEM with logarithmic IEM-water partition coefficients (log $K_{IEM/w}$) >2.3 (see also Table 1). Sorption equilibrium was established quickly, within several hours for the FAS membrane and within 1-3 days for the FKS membrane.

Table 1 Determined Partition Coefficients at 37°C (pH 7.4 in HBSS with 10 mM Tris).

Chemical	IEM	log $K_{IEM/w}$	log $K_{BSA/w}$	
		C_{free}=0.01 mmol/L	Passive Sampling	Equilibrium Dialysis
2,4-dichlorophenoxyacetic acid	FAS-30	3.62	3.46 ± 0.02	3.28 ± 0.10
4-ethylbenzene-1-sulfonate	FAS-20	3.19	3.19 ± 0.04	3.17 ± 0.03
pentachlorophenol	FAS-20	>6[a]	nd	5.27 ± 0.10
verapamil	FKS-30	2.32[b]	2.96 ± 0.35[b]	0.97 ± 0.13
nicotine	FKS-20	2.70	<0.5[c]	<0.5[c]
difenzoquat	FKS-30	4.40	<0.5[c]	<0.5[c]

[a] Calculated from the lowest quantifiable water concentration.

[b] Determination of $K_{IEM/water}$ and $K_{BSA/water}$ is not reliable, because of possible saturation of FKS membrane with verapamil.

[c] Concentration reduction in the IEM (passive sampling) or in the buffer phase (equilibrium dialysis) due to the binding to BSA was too small (<10%).

The influence of pH value and salt concentration (i.e., co-existing inorganic ions) on $K_{IEM/w}$ was also investigated. The pH-dependence was small (<0.08 log units) for the sorption of 4-ethylbenzene-1-sulfonate and difenzoquat to FAS and FKS membrane, respectively. This result indicates that partition coefficients for ionic species measured at a single pH value can represent those for other pH values as well. The measurements at different salt concentrations clearly showed that types and concentrations of salts have significant influences on the partition coefficients of the tested organic ions. For example increasing Cl^- concentration by a factor of 3 decreases $K_{IEM/w}$ of 4-ethylbenzene-1-sulfonate by a factor of 2.4.

Isotherm experiments were performed for 2,4-dichlorophenoxyacetic acid, 4-ethylbenzene-1-sulfonate, nicotine, difenzoquat and verapamil. A moderately non-linear sorption behavior to both types of membrane was found. Hence, $K_{IEM/w}$ is dependent on the concentration of the test chemicals. For verapamil a nearly constant concentration

was measured in the IEM at any measured water phase concentration, indicating that the IEM was already saturated with the test chemical at the lowest water concentration tested.

For the determination of $K_{BSA/w}$ IEMs were exposed to solutions of BSA in HBSS (1-10 mL, BSA concentration 15-50 g/L) that were previously spiked with the test chemical. The same equilibration times as determined for IEM-water partitioning experiments were applied. The membranes were taken out, cleaned thoroughly with lint-free tissues and extracted. The concentration of the test chemical in the extract was measured to determine the concentration in the IEM. As shown in Table 1 $K_{BSA/w}$ determined by passive sampling with IEMs agree well with those determined by equilibrium dialysis.

The results of this study indicate that IEMs exhibit the potential to measure freely dissolved concentrations of organic ions in a simple and time-saving manner. Because the measured salt concentration dependence of $K_{IEM/w}$ and the non-linear sorption behavior of the test chemicals complicate the IEM passive sampling experiments with protein, the equilibrium dialysis method was finally selected for the determination of protein-water partition coefficients in this study.

1.3.3 Instrumental analysis

For the majority of the test chemicals an HPLC system from JASCO was used, equipped with an Eclipse Plus C18 column (4.6 mm × 100 mm, 5 µm particle size) or a ZORBAX Extend C18 column (4.6 mm × 150 mm, 5 µm particle size), both from Agilent. Chemicals were detected using either a UV detector (UV-970 M, JASCO) or a fluorescence detector (RF-10AXL, Shimadzu). Gradient or isocratic elution of acetonitrile and water (both containing 0.1 % orthophosphoric acid) at a flow rate of 1 mL/min was applied. For verapamil all samples and standards in HBSS were diluted 1:1 with methanol before the measurement for better sensitivity. For 4-ethylbenzenesulfonate some samples with low concentrations had to be enriched with SPE prior to analysis. Propranolol and nicotine were measured with a Shimadzu HPLC system equipped with a diode array detector (SPD-M10AVP) and a Phenomenex Luna HILIC column (4.6 mm × 100 mm, 5 µm particle size). The mobile phase was a mixture of acetonitrile and water (90:10 or 10:90) both with 5 mM ammonium acetate. A flow rate of 1 mL/min was applied.

For chemicals that needed a sensitive quantification method, LC-MS/MS measurements were performed with three different instruments. First, an Acquity UPLC i-Class system equipped with a Xero TQ-S mass spectrometer and an Acquity UPLC HSS T3 column (100 mm × 2.1mm, 1.8 μm particle size), all purchased from Waters. Gradient elution of methanol and water, both containing 0.1 % formic acid, was applied. The flow rate was 0.6 mL/min. Second, an Acquity UPLC system from Waters with a Xevo TQ mass spectrometer operating in negative ion mode. The test chemicals were separated on an Acquity BEH C18 column (100 × 2.1 mm, 1.7 μm particle size, Waters). Gradient elution of acetonitrile and water (with 10 mM ammonium acetate) was applied at a flow rate of 0.2 mL/min. Third, an UPLC system from Agilent Technologies (1290 Infinity Series) equipped with a 6400 Triple Quad mass spectrometer operating in negative ion mode and a Poroshell 120 EC C18 column (50 × 4.6 mm, 2.7 μm particle size, Agilent Technologies). The mobile phase was a mixture of acetonitrile and water (both with 0.1 % formic acid) and a flow rate of 1 mL/min was applied.

1.3.4 Quality assurance

As discussed in the introduction, data for protein binding are often published without sufficient quality control. To assure comparability and consistency of the protein binding data of this study, the influence of different experimental parameters on $K_{BSA/w}$ and $K_{MP/w}$ was investigated.

Leaking of dialysis membrane

In preliminary experiments the amount of protein that passes the dialysis membrane was determined using the Bradford assay. For both proteins only 0.01 % of the total protein in the dialysis cell was found to diffuse through the membrane, which is not expected to influence the determination of $K_{BSA/w}$ and $K_{MP/w}$ in our experiments.

Volume shift

At high concentrations dissolved macromolecules like proteins possibly cause a colloid osmotic pressure that leads to a volume shift in the dialysis cell. However, this was not observed in the experiments with BSA and was found to be small for muscle protein (only up to 10% of the total volume).

Reversibility of protein binding

Because irreversible binding of the test chemicals to the tested protein would complicate the measurement of partition coefficients, reversibility of BSA and muscle protein binding was determined using the following method: dialysis cells with BSA and muscle protein were prepared as described above and the dialysis experiment was performed. After three-day equilibration, the buffer containing half-cell was emptied completely and was refilled with fresh buffer. Additional three days were given for equilibration and the buffer phase was sampled again. If binding to the proteins is completely reversible and no mass loss of the chemicals occurred, the partition coefficients calculated from the equilibrium concentrations measured after three and six days should be the same. This was the case for both proteins. Therefore we assume that the interactions of the test chemicals with BSA and muscle protein are non-covalent and reversible.

Sorption isotherms

Because log $K_{MP/w}$ and log $K_{BSA/w}$ for the test chemicals of this study were measured at different concentration levels, it is important for their comparability that the sorption to BSA and muscle protein is linear in the tested concentration range (i.e., the partition coefficients are independent of the concentration of the chemical) or that the degree of non-linearity is known. For the experiments with BSA the amount of the bound test chemicals was kept well below the amount of BSA (i.e., ≤ 0.1 mol/mol at equilibrium), to avoid saturation of the binding sites of BSA. For muscle protein sorption isotherms were measured for six test chemicals. All isotherms were fitted using the log-transformed Freundlich model, $\log C_{MP} = n_f \cdot \log C_{free} + \log K_{Fr}$, where n_f and K_{Fr} are the Freundlich exponent and Freundlich coefficient, respectively. The Freundlich exponent is 1.00 ± 0.08 (mean ± standard deviation) for the tested chemicals, indicating linear sorption to muscle protein.

pH and salt concentration dependence

The partitioning of IOCs to proteins may be influenced by pH value and salt concentration. For log $K_{BSA/w}$ the observed pH dependence was relatively small (difference ≤0.2 log units measured for 2,6-dichlorobenzoic acid, pH 6-8). In contrast, the results for the measurements at different salt concentrations for 2,6-dichlorobenzoic acid show a clear competition effect (e.g., increasing the Cl- concentration by a factor of

50 decreases $K_{BSA/w}$ by a factor of 17). As a consequence, all data for BSA binding discussed in the following sections were measured with a buffer of the same composition. For binding to muscle protein increasing the pH value from 6 to 9 decreased log $K_{MP/w}$ for 3,4-dichlorobenzoic by 0.5 log units, while for benzyldimethyloctylammonium log $K_{MP/w}$ increased by 0.4 log units. A factor of 10 difference in the K^+, phosphate or lactate concentration caused only a factor of ≤1.6 difference in $K_{MP/w}$ (or ≤0.2 log units) for the chemicals tested. These results indicate that minor variations in experimental pH and salt concentrations do not influence $K_{MP/w}$. Nevertheless, some control is still needed, because shifts in pH value can change the speciation of ionizable chemicals, and neutral and ionic species of a chemical can exhibit different partitioning behavior toward the protein.

1.4 Experimentally determined partition coefficients to selected proteins

1.4.1 Bovine serum albumin (BSA)

BSA-water partition coefficients ($K_{BSA/w}$) were successfully measured for 45 anionic and 4 cationic organic chemicals. The determined log $K_{BSA/w}$ range from 0.97 to 5.27 (Table 3, Appendix). The organic anions measured in this study tended to show high affinities for BSA. In contrast, binding of the tested cations to BSA was often too weak to be measureable (i.e., fraction bound <20 % in our dialysis experiments) and log $K_{BSA/w}$ was finally only determined for four cationic chemicals. From the dataset presented in Table 3 (Appendix), it can be seen that addition of a CH_2 group or a longer chain to the molecule increases log $K_{BSA/w}$ by, on average, 0.28-0.62 log units per CH_2 unit. For example, a linear increase of log $K_{BSA/w}$ was measured for a homologous series of 4-alkylbenzoic acids with a slope of 0.31 log units/CH_2.

An interesting outcome of this work is that, for a series of anionic substituted benzenes and naphthalenes measured in this study, a remarkably high influence of the substitution pattern of the molecules on BSA binding was found. As presented in Figure 2, data for benzoic acids show that substitution in direct vicinity to the charged carboxylate group lowers the partition coefficient substantially. For example, chlorinated benzoic acids show the following trends: 3-chlorobenzoic acid and 4-chlorobenzoic acid have similar partition coefficients (log $K_{BSA/w}$ is 3.22 and 3.21, respectively), while log $K_{BSA/w}$ for 2-chlorobenzoic acid is only 1.84. For the two constitutional isomers, 3,4-dichlorobenzoic

acid and 2,6-dichlorobenzoic acid, a log $K_{BSA/w}$ of 4.06 and 1.65 was determined, respectively, which means a difference of more than two orders of magnitude. A similar trend was found for methylated benzoic acids: log $K_{BSA/w}$ is 2.67 for 4-methylbenzoic acid, but only 1.99 for 2-methylbenzoic acid. It is also interesting that log $K_{BSA/w}$ of 2,4,6-trimethylbenzoic acid is just 2.26, although it has two carbon atoms more than 4-methylbenzoic acid (2.67). These consistent decreases of $K_{BSA/w}$ upon ortho-substitutions on benzoic acid suggest steric effects on the BSA binding, e.g., reduced accessibility to binding sites.

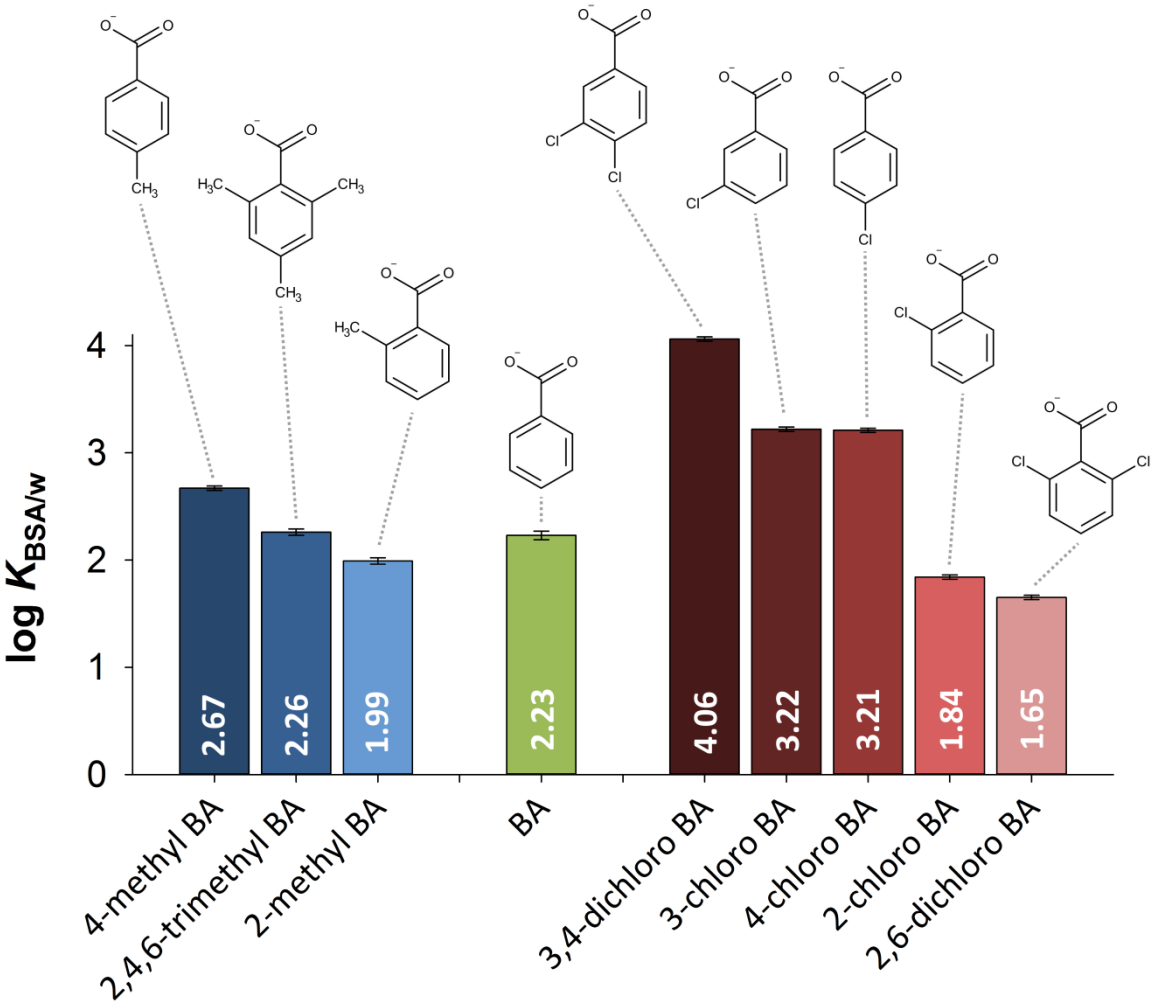

Figure 2 Influence of substitution position on log $K_{BSA/w}$ (BA - benzoic acid).

Another notable finding is that 1-naphthoic acids consistently have much lower BSA-water partition coefficients than corresponding 2-naphthoic acids (Figure 3). The determined log $K_{BSA/w}$ of 1-naphthoic acid is 2.81, while it is 4.36 for 2-naphthoic acid; 1-naphthaleneacetic acid and 2-naphthaleneacetic acid have log $K_{BSA/w}$ of 3.43 and 4.77,

respectively and 2-methoxy-1-naphthoic acid and 3-methoxy-2-naphthoic acid of 2.16 and 2.86, respectively. The difference might be explained by the 3D shape of these chemicals, because 2-naphthoic acids are more linear, whereas the 1-naphthoic acids have a more bulky structure.

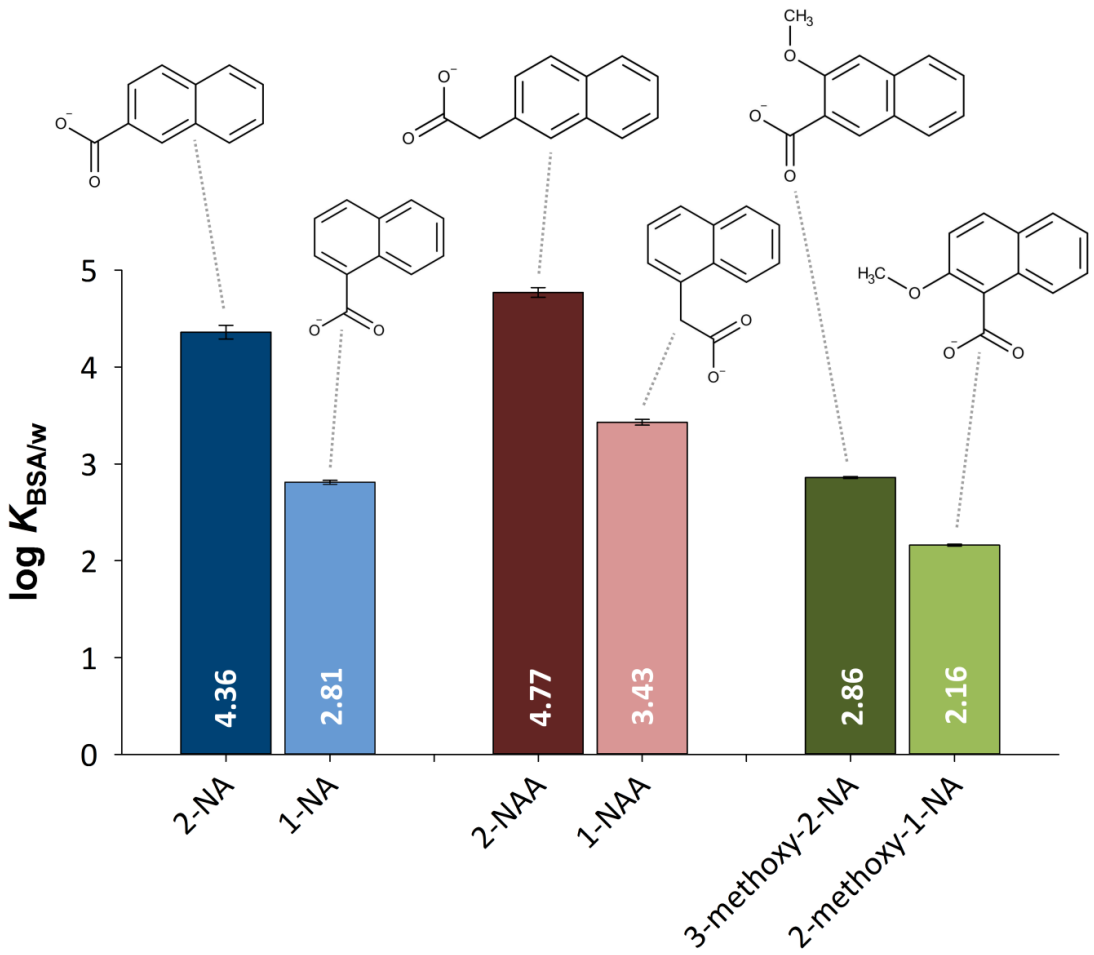

Figure 3 Log $K_{BSA/w}$ of selected substituted naphthalenes (NA - naphthoic acid, NAA - naphthaleneacetic acid).

In the dataset, we included four pairs of chemicals that have the same non-ionic substructure but different charged functional groups (i.e., sulfonate vs carboxylate): 4-ethylbenzenesulfonate and 4-ethylbenzoic acid, naphthalene-2-sulfonate and 2-naphthoic acid, 4-bromobenzenesulfonate and 4-bromobenzoic acid, and 2,4,6-trimethylbenzenesulfonate and 2,4,6-trimethylbenzoic acid. For the first three pairs, the type of the charged functional group has only a minor influence on log $K_{BSA/w}$ (0.14-0.35 log units). However, a large difference (1.97 log units) was found between 2,4,6-trimethylbenzenesulfonate and 2,4,6-trimethylbenzoic acid, which cannot easily be

explained. As already mentioned above, 2,4,6-trimethylbenzoic acid has two methyl substitutions in direct vicinity of the charged group and its $K_{BSA/w}$ value is even lower than that of benzoic acid most likely due to a steric effect. In contrast, 2,4,6-trimethylbenzenesulfonate appears not to experience such a steric effect, as is indicated by the $K_{BSA/w}$ value being greater than that of 4-ethylbenzenesulfonate.

1.4.2 Muscle protein

Muscle protein-water partition coefficients (log $K_{MP/w}$) were determined for 41 anionic and 10 cationic test chemicals on a single concentration level (Table 3, Appendix). In general, the measured partition coefficients are rather low. For the majority of the test chemicals (38 out of 51) log $K_{MP/w}$ is smaller than 2 and many chemicals could not be measured because of too weak sorption (fraction bound <20 %, which corresponds to log $K_{MP/w}$ ≤0.6). Two primary amines (4-phenylbutylamine and 1-naphthylmethylamine) had to be removed from the dataset, because results for log $K_{MP/w}$ were not reproducible.

Especially compared to the partition coefficients measured for BSA, log $K_{MP/w}$ of this study fall within a lower range (0.70 to 3.36). Furthermore, for sorption to muscle protein steric effects are less evident. For BSA a much stronger sorption of 2-naphthoic acids compared to 1-naphthoic acids was found, whereas for muscle protein all naphthoic acids have similar partition coefficients. As was observed with BSA, sorption to muscle protein for ortho-substituted benzoic acids seems to be lower than for other benzoic acids. For example, log $K_{MP/w}$ for 3-chlorobenzoic acid, 4-chlorobenzoic acid, and 3,4-dichlorobenzoic acid is 0.72, 0.72, and 1.31, respectively, while binding of 2-chlorobenzoic acid, 2,6-dichlorobenzoic acid, and 2,4,6-trimethylbenzoic acid was too weak to be measurable (fraction bound <20%, log $K_{MP/w}$ <0.6). The dataset for muscle protein includes three pairs of chemicals with the same non-ionic substructure, but with either a sulfonate or a carboxylate group as charged functional group: 4-bromobenzenesulfonate and 4-bromobenzoic acid, naphthalene-2-sulfonate and 2-naphthoic acid, and 1-pyrene sulfonate and 1-pyrenecarboxylic acid. The difference between sulfonates and carboxylates is small (≤0.1 log units), which is again similar to the observations with BSA.

1.4.3 Comparison of serum albumin and muscle protein

Figure 4 shows a comparison of experimental log $K_{MP/w}$ and log $K_{BSA/w}$. For 31 anionic and 4 cationic chemicals of this work partition coefficients to both proteins are available. Data for 39 neutral chemicals from Endo et al.[8, 9] were included for comparison. While the data scatter considerably, some clear trends do exist. For the neutral chemicals log $K_{MP/w}$ is on average one log unit smaller than log $K_{BSA/w}$. For the anionic chemicals log $K_{MP/w}$ is much smaller than log $K_{BSA/w}$ by up to 3.7 log units (for 2-naphthaleneacetic acid and naphthalene-2-sulfonate). For the four cations log $K_{MP/w}$ and log $K_{BSA/w}$ are similar.

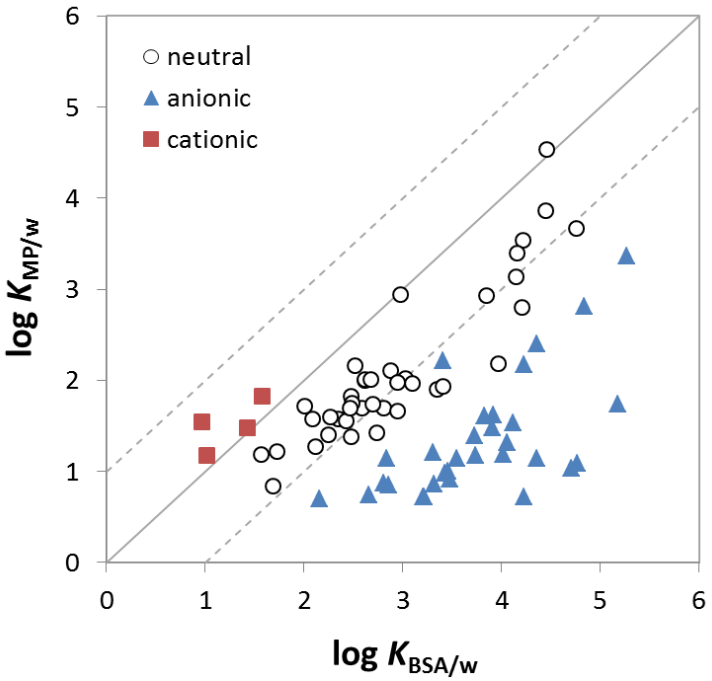

Figure 4 Comparison of experimentally determined muscle protein-water partition coefficients (log $K_{MP/w}$) and BSA-water partition coefficients (log $K_{BSA/w}$).

Combining the results for BSA and muscle protein we shortly summarize here the similarities and differences in sorption properties between BSA and muscle protein. First, anionic chemicals sorb stronger to BSA than to muscle protein, while cationic chemicals seem to show similar partition coefficients to both proteins. Here, it should be noted that a comparison of both proteins was only possible for four cationic chemicals (two secondary and two tertiary amines) and could be specific for these chemicals. Second, a high influence of the 3D molecular structure was found for sorption to BSA, while this was of minor importance for muscle protein. Third, sorption to BSA is influenced by the concentration of competing ions, whereas sorption to muscle protein is not.

1.5 Modeling and application of binding data

1.5.1 Comparison with octanol-water partition coefficients

As a first step towards developing a predictive model for partitioning of organic ions to serum albumin and structural proteins, we plotted log $K_{BSA/w}$ and log $K_{MP/w}$ determined in this study against various other partition coefficients that can be derived more easily than the protein-water partition coefficients themselves. For example, the data were compared with logarithmic octanol-water partition coefficients of the neutral species of the test chemicals (log K_{ow} (neutral)), although we are not hypothesizing that there is a mechanistic relationship between our measured protein-water partition coefficients and log K_{ow} (neutral). Log K_{ow} (neutral) data are readily available from the literature and many environmental models are based on correlations with log K_{ow} (neutral), although it has been shown that such models are purely on an empirical basis and can be inaccurate even for neutral chemicals.[5] All log K_{ow} values shown in Figures 5 and 6 are taken from the EPI-Suite data base (version 4.1) provided by the U.S. Environmental Protection Agency. If no experimental log K_{ow} (neutral) was available, the value calculated from EPISuite was used instead. For comparison, BSA binding data for 83 neutral chemicals and $K_{MP/w}$ data for 46 neutral chemicals from Endo et al.[8, 9] are included in Figures 5 and 6, respectively.

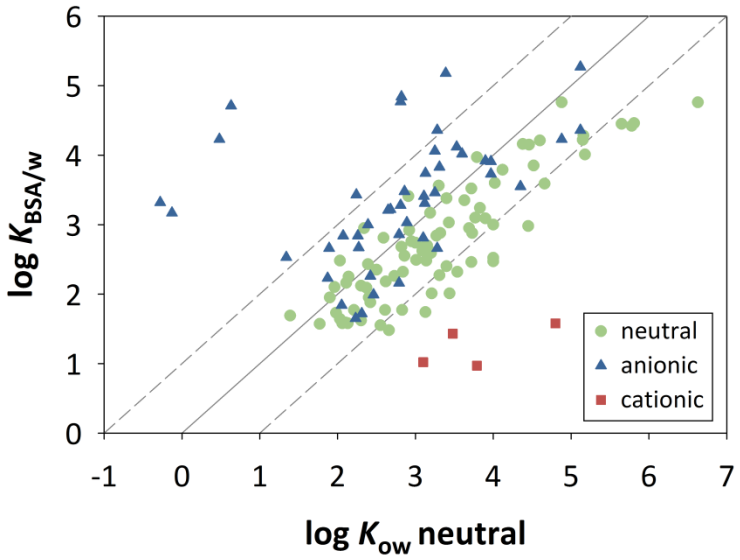

Figure 5 Comparison of experimentally determined BSA-water partition coefficients (log $K_{BSA/w}$) and octanol-water partition coefficients of the neutral species (log K_{ow} neutral).

For BSA binding the following trends were observed: for the neutral chemicals, there is a relatively weak but clear positive trend between log $K_{BSA/w}$ and log K_{ow} (R^2 = 0.75).[8] In contrast, for the organic anions and cations of this study, there is no overall trend (R^2 = 0.13 for all anions). The high scatter appears to result from several reasons. First, according to EPI-Suite, sulfonates have extremely small log K_{ow} (neutral), except for naphthalene-2-sulfonate, which is the only sulfonate for which an experimental log K_{ow} (neutral) was available. The reliability of log K_{ow} (neutral) calculated by EPI-Suite for sulfonates is unclear, because experimental log K_{ow} for neutral species of sulfonates are difficult to measure. Second, for the cationic test chemicals, the relationship between log $K_{BSA/w}$ (ion) and log K_{ow} (neutral) appears to be substantially different from that of anions. Third, while log K_{ow} (neutral) captures the increase of log $K_{BSA/w}$ by addition of CH_2 increments, the other trends that were found in the dataset for benzoic and naphthoic acids of this study (e.g., influence of substitution position and differences between 1- and 2-naphthoic acids) cannot be depicted with log K_{ow} (neutral).

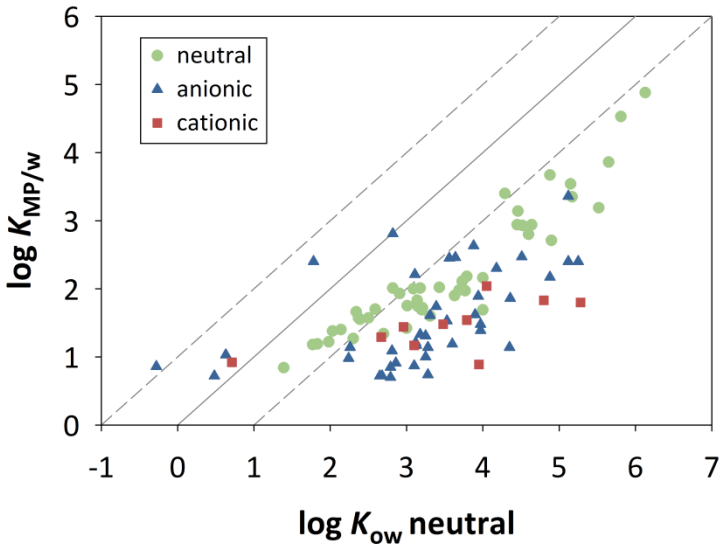

Figure 6 Comparison of experimentally determined muscle protein-water partition coefficients (log $K_{MP/w}$) and octanol-water partition coefficients of the neutral species (log K_{ow} neutral).

The binding data for muscle protein show a better correlation with log K_{ow}, compared to log $K_{BSA/w}$ (Figure 6, R^2 = 0.53 and RMSE = 0.41 for all chemicals). Nevertheless, the overall correlation may still be too unsatisfying to be considered as a predictive model for muscle protein binding.

1.5.2 Polyparameter linear free energy relationships (PP-LFERs)

For neutral organic chemicals PP-LFER models for partitioning to BSA and muscle protein based on eq 2 are already available from the literature (eq 4 + 6 in Table 2).[8, 9]

$$\log K_{protein/w} = c + eE + sS + aA + bB + vV \qquad (2)$$

In eq 2 the capital letters represent the substance properties and the small letters the corresponding system properties. *E* is the excess molar refraction, *S* is the polarizability/dipolarity parameter, *A* represents the H-bond donor properties, *B* the H-bond acceptor properties, and *V* is the molar volume. This PP-LFER approach is applicable for neutral chemicals only.

Abraham et al.[36, 37] have proposed a PP-LFER equation that can be used for ionic chemicals as well (eq 3).

$$\log K_{protein/w} = c + eE_i + sS_i + aA_i + bB_i + vV_i + j^+J_i^+ + j^-J_i^- \qquad (3)$$

Note that in this approach ionic chemicals do not only have specific descriptor values for *E*, *S*, *A*, *B* and *V* (denoted with subscript *i*), but also require two additional descriptors for their charge (J_i^+ and J_i^-). Descriptors for the neutral species of all test chemicals (*E*, *S*, *A*, *B*, *V*) were required for the calculation of the descriptors of the corresponding ionic species. If available, the experimentally determined descriptors of neutral species collected in the UFZ-LSER database[38] were used. For many chemicals no experimental substance descriptors were found and the descriptors for such chemicals were predicted using the ABSOLV module from the ACD/Percepta software (2015 release). Using the empirical equations from Abraham et al.[36, 39] and the descriptors of the neutral species, descriptors of the ionic species (E_i, S_i, A_i, B_i, V_i, J_i^+, J_i^-) were calculated. Because such equations are only available for phenols, carboxylic acids, pyridines, and amines, several chemicals had to be excluded from the calculation (e.g., sulfonates, coumarines, quaternary ammonium compounds).

Table 2 Regression equations derived for log $K_{BSA/w}$ [L_{water}/kg_{BSA}] and log $K_{MP/w}$ [L_{water}/kg_{MP}], values in parentheses denote standard errors.

Eq	*e*	*s*	*a*	*b*	*v*	*c*	j^-	j^+	*n*	R^2	RMSE
serum albumin											
eq 4[a]	0.36 (0.12)	-0.26 (0.21)	0.37 (0.24)	-3.23 (0.31)	2.82 (0.25)	0.14 (0.25)	-	-	82	0.78	0.42
eq 5	0.634 (0.081)	-0.630 (0.074)	-0.054 (0.222)	-2.085 (0.188)	2.059 (0.197)	0.850 (0.189)	3.133 (0.280)	-1.156 (0.273)	123	0.723	0.498
muscle protein											
eq 6[a]	0.51 (0.10)	-0.51 (0.17)	0.26 (0.17)	-2.98 (0.24)	3.01 (0.21)	-0.79 (0.25)	-	-	46	0.95	0.22
eq 7	0.675 (0.067)	-0.764 (0.083)	-0.196 (0.146)	-2.285 (0.120)	2.511 (0.143)	-0.237 (0.157)	2.890 (0.209)	-0.682 (0.188)	86	0.887	0.288

[a] equations taken from Endo et al.[8, 9] calibrated with neutral chemicals only.

First, a PP-LFER model was fitted to the BSA binding data of this study (37 anions and 4 cations), including 82 neutral chemicals from Endo et al.[8] The derived PP-LFER equation (eq 5) gives a good correlation for log $K_{BSA/w}$ (R^2 = 0.72, RMSE = 0.50, Figure 7), but important trends in the dataset such as the influence of substitution position and differences between 1- and 2-naphthoic acids are not captured correctly. This deficiency of the model is not surprising, because the solute descriptors used for PP-LFER models incorporate only the volume of the solute, but not the specific 3D structure.[5] Because there may well be unknown structural effects that cause even larger errors, we do not recommend a general use of the PP-LFER equation derived within this study to predict sorption of organic ions to serum albumin.

A second PP-LFER model was fitted to the binding data for muscle protein. Again we added $K_{MP/w}$ data for 46 neutral chemicals from Endo et al.[9] for extension of the dataset. All chemicals (46 neutral, 34 anionic, 6 cationic, 86 in total) were used to derive all system descriptors (*e*, *s*, *a*, *b*, *v*, j^+, j^-), leading to eq 7 (Table 2). As depicted in Figure 7, eq 7 fits the data well for all chemicals (R^2 = 0.89, RMSE = 0.29).

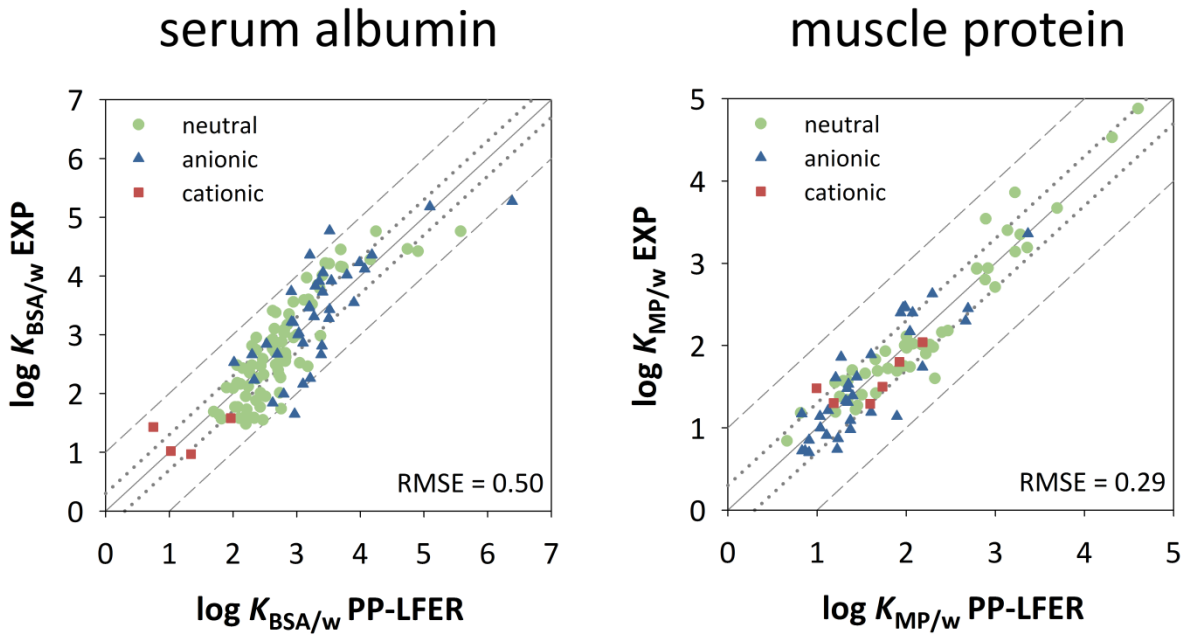

Figure 7 Comparison of experimentally determined partition coefficients with PP-LFER fitted partition coefficients for BSA and muscle protein; the solid line denotes the 1:1 line, dotted and dashed lines indicate a deviation of 0.3 and 1 log unit, respectively.

The good performance of eq 5 + 7 is surprising, keeping in mind that empirical formulas were used to calculate the descriptors of ionic species from the descriptors of neutral species and that the descriptors of the neutral species themselves are also predicted for many chemicals (22 out of 41 ionic chemicals for BSA, 23 out of 40 ionic chemicals for muscle protein).

1.5.3 Implications for partitioning of IOCs in vivo

As an application of the dataset obtained in this study, partition coefficients between muscle tissue and plasma were estimated for different chemicals based on their partitioning to muscle protein and serum albumin. In the literature[40] it has been reported that in vivo measured muscle tissue-plasma steady-state distribution ratios ($K_{muscle/plasma}$) show the following trend: cationic chemicals have the highest values for $K_{muscle/plasma}$ (0.5 - 29), followed by neutral chemicals (0.4 - 6), while anionic chemicals show relatively small $K_{muscle/plasma}$ (< 0.2). In Figure 8 selected literature data are plotted for 11 neutral chemicals and 42 acids and bases that are predominantly present in their ionic form (>95 %) and have bound fractions of more than 20% in plasma.

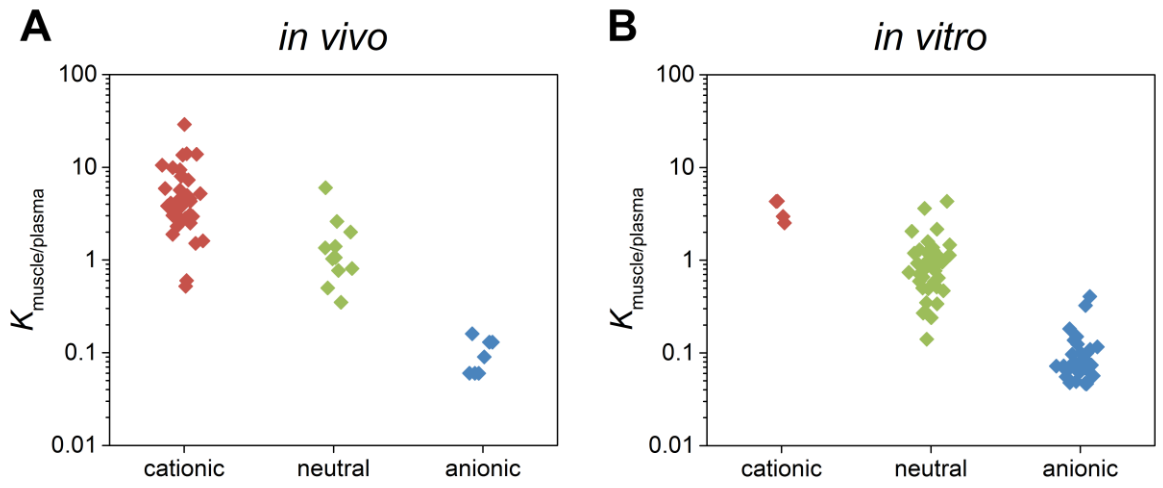

Figure 8 A) Experimental muscle tissue-plasma partition coefficients ($K_{muscle/plasma}$) of selected chemicals from reference 40 and B) calculated $K_{muscle/plasma}$ for the chemicals of this work and a previous work.[8, 9]

Using a simple model (eq 8) equilibrium $K_{muscle/plasma}$ were estimated for all chemicals for which experimental data for $K_{BSA/w}$ and $K_{MP/w}$ were available (4 cationic and 31 anionic chemicals of this study and 39 neutral chemicals from Endo et al.[8, 9]).

$$K_{muscle/plasma} = \frac{f_{SA,muscle} \cdot K_{BSA/w} + f_{MP,muscle} \cdot K_{MP/w} + f_{w,muscle}}{f_{SA,plasma} \cdot K_{BSA/w} + f_{protein,plasma} \cdot K_{MP/w} + f_{w,plasma}} \qquad (8)$$

In the calculation it is assumed that the only relevant sorption phases in muscle tissue and plasma are structural proteins, serum albumin, and water. The volume fractions of serum albumin, muscle protein and water in muscle and plasma were calculated from physiological data collected in refs 17, 18 and 41, and are denoted with f_{SA}, f_{MP}, and f_w, respectively; $f_{protein,plasma}$ is the fraction of other serum proteins. Serum proteins other than albumin are represented by muscle protein, because partition coefficients to these proteins are not available for the test chemicals of this study. The calculated values for $K_{muscle/plasma}$ are presented in Figure 8. Interestingly, these theoretical partition coefficients show the same trend and dimension as the literature data for $K_{muscle/plasma}$. The highest values for $K_{muscle/plasma}$ were calculated for the four cationic chemicals (2.5 - 4.3). Neutral chemicals generally show lower values than cations (0.1 - 4.3) and for all anionic chemicals a very low $K_{muscle/plasma}$ was calculated ($\leq$ 0.4). Experimental log $K_{muscle/plasma}$ for (S)-(-)-propranolol and verapamil are 0.63 and 0.54, respectively, and agree well with the calculated partition coefficients of 0.47 and 0.64, respectively.

It should be noted that the calculation of $K_{muscle/plasma}$ above is very simplified, because it considers only serum albumin and muscle protein. Significant partitioning to phospholipids would increase the sorptive capacity of both plasma and muscle tissue. Because muscle tissue has more phospholipids than plasma,[41] $K_{muscle/plasma}$ would increase with increasing sorption to phospholipids. Specific sorption to plasma proteins other than serum albumin would increase the sorptive capacity of the plasma and thus decrease $K_{muscle/plasma}$. If α_1-acid glycoprotein binding is significant, for example, a lower $K_{muscle/plasma}$ is expected for neutral and cationic chemicals.

1.6 Conclusions and outlook

Within this study partition coefficients to BSA and muscle protein were successfully determined for systematically selected organic anions and cations. The obtained results do support the initial statement that binding to proteins can substantially influence the in vivo distribution of organic ions. For example anionic chemicals measured in this study tended to show high affinities for BSA. Given that serum albumin is present with a fraction of 2.9 vol%[42] and is the dominant sorption phase in blood plasma, the majority of the anions tested (38 out of 45) are expected to be more than 90 % bound to serum albumin in plasma. From the derived dataset it is also evident that sorption to muscle protein and serum albumin can differ substantially (particularly for anionic and neutral chemicals) and that for partitioning models (e.g., for the assessment of the bioaccumulation potential of chemicals) both proteins should be approached separately, instead of considering the bulk protein fraction of an organism as one sorption phase.

For BSA binding a large influence of the 3D structure of the chemicals was observed. Established modeling strategies, e.g., based on log K_{ow} or PP-LFERs were found to be not suitable for modeling BSA binding of organic ions, because they do not capture the 3D structure effects. Finding alternative modeling approaches that explicitly consider the 3D structure of BSA, for example 3D quantitative structure activity relationships (3D-QSARs) may be an interesting area for further research. For binding to muscle protein a PP-LFER model was successfully established. The derived equation may provide a predictive tool for partitioning of both neutral and ionic chemicals to structural proteins.

We finally evaluated if the in vitro measured protein-water partition coefficients of this study can explain muscle tissue-plasma partition coefficients that were measured in vivo. While more data are clearly required to assess partitioning of IOCs into biological tissues, the good prediction of the trends for in vivo $K_{muscle/plasma}$ is encouraging, as it suggests that the in vitro partitioning data for isolated serum albumin and muscle protein are relevant for the assessments of the in vivo behavior of IOCs. It should be noted that the $K_{MP/w}$ measured in this study represent a mixture of the different structural proteins found in muscle tissue. For a future extension of the modeling approach discussed above to other tissues than muscle tissue, partitioning behavior of organic ions towards other types of structural protein (e.g., keratin and collagen) should also be investigated.

1.7 References

1. Endo, S.; Brown, T. N.; Goss, K.-U., General Model for Estimating Partition Coefficients to Organisms and Their Tissues Using the Biological Compositions and Polyparameter Linear Free Energy Relationships. *Environ. Sci. Technol.* **2013,** *47*, (12), 6630-6639.

2. van der Heijden, S. A.; Hermens, J. L. M.; Sinnige, T. L.; Mayer, P.; Gilbert, D.; Jonker, M. T. O., Determining High-Quality Critical Body Residues for Multiple Species and Chemicals by Applying Improved Experimental Design and Data Interpretation Concepts. *Environ. Sci. Technol.* **2015,** *49*, (3), 1879-1887.

3. Escher, B. I.; Cowan-Ellsberry, C. E.; Dyer, S.; Embry, M. R.; Erhardt, S.; Halder, M.; Kwon, J.-H.; Johanning, K.; Oosterwijk, M. T. T.; Rutishauser, S.; Segner, H.; Nichols, J., 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.* **2011,** *24*, (7), 1134-1143.

4. Poole, C. F.; Ariyasena, T. C.; Lenca, N., Estimation of the environmental properties of compounds from chromatographic measurements and the solvation parameter model. *J. Chromatogr. A* **2013,** *1317*, 85-104.

5. Endo, S.; Goss, K.-U., Applications of Polyparameter Linear Free Energy Relationships in Environmental Chemistry. *Environ. Sci. Technol.* **2014,** *48*, (21), 12477-12491.

6. Geisler, A.; Endo, S.; Goss, K.-U., Partitioning of Organic Chemicals to Storage Lipids: Elucidating the Dependence on Fatty Acid Composition and Temperature. *Environ. Sci. Technol.* **2012,** *46*, (17), 9519-9524.

7. Endo, S.; Escher, B. I.; Goss, K. U., Capacities of Membrane Lipids to Accumulate neutral Organic Chemicals. *Environ. Sci. Technol.* **2011,** *45*, 5912-5921.

8. Endo, S.; Goss, K. U., Serum Albumin Binding of Structurally Diverse Neutral Organic Compounds: Data and Models *Chem. Res. Toxicol.* **2011,** *24*, (12), 2293-2301.

9. Endo, S.; Bauerfeind, J.; Goss, K.-U., Partitioning of Neutral Organic Compounds to Structural Proteins. *Environ. Sci. Technol.* **2012,** *46*, (22), 12697-12703.

10. Armitage, J. M.; Arnot, J. A.; Wania, F.; Mackay, D., Development and evaluation of a mechanistic bioconcentration model for ionogenic organic chemicals in fish. *Environ. Toxicol. Chem.* **2013,** *32*, (1), 115-128.

11. Peters Jr, T., *All About Albumin*. Academic Press: San Diego, 1995.

12. Kragh-Hansen, U., Molecular aspects of ligand binding to serum albumin. *Pharmacol. Rev.* **1981,** *33*, (1), 17-53.

13. Fanali, G.; di Masi, A.; Trezza, V.; Marino, M.; Fasano, M.; Ascenzi, P., Human serum albumin: From bench to bedside. *Mol. Asp. Med.* **2012,** *33*, (3), 209-290.

14. Fasano, M.; Curry, S.; Terreno, E.; Galliano, M.; Fanali, G.; Narciso, P.; Notari, S.; Ascenzi, P., The extraordinary ligand binding properties of human serum albumin. *IUBMB Life* **2005,** *57*, (12), 787-796.

15. Kremer, J. M.; Wilting, J.; Janssen, L. H., Drug binding to human alpha-1-acid glycoprotein in health and disease. *Pharmacol. Rev.* **1988,** *40*, (1), 1-47.

16. Nilsson, L. B., The bioanalytical challenge of determining unbound concentration and protein binding for drugs. *Bioanalysis* **2013,** *5*, (24), 3033-3050.

17. ICRP, Report of the Task Group on Reference Man. *ICRP Publication 23* **1975**.

18. ICRP, 2002. Basic Anatomical and Physiological Data for Use in Radiological Protection Reference Values. ICRP Publication 89. *Ann. ICRP 32 (3-4).*

19. Xing, B.; McGill, W. B.; Dudas, M. J., Sorption of benzene, toluene, and o-xylene by collagen compared with non-protein organic sorbents. *Can. J. Soil Sci.* **1994,** *74*, (4), 465-469.

20. Hansen, S.; Selzer, D.; Schaefer, U. F.; Kasting, G. B., An extended database of keratin binding. *J. Pharm. Sci.* **2011,** *100*, (5), 1712-1726.

21. Wang, L.; Chen, L.; Lian, G.; Han, L., Determination of partition and binding properties of solutes to stratum corneum. *Int. J. Pharm.* **2010,** *398*, (1–2), 114-122.

22. Sudlow, G.; Birkett, D. J.; Wade, D. N., The Characterization of Two Specific Drug Binding Sites on Human Serum Albumin. *Mol. Pharmacol.* **1975,** *11*, (6), 824-832.

23. Vaes, W. H. J.; Urrestarazu Ramos, E.; Verhaar, H. J. M.; Seinen, W.; Hermens, J. L. M., Measurement of the Free Concentration Using Solid-Phase Microextraction: Binding to Protein. *Anal. Chem.* **1996,** *68*, (24), 4463-4467.

24. Poerschmann, J.; Zhang, Z.; Kopinke, F.-D.; Pawliszyn, J., Solid Phase Microextraction for Determining the Distribution of Chemicals in Aqueous Matrices. *Anal. Chem.* **1997,** *69*, (4), 597-600.

25. Mayer, P.; Vaes, W. H. J.; Wijnker, F.; Legierse, K. C. H. M.; Kraaij, R.; Tolls, J.; Hermens, J. L. M., Sensing Dissolved Sediment Porewater Concentrations of Persistent and Bioaccumulative Pollutants Using Disposable Solid-Phase Microextraction Fibers. *Environ. Sci. Technol.* **2000,** *34*, (24), 5177-5183.

26. Heringa, M. B.; Hermens, J. L. M., Measurement of free concentrations using negligible depletion-solid phase microextraction (nd-SPME). *TrAC Trends in Analytical Chemistry* **2003,** *22*, (9), 575-587.

27. Vaes, W. H. J.; Urrestarazu Ramos, E.; Hamwijk, C.; van Holsteijn, I.; Blaauboer, B. J.; Seinen, W.; Verhaar, H. J. M.; Hermens, J. L. M., Solid Phase Microextraction as a Tool To Determine Membrane/Water Partition Coefficients and Bioavailable Concentrations in in Vitro Systems. *Chem. Res. Toxicol.* **1997,** *10*, (10), 1067-1072.

28. Ohlenbusch, G.; Kumke, M. U.; Frimmel, F. H., Sorption of phenols to dissolved organic matter investigated by solid phase microextraction. *Sci. Total Environ.* **2000,** *253*, (1–3), 63-74.

29. Escher, B. I.; Berg, M.; Muhlemann, J.; Schwarz, M. A. A.; Hermens, J. L. M.; Vaes, W. H. J.; Schwarzenbach, R. P., Determination of liposome/water partition coefficients of organic acids and bases by solid-phase microextraction. *Analyst* **2002,** *127*, (1), 42-48.

30. Broeders, J. J. W.; Blaauboer, B. J.; Hermens, J. L. M., Development of a negligible depletion-solid phase microextraction method to determine the free concentration of chlorpromazine in aqueous samples containing albumin. *J. Chromatogr. A* **2011,** *1218*, (47), 8529-8535.

31. Haftka, J. J. H.; Scherpenisse, P.; Jonker, M. T. O.; Hermens, J. L. M., Using Polyacrylate-Coated SPME Fibers To Quantify Sorption of Polar and Ionic Organic Contaminants to Dissolved Organic Carbon. *Environ. Sci. Technol.* **2013,** *47*, (9), 4455-4462.

32. Peltenburg, H.; Bosman, I. J.; Hermens, J. L. M., Sensitive determination of plasma protein binding of cationic drugs using mixed-mode solid-phase microextraction. *J. Pharm. Biomed. Anal.* **2015,** *115*, 534-542.

33. Peltenburg, H.; Droge, S. T. J.; Hermens, J. L. M.; Bosman, I. J., Sorption of amitriptyline and amphetamine to mixed-mode solid-phase microextraction in different test conditions. *J. Chromatogr. A* **2015,** *1390*, 28-38.

34. Peltenburg, H.; Groothuis, F. A.; Droge, S. T. J.; Bosman, I. J.; Hermens, J. L. M., Elucidating the sorption mechanism of "mixed-mode" SPME using the basic drug amphetamine as a model compound. *Anal. Chim. Acta* **2013,** *782*, (0), 21-27.

35. Peltenburg, H.; Timmer, N.; Bosman, I. J.; Hermens, J. L. M.; Droge, S. T. J., Sorption of structurally different ionized pharmaceutical and illicit drugs to a mixed-mode coated microsampler. *J. Chromatogr. A* **2016,** *1447*, 1-8.

36. Abraham, M. H.; Acree, W. E., Equations for the Transfer of Neutral Molecules and Ionic Species from Water to Organic phases. *J. Org. Chem.* **2010,** *75*, (4), 1006-1015.

37. Abraham, M. H.; Zhao, Y. H., Determination of Solvation Descriptors for Ionic Species: Hydrogen Bond Acidity and Basicity. *J. Org. Chem.* **2004,** *69*, (14), 4677-4685.

38. Endo, S.; Watanabe, N.; Ulrich, N.; Bronner, G.; Goss, K.-U., UFZ-LSER database v 2.1 [Internet]. In Helmholtz Centre for Environmental Research-UFZ: Leipzig, Germany, 2015; Vol. v 2.1.

39. Abraham, M. H., The permeation of neutral molecules, ions, and ionic species through membranes: Brain permeation as an example. *J. Pharm. Sci.* **2011,** *100*, (5), 1690-1701.

40. Yun, Y. E.; Edginton, A. N., Correlation-based prediction of tissue-to-plasma partition coefficients using readily available input parameters. *Xenobiotica* **2013,** *43*, (10), 839-852.

41. White, D. A., The phospholipid composition of mammalian tissues. In *Form and function of phospholipids*, Ansell, G. B.; Hawthorne, J. N.; Dawson, R. M. C., Eds. Elsevier Scientific Publishing Company: Amsterdam, London, New York, 1973.

42. Peyret, T.; Poulin, P.; Krishnan, K., A unified algorithm for predicting partition coefficients for PBPK modeling of drugs and environmental chemicals. *Toxicol. Appl. Pharmacol.* **2010,** *249*, (3), 197-207.

1.8 Abbreviations

BSA	bovine serum albumin
eq	equation
FAS	anion exchange membrane used in this study
FKS	cation exchange membrane used in this study
HBSS	Hanks' Balanced Salt Solution
IEM	ion exchange membrane
IOC	ionogenic organic chemical
K	partition coefficients
MP	muscle protein
nd	not determined
PA	polyacrylate
PP-LFER	polyparameter linear free energy relationship
PTFE	polytetrafluoroethylene
RC	regenerated cellulose
RMSE	root-mean-square error
rpm	revolutions per minute
SD	standard deviation

1.9 Appendix

Table 3 Bovine serum albumin-water ($K_{BSA/w}$) and muscle protein-water partition coefficients ($K_{MP/w}$) determined in this study at 37°C in HBSS buffer at pH 7.4 (for BSA) and in 30 mM phosphate buffer at pH 7.0-7.1 (for muscle protein).

CAS-No.	Chemical	log $K_{BSA/w}$ [L_{water}/kg_{BSA}]	SD	log $K_{MP/w}$ [L_{water}/kg_{MP}]	SD
935-95-5	2,3,5,6-tetrachlorophenol			2.63	0.02
87-86-5	pentachlorophenol	5.27	0.10	3.36	0.20
88-85-7	dinoseb			2.45	0.02
1420-07-1	dinoterb			2.46	0.04
1689-84-5	bromoxynil	5.18	0.06	1.74	0.02
122-59-8	2-phenoxyacetic acid	2.53	0.07	<0.5	
94-75-7	2,4-D	3.28	0.10		
94-82-6	2,4-DB	4.12	0.03	1.53	0.01
93-76-5	2,4,5-T	3.83	0.02	1.61	0.03
7085-19-0	mecoprop	3.74	0.06	1.17	0.02
81-82-3	coumachlor	3.41	0.02	2.21	0.02
117-52-2	coumafuryl	2.84	0.02	1.14	0.07
99105-77-8	sulcotrione	1.72	0.06	<0.6	
15687-27-1	ibuprofen	3.91	0.02	1.48	0.04
29679-58-1	fenoprofen	3.92	0.02	1.62	0.03
22071-15-4	ketoprofen	3.31	0.01	1.21	0.02
22204-53-1	naproxen			1.33	0.02
15307-86-5	diclofenac			2.47	0.08
91-40-7	N-phenylanthranilic acid			1.86	0.04
530-78-9	flufenamic acid			2.40	0.07
61-68-7	mefenamic acid	4.36	0.02	2.40	0.02
65-85-0	benzoic acid	2.23	0.04	<0.5	
118-91-2	2-chlorobenzoic acid	1.84	0.02	<0.5	
535-80-8	3-chlorobenzoic acid	3.22	0.02	0.72	0.02
74-11-3	4-chlorobenzoic acid	3.21	0.02	0.72	0.03
51-44-5	3,4-dichlorobenzoic acid	4.06	0.02	1.31	0.02
50-30-6	2,6-dichlorobenzoic acid	1.65	0.02	<0.5	
456-22-4	4-fluorobenzoic acid	2.84	0.03	<0.5	
62-23-7	4-nitrobenzoic acid	2.66	0.02	<0.5	
586-76-5	4-bromobenzoic acid	3.48	0.02	0.91	0.01
99-94-5	4-methylbenzoic acid	2.67	0.02	<0.5	
118-90-1	2-methylbenzoic acid	1.99	0.03	<0.5	
619-64-7	4-ethylbenzoic acid	3.03	0.04	<0.6	
20651-71-2	4-butylbenzoic acid	3.73	0.02	1.39	0.01

21643-38-9	4-hexylbenzoic acid	4.23	0.02	2.17	0.04
480-63-7	2,4,6-trimethylbenzoic acid	2.26	0.03	<0.5	
97023-48-8	2-cyclohexylbenzoic acid	3.55	0.04	1.14	0.02
93-09-4	2-naphthoic acid	4.36	0.07	1.14	0.01
581-96-4	2-naphthaleneacetic acid	4.77	0.05	1.09	0.02
86-55-5	1-naphthoic acid	2.81	0.02	0.87	0.02
86-87-3	1-naphthaleneacetic acid	3.43	0.03	0.98	0.02
20717-79-7	1-bromo-2-naphthoic acid	4.02	0.03	1.19	0.02
5773-80-8	6-bromo-2-naphthoic acid			1.89	0.02
883-62-5	3-methoxy-2-naphthoic acid	2.86	0.01	0.85	0.02
947-62-6	2-methoxy-1-naphthoic acid	2.16	0.01	0.70	0.01
2224-00-2	2-ethoxy-1-naphthoic acid	2.66	0.02	0.74	0.02
573-03-5	4-fluoro-1-naphthoic acid	3.46	0.01	1.00	0.02
938-94-3	α,4-dimethylphenylacetic acid	3.00	0.03		
19694-02-1	1-pyrenecarboxylic acid			2.30	0.02
14995-38-1	4-ethylbenzene-1-sulfonate	3.17	0.03	<0.5	
6148-75-0	2,4,6-trimethylbenzenesulfonate	4.23	0.05	0.72	0.02
6149-03-7	4-n-octylbenzenesulfonate	4.84	0.02	2.81	0.04
79326-93-5	4-bromobenzenesulfonate	3.32	0.04	0.86	0.04
532-02-5	naphthalene-2-sulfonate	4.71	0.04	1.03	0.05
59323-54-5	pyrene sulfonate			2.40	0.03
525-66-6	(S)-(-)-propranolol	1.43	0.05	1.48	0.02
13655-52-2	alprenolol	1.02	0.07	1.17	0.06
2403-22-7	N-benzyl-N-butylamine			<0.5	
589-08-2	N-methyl-phenethylamine			<0.6	
54910-89-3	fluoxetine			2.04	0.01
103-49-1	dibenzylamine	<0.5		1.29	0.02
50-49-7	imipramine	1.58	0.09	1.83	0.03
52-53-9	(±)-verapamil	0.97	0.13	1.54	0.07
43222-48-6	difenzoquat	<0.5		0.92	0.07
23616-79-7	benzyltributylammonium	<0.5		0.89	0.09
959-55-7	benzyldimethyloctylammonium	<0.5		1.44	0.02
2001-45-8	tetraphenylphosphonium			1.80	0.01

2 Abstracts of the original publications

2.1 Ion exchange membranes as novel passive sampling material for organic ions: Application for the determination of freely dissolved concentration

Many studies in pharmacology, toxicology and environmental science require a method for determining the freely dissolved concentration of a target substance. A recently developed tool for this purpose is equilibrium passive sampling with polymeric materials. However, this method has rarely been applied to ionic organic substances, primarily due to limited availability of convenient sorption materials. This study introduces ion exchange membranes (IEMs) as a novel passive sampling material for organic ions. The partitioning of 4-ethylbenzene-1-sulfonate, 2,4-dichlorophenoxyacetic acid and pentachlorophenol to one anion exchange membrane (FAS) and of difenzoquat, nicotine and verapamil to one cation exchange membrane (FKS) was investigated. All test substances exhibited a sufficiently high affinity for the respective IEM with logarithmic IEM–water partition coefficients >2.3. Sorption equilibrium was established quickly, within several hours for the FAS membrane and within 1–3 days for the FKS membrane. For permanently charged substances the partitioning to the IEMs was independent of pH, but was influenced by the salt composition of the test solution. For all test substances sorption to IEM was dependent on the substance concentration. Bovine serum albumin–water partition coefficients determined by passive sampling with IEMs agree well with those determined by the conventional dialysis method. The results of this study indicate that IEMs exhibit the potential to measure freely dissolved concentrations of organic ions in a simple and time-saving manner.

2.2 Equilibrium Sorption of Structurally Diverse Organic Ions to Bovine Serum Albumin

Reliable partitioning data are essential for assessing the bioaccumulation potential and the toxicity of chemicals. In contrast to neutral organic chemicals, the partitioning behavior of ionogenic organic chemicals (IOCs) is still a black box for environmental scientists. Partitioning to serum albumin, the major protein in blood plasma, strongly influences the freely dissolved concentration of many chemicals (including IOCs), which affects their transport and distribution in the body. Because consistent data sets for partitioning of IOCs are rarely available, bovine serum albumin-water partition coefficients ($K_{BSA/w}$) were measured in this study for 45 anionic and 4 cationic organic chemicals, including various substituted benzoic and naphthoic acids, sulfonates and several pesticides and pharmaceuticals. The results of this study suggest that binding to BSA is substantially influenced by the three-dimensional structure of the chemicals and the position of substitutions on the sorbing molecules. For example, we found a difference of >1.5 log units between isomeric chemicals such as 3,4-dichlorobenzoic acid and 2,6-dichlorobenzoic acid, and 1-naphthoic acid and 2-naphthoic acid. Conventional modeling approaches (e.g., based on octanol−water partition coefficients) poorly predict log $K_{BSA/w}$ of organic ions ($R^2 \leq 0.5$), partially because they do not capture the observed steric effects. Hence, alternative modeling strategies will be required for accurate prediction of serum albumin-water partition coefficients of organic ions.

2.3 Partitioning of Organic Ions to Muscle Protein: Experimental Data, Modeling and Implications for in vivo Distribution of Organic Ions

The in vivo partitioning behavior of ionogenic organic chemicals (IOCs) is of paramount importance for their toxicokinetics and bioaccumulation. Among other proteins, structural proteins including muscle proteins could be an important sorption phase for IOCs, because of their high quantity in the human and other animals' body and their polar nature. Binding data for IOCs to structural proteins are, however, severely limited. Therefore, in this study muscle protein−water partition coefficients ($K_{MP/w}$) of 51 systematically selected organic anions and cations were determined experimentally. A comparison of the measured $K_{MP/w}$ with bovine serum albumin (BSA)−water partition coefficients showed that anionic chemicals sorb more strongly to BSA than to muscle protein (by up to 3.5 orders of magnitude), while cations sorb similarly to both proteins. Sorption isotherms of selected IOCs to muscle protein are linear (i.e., $K_{MP/w}$ is concentration independent), and $K_{MP/w}$ is only marginally influenced by pH value and salt concentration. Using the obtained data set of $K_{MP/w}$ a polyparameter linear free energy relationship (PP-LFER) model was established. The derived equation fits the data well (R^2 = 0.89, RMSE = 0.29). Finally, it was demonstrated that the in vitro measured $K_{MP/w}$ values of this study have the potential to be used to evaluate tissue-plasma partitioning of IOCs in vivo.

Curriculum Vitae

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03/2015 - 04/2015 **Intern at the Safety & Environmental Assurance Centre (SEAC), Unilever, Colworth:** Developed a method for measuring partitioning behavior of chemicals in complex personal care formulations

05/2012 - 08/2012 **Research Assistant at The Helmholtz Centre for Environmental Research (UFZ), Leipzig:** Developed a method and measured partitioning of hydrophobic substances and mycotoxins to storage lipid

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Publication list

Publications

Henneberger, L.; Goss, K. U.; Endo, S., Partitioning of Organic Ions to Muscle Protein: Experimental Data, Modeling, and Implications for in Vivo Distribution of Organic Ions. Environ. Sci. Technol. 2016, 50, (13), 7029-36.

Henneberger, L.; Goss, K. U.; Endo, S., Equilibrium Sorption of Structurally Diverse Organic Ions to Bovine Serum Albumin. Environ. Sci. Technol. 2016, 50, (10), 5119-26.

Geisler, A.; **Oemisch**, L.; Endo, S.; Goss, K.-U., Predicting Storage–Lipid Water Partitioning of Organic Solutes from Molecular Structure. Environ. Sci. Technol. 2015, 49, (9), 5538-5545.

Oemisch, L.; Goss, K.-U.; Endo, S., Ion exchange membranes as novel passive sampling material for organic ions: Application for the determination of freely dissolved concentrations. J. Chromatogr. A 2014, 1370, 17-24.

Oemisch, L.; Goss, K.-U.; Endo, S., Determination of oil–water partition coefficients of polar compounds: silicone membrane equilibrator vs. SPME passive sampler. Anal. Bioanal. Chem. 2013, 405, (8), 2567-2574.

Conference presentations

Platform presentation: "Partitioning of ionic organic chemicals to proteins: Influences of solute molecular structure", ICCE 2015, Leipzig, Germany, September 2015.

Platform presentation: "Passive sampling of organic ions with ion exchange membranes - Application for the determination of freely dissolved concentrations in protein binding experiments", SETAC Europe 24th Annual Meeting. Basel, Switzerland, May 2014.

Poster presentation: SETAC-GLB. Duisburg-Essen, Germany, September 2013.

Poster presentation: SETAC-GLB. UFZ Leipzig, Germany, September 2012.