Sorption to soil of hydrophobic and ionic organic compounds: measurement and modeling

Sorptie van hydrofobe en ionogene organische stoffen aan bodem: meten en modelleren

(met een samenvatting in het Nederlands)

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"when he was six he believed that the moon overhead followed him
by nine he had deciphered the illusion, trading magic for fact
no tradebacks"
(Eddy Vedder)

Contents

Chapter 1

Introduction

Introduction

Scope

Numerous organic chemicals are introduced into the environment by natural (e.g. forest fires, volcanic activity, biological processes) and human activities (e.g. industrial activities, agriculture, traffic, heating). The sorption of organic chemicals to soil or sediments is important in the determination of the fate and effects of these compounds, since it affects the leaching of compounds to the groundwater, transport into and in surface waters and availability for chemical degradation (*1*). Furthermore, the sorption also affects the biological availability, thereby influencing the bioaccumulation, biodegradation and potential toxic effects for organisms (*2, 3*). Consequently, knowledge about the sorption processes, and models predicting the sorption behavior to soils and sediments are essential for evaluating the risk of these compounds for the environment.

The scope of this thesis is to study the sorption of hydrophobic, polar and ionic organic chemicals to soil (and sediment), and to investigate how analytical methods and modeling can be improved for a more realistic estimation of sorption and subsequently environmental fate and risks.

Soil sorption

Soil is a complex matrix consisting of a mineral and organic fraction. The mineral fraction consists of various silicates that can be coated with various metal oxides/hydroxides of aluminum, iron, and manganese, and with organic matter. The composition of the minerals and their coatings reflects the parent rock material and the degree of weathering (*4*). The organic fraction can consist of unaltered debris, degraded organic materials (humus), combustion residues (e.g. soot) and non-aqueous phase liquids (e.g. oil, tar). Furthermore, a soil contains air and pore water with dissolved organic matter, various dissolved ions and suspended minerals (*5*).

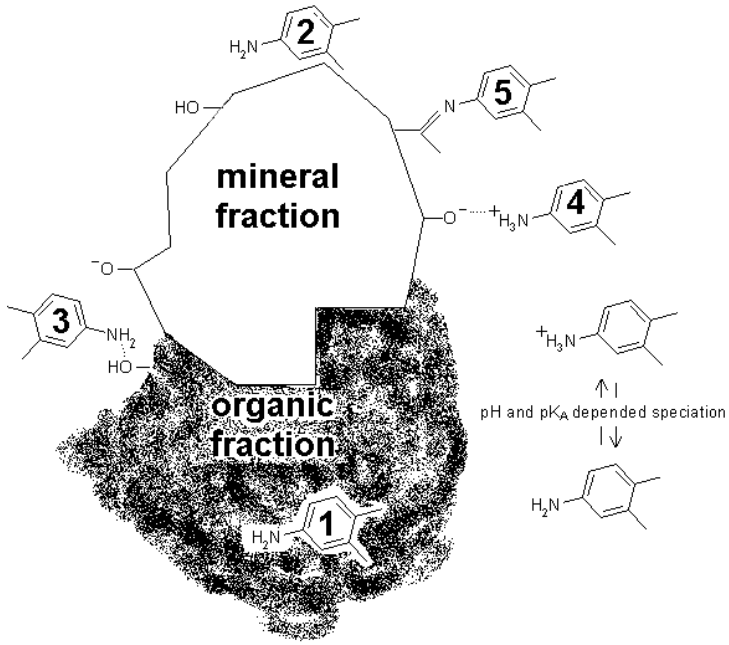
The sorption of a compound to soil can be described by the soil sorption coefficient (K_D, L/kg) defined as the concentration bound to soil (C_s, mg/kg) divided by the concentration in the aqueous phase (C_{aq}, mg/L) (Equation 1).

$$K_D = \frac{C_s}{C_{aq}} \qquad (1)$$

In this thesis, the concentration in the aqueous phase is defined as the freely dissolved aqueous concentration, all compounds associated with particulate and dissolved (organic) matter in the pore water are considered bound.

Organic chemicals can be associated with solid phases (soils) via adsorption or absorption. Adsorption is when molecules attach to a two-dimensional surface, while absorption is when molecules penetrate (dissolve) into a three-dimensional matrix. The significance of

the different sorption processes for the apparent sorption coefficient to a soil depends on both the sorbate and sorbent properties. Different conceptual sorption mechanisms are shown in Figure 1.



code	Sorption mechanism	Co-varying sorbate and sorbent properties
1	Neutral sorbate escapes water and absorbs in to the organic material of the soil	Octanol water partition coefficient Aqueous solubility Molecular weight / size
2	Neutral sorbate adsorbs by van der Waals interactions to organic and mineral soil surfaces	Surface of sorbate
3	Neutral sorbate adsorbs by hydrogen bonds to mineral and organic soil surfaces	Hydrogen accepting and / or hydrogen donating properties of sorbent and sorbate
4	Charged sorbate adsorbs by non-specific electrostatic attraction (outer sphere complexation) or specific (ionic) bonding (inner sphere complexation) to mainly mineral soil surfaces with to oppositely charged surface groups (*5*)	Charge of sorbate Charge of sorbent Ionic strength Specific ionic sites on sorbate and Specific ionic sites on sorbent
5	Reactive moiety of sorbate covalently bonds with reactive moiety of mineral or organic sorbent	Reactive groups on sorbate Reactive groups on sorbent

Figure 1: Various sorbate-sobent interactions that can control the association of a chemical with natural solids. The figure is based on Schwarzenbach et al. (*1*) and Sposito (*5*).

Absorption or partitioning between the aqueous phase and the soil usually takes place in the organic fraction of a soil. This sorption process is driven by the difference in energy that is needed to form a cavity in the aqueous phase and in the organic phase (*6*). The larger the compound, the more hydrogen bonds it has to disturb to form a cavity in the aqueous phase, so the higher the sorption coefficient to the organic phase is. This type of interaction is often the most important factor that determines the sorption of non-polar hydrophobic organic compounds. Since the chemical is absorbed in the organic fraction of a soil, the sorption coefficient (K_{OC}) is often normalized to the organic carbon fraction (f_{OC}).

$$K_{OC} = \frac{K_D}{f_{OC}} \tag{2}$$

Absorption or partitioning processes are considered to be independent of the concentration, because the compounds do not compete for a limited amount of sorption sites (*7*) (Figure 2a).

Adsorption (Figure 1) can take place on the surfaces of various soil constituents. Polar organic chemicals with hydrogen accepting and/or donating groups can form hydrogen bonds with water but also with mineral and organic surfaces of a soil or sediment. Furthermore ionized organic compounds can adsorb to surfaces with an opposite charge by non-specific electrostatic attraction, these interactions are called outer sphere complexes. Besides that, ionized compounds can also sorb via specific interactions, these interactions are called inner sphere complexes (sorbent and sorbate are so close the electron-orbitals overlap, and ionic / covalent bonds are formed). Moreover, compounds can also sorb by chemical reactions also leading to covalent bonds (*4, 5*).

The sorption coefficients of the various adsorption processes listed above are often affected by the compound concentration, since compounds compete with each other and other molecules for a limited sorption surface or a limited number of sorption sites (*1, 4, 5*). As a result, nonlinear sorption isotherms are often observed in literature (*5*). A common phenomenological mathematical approach to fit the changing sorption coefficients with concentration is the Freundlich isotherm:

$$C_s = K_D * C_{aq}^{\ n} \tag{3}$$

where the K_D is the sorption coefficient at a defined aqueous concentration (e.g. 1 µg/L) and n is the parameter describing the sorption linearity (Figure 2b). If n is larger than one, the sorption coefficient increases with increasing aqueous concentration and if n is smaller than one the sorption coefficient decreases with increasing sorption coefficient. Decreasing sorption coefficients with increasing concentrations are more often observed in

literature. The mechanistic Langmuir model assumes adsorption of molecules at homogeneous surfaces with equal adsorption energies and a limited number of sorption sites (Γ_{max}) (Figure 2c):

$$C_s = \frac{\Gamma_{max} * K_D * C_{aq}}{1 + K_D * C_{aq}} \qquad (4)$$

The sorption coefficient (K_D) in this model is the "initial sorption coefficient" when the aqueous concentration is extrapolated to zero.

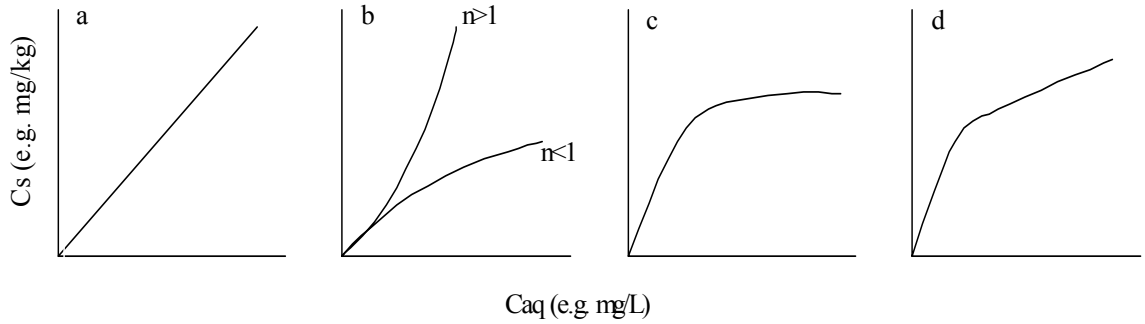


Figure 2: Various types of observed relationships between sorbed and free aqueous concentrations. Figure A shows a linear relationship, Figure B a nonlinear relationship (Equation 3) with a Freundlich n that is larger and smaller than 1. Figure C shows a nonlinear Langmuir isotherm (Equation 4) with a maximum sorption capacity of the sorbent and Figure D is a combination of a Langmuir and a linear sorption isotherm (*1*).

In some cases, the relationship between the sorbed and aqueous concentration cannot be described by one single sorption model, and combinations of the different models should be applied (*8*) (Figure 2d), since organic compounds can undergo various sorption interactions (Figure 1) with the soil different constituents (e.g. sand, clay, organic matter and aluminum and iron oxide/hydroxide coatings, Figure 3).

Not only the composition of the soil, but also the aqueous chemistry can affect the sorption. The pH and ionic strength have, for example, been demonstrated to affect soil and sediment sorption as well (*9-11*). Changing the pH leads to protonation or deprotonation of ionizable compounds, thereby shifting the speciation equilibrium between the different species, each of which sorb according to their respective physical-chemical properties, leading to a different apparent sorption coefficient. Furthermore, the pH also induces protonation and deprotonation of acidic (and basic) groups on the soil surface, thereby affecting surface charge and the solubility of humic matter. Changing the ionic strength can influence sorption of ionized compounds by changing the interfacial potential of soils surfaces, by competing for sorption (ion-exchange) sites and by forming complexes with the compounds (*5*). In addition, increasing ionic strength can also lead to

increased sorption of neutral hydrophobic organic compounds by increasing the energy that is needed to form a cavity in the (salty) aqueous phase (salting-out effect (*1, 12*)).

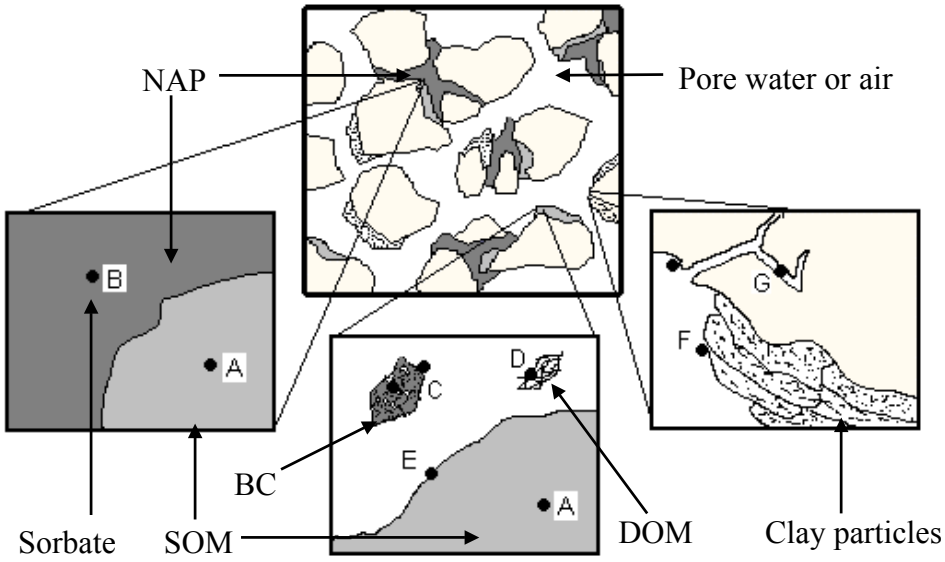


Figure 3: Conceptual model of soil sorption domains. (A) Refers to absorption in to soil organic matter (SOM), (B) refers to absorption into non-aqueous phase liquid (NAPL, e.g. oil, tar), while (C) refers to absorption and adsorption to "black carbon" (BC, condensed combustion residues like soot) and (D) refers to adsorption or absorption to dissolved organic matter (DOM). (E) Refers to adsorption to soil organic matter, (F) refers to adsorption to clay surfaces (with metal oxide/hydroxide coatings) and (G) refers to adsorption to mineral surfaces or to mineral pore-surfaces. The figure is based on Luthy et al. (*7*).

Determining freely dissolved concentrations and soil sorption

Generally, two experimental approaches are available to study sorption. Batch equilibrium experiments in which the test chemical is partitioned between soil/sediment and an aqueous solution are most frequently to obtain sorption coefficient. The simplest and most common method is to separate the solid and liquid phase by centrifugation, and to measure the concentration in both phases. Furthermore, column-leaching experiments have been used to determine sorption coefficients. These columns mimic the field situation more closely, but they are subject to various experimental artifacts like: failure to establish equilibriums (locally), leakage via the test system walls and (macro) pores in the soil and losses of particles and dissolved organic matter (*13*). Both column leaching and centrifugation, however, do not remove dissolved organic matter (DOM) and very fine suspended matter from the pore water. In order to quantify sorption coefficients to soil, information is needed about the concentration in the pore water. The concentration in the pore water is defined as the molecules that are freely dissolved in this aqueous phase. These freely dissolved concentrations are difficult to assess in complex matrices like soils or sediments (*14, 15*). Various techniques have been developed to determine (freely

dissolved) pore water concentration in soils and sediments. Compounds sorbed to dissolved and suspended matter can lead to an overestimation of the aqueous concentration and subsequently to an underestimation of the sorption coefficient. Systematic errors due to incomplete phase separation and the consequent overestimation of the freely dissolved aqueous concentration are higher for the more hydrophobic chemicals (*14*). Various techniques have been applied to determine the free aqueous concentration. Dissolved organic phases in the water have been removed actively by flocculating dissolved organic phases (*16*), or passively by a dialysis membrane (*17*). Additionally, fluorescence quenching has been applied to determine free concentrations in DOC solutions (*18*). Furthermore, various passive sampling techniques using partitioning to gaseous, liquid or solid phases (*19-32*) can be applied to assess pore water concentrations in complex matrices like soil or sediment. These samplers only sense the freely dissolved concentration. If they are equilibrated with the matrix without depleting the system, their equilibrium concentration can directly be used to calculate the freely dissolved concentration using sampler-water partition coefficients (*27, 33, 34*).

Modeling soil sorption

The sorption of organic compounds is dependent on both sorbent and sorbate properties. The most common quantitative structure activity relationship (QSAR) model to describe (or estimate) soil or sediment sorption of hydrophobic organic compounds is the relation of the octanol-water partition coefficient (K_{OW}) and the organic carbon normalized sorption coefficient (K_{OC}) (Equation 5).

$$\log K_{OC} = a * \log K_{OW} + b \tag{5}$$

Karickhoff et al. (*35*) was one of the first to make such a relationship in the late seventies:

$$\log K_{OC} = \log K_{OW} - 0.21 \tag{6}$$

With the ever growing amount of data on compound properties like octanol-water partition coefficients, olive oil-water partition coefficients, aqueous solubility, HPLC retention times, hydrogen bonding and accepting properties, molecular topology and sorption coefficients to soils, sediments or specific organic materials (e.g. dissolved organic matter, DOM), numerous QSARs have been developed during the last decades (*17, 36-39*).

The descriptive and predictive power of these QSARs is first of all dependent on the quality of the data employed for model development (e.g. log K_{OW} and the sorption coefficient). This seems rather trivial, but the determination of freely dissolved aqueous concentrations of compounds in soil slurry or a solvent-water system (e.g. octanol-water) becomes more difficult with increasing sorption or partition coefficients as described above. For example, the standard deviation (SD) of the log K_{OW} of phenanthrene (4.54

±0.35, n= 26) is much smaller than the SD of the more hydrophobic benzo[ghi]perylene (6.70 ±0.84, n= 15). The same holds for partitioning data to more complex hydrophobic phases like DOM, soils and sediments (*17, 40*).

The descriptive and predictive power of a QSAR also depends on the physical-chemical variability of the chosen group of compounds and to what extent the predictive variable correlates with the sorption mechanisms. Literature shows that these QSARs can describe or predict sorption of (non-polar) hydrophobic organic chemicals relatively well when applied to a group of similar compounds (freshly) spiked to standard soils or sediments (*37*). More general models describing a large variety of hydrophobic organic compounds show poorer correlation. Mechanistic multi-parameter models, using various compound properties (e.g. van der Waals interactions, molecular volume, H-donating and accepting properties) are more appropriate to describe the partitioning of a more heterogeneous group of compounds to solvents (*39*).

The prediction of sorption of less hydrophobic and especially ionizable compounds is more difficult, since various sorption processes can occur simultaneously (*1, 41*). Tolls (*41*) shows that the sorption of various (ionizable) pharmaceuticals is largely underestimated by the QSAR of Karickhoff (*42*). Furthermore, the sorption is dependent on various soil properties and can be strongly affected by the ionic composition of the soil solution (*5, 9-11, 43, 44*). This makes the modeling of sorption coefficients rather difficult. Modeling of ionizable compounds is usually more descriptive than predictive, since it is limited to single compounds or a group of similar compounds and can differ from soil to soil.

The effect of pH is often described by different sorption coefficients for the different species that are formed at different pH (Equation 7):

$$K_{D'} = K_{D1} * f_1 + K_{D2} * f_2 \qquad (7)$$

The apparent sorption coefficient (K_D') is defined as the fractions of different species f_1 and f_2 times their specific sorption coefficients (K_{D1} & K_{D2}). The fractions of the species are determined by the pK_A value of the compound and the pH of the soil solution. Species-specific sorption has to be determined by determining sorption coefficients at various pHs.

Objectives and outline

In this thesis, the sorption of hydrophobic chemicals and ionizable veterinary pharmaceuticals to geosorbents is studied in order to improve the scientific instruments for assessing the impact of sorption on the risk of chemicals in the environment. To that end, different experimental approaches are developed for the determination of freely dissolved concentrations and sorption coefficients of (very) hydrophobic compounds in the presence of soil, sediment and DOM based on the principle of passive sampling approach. Contrastingly, there is less data available on the sorption of (veterinary) pharmaceuticals

to soil. Three veterinary antibiotics, extensively used in veterinary practice, were selected. Sorption coefficients were determined in dependence of pH, ionic strength, ionic composition, and in different soil properties. The experimental results were employed to develop models. Finally, the predictive power of these models was evaluated in order to elucidate to which degree these model can contribute to assessing the environmental risk of veterinary pharmaceuticals.

Chapter 2 describes a passive dosing and sampling technique that is used to study the sorption to dissolved organic matter. In this study, a hydrophobic phase (poly(dimethylsiloxane), PDMS-coated glass fiber) is "loaded" with compounds, and the depletion at different concentrations of dissolved organic material is used to determine the sorption coefficient to the organic phase. In Chapter 3, sediment containing some hydrophobic organic compounds is diluted with water and a negligible depletive solid phase microextraction technique is applied to determine the freely dissolved aqueous concentration. The decrease of the free concentration with increasing aqueous concentration is used to determine the sorption coefficient to the sediment. Chapter 4 and 5 are both focused on the determination of freely dissolved pore water concentrations in soil slurry. Chapter 4 focuses on the validation of the technique that was initially developed by Mayer and Van Der Wal (*27, 30*). This technique is used to determine freely dissolved aqueous concentrations and sorption coefficients of various PAHs in spiked, aged and field-contaminated soils. Chapter 5 studies the free concentrations and sorption of PAHs at a range of soil concentrations (as is often used in soil testing).
Chapter 6 and 7 are both focused on the sorption of three commonly used ionizable veterinary pharmaceuticals to soil. Chapter 6 studies the effect of pH and ionic strength on the sorption of these ionizable compounds in two soils. Chapter 7 studies the sorption of the same compounds in 11 soils that were selected on basis of their variable properties. The sorption to the different soils was analyzed using various statistical techniques.

Chapter 2

A solid phase dosing and sampling technique to determine partition coefficients of hydrophobic chemicals in complex matrices

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Abstract

Determination of polymer-water and dissolved organic carbon (DOC)-water distribution coefficients of very hydrophobic chemicals (log K_{OW} >6) is not straightforward. Poor water solubility of the test-compounds complicates the spiking and analysis of actual freely dissolved concentrations. By dosing a system via a PDMS-fiber and monitoring the depletion in the polymer, spiking and analysis of concentrations in the aqueous phase are avoided, and sorption to the polymer and other hydrophobic phases can be determined easily and accurate. In this publication we report the determination of poly(dimethylsiloxane)-water, and Aldrich humic acid-water distribution coefficients for six PAHs with log K_{OW} values varying from 4.56 to 6.85. The distribution coefficients to a PDMS fiber (log K_f) and the DOC (log K_{DOC}) range from 3.86 to 5.39 and 4.78 to 7.43, respectively. Even for the most hydrophobic compounds, the distribution coefficients show small standard errors (≤0.05 log units). Therefore, this method might be applied to determine sorption coefficients of numerous, even more hydrophobic compounds, to humic acids as well as other dissolved hydrophobic matrices.

Introduction

The bioavailability and fate of hydrophobic organic chemicals is influenced by binding to dissolved and particulate hydrophobic phases (*45-50*). Therefore, accurate sorption coefficients to soils, sediments and dissolved organic matrices are needed (*17, 40, 51*). The determination of these partition coefficients tends to become increasingly difficult when compounds are more hydrophobic. Increasing hydrophobicity usually coincides with higher partition coefficients and low aqueous solubility, making spiking procedures of the aqueous phase and detection of freely dissolved concentrations complicated (*40, 52*). In addition, systematic errors due to incomplete phase separation and the consequent overestimation of the free dissolved aqueous concentration are higher for the more hydrophobic chemicals. This can be observed for the commonly used octanol-water partition coefficient (K_{OW}). For example, the standard deviation (SD) of the log K_{OW} of phenanthrene (4.54 ±0.35, n= 26) is much smaller than the SD of the more hydrophobic benzo[ghi]perylene (6.70 ±0.84, n= 15) (*53*). The same holds for sorption to complex hydrophobic phases such as soils, sediments, particulate organic carbon and dissolved organic carbon (DOC) (*40*).

The determination of DOC-sorption is complicated, because this hydrophobic phase is dissolved in the aqueous phase, and a classical separation of the two phases without disturbing the equilibrium is not easy to achieve (*1*). Therefore, sorption behavior of hydrophobic substances to DOC is often studied by measuring the free concentration without active separation of the two phases. These techniques include dialysis (*54*),

headspace equilibration, fluorescence quenching (*18, 55, 56*), or partitioning to a well defined hydrophobic phase such as thin polymer films (*28, 29*) and negligible depletion solid-phase microextraction (nd-SPME) (*48, 57-61*).

Besides the complications with complete separation of the two phases, the spiking procedure of aqueous solutions is not always straightforward. Classical spiking procedures of hydrophobic chemicals with an organic solvent often lead to instable and variable concentrations and solutions containing not completely dissolved substances. The application of a generator column (*62, 63*) or a partitioning driven administrator (*64, 65*) to prepare aqueous solutions is an improvement, since it generates "real" solutions with only dissolved molecules. The two phenomena (incomplete separation of phases and unstable aqueous solutions) lead to a large variability in partition and sorption coefficients in the literature (*40, 51, 66*).

This study attempted to overcome these problems by applying another dosing system as an alternative to regular spiking procedures. The new aspect in the approach is that the dosing system is also used to indirectly monitor sorption to a dissolved hydrophobic phase (*67*). A hydrophobic phase (poly(dimethysiloxane), PDMS) is "loaded" with compounds, and the depletion of this phase is monitored at different dilution factors (DF, volume water / volume PDMS) or with different concentrations of DOC. This procedure enables the determination of partition coefficients to PDMS and sorption coefficients to DOC without using a carrier-solvent to spike the water and analyzing water samples. Figure 1 shows a conceptual picture of the experimental setup to determine the partition coefficient to PDMS (Figure 1a & 1c) and the sorption to DOC (Figure 1b & 1d).

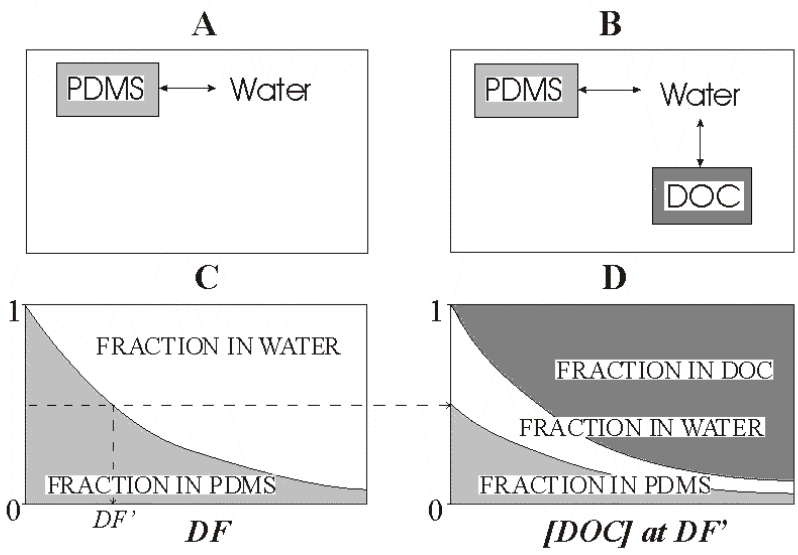

Figure 1: A conceptual picture of the test systems without DOC (A) and with (B) DOC, and the expected fraction of the test compound in the different phases as a function of the dilution factor (DF) (C) and the dissolved organic carbon concentration, [DOC] at a single dilution factor (DF') (D).

Materials and methods

Chemicals, fibers and solvents

The PAHs (Table 1) and Aldrich humic acid used were all purchased at Sigma Aldrich Chemie BV (Zwijndrecht, The Netherlands). Glass fibers with a core diameter of 110 μm and a 28.5μm poly(dimethylsiloxane) (PDMS) coating (volume 12.4 μl/m) were obtained from Poly Micro Industries (Phoenix, AZ, USA). Acetonitrile, acetone, methanol (Lab-Scan, Dublin, Ireland) and n-hexane (Baker BV, Deventer, The Netherlands) used, were of analytical grade and highly pure deionized water (R ≥18 MΩ) was prepared by a Millipore water purification system, equipped with organic free kit (Millipore Waters, Amsterdam, The Netherlands).

Table 1: The test compounds and some of their properties. The reference numbers are given between brackets.

Compound	log K_{OW} (@ 25ºC) (*53*)	Aqueous solubility (mg/L) (*53*)	Purity (%)
phenanthrene (Phe)	4.56	1.15	not given
fluoranthene (Fla)	5.16	0.26	98
pyrene (Pyr)	5.18	0.135	99
benz[a]anthracene (BaA)	5.91	0.0094	95
benzo[b]fluoranthene (BbF)	6.20[a]	0.0015	99
benzo[k]fluoranthene (BkF)	6.20[a]	0.00080	98
benzo[ghi]perylene (BghiP)	6.85[a]	0.00026	not given

[a] Selected values.

PDMS-water partition coefficients

PDMS-water partition coefficients (K_f) were determined using a "classical" and the new "fiber depletion" method. Both experiments were performed at 21 (±1)°C in the dark. Before use, all fibers were cut to a length of 5.0 cm (0.62 μL PDMS per fiber) and thermally cleaned at 275°C for 16 h under a constant helium flow of ~35 ml/min.

In the "classical" experiment, 16 clean fibers of 5 cm (9.92 μL PDMS in total) were exposed to 100 mL water that was spiked with 0.1% acetone containing 5-20 mg/L phenanthrene, fluoranthene, pyrene, benz[a]anthracene and benzo[b]flouranthene. A 0.005 mM NaN_3 (Merck, Amsterdam, The Netherlands) solution was added to inhibit bacterial degradation. Fibers were sampled after 7 and 14 days of gentle stirring at 120 rpm. There was no significant difference between the 1 and 2 week exposed fibers indicating that equilibrium was reached in the fiber-water system. After exposure, fibers were gently blotted dry with a tissue, cut into two pieces and transferred to a 1.8 mL autosampler vial containing 1.0 mL acetonitrile within 30 s. An additional fiber-air depuration study showed that no significant amount of the compounds in the fiber coating were lost during a 30 s transfer-period, as half-lives of the test compounds exposed to a gentle stream of air in a fume hood ranged from 1.4 h to much more than

24 h (Appendix 1, Figure A). Aqueous concentrations were determined by sampling 10 mL of water (n= 3) and extracting these samples with 1 mL of n-hexane three times. After extraction the 3 mL hexane was evaporated to ~0.5 mL using a gentle stream of N_2. Subsequently 1 mL of acetonitrile was added, and the mixture was evaporated to ~0.3 mL. The extraction recovery obtained from a parallel experiment with spiked n-hexane was 100% (RSD 2%). Final aqueous concentrations ranged from 10 µg/L (phenanthrene) to 0.9 µg/L (benzo[b]fluoranthene).

In the "fiber depletion" experiment, the aqueous phase was dosed via a "loaded" PDMS-fiber. Clean fibers (n= 60-80) were "loaded" by exposing them for 24 h on a "rock and roller" shaker to a 30 mL methanol-water mixture spiked with 0.1 - 0.5 mg/L PAHs. Partition coefficients between the fibers and the methanol-water mixture (Appendix 1, Table A) were determined using the "classical" approach, measuring concentrations in both phases. This classical approach was chosen because concentrations and solubility in the MeOH-water mixture are high enough to accurately measure partition coefficients (*68*). Initial concentrations in the PDMS ranged from 10 to 20 mg/L per compound. The loading of the fiber via methanol-water is highly reproducible as standard deviations of the concentrations in the PDMS coating were smaller than 6% for all compounds (n= 5).

Loaded fibers were exposed to water (0.005 M NaN_3) in different sized flasks. The water-volume varied from 6.5 to 1100 mL and the PDMS-volume varied from 0.6 to 2.5 µL, thereby creating eight different dilution factors (volume water / volume PDMS), ranging from 2760 to 920000. All bottles were gently shaken using a two-dimensional shaker (KS501, IKA Labortechnik, Staufen, Germany) at 190 rpm. Fibers were sampled directly after loading (n= 5) and after 68.5, 168 and 336 h (n= 2). For the two largest flasks, a 672-h exposure time had to be included in order to reach equilibrium (n= 2). After exposure, fibers were extracted in a 1.8 mL autosampler vial, containing a 250 µL insert with 200 µL acetonitrile. All fibers were extracted for at least 1 day and stored at -20°C (three subsequent extractions showed an extraction recovery of 99.6% ±0.1% in the first extraction).

DOC-water sorption coefficients

Loaded fibers were exposed to 5 mL water containing 0, 0.5, 1, 5, 10, 25, 50 and 98 mg/L Aldrich humic acid sodium salt (38.25% organic carbon, Sigma Aldrich) at 21 ±1°C. Five fibers were sampled directly (exposure time "0"), and a single fiber was sampled after 2, 4, 8, 24, 72 and 168 h shaking on the "rock and roller" (Snijders Scientific, Tilburg, The Netherlands) for every DOC concentration. Loading, sampling and extraction procedures of the fibers were identical to the PDMS-water partition coefficient experiments.

Analysis of samples

The concentrations in the fiber extracts were determined by HPLC-fluorescence detection. The system consisted of a Shimadzu DGU 14A degasser (Den Bosch, The

Netherlands), a Varian Prostar 420 autosampler (Bergen op Zoom, The Netherlands), a Gynkotek P580 HPG HPLC pump (Gemering, Germany), and a Jasco FP-920 fluorescence detector (Maarssen, The Netherlands). Separation was performed using a Supelcosil (Supelco, Bellefonte, CA, USA) LC-PAH column (length 100 mm, ø 4.6 mm, particles 3 μm) that was operated at 28°C. All analyses were performed with a flow rate of 1000 μL/min and an injection volume of 20 μL. The compounds were separated using gradient elution starting with 40% H_2O for two min followed by an increase of the acetonitrile-fraction to 100% in 9.5 min, where it was kept for another 7.5 min before returning to the initial solvent composition for 6 min. The excitation and emission wavelengths (nm) of Phe, Fla and Pyr were 255/405, those of BaA were 277/393, while BbF and BkF concentrations were determined at 260/420 and BghiP at 295/425, with detection-limits of 1.0, 2.0, 0.5, 0.1, 0.3, 0.1 and 0.2 μg/L respectively. Quantification was done using standards containing 16 PAHs (Supelco, Bellefonte, CA, USA, selected by the EPA) diluted in acetonitrile. Chromatograms were analyzed using Chromcard version 1.21 (Milan, Italy), and corrected by hand if necessary. Quantification was done by a series of 7 standards, with concentrations ranging from 2 to 200 μg/L.

Determination of K_f

Two methods were used to determine the partition coefficient to the PDMS-fiber (K_f). In the "classical" experiment the fiber concentration (C_f) was divided by the aqueous concentration (C_{aq}) measured at the end of the exposure (Equation 1).

$$K_f = \frac{C_f}{C_{aq}} \tag{1}$$

In the "fiber depletion" experiment, a one phase exponential decay model (Equation 2) was applied to the concentration in the fiber in time ($C_{f(t)}$), and the elimination rate constant (k_2 in h^{-1}) and the equilibrium concentration in the fiber ($C_{f(\infty)}$) were determined.

$$C_{f(t)} = (C_{f(initial)} - C_{f(\infty)}) * \exp^{(-k_2 * t)} + C_{f(\infty)} \tag{2}$$

The model was fitted using Graphpad, version 3.0 (*69*). If the decrease of the concentration in the fiber was insufficient (($C_{f(initial)}$ - $C_{f(\infty)}$) / $C_{f(initial)}$ >5%) for a proper fit of Equation 2, the average of the longest exposure-time was taken as the equilibrium concentration (n ≥3). Subsequently, the percentage recovered from the fiber at equilibrium ($C_{f(\infty)}$ / $C_{f(initial)}$,%) was plotted against the volume water / volume PDMS ratio (DF), and the PDMS-water partition coefficient (K_f) was calculated by fitting Equation 3 the data using Graphpad (see Appendix I for the derivation of Equation 3).

$$\frac{C_{f(\infty,DF)}}{C_{f(initial)}}(\%) = \frac{100\%}{1+\frac{DF}{K_f}} \qquad (3)$$

The determination of K_{DOC}

The sorption coefficient to dissolved organic material; normalized for organic carbon content (K_{DOC}), was determined by monitoring the depletion of PAHs at different Aldrich humic acid concentrations. Equation 2 was also used to describe the depletion of the DOC exposed fibers, and to calculate the equilibrium concentration in the fiber ($C_{f(\infty)}$). The percent recovery from the exposed fiber ($C_{f(DOC)}$ / $C_{f(initial)}$,%) was plotted against the DOC concentration ([DOC]). Together with a constant DF (8064) and known K_f-values from the previous experiment, K_{DOC} –values could be determined by the following equation, using Graphpad (see Appendix I for the derivation of Equation 4).

$$\frac{C_{f(DF)}}{C_{f(initial)}}(\%) = \frac{100\%}{1+\frac{DF}{K_f}(1+[DOC]*K_{DOC})} \qquad (4)$$

Results & discussion

Determination of K_f

The depletion of the fibers exposed to water was fitted by Equation 2. Figure 2 provides the data and fitted curves for phenanthrene and benzo[ghi]perylene, the other compounds can be found in Appendix I (Figure B).

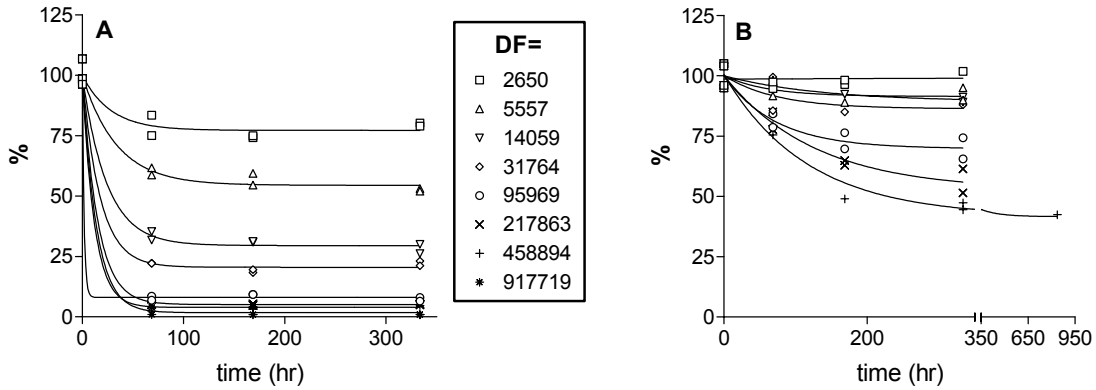

Figure 2: The depletion of phenanthrene (A) and benzo[ghi]perylene (B) from the PDMS fiber at different water-fiber volume ratios (DF) in time. A one-phase exponential decay curve is fitted through the data (n= 7-10 per DF). The percentage was calculated as: measured concentration in the fiber at time t divided by the concentration in the fiber at t=0.

It can be observed that the 2 to 4 week equilibration period was sufficient to reach or almost reach equilibrium for all compounds. The percentage recovered from the fibers at equilibrium is plotted against the dilution factor in Figure 3.

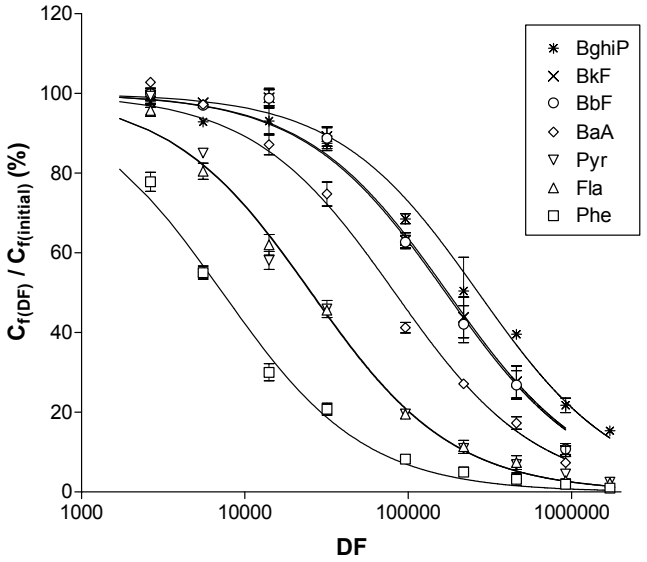

Figure 3: Depletion of the SPME fiber (at equilibrium) as a result of the increasing water-PDMS ratio (DF) and their standard errors (error bars). Equation 3 is fitted through the data (lines) to obtain the fiber-water partition coefficient (K_f).

The PDMS-water partition coefficients were calculated from this data using Equation 3. Table 2 displays the calculated log K_f-values from the "classical" and "fiber depletion" experiment, as well as partition coefficients to different PDMS coated fibers presented in literature.

A comparison of the data with partition coefficients from literature, that sometimes use different coating thicknesses, is legitimate because it is assumed that the sorption of hydrophobic compounds to PDMS is an absorption process (*25, 70-73*). Therefore, partition coefficients to this material are thought to be independent of concentration and dimensions of the fiber used. The determined K_f-values with both methods are comparable to literature data, even though the variation within literature data is generally large, and K_f-values of the larger PAHs are scarce. The high r^2 ($\geq$0.99) of the fit of Equation 3 and the small standard errors ($\leq$0.04 log units) of the determined log K_f-values, indicate that the model fits the data well, and can generate K_f-values with high accuracy.

Table 2: PDMS-water partition coefficients (log K_f) of 7 PAHs.

Compound	*Experimental*		*Literature*		
	Log K_f (SD, n) "Classical method"	*Log K_f (SE, n) "Fiber depletion method"*	*Log K_f Average (SD, n)*	*Log K_f Range*	*References*
Phe	3.73 (±0.05, 15)	3.86 (±0.03, 52)	3.82 (±0.39, 10)	3.25-4.42	(*65, 70, 73-77*)
Fla	4.18 (±0.05, 15)	4.40 (±0.02, 53)	4.22 (±0.31, 11)	3.72-4.71	(*65, 70, 73-77*)
Pyr	4.22 (±0.05, 15)	4.41 (±0.04, 55)	4.42 (±0.42, 7)	3.80-4.86	(*65, 73, 74, 76, 77*)
BaA	4.59 (±0.05, 15)	4.92 (±0.03, 54)	4.70 (±0.57, 5)	3.83-5.26	(*74, 75, 77, 78*)
BbF	4.77 (±0.06, 15)	5.28 (±0.04, 46)	5.17	-	(*77*)
BkF	-[a]	5.29 (±0.04, 46)	5.33	-	(*77*)
BghiP	-[a]	5.39 (±0.03, 38)	4.28	-	(*77*)

[a] no data.

Determination of K_{DOC}

Similar to the "fiber depletion" experiment, the depletion of the Aldrich humic acid exposed fibers was monitored in time (Figure 4 and Figure C of Appendix 1).

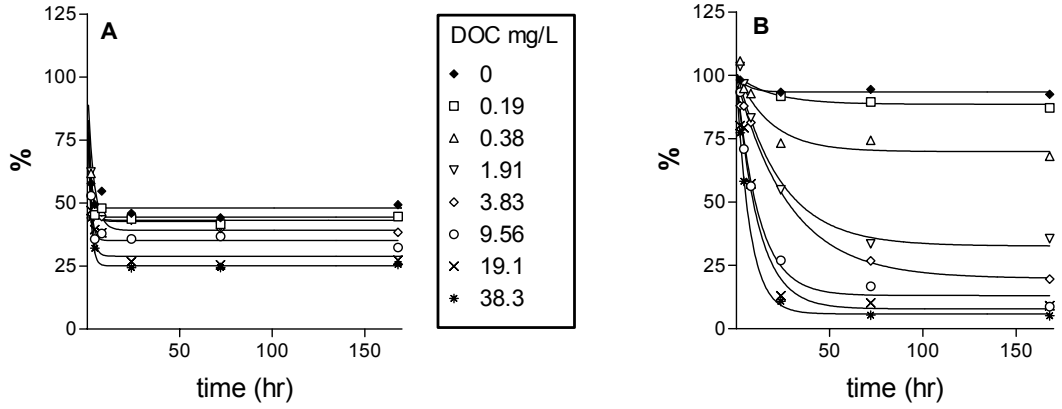

Figure 4: The depletion of phenanthrene (A) and benzo[ghi]perylene (B) from the PDMS fiber at different DOC concentrations in time. A one-phase exponential decay curve is fitted through the data (*n*= 9-12 per DOC concentration). The percentage was calculated as: measured concentration in the fiber at time t divided by the concentration in the fiber at t=0.

Both the desorption from the fiber and sorption to the DOC might influence the equilibration kinetics (Figure 1b), but since DOC-sorption is considered extremely fast (*18, 54, 56, 79, 80*), the exchange between water and PDMS-fiber was thought to be the

rate limiting step. Therefore, equilibrium concentrations in the fiber could be obtained from the plateau of a one-phase exponential decay-curve (Equation 2). For larger particulate hydrophobic phases with slow sorption kinetics, a two-phase exponential decay curve might be used to obtain the equilibrium concentration in the fiber. In those cases, equilibration times can become much longer. The percentage recovered from the equilibrated fiber was and plotted against the DOC concentrations in Figure 5. Sorption coefficients to the DOC (K_{DOC}) were determined by fitting Equation 4 on the data (see fitted lines) using K_f-values determined in the previous experiment.

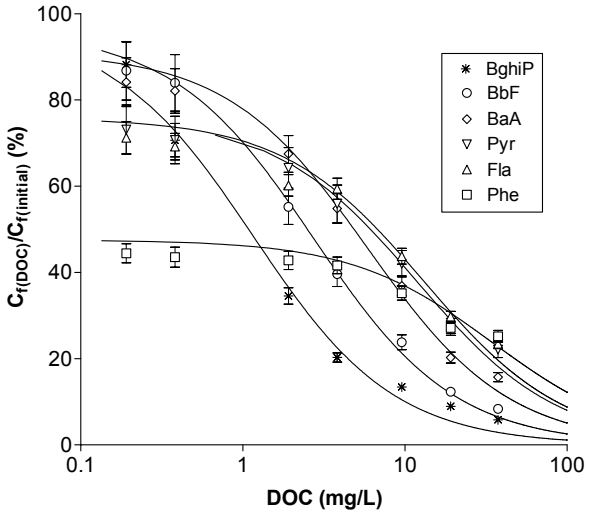

Figure 5: The depletion of the SPME-fiber at a fixed DF as a result of increasing DOC concentrations, the error bars represent the standard errors. The line represents the fit of equation 4, using a fixed fiber-partition coefficient (K_f) obtained from the fiber depletion experiment (Table 2).

Detailed discussion of calculated K_{DOC}-values

The calculated K_{DOC}-values are shown in Table 3. Even though the determined K_{DOC}-values have small standard errors (≤0.05 log units), a closer look at Figure 5 shows that the fiber-concentrations of the analytes (particularly BghiP) were systematically above the fitted curve at the highest DOC concentrations.

Table 3: DOC-water sorption coefficients (log K_{DOC}) to Aldrich humic acid of 6 PAHs.

Compound	*Experimental Log K_{DOC} (L/kg) (SE, n) (Aldrich HA)*	*Literature Log K_{DOC} (L/kg) Average (SD, n) (Aldrich HA)*	*Literature Log K_{DOC} (L/kg) Range*	*References*
Phe	4.78 (±0.05, 32)	4.43 (±0.50, 23)	3.66-5.84	(*16, 19, 55, 58-60, 79, 81-87*)
Fla	5.50 (±0.04, 27)	4.90 (±0.43, 11)	3.92-5.32	(*16, 31, 55, 58, 82, 87-89*)
Pyr	5.55 (±0.03, 33)	4.83 (±0.55, 26)	3.48-5.69	(*16, 32, 56, 58-60, 74, 79, 82, 85, 87, 89-93*)
BaA	6.28 (±0.03, 37)	5.36 (±0.18, 6)	5.18-5.62	(*19, 31, 54, 84, 87*)
BbF	6.96 (±0.02, 42)	5.85 (±0.21, 2)	5.09-5.66	(*31, 87*)
BghiP	7.43 (±0.04, 39)	5.83 (±0.20, 3)	5.79-6.05	(*31, 32, 87*)

This deviation is more clearly shown in Figure 6, where the sorption coefficients at a particular DOC level slightly decreased with increasing DOC concentration.

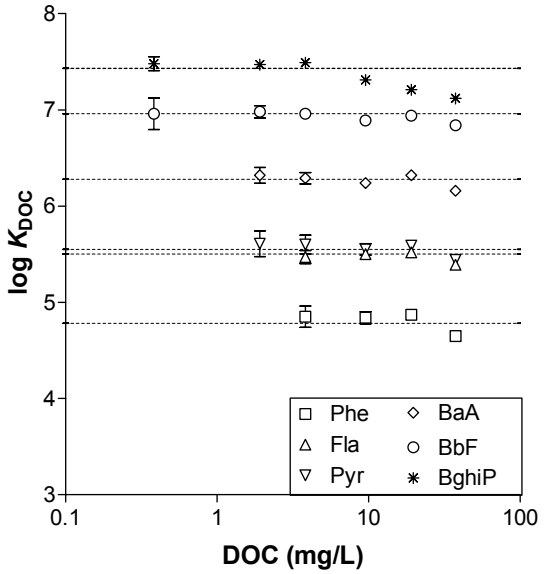

Figure 6: The separately calculated K_{DOC}-values with their standard errors at different DOC-concentrations. The broken lines are the sorption coefficients obtained from the fits of Figure 5.

Nonlinear sorption behavior of the compounds to DOC might explain this effect, but this does not seem plausible, since DOC-sorption coefficients of hydrophobic organic chemicals (PAHs) are generally close to linearity (*17, 40, 51, 74*). Even if sorption is nonlinear, sorption coefficients usually increase with decreasing free concentrations.

Alternatively, fouling of DOC on the fiber surface might occur, creating an increasing hydrophobic phase with increasing DOC concentrations. The exposed fibers were cleaned with a tissue to minimize fouling effects. If fouling was responsible for the observed deviation of BghiP, almost 10% of the DOC in the system should be adsorbed to the fiber to create this effect. This would have led to severe change of color of the fiber, which was not observed. Furthermore, substantial fouling effects have not been observed for SPME fibers exposed to various hydrophobic compounds via an aqueous phase containing high levels of dissolved organic matrices (*34, 48*).
Another, more plausible explanation might be that the addition of Aldrich humic acid sodium salt altered the aqueous chemistry. The pH and conductivity were monitored in the DOC solutions and while the conductivity hardly changed (0.74 – 0.80 mS/cm), the pH increased from 7.03 to 7.82 with increasing DOC concentration (Table B, Appendix 1). Literature shows that the sorption coefficients of PAHs to fulvic and humic acids can decrease slightly with increasing pH (*56, 74, 82, 93, 94*). This phenomenon is explained by the deprotonation of the macromolecules, changing their shape (and interaction with each other (*95*)), increasing their polarity and thereby decreasing their ability to sorb hydrophobic molecules such as PAHs (*56*). This could have happened in our experiments, explaining the slightly decreasing sorption coefficient of especially BghiP with increasing DOC concentration and pH. Although a pH effect cannot be ruled out, the variation of the sorption coefficients of the PAHs was only minor (0.4 log units), so the fits of Figure 5, from which the K_{DOC} was derived, still describe the data well.

Observed K_{DOC} values vs. literature data
Sorption coefficients of the low molecular weight PAHs (Phe, Fla, Pyr, BaA) to Aldrich humic acid from literature are highly variable, while data on high molecular weight PAHs (BbF, BghiP) are very scarce (Table 3). The observed K_{DOC} values of the PAHs with a lower molecular weight are slightly higher than literature values, but as hydrophobicity increases differences increase up to 1.5 log units for BghiP. This discrepancy can probably be attributed to the overestimation of freely dissolved aqueous concentrations in other studies (*66*). It seems that the presented method results in accurate and reproducible partition coefficients, and might also be applicable to more hydrophobic compounds, because the "partitioning driven administration" method provides and samples only true solutions (*64, 65*). In addition, very low aqueous concentrations can be determined due to the high concentration gradient between PDMS and the aqueous phase (*33*). When testing more hydrophobic compounds, increasing equilibration times to obtain K_f and K_{DOC} could become a limiting factor. Thinner polymer coatings, thereby increasing the surface volume ratio, and better agitation can overcome part of this problem. Monitoring the equilibration process remains essential, since dissolved matrices might accelerate kinetics of the partition process due to interference in the aqueous diffusion layer around the fiber (*79, 96*), and larger

particulate hydrophobic phases (e.g. tar) might result in slower kinetics than the fiber-water exchange process, thereby slowing down the equilibration process (*97*).

Mass balance considerations

A crucial assumption in the presented method is a 100% mass balance and no sorption to other phases than described by the models used (Equation 3 and 4). If this assumption is not met, the method will fail to give accurate distribution coefficients.

Sodium azide (NaN_3) was added to inhibit biological degradation (*98*), and photo degradation was prevented by performing experiments in the dark. Any degradation or leakage losses would result in a continuous decrease of fiber concentration in time. However, the time curves of both experiments reach a clear equilibrium level (see Figures 2 and 4, and Figures B and C of Appendix 1), so degradation and leakage were considered insignificant.

Two additional processes, affecting free concentrations could occur: evaporation to the headspace and sorption to the vial wall. Evaporation to the headspace seems insignificant as calculated amounts in the headspace, using air-water partition coefficients (*99*), were always lower than 0.016% and 0.056% in the K_f and K_{DOC} experiment, respectively. Sorption to other phases like (hydrophobic) impurities and the vial walls is of special interest in the fiber partition experiment, since a variety of bottles was used. Their surface volume ratio ranged from 4 cm^{-1} (7.4 mL vials) to 0.6 cm^{-1} (1.1 L bottles). In order to remove hydrophobic impurities, the bottles were cleaned thoroughly with soap, rinsed with hot tap water (3 times), millipore water (3 times), acetone (analytical grade, 3 times), and again with millipore water. However, sorption to glass walls cannot be ruled out. Losses and subsequently underestimation of K_f-values are expected to become important at lower dilution factors, since the surface volume ratio increases with decreasing bottle size. No trends were observed (Figure 7) and therefore sorption to glass walls was considered insignificant as well.

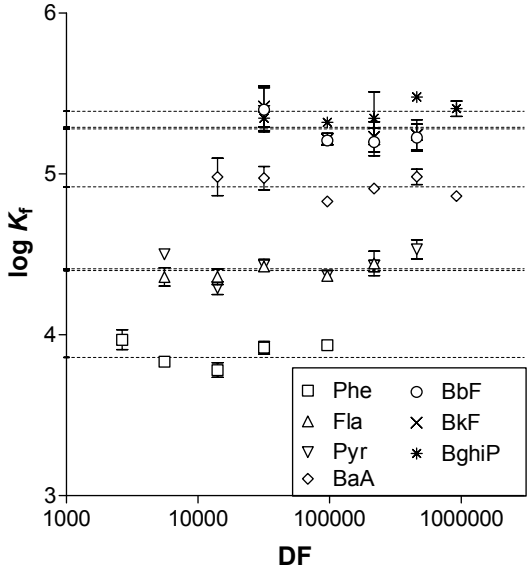

Figure 7: The separately calculated K_f-values and their standard errors plotted against the DF. The broken lines are the partition coefficients obtained from the fits of Figure 3.

Implications and relevance

Sorption behavior to DOC and other hydrophobic matrices is relevant, because it influences the fate of compounds in the environment and their bioavailability (*45-50*). Current models used in risk assessment usually calculate sorption coefficients to DOC from their octanol water partition coefficient (log K_{DOC} = a*log K_{OW} + b), while various researchers have shown that the type of DOC and aqueous chemistry influence the sorption behavior (*74, 90, 91, 95*). However, as long as the variation of reported octanol water partition coefficients and sorption coefficients to DOC remains large, modeling the sorption of very hydrophobic compounds remains difficult.

The "solid phase dosing and sampling technique" enables the measurements of partition coefficients without having to spike aqueous phases with a co-solvent or to separate the aqueous and matrix phases, and it can detect very low aqueous concentrations. In this study, the method is applied to measure partition coefficients to Aldrich humic acid, but in principle, the method can be used to determine sorption coefficients of all kinds of dissolved and fine suspended matrices, including natural DOC, sediment, cell membranes and proteins. The only requirements are; known polymer water partition coefficients, measurements performed at equilibrium, no substantial fouling and careful considerations of mass balance.

Acknowledgements

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Chapter 3

A sediment dilution method to determine sorption coefficients of hydrophobic organic chemicals

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Environmental Science & Technology (2005) 39: 4220-4225

Abstract

Sorption coefficients of hydrophobic organic chemicals (HOC) to sediments and soils can easily be underestimated in traditional batch experiments, especially because analysis of the aqueous concentration often includes compounds sorbed to colloidal organic matter. In this work a "sediment dilution approach" has been combined with measurements of freely dissolved concentrations in order to determine sorption coefficients of five chlorobenzenes and two chloroanilines in spiked sediment, and of two unknown chemicals in field-contaminated sediment. A range of sediment suspensions with different sediment-water ratios was made. Freely dissolved concentrations in these suspensions were measured by negligible depletion solid phase microextraction (nd-SPME). Sorption coefficients (K_D) were derived from the decrease of the freely dissolved concentrations as a function of the "dilution factor" (DF = volume water / mass sediment). The determined sorption coefficients were very similar to literature values.
The experimental set-up provides sorption coefficients without the need for total extractions and the negligible depletion SPME technique does not require need phase separation. The proposed method might be an alternative for batch equilibrium experiments to determine sorption coefficients.

Introduction

The determination of sorption coefficients to sediment normally requires measurements of the concentration in the sediment as well as the aqueous concentration. Determination of the concentration in sediment can be performed relatively easy by exhaustive extractions using a solvent or a solvent mixture (*100*), whereas the determination of aqueous concentrations is more difficult. Numerous methods are applied to determine these aqueous concentrations. Some of these methods separate the sediment and the water actively by centrifugation, or passively by a dialysis membrane (*17*). Often, complete phase separation is not feasible, particularly when solutions contain dissolved organic matter. This incomplete separation may lead to an overestimation of the actually dissolved concentration and to a consequent underestimation of sorption coefficients (*14, 66*). These systematic errors increase with the hydrophobicity of compounds. Various techniques exist that do not need phase separation. These techniques include solubility enhancement (*101*) (usually applied to dissolved matrices), headspace equilibration, fluorescence quenching (*18, 55, 56*), or partitioning based methods, applying a well defined hydrophobic phase such as thin polymer films (*28, 29*) or polymer coated glass fibers (*48, 57-59, 61*).
The present study uses a different approach to determine sorption coefficients, similar to setups used to determine solvent-air partition coefficients (*102*) and sorption to dissolved

organic carbon (*61*). The sediment is diluted with water and the sorption coefficients are deduced from the decrease of freely dissolved concentrations.

Experimental section

Theoretical considerations

When contaminated sediment is diluted with water, part of the compounds associated with the sediment will desorb into the aqueous phase. At equilibrium, the partitioning between the sediment (C_{sed}, mg/kg) and the aqueous phase (C_{aq}, mg/L) is described by the sorption coefficient (K_D, L/kg).

$$K_D = \frac{C_{sed}}{C_{aq}} \tag{1}$$

The free fraction (ff) is the ratio of the freely dissolved amount (C_{aq}*V_{aq}) and the total amount in the suspension (C_{aq}* V_{aq}+ C_{sed}* M_{sed}). In this equation V_{aq} is the aqueous volume in L and M_{sed} is the mass of sediment in kilograms. At a certain dilution factor (DF = V_{aq} / M_{sed}, L/kg), this free fraction can be used to determine the sorption coefficient (K_D) to the solid phase (*57*) using the following equations:

$$ff = \frac{C_{aq} * V_{aq}}{C_{sed(DF=0)} * M_{sed}} = \frac{C_{aq} * V_{aq}}{C_{aq} * V_{aq} + C_{sed} * M_{sed}} = \frac{1}{1 + K_D * \frac{1}{DF}} \tag{2}$$

When the initial sediment concentration ($C_{sed(DF=0)}$) and the theoretical initial aqueous concentrations ($C_{aq(DF=0)}$) are used, Equation 2 can be rewritten as:

$$C_{aq} = \frac{\frac{1}{DF} * C_{sed(DF=0)}}{1 + K_D * \frac{1}{DF}} = \frac{C_{sed(DF=0)}}{DF + K_D} = \frac{C_{aq(DF=0)} * K_D}{DF + K_D} = \frac{C_{aq(DF=0)}}{1 + \frac{DF}{K_D}} \tag{3}$$

In this study, freely dissolved concentrations were measured using negligible depletion solid phase microextraction (nd-SPME) (*34*). Fiber concentrations (C_f) can then be used to calculate the K_D with Equation 4.

$$\frac{C_f}{C_{f(DF=0)}} = 1 - \frac{DF}{K_D + DF} \qquad (4)$$

In a hypothetical case where contaminated sediment, soil or another matrix containing a hydrophobic phase is diluted with water, the free fractions of compounds can be written as in Equation 4, and K_D can be calculated as long as there is a sufficient decrease of the freely dissolved concentration. The relation between the fiber concentration and the DF is plotted in Figure 1. In Equation 4, it is assumed that the sorption coefficient is constant within the relevant concentration range of the experiment (e.g. 10 – 100% of initial concentration). This assumption is probably correct since sorption isotherms of hydrophobic compounds to sediments are generally close to linearity (*17*).

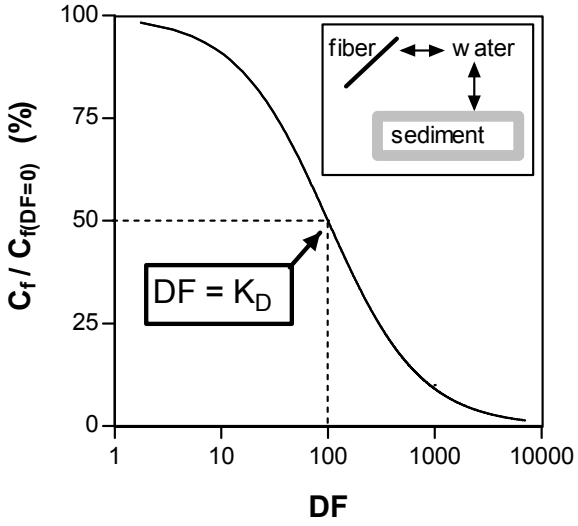

Figure 1: A schematic picture of the dilution test systems. Expected fiber concentrations (C_f) relative (%) to the initial fiber concentration ($C_{f(DF=0)}$) as a function of "dilution factor" (DF), for a hypothetical compound with a K_D of 100.

Sediments, chemicals, fibers and solvents

Uncontaminated freshwater sediment was sampled at lake Oostvaarders plassen and contaminated brackish sediment was sampled at the Zeehavenkanaal estuary near the harbour of Delfzijl (both The Netherlands). The sediments were stored at 4°C. Table 1 shows the properties of both sediments. Five chlorobenzenes and two chloroanilines were used in this study. 1,2,3 trichlorobenzene (TriCB), 1,2,3,5 tetrachlorobenzene (TeCB), pentachlorobenzene (PeCB), hexachlorobenzene (HeCB), 2,3,5,6 tetrachloroaniline (TeCA) and pentachloroaniline (PeCA) were purchased at Sigma-Aldich BV (Zwijndrecht, The Netherlands), while 1,4 dichlorobenzene (DiCB) came from the British Drug House (Poole, UK).

Table 1: Properties of the spiked and field contaminated sediment.

Property	*Clean sediment (reference)*	*Contaminated sediment*
fraction particle size <2μm%	24 (*103*)	-[a]
fraction particle size <16μm%	43 (*103*)	-[a]
pH	7.49	7.01
Organic Carbon%	2.7 (*103*)	6.3
N%	0.27 (*103*)	-[a]
dry weight of sediment samples%	34.9	25.9

[a] Not measured.

Octanol-water and fiber-water partition coefficients of the compounds are listed in Table 2. The 7 μm poly(dimethylsiloxane) (PDMS) coated SPME fiber was purchased at Supelco (Bellefonte, USA). Acetone (Labscan, Dublin, Ireland) and n-hexane (Baker BV, Deventer, The Netherlands) were of analytical grade, and highly pure water (R ≥18 MΩ) was prepared by a Millipore purification system equipped with organic free kit (Millipore waters, Amsterdam, The Netherlands). NaN_3 and NaCl were purchased at Merck, VWR International BV (Amsterdam, The Netherlands).

Table 2: The test compounds and some properties.

Compound	*Purity %*	*log K_{OW} De Bruijn (104)*	*log K_f log $K_{PDMS:WATER}$ (reference)*
1,4 dichlorobenzene (DiCB)	not given	3.43	2.44 (*70*)
1,2,3 trichlorobenzene (TriCB)	>99.8	4.05	3.14 (*105*)
1,2,3,5 tetrachlorobenzene (TeCB)	>99	4.64	3.85 (*105*)
pentachlorobenzene (PeCB)	>99	5.18	4.42 (*105*)
hexachlorobenzene (HeCB)	>99	5.71	4.87 (*105*)
2,3,5,6 tetrachloroaniline (TeCA)	>98	4.46	3.18 (*105*)
pentachloroaniline (PeCA)	>99	5.08	4.17 (*70*)

Spiking the clean sediment

5.0 grams of the clean sediment was oven dried at 200°C, homogenized with a mortar, sieved (1 mm mesh size) and spiked with 5.0 mL acetone containing an equimolar (0.110 mM) mixture of the seven test compounds listed in Table 2. The acetone was evaporated by rotating the sediment-acetone mixture for 90 minutes at a temperature of 35-40°C and a pressure of 700 mb using a Rotavapor (Büchi Laboratortechnik, Hendrik Ido Ambacht, The Netherlands). The spiked sediment was added to 129 grams of wet clean sediment (34.9% dry weight) and 152.6 mL salt solution (0.93 M NaCl and 15.5 mM NaN_3 to inhibit bacterial activity). The obtained sediment suspension was homogenized by stirring at 600 rpm for 15 minutes, and stored at 5°C for 15 days to equilibrate. The spiked sub-sample (10%) was mixed with untreated sediment (90%) to leave the sorption properties of the whole sediment as unaltered as possible.

Diluting the sediments to measure sorption coefficients

Sediment suspension dilutions were made in saline solution containing 0.6 M NaCl and 10 mM NaN_3. The clean and contaminated sediment suspensions were placed on a magnetic stirrer for 15 minutes. A vortex of ~50% of the suspension-level was created and a sample of 4.0 mL was taken and added to 4.0 mL of salt solution. The freshly diluted sediment-suspensions were mixed, by flushing the pipette 4 times before a new sample of 4.0 mL was taken, and put in the next vial containing 4.0 mL of salt solution. This step was repeated 12 times, creating 13 different sediment-water ratios. The dilutions were prepared in triplicate. To all dilutions, 8.0 mL of 0.6 M NaCl solution was added, so all vials contained 12 mL of sediment suspension. Final dilution factors (DF, L/kg) varied from 14.2 to 59722 for the clean sediment and from 23 to 94980 for contaminated sediment. An identical "blank" dilution series was made for the clean sediment, to check for background contamination.

After dilution, the sediment suspensions were stored at 5°C in the dark for 126 to 160 hours to equilibrate. Free concentrations were measured using PDMS coated SPME fibers. The peak areas (PA) derived from the chromatograms were plotted against the dilution factor (DF). The relative standard deviation (n= 3) of the peak areas at the lowest DF (14.2) were 4.6%, 8.6%, 5.4%, 1.2%, 2.5%, 3.8% and 3.3% for DiCB, TriCB, TeCB, PeCB, HeCB, TeCA and PeCA respectively. Equation 4 was adapted and both the peak area at DF = 0 ($PA_{(DF=0)}$) and the "log" K_D was obtained from fitting equation 5 to the experimental data. The K_D was logarithmized to obtain symmetric standard deviations in log K_D.

$$\frac{PA}{PA_{(DF=0)}} = 1 - \frac{DF}{DF + 10^{\log K_D}} \qquad (5)$$

Graphpad version 3.0 (San Diego, CA, USA (*69*)) was used to fit the data. Only peak areas within the linear range of the standard series (determined with an external solvent injected standard series) were used in the fitting.

Chemical analysis

A 1.0 cm long 7 μm PDMS coated fiber (PDMS-volume = 25.73 nL) was exposed to the sediment suspensions for 2 minutes by a Varian 8200 CX Autosampler (Varian, Palo Alto, USA) with an SPME-agitation device. Fibers were desorbed in the 1078 Universal Capillary Injector (inlet liner developed for SPME use, diameter 0.8mm) of a Varian 3600 CX gas chromatograph. The initial injection temperature was 200°C and after 5 minutes, it was increased to 250°C with a temperature rate of 200°C per minute, where it was kept for 4.75 minutes. The total desorbing time was 10 minutes. Helium was used as carrier gas. The split valve was opened 5 minutes after injection (with a bottom split of 100 mL/min). The desorbed chemicals were focused at 50°C on a 30 m * 0.32 mm fused

silica DB5.625 column (J&W Scientific, Folson CA, USA) with a film thickness of 0.25 μm. After 5 minutes the initial column-temperature was increased to 250°C with a rate of 10°C/min for the spiked clean sediment samples and with 20°C/min for the contaminated sediment samples. Compounds were detected using a ^{63}Ni Electron Capture Detector (ECD, Varian) at 325°C isothermally. The stability of the ECD signal was monitored every 5-10 samples by an external standard solution. Chromatograms were analyzed using Varian Star workstation version 5.31 software, and checked by hand after automatic integration.

Results and discussion

Negligible depletion SPME

The concentration in the polymer coating of an SPME-fiber is directly proportional to the initial freely dissolved concentration if the extracted amount by the fiber is negligibly small (<5%) (*57, 58, 106, 107*). In the present study this was achieved by a very short extraction time of only 2 minutes. The amount that the fiber extracted within this period was calculated to be smaller than 5.6% of the freely dissolved fraction (*48, 105*). This was confirmed by three subsequent fiber extractions within the same sample, which showed no significant decrease in concentration. The applied SPME method can thus be considered non-depletive, which makes it suitable to measure freely dissolved concentrations. All obtained peak areas were within the linear range of the detector and were thus directly proportional to the freely dissolved concentration.

Sorption coefficients obtained with the dilution method

All peak areas were normalized to the peak area at a DF of 0 and then plotted against the DF (Figure 2). The data were fitted to equation 5 and corrected for the organic carbon content of the sediment in order to determine log K_{OC}-values. It can be observed that the equation fits the data generally well, with regression coefficients larger than 0.90. Schrap et al. (*14*) and Cornelissen et al. (*108*) determined sorption coefficients of various chlorobenzenes to the same sediment, using a classical batch equilibrium approach. Additionally, Cornelissen measured sorption coefficients after 2 and 34 days shaking, and corrected sorption coefficients for the sorption to DOC present in the supernatant. Schrap (*109*) also determined uptake and elimination rate constants for some chloroanilines, that could be used to calculate their sorption coefficients. The sorption coefficients of Schrap and especially the data of Cornelissen (2 days shaking) were almost identical to our data (Table 3).

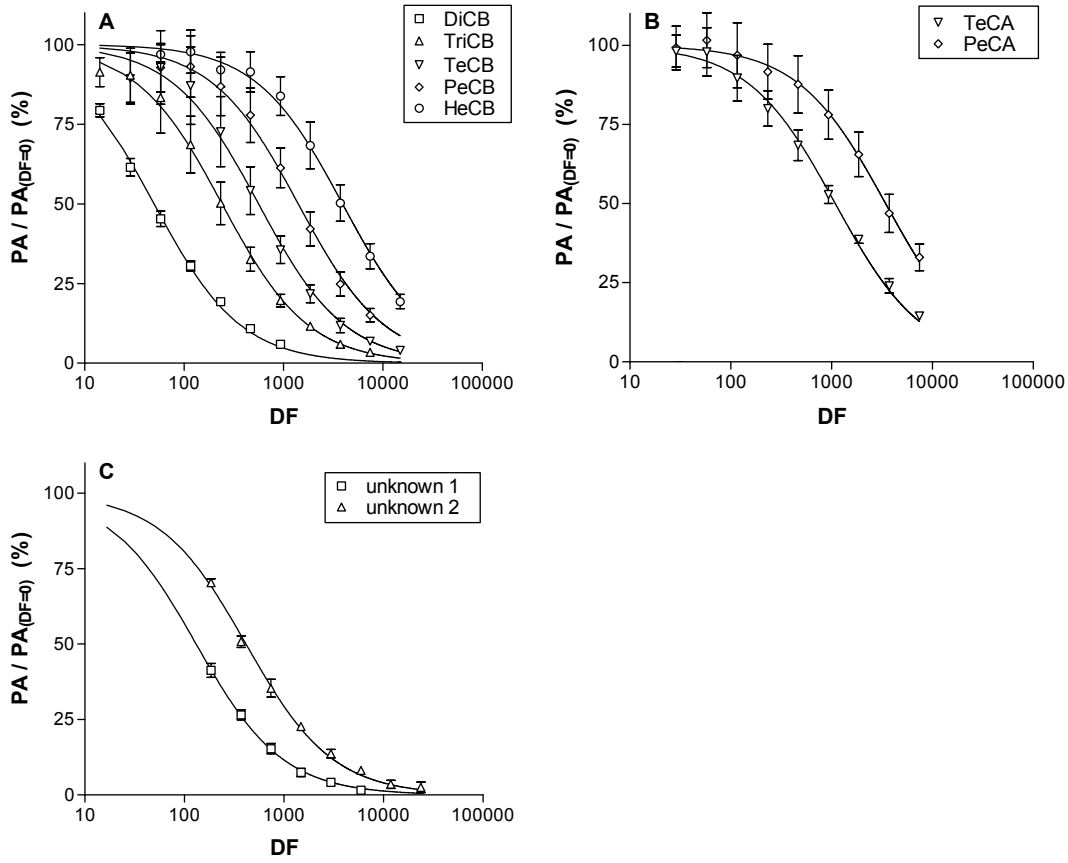

Figure 2: The peak area (PA) as a percentage of the initial peak area ($PA_{(DF=0)}$) as a function of the dilution factor (DF). The lines represent the fit of Equation 5 to the data. Figure 2a shows the results of the chlorobenzenes and Figure 2b the results of the chloroanilines in the clean sediment. Figure 2c shows the results of the field sediment, giving data on two unidentified chemicals.

Table 3: Sorption coefficients (K_{OC}, L/kg) to the clean Oostvaarders plassen sediment measure in this study and data from the literature.

Compounds	*log K_{OC} (±SE) of this study*	*log K_{OC} (SD) from reference (14, 109)*	*DOC corrected log K_{OC} after 2 d incubation from reference (108)*	*DOC corrected log K_{OC} after 34 d incubation from reference (108)*
1,4 DiCB	3.27 (±0.04)			
1,2,3 TriCB	3.95 (±0.08)	3.80 (3.44 – 4.00) [a]		
1,2,3,4 TeCB	-	4.26 (4.06 – 4.40) [a]	4.27	4.66
1,2,3,5 TeCB	4.32 (±0.09)			
PeCB	4.72 (±0.08)	4.68 (4.51 – 4.81) [a]	4.67	5.07
HeCB	5.17 (±0.06)	4.98 (4.56 – 5.19) [a]	5.19	5.47
2,3,5,6 TeCA	4.61 (±0.04)	4.82 [b]		
PeCA	5.12 (±0.06)	5.04 [b]		

[a] The interval of one standard deviation below and one standard deviation above the log K_{OC} is given.
[b] Values are calculated from uptake and elimination rate constants of reference (*109*).

In Figure 3, the organic carbon normalized sorption coefficients to the clean sediment are plotted against the log K_{OW} of the compounds. This figure shows that sorption coefficients correlate well with the octanol-water partition coefficients. In addition, two log K_{OW}-based QSAR relationships for sorption coefficients (log K_{OC}) from literature (*35, 110*) are plotted in Figure 3 and the observed sorption coefficients are very close to the model predictions. A more detailed look to the data shows that the more polar chloroanilines have significantly higher sorption coefficients than the non-polar chlorobenzenes. Higher sorption coefficients of the polar compounds are often observed in literature (*17, 110*), and can be explained by more specific polar interactions (H-bonds) with H-bond accepting and donating functional groups in the sediment (*1, 39*).

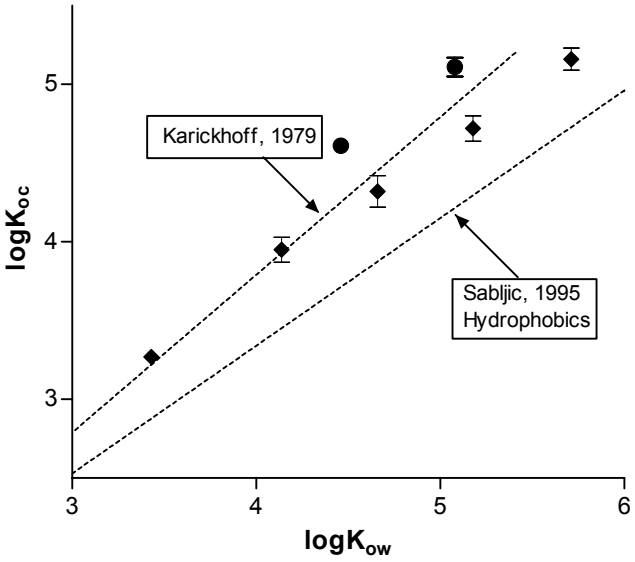

Figure 3: Measured log K_{OC} vs. log K_{OW} of compounds investigated in this study (Table 2). The triangles represent the chlorobenzenes and the circles the two anilines (error bars represent standard errors). The two broken lines represent two QSARs of Karickhoff et al. (*35*) and Sabljić et al. (*110*).

Evaluation of the dilution method

The sediment dilution method is a simple method to determine sorption coefficients of a large number of non-ionic hydrophobic compounds in spiked and field-contaminated sediments. This method has a number of advantages. Firstly, the sampling at a series of sediment-water ratios enables the determination of sorption coefficients (of mixtures) of chemicals at the most sensitive range, thereby increasing the accuracy of the data (*17*). This can be observed from Figures 4a and 4b, where sorption coefficients calculated from the series of sediment-water ratios generally have lower standard errors than those calculated from the individual dilutions. Secondly, the dilution method uses the relative decrease of peak areas (Equation 5), making quantification unnecessary. Note that this procedure is only valid as long as concentrations fall within the linear range of the

detector. Thirdly, the partitioning based sampling method (nd-SPME) is sensitive due to high partition coefficients between the PDMS and water (*33*), and it does not require the separation of aqueous and solid phases, because it only samples the freely dissolved fraction in the aqueous phase (*25, 34, 52, 57*). Finally, the method allows the determination of sorption coefficients for native pollutants and even unknown compounds at an environmentally relevant range.

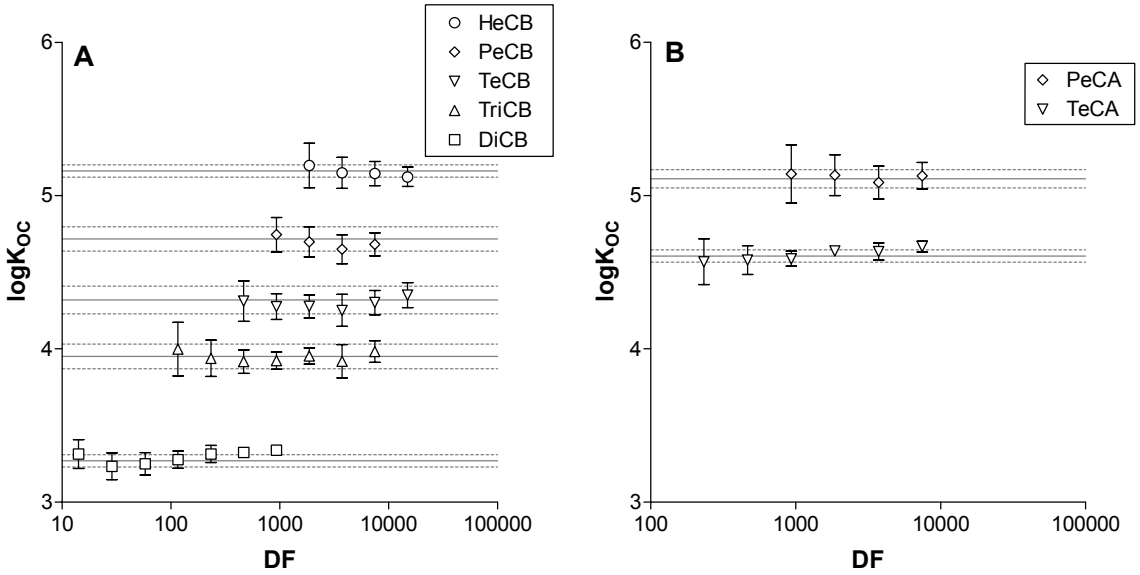

Figure 4: Measured K_{OC}-values calculated at individual dilution factors of the chlorobenzenes (4a) and chloroanilines (4b). The error bars represent their standard errors, and the horizontal lines represent the K_{OC}-values calculated using Equation 5 (solid lines) and their standard errors (broken lines).

An example is given in Figure 2c, where the sediment dilution method was applied to a field-contaminated sediment and the SPME analysis showed two peaks that clearly decreased with dilution. Even though the ECD-detector could not identify the compounds, sorption coefficients to the sediment were calculated (unknown 1: log K_{OC} = 3.32, SE = 0.02; unknown 2: log K_{OC} = 3.82, SE = 0.05).

Although the presented method has advantages over classical methods in determining sorption coefficients, it is built on some assumptions and has some potential limitations and pitfalls as well. First of all, the presented approach is only suited to determine sorption coefficients at the actual concentration level in the tested sediment. These sorption coefficients should be applied with care at other concentration levels, since nonlinear sorption has been observed, even for non-polar hydrophobic organic compounds (*1, 111-114*). In principle, however, Equation 4 and 5 can be rewritten for nonlinear sorption behavior.

Additionally, Equation 5 (used to calculate sorption coefficients) assumes that there is no significant loss of compounds during dilution and subsequent equilibration (100% mass balance) and that the compounds are also not lost by sorption to vial walls. In general,

the freely dissolved fraction only is considered to be (most) vulnerable for losses due to evaporation, (biological) degradation or sorption to glass. Thus, the largest relative losses can be expected at the higher dilution factors, thereby leading to an underestimation of the sorption coefficient. In order to reduce evaporation, the headspace in the test vials was small (8%). It was calculated that even for the compound with the highest air-water partition coefficient (1,2,3,5 TeCB) (*1*) the amount in the headspace was less than 3% of the amount that was freely dissolved in the water. Losses due to biodegradation were prevented by the addition of the bactericide NaN_3, and recycling pipette tips for 4 dilution steps reduced potential losses due to sorption to the polypropylene material. Sorption to vial walls cannot be ruled out completely. However, we believe that this did not have a significant effect on our measurements, since the data are perfectly in line with literature values where no mass balance approach was used (Table 3).

Furthermore, the compounds in the sediment-water system should be at equilibrium. As equilibration times between sediment and the aqueous phase will increase at higher dilution factors, especially for the more hydrophobic compounds (*108*), equilibrium might not have been reached. Non-equilibrium situations will reduce free concentrations at higher dilution factors. In order to overcome this problem, all sediment suspensions were equilibrated for 126 to 160 hours. Figure 4a and 4b show that the sorption coefficient determined with Equation 5 does not depend on the dilution factor, and we may conclude that desorption kinetics did not affect our measurements.

Finally, dissolved organic material (DOM) can affect the uptake in the SPME fiber by fouling to the fiber material or by influencing uptake kinetics. Fouling is thought to be insignificant since the peak areas of the more hydrophobic compounds (HeCB and PeCA) did not show a decrease at a dilution range of 14 to 200, where DOM concentrations are high and decrease steeply (Figure 2). The presence of dissolved organic matter (DOM) may affect the uptake kinetics to the fiber (*33, 34, 79, 96*), as long as the diffusion through the so called "unstirred boundary-layer" (UBL) is the rate limiting step. However, this phenomena requires very high DOM concentrations of hundreds of milligrams per liter (*48, 74, 79*), which is higher than expected in (diluted) soil and sediment suspensions. In addition, the autosampler with an agitation device applied in this study is thought to achieve such good stirring that kinetics are determined by the diffusion in the polymeric phase (*48*), so kinetics could not have been influenced by the DOC in the aqueous phase.

All phenomena mentioned above will lead to an underestimation of the real freely dissolved aqueous concentration (at equilibrium) in particular at the higher dilution factors. However, the sorption coefficients calculated with Equation 5 (see Figure 4) do not show a significant trend over the range of dilution factors, and observed sorption coefficients agree very well with other independent studies (*14, 108, 109*). Therefore, potential artifacts addressed above did not seem to have influenced our measurements.

The study shows that, a passive non-depletive sampler and a dilution set-up of a sorbent can supply accurate sorption coefficients to spiked and field-contaminated sediments, and is a suitable alternative for classical batch equilibrium methods.

Acknowledgements
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Chapter 4

Freely dissolved pore water concentrations and sorption coefficients of PAHs in spiked, aged and field-contaminated soils

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Manuscript in preparation

Abstract

Freely dissolved aqueous concentrations in the soil pore water represent an important aspect of bioavailability and risk assessment of contaminated soils. Pore water concentrations of hydrophobic organic compounds are difficult to assess, so risk assessors generally estimate these concentrations from total soil concentrations using equilibrium partitioning (EqP) models. Strong sorption to soot, coal and weathered oil, as well as so-called aging effects hampers a good estimation of these pore water concentrations. Various chemical methods like Supercritical Fluid Extraction (SFE), Tenax extraction and equilibrium partitioning based extraction methods have been developed to measure site specific bioavailable, bioassessable, or freely dissolved concentrations in contaminated soils.
In this study, a negligible depletion, partitioning based, sampling technique was validated and applied to measure free concentrations of polycyclic aromatic hydrocarbons (PAHs) in spiked, aged and field-contaminated soils. Detailed kinetic studies were performed to select appropriate equilibration times. Freely dissolved aqueous concentrations in the pore water were compared to total concentrations, and sorption coefficients were calculated. Results show that EqP-models from literature can predict sorption coefficients of freshly spiked and lab-aged soils with an accuracy of less than a one order of magnitude. The effects of aging (up to 550 days) are rather minor, leading to an increase in the sorption coefficient of less a factor 3. Contrastingly, pore water concentrations of field-contaminated soils are often highly overestimated. Freely dissolved concentrations in the pore water were up to more than two orders of magnitude lower than what was expected from EqP-models, and consequently, risks can be highly overestimated with these models. The partitioning based sampling technique used in this study is a simple and sensitive tool to measure pore water concentrations, and could therefore be applicable in site-specific risk assessment of field-contaminated soils.

Introduction

Hydrophobic organic chemicals like polycyclic aromatic hydrocarbons (PAHs) are common micro-pollutants in soils (*115*). PAHs are produced by incomplete combustion processes and can be of natural and anthropogenic origin. Industrial soils of manufacturing gas plants, petroleum refineries and wood preservation plants tend to have very high PAH-concentrations up to thousands of milligrams per kilogram soil. Freely dissolved concentrations in the pore water and sorption coefficients are two important and relevant entities in the analysis and discussion of bioaccessibility and bioavailability.
The generic environmental risk assessment procedures of organic chemicals like PAHs in soils are based on total soil concentrations or pore water concentrations estimated from total soil concentrations and organic carbon normalized partition coefficients (*17,*

35, 37, 110, 116). These partition coefficients are generally based on experiments with freshly spiked standard soils and sediments. They do not consider so-called aging effects, where sorption may slowly increases in time, due to for example slow diffusion of compounds into micro-pores or inflexible organic materials (*3, 7, 117-119*). Besides that, these models also disregard the heterogeneity of the organic carbon in soil. Standard tests soils and sediments usually contain lower amounts of strong sorbing matrices like soot, coal or tar than are found at contaminated industrial sites (*112, 120-124*) and in those cases the matrix itself is often the main source of PAHs. Aging effects as well as and strong sorption these matrices will lead to higher sorption coefficients and lower pore water concentrations in field sediment and soil. Higher sorption may lead to a reduction in the bioavailability and bioaccessibility, and subsequently to lower risks.

Various chemical techniques have been developed to study bioaccessibility and bioavailability. One series of approaches is focused on extraction only the weakly bound fraction in soil and sediment. Several solvents (mixtures) (*125, 126*) and sorbents like Tenax (*108, 127-129*) or XAD-2 (*130*) are used for this purpose. Supercritical Fluid Extraction (SFE) is somewhat similar, but this method uses a highly pressurized gas (CO_2) in the liquid phase (*131*).

Other approaches focus on the freely dissolved concentrations in the pore water. Freely dissolved aqueous concentrations can be determined by equilibrium dialysis (*54*), by a gas purge method (*24, 132, 133*), or by negligible-depletive passive samplers such as semi permeable membrane devices (SPMD) (*20, 22, 26, 134*), poly(oxymethylene) solid phase (POM) (*28*), polymer coated glass sheets or fibers (*27, 29, 34, 57*). These passive samplers are equilibrated with the contaminated soil or sediment, and free aqueous concentrations can be calculated with known partition coefficients between the passive sampler and water. If the sampler is not at steady state yet, kinetic data are needed to estimate freely dissolved concentrations. As pointed out by Mayer et al. (*33*) equilibrium based methods are often more reliable.

The objective of this study was to measure pore water concentrations and soil sorption coefficients in freshly spiked soils, and to study how aging affects these concentrations. Similar studies were performed in a series of contaminated field soils, to determine site-specific sorption coefficients and to analyze the variability in these sorption coefficients. Poly(dimethylsiloxane) coated glass fibers were applied as passive samplers to measure free aqueous pore water concentrations in the different soils. For the development of the method, fiber-water sorption coefficients were measured for a broad range of PAHs, and detailed kinetic studies were performed in soil suspensions. Furthermore, the results will be shortly discussed in relation to the risk assessment of contaminated soils.

Experimental Section

Chemicals, fibers, solvents and soils

Phenanthrene (Phe), fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), benzo[b]fluoranthene (BbF), benzo[k]fluoranthene (BkF) and benzo[ghi]perylene (BghiP) used for spiking soils were all purchased at Sigma Aldrich Chemie BV (Zwijndrecht, The Netherlands). Disposable glass fibers with a core diameter of 110 μm and a 28.5μm poly(dimethylsiloxane) (PDMS) coating (volume 12.4 μl/m) were obtained from Poly Micro Industries (Phoenix, AZ). Acetonitrile, acetone, methanol, ethylacetate (Lab-Scan, Dublin, Ireland) and n-hexane (Baker BV, Deventer, The Netherlands) used, were of analytical grade. Highly pure de-ionized water (R ≥18 MΩ) was prepared by a Millipore water purification system, equipped with organic free kit (Millipore Waters, Amsterdam, The Netherlands). Table 1 shows the origin and organic carbon contents of the five clean field soils and six contaminated field soils that were used in this study.

Table 1: The organic carbon (OC) content and the total PAH concentrations of the selected test soils. See Table A and B fore more detailed information on the PAH concentrations in the soils.

Soil	*% TOC (ref)*	*Additional information on the soils*	*ΣPAH mg/kg soil (dw)*
		Clean soils used for spiking studies	
Askov	1.39 (*135*)	Sandy loam soil (Denmark)	113.9 [a]
Borris-2	1.67 (*135*)	Sandy loam soil (Denmark)	105.4 [a]
Kettering	2.09 (*135*)	Sandy clay loam soil (UK)	105.4 [a]
Waschbach	2.29 (*135*)	Silt loam soil (Austria)	105.4 [a]
Norway	5.49 (*135*)	Forest soil (Norway)	105.4 [a]
		Contaminated soils	
Andujar-B2	3.3 (*136*)	Railway station site, air dried clayish silt 2003 (Spain)	4560
E6068-K	4.7 (*136*)	Industrial soil, piled since 1994, straw and sewage sludge added (Denmark)	332.1
K3840	1.2 (*136*)	Gasoline station site, sand soil piled since 2000 (Denmark)	16.7
Olst-J	4.1 (*136*)	Industrial soil (The Netherlands)	16.0
Skaegen	1.97 (*135*)	Sandy soil contaminated with tar by fishnet dipping (Denmark)	522
TP44	3.0[b]	Soil from a gas manufacturing plant (UK)	1020

[a] Nominal concentration spiked to the clean soils.

[b] CO_2-analysis after CO_3 removal by Jordforsk (Centre for Soil and Environmental Research, Norway).

Sorption isotherms to PDMS

Fiber water partitioning were determined with a relatively new method that is based on the depletion of pre-loaded fibers with selected amounts of water (*137*). The advantage

of this method is that it avoids difficulties that are often encountered with spiking of aqueous solutions with hydrophobic chemicals. The PDMS coated fibers were cut into 5.0 or 3.0 cm pieces and cleaned by heating at 275°C for 16 hr under a constant helium flow of 30-35 mL/min. Clean fibers were "loaded" by exposing them to a 1:1 methanol-water mixture (~6.2 µL PDMS in 5 mL methanol-water) spiked with seven PAHs at seven concentration levels. The loaded fibers were placed in three different volumes of water (6.2, 38 and 102 mL), to obtain three different volume water / volume PDMS ratios (11000, 102000 and 272000). The concentration of sodium azide (NaN_3, Merck, Amsterdam, The Netherlands) in these solutions was 10 mM in order to inhibit bacterial degradation. The flasks were shaken for 28 d (which is sufficient to reach equilibrium (*137*)) in the dark at 20 ± 1°C. PAH in the loaded and water-exposed fibers were extracted with 0.20 to 20 mL acetonitrile. Initial concentrations in the PDMS coating ranged from 0.7 to 5440 mg/L, 1.0 to 2380 mg/L, 0.5 to 1620 mg/L, 0.5 to 79 mg/L, 0.5 to 105 mg/L, 0.6 to 63 mg/L and 0.5 to 14 mg/L, for Phe, Fla, Pyr, BaA, BbF, BkF and BghiP, respectively. Aqueous concentrations were estimated by a mass-balance approach, assuming that all compounds depleted from the fiber were dissolved in the aqueous phase (Equation 1),

$$\frac{C_f}{C_{f\,(initial)}} = \frac{1}{1 + \frac{V_{aq}}{V_f * K_f}} \qquad (1)$$

where $C_{f\ (initial)}$ is the initial concentration in the loaded fiber, C_f and C_{aq} are the concentrations in the PDMS and aqueous phase at equilibrium, V_f and V_{aq} are the volumes of the PDMS and aqueous phase, and K_f is the fiber (PDMS)-water partition coefficient. In this approach it is assumed that the mass balance is 100%. Furthermore, aqueous concentrations were determined in a selection of the test vials (n= 13), by extraction a sample of the aqueous phase was extracted with n-hexane three times. Subsequently, the hexane was evaporated under a gentle stream of nitrogen, while adding acetonitrile.

Spiking and analyzing soils

All soils (Table 1) were stored at 4°C until use. Clean field soils were spiked according to Brinch and coworkers (*138*) with some adaptations. Before spiking, the clean soils were dried at 25 ±1°C until a constant weight (2-4 days) and gently grounded and sieved (1 mm mesh size). A sub-sample of 10% was spiked with the selected PAHs dissolved in acetone (soil / acetone ratio = 2 / 1 (w/v)) and was mixed by hand. After evaporation of the acetone (overnight at room temperature), the sub-sample (10%) was mixed with the rest (90%) of the soil, and shaken thoroughly for 1 hour with a one-dimensional shaker. After mixing, water (10 mM sodium azide) was added to ~60% of the water holding

capacity, and the soil was incubated for three weeks at 20 ±1°C. Final soil concentrations varied from 7.2 - 28.9 mg/kg per compound (Appendix II, Table A).
Total concentrations of spiked and field-contaminated soils were determined after a 16-hour ethylacetate soxhlet-extraction using 45 mL ethylacetate per 2 grams of soil. The field-contaminated soils were gently grounded and homogenized before extraction. The ethylacetate extraction has shown similar results as classical n-hexane / acetone extractions (*100*). Furthermore, a second soxhlet extraction of the field-contaminated Skaegen soil recovered less than 0.5% of the initially extracted PAHs. The initial extraction was therefore considered exhaustive. Additional information on the PAH concentrations of the field-contaminated soils can be found in Table B of Appendix II.

Exposing fibers to soil
Figure 1 gives a schematic picture of the fiber exposure. One to three thermally cleaned fibers (5 cm long, 0.62 µL PDMS) were exposed to 2 g (ww) aliquots of spiked or field-contaminated soil in 7 mL amber vials with 2 mL 10 mM sodium azide solution. The field-contaminated soils were gently grounded and sieved (1 mm mesh size) before fiber exposure. The vials were shaken on a "rock and roller" shaker (Snijders Scientific, Tilburg, The Netherlands). Exposed fibers were gently wiped with a wet tissue, and extracted with acetonitrile.

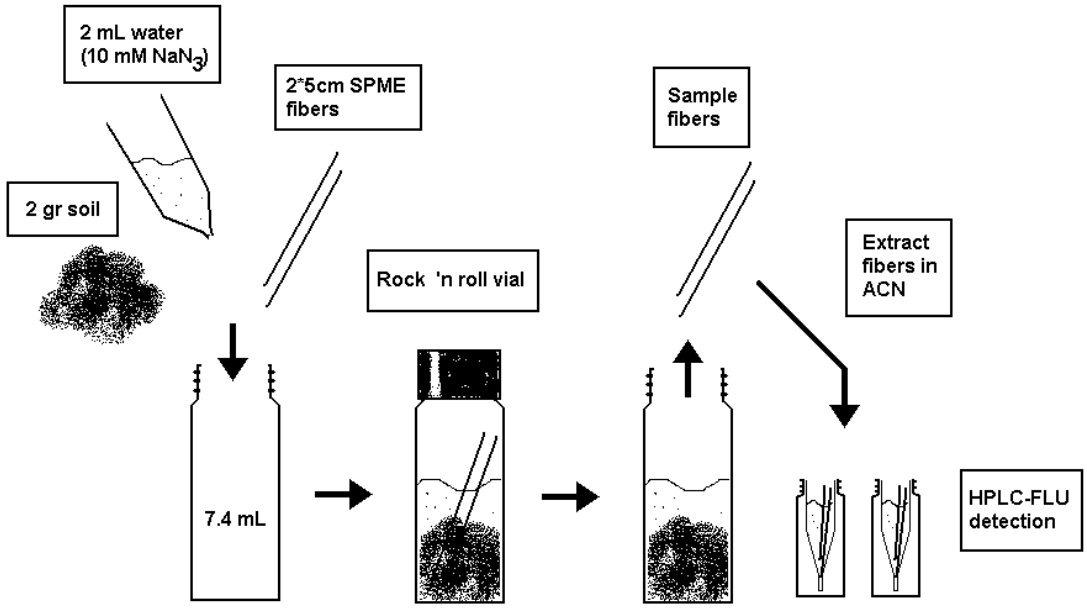

Figure 1: A schematic picture of negligible depletion passive samplers to measure freely dissolved aqueous concentrations of contaminants in soil.

The uptake kinetics of the fiber in the soil system was determined by analyzing fibers that were exposed to Askov soil spiked with Phe Fla, Pyr, BaA, BbF, BkF and BghiP, and field-contaminated Skaegen and TP44 soil for a variety of exposure times varying

between 0.5 and 7778 h. Equilibrium concentrations in the fiber ($C_{f(\infty)}$) and rate constants (k) were estimated from concentrations in the fiber in time ($C_{f(t)}$) with a one-compartment model using Graphpad Prism™ 3.0 (San Diego, CA):

$$C_{f(t)} = C_{f(\infty)} * (1 - e^{-k * t}) \quad (2)$$

If sorption to the fiber is the rate-limiting step, the rate constant k is equal to the elimination rate constant of the fiber ($k_{e\text{-fiber}}$). Based on this study, a minimum exposure time of 72 h was chosen.

Free aqueous concentrations (C_{aq}) in the soil pore water were calculated by dividing the concentration in the fiber at equilibrium ($C_{f(\infty)}$) by the fiber-water partition coefficient (K_f).

$$C_{aq} = \frac{C_{f(\infty)}}{K_f} \quad (3)$$

Analysis of samples

The quantification of PAH-mixtures in soil extracts, fiber extracts and water extracts were performed with a standard solution containing 16 PAHs (Supelco, Bellefonte, CA, selected by the EPA) in acetonitrile. The system consisted of a Shimadzu DGU 14A Degasser (Den Bosch, The Netherlands), a Spark Marathon autosampler (Emmen, The Netherlands), a Gynkotek p580 HPG HPLC pump (Gemering, Germany) and a Jasco FP-920 fluorescence detector (Maarssen, The Netherlands). Separation was performed using a Supelcosil (Supelco, Bellefonte, CA) LC-PAH column (length 100 mm, ø 4.6 mm, particles 3 μm) that was operated at 26°C. All analyses were performed with a flow rate of 1000 μL/min and an injection volume of 20 μL. The compounds were separated using gradient elution starting with 40% H_2O for two minutes followed by an increase of the acetonitril-fraction to 100% in 9.5 minutes, where it was kept for another 7.5 minutes before returning to the initial solvent composition for 6 minutes. The excitation and emission wavelengths (nm) of naphthalene were 221/337, fluorene was analyzed at 227/315, phenanthrene, anthracene, fluoranthene and pyrene were analyzed at 255/405 or 240/405, benz[a]anthracene and chrysene were analyzed at 277/393 or 271/386, while benzo[b]fluoranthene, benzo[k]fluoranthene and benz[a]pyrene concentrations were determined at 260/420 and benzo[ghi]perylene and dibenz[ah]anthracene at 295/425. Chromatograms were analyzed using Chromcard version 1.21 (Milan, Italy), and corrected by hand if necessary.

Results and discussion

Partitioning to PDMS at different concentrations

The relation between the concentration in the PDMS coating of the fiber (C_f, µg/L) and the aqueous phase (C_{aq} µg/L) was fitted by a Freundlich isotherm (Equation 4).

$$C_f = K_f * C_{aq}^{\ n} \tag{4}$$

The K_f is the ratio between concentrations in the fiber coating at an aqueous concentration of 1 µg/L, and n is the parameter that determines the sorption linearity. The obtained n-values did not significantly differ from 1.0 ($n_{Freundlich}$ values varied from 0.98 to 1.07), so the sorption to the PDMS material is concentration-independent, and a single K_f could be calculated. The data and the fits are shown in Figure 2, and the obtained partition coefficients are listed in Table 2.

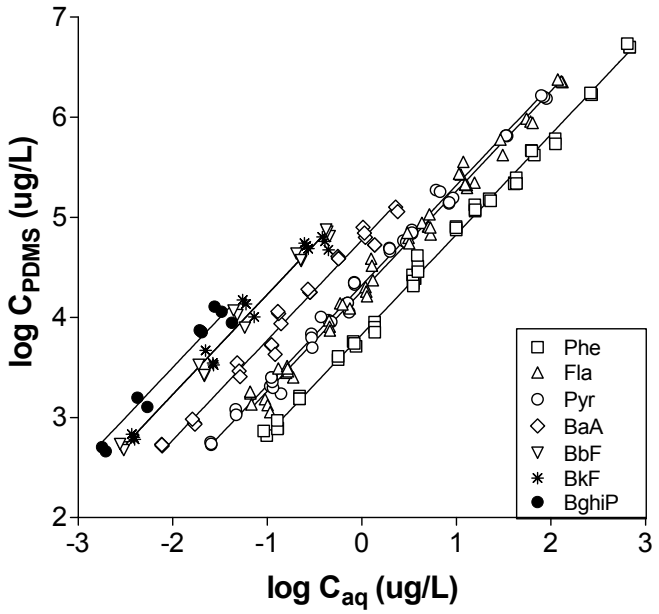

Figure 2: The concentrations in the fiber coating (log C_f) vs. the aqueous concentration (log C_{aq}). Aqueous concentrations are estimated using a 100% mass balance approach.

Table 2 also shows K_f values calculated from measured aqueous concentrations. These values are slightly higher (0.11 ± 0.04 log units) than the values obtained with the 100% mass balance approach, since recoveries varied from 87 to 95%. The corresponding fits of these data are shown in Appendix II, Figure A. Furthermore, it can be observed that obtained partition coefficients were very similar to literature values (*73, 137*) and a log K_{OW} based QSAR developed by Mayer et al. (*70*) (Figure 3).

Table 2: The PDMS-water partition coefficients (K_f) of a series of PAHs.

Comp.[a]	*log K_{OW} (ref)*	*Data from this study*		*Data from literature*			*log K_f Selected*[e]
		log K_f (SE, n) 100% MB[b]	*log K_f (SE, n) Measured C_{aq}*[c]	*log K_f Data from ref (73)*	*log K_f (SE) Data from ref (137)*	*log K_f (SD, n) Data from Jonker et al*[d]	
Naph	3.33 (*99*)			2.91			2.91
Flu	4.18 (*104*)			3.72			3.72
Phe	4.56 (*104*)	3.83 (0.01, 50)	3.88 (0.01, 13)	3.98	3.86 (0.03)	3.84 (0.04, 4)	3.83
Anth	4.63 (*139*)			4.17		3.84 (0.04, 4)	3.84
Fla	5.16 (*104*)	4.26 (0.01, 49)	4.35 (0.02, 13)	4.52	4.40 (0.02)	4.20 (0.03, 4)	4.26
Pyr	5.22 (*139*)	4.32 (0.01, 44)	4.41 (0.01, 13)	4.63	4.41 (0.04)	4.27 (0.02, 4)	4.32
BaA	5.91 (*99*)	4.77 (0.02, 28)	4.92 (0.01, 13)		4.92 (0.03)	4.77 (0.03, 4)	4.77
Chr	5.81 (*99*)			5.19		4.69 (0.03, 4)	4.69
BbF	6.20 (*140*)	5.23 (0.02, 15)	5.36 (0.02, 12)		5.28 (0.04)	5.21 (0.04, 4)	5.23
BkF	6.20 (*140*)	5.23 (0.03, 15)	5.37 (0.03, 13)		5.29 (0.04)	5.25 (0.04, 4)	5.23
BaP	6.13 (*99*)					5.24 (0.04, 4)	5.24
BghiP	6.85 (*139*)	5.50 (0.04, 10)	-[f]		5.39 (0.03)	5.08 (0.04, 4)	5.50
DahA	6.20 (*140*)					4.83 (0.04, 4)	4.83

[a] Abreviations of compounds: naphtalene (Naph), fluorene (Flu), phenanthrene (Phe), anthracene (Anth), fluoranthene (Fla), pyrene (Pyr), benz[a]anthracene (BaA), chrysene (Chr), benzo[b]fluoranthene (BbF), benzo[k]fluoran-thene (BkF), benz[a]pyrene (BaP), benzo[ghi]perylene (BghiP), dibenz[ah]anthracene (DahA).

[b] Partition coefficients were calculated assuming a 100% mass balance (Figure 2).

[c] Partition coefficients were calculated assuming with measured aqueous concentrations (Figure A, Appendix II).

[d] Unpublished results of M. T. O. Jonker and S. A. van der Heijden.

[e] These selected K_f values have been used in all further calculations.

[f] Aqueous concentrations were not quantifiable; therefore partition coefficients could not be calculated.

The constant K_f over a broad range of concentrations (4 orders of magnitude for phenanthrene), up to solubility in the aqueous phase and up to very high concentrations in the PDMS phase (ΣPAH >10000 mg/L), gives strong evidence that the sorption to the

30 μm PDMS of the disposable fibers is a partitioning process. Similar conclusion were also drawn by Mayer et al. (*70*), Poerschmann et al. (*73*) and Vaes et al. (*71*).

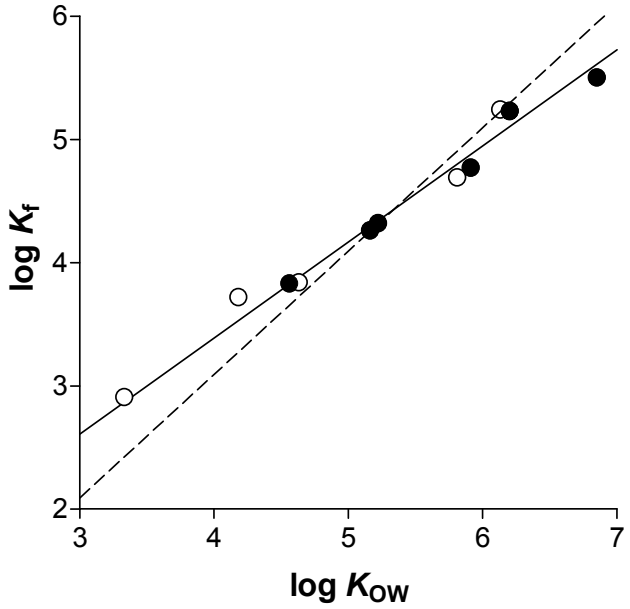

Figure 3: The PDMS partition coefficient (log K_f) plotted against the log K_{OW}. The solid symbols are the partition coefficients obtained in this study and the open symbols are selected partition coefficients from literature (see Table 2). The solid line represents the fit of the data obtained in this study (log K_f = 0.78 * log K_{OW} + 0.56) and the broken line is a log K_f – log K_{OW} relationship from Mayer et al. (*70*) obtained for a series of hydrophobic compounds (log K_f = log K_{OW} - 0.91).

Uptake kinetics of the fiber in a soil suspension: Askov and TP44 soil

The kinetics of the fiber exposure in soil was studied, since the concentration in the fiber can only be used to estimate the freely dissolved concentration in the pore water correctly when the soil, pore water and fiber are at equilibrium (*27, 30*). Figure 4 shows the uptake profile of the fibers exposed to Askov soil spiked with seven PAHs, at 10-30 mg/kg per compound, and the field-contaminated TP44 soil. It can be observed that concentrations in the fibers reached a steady state within the first three days for the Askov soil (aged for 21 (4a) and 553 (4b) days) and the TP44 soil (4c). A one-compartment model was fitted through the data (Equation 2), and equilibrium concentrations in the fiber ($C_{f(\infty)}$) and elimination rate constants (k_e) were determined. Table 3 lists the calculated elimination rate constants of the experiments with these soils. It can be observed that the rate constants measured in the TP44 soil and the freshly spiked and aged Askov soil are similar.

Table 3: Organic carbon normalized sorption coefficients (K_{OC}) and elimination rate constants (k_e) of spiked (aged) Askov soil and field-contaminated TP44 and Skaegen soil.

Compound	*Spiked Askov soil*				*TP44 soil*		*Skaegen soil*		
	aged for 21 days		*aged for 553 days*						
	log K_{OC} (SE, n)	*log $k_{e\text{-}fiber}$ (SE, n)*	*log K_{OC} (SE, n)*	*log $k_{e\text{-}fiber}$ (SE, n)*	*log K_{OC} (SE, n)*	*log $k_{e\text{-}fiber}$ (SE, n)*	*log K_{OC} (SE, n)*	*log $k_{e\text{-}fiber}$ (SE, n)*	*log $k_{e\text{-}slow}$ (SE, n)*
Phe	4.31 (0.05, 43)	0.170 (0.032, 59)	-[a]	-[a]	5,20 (0.01, 42)	0.140 (0.031, 42)	5.14 (0.11, 17)	0.046 (0.070, 59)	-2.677 (0.045, 59)
Anth					5,23 (0.01, 39)	0.074 (0.022, 39)	5.31 (0.12, 17)	-0.070 (0.057, 59)	-2.765 (0.044, 59)
Fla	4.89 (0.05, 43)	-0.099 (0.016, 59)	-[a]	-[a]	6,14 (0.01, 42)	-0.198 (0.011, 42)	6.05 (0.12, 17)	-0.313 (0.029, 55)	-2.907 (0.050, 55)
Pyr	5.03 (0.05, 42)	-0.128 (0.016, 59)	-[a]	-[a]	5,94 (0.01, 42)	-0.244 (0.010, 42)	6.14 (0.12, 17)	-0.264 (0.036, 59)	-2.993 (0.042, 59)
BaA	5.73 (0.05, 35)	-0.450 (0.005, 56)	5,94 (0.03, 43)	-0.521 (0.044, 45)	6,81 (0.01, 39)	-0.690 (0.005, 39)	6.91 (0.11, 17)	-0.437 (0.029, 59)	-3.050 (0.061, 59)
Chr					6,71 (0.01, 39)	-0.725 (0.004, 39)	6.47 (0.23, 17)	-0.209 (0.176, 59)	-3.314 (0.041, 59)
BbF	6.43 (0.05, 31)	-0.774 (0.003, 59)	6,70 (0.02, 43)	-0.836 (0.014, 45)	7,52 (0.01, 39)	-0.941 (0.002, 39)	7.71 (0.14., 17)	-0.375 (0.096, 59)	-3.096 (0.123, 59)
BkF	6.39 (0.05, 31)	-0.817 (0.003, 59)	6,59 (0.01, 43)	-0.889 (0.007, 47)	7,55 (0.01, 39)	-1.014 (0.002, 39)	7.67 (0.13, 17)	-0.604 (0.015, 59)	-3.033 (0.111, 59)
BaP					7,59 (0.01, 39)	-0.981 (0.002, 39)	7.85 (0.17, 17)	-0.172 (0.093, 59)	-3.283 (0.140, 59)
BghiP	6.94 (0.05, 27)	-1.099 (0.002, 59)	7,22 (0.02, 43)	-1.180 (0.004, 47)	8,08 (0.01, 36)	-1.248 (0.001, 36)	8.15 (0.35, 17)	-0.632 (0.030, 52)	-3.648 (0.440, 52)
DahA					7.33 (0.01, 33)	-1.365 (0.002, 33)	7.41 (0.47, 17)	-[a]	-[a]

[a] Quantification of compound of insufficient quality for determination of kinetic parameters.

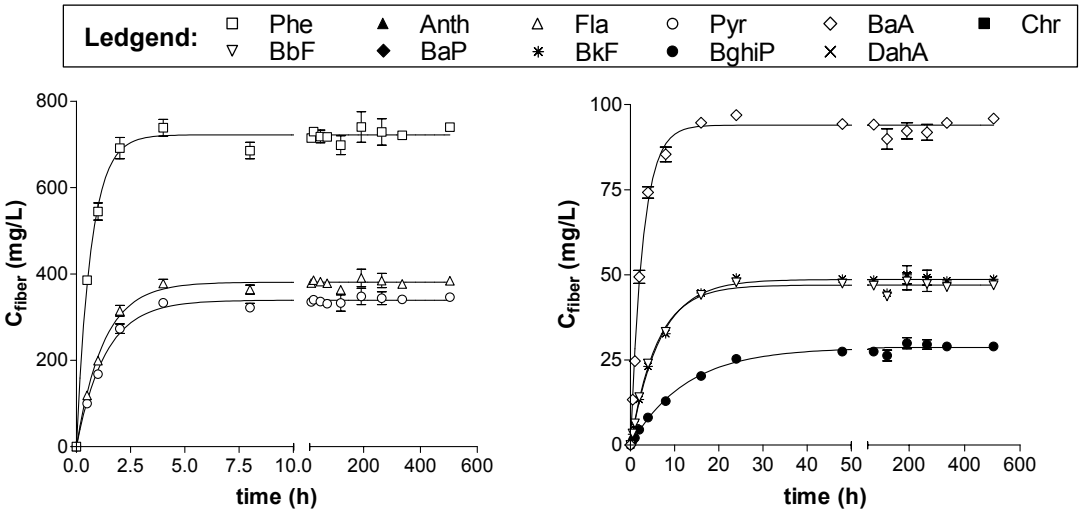

Figure 4a: Uptake profiles of fibers exposed to spiked Askov soil after 21 days. The lines in represent the fits of Equation 2.

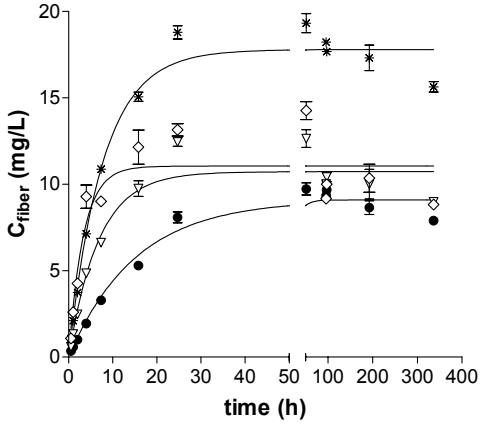

Figure 4b: Uptake profiles of fibers exposed to spiked Askov soil after 553 days of aging. The lines represent the fits of Equation 2.

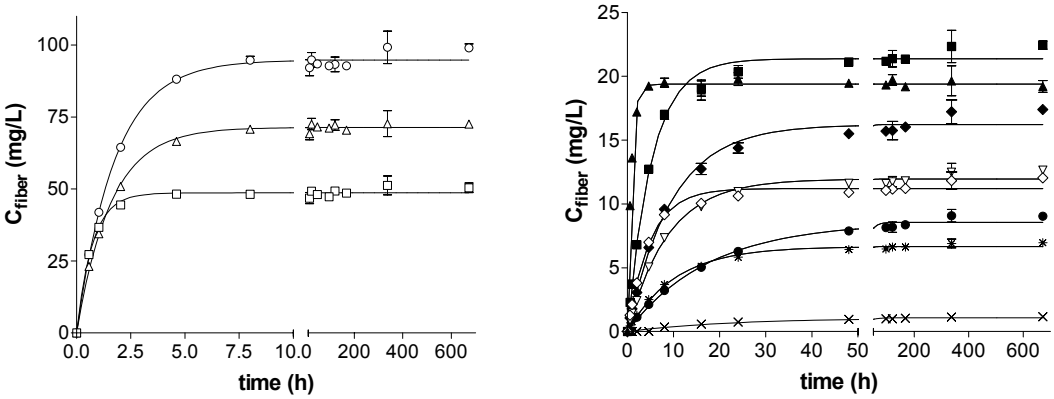

Figure 4c: Uptake profiles of fibers exposed to field-contaminated TP44 soil. The lines represent the fits of Equation 2.

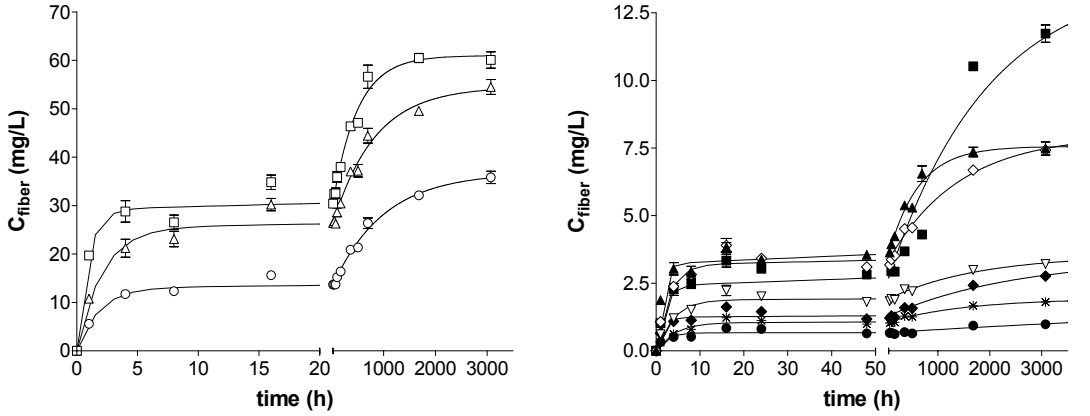

Figure 4d: Uptake profiles of fibers exposed to Skaegen soil. The lines in represent the fits of Equation 6.

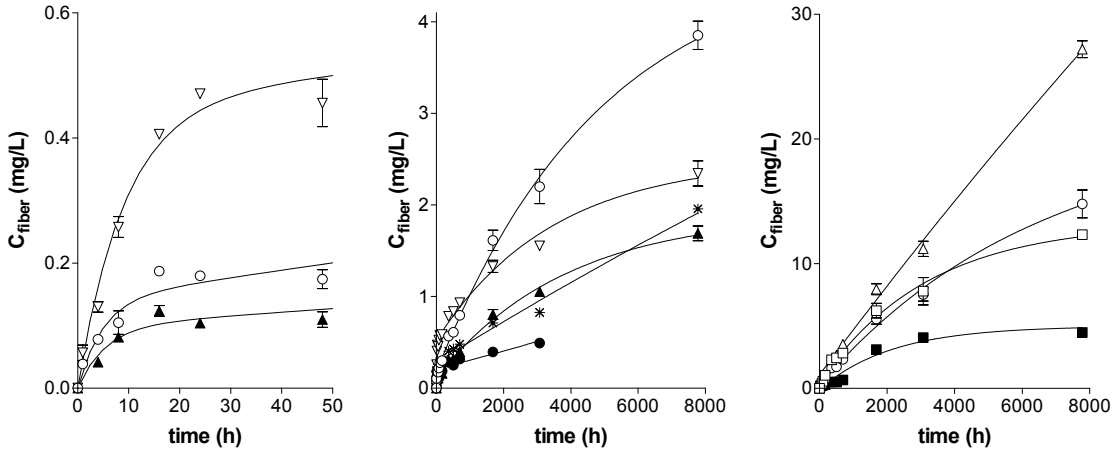

Figure 4e: Uptake profiles of fibers exposed to untreated Skaegen soil. The lines represent the fits of Equation 6.

The interpretation of this rate constant is not unambiguous because several kinetic processes occur in the exchange between soil, soil pore water and the fiber (see Figure 5 for a schematic overview). We strongly believe that, at least in these two soils, the fiber-water exchange is the rate-limiting step. Arguments for the validity of this assumption are, that the experimental data follow the one compartment model precisely (see Figure 4a, 4b & 4c), and that earlier studies, including modeling exercises, have shown that the flux from soil to water is usually too high to be rate limiting (*30, 108, 141*). If we assume that the fiber-water exchange is the rate-limiting step, Equation 2 can be rewritten as:

$$C_{f(t)} = \frac{k_{u-fiber}}{k_{e-fiber}} * C_{aq} * (1 - e^{-k_{e-fiber}*t}) \qquad (5)$$

In this equation, $k_{u\text{-fiber}}$ is the uptake rate constant from pore water to fiber, and $k_{e\text{-fiber}}$ is the elimination rate constant from the fiber to water. The ratio of the uptake rate constant and the elimination rate constant (k_u/k_e) is the fiber-water partition coefficient (K_f). A plot of the uptake and elimination rate constant versus the fiber partition coefficient will supply information about the rate-limiting step in the exchange process between fiber and water (*34, 142-145*). The $k_{u\text{-fiber}}$ will be constant if the diffusion in the aqueous phase around the fiber, also known as the unstirred boundary layer (UBL), is rate limiting. If the diffusion in the PDMS coating is rate limiting, the $k_{u\text{-fiber}}$ will increase linearly with increasing fiber water partition coefficients.

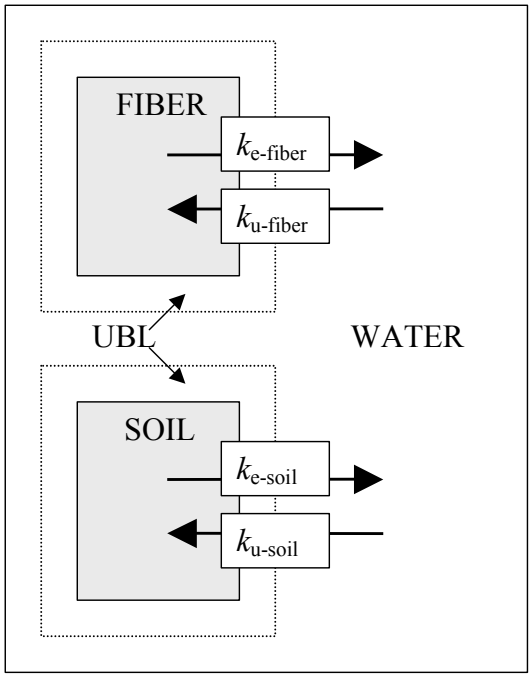

Figure 5: A schematic picture of the soil water fiber system, with uptake (k_u) and elimination (k_e) rate constants of the PDMS coated fiber and the sorbent in the soil. UBL stands for the unstirred boundary layer, a stagnant layer of water that surrounds the fiber or soil particle.

The shift from the rate limiting step from PDMS to the aqueous diffusion layer is often found at a K_f of 10^3 to 10^4 (*34, 143, 144*). However, this break point also depends on the agitation of the system and the surface to volume ratio of the fiber. Strong agitation can move the break point to a K_f above 10^5 (*48*). A plot of the uptake ($k_{u\text{-fiber}}$) and elimination ($k_{e\text{-fiber}}$) rate constant, versus the K_f is given in Figure 6a. The $k_{u\text{-fiber}}$ (h^{-1}) is constant at a level of $10^{4.3}$ at a $K_f > 10^{4.5}$ for the Askov and TP44 soil. This suggests that the diffusion in the UBL is rate limiting above this K_f. The reduction of the $k_{u\text{-fiber}}$ at a lower K_f can be attributed to a shift in the rate limiting process from diffusion in the UBL towards the diffusion in the PDMS coating.

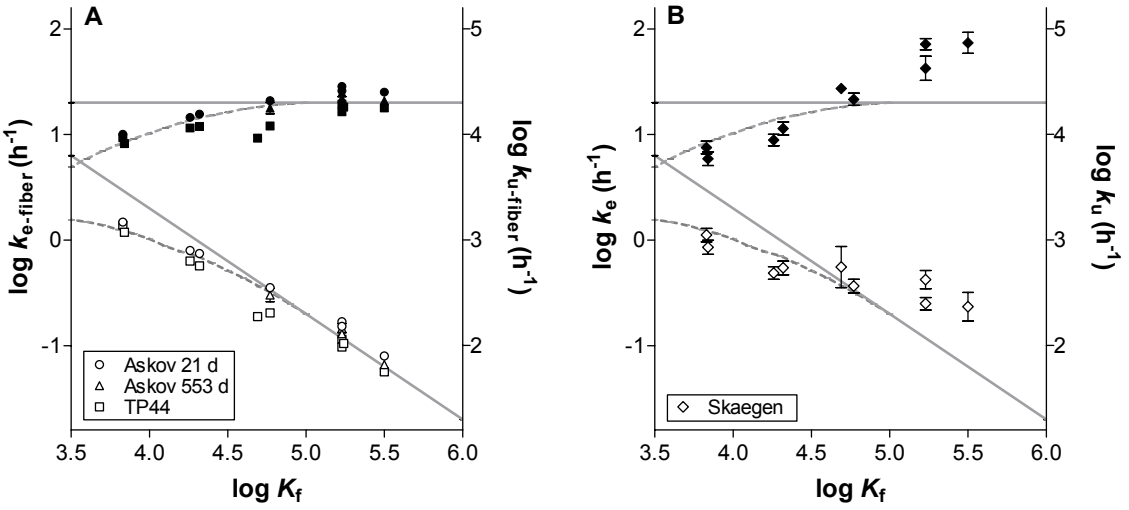

Figure 6: The elimination ($k_{e\text{-fiber}}$, open symbols, left Y-axes) and uptake ($k_{u\text{-fiber}}$, solid symbols, right Y-axes) and rate constants plotted against the fiber-PDMS partition coefficient (K_f). Figure 6a shows the data for the spiked Askov soil incubated for 21 and 553 days and the field-contaminated TP44 soil. Figure 6b shows the data for the field-contaminated Skaegen soil. Error bars represent standard errors.

The small but systematic variation in the $k_{u\text{-fiber}}$ (and $k_{e\text{-fiber}}$) observed between the Askov soil and TP44 soil (~0.14 ± 0.07 log units) can be due to different physical properties of the soil slurry (e. g. viscosity, dissolved organic matter (DOM) concentration). Differences in viscosity can affect the mixing of the soil slurry and the movement of the fiber in the soil slurry, thereby influencing the thickness of the UBL. This is difficult to quantify and therefore an excess of water (far beyond the water holding capacity) was added to create more standardized conditions. The presence of DOM in the aqueous diffusion layer may also affect the kinetics (*79, 96*). This phenomenon will become more important at increasing hydrophobicity. Figure 6a shows no clear increase of the $k_{u\text{-fiber}}$ above a K_f of $10^{4.5}$, therefore the contribution of DOM on the kinetics of the fiber is considered insignificant. This observation is in line with literature, where only very high DOM concentrations (>100 mg/L) affected the kinetics significantly (*79, 146*).

Uptake kinetics of the fiber in a soil suspension: Skaegen soil

The fiber uptake kinetics was also studied in Skaegen soil. The uptake profile of the fibers exposed to the Skaegen soil was different from the fibers exposed to the spiked Askov and field contaminated TP44 soil. A pseudo-equilibrium was reached within the first days of exposure, after which the concentration slowly increased over a period of weeks to months (Figure 4d). A two-phase uptake model distinguishes a "fast equilibrating fraction" (fef) and a "slow equilibrating fraction" (1-fef) of the final equilibrium level in the fiber. The kinetics of the fast equilibrating fraction was very similar to those in the spiked Askov soil and field contaminated TP44 soil. We therefore assume that the initial uptake represents the kinetics of the fiber-water exchange, described by the fiber elimination rate constant ($k_{e\text{-fiber}}$). The orders of magnitude slower

kinetics of the second, slow equilibrating fraction is described by a second elimination rate constant ($k_{e\text{-}slow}$) and might be related to slow desorption from the soil.

$$C_{f(t)} = C_{f(\infty)} * fef * (1 - e^{-k_{e-fiber} * t}) + C_{f(\infty)} * (1 - fef) * (1 - e^{-k_{e-slow} * t}) \quad (6)$$

Equation 6 was used to fit the concentrations in the fiber exposed to the Skaegen soil. The $k_{e\text{-}fiber}$ values are summarized in Table 3. Figure 6b shows both the $k_{e\text{-}fiber}$ values and the $k_{u\text{-}fiber}$ values (calculated by replacing $C_{f(\infty)}$ by C_{aq} * $k_{u\text{-}fiber}$ / $k_{e\text{-}fiber}$, see Equation 6). It can be observed that the $k_{e\text{-}fiber}$ values of the less hydrophobic PAHs (K_f <$10^{4.5}$) are comparable to the $k_{e\text{-}fiber}$ values of the other soils. Therefore, also the pseudo-equilibrium is limited by diffusion in the aqueous layer around the fiber. Both the $k_{e\text{-}fiber}$ and $k_{u\text{-}fiber}$ values of the more hydrophobic PAHs (K_f >$10^{4.5}$) are higher than the $k_{e\text{-}fiber}$ values of the other soils, resulting in a (up to 4 times) shorter equilibration time of the fiber. This is probably a result of the (temporary) depletion of the 2 mL soil pore water, since depleting the aqueous phase during uptake, leads to a reduction in equilibration times (*141*).
The slow uptake kinetics of the fibers exposed to the Skaegen soil is not typical for field soils. The other field soil (TP44) behaves similar to spiked soils, in the sense that the fiber-water exchange is the rate-limiting step for fiber-uptake in soil suspensions. The "abnormal" behavior in the Skaegen soil is likely due to the type of sorbent. The PAHs in this soil are mainly incorporated or captured in tar particles. The PAHs will diffuse very slowly out of this matrix (*147*) and a new equilibrium with the depleted fast desorbing fraction, the pore water and the fiber will be established very slowly. The observed equilibration times of several months are in line with slow diffusion / desorption rates found for tar, soot and coal matrices or soils and sediments contaminated with these materials (*128, 147, 148*).

Effects of soil pretreatment on uptake kinetics of exposed fibers in the Skaegen soil
The equilibrium kinetics of the fibers exposed to the Skaegen soil seemed to depend on the desorption rate of the soil. Grounding and sieving a soil breaks up organic matrices, enlarges their surface-volume ratio and affect the desorption rate of the contaminants from these matrices. In Figure 4d, the fibers were exposed to gently treated (grounded and sieved) Skaegen soil. Figure 4e shows the uptake profile of the fiber exposed to Skaegen soil that was untreated. It can be observed that the fast equilibrating fraction in this untreated soil was very small (BaA, BbF and BaP) or not even quantifiable (other compounds), and that steady state was not even fully reached after 324 days (7778 hours). Because the system was still far from equilibrium, steady state concentrations in the fibers could not be estimated accurately for the untreated Skaegen soil.
The observed effect of grounding and sieving clearly shows that pretreatment of soils may speed up the uptake into the SPME fiber. Similar effects might be observed for

uptake in organisms, and processes such as (bio)degradation and leaching might be affected. Therefore, pretreatment of soils in testing for risk assessment should be considered with great care.

Sorption coefficients of spiked aged soils

Kinetic studies have shown that a 2 to 3 d equilibration period is sufficient to equilibrate the soil-water-fiber system in those cases where desorption from the soil is not the rate limiting step. Therefore, a standard exposure time of 72 h was selected. Concentrations in the pore water were calculated from concentrations in the fiber after 72 h exposure ($C_{f(72)}$), and fiber-water partition coefficients (K_f) reported in Table 2. Organic carbon normalized soil sorption coefficients (K_{OC}) were calculated from measured pore water concentrations and measured total organic carbon normalized concentrations in the soil (C_{OC}).

$$K_{OC} = \frac{C_{OC}}{\frac{C_{f(72)}}{K_f}} \tag{7}$$

The soil concentrations were determined from samples that were taken at the start of the fiber exposure and are reported in Table C of Appendix II. Even though the soils were sterilized using 10 mM sodium azide, concentrations in soil decreased during the 553-day storage period. The concentrations of most of the PAHs in the soil remained constant, only the low molecular decreased significantly during storage. However, sorption coefficients could still be calculated at the different aging periods, because concentrations in soil were measured. Another, more critical, assumption is the stability of the test compound during the fiber exposure. In an additional study it was observed that the total concentration of the soils aliquots used to expose the fibers did not show a significant decrease during 504 hours of fiber exposure (95% to 99% was recovered after 504 h fiber exposure compared to 0.5 - 2 h fiber exposure (see Figure B of Appendix II).
Figure 7 shows the sorption coefficients of seven PAHs that were spiked to five clean field soils after different aging periods. Soil sorption coefficients were determined after 21, 240 and 553 days aging for the Askov soil and 18, 77 and 171 days for Borris-2, Kettering, Waschbach, and Norway soil, and plotted against the octanol water partition coefficient of the compounds. It can be observed that the sorption coefficients of the smaller PAHs are comparable to the QSAR-prediction of Karickhoff (*35*), and the sorption of the larger PAHs is slightly higher than predicted. The small deviation is most likely an effect of the overestimation of free pore water concentrations (due to binding of compounds to dissolved organic carbon), in the data Karickhoff used to develop this QSAR (*66, 149*). One of the objectives of our study was to analyze effects of aging on freely dissolved concentrations and sorption coefficients. The sorption coefficients only

slightly increase during the 177 or even 553 days aging periods (less than a factor 3). The small increase in sorption coefficients might be explained by slow absorption into so-called "hard" organic polymeric matrices or slow diffusion into micro-pores (*7*). The (relative) fraction that is strongly sorbed might be enlarged by degradation of more accessible fractions, resulting in a higher (apparent) sorption coefficient. This process is often thought to be responsible for the increased sorption coefficients and incomplete removal of contaminants from remediated contaminated sites (*150*).

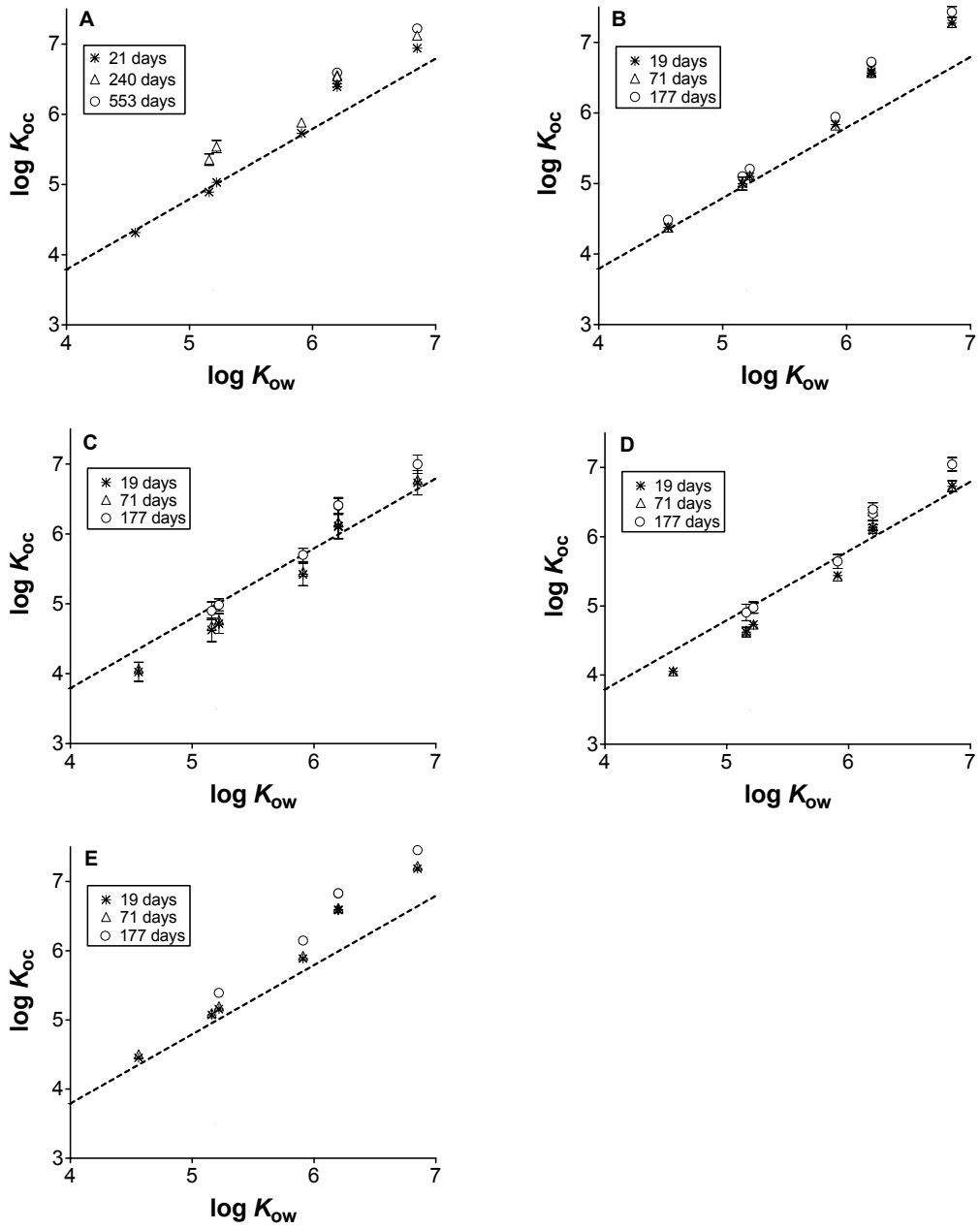

Figure 7: Organic carbon normalized sorption coefficients (log K_{OC}) plotted against the octanol water partition coefficient (log K_{OW}) for spiked Askov (a) soil aged for 21, 240 and 553 days, and the Borris-2 (b), Kettering (7c), Waschbach (d) and Norway (e) soil, aged for 19, 77 and 177 days. The sorption coefficients are only shown when less than 50% of the spiked concentration was recovered. The broken line represents: log K_{OC} = log K_{OW} - 0.21 of Karickhoff (*35*).

Concluding, the contact time of the selected compounds with the selected clean field soils have only a small effect on the sorption coefficient. A factor 3 reduction in freely dissolved concentration in pore water is not really high in light of the risk assessment process. These observations are in line with literature findings. Sorption coefficients of hydrophobic organic chemicals spiked to soils did not increase severely in time (*151, 152*), and bioavailability and extractability by mild solvents (*125, 126*) or Tenax (*153*), decreased only slightly (*153*).

Sorption coefficients of field-contaminated soils

Sorption coefficients were also determined for a series of field-contaminated soils. The chosen equilibration time of the fiber exposure to determine the aqueous concentration was 72-74 hours for all soils. This exposure is sufficient for laboratory-spiked soils and probably also for most of the field-contaminated soils. Only for the Skaegen soil, contaminated with tar, the exposure time is too short to reach steady state, leading to an overestimation of the sorption coefficient. Figure 8a shows the sorption coefficients of 5 field contaminated industrial soils. It can be observed that sorption coefficients of the field contaminated soils are variable, and sorption coefficients can be up to two orders of magnitude higher than what is expected from the log K_{OW} - log K_{OC} relationship of Karickhoff (*35*). In Figure 8b sorption coefficients of the Skaegen soil are plotted against the log K_{OW} of the compounds. The sorption coefficients were calculated from fibers exposed to untreated and treated Skaegen soil for 72 hours as well as by estimation of the equilibrium concentration calculated from uptake profiles (Figure 4d) of the treated soil. The sorption coefficients calculated from the 72 h exposure are generally 0.3 log units (a factor 2) higher than sorption coefficients calculated at equilibrium. The overestimation of sorption coefficients estimated from the fiber exposed to the untreated soil for 72 h is much more severe (one to two orders of magnitude).

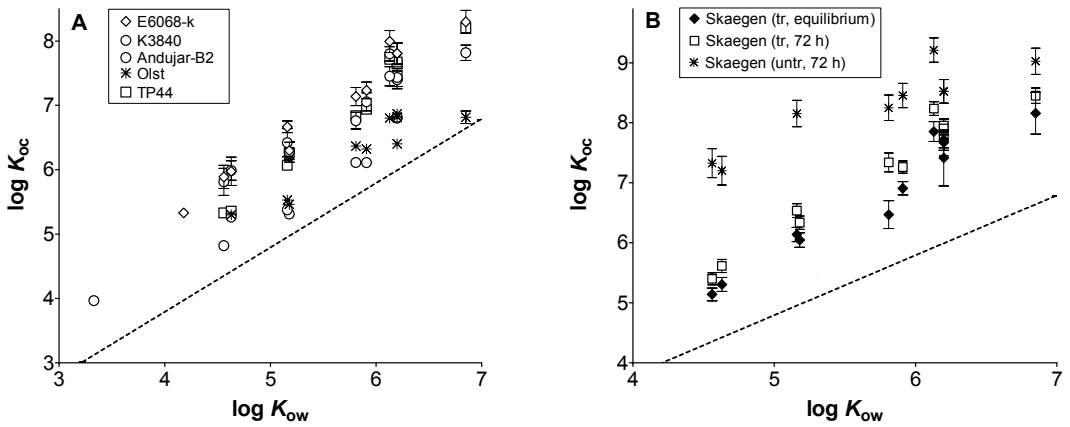

Figure 8: Organic carbon normalized sorption coefficients with their standard deviations (log K_{OC}) are plotted against the octanol water partition coefficient (log K_{OW}) for 6 field-contaminated soils. Figure 8a shows the sorption coefficients of five soils and Figure 8b the calculated sorption coefficients of treated and untreated Skaegen soil after 72 h and treated skaegen soil at equilibrium. The broken line represents a QSAR (log K_{OC} = log K_{OW} - 0.21) of Karickhoff (*35*).

Evaluation of the negligible depletion-SPME technique to measure freely dissolved concentration and sorption coefficients

Requirements for accurate measurements of freely dissolved pore water concentrations (and soil sorption coefficients) via a passive sampler such as the SPME fiber are: (i) the sampler is in equilibrium with the soil-water system, (ii) partition coefficients to the fibers are known, and (iii) the sampler may not affect the concentration in soil. The exposure time of the passive sampler is therefore crucial. Because elimination rate constants for the fiber-water exchange are related to the partition coefficient of the fiber (*30*), equilibration times can be predicted relatively easily. Adjusting the surface - volume ratio or the material (partition coefficient) of the passive sampler can change equilibration times to practical periods.

If, however, desorption of the soil becomes rate limiting, because compounds are sequestered in condensed organic matrices, the equilibration times become longer, and are more difficult to predict. Grinding and sieving a soil might increase desorption kinetics, by enlarging surface-volume ratios of the sorbing materials in the soil. However, it is the question whether or not these changes in the properties of the soil still will lead to realistic numbers from a risk assessment perspective. Another option is to increase the soil – passive sampler ratio, thereby increasing the desorption capacity of the soil compared to the amount sampled by the passive sampler. Furthermore, the larger this ratio the smaller the relative amount sampled by the passive sampler, so the smaller the effect of potential desorption nonlinearity of the test soil, and the closer the sorption coefficient is to the sorption coefficient in the field situation (*154*). In order to check whether desorption from soil is rate limiting, the uptake kinetics of a passive sampler should always be monitored in new samples by measuring concentrations at a (small) series of exposure times.

With the suggested adaptations, the pore water concentration and sorption coefficient can be determined. This pore water concentration might however be different from the field situation, since free concentrations in the field can also be affected by biological (and chemical) degradation and losses due to evaporation or leaching (*155-159*). If these processes are faster than desorption from the soil, free pore water concentrations will continuously be at a steady state below the chemical equilibrium as determined under sterile, controlled conditions in the laboratory. Especially soils with very slow desorption kinetics are prone to be affected by these processes. In vivo measurements of pore water concentrations using passive samplers might therefore be considered estimates of 'potential' pore water concentrations in the field. The pore water concentrations in the field might also be assessed by in situ exposure of passive samplers (*160*). This is however more difficult, especially in a soil, since exposure concentrations might vary in time, samplers are not agitated (leading to longer equilibration times, and possibly depletion of the local environment), and environmental conditions (temperature, the amount and chemical composition of the water in the soil) cannot be controlled.

Free concentrations and site-specific sorption coefficients in risk assessment

It is clear that simple generic modeling of sorption is not sufficient to estimate the risks of hydrophobic contaminants like PAHs in soil. In actual field samples, pore water concentrations can be much lower and soil sorption coefficients can be much higher than predicted by K_{OW}-based QSAR models. Therefore, site-specific risk assessment should be applied. The tool presented in this study can contribute to site-specific risk assessment by determining site-specific (potential) pore water concentrations and sorption coefficients. The proposed tool is relatively simple and cheap, and might therefore be implemented as a screening tool. If the results are pivotal, one can decide to expand the research to bioassays, and other tests.

It is beyond doubt that the presented technique is only valid for hydrophobic organic contaminants. The negligible depletion passive sampling approach needs development and testing before it can be applied for a wider range of organic (polar and ionizable) and possibly also inorganic compounds in soils (*33*).

Aknowledgements

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Chapter 5

Freely dissolved concentrations of PAHs in soil pore water: measurements via solid phase extraction and consequences for soil tests

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Submitted

Abstract

Freely dissolved pore water concentrations are difficult to assess in complex matrices like soils or sediments. In this study a negligible-depletion partitioning based sampling technique was applied to measure freely dissolved pore water concentrations. A PDMS (poly(dimethylsiloxane)) coated glass fiber was exposed to a slurry of a soil spiked with several PAHs at concentrations ranging from 2 to 2000 mg/kg. Concentrations in the PDMS coating increased linear with the total soil concentration until a certain maximum was reached. Freely dissolved pore water concentrations were calculated using PDMS-water partition coefficients, and the observed maximum pore water concentrations were very similar to aqueous solubility of the tested PAHs. Estimated detection limits of pore water concentrations of the PAHs are very low and range from 0.2 to 10 ng/L. The sampling technique can measure pore water concentrations over a broad range of soil concentrations. Freely dissolved pore water concentrations are an important dose parameter for the exposure of organisms in soil. Therefore, increasing soil concentrations above a certain level, where aqueous solubility is reached in the pore water, might be irrelevant, and must at least be noticed.
Sorption coefficients that were calculated from the freely dissolved concentrations were slightly higher that estimates based on the octanol water partition coefficient. These differences are discussed in relation to the effects of dissolved organic matter in soil pore water on the determination of sorption coefficients.

Introduction

The freely dissolved concentration in soil pore water is an environmentally relevant parameter for various processes in soil, including for example evaporation to air, biological and chemical degradation and accumulation in soil-biota (*3, 47, 161*). These freely dissolved concentrations, however, are difficult to determine, especially when the compounds are very hydrophobic and sorb strongly to the soil and the dissolved organic matter in the pore water. Information about freely dissolved concentrations is also relevant for the accurate measurement of sorption coefficients, as the presence of dissolved organic matter (DOM) may affect the measured values (*14, 108*). Various techniques have been developed to determine (freely dissolved) pore water concentration in soils and sediments. The simplest method is to separate the solid and liquid phase by centrifugation, and subsequently extract the liquid phase with a solvent and measure the concentration. Centrifugation, however, does not remove dissolved organic matter (DOM) from the pore water. Since DOM has a high affinity for hydrophobic chemicals, free pore water concentrations can also be overestimated by this method (*66*). The freely dissolved pore water concentration can be estimated from the pore water extract by correcting for the DOM-associated compounds (*15, 108, 162-164*). In that case,

information is needed for the DOM concentration in the pore water and the sorption coefficient to the specific DOM material. Another option is to remove the DOM by flocculation (*16*). Flocculation might, however, disturb the equilibrium of the compounds between the DOM and the aqueous phase, thereby biasing the measurement of the free concentration. The DOM and water can also be separated passively by a dialysis membrane (*17*), since freely dissolved compounds will diffuse through this membrane while it is impermeable for the soil matrix and DOM. However, large amounts of water inside the dialysis membrane are necessary to detect and quantify very hydrophobic chemicals, and the technique is rather laborious. Additionally, various passive sampling techniques based on gas purging (*23, 24*) semi-permeable membrane devices (SPMD (*20, 22, 26*)), poly(oximethylene) sheets (POM (*28*)), polymer coated glass surfaces (*29*) or solid phase microextraction (SPME) fibers (*27, 30*) have been applied to assess pore water concentrations in complex matrices like soil or sediment. SPME has been developed by Pawliszyn and co-workers, as a very useful sampling technique (*21, 25*). These samplers only sense the freely dissolved concentration, and if they are equilibrated with the matrix without depleting the system, their equilibrium concentration can directly be used to calculate the freely dissolved concentration (*27, 33, 34*). The dimensions, properties, and agitation of the exposure vessel and the size of the passive sampler can be adjusted to sample detectable amounts of a test compound in a practical time span.

The objective of this study was to measure soil pore water concentrations of a series of PAHs at increasing concentrations in soil. We were interested in the trend of pore water concentration at high concentrations in soil as these are used for example in soil toxicity testing. Sorption coefficients were calculated as well and the linearity of the sorption process was analyzed. Moreover, potential effects of the presence of dissolved organic matter (DOM) in soil pore water on the measurements of sorption coefficients are discussed.

Negligible depletion SPME was applied to measure these pore water concentrations and another objective was to determine the detection limits of this technique. Disposable glass fibers with a 28.5 μm poly(dimethylsiloxane) (PDMS) coating were exposed to a soil, separately spiked with five PAHs, at a wide range of concentrations. The PDMS coated fibers were equilibrated with the soil slurry, and free pore water concentrations were calculated from PDMS-water partition coefficients. The results are also discussed from the perspective of soil (toxicity) testing.

Experimental Section

Soil, chemicals, fibers and solvents

Clean sandy agricultural soil (Borris-2) was collected in Denmark in 2001. The soil was stored at 4°C. Before use, the soil was dried to constant weight (at 25 ± 1°C), gently homogenized with a mortar, sieved (1 mm mesh size) and stored at room temperature.

Table 1 shows some properties of the test soil. Phenanthrene (Phe), pyrene (Pyr), benz[a]anhracene (BaA) benzo[b]fluoranthene (BbF) and benzo[ghi]perylene (BghiP), used for spiking the soils and making standard series, were all purchased at Sigma Aldrich Chemie BV (Zwijndrecht, The Netherlands). Glass fibers with a core diameter of 110 μm and a 28.5 μm poly(dimethylsiloxane) (PDMS) coating (volume 12.4 μl/m) were obtained from Poly Micro Industries (Phoenix, AZ, USA). Acetonitril, ethylacetate and acetone (Lab-Scan, Dublin, Ireland) used were of analytical grade, and highly pure water (R ≥18 MΩ) was prepared by a Millipore purification system equipped with organic free kit (Millipore waters, Amsterdam, The Netherlands).

Table 1: Soil properties of the Borris-2 soil (obtained from ref (*135*)).

pH	6.7
Sand (50-2000 μm, g/100g)	69.8
Silt (2-63 μm, g/100g)	20.5
Clay (<2 μm, g/100g)	6.9
Total organic carbon (g/100g)	1.67
Total organic matter (g/100g)	2.80
Dissolved organic carbon in pore water (mg/L)	20.9

Spiking Borris-2 soil

10 gram aliquots of air dried soil were put in 50 mL erlenmeyers and spiked with 2, 5, 10, 20, 50, 100, 200, 500 and 1000 mg/kg soil of Phe, Pyr, BaA, BbF and BghiP, separately. Additionally, Phe was spiked at 2000 mg/kg, and Pyr and BbF at 1500 mg/kg soil. All concentrations were spiked with 1 mL of acetone except for BghiP, where 2, 5 and 10 mL of acetone were necessary to dissolve and spike 200, 500 and 1000 mg/kg, respectively. The soil aliquots with acetone-spike were closed for 1 hour to let the acetone disperse, and reopened to let the acetone evaporate overnight at room temperature under a gentle stream of N_2. Subsequently, 1.25 mL water (~60% of the water holding capacity), with 10 mM sodium azide (NaN_3, Merck, Amsterdam, The Netherlands) to inhibit bacterial degradation, was added to the soil, and the soil was incubated for 28 days at 4°C.

Measurement of partition coefficients to PDMS

Fiber water partitioning were determined with a relatively new method that is based on the depletion of pre-loaded fibers with selected amounts of water (*137*). The advantage of this method is that it avoids difficulties that are often encountered when spiking aqueous solutions with hydrophobic chemicals. The PDMS coated fibers were cut into 5.0 or 3.0 cm pieces and thermally cleaned at 275°C for 16 hr under a constant helium flow of 30-35 mL/min. Fibers were stored in millipore water until use. Clean fibers were "loaded" by exposing them to a 1:1 methanol-water mixture (~6.2 μL PDMS in 5 mL methanol-water) spiked with Phe, BaA and BghiP separately at six concentration levels.

The Phe, BaA and BghiP loaded fibers were exposed to 6.2, 38 and 102 mL water (10 mM sodium azide) respectively, thereby creating volume water-volume PDMS ratios of 11000, 102000 and 272000. The flasks were shaken for 27 d which is sufficient to reach equilibrium (*165*) in the dark at 20 ± 1°C. Loaded and water-exposed fibers were extracted with various volumes of acetonitrile (200 µL to 20 mL, depending on expected concentrations in the coating) for at least one day. The initial concentrations in the PDMS coating ranged from 1.7 to 4500 mg/L, 0.09 to 600 mg/L and 0.09 to 16.6 mg/L, for Phe, BaA, and BghiP respectively. The assumption behind this method is that the mass balance (Equation 1) was 100% and the validity of this assumption has been proved in (*165*),

$$C_{f\,(initial\ )} * V_f = C_f * V_f + C_{aq} * V_{aq} \qquad (1)$$

where $C_{f\,(initial)}$ is the initial concentration in the loaded fiber, C_f is the concentration in the exposed fiber, V_f is the PDMS volume, C_{aq} is the aqueous concentration and V_{aq} is the aqueous volume. The fiber-water partition coefficient (K_f) can then be calculated from:

$$\frac{C_f}{C_{f\,(initial)}} = \frac{1}{1 + \frac{V_{aq}}{V_f * K_f}} \qquad (2)$$

Measurement of total and pore water concentrations in soil

Total soil concentrations were determined by a soxhlet extraction according to Szolar et al. (2002) (*100*). 2 grams of soil were sampled (triplicate per concentration) and extracted with 45 mL ethylacetate for 16 hours. The extract was diluted with acetonitrile (6 to 1000 times depending on soil concentration) and no further cleanup treatments were necessary. Parallel to the soxhlet extractions, 2 grams soil (triplicate per concentration), 2 mL of water (10 mM sodium azide), 2 thermally cleaned fibers of 5 cm were put in a 7.4 mL amber vial for SPME analysis (*30, 163*). The soil slurry was shaken for 48 hours on a "rock and roller" shaker (Snijders Scientific, Tilburg, The Netherlands). Earlier studies have shown that 48 hours rock and rolling is sufficient to reach ≥95% of the equilibrium for the selected compounds (*30, 166*). After exposure, the fibers were sampled and cleaned with a moist tissue and extracted in various volumes of acetonitrile (200 µL to 10 mL), depending on the expected concentrations in the fibers. Freely dissolved pore water concentrations (C_{aq}) in the soil were calculated using the concentrations in the fiber coating (C_f) and PDMS-water partition coefficients (K_f) according to Equation 3.

$$C_{aq} = \frac{C_f}{K_f} \quad (3)$$

Analysis of samples

The concentrations in the soil- and fiber-extracts were determined by HPLC-fluorescence detection. The system consisted of a Shimadzu DGU 14A degasser (Den Bosch, The Netherlands), a Varian Prostar 420 autosampler (Bergen op Zoom, The Netherlands), a Gynkotek P580 HPG HPLC pump (Gemering, Germany), and a Jasco FP-920 fluorescence detector (Maarssen, The Netherlands). Separation was performed using a Supelcosil (Supelco, Bellefonte, CA, USA) LC-PAH column (length 100 mm, ø 4.6 mm, particles 3 μm) that was operated at 26°C. All analyses were performed isocratically with a flow rate of 1.0 mL/min and an injection volume of 20 μL. Phe was eluted with a acetonitrile-H_2O ratio of 70%:30% Pyr was eluted at 80%:20%, BaA at 85%:15% and BbF at 90%:10%, while BghiP was eluted with 100% acetonitrile. The excitation and emission wavelengths (nm) of Phe were 255 and 355, Pyr was analyzed at 274/400, BaA at 280/390, BbF at 260/420 and BghiP at 295/415. Chromatograms were analyzed using Chromcard version 1.21 (Milan, Italy), and corrected by hand if necessary. Detection limits (peaks ≥5 times background noise) ranged from 0.05 to 0.2 μg/L for the selected PAHs.

Results and Discussion

Determining PDMS sorption isotherms

A Freundlich isotherm (Equation 4) was fitted to test the linearity of the relation between the concentration in the fiber coating (C_f, μg/L) and the aqueous phase (C_{aq}, μg/L).

$$C_f = K_f * C_{aq}^{\ n} \quad (4)$$

The K_f is the PDMS-water partition coefficient at an aqueous concentration of 1.0 μg/L, and n is the parameter describing the sorption linearity. The obtained n-values (1.01 ± 0.01, 1.01 ± 0.02 and 1.05 ± 0.03 for Phe, BaA and BghiP respectively) did not differ significantly from 1, so the sorption to the PDMS material can be considered linear, and a single concentration-independent K_f could be calculated. Figure 1 shows the concentrations in the fiber coating (C_f) plotted against the aqueous concentrations (C_{aq}). The lines represent the fit of Equation 4 with n fixed at 1. The obtained K_f-values are listed in Table 3. There has been some debate about the process of sorption to PDMS coated SPME fibers (*71, 73, 167*). Concentration-independent K_f-values over a broad range of concentrations up to 4 orders of magnitude, give strong evidence that the sorption to the PDMS polymer on the disposable fibers used is a partitioning process. A

similar conclusion was drawn by Mayer et al. (*70*), Poerschmann et al. (*73*) and Vaes et al. (*71*).

Table 2: Detection limits of pore water analysis using nd-SPME.

Comp.	*Detection limits in acetonitrile (µg/L)*	*Detection limits in pore water (ng/L)* [a]	*Corresponding organic carbon normalized concentration in the soil (µg/kg)* [b]	*Volume of pore water sampled by a 5 cm fiber (mL)* [c]	*Depletion of 2 grams of Borris-2 soil by the addition of 2*5 cm fiber (%)*
Phe	0.20	10	66	4.9	0.55
Pyr	0.30	3.7	95	15.9	0.53
BaA	0.1	0.51	31	37.4	0.14
BbF	0.30	0.51	97	118.1	0.14
BghiP	0.20	0.20	76	187.2	0.07

[a] A 5 cm fiber extracted in 200 µL acetonitrile and the analytical methods described in paragraph "Analysis of Samples" were used.
[b] Detection limits in field soils can be higher if sorption is higher than in spiked soils (*28, 166, 168*).
[c] Calculation with: $V_{fiber} * K_{fiber} = V_{aq}$.

Table 3: PDMS-water partition coefficients (K_f) and aqueous solubility, and soil sorption coefficients of the test compounds.

Comp.	*Log K_{OW}*	*Log K_f (SE, n)*	*Aq. sol. µg/L from lit.*	*Max. pore water conc. µg/L (SD, n)*	*Log K_{OC} at 1000 mg/kg OC (SE, n)*	*$n_{Freundlich}$ (SE)*	*Log K_{OC} from lit.* [h]	*Ratio between measured and estimated K_{OC}-values*
Phe	4.56[a]	3.82 (0.01, 15)[e]	1100 [g], 823 [c]	912 (39, 12)	4.65 (0.03, 22)	0.84 (0.01)	4.45	1.6
Pyr	5.22[b]	4.41 [f]	131 [g]	99.0 (4.0, 11)	5.26 (0.02, 24)	0.90 (0.02)	5.01	1.8
BaA	5.91[c]	4.78 (0.03, 17)[e]	13.0 [c]	7.76 (0.19, 8)	6.21 (0.01, 20)	0.89 (0.01)	5.70	3.2
BbF	6.20[d]	5.28 [f]	15.1 [g], 1.09 [c]	5.34 (0.53, 8)	6.72 (0.01, 20)	0.89 (0.01)	5.99	5.4
BghiP	6.85[b]	5.48 (0.03, 11)[e]	0.27 [g], 0.137 [c]	0.329 (0.03, 20)	7.18 (0.06, 20)	0.88 (0.05)	6.64	3.5

[a] Data from De Bruijn et al. (*104*).
[b] Data from Yalkowsky et al. (*139*).
[c] Data from De Maagd et al. (*169*).
[d] Data from Ma et al. (*140*).
[e] Data obtained from this study, Figure 1.
[f] Data from Ter Laak et al. (*165*).
[g] Data selected by Mackay et al. (*53*).
[h] Calculated from a QSAR of from Karickhoff et al. (*35*): $\log K_{OC} = \log K_{OW} - 0.21$.

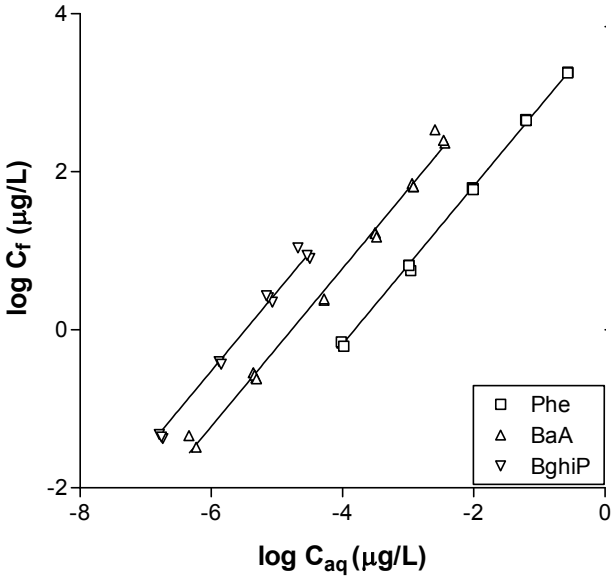

Figure 1: The concentrations in the fiber coating (log C_f) vs. the aqueous concentration (log C_{aq}) of three PAHs. Aqueous concentrations are calculated using a 100% mass balance approach.

Measuring aqueous concentrations in soil pore water and determining soil sorption isotherms

The large partition coefficients, and clean fiber extracts lead to low detection limits of aqueous concentrations in soil pore water (*33*). Table 2 shows the detection limits using the current analytical set-up, using 5 cm fibers extracted with 200 µL acetonitrile, and an injection volume of 20 µL. The exposed fibers did not deplete the soil slurry as they sampled 0.55% or less of the total amount in the system (Table 2). This is important information because, negligible depletion is a critical assumption behind the measurement of freely dissolved pore water concentrations (*34*). Increasing the fiber volume, decreasing the acetonitrile volume and improving analytical conditions and equipment could lower the detection limits even further. Pore water concentrations could be detected in the ng/L-range, with only using 2 grams of soil, and without any cleanup. Five to 187 mL pore water should be extracted and transferred into 200 µL acetonitrile to obtain similar detection limits from pore water extractions. Collecting these volumes of pore water requires large amounts of soil and the procedure for the collection of pore water is rather laborious and time consuming. Another complicating factor in the analysis of pore water concentrations in soil is the presence of dissolved organic matter (DOM). A more detailed quantitative discussion of the effects of DOM is given below.

Concentrations of DOM can range from single milligrams per liter to hundreds of milligrams per liter, depending on the soil type and soil pH. The sorption of hydrophobic compounds to DOM can lead to an overestimation of the freely dissolved concentration in exhaustive liquid-liquid extractions (*14, 17, 40, 51, 108*). Generally, the more hydrophobic the compound, the higher the sorption coefficients to the DOM, and therefore the larger the overestimation of the free concentration (*66*). The overestimation of the aqueous concentration would be a factor of 1.1 for Phe to 11.3 for BghiP for a soil

with 10 mg/L dissolved organic carbon (DOC) in the pore water (log K_{DOC}-values are based on the octanol-water partition coefficient and a QSAR specific for pore water-DOC (*40*)). The removal of DOM without disturbing the equilibrium of the compounds in the pore water is difficult (*1*). Flocculation (*16, 168*) can remove the DOM from the aqueous phase, but the addition of e.g. aluminum potassium sulfate, and adjusting the pH might affect the equilibrium of the freely dissolved and sorbed compounds (*74, 90, 91*). Passive separation by a dialysis membrane might be a better option, but both techniques still need large aqueous volumes to obtain sufficient sensitivity.

The used negligible depletion passive sampler is a good alternative to determine free aqueous concentrations in complex matrices like soil or sediment, as has also been shown by others (*27, 30, 170*). The technique does not need separation of the aqueous and matrix phase. It can also detect very low aqueous concentrations, since the partition coefficient increases with increasing hydrophobicity and decreasing solubility of the test compounds. Detection limits are in the range of 0.2 to 10 ng/L in pore water, corresponding with ~1 to 10 μg/kg in a soil with 2% organic carbon and sorption coefficients estimated from the octanol-water partition coefficient and a QSAR of Karickhoff et al. (*35*). In addition, the extracts are very clean and, therefore, no clean up steps are needed, contrary to what is often the case in soil analysis. The only requirements are: no depletion of the system by the passive sampler, known partition coefficients between the passive sampler and water, measurements performed at equilibrium and no substantial fouling on the fiber surface. The selection of an appropriate set-up and dimensions of the system can easily meet the first three requirements, but fouling may affect uptake kinetics or increase the sorption capacity (*171*). Several studies have shown that this does not occur to an extent that it influences the measurements (*48, 79, 172*). In addition, disposable fibers used in this study are exposed and extracted only once, so the disturbing fouling effects are probably less than for the repetitive exposure and thermal desorption of commercial SPME fibers (e.g. at 275°C).

The relation between the organic carbon normalized soil concentration (C_{OC}) and the concentration in the PDMS coating of the fiber (C_f) is shown in Figure 2. It can be observed that the concentration in the fiber increase with the soil concentration until a certain threshold-value. This threshold might be an effect of saturation of the PDMS phase or the aqueous phase. The compounds are thought to partition into the "rubbery liquid" (*173*) PDMS material (*25, 70, 71, 73*). The sorption to the PDMS is linear over a broad range of concentrations, so this assumption is probably correct. In true partitioning processes, the ratio of the solubility of a compound in phase A (PDMS) and phase B (water) should be equal to their partition coefficient.

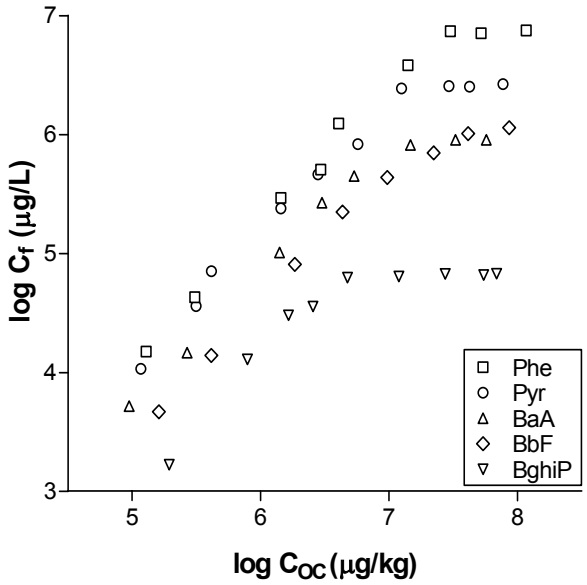

Figure 2: The logarithm the PAH-concentration in the fiber coating (log C_f) plotted against the logarithm of the organic carbon normalized PAH-concentration in the soil (log C_{OC}).

Figure 3 shows the relation between the soil concentrations (C_{OC}) and the free pore water concentration (C_{aq}), calculated from fiber concentrations (C_f) and fiber partition coefficients (K_f) using Equation 3.

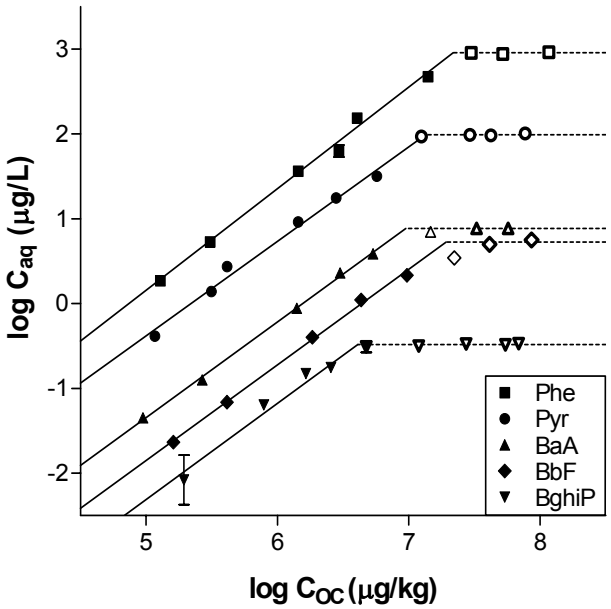

Figure 3: The logarithm of the freely dissolved concentration in the pore water (log C_{aq}) plotted against the logarithm of the organic carbon normalized concentration in the soil (log C_{OC}). The solid lines represent the Freundlich isotherms, and are fitted on the solid symbols. The maximum freely dissolved concentrations measured in the pore water (broken lines), were determined with the thick open symbols (the maximum range of selected values was 0.10 log units). Error bars represent the standard deviations, and are in most cases too low to be visible.

Freely dissolved pore water concentrations increase with increasing soil concentration up to a certain maximum (threshold). The observed maximum in soil pore water concentrations are very similar to aqueous solubility data obtained from literature (*53, 99*).

The observed threshold-value is very likely determined by the aqueous solubility of the PAHs (Table 3, Figure 4). As a spin off of this study, we suggest that the fiber method can also be used as a tool to estimate aqueous solubility as long as; the large amounts of PAHs (up to 7.0 g/L PDMS) do not affect the properties of the PDMS and all compounds extracted from the PDMS coating are dissolved (no crystals in the PDMS material or on its surface).

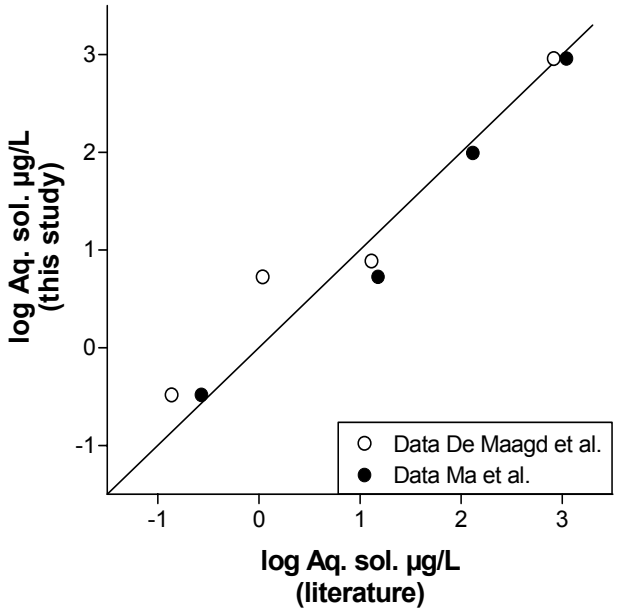

Figure 4: Estimated aqueous solubility of the tested PAHs by maximum observed concentrations in the PDMS coating, plotted against aqueous solubility determined by De Maagd et al. (*99*) and selected by Ma et al. (*140*). The line represents a 1:1 relationship.

Figure 3 also shows a Freundlich isotherm (Equation 5) that describes the relation of the organic carbon normalized soil concentration (C_{OC}) and the freely dissolved aqueous concentrations (C_{aq}). Only values below the threshold level were used.

$$C_{OC} = K_{OC} * C_{aq}^{\ n} \qquad (5)$$

The K_{OC} is the organic carbon normalized sorption coefficient at an aqueous concentration of 1.0 µg/L, and n is the Freundlich linearity parameter. Table 3 displays the calculated sorption coefficients at a concentration of 1000 mg/kg organic carbon, and the $n_{Freundlich}$. The sorption is close to linearity ($n_{Freundlich}$ ranges from 0.84 to 0.90),

suggesting that a non-specific hydrophobic absorption process into relatively amorphous "soft" natural organic matter (*7*) is the most important sorption process**.** The rather linear sorption isotherm could be expected, since the Borris-2 soil was freshly spiked and does not contain large amounts of (xenobiotic) organic materials like soot, coal or tar that could have led to nonlinear sorption isotherms due to slow absorption and strong adsorption interactions (*7, 112, 113, 174*).

Effects of dissolved organic matter on soil sorption coefficient determination

The obtained sorption coefficients are higher (a factor 1.6 to 5.4) than estimated according to a QSAR of Karickhoff et al. (*35*) (see Table 3). Generally, this factor increases with the hydrophobicity of the PAH. The difference might be an effect of slight overestimations of the concentration in the aqueous phase, due to DOM sorption, in the original sorption studies. Other explanations are related to potential differences in sorbent properties between the two studies or differences in the concentration level at which the sorption studies were performed. The potential effect of dissolved organic matter on the measurement of sorption coefficients has been recognized earlier by Schrap et al. (*14*). The ratio of the actual freely dissolved concentration in soil pore water (C_{aq}) and the total concentration in solution (C_{total}, including the DOM bound fraction) can be estimated from Equation 6.

$$\frac{C_{aq}}{C_{total}} = \frac{1}{1 + K_{DOC} * C_{DOC}} \qquad (6)$$

Where K_{DOC} is the sorption coefficient and C_{DOC} is the dissolved organic carbon concentration. Exact DOC or DOM concentrations and specific sorption coefficients are unknown in our experiments. Therefore, the effect of DOC is illustrated, using a pore water specific log K_{OW}-based relationship of Burkhard (*40*) and a relatively low DOC concentration of 10 mg/L (Table 4).

Table 4: Potential overestimation of aqueous concentrations by sorption to DOC.

Compound	*Log K_{OW}*	*Log K_{DOC}* [e]	*Fraction freely dissolved at 10 mg/L DOC*	*Overestimation-factor of pore water concentration at 10 mg/L DOC*
Phe	4.56 [a]	3.93	0.92	1.1
Pyr	5.22 [b]	4.53	0.75	1.3
BaA	5.91 [c]	5.16	0.41	2.4
BbF	6.20 [d]	5.42	0.27	3.6
BghiP	6.85 [d]	6.01	0.09	11.3

[a] Data from De Bruijn et al. (*104*).
[b] Data from Yalkowski et al. (*139*).
[c] Data from De Maagd et al. (*169*).
[d] Data from Ma et al. (*140*).
[e] Calculated from a pore water-DOC specific QSAR (*40*): $\log K_{DOC} = 0.91 * \log K_{OW} - 0.22$.

It is obvious from this simulation that measurement of total concentrations in pore water can be highly overestimated, leading to systematic errors in the determination of soil sorption coefficients. The estimated fractions listed in Table 4 are in the same range as observed in our experiment (Table 3). These systematic errors can be avoided by actual measurements of free concentrations.

Relevance for soil testing

The free concentration in soil pore water is a relevant entity for all kinds of processes in soil, including evaporation to air, (bio)degradation and accumulation in biota living in the soil or sediment (*3, 47, 159, 161, 175*). The freely dissolved concentration of a compound is generally thought to be decisive in determining the internal concentration and subsequent toxic effects in small soil dwelling deposit feeders (*47, 163, 170, 176, 177*). Due to their limited aqueous solubility, the larger PAHs (≥4 rings) can't evoke lethal body burdens (LBB) in the organisms, and therefore, these chemicals are generally found to be non-toxic (*178-180*). In soil toxicity tests, however, organisms are generally exposed to a series of increasing concentrations in soil, even above the aqueous solubility in the pore water. Effects are then usually expressed on basis of total soil concentrations, or sometimes to estimated pore water concentrations (*176, 179, 180*). Freely dissolved concentrations in the pore water are hardly ever measured, and the saturation of the aqueous phase is often disregarded. Results from this study show that the aqueous phase can get saturated in a soil toxicity test set-up at concentrations of 80 to 400 mg/kg (at an organic carbon content of 2%). Negligible depletive passive samplers like PDMS coated fibers and also other partition based sampling methods might be applied to monitor freely dissolved concentrations (and saturation of the aqueous phase) in soil and sediment toxicity set-ups. Information on these pore water concentrations may be extremely useful in interpreting the outcome of these soil tests.

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Chapter 6

The effect of pH and ionic strength on the sorption of sulfachloropyridazine, tylosin and oxytetracycline to soil

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Abstract

Anti-microbial agents are the most heavily used pharmaceuticals in intensive husbandry. Their usual discharge pathway is application to agricultural land as constituents of animal manure, which is used as fertilizer. Many of these compounds undergo pH-dependent speciation and therefore might occur as charged species in the soil environment. Consequently, their sorption behavior and its pH-dependence is relevant for the assessment of the environmental mobility and the availability to soil organisms. Hence, the influence of the pH and ionic strength of the soil suspension on the sorption of three anti-microbial agents, sulfachloropyridazine (SCP), tylosin (TYL), oxytetracycline (OTC) was investigated. Their respective sorption coefficients in two agricultural soils range from 1.5 to 1800 L/kg. Sorption coefficients were greater under acidic conditions. Addition of an electrolyte to the solution led to decreased sorption of OTC and TYL with a factor of three to fifty, but did not influence the sorption of SCP. This behavior was analyzed by accounting for the pH dependent speciation of TYL and OTC and considering the presence of OTC-Ca complexes. It appears that the decreased sorption of TYL and OTC with increasing ionic strength is due to competition of the electrolyte cations with the positively charged TYL species and the positively charged OTC-complexes. A model linking sorbate speciation with species-specific sorption coefficients can describe the pH-dependence of the apparent K_D-values. This modeling approach is proposed for implementation in the assessment of sorption of ionizable compounds.

Introduction

Anti-microbial agents are physiologically highly active compounds. They are used as veterinary pharmaceuticals to treat infections, prevent animals from infectious diseases or promote growth. In 1999 their use for veterinary purposes amounted to approximately 3900 tons (*181, 182*) in the European Union, thus accounting for 70% of the total veterinary pharmaceutical use (*183-185*). Since 1997, veterinary pharmaceuticals (VPs) are submitted to an environmental risk assessment as part of the registration or the re-registration procedure (*186, 187*). This process has to account for the particular fate of VPs. Upon administration, VPs can be metabolized within the animal, however, the treated animals excrete substantial fractions of the compounds unchanged, as conjugates or as active metabolites via urine and feces (*188-193*). Further transformation of the compound can occur during manure storage, but still large amounts of the veterinary antibiotics can reach the soil environment via manure application (*194*). As a result, several pharmaceuticals have been found in agricultural soils, groundwater, surface waters and even drinking water (*195-198*). The soils' capacity to retain VPs is dependent on the tendency of these chemicals to sorb to soil particles. This tendency is quantified as

the sorption coefficient (K_D). The K_D is an important parameter for the assessment of the risk of leaching to the groundwater and transport to surface waters.
According to the current risk assessment paradigm, log K_{OW}-based models are employed to predict organic carbon normalized sorption coefficients (log K_{OC}). This approach appears applicable to non-polar compounds for which sorbate-soil interactions are mainly driven by hydrophobicity and for which strong correlations exist between the sorption coefficients and octanol-water partition coefficients (*42*). However, literature data show that these models cannot predict sorption of anti-microbial agents and other pharmaceuticals to soil (*41, 182*). This can be explained by the relatively complex structures of these compounds, which often contain more than one functional group and are frequently ionizable. The pH and ionic strength have been demonstrated to affect the partitioning of ionizable chemicals in several processes including soil and sediment sorption (*5, 9-11, 43, 44*) octanol-water partitioning (*199, 200*) membrane partitioning (*201*) and protein adsorption (*202*). Changing the pH leads to protonation or deprotonation of ionizable compounds like various veterinary medicines, thereby changing the physical chemical properties and subsequently sorption of a compound. Ionic strength can influence sorption of ionized antibiotics by changing the interfacial potential and by competing for ion-exchange sites. Most soil surfaces carry a net negative charge. When ionic strength increases, the cations will be electrostatically attracted by the negative soil surfaces thereby replacing sorbed cationic organic compounds (*10*) and reducing the negative surface charge. This in turn might increase sorption of anionic organic compounds (*43, 203-205*).

Scope
For risk assessment purposes it needs to be known to what extent pH and ionic strength influence the sorption coefficients, whether it is necessary to account for the influence of pH an ionic strength, and how that can be achieved. To that end, the effect of pH and ionic strength on the sorption behavior of three anti-microbial agents in two agricultural soils was investigated. They represent three major classes of veterinary anti-microbial agents (tetracyclines, sulfonamides and macrolids) used in Europe. In The Netherlands (1999) and the Weser-Ems district in Germany (1997), more than 50% of the veterinary anti-microbial agents used were tetracyclins, about 20% were sulfonamides and 12% were macrolids (*184, 188, 189*).The three VPs differ significantly in their physical-chemical properties. SCP is a weak acid, TYL a weak base, while OTC is amphoteric with three moieties undergoing protonation and deprotonation reactions. On top of that, OTC forms complexes with various di- and trivalent metal ions (*206-209*). Among them is Ca^{2+} ion, that is abundant in the soil solution of agricultural soils (*5, 210*), and is present at a concentration of 10 mM in the solution of the batch equilibrium experiments according to OECD technical guideline 106 (*211*). The experimental data obtained in batch sorption experiments performed at different values of soil solution pH and ionic strength were analyzed in terms of aqueous speciation, in order to gain more insight into

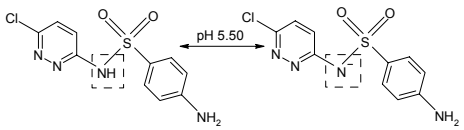
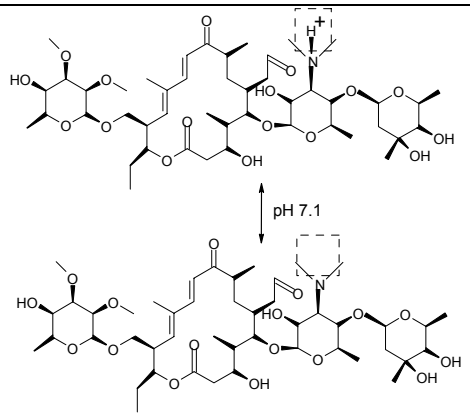
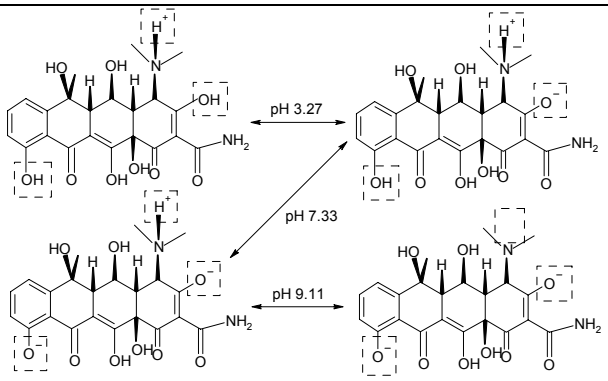
the processes involved in sorption of the compounds. The results of the experimental work are discussed with regard to their relevance for the assessment of environmental exposure of veterinary pharmaceuticals.

Experimental section

Chemicals

The test chemicals sulfachloropyridazine (SCP), tylosin hemitartrate dihydrate (TYL), oxytetracycline hydrochloride (OTC) and erythromycin A dihydrate that was used as internal standard for the tylosin analysis, were all purchased from Riedel-de Haën (Darmstadt, Germany) and used as received.

Table 1: Some physicochemical properties, the molecular structure and the different species of SCP, TYL and OTC. The reference numbers are given between brackets.

	SCP	
MW (g/mol)	284.72	
Solubility (g/L)	7.0 (*212*)	
log K_{OW}	0.31 (*213*)	
pK_A	5.5 (*214*)	
	TYL	
MW (g/mol)	917.14	
Solubility (g/L)	5.0 (*215*)	
log K_{OW}	2.50 (*215*) 1.63 (*216*)	
pK_A	7.1 (*212*)	
	OTC	
MW (g/mol)	460.44	
Solubility (g/L)	1.0 (*217*)	
log K_{OW}	-1.12 (*215*)	
pK_A	3.27, 7.33, 9.11 (*217*)	
Log $pK_{OTC\text{-}Ca}$	OTC^{2-} = 3.81 (±0.07) (*207*) OTC^{-} = 2.93 (±0.02) (*207*) OTC^{0} = 2.42 (±0.02) (*218*)	

2-Morpholinoethanesulfonic acid (MES) buffer was obtained from Fluka (Zwijndrecht, The Netherlands), $CaCl_2$, NaCl and NaN_3 were supplied by Merck (Amsterdam, The Netherlands). Highly pure water (R ≥18 MΩ) was prepared by a Millipore purification system (Millipore waters, Amsterdam, The Netherlands) and employed throughout the experiments.

Soils

Two agricultural soils (arable land) were sampled on 09/10/01. A clay loam soil was obtained from the Woodsite farm, Orgathorpe, Leicestershire, UK (C). The specimen collected at Hall Farm, Lockington, Leicestershire, UK (S) was a sandy loam soil. Both samples were stored at 4°C, until drying to constant weight (at 25 ±1 °C). Afterwards the soils were sieved (1 mm mesh size) and stored, Table 2 shows the soil properties.

Table 2: Properties of the test soils.

Property	*Clay loam soil (C)*	*Loamy sand soil (S)*
Soil type	clay loam	loamy sand
Sampling depth	0-37 cm depth	0-30 cm depth
63μm – 2mm (sand)	42.6%	69.2%
2μm – 63μm (silt)	32.3%	20.5%
<2μm (clay)	25.1%	10.3%
pH (0.01M $CaCl_2$)	6.8	6.6
CEC mEq/100g	22.4	11.4
Organic Carbon%	3.08	2.18
N%	0.169	0.109

Sorption experiments

In batch sorption experiments performed according to OECD technical guideline 106 (*211*) soil was pre-incubated (shaken) for 48 hours with an electrolyte solution containing 10 mM $CaCl_2$ and 10 mM NaN_3 in 20 mL vials containing sufficient headspace (>7 mL) to enable thorough shaking. After pre-incubation, OTC and TYL were spiked at 3.0 mg/L, and SCP at 1.5 mg/L, using 1000 mg/L stock solutions in residue analyzed MeOH (Baker-Mallinckrodt, Deventer, The Netherlands) and were shaken for another 48 hrs. Experiments were performed at 25 ± 1°C in the dark.

Deviating from the OECD protocol, the soil–water (kg/L) ratio was adapted such that 50 to 90% of the substance was sorbed to the soil. The chosen ratios for soil C were $^1/_2$, $^1/_{25}$, and $^1/_{300}$ for SCP, TYL and OTC respectively. The corresponding ratios for soil S amounted to $^1/_2$, $^1/_2$, and $^1/_{150}$. A blank without soil (triplicate) was used to confirm the initial amount of compound and to correct for sorption to the vial walls. The soil-water partition coefficient (K_D) of the test substance in a single vial was calculated using the following equation:

$$K_D = \frac{C_{soil}}{C_{water}} \quad (1)$$

Sorption coefficients are not always the same at different concentrations so the Freundlich isotherm (Equation 2) was employed to test the linearity of the soil sorption coefficient over a range of concentrations,

$$C_s = K_{Freundlich} * C_W^n \quad (2)$$

C_S is the concentration of the compound in the soil in mg/kg, $K_{Freundlich}$ is the partition coefficient of the substance between soil and water at a water-concentration of 1.0 mg/L, C_W is the concentration in the water in mg/L and n is the non-linearity constant, or the Freundlich constant. If n is significantly lower or higher than 1, sorption is nonlinear. n-Values lower than 1 indicate a decrease in sorption with increasing concentration in the water and n-values higher than 1 indicate the opposite.

Adjustment of pH

The ambient pH value at a soil-water ratio of $^1/_2$ was 7.10 (± 0.0) and 6.54 (± 0.0) for soil C and S respectively. The pH was adjusted using various amounts of NaOH or HCl solution up to a maximum nominal concentration of 30 mM. The ion concentration in the system was kept constant by adding NaCl.

The pH-dependent speciation of an ionizable compound is determined by its pK_A-value(s). In case of a monoprotic compound the observed sorption coefficient (K_D') can be written as the sum of the species specific sorption coefficients (K_{D1} & K_{D2}) weighted with the fraction (α & (1- α)) of the respective species present in the soil solution, Equation 3 describes this relationship:

$$K_{D'} = K_{D1} * \alpha + K_{D2} * (1 - \alpha) \quad (3)$$

Because the species composition is determined by aqueous pH and the pK_A of the compound, the equation can be written in the following manner:

$$K_{D'} = \frac{K_{D1}}{1 + 10^{pH - pKa}} + \frac{K_{D2}}{1 + 10^{pKa - pH}} \quad (4)$$

The pK_A values used were obtained from literature (Table 1). A nonlinear regression line was fitted to the data with Equation 4 using Graphpad (GraphPad Software, San Diego, CA, USA), version 3.0. Given that the pH range of the present study included only one

pK_A-value, neglecting those species occurring in minor fractions (SCP^+, OTC^+ and OTC^{2-}) made Equation 4 applicable for the purpose of the present study (*1, 219*).

Adjustment of ionic strength

NaCl and $CaCl_2$ were added at different concentrations (ranging from 0.0 to 0.2 M) to test the effect of ionic strength (I) on sorption of SCP, TYL and OTC to the two English soils. In these experiments NaN_3 was not used, such that low values of ionic strength could be established. The initial conductivity of the soil suspension (without the addition of any electrolyte) at a soil-water ratio of ½ was 0.74 and 0.46 mS/cm for soil C and S respectively corresponding to a $CaCl_2$ concentration of 0.51 and 0.32 mM. The supernatant contains other cations besides Ca^{2+}, however Ca^{2+} is often the most important cation in soil (*210*), and actual concentrations will be near 0.51 and 0.32 mM for soil C and S respectively. Kinetic studies showed that 24 hours was sufficient to reach equilibrium, so the equilibration-time was shortened to 24 hours to minimize biodegradation of the compounds. The conductivity was measured and calibrated with three standard solutions to obtain the ion concentrations. The ion concentrations were multiplied by the valence (v) of the different ions in solution ((NaCl =½*(v_{Na}^2*[Na] + v_{Cl}^2*[Cl]) and CaCl =½* (v_{Ca}^2 * [Ca] + v_{Cl}^2 * [Cl])) to determine the ionic strength of both $CaCl_2$ and NaCl.

Ca^{2+}-Complexation of OTC

The complexation of the zwitterionic species of OTC and Ca^{2+} was studied by adding $CaCl_2$ to 6.5 μM OTC in Millipore water in the following series: 0, 0.00001, 0.0001, 0.001, 0.003, 0.01, 0.03, 0.1 and 0.5 M in volumetric flasks. The pH was adjusted to 5.9 (SD = 0.3) using 0.002 M MES buffer, at this pH OTC^0 was the prevalent species. The solutions were equilibrated in the dark for 75 min. at 4°C. Afterwards the absorption was measured in a 1.0 cm quartz cuvet using a spectrophotometer (UV 160 A, Shimadzu, Den Bosch, The Netherlands). Upon varying the wavelengths from 200-450 nm in 10 nm intervals a wavelength of 380 nm was found to be optimal to analyze complexation constant(s) of OTC (*207, 220*). The apparent affinity constant of OTC^0 for Ca^{2+} ($K_{OTC\text{-}Ca}$') assuming a 1 to 1 complex stoichiometry, was calculated by fitting the measured absorption of 380 nm as a function of the molar Ca^{2+} concentration:

$$A = A_{\max} - (A_{\max} - A_{\min}) * \frac{1}{1 + K_{OTC-Ca}' * [Ca^{2+}]} \qquad (5)$$

by nonlinear regression using Graphpad, version 3.0 (GraphPad Software, San Diego, CA, USA). A is the observed absorbance at different concentrations of Ca^{2+}; A_{max} is the absorbance where all OTC complexes with Ca^{2+}, and A_{min} is the absorbance in the

absence of Ca^{2+}. The small fractions of OTC species other than OTC^0 at pH of 5.9 (± 0.3) were considered negligible for the data analysis (*221*).

HPLC-analysis

The concentrations of SCP and TYL were determined by reversed phase HPLC using a Discovery (Supelco, Zwijndrecht, The Netherlands) C_{18}-column (length 150 mm, ø 4.5 mm, particles 5 µm) that was operated at 28°C, and OTC was analyzed with an YMC (Fisher Scientific, Landsmeer, The Netherlands) C_{18}-column (length 50 mm, ø 4.0 mm, particles 3 µm). For the analysis of SCP and OTC, the solvent delivery system consisted of a Separations GT-103 degasser (Separations, HI-Ambacht, The Netherlands), a Varian ProStar 420 autosampler, a Varian 9012 solvent pump (Varian, Bergen op Zoom, The Netherlands). All analyses were performed at a flow-rate of 700 µL/min with mixtures of water-acetonitrile (CH_3CN, HPLC-Grade, Lab-Scan Analytical Science) containing 0.1% formic acid (Fluka, purity 98%) to adjust the pH to 2.6. SCP and OTC were analyzed isocratically with a flow rate of 700 µL/min of a 70%:30% and 85%:15% solvent mixture, respectively. TYL was chromatographed using gradient elution starting with 100% H_2O (0.1% formic acid) for one minute followed by an increase of the CH_3CN-fraction to 30% in 3 minutes and to 95% in the subsequent 3 minutes, where it was kept for another three minutes before returning to the starting conditions. The solvents were delivered by a Perkin Elmer series 200 LC-system (Perkin Elmer, Oosterhout, The Netherlands). OTC and SPC were detected by UV-absorption (Shimadzu SPD-10AV, Den Bosch, The Netherlands) at wavelengths of 360 nm and 260 nm, respectively. TYL and erythromycin (internal standard) were detected by electrospray ionization mass spectrometry (Perkin Elmer Sciex API 365) in the single monitoring mode at m/z 916 and 734, respectively. To that end, 25% of the column effluent was diverted to the electro-spray ion source, which was operated in the positive ion mode.

Results and Discussion

Freundlich isotherms

Table 3 shows the results of the sorption isotherms recorded for SCP, TYL and OTC with both 10 mM NaCl and $CaCl_2$ as background electrolyte. Freundlich isotherms (Equation 2) were fitted over a concentration range from less than 0.05 to more than 5 mg/L. The resulting $K_{Freundlich}$ (at C_W = 1.0 mg/L) values of OTC, TYL and SCP vary by orders of magnitude. The $K_{Freundlich}$ sorption coefficients of OTC are 1814 and 655 for soil C and S respectively, while those of TYL are 85 (C) and 6.8 (S), and those of SCP are 2.5 (C) and 1.5 (S). Langhammer (*222*), Fontaine et al. (*223*), Thurman et al. (*224*) and Thiele-Bruhn et al. (*225*) found comparable low sorption coefficients (K_D ranged from 0.2 to 7 L/kg) for various sulfonamides at natural soil pH. Rabølle and Spliid (*226*)

found comparable K_D-values for TYL and OTC in four Danish soils ranging from 8 to 128 for TYL and 417 to 1026 for OTC. It can be observed that the clay loam soil (C) sorbs all compounds stronger than the loamy sand soil (S). The differences are less than a factor of three for OTC and SCP and they correspond with the differences in CEC and the organic carbon content (Table 2), while the sorption coefficient of TYL to the clay loam soil is more than one order of magnitude higher.

Table 3: Freundlich sorption coefficients, $n_{Freundlich}$ values at neutral pH, and the fitted K_D-values of the different species of SCP, TYL and OTC to the two English soils.

	Soil	*$K_{Freundlich}$ (L/kg)[a] (95%CL)*	*$n_{Freundlich}$ (95% CL)*	*pH (± SD)*	*r^2*	*K_D (L/kg) (± SD)*	*K_D (L/kg) (± SD)*	*r^2*
SCP	*Species*					[0]	[-]	
	C	2.5 (2.3-2.9)	0.93 (0.84-1.03)	7.10 (± 0.01)	0.98	16.6 (± 0.6)	1.1 (± 0.3)	0.94
	S	1.5 (1.3-1.8)	0.95 (0.85-1.04)	6.54 (± 0.01)	0.98	8.1 (± 0.5)	0.0 (+ 0.3)	0.94
TYL	*Species*					[+]	[0]	
	C	85 (72-102)	0.96 (0.82-1.11)	6.98 (± 0.03)	0.94	156 (± 8)	32 (± 8)	0.96
	S	6.8 (6.4-7.3)	1.07 (1.00-1.13)	6.82 (± 0.21)	0.99	8.9 (± 0.4)	3.0 (± 0.3)	0.93
OTC	*Species*					[+00 & +-0]	[+-- & 0--]	
	C	1814 (1667-1945)	0.65 (0.61-0.69)	7.40 (± 0.11)	0.96	4740 (± 140)	630 (± 150)	0.87
	S	655 (585-733)	0.68 (0.62-0.75)	7.47 (± 0.04)	0.94	4200 (± 160)	310 (± 200)	0.90

[a] The $K_{Freundlich}$ calculated at a aqueous concentration 1.0 mg/L. The $K_{Freundlich}$, $n_{Freundlich}$, and r^2 were obtained by fitting a linear regression trough the logarithmically transformed water and soil concentrations ($\log C_{soil} = n * \log C_{water} + \log K_{Freundlich}$).

The non-linearity parameter n (Equation 2) did not deviate significantly from 1 for SCP and TYL, indicating that sorption of these two compounds can be described with linear isotherms and thus is independent of concentration. In that regard, the results are similar with nearly linear sorption isotherms found in literature (n ranged from 0.90 to 0.97 for SCP (*227*) and from 0.83 to 0.94 for TYL (*226*)). Contrastingly, the values of n for OTC were significantly lower than 1.0 (0.65 and 0.68 for soil C and S respectively). This means that the sorption coefficient decreases with increasing concentration of the compound in the water-phase. Similar nonlinear sorption of OTC is also observed in sorption experiments with pure clays (*228, 229*) and organic material (*230*).

Influence of pH

In Figure 1 the sorption coefficients of SCP, TYL and OTC are plotted against pH. For all three chemicals it can be observed that increasing pH led to a decrease of the sorption

coefficients. Upon fitting Equation 4 to the data with K_{D1} and K_{D2} as the adjustable parameters, the solid and broken lines represent the best fit obtained for the clay and the sandy soil, respectively. Table 3 displays the results of these fits.

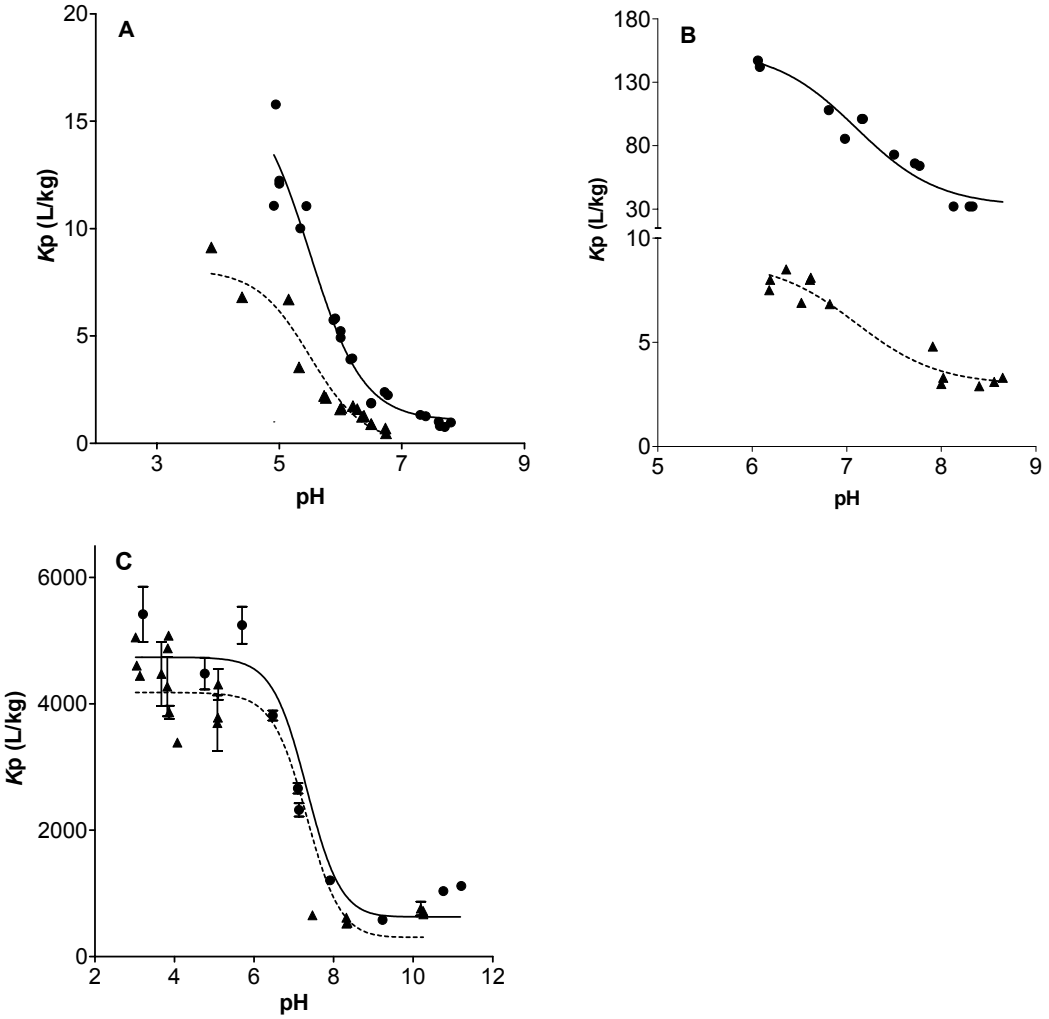


Figure 1: The sorption coefficient of SCP (2a) TYL (2b) and OTC (2c) is plotted against the pH. The circles (●) are the data of soil C and the triangles (▲) show the data of soil S. The solid line (soil C) and the dotted line (soil S) display the fit of Equation 4 through the data.

For SCP the K_D'-values showed the strongest relative decrease with increasing pH. The respective values of K_{D1} and K_{D2} for SCP were 16.6 and 1.1 for soil C (*205*) and 8.1 and 0.003 for soil S, demonstrating that the SCP-anion has a weak tendency to sorb to soil (Figure 1a, Table 3). This is presumably due to a combination of increased occurrence of the more water-soluble SCP-anion and its electrostatic repulsion from increasingly negatively charged soil surfaces. Similar pH effects have been observed for various organic acids sorbing to soils and sediments (*9, 43, 44, 223, 231-233*).

The K_D'-values of TYL showed the smallest variation with pH. Sorption coefficients decreased from 156 to 32 for soil C and from 8.9 to 3.0 for soil S (Figure 1b, Table 3).

The increased sorption of the TYL with decreasing pH can be explained by a shift of the speciation towards the positively charged TYL-cation and subsequent electrostatic attraction to negatively charged soil surfaces. Hence, the data indicate that the increased solubility in the soil solution of the TYL-cation is more than compensated for by the effect of electrostatic attraction of the TYL-cation, resulting in higher sorption coefficients with decreasing pH. Similar decreasing sorption of organic bases with increasing pH have been observed for methyl anilines (*200*) and ciprofloxacin (*234*).
In both soils, the sorption coefficients of OTC showed a similar decrease with increasing pH. The K_D' of soil C decreased from 4740 to 630 and soil S from 4200 to 310. In fitting the Equation 4 to the OTC, the model was simplified by the assumption that in the pH-range considered, sorption can be represented by two K_D-values: One for the positive and neutral species of OTC ([+00] & [+0-]), and the second for the OTC species carrying a net negative charge ([+--] & [0--]). As shown in Figure 1c, the model fits the pH-dependent K_D'–data of OTC well. Literature shows that sorption of OTC to clay (*228, 229*) or organic material (*228*) also decreases with increasing pH, while sorption to Fe-oxides increased with increasing pH (*235*) (between pH 4 and 7). In conclusion, the variation of K_D' with pH is closely related with the speciation of the compounds (r^2 >0.87), indicating that the apparent K_D' is determined by the sorbate speciation.

Ionic strength and sorption of SCP and TYL
In Figure 2 the sorption coefficients of SCP, TYL and OTC are plotted against the logarithm of the ionic strength (I). Figure 2a shows that ionic strength did not influence SCP sorption significantly, except for the $CaCl_2$ treatment in soil C where sorption increased by a factor of 2 when the $CaCl_2$ concentration was increased from 0.006 to 0.2 M. This increase in sorption by a factor 2 is probably a result of the increase of the neutral SCP-species from 3.3% to 8.3% due to decreasing pH. The pH is decreased the displacement of protons from the cation exchange sites as a result of increased ionic strength. The effects of ionic strength on pH and the speciation of the model compounds are illustrated in Figure 3a-c for the model compounds. Alternatively, increased sorption can be explained by decreased electrostatic repulsion of negatively charged sorbate molecules due to increased cation concentrations near the negatively ciharged soil surfaces. This phenomenon has been observed in sorption studies with other organic anions such as 2,4-dinitro-o-cresol (DNOC), 2-(2,4,5-trichlorophenoxy)propanoic acid (Silvex) (*43*), Brilliant Bleu FCF (pK_A1 = 5.83 & pK_A2 = 6.58) (*204*) and linear alkylbenzenesulfonates (LAS, $pK_A \sim 2$) (*203, 236*).

In the TYL-experiments the increase of $CaCl_2$ or NaCl decreased the pH of the soil solution (7.6 → 6. 9 and 6.8 → 6.4 for soil C and S respectively), such that on the basis of the pH-dependence of TYL-sorption (Figure 1b), an increase in sorption was expected. However, at higher ionic strength, the opposite was observed (I >0.03, Figure 2b), indicating that the observed effect must have been directly linked to the changing

ionic strength. The decreased sorption can be attributed to competitive sorption of Ca^{2+} or Na^{+} with TYL^{+} for negative soil surfaces in the diffuse double layer near the negatively charged soil surfaces (*5*). Brownawell et al. (*10*) also observed a decrease of the sorption of the dodecylpiridinium cation when the Na^{+} and Ca^{2+} concentrations increased.

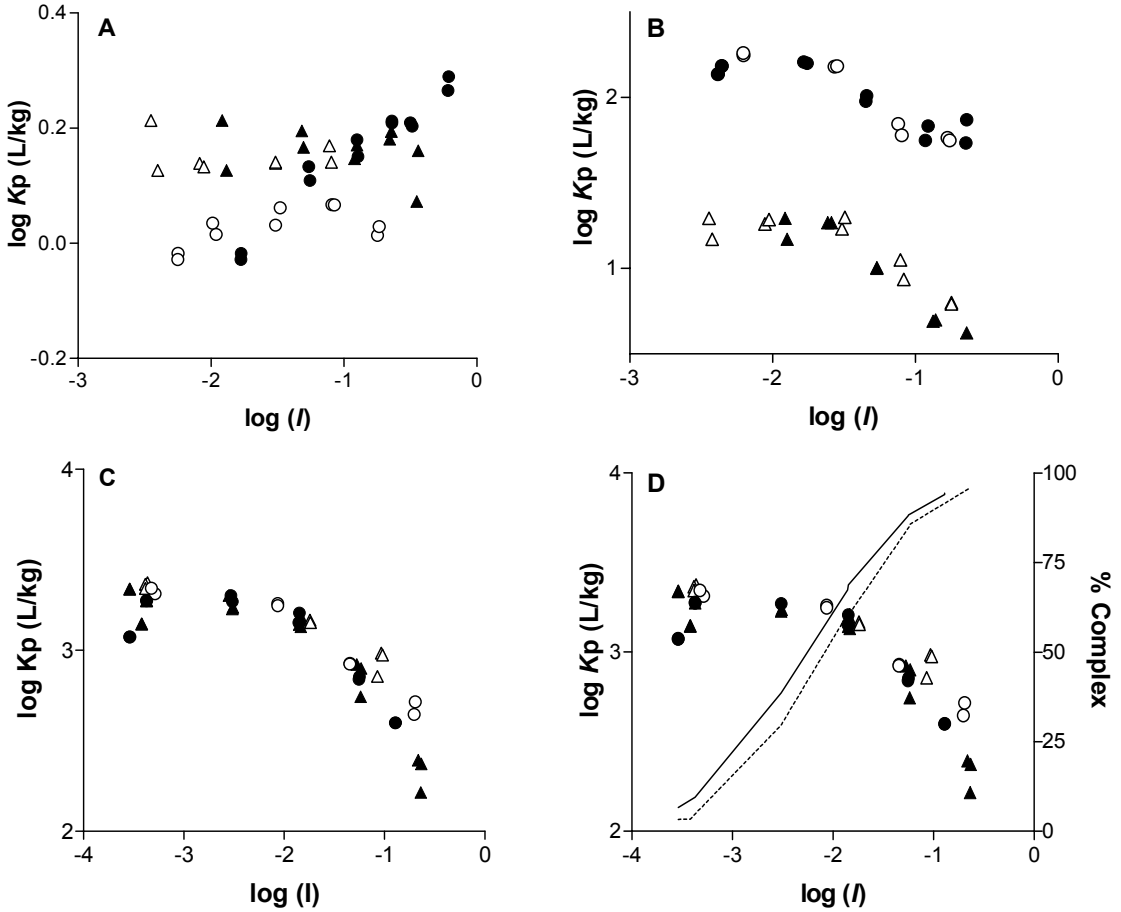


Figure 2: Measured sorption coefficients of SCP (3a), TYL (3b) and OTC (3c) are plotted against the conductivity, Figure 2d is similar to Figure 2c but on the second Y-axes the calculated percentage of OTC-calcium complexes are also plotted against conductivity. The circles display sorption of soil C (● = $CaCl_2$ added, ○ = NaCl added) and the triangles display sorption coefficients of soil S (▲ = $CaCl_2$ added, Δ = NaCl added). Note that there are two Y-axes in Figure 2b, the left axes is for soil C and the right one for soil S.

Ionic strength and complexation of OTC

Ca^{2+} complexation of OTC - OTC and other tetracyclines have a strong tendency to form complexes with various bivalent and trivalent metal ions (*206-209*). The complexation of OTC with Ca^{2+} is of interest because Ca^{2+} is the most abundant cation in soil (*5*). OECD guideline 106 (*211*) prescribes the use of 10 mM $CaCl_2$ and 10 mM NaN_3 (I = 0.04) while pore-water concentrations of Ca^{2+} in neutral soils are usually lower (*5, 210*). Martin (*207*) investigated the affinity constant of OTC and Ca^{2+} of the species OTC^{2-}

(log $K_{OTC\text{-}Ca}$ = 3.81 (±0.07)) and OTC^- (log $K_{OTC\text{-}Ca}$ = 2.93 (±0.02)). But to our knowledge, the affinity constant of the zwitterionic OTC^0, which account for a significant portion of the overall OTC in the pH range between pH 3.3 and 7.3, was unknown. Figure 4 shows that the absorption of 6.5 μM OTC^0 at 380nm increased with increasing $CaCl_2$ concentration. The lines represent the best fit of Equation 5 to the data, yielding a value of 2.42 for the logarithm of the complexation constant (Log $K_{OTC\text{-}Ca}$). Consequently, a large part of the OTC will be complexed with calcium in batch equilibrium sorption experiments according to the OECD guideline (*211*).

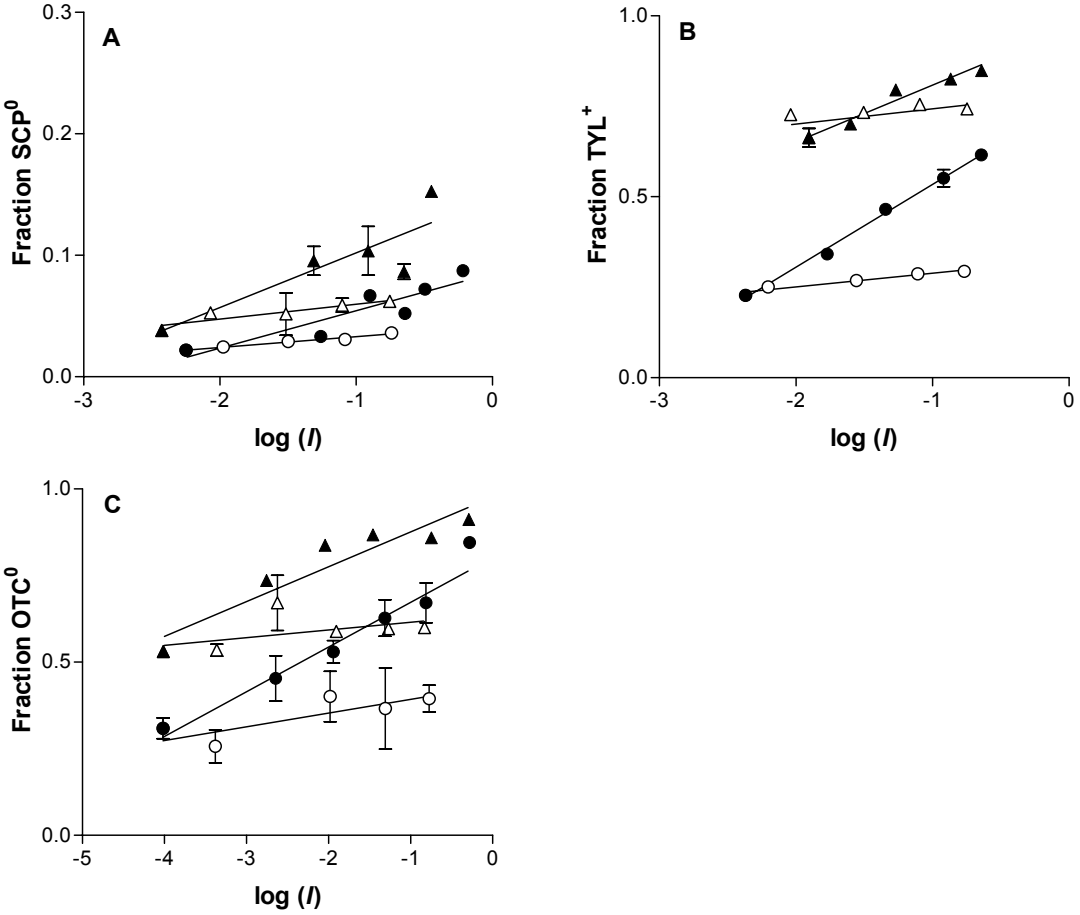


Figure 3: The calculated fractions of the different SCP (3a), TYL (3b) and OTC (3c)-species at the conductivity treatments. The circles display the fraction of the compound using soil C (● = $CaCl_2$ added, ○ = NaCl added) and the triangles display the fraction of the compound using soil S (▲ = $CaCl_2$ added, Δ = NaCl added).

The effect of ionic strength on sorption of OTC - Analogous to TYL, OTC sorption coefficients stay stable at low ionic strength and decrease at higher ionic strength. The addition of $CaCl_2$ and NaCl changed the pH, and subsequently increased the fraction of OTC^0 as is shown in Figure 3c. Based on results of the pH-experiments, this increase of the OTC^0 species is expected to lead to an increase in sorption, but the opposite was observed (Figure 1c). Hence, the effects can be credited to ionic strength. Even though

OTC^0 bears no net charge, its sorption behavior resembles that of cations. Considering that OTC^0's positively charged moiety might be attracted by negatively charged soil surfaces, the adsorption might be attributed to electrostatic interactions similar to cations (*5, 229*) such as TYL^+.

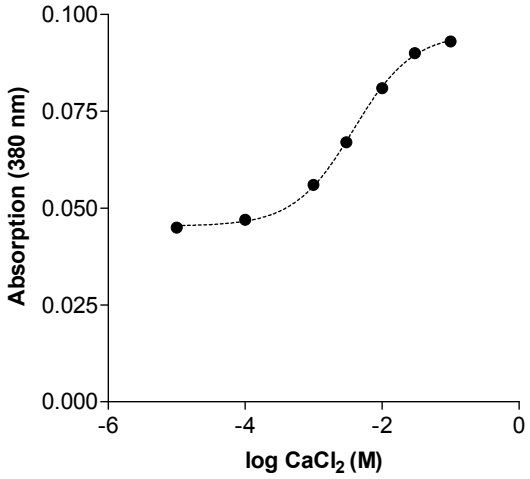


Figure 4: The absorption of OTC and the OTC-Ca complex at 380 nm light plotted against the $CaCl_2$ concentration. The dotted line displays the best fit of Equation 5 to the experimental data. The calculated "p$K_{Calcium}$" (log $K_{OTC\text{-}Ca}$) of OTC^0 was -2.42.

Sorption of OTC complexes – Taking complexation of OTC into account, Ca^{2+} can interfere with OTC-sorption via three processes. Besides competing for cationic exchange sites, OTC complexes with bivalent and trivalent metal ions (like Ca^{2+}) in the soil solution. If these complexes have lower sorption coefficients, or when they also compete with freely dissolved cations for cation exchange sites, overall sorption decreases with increasing Ca^{2+} concentrations. Furthermore, specific "surface-bridging" mechanisms could be involved (*5, 228, 229*), in which Ca^{2+} or autochthonous metal ions form a bridge between the negative mineral surfaces and the negative OTC-site.

For the evaluation of these explanations the percentages of OTC-Calcium complexes are plotted for $CaCl_2$ treatments of the two soils (Figure 2d). The percentages were calculated using the respective Ca^{2+} concentrations, the affinity constants of the different species and the species composition. Sorption of OTC is unaffected up to I of ~0.005 M (Figure 2c). Above this ionic strength, a decrease of the K_D is observed for both the $CaCl_2$ and the NaCl treatment. As only Ca^{2+} forms complexes with OTC (Figure 2c), and already >50% of the OTC is complexed at $CaCl_2$ induced ionic strength of 0.005 M (Figure 2d) complexation does not seem to decrease sorption directly.

Figueroa et al. (*229*) observed that OTC-sorption to pure clay minerals was higher in Ca^{2+}- than in Na^+-exchanged clays and took this as evidence for a surface-bridging mechanism. However, in another study by the same group, the sorption of OTC to clay surfaces was thought to be mainly a cation exchange mechanism because of its

reversibility (*237*). This latter conclusion is in line with the findings obtained in the present study with whole soils.

Implications for experimental test design and environmental fate modeling

The mobility of the test compounds in soil increases in the order SCP >TYL >OTC. OTC is immobile in the both soils, TYL is immobile in soil C and relatively mobile in soil S, while SCP is very mobile in both soils. Regarding the environmental risk assessment it appears that the effect of ionic strength on soil sorption within the environmentally relevant range can be neglected.

In this regard it is particularly important that veterinary pharmaceuticals reach the soil environment as constituent of manure, which contains high ammonium concentrations and hence provides an input of base into the soil solution, leading to an increase of the soil solution pH. Subsequent to manuring the soil, the ammonium nitrogen will undergo biological conversion to nitrate, a process which releases protons and thus decreases soil solution pH (*210*). Hence, this important determinant of sorption behavior of veterinary pharmaceuticals can vary considerably. Therefore, it appears sensible to evaluate the pH-dependence of a given pharmaceutical if the pH-variations may cause a significant change in soil mobility, as is the case for SCP.

To that end, we suggest obtaining sorption data at different pH-values, such that the speciation parameter α assumes values of <0.2, about 0.5 and >0.8, as long as species occur at environmentally relevant pH ranges. Species-specific sorption coefficients can then be estimated from the intercept and the slope of a linear regression of the apparent sorption coefficient versus the speciation parameter α according to Equation 3. This demonstrates that the process-based research of sorption of veterinary antibiotics not only yielded deeper insight into the mechanisms involved in sorption of these chemicals. It also resulted in an approach, which is applicable to sorption of ionisable chemicals in general and therefore contributes to improving the risk assessment of environmental chemicals.

Acknowledgments

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Chapter 7

Prediction of soil sorption coefficients of veterinary pharmaceuticals in dependence of soil properties by partial least squares regression: An evaluation

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Submitted

Abstract

Environmental exposure assessment of veterinary pharmaceuticals requires estimating the soil sorption coefficient (K_D). The present study investigates the correlation between soil properties (pH, organic carbon content, clay content, cation exchange capacity, aluminum-oxyhydroxide content and iron-oxyhydroxide content) and the sorption coefficient. To that end, sorption coefficients of three ionizable model anti-microbial agents, oxytetracycline, tylosin, and sulfachloropyridazine were determined in 11 soils in relation to soil solution pH. The ranges of apparent K_D-values are 950-7200 L/kg (oxytetracycline), 10-370 L/kg (tylosin), and 0.4 to 35 L/kg (sulfachloropyridazine). The organic carbon content explains <30% of the variation in K_D-values and is therefore unsuitable as sole prediction parameter. Partial least squares (PLS) models considering the soils' cation exchange capacity, the pH and the contents of organic carbon, clay, and aluminum and iron oxyhydroxides explain up to 78% of the variation in K_D, with confidence intervals spanning a factor of thirty. We found species-specific K_D of oxytetracycline and sulfachloropyridazine, demonstrating the influence of soil pH and subsequent speciation. Upon applying PLS to the separate species, the width of the confidence intervals of the K_D estimates are reduced to less than one order of magnitude for both tylosin species and the negatively charged oxytetracycline and sulfachloropyridazine species. However, the species-specific sorption models did not improve the estimation of apparent sorption coefficients of species-mixtures.
Given the complexity of sorbate-soil interactions for veterinary pharmaceuticals, we suggest that the applicability of the PLS regression models as well as information from more mechanistically oriented sorption studies must be investigated for the estimation of sorption coefficients. Possibly, this information can be used at lower tier levels, to decide whether further sorption studies are necessary.

Introduction

Veterinary pharmaceuticals (VPs) are widely used to protect animals from diseases or promote growth (*189*). They are currently the subject of growing concern, because residues have been found in agricultural soils, groundwater, surface waters and even drinking water (*195-197*). More than 70% of the veterinary pharmaceuticals used in the EU are anti-microbial agents (*183-185*). In 1999 approximately 3900 tons (*181, 182*) of anti-microbial agents were sold in the EU for veterinary purposes. Since 1997 (*186, 187*) an environmental risk assessment is part of the registration procedure.
The risk assessment includes the fate of VPs to predict concentrations in the environmental compartments of interest. For most VPs, substantial fractions leave the treated animals unchanged, as conjugates or as active metabolites via urine and feces (*188-191, 193*). While degradation can occur during manure storage, considerable

fractions of the compounds can reach the soil environment via manure application. Hence, sorption of VPs in the soil is one of the key processes once these compounds have entered the environment. The degree of binding to soil particles is quantified using the sorption coefficient K_D (L/kg), which is defined as the ratio of the concentration in dry soil (mg/kg) divided by the concentration in the aqueous phase (mg/L) after equilibration. Higher tier assessment of the environmental risk of VPs requires the experimental determination of this parameter according to standard protocols (*211, 238*). The sorption coefficient partially influences how fast veterinary pharmaceuticals are transported through the soil column into the groundwater or surface water. In addition, VPs with a high tendency to sorb to soil are to a much lesser degree biologically available for exerting adverse effects and for biodegradation. As a consequence, it is necessary to have the tools to arrive at a realistic assessment of the sorption behaviour and associated risk of veterinary pharmaceuticals.

Many VPs are ionizable compounds with pK_A-values within the pH range of natural soils. Hence, they can occur in the environment as negative, neutral, zwitterionic and positively charged species, depending on the environmental conditions. These species have different chemical properties and thus different sorption interactions with soil or sediment. Soil mineral surfaces are generally negatively charged. Hence, cationic species will be elecrostatically attracted while anionic species will be repulsed from these surfaces. However, if anions and zwitterions can complex with divalent and trivalent cations, the complexes can sorb to these negative soil surfaces via electrostatic attraction or more specific surface-bridging mechanisms. Furthermore, all VPs with polar functional groups can undergo sorption via polar interactions like hydrogen bonding with mineral and organic fractions of the soil (*5, 228, 230*). In addition, neutral species can absorb via hydrophobic interactions with the organic material in the soil.

The current risk assessment paradigm assumes that the soil-water partitioning of organic compounds is dominated by hydrophobic interactions of the sorbate molecules with water and the organic carbon fraction of the soil. As a result, it is present practice to predict sorption coefficients to soil organic matter (K_{OC}) on the basis of the VP octanol-water partition coefficient. Subsequently, soil-specific K_D-values are estimated by accounting for the organic carbon content of the soil of interest. However, this approach is not applicable to ionizable and polar compounds like surfactants (*203, 239*), some pesticides (*10, 204, 223, 240*) and veterinary pharmaceuticals (*41, 182, 225, 229, 237*) because the sorption does not seem to be dominated by hydrophobic interactions. As a consequence, available tools that enable to extrapolate soils sorption coefficients from one soil to another do not exist.

The present investigation evaluates whether a multivariate statistical technique can contribute to the risk assessors ability to predict the complex process of sorption of VP. To that end, we chose oxytetracycline (a tetracyclin), sulfachloropyridazin (a sulfonamide) and tylosin (a macrolide) as model compounds representing the most important classes of VPs in the EU. In the Netherlands (1999) and the Weser-Ems

district in Germany (1997), more than 50% of the veterinary anti-microbial agents used were tetracyclins, 21% were sulfonamides and 12% were macrolids (*184, 188, 189*). Soil sorption coefficients of these chemicals were obtained for 11 soils which had been selected based on a multivariate analysis of a wide array of soil characteristics to represent a wide range of properties (*241*). Furthermore, the pHs of the soils were artificially varied, to investigate sorption of the different species separately. Partial least squares regression modeling was employed to develop relationships between the soil properties and the sorption coefficients. The resulting regression models were tested for their ability to accurately predict sorption coefficients.

Experimental section

Chemicals

Methanol (CH_3OH) and acetonitrile (CH_3CN) used were of HPLC-grade (respective suppliers: Baker Mallinckrodt, Deventer, The Netherlands and Boom, Meppel, The Netherlands). Sulfachloropyridazine (SCP), tylosin hemitartrate dihydrate (TYL), oxytetracycline hydrochloride (OTC) and erythromycin A dihydrate were all purchased from Riedel-De Haën (Darmstadt, Germany) and used as received. Their respective purities were 98.8%, 91.4%, 96.2% and 94.4%. Table 1 shows the structural formulas and the sorption relevant physical-chemical properties. Stock solutions (1000 mg/L) were prepared in HPLC-grade methanol (Baker Mallinckrodt, Deventer, The Netherlands). 2-Morpholinoethanesulfonic acid (MES) buffer and formic acid (>98% purity) were obtained from Fluka (Zwijndrecht, The Netherlands), $CaCl_2 \times 2H_2O$ and NaCl and NaN_3 were supplied by Merck (Amsterdam, The Netherlands). Water purified to a resistance of ≥18 MΩ (Millipore Purification System, Waters, Amsterdam, The Netherlands) was employed throughout the experiments.

Soils and soil selection

Table 2 shows the sources of the 11 field soils and some of their properties. The soils were sampled between September and December 1997 by the Dutch Institute of Public Health and the Environment (RIVM), and were stored at 4°C until drying to a constant weight at 25 ±1 °C. Afterwards soils were sieved (1 mm mesh size) and stored at 21 ± 2 °C. The soils are a subpopulation of about 35 Dutch soils which have been characterized by the RIVM and analyzed by principal component analysis with regard to the soil properties (*241*). For the purpose of the present study, four soils were selected such that each of those represents an extreme of the first and second principal component. Those four soils representing the extreme points on the diagonals of the plane spanned by the first and second principal component were also selected. In addition, three soils close to the intersection of the two principal components were included in the present

investigation. In this manner it is possible to study a wide variety of soil properties with a rather limited number of soils.

Table 1: Some physicochemical properties, the molecular structure and the different species of SCP (1a), TYL (1b) and OTC (1c). The reference numbers are given between brackets.

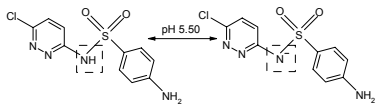
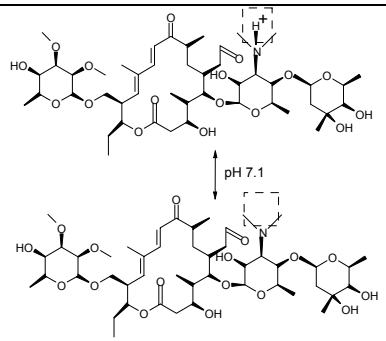
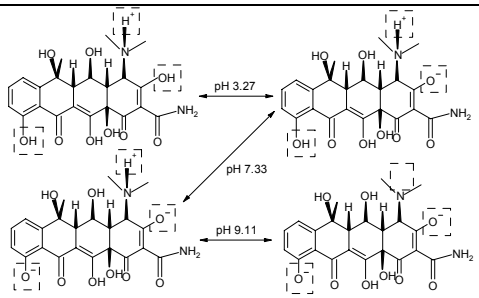
	SCP	
MW (g/mol)	284.72	
Solubility (g/L)	7.0 (*212*)	
log K_{OW}	0.31 (*213*)	
pK_A	5.5 (*214*)	
	TYL	
MW (g/mol)	917.14	
Solubility (g/L)	5.0 (*215*)	
log K_{OW}	2.50 (*215*), 1.63 (*216*)	
pK_A	7.1 (*212*)	
	OTC	
MW (g/mol)	460.44	
Solubility (g/L)	1.0 (*217*)	
log K_{OW}	-1.12 (*215*)	
pK_A	3.27, 7.33, 9.11 (*217*)	
Log pK_{OTC-Ca}	OTC^{2-} = 3.81 (±0.07) (*207*) OTC^{-} = 2.93 (±0.02) (*207*) OTC^{0} = 2.42 (±0.02) (*218*)	

Table 2: The origin and properties of the 11 test soils. The pH was measured in a 10 Mm $CaCl_2$ solution, OC and CEC stand for the organic carbon content (CO_2 evolution during combustion) and the cation exchange capacity, respectively. The soil content of amorphous iron and aluminum oxyhydroxides are abbreviated as Fe-Ox and Al-Ox, respectively.

Code	*Origin*	*pH (CaCl$_2$)*	*OC %*	*Clay %*	*CEC mM/ 100g*	*Fe-Ox mmol/ kg*	*Al-Ox mmol/ kg*
1	Boxtel (river bank)	6.09	2.2	5.8	8.91	134.18	18.19
2	Eendenkooi	5.59	2.2	51.6	24.84	111.14	63.95
3	Eijsden (river bank)	7.24	3.2	10	13.07	184.80	37.86
4	Eijsden	7.38	3.9	13.3	20.52	43.13	28.14
5	Ermelo	3.41	2.5	0.2	1.61	5.37	13.22
6	Hank (Estuarine river bank)	7.36	5.9	8.2	39.33	214.41	66.13
7	Lheembroekerzand	3.59	7.0	1.4	2.74	24.14	79.98
8	Maatheide	6.33	3.1	1.2	4.18	133.33	61.98
9	Nieuwerkerk	7.35	2.6	11.2	12.47	34.28	6.92
10	Oudekerk a/d IJssel	4.88	12.2	27.3	39.04	234.54	247.96
11	Valkenswaard	4.55	4.5	1.3	2.39	8.25	20.72

Sorption studies of SCP, TYL and OTC

Batch sorption experiments were performed according to OECD technical guideline 106 (*211*). The soil was pre-incubated for 24 - 48 hours in 10 mL (SCP & TYL) or 20 mL (OTC) vials containing 5 or 15 mL electrolyte solution (10m $CaCl_2$ and 10mM NaN_3). After pre-incubation, sulfachloropiridazin (SCP) was spiked at 1.0 to 3.0 mg/L, Tylosin (TYL) was spiked at 3.0 to 7.5 mg/L, and oxytetracycline (OTC) at 7.5 to 15 mg/L, and soils were incubated for 2 days. Experiments were performed at 25 ±1 °C. The soil/water ratio was adjusted such that free dissolved aqueous concentrations usually were in a range of 0.1 to 2 mg/L, and a significant part of the substance was sorbed to the soil. In order to obtain the appropriate sorbent-solution ratios, experiments were repeated 2 or 3 times, each in triplicate. The chosen ratios (dw soil/volume water, kg/L) varied from $^1/_{10}$ to $^1/_2$, $^1/_{150}$ to $^1/_2$ and $^1/_{500}$ to $^1/_{50}$ for SCP, TYL and OTC respectively. A control without soil (triplicate) was used to confirm the initial amount of compound and to correct for sorption to the vial walls. The soil-water sorption coefficient (K_D) was the concentration of the test substance in the soil (C_{soil}) divided by the concentration in the aqueous phase (C_{water}) after equilibration (Equation 1):

$$K_D = \frac{C_{soil}}{C_{water}} \qquad (1)$$

Adjustment of pH

Besides the sorption experiments at ambient soil pH, pH was also adjusted by adding NaOH or HCl. The added amount was chosen on basis of the soil pH, the added amount of soil and the pK_A of the compound (for OTC the pH was only varied to obtain data of the zwitterionic and single negative species, as these species mainly occur at natural soil

pH). Acid or base was added to a maximum concentration of 30 mM and the conductivity was kept constant by adding NaCl (5.6 (± 1.6) mS/cm). We aimed to obtain 3 partition coefficients (in triplicate) at ~pK_A and >0.5 pH-units above and below the pK_A. From the pH measured in the supernatant obtained after centrifugation of the soil suspension of the fraction of species 1 (α) and 2 (1-α) were calculated with $\alpha = 1/(10^{pH-pKa})$.

Chemical analysis

Supernatant samples were filtered using a 0.45 µm PVDF w/GMF filter (Whatman, Maidstone, UK), when filtrate contained too much particulate matter. The filter was pre-flushed with at least 1 mL supernatant solution to minimize losses due to sorption to the filter. Directly after samping, pH and conductivity were measured, and the samples were stored at 4ºC in the dark for 7 days at maximum. Re-injection of samples after different storage times showed no degradation within this period.

The concentrations of SCP, TYL and OTC were determined by reversed phase HPLC using a variety of C_{18}-columns. For the analysis of SCP and OTC, the solvent delivery system consisted of a Separations GT-103 degasser (Separations, HI-Ambacht, The Netherlands), a Varian ProStar 420 autosampler, a Varian 9012 solvent pump (Varian, Bergen op Zoom, The Netherlands). All analyses were performed with an injection volume of 20 µL and at a flow-rate of 700 µL/min with mixtures of water - CH_3CN containing 0.1% formic acid to adjust the pH to 2.6. SCP and OTC were analyzed isocratically using a 70%:30% and 85%:15% solvent mixture, respectively. TYL was analyzed using gradient elution starting with 100% H_2O (0.1% formic acid) for one minute followed by an increase of the CH_3CN-fraction to 30% in 3 minutes and to 95% in the subsequent 3 minutes, where it was kept for another three minutes before returning to the starting conditions. The solvents were delivered by a Perkin Elmer series 200 LC-system (Perkin Elmer, Oosterhout, The Netherlands). OTC and SPC were detected by UV-absorption (Shimadzu SPD-10AV, 's-Hertogenbosch, The Netherlands) at wavelengths of 360 nm and 260 nm, respectively. TYL and erythromycin (internal standard) were detected by electrospray ionization mass spectrometry (Perkin Elmer Sciex API 365) in the single monitoring mode at m/z 916 and 734, respectively. To that end, 25% of the column effluent was diverted to the electrospray ion source, which was operated in the positive ion mode.

Data analysis

Apparent sorption coefficients (K_D') were determined for each individual sorption experiment (performed in triplicate). K_D' can be considered as sum of the species-specific coefficients K_{D1} and K_{D2} weighted with their respective fractions α and 1-α according to equation 2 (*223*).

$$K_D' = K_{D1}*\alpha + K_{D2}*(1-\alpha) \quad (2)$$

Upon rearranging Equation 3 is derived, which facilitates estimation of K_{D1} and K_{D2} by linear regression of K_D' against α.

$$K_D' = K_{D2} + \alpha*(K_{D1} - K_{D2}) \quad (3)$$

K_{D2} can be obtained as the regression intercept, and K_{D1} can be estimated as the sum of slope and intercept (the standard errors can be estimated as well).
For the evaluation of the relationships between sorbent properties and the sorption coefficients the K_D data were log transformed such that normal-distributed data were obtained (*69*). The relationships between sorption coefficients (response variable) and soil characteristics (predictor variables) were established using partial least squares (PLS) regression (Scan 1.1(*242*)) by fitting the experimental data to Equation 4,

$$LogK_D' = C + \sum c_i * p_i \quad (4)$$

with p_i and c_i being a soil property and its respective partial least squares regression coefficient and C is the regression constant.
The predictive power of the PLS model was calculated using the "leave one out method". Besides the analysis of the total dataset, the calculated sorption coefficients of the separate species (Equation 3) were also analyzed. For every sorption coefficient of a single species to a single soil the average and a maximum and minimum value that represented the confidence interval of the calculated species-specific sorption coefficient was used.

Results & Discussion

Sorption data
Table 3 summarizes the experimentally determined sorption coefficient data determined in the eleven Dutch soils at natural pH. The average K_D'-value for OTC is 3100 L/kg with a minimum and maximum value of K_D' of 950 and 7200 L/kg. The corresponding data for TYL are 130, 10 and 372 L/kg, and for SCP 6.9, 0.4 and 34.8 L/kg.

Table 3: Apparent sorption coefficients (K_D') for the three model VPs in the test soils recalculated to an equilibrium concentration of 0.5 mg/L in the supernatant using isotherms that were fitted on aqueous concentrations around a 0.5 mg/L level (maximum concentration range: 0.03 - 3 mg/L).

Soil-Nr	*K_D OTC (L/kg) (at C_W= 0.5 mg/L, ± SE)*	*K_D TYL (L/kg) (at C_W= 0.5 mg/L, ± SE)*	*K_D SCP (L/kg) (at C_W= 0.5 mg/L , ± SE)*
1	3913 (±173)	125.4 (±6.1)	2.4 (±0.06)
2	5257 (±109)	372.4 (±6.4)	3.7 (±0.05)
3	1126 (±64)	49.9 (±0.3)	0.9 (±0.02)
4	946 (±57)	76.2 (±1.2)	0.9 (±0.02)
5	2708 (±242)	16.6 (±2.5)	8.1 (±0.14)
6	4687 (±61)	293.4 (±22)	2.4 (±0.13)
7	7199 (±513)	12.4 (±0.4)	15.0 (±0.06)
8	960 (±95)	44.4 (±3.7)	5.1 (±2.0)
9	1626 (±82)	387.0 (±13)	0.4 (±0.04)
10	3347 (±1345)	36.6 (±0.57)	34.8 (±1.3)
11	2630 (±261)	10.4 (±0.4)	2.3 (±0.02)
Statistics			
Average	3127	129.5	6.9
St. Dev.	2009	147.6	10.2
RSD (%)	64	114	147

While the amplitude of the K_D'-values is less than one order of magnitude for OTC, the variability in the sorption data is much larger for TYL and SCP, and amounts to a factor of 37 (TYL) and 87 (SCP). Hence, there is a considerable variation in the sorption coefficients of the three model compounds in dependence of the sorbent.

Normalization to organic carbon

Figure 1 presents the sorption data in the way that is usually employed in organic carbon normalization. The logarithmized values of K_D' are plotted against the logarithm of the organic carbon content of the soils. For none of the compounds an obvious relationship between the values of K_D' and the OC-content of the soils appears to exist. This confirms that organic carbon content is not a suitable normalization basis for VP soil sorption coefficients (*41*). In addition, when comparing the sorption data of the three model compounds per soil, no consistent variation of the K_D'–values with the different soils can be discerned. This can be expected, because the soils have different pH-values, and compounds will have different species-compositions, with different sorption properties and sorption mechanisms.

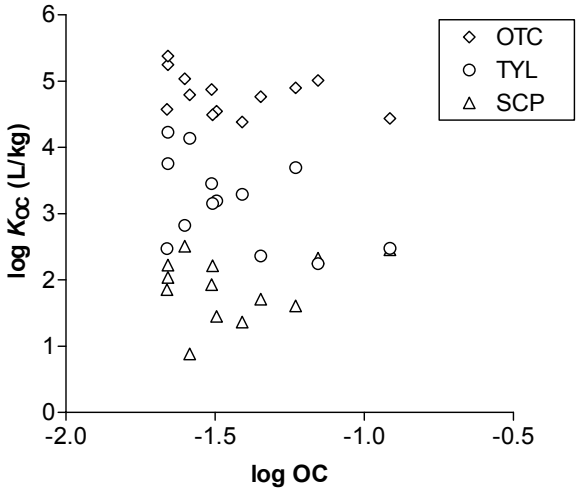


Figure 1: The logarithm of the apparent sorption coefficients of the three model compounds against the logarithm of the organic carbon content of the soils under investigation.

Species-specific sorption coefficients

In a first attempt to reduce the variation in the data we determined the sorption coefficient for the different species for each of the VPs. The respective data are presented in Table 4 and reveal that the sorption coefficients of the prevalent species under alkaline conditions are significantly lower than those of the species under acidic conditions for OTC (average OTC^{+} & OTC^{0} = 1180 vs. OTC^{-} & OTC^{2-}= 3930 L/kg) and SCP (average SCP^{-}= 2.1 vs. SCP^{0}= 19.1). In addition, the values for the species-specific sorption coefficients K_{D1} and K_{D2} display less variation than K_D' except for the negative OTC-species, as is evidenced by the value of the relative standard deviation. In contrast to OTC and SCP, the averages of, TYL^{+} (140) and TYL^{0} (114) do not differ significantly, and the relative standard deviations of K_{D1} and K_{D2} are similar to that of K_D'. Hence consideration of speciation did not reduce the variability. Contrary to the consistent increase in K_D' with decreasing pH observed for OTC and SCP, K_D' decreased with decreasing pH in soils 2, 6, 10 and 11.

Table 4: The species-specific sorption coefficients (K_{D1} & K_{D2}) of the three model compounds in the soils.

Soil-Nr	K_{D1} OTC^+ & OTC^0 *(L/kg± SE)*	K_{D2} OTC^- & OTC^{2-} *(L/kg± SE)*	K_{D1} TYL^+ *(L/kg± SD)*	K_{D2} TYL^0 *(L/kg± SD)*	K_{D1} SCP^0 *(L/kg± SD)*	K_{D2} SCP^- *(L/kg± SD)*
1	3925 (±371)	472 (±76)	65.0 (±5.3)	4.7 (±0.5)	7.0 (±0.4)	0.71 (±0.10)
2	5312 (±218)	432 (±43)	341 (±10.8)	440 (±14)	7.5 (±0.6)	1.04 (±0.46)
3	- [a]	- [a]	52.3 (±3.7)	5.0 (±0.3)	29.1 (±6.0)	0.48 (±0.30)
4	887 (±26)	906 (±64)	86.3 (±4.1)	70.7 (±5.0)	35.3 (±2.9)	0.57 (±0.14)
5	2340 (±104)	578 (±36)	- [a]	- [a]	10.8 (±1.0)	1.50 (±0.18)
6	5329 (±157)	4087 (±184)	49.7 (±4.2)	73.1 (±4.5)	26.9 (±2.1)	2.13 (±0.36)
7	6455 (±298)	1236 (±103)	13.5 (±1.7)	11.7 (±1.8)	16.1 (±0.5)	5.12 (±0.43)
8	1539 (±99)	247 (±28)	- [a]	- [a]	15.0 (±1.0)	5.25 (±0.52)
9	4169 (±368)	942 (±117)	694 (±14)	351 (±17)	- [a]	- [a]
10	3611 (±124)	1741 (±96)	26.8 (±2.1)	115 (±11)	40.8 (±1.5)	3.03 (±1.31)
11	- [a]	- [a]	5.9 (±0.2)	7.5 (±0.3)	2.8 (±0.2)	0.77 (±0.06)
Statistics						
Average	3730	1182	140	114	19.1	2.1
St. Dev.	1856	1183	232	166	13.1	1.8
RSD (%)	50	100	166	146	68	89
K_D of species different? (T-test)	Yes (p= 0.0024)		No (p= 0.52)		Yes (p= 0.0022)	

[a] α was varied by less than 0.5 units, as a result, regression was not possible.

OLS-modeling of sorption coefficients

In a first attempt to evaluate predictive relationships between soil properties and sorption coefficients, ordinary least squares (OLS) regressions were employed to establish monovariate models. The results for the K_D´ data are detailed in Table 5 and those for the species-specific K_D data in Table 6. The results of the OLS regression for the individual soil properties reveal a number of interesting details. The sorption of TYL mainly correlates with the clay content and the related CEC, 50% and 39% of the variability of the K_D' of TYL is explained by these factors respectively (Table 5). This is primarily reflected by the TYL cation (Table 6), indicating that a cation exchange mechanism at mineral (clay) surfaces is important for the sorption of TYL, which is in line with literature data on other weak organic bases (*5, 10, 234*). The sorption of TYL^0 shows no relation with the organic carbon content so hydrophobic interactions do not seem to be important, even for this relatively hydrophobic compound (Table 1). Polar interactions with various polar surfaces likely determine the sorption of TYL^0 (Table 6). The sorption of OTC and SCP, occurring as neutral or anionic species under natural soil pH, seems to be affected by other soil properties. Speciation parameter α can explain 54% and 32% of the variation for OTC and SCP respectively. This is in line with the significantly lower sorption coefficients of the anionic species, observed in Table 4 and literature (*182, 205,*

225, 228-230). Anionic species usually have low sorption coefficients, as they are repulsed by negatively charged mineral surfaces of a soil (*5, 43, 223*). The sorption of the anionic species of OTC primarily correlates with the organic carbon content (49%) and the cation exchange capacity (27%). The correlation with the organic carbon content might be due to polar interactions with the organic carbon surfaces (*1, 230*) and the correlation with the CEC might be due to sorption by surface-bridging mechanisms with cations to negatively charged surfaces (*11, 228, 229*).

Table 5: Results of the partial least squares (PLS)-modeling of the sorption data for SCP, TYL, and OTC. The model has the form: $\log K_D' = c_1 \times \alpha + c_2 \times \log OC + c_3 \times \log clay + c_4 \times \log CEC + c_5 \times \log [Fe\text{-}ox] + c_6 \times \log [Al\text{-}ox] + C$. The parameter r^2 is employed to indicate the fraction of variability in $\log K_D'$ as explained by the overall PLS model and by ordinary least squares (OLS) regression of $\log K_D'$ against the individual independent variables.

Overall PLS	*OTC*		*TYL*		*SCP*	
r^2	0.69		0.68		0.78	
r^2 *–cross validated*	0.66		0.64		0.74	
Regression Details	coefficient	r^2 (OLS)	coefficient	r^2 (OLS)	coefficient	r^2 (OLS)
α	1.138	0.54	409	0.003	0.537	0.32
log OC	0.752	0.26	-1.196	0.16	-0.051	0.29
log Clay	0.160	0.06	0.528	0.50	-0.438	<0.01
log CEC	0.186	0.08	0.559	0.39	0.341	<0.01
log [Fe-Ox]	-0.089	0.05	0.168	0.20	0.053	0.05
log [Al-Ox]	0.001	0.24	-0.424	0.01	0.886	0.51
C	4.008		0.074		-2.104	

The greatest value of r^2 for the OLS-models amounts to 0.54, indicating that the models explain hardly more than 50% of the variation in the data. Hence, the predictive capability of the OLS models is insufficient for estimating sorption coefficients in dependence of soil properties. In addition, it should be noted that some soil properties are highly correlated (e.g. clay content and CEC, r^2: 0.83). Therefore, the results of the ordinary least squares (OLS) of the three compounds (Table 5) and their species (Table 6) should be interpreted with care when they are used to identify mechanisms underlying the sorption of the different species of the VPs.

Table 6: Results of the partial least squares (PLS)-modeling of the sorption data for $OTC^{+\ \&\ 0}$, $OTC^{-\ \&\ 2-}$, TYL^{+}, TYL^{0}, SCP^{0} and SCP^{-}. The model has the form: log K_D = c_2×log OC + c_3×log clay + c_4×log CEC + c_5×log [Fe-ox] + c_6×log [Al-ox] + C. The parameter r^2 is employed to indicate the fraction of variability in log K_D as explained by the PLS model and by ordinary least squares (OLS) regression of log K_D against the individual independent variables.

PLS	*OTC^{+} & OTC^{0}*		*OTC^{-} & OTC^{2-}*		*TYL^{+}*		*TYL^{0}*		*SCP^{0}*		*SCP^{-}*	
r^2 (OLS)	0.15		0.89		0.83		0.78		0.38		0.58	
r^2 (OLS)	-[a]		0.84		0.74		0.67		0.10		0.40	
Regression Details	coef.	r^2 (OLS)	coef.	r^2 (OLS)	coef.	r^2 (OLS)	coef.	r^2 (OLS)	coef.	r^2 (OLS)	coef.	r^2 (OLS)
log OC	0.058	0.04	1.542	0.49	-0.776	0.28	-3.878	0.01	0.268	0.18	-1.371	0.29
log Clay	0.018	0.04	-0.241	0.09	0.565	0.52	-0.909	0.16	0.071	0.07	-1.188	0.12
log CEC	0.017	0.02	0.786	0.27	0.437	0.32	2.017	0.21	0.109	0.11	1.501	0.03
log [Fe-Ox]	0.020	0.03	-0.169	0.07	-0.070	0.05	-1.126	0.14	0.129	0.16	-0.689	0.01
log [Al-Ox]	0.012	0.02	-0.315	0.12	-0.521	0.17	3.370	0.31	0.254	0.31	1.920	0.28
C	3.529		4.848		1.796		-10.56		0.829		-6.597	

[a] Could not predict any of the variation.

PLS modeling of K_D'

In view of the inappropriateness of the monovariate OLS-models, multivariate models, which allow for multiple independent predictor variables, are a logical choice for establishing relationships for prediction of soil sorption coefficients from soil property data. In this investigation the partial least squares (PLS) regression technique is employed, because it eliminates statistical artifacts due to inter-correlation of the soil properties. The ionization parameter α is used in the model to reflect the influence of the soil solution pH on the ionization of the VP molecules. Cation exchange is an important process in the sorption of cationic and amphoteric molecules or complexes to negatively charged mineral surfaces (*5, 10, 228, 229, 243*). Hence, the cation exchange capacity (CEC) and the contents of clay and amorphous Fe- and Al-oxyhydroxides (Fe-Ox and Al-Ox) were included in the set of soil properties. In addition, hydrophobic sorption mainly occurs in the organic part of the soil, so the organic carbon content (OC) is also used as an independent variable in the PLS model.

A total of 146, 114, and 99 experimental sorption data for OTC, TYL, and SCP in eleven soils at different pH-values were used for the model development. Table 5 details the coefficients of the PLS regression equation along with the r^2- and the cross-validated r^2-values as well as the r^2-values of the ordinary least squared regression of log K_D' versus the individual independent variables. A total of 68% to 78% of the variation observed in the partition coefficient can be explained by the selected variables. The predictive capabilities of the regression models is reflected by the values of the leave-one-out cross validated r^2, which amounts to 66%, 64%, and 74% for OTC, TYL, and SCP, respectively. A comparison of the measured and predicted sorption coefficients is given in Figure 2. The predicted sorption coefficients have uncertainty intervals of a factor 30 (OTC, TYL), and 7 (SCP). The overall variation in the experimental sorption data

(including sorption coefficients of soils with adjusted pH) is a factor of 630, 1100 and 90 for OTC, TYL and SCP respectively. Hence, the prediction models narrow down the variation by more than one order of magnitude. However, the prediction of K_D' is still accompanied with some degree of uncertainty.

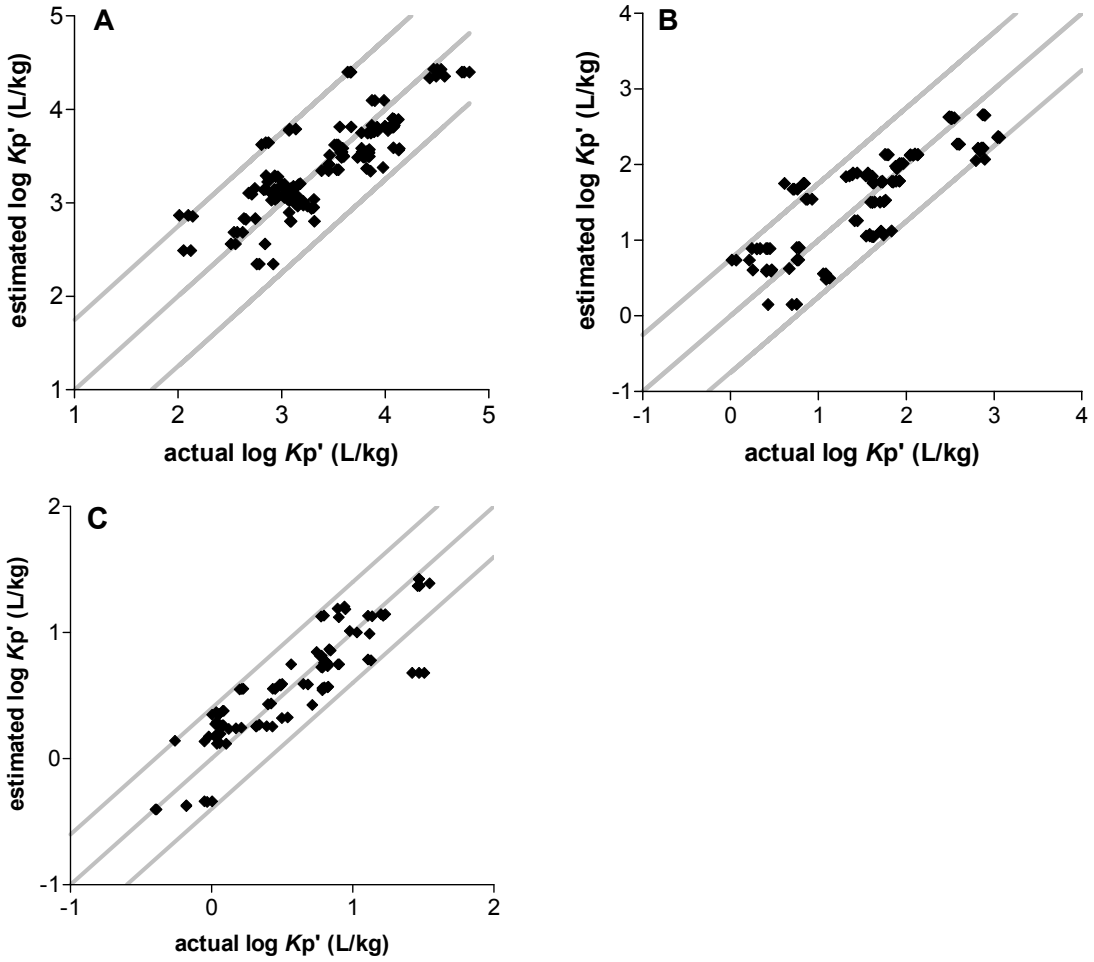


Figure 2: Evaluation of the predictive value of the PLS-models developed for oxytetracycline (a), tylosin (b) and sulfachloropyridazine (c) in a plot of the predicted versus the measured value. The outer lines correspond to the predicted value plus and minus 0.75, 0.75, and 0.4 log units for OTC, TYL, and SCP, respectively.

PLS modeling of species-specific K_D-data

To improve the model, sorption coefficients of the separate species (Table 6) were analyzed using PLS-fitting. 89%, 83%, and 78% and 58% of the variation observed could be explained by the developed PLS model for OTC^- (& OTC^{2-}), TYL^+, TYL^0 and SCP^- respectively, and the predictive capabilities were 84%, 74%, 67% and 40% (Figure 3a, 3b & 3c).

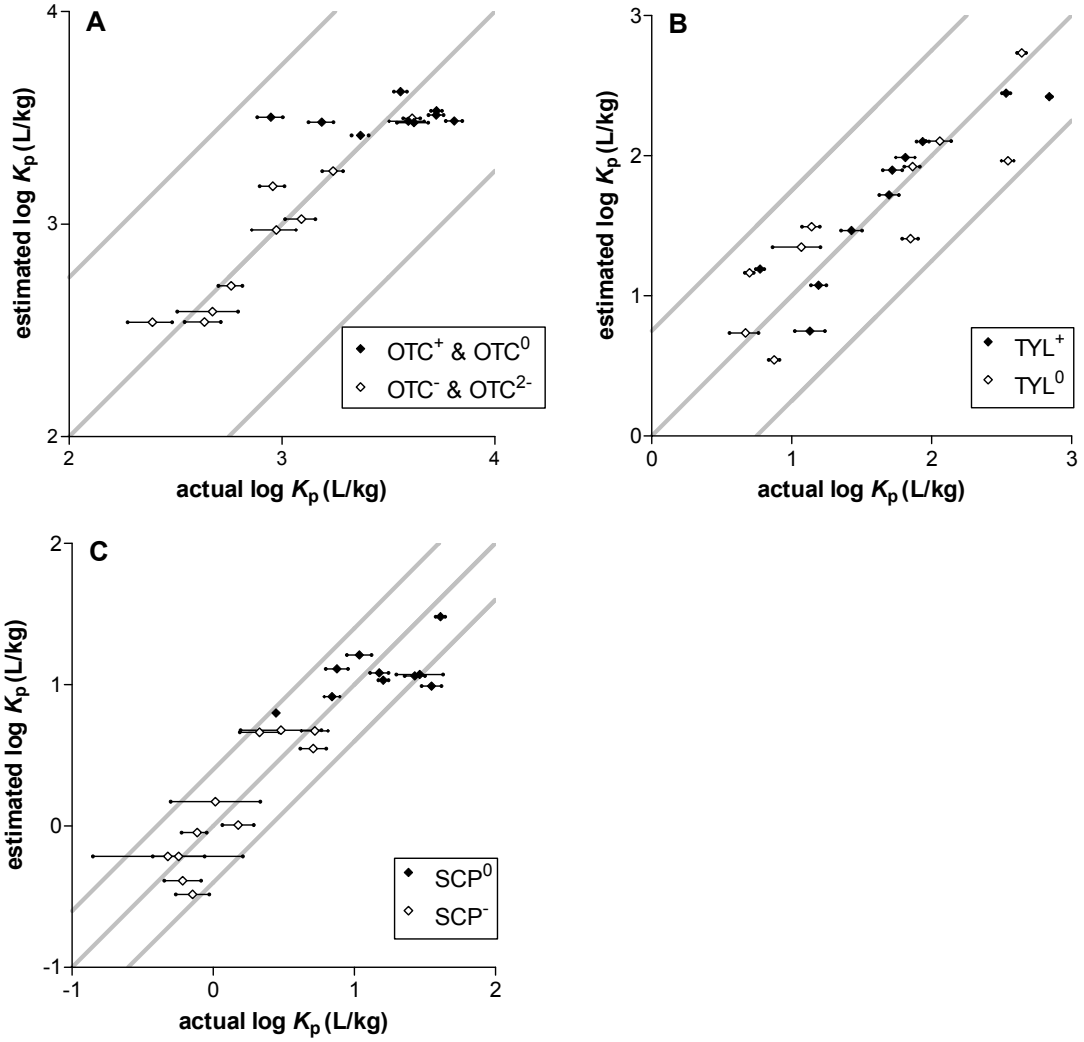


Figure 3: Evaluation of the predictive value of the species-specific PLS-models developed for the species of tetracycline (a), tylosin (b) and sulfachloropyridazine (c) in a plot of the predicted versus the measured value (error bars represent standard deviations). Sorption coefficients were normalized to an aqueous concentration of 0.5 mg/L. The outer lines correspond to the predicted value plus and minus 0.75, 0.75, and 0.4 log units for OTC, TYL, and SCP, respectively.

The small variation of the sorption coefficients of OTC^0 (& OTC^+) and SCP^0 could not be reduced further by the developed PLS-model (r^2: 0.15 & 0.39, r^2-crossvalidated: <0.00 and 0.10 respectively). The predicted species-specific sorption coefficients have confidence intervals of one order of magnitude or less. Consequently, the species-specific model predicted sorption coefficients with similar (SCP, Figure 3c) or higher accuracy (OTC & TYL, Figure 3a & 3b) than the generic sorption model (Figure 2). Nevertheless, the species-specific models did not improve the prediction of observed sorption coefficients (K_D', Equation 2) of species mixtures (Figure 4). This can be attributed to the data-reduction that was associated with calculating species-specific sorption coefficients. First of all, the calculation of species-specific sorption coefficients resulted in only one sorption coefficient (with confidence intervals) per species, per soil. Furthermore, the calculation needed sorption coefficients that were normalized for the

aqueous concentration (0.5 mg/L was chosen, because this concentration was covered by all compounds and most soils). Sorption isotherms of polar and especially ionic species are typically nonlinear (*5, 226*). The variation of the aqueous concentrations of SCP and TYL were relatively small, 0.39 - 4.85 mg/L and 0.15 - 2.41 mg/L respectively, with variations within a soil that are generally smaller than a factor of 5, while the variation of the aqueous concentration of OTC were larger, ranging from 0.02 to 4.10 mg/L over all soils, and often more than a factor of 10 within one soil. The sorption coefficients (K_D') that were obtained with aqueous lower than 0.1 mg/L are marked with open diamonds, and it can bee seen that sorption coefficients determined at these aqueous concentrations are mostly underestimated by the model. These results indicate how important the concentration is for the outcome of a sorption experiment.

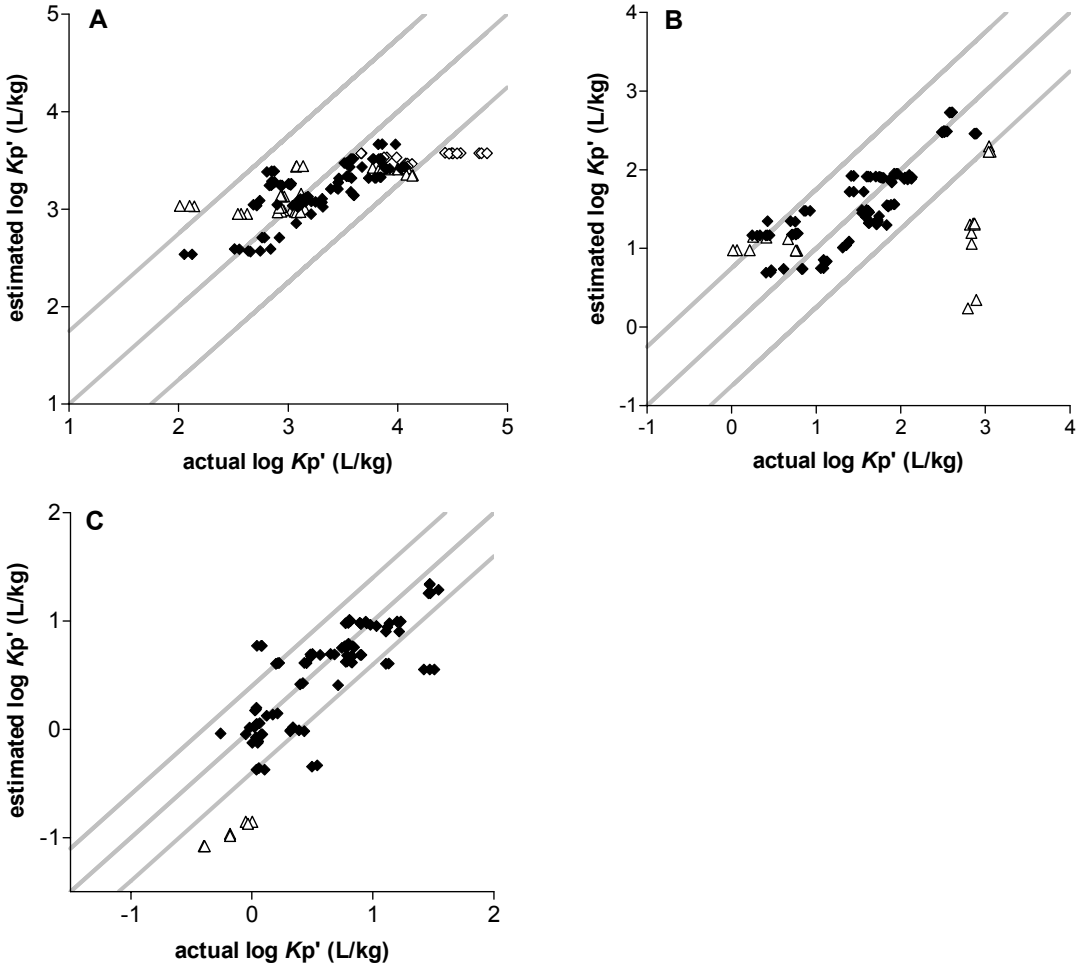


Figure 4: Evaluation of the predictive value of the species-specific PLS-models for the observed sorption coefficients (of species-mixtures) of oxytetracycline (a), tylosin (b) and sulfachloropyridazine (c). The outer lines correspond to the predicted value plus and minus 0.75, 0.75, and 0.4 log units for OTC, TYL, and SCP, respectively. The diamonds represent the data that was used to fit the species-specific models (Figure 3) (The open diamonds in Figure 4a represent the sorption coefficients that were obtained at an aqueous concentration that was lower than 0.10 mg/L). The triangles represent the data that was not used in the models.

Concluding, the species-specific models that have been developed are more correct from a mechanistic perspective, as they separate the species and the different soil properties that affect their sorption. However, the species-specific sorption models could not improve the predictability of observed sorption coefficients, because they were based on a reduced dataset.

Conclusions and implications for environmental risk assessment

The above discussion shows that the sorption of the tested veterinary pharmaceuticals are not (solely) determined by hydrophobic interactions with the organic carbon in a soil, therefore, their sorption cannot be predicted by organic carbon based models currently in use to assess risks of organic compounds in soils (*41*). Literature data (*43, 44, 199, 200, 204, 205, 219, 231-233, 244*) has shown that the soil sorption behavior of ionizable organic compounds dependent on pH and ionic strength, and can be reasonably well described by accounting for the speciation behavior in the soil solution. However, the present investigation shows that various soil properties influence the sorption behavior of the model VP besides sorbate speciation. PLS-regression modeling was employed to model sorption coefficients on the basis of soil properties. The model could predict the apparent sorption coefficients for a specific soil with an uncertainty interval of a factor 7 (SCP) to 30 (OTC and TYL) thereby reducing the variation of observed sorption coefficients more than one order of magnitude. The species-specific PLS-models could not predict the apparent sorption coefficients of species mixtures better.

The outcome of the modeling exercise therefore suggests a tiered approach for assessing the sorption coefficients. On an initial tier, a rather limited set of experimental data can be extrapolated on the basis of PLS modeling of the apparent K_D-values and taking the uncertainty of the prediction into account. A more accurate assessment of the sorption coefficients might be required, when this parameter is pivotal for the risk assessment. At this tier of the soil sorption assessment, species-specific K_D-values can be determined and subsequent PLS-modeling might provide sufficiently accurate predictions. A further refinement is possible by experimental determination of soil sorption coefficient and its concentration dependence in soils or soil types for which the VP under investigation poses an apparent risk, based on the lower tier assessments. In conclusion, the present investigation shows that sorption of VPs is strongly influenced by soil properties. PLS-regression-modeling provides for a tool to predict sorption coefficient in dependence of soil properties and can be implemented in a tiered risk assessment strategy.

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Chapter 8

Summary and discussion

Summary

The sorption of organic compounds to soil, sediments and dissolved organic matter affects the fate of organic compounds. Given the central role of this process in environmental transport, distribution, and (bio)degradation processes, it needs to be well-understood and well represented in risk assessments of chemicals in the environment. In this thesis soil-sorption of various organic compounds was studied in order to improve the scientific basis for representing sorption in risk assessment. Chapters 2 to 5 study the sorption of hydrophobic organic chemicals. They are mainly focused on the development and validation of passive sampling techniques to simplify the determination of freely dissolved concentrations and sorption coefficients. Chapters 6 and 7 explore the sorption of ionizable organic antimicrobial agents (often used in veterinary practice) with different soils and pore water composition. A short summary of the experimental findings will be followed by a discussion that relates the main findings of this thesis with current knowledge.

Chapter 2 describes the development and validation of a "solid phase dosing and sampling technique" to determine partition coefficients to a dissolved organic matrices. For this purpose, a PDMS (poly(dimethylsiloxane))-coated fiber was spiked with a series of polycyclic aromatic hydrocarbons (PAHs) and equilibrated with various concentrations of Aldrich humic acid. The depletion of the fiber was used to determine the partitioning to this dissolved phase. The sorption coefficients obtained are generally greater than those reported in the literature (*40*). The developed technique is a promising experimental approach for the determination of sorption coefficients to dissolved phases, since it does not need phase separation or spiking by a co-solvent.
In Chapter 3, a sediment dilution approach was used to determine sorption coefficients of various chlorobenzenes and chloroanilines to sediment. Contaminated sediment was diluted with various amounts of water, and a PDMS coated fiber was used to determine the reduction of the freely dissolved concentration. Sorption coefficients calculated from these data compared favorably to literature values (*14, 108*), indicating that this technique might be applied as an alternative for standard batch equilibrium techniques.
In Chapter 4 the sorption coefficients of PAHs to spiked, aged and field-contaminated soils were studied. It was observed that aging of a spiked soil over more than a year resulted in an increase of the sorption coefficient less than a factor 3. Sorption coefficients in field-contaminated industrial soils were up to two orders of magnitude higher. The objective of Chapter 5 was to measure soil pore water concentrations of a series of PAHs at increasing concentrations in soil. Pore water concentrations increased rather linear with soil concentrations until a certain maximum was reached. This maximum level corresponded very well with the aqueous solubility of the selected PAHs, showing that the fiber actually only measures the freely dissolved concentration and might be applied to determine aqueous solubility. More importantly, this also reveals

that this phenomenon should be considered in soil testing, since soils are often spiked to levels where aqueous solubility can be reached in the pore water.

Chapter 6 and 7 study the soil sorption of three veterinary pharmaceuticals (sulfachloropyridazine (SCP), tylosin (TYL), oxytetracycline (OTC)) to soil. These compounds are rather soluble in water and ionizable at environmentally relevant pH-values. The pH dependent speciation is relevant for their sorption behavior. Hence, the influence of the pH and ionic strength of the soil suspension on the sorption of these anti-microbial agents was investigated in Chapter 6. It was observed that all three compounds sorbed more strongly under acidic than under basic conditions. Furthermore, increasing the ionic strength led to a decrease in sorption of TYL and OTC, both of which contain basic functional groups, while the sorption of SCP, a weak organic acid, was not affected to a significant extent. In Chapter 7 sorption of these compounds to a series of soils was investigated in order to relate sorption coefficients to soil properties. The sorption of the different compounds correlate with different combinations of soil properties (e.g. soil pH, cation exchange capacity, clay content, organic carbon content and aluminum and iron oxides/hydroxides) indicating that multiple interactions are involved in sorption of these compounds. Accounting for the pH-dependent speciation of the three sorbates improved the predictive power of the regression models. However, a considerable fraction of the variation remained unexplained, rendering model predictions relatively inaccurate.

Discussion

Determining freely dissolved concentrations in complex matrices

The "freely dissolved" or "active" concentration is an important entity, which determines the bioavailability and fate of organic chemicals in the environment (*45-50*). Its accurate determination is a prerequisite for obtaining valid sorption coefficients to various environmental matrices. It is increasingly difficult to measure as the hydrophobicity of the test chemical increases and aqueous solubility decreases (*40, 52*). In addition, systematic errors due to incomplete phase separation and the consequent overestimation of the freely dissolved aqueous concentration are higher for the more hydrophobic chemicals. Figure 1 shows the ratio of the aqueous concentration measured by a classical liquid-liquid extraction of an aqueous phase that contains 1, 10 and 100 mg/L dissolved organic matter (DOM) as a function of the DOM sorption coefficient (K_{DOM}). It can be observed that even a DOM concentration of 10 mg/L (a concentration that is often exceeded in surface water and soil pore water) in an aqueous solution leads to an overestimation of the aqueous concentration of one order of magnitude for a compound with a log K_{DOM} of 10^6. As a result, overestimation of the aqueous concentration will lead to an underestimation of the sorption coefficient of the environmental matrix that is tested.

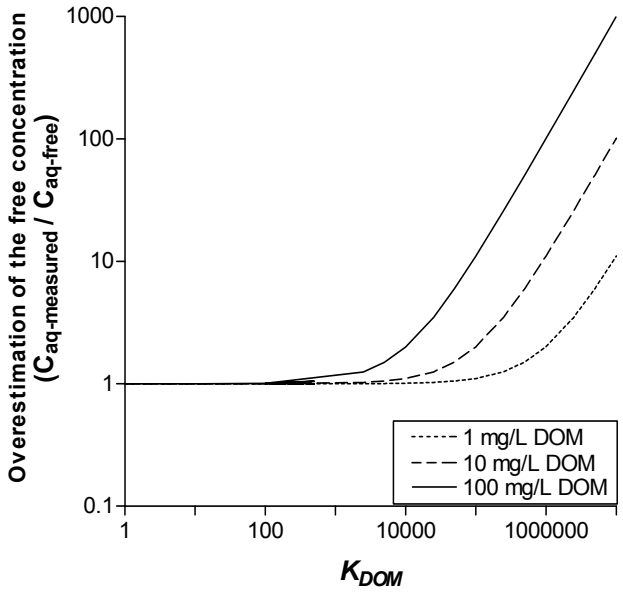


Figure 1: The overestimation of the freely dissolved aqueous concentration due to dissolved organic matter (DOM) in the aqueous phase as a function of the partition coefficient to the DOC.

Various approaches have been applied to determine free aqueous concentrations and sorption to DOM. Among them are techniques to remove the DOM from the aqueous phase for a better determination of the freely dissolved concentration in the aqueous

phase (e.g. flocculation of DOM (*16*), dialysis separation of DOM and water (*17*)). Alternatively, solid phase extraction has been employed to extract the freely dissolved fraction only (*19, 31, 32*). Additionally, various passive sampling techniques have been applied to assess freely dissolved pore water concentrations in complex environmental matrices without any phase separation (*20, 23, 27-29*). The passive samplers are considered sensors of the fraction of a chemical in a system, which participate in partitioning processes. Since only this fraction is involved in establishing freely dissolved concentration in water. The passive samplers only sample the freely dissolved molecules (*25*). Furthermore, passive samplers are sensitive, since partition coefficients are rather high and increase with increasing sorption to soil and decreasing aqueous solubility (*33*). Requirements for accurate measurements of freely dissolved concentrations are: (i) the sampler is in equilibrium with the system, (ii) partition coefficients to the fibers are known, and (iii) the sampler may not deplete the system.

Chapters 2 to 5 demonstrate that the partitioning of hydrophobic chemicals to PDMS coated fibers is sufficiently well understood such that they can be employed as versatile probes for studying partitioning processes. They were used were used as a passive sampler to assess freely dissolved aqueous concentrations and sorption coefficients. Figure 2A shows how a passive sampler is used to assess the free aqueous concentration in the pore water by negligible depletive equilibrium extraction. In Chapters 4 and 5 the concentration independent partition coefficient to the PDMS material were employed to assess free concentrations in complex soil matrices. Determination of the chemical concentration in the soil via total soil extractions was performed to determine the sorption coefficients. Contrastingly, in chapter 3, PDMS coated fibers were applied under non-equilibrium conditions. Total extractions were not performed (Figure 2B). Therefore, free concentrations could not be identified, but the relative decrease of the free concentration with increasing water / sediment ratio still enabled us to determine sorption coefficients to sediment. In chapter 2, the fiber was applied as a partitioning driven administrator (*64, 65*) and sampler at the same time (Figure 2C).

Altogether, these studies show that PDMS coated fibers can be applied in different ways, to determine free concentrations or sorption coefficients.

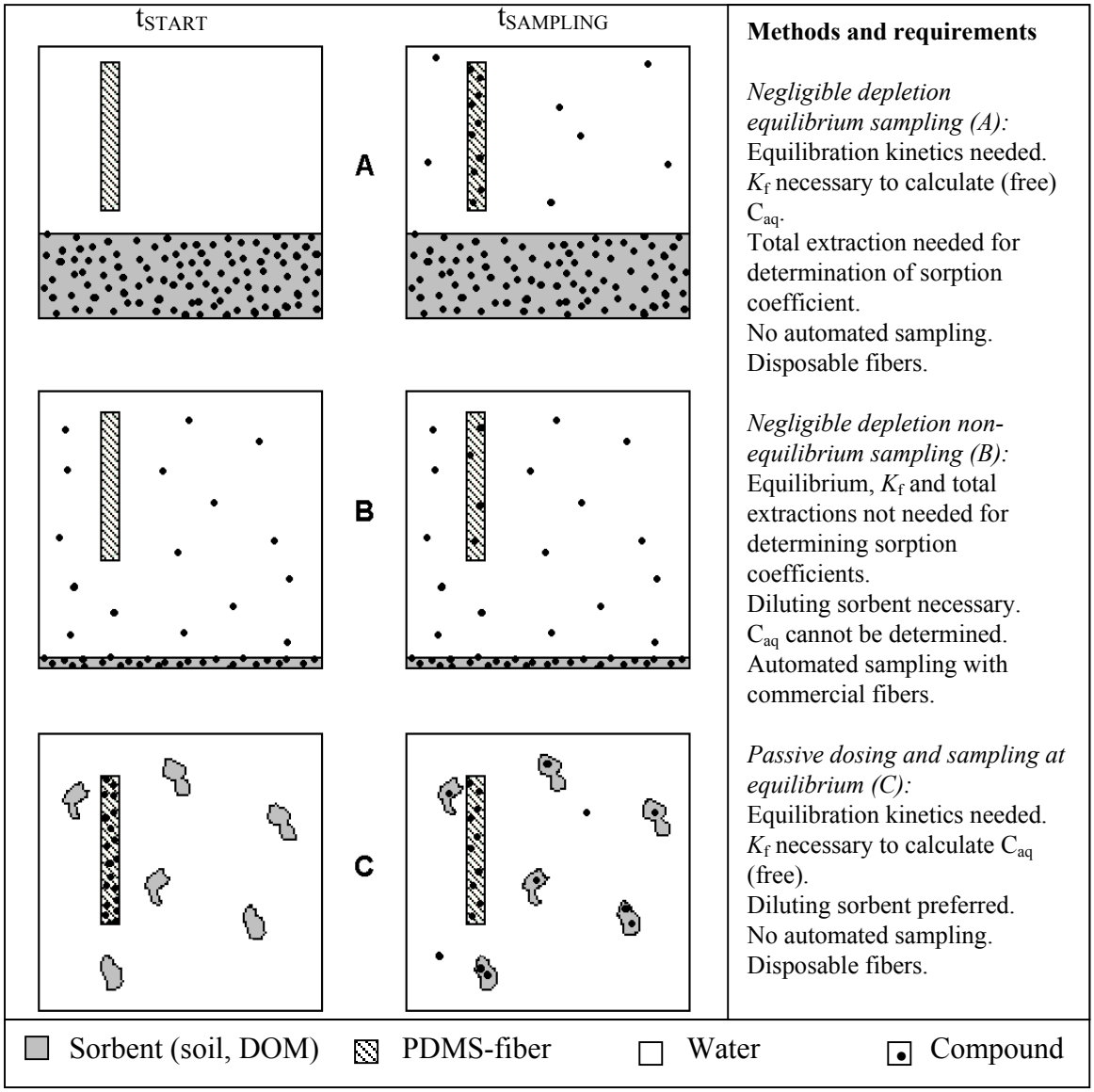


Figure 2: Different approaches to study free concentrations or sorption coefficients using a PDMS coated fiber as a passive sampler. The initial distribution of the contaminants (t_{START}) and the distribution after exposure of the PDMS coated fiber ($t_{SAMPLING}$).

The selection of a particular method depends on the properties of the chemical and matrix, the dimensions of the system, the knowledge of the partition coefficient to the passive sampler and the specific scientific question. In conclusion, free concentration of a hydrophobic chemical can be determined best by measuring the equilibrated fiber concentration and knowing the partition coefficient to the fiber-phase. This information can be combined with the concentration in the sorbent phase to determine the sorption coefficient. The passive dosing and sampling approach, described in Chapter 2, is an alternative for very small homogenous hydrophobic phases concentration (e.g. humic acids, biological fluids, or microorganism suspensions), because spiking these phases might be difficult and the passive sampler might be too large to be applied without depleting the system or making a system large and impractical. Both negligible depletive

equilibrium sampling and the passive dosing and sampling technique need an equilibrated system, and the partition coefficient to the fiber material has to be known. Equilibration times might become exceedingly long for the more hydrophobic compounds, since the equilibration time increases linearly with increasing K_f (when unstirred boundary layer (UBL) is rate limiting, (Chapter 3, (*25, 142*)). Thinner polymer coatings thickness and increased agitation reduce equilibration times of very hydrophobic compounds (log K_f >6) to weeks or even days (*27*). The experimental setup of Chapter 3 is less laborious, can be automated, does not require equilibrium K_f-values, and facilitates accurate determination of sorption coefficients. Stressed that, due to small amounts of sorbent employed in the experimental setup, careful homogenization is required, especially when sorption coefficients increase. Furthermore, certain conditions must be fulfilled when employing non-equilibrium extractions for determining partition coefficients (*96*). This approach is less sensitive, and free concentrations cannot be determined (*33*).

Determining and modeling sorption of hydrophobic organic chemicals

The sorption of hydrophobic chemicals is mainly occurring in the organic fraction of a soil. Various models have been developed that relate compound properties (e.g. K_{OW}, aqueous solubility) to the organic carbon normalized sorption coefficient (K_{OC}) (*17, 35, 37, 110, 116*). These models are usually based on sorption studies performed with spiked test soils, and can therefore predict the sorption of hydrophobic chemicals to these soils relatively well. The models disregard so-called aging effects (increasing sorption in time) due to for example slow diffusion of compounds into micro-pores or inflexible organic materials (*3, 7, 117, 118*) and the heterogeneity of the organic carbon in soil. Numerous studies showed that aging reduced biodegradability and extractability of PAHs and other hydrophobic chemicals from soils and sediments (*117, 126, 161, 245-247*). Chapter 4 and results from literature (*119, 151, 152*) show however, that the sorption coefficients after months or years of aging do not increase dramatically (less than a factor 3). Contrastingly, organic materials like soot, coal and weathered oil (tar) can have orders of magnitude higher sorption coefficients than natural organic matter (NOM) for certain hydrophobic chemicals (e.g. PAHs), thereby affecting sorption to soil and sediment significantly (*122, 248-251*).

Aging effects and strong sorption to certain matrices will lead to higher sorption coefficients and lower pore water concentrations in field-contaminated soil and sediment. Higher sorption may lead to a reduction in the bioavailability and bioaccessibility, and subsequently to lower risks. However, these interactions are difficult to predict, and this complicates the estimation of exposure of environmental biota. Current sorption models and toxicological tests often overestimate risks in field-contaminated soils (and sediments) and might be seen as a worst-case estimate of environmental risks. A more realistic assessment of the environmental risk can be performed by refining sorption models with specific sorption coefficients for different sorbents in soil or sediment (*112,*

113, 252). This, however, requires an appropriate characterization of the different sorbents, and will lead to more complicated modeling, which is more difficult to apply in risk assessment. Rather than estimating risk on the basis of predicted sorption coefficients, experimentally determined input values can be employed. Various techniques developed to study freely dissolved or weakly bound fractions in soil are available. Weakly bound fractions in soil and sediment can be assessed by mild solvent extractions (*126*), Supercritical Fluid Extraction (SFE) (*131*), dissolved sorbents such as cyclodextrin (*253*) or solid sorbents like XAD-2 (*130*) and Tenax (*108, 127, 128*). These techniques have been shown to correlate relatively well with bioavailability and biodegradation potential (*123, 129, 253, 254*). However, these methods operationally define the availability of a chemical due to selection of the experimental conditions. As a result they are arbitrary measures.
In contrast, the results obtained by negligible depletive passive sampling approaches are independent of the experimental conditions, since only sample freely dissolved molecules are probed. As described earlier, these samplers can be applied to measure freely dissolved (active) concentrations when they are applied non-depletive and at equilibrium (*33, 34*). Since an organism living in the soil or sediment is thought to be exposed to this free concentration, the freely dissolved concentration also constitutes a measure for exposure (*30, 105*). Negligible depletive passive sampling techniques can, however, not be used to determine weakly and more strongly sorbed fractions in soil and desorption kinetics (e.g. like Tenax is used for (*108*)). It should therefore be stressed that these different approaches measure different entities of the accessibility and availability of compounds in soils and sediments.

Determining and modeling sorption of polar ionizable organic chemicals
Veterinary pharmaceuticals such as the antimicrobial agents studied in Chapters 5 and 6 are polar ionizable, highly soluble organic compounds. As a result of their relatively high aqueous solubility, there is no energy gain when they partition into a non-polar organic phase. Therefore, their partition coefficients into hydrophobic phases like octanol, PDMS or organic carbon in soils will be rather low (*255*), and incomplete phase separation of the soil and the aqueous phase is not likely to affect the determination of the freely dissolved aqueous concentration. Consequently, a classical batch equilibrium experiment with separation by centrifugation is a (generally) suitable method to determine free aqueous concentrations and sorption coefficients for these compounds. If sorption to dissolved organic matter is high (*219, 230*), a dialysis approach might be a suitable solution.
The polar and ionic characteristics cause these compounds to (ad)sorb to various soil constituents (e.g. organic fractions, various clay minerals, and metal oxide/hydroxide coatings), by various interactions (e.g. hydrogen bonding, electrostatic attraction, and surface reactions) as listed in the introduction. These kinds of sorption interactions are likely to be more prominent than hydrophobic partitioning. Consequently, sorption to

soil does not correlate with hydrophobicity related properties (e.g. K_{OW}, aqueous solubility) (*41*). This can clearly be observed in Figure 3 where sorption coefficients of three veterinary antibiotics (oxytetracycline, tylosin, and sulfachloropyridazine) studied in Chapters 6 and 7 do not show any correlation with the octanol water partition coefficient. The sorption of these compounds is clearly underestimated by a log K_{OW} based sorption model (*35*) that was based on hydrophobic neutral (hydrophobic) organic chemicals.

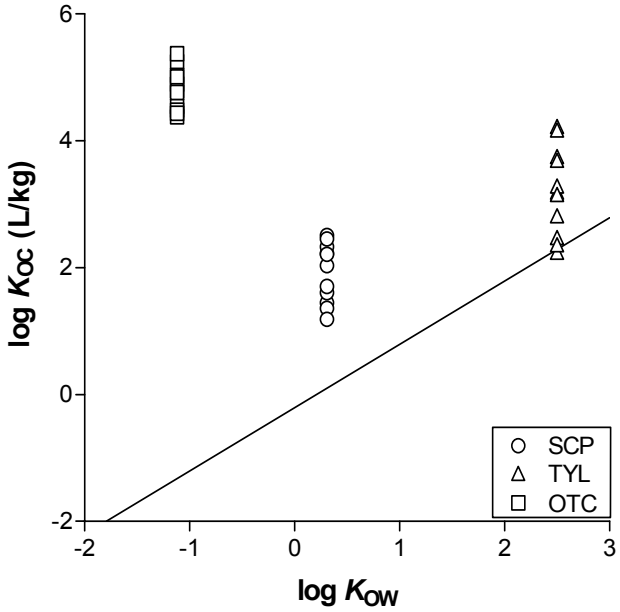


Figure 3: The organic carbon normalized sorption coefficients of sulfachloropyridazine (SCP), tylosin (TYL) and oxytetracycline (OTC) in eleven soils, plotted against their octanol water partition coefficient (*213, 215*). The straight line is a log K_{OC} - LogK_{OW} relationship of Karickhoff (*35*) that was developed with hydrophobic organic chemicals.

The variety of processes involved in sorption of veterinary antibiotics, the variable composition of soils and sediments, the pH dependent speciation and the concentration dependent sorption behavior make the sorption of these compounds difficult to model and predict (chapter 7). Several studies focus on the sorption to specific constituents in soil (*10, 228, 230, 243, 256-258*), thereby revealing different sorption processes. It is however, not straightforward to predict the sorption to soil based on information for individual constituents, since different constituents can interact, thereby affecting the overall sorption coefficient of a soil (e.g. organic matter and iron oxide/hydroxides are typically present in soil as particle coatings at the sorbent-water interface) (*5*). Hence, soil characterization on the basis of the bulk composition may not be representative of the sorption determining properties of a soil.

The pH can strongly affect sorption of ionizable compounds since it can shift the equilibrium between the different species. The pH effect on the apparent sorption

coefficient (K_D') can be modeled by describing the fractions of the different species (f_1, f_2) and their specific sorption coefficients (K_{D1}, K_{D2}), (Equation 1).

$$K_{D'} = K_{D1} * f_1 + K_{D2} * f_2 \quad (1)$$

The pH-dependent speciation of an ionizable compound is determined by its pK_A-value(s). Moreover, the pH can also affect the properties (humic acid (de)protonation) of the sorbent (soil). However, both literature (*5, 9, 43, 200*) and chapter 6 show that the pH-dependent speciation is often the most important factor in explaining the observed variation in sorption with pH.

The ionic strength also has a considerable influence on the sorption. It can largely be explained by the competition of the compounds and ions for cation exchange sites on the soil surface (*5, 10, 11*) (chapter 6). Chapter 7 shows that the implementation of pH-dependent species-specific sorption coefficients of ionizable antimicrobial agents reduces the variation of sorption coefficients in the eleven selected soils. However, the variability of the species-specific sorption coefficients still remains rather large. PLS-regression models were developed for a single compound in order to account for the influence of soil characteristics such as pH, cation exchange capacity, organic carbon content, clay content, and aluminum and iron oxide/hydroxide content on sorption coefficients. They allow for predicting the soil sorption coefficients by about one order of magnitude.

Implications and relevance for risk assessment

Soils and sediments are important sinks for organic contaminants in the environment, and sorption reduces the mobility in soil and the availability for organisms. Therefore describing sorption is an important aspect of the assessment of the environmental risks of these compounds.

The sorption of hydrophobic organic chemicals (e.g. PAHs, PCBs, chlorobenzenes, various pesticides and flame retardants) to these to soil and sediments has been studied intensively for several decades (*17*). Various models have been developed to describe sorption of hydrophobic compounds to soils and sediment, organic fractions or more specific types of organic materials (*17, 40, 51*). Recently, organic carbon based sorption-models to soils and sediments have been criticized because they do not consider the heterogeneity of the soil organic matter as well as other interactions than hydrophobicity driven partitioning (*112, 252*). Various studies have shown that organic materials like soot, coal and tar can have very high sorption coefficients. If we, however, look at the sorption to more homogenous organic materials like for instance dissolved humic material (*40, 51*) or more specifically to Aldrich humic acid, it can be observed that these sorption coefficients also show a rather large variation (Figure 4). This cannot be attributed to sorbent heterogeneity, presumably the selected method and test circumstances, have led to dissimilar data of variable quality.

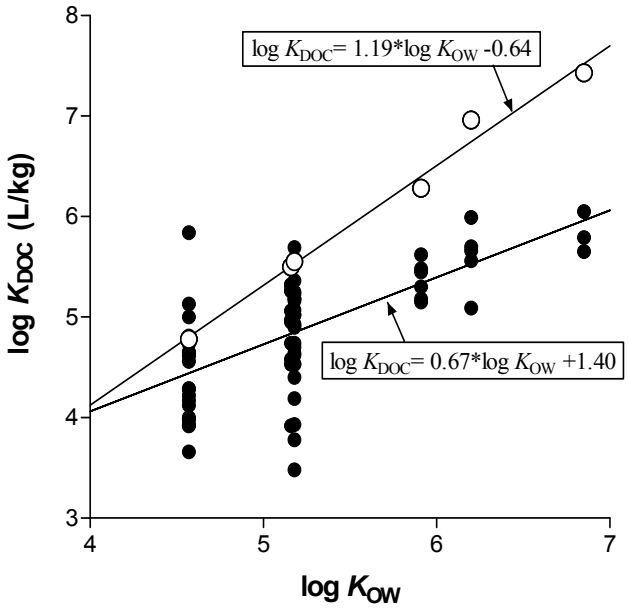


Figure 4: The organic carbon normalized sorption coefficients of various PAHs to Aldrich humic acid. The solid symbols represent literature data obtained from references (*16, 19, 31, 32, 54-56, 58-60, 74, 79, 81-93*), and the open symbols represent data from this thesis (Chapter 2, (*137*)).

Total concentrations of hydrophobic chemicals in soils and sediment are generally simple to measure. Aqueous concentrations are more difficult to determine due to detection problems and incomplete phase separation as discussed earlier. Incomplete separation of sorbent and aqueous phase, will bias data of the more hydrophobic compounds (*66*). The improvement of estimating sorption coefficients should therefore not only be focused on heterogeneity of the sorbent, but also on the improvement of analytical methods to obtain high quality data.

In contrast, far less knowledge is available on the sorption of the diverse group of polar ionizable veterinary pharmaceuticals (*41, 182*). This lack of data and the current lack of understanding render the modeling of the sorption of these compounds currently impossible. Mechanistic investigations to separate soil constituents are needed to understand sorption processes, and compare processes and data with other organic ionizable compounds (e.g. pesticides). Besides that, more sorption studies need to be performed to various complete soils to generate sorption coefficients and information on their variation between soils. However, the large variety of functional groups present in veterinary pharmaceuticals results in a multitude of interactions being involved in sorption of these compounds. In addition, soil and sediment properties relevant to sorption may not be linearly related with the soil compositions. As a result, the development of models for accurate prediction of sorption coefficients for theses chemicals continues to be a challenge, since modeling approaches have to be developed which can handle the wide variety of chemical properties observed within veterinary pharmaceuticals and the variety of soil properties. It appears more feasible to develop

models for specific compound classes, but this still needs a large amount of information on soil characteristics. Furthermore, this level of detail may be undesirable in generic risk assessment. I therefore suggest generating experimental sorption data on various soils that cover the range of properties of soils that are at risk (agricultural land) of the environmentally relevant species and use this range of sorption coefficients in qualitative risk assessment.

Concluding remarks

The sorption of neutral organic (hydrophobic) compounds has been studied intensively for several decades. Difficult determination of freely dissolved concentrations and the heterogeneity of soil organic material can complicate the measurement and modeling of sorption coefficients for these compounds. The simple and sensitive (negligible depletive) solid phase microextraction, presented in this thesis can, however, measure freely dissolved concentrations in complex matrices such as soil. This passive sampling technique should therefore be applied to generate unbiased sorption coefficients for modeling purposes. Moreover, the simplicity and sensitivity of the technique strongly advocates for application in site-specific monitoring and risk assessment.
Contrastingly, there is limited knowledge on the soil sorption of polar ionizable organic compounds such as veterinary pharmaceuticals. Hence, more research is needed on the sorption of these compounds. The present thesis has demonstrated that soil and aqueous phase properties significantly influence sorption of these compounds. Modeling the influence of these factors allows prediction of sorption with limited accuracy. Modeled sorption data should therefore be taken as qualitative indicators of the sorption behavior. Consequently, experimental data are preferred for site-specific risk assessment. Clearly, more research is needed in order to improve our understanding and modeling of the sorption of these compounds.

Samenvatting in het Nederlands

Inleiding

Zowel door natuurlijke processen (vulkanische activiteit, bosbranden en biologische processen) als menselijke activiteiten (industrie, landbouw en verkeer) zijn verscheidene organische stoffen in het milieu terechtgekomen. Binding (sorptie) van deze stoffen aan bodem en sediment is bepalend voor de verspreiding, de mogelijkheid tot afbraak (zowel chemisch als biologisch) en de blootstelling van dieren, planten en micro-organismen in het milieu (*1-3*). De mate van sorptie aan bodem wordt meestal uitgedrukt met de sorptiecoëfficiënt (K_D), het quotiënt van de concentratie in de bodem (C_s) en de vrij opgeloste concentratie in het poriewater[a] (C_{aq}).

$$K_D = \frac{C_s}{C_{aq}} \qquad (1)$$

Deze sorptiecoëfficiënt is een belangrijke parameter voor het schatten van de concentratie van stoffen in verschillende milieucompartimenten en het hiervan afgeleide milieurisico. Het is niet haalbaar voor elke stof en elk type bodem of sediment de sorptiecoëfficiënt te bepalen. Men gebruikt daarom schattingsmodellen, waarbij aan de hand van stof- en bodemeigenschappen de sorptiecoëfficiënt wordt voorspeld. De kwaliteit van deze voorspelling is echter afhankelijk van de kwaliteit van de gegevens waarop het model is gebaseerd en de gelijkenis tussen de bodems, sedimenten, omstandigheden en stoffen die in het model zijn gebruikt. In dit proefschrift is de sorptie van *hydrofobe* (watervrezend) en *hydrofiele* (waterminnend) organische verbindingen aan bodem en sediment bestudeerd.

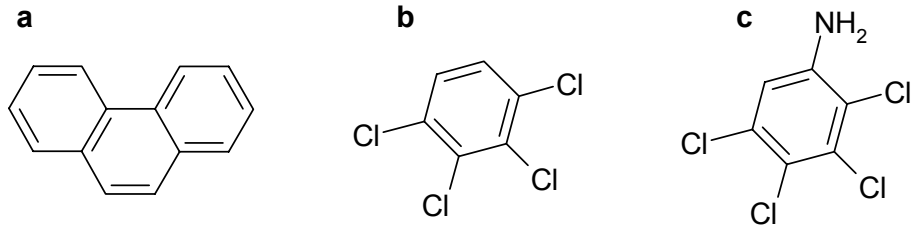
Hydrofobe stoffen

In de hoofdstukken 2 tot 5 is de sorptie van hydrofobe stoffen aan bodem sediment en opgelost organisch materiaal bestudeerd. In de hoofdstukken 2, 4 en 5 is met verschillende polycyclische aromatische koolwaterstoffen (PAKs) gewerkt en in hoofdstuk 3 is een aantal chloorbenzenen (CBs) en chlooranilines (CAs) gebruikt. In Figuur 1 is de structuurformule van een vertegenwoordiger van deze drie stoffengroepen weergegeven. Deze hydrofobe stoffen lossen slecht op in water en sorberen sterk aan of in het organische materiaal in een bodem. Het gevolg hiervan is dat deze stoffen hoge sorptiecoëfficiënten aan organisch materiaal in bodem of sediment hebben.

Er is relatief veel wetenschappelijke literatuur over de sorptie van hydrofobe stoffen aan bodem en sediment. Voor het bepalen van een sorptiecoëfficiënt is het noodzakelijk de vrij opgeloste concentratie in het poriewater te meten. Hiervoor zijn in de literatuur veel

[a] Poriewater is het water tussen de bodem- of sedimentdeeltjes.

verschillende methoden gebruikt (*17, 18, 54-56*). Als de oplosbaarheid van de stof in water erg laag is en de stof sterk aan opgelost organisch materiaal zoals humuszuren[b] sorbeert (*14, 108*), dan blijken de meeste van deze methoden ontoereikend om de poriewater concentratie nauwkeurig te bepalen. Dit heeft tot gevolg, dat sorptiecoëfficiënten uit de literatuur niet altijd correct zijn. Deze sorptiecoëfficiënten vormen echter wel de basis van modellen, die de sorptie van deze stoffen voorspellen. Buiten de kwaliteit van de gegevens waarop een sorptiemodel is gebaseerd kunnen ook andere factoren de voorspellende waarde van een model beïnvloeden. Het blijkt namelijk dat de sorptiecoëfficiënt in de tijd kan toenemen als gevolg van "veroudering" (*3, 7, 117-119*). Dit soort verouderingsprocessen worden eigenlijk nooit meegenomen in schattingsmodellen. Daarnaast blijken sommige (antropogene) organische materialen in bodem en sediment, zoals roet, teer en houtskool, bepaalde hydrofobe stoffen (vooral PAKs) nog sterker te sorberen dan het van planten afkomstige organische materiaal (*112, 120-124*).
Het model zal de sorptie van de hydrofobe stoffen niet (voor elk type bodem) kunnen voorspellen als de gegevens waarop een model is gebaseerd niet goed zijn en als er geen rekening wordt gehouden met de specifieke sorptie-eigenschappen van verschillende typen organisch materiaal en veranderende sorptie in de tijd.



Figuur 1: Een voorbeeld van een (a) polycyclische aromatische koolwaterstof (fenantreen), (b) een chloorbenzeen (1,2,3,4 tetrachloorbenzeen) en (c) een chlooraniline (2,3,4,5 tetrachlooraniline).

In dit proefschrift (hoofdstuk 2 tot 5) is de relatief nieuwe "solid phase microextraction" (SPME) techniek gebruikt om de vrij opgeloste concentratie in het poriewater beter en makkelijker te kunnen bepalen (*30, 33, 34*). Deze techniek maakt gebruik van een glasvezel (fiber) gecoat met een dun laagje polymeer[c]. Alleen vrij opgeloste stoffen kunnen door middel van passieve diffusie in deze coating terechtkomen. Dit maakt het mogelijk de vrij opgeloste concentratie in het water te bepalen, mits de verdelingscoëfficiënt tussen de coating en water bekend is. In dit proefschrift is deze techniek op verschillende manieren gebruikt.

[b] Humuszuren zijn grote organische moleculen, gevormd door de verwering (humificatie) van met name plantenresten.

[c] De fiber is gecoat met een 7 of 30 µm dik laagje poly(dimethylsiloxaan) in de volksmond heet dit materiaal siliconenkit.

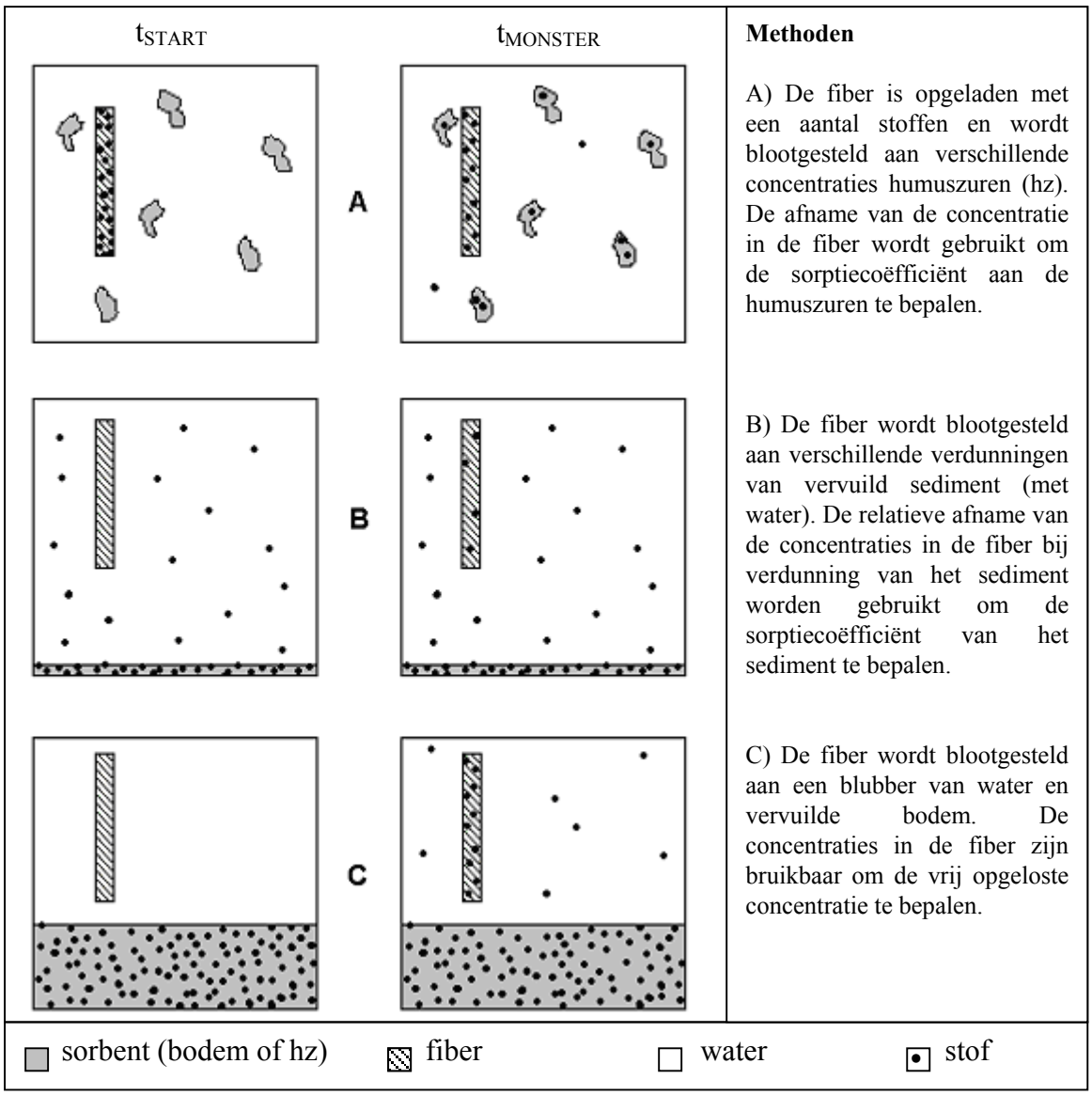
Hoofdstuk 2 beschrijft de ontwikkeling van een methode waarbij de fiber tegelijk als doseer- en meetsysteem wordt gebruikt. De coating van de fiber wordt "opgeladen" met een aantal verschillende PAKs en wordt blootgesteld aan water met verschillende concentraties humuszuren. De afname van de concentratie PAKs in de fibercoating met toenemende concentratie humuszuren wordt gebruikt om de sorptiecoëfficiënt van de PAKs aan de humuszuren te bepalen (Figuur 2A). De gemeten sorptiecoëfficiënten zijn over het algemeen hoger dan waarden uit de literatuur (*40*). Dit is waarschijnlijk het gevolg van in de literatuur gebruikte technieken, die veelal de vrij opgeloste concentratie overschatten en daardoor de sorptiecoëfficiënt onderschatten (*66*). De ontwikkelde techniek lijkt veelbelovend voor het bepalen van sorptiecoëfficiënten aan opgeloste fases zoals humuszuren, en kan mogelijk ook worden toegepast in biologische vloeistoffen zoals eiwitoplossingen.

In hoofdstuk 3 is een sediment, vervuild met een aantal chloorbenzenen en chlooranilines, steeds verder met water verdund. De fiber is gebruikt om de afname van de vrij opgeloste concentratie te meten en de sorptiecoëfficiënt van de stoffen aan het sediment te bepalen (Figuur 2B). De gemeten sorptiecoëfficiënten bleken zeer vergelijkbaar met literatuurgegevens te zijn (*14, 108*). Dit laat zien dat deze verdunningsmethode, waarbij het niet nodig is de totale concentratie in het sediment te meten, kan worden gebuikt om sorptiecoëfficiënten te bepalen.

In hoofdstuk 4 is de sorptie van PAKs aan in het laboratorium en door industriële activiteiten vervuilde bodems onderzocht. De totale concentratie in de bodem is bepaald met een oplosmiddelextractie en de vrije concentratie in het poriewater is bepaald met de fiber (Figuur 2C). Modellen uit de literatuur konden de sorptie van PAKs aan de lab-vervuilde bodems redelijk goed voorspellen. Zelfs als deze bodems tot twee jaar werden bewaard, waren de sorptiecoëfficiënten slechts marginaal toegenomen. De sorptiecoëfficiënten van de industriële bodems waren daarentegen tot wel een factor 100 hoger dan de in het lab vervuilde bodems en de voorspellingen van de modellen. De onderschatting van deze sorptiecoëfficiënten door de modellen zijn waarschijnlijk het gevolg van sterk sorberende materialen zoals roet en teer, die veel voorkomen in industriële bodems. Dit betekent dat het milieurisico van de vervuiling in deze bodems veel lager is dan op basis van de sorptiemodellen wordt voorspeld, omdat de stoffen veel sterker aan de bodem zijn gesorbeerd dan verwacht.

In hoofdstuk 5 zijn de poriewater concentraties bestudeerd bij toenemende bodemconcentraties (Figuur 2C). De poriewater concentraties bleken lineair toe te nemen met de bodemconcentratie tot er een bepaald maximum werd bereikt. Het maximum correspondeerde met de oplosbaarheid van de stoffen in water. Dit laat zien dat de fiber enkel de vrij opgeloste waterconcentratie in het poriewater meet tot het moment dat het is verzadigd met de stof. Bij het testen van de giftigheid van hydrofobe stoffen zoals PAKs

voor bodemorganismen wordt over het algemeen geen aandacht geschonken aan de mogelijke verzadiging van het poriewater, terwijl de gebruikte bodemconcentraties dikwijls tot verzadiging van het poriewater zullen leiden.



Figuur 2: Verschillende manieren om vrije concentraties te meten en sorptiecoëfficiënten te bepalen met behulp van een fiber met een polymeercoating. t_{START} is de initiële verdeling van stoffen over het systeem en $t_{MONSTER}$ is de verdeling van stoffen over het systeem op het moment van bemonsteren.

Hydrofiele verbindingen

In hoofdstuk 6 en 7 is de bodemsorptie van drie antibiotica, oxytetracycline (OTC), tylosine (TYL) en sulfachloropyridazine (SCP), bestudeerd. Deze stoffen worden veel gebruikt als diergeneesmiddel en kunnen via de mest in de bodem terechtkomen (*192, 259, 260*). Op dit moment is er nog relatief weinig onderzoek gedaan naar de sorptie van

diergeneesmiddelen aan bodem. Het bepalen van sorptiecoëfficiënten van deze hydrofiele verbindingen is niet zo moeilijk, omdat deze stoffen goed in water oplossen en over het algemeen minder sterk aan bodem sorberen. Het schatten van de sorptie is echter veel moeilijker, omdat deze stoffen op verscheidene manieren interacties kunnen aangaan met verschillende componenten van de bodem. Daarnaast kan de sorptiecoëfficiënt afhankelijk zijn van de concentratie van de stof in het poriewater. Bovendien zijn deze stoffen ioniseerbaar, dat wil zeggen dat ze afhankelijk van de pH neutraal of geladen (positief of negatief) kunnen zijn. Deze lading heeft invloed op de oplosbaarheid in het water en de mogelijkheid tot elektrostatische interacties met het bodemoppervlak.

In hoofdstuk 6 is het effect van de pH en het zoutgehalte van het poriewater op de bodemsorptie onderzocht. Onder zure omstandigheden was de sorptie hoger dan onder basische omstandigheden. De relatie van de pH en de sorptiecoëfficiënt kon grotendeels worden teruggevoerd op de verhouding van de geladen en ongeladen stoffen. Het toevoegen van zout leidde tot een afname van de sorptiecoëfficiënt voor OTC en TYL die bij de gebruikte pH een positief geladen groep hebben, terwijl de sorptie van SCP, dat bij de gebruikte pH neutraal of negatief geladen, nauwelijks werd beïnvloed.

In hoofdstuk 7 is de sorptie van de drie antibiotica aan 11 bodems onderzocht. Er is gekeken in hoeverre de eigenschappen van de bodems (pH, kleigehalte, organisch materiaal gehalte, kation-uitwisselingscapaciteit, hoeveelheid aluminium oxy-hydroxides en ijzer-oxyhydroxides) correleerden met de bodemsorptie. Uit deze inventarisatie bleek dat de pH de sorptiecoëfficiënt beïnvloedde. Een ingewikkeld model waarin alle bodemeigenschappen zijn verdisconteerd kon de sorptiecoëfficiënt nog steeds niet nauwkeurig schatten. Dit geeft aan dat het modelleren van de sorptie van deze stoffen zeer ingewikkeld is. Gezien het grote aantal bodemeigenschappen die nodig zijn om de sorptiecoëfficiënt enigszins te kunnen schatten zijn deze modellen in de praktijk niet goed toepasbaar.

Conclusie

De vrij opgeloste concentratie van neutrale hydrofobe stoffen is moeilijker te meten naarmate de oplosbaarheid van een stof naar beneden gaat, en de sorptiecoëfficiënt van (opgelost) organisch materiaal omhoog gaat. In dit proefschrift is de solid phase microextraction (SPME) techniek op verschillende manieren toegepast. Met deze techniek is de vrije concentratie in complexe matrices -zoals bodem- goed te meten, omdat de polymeercoating zeer selectief alleen vrij opgeloste moleculen bemonstert. Daarnaast is de techniek ook zeer gevoelig, doordat verdelingscoëfficiënten tussen de coating en het water hoger worden met afnemende oplosbaarheid van een stof (*33*). Deze techniek kan daarom gebruikt worden om op een correcte wijze sorptiecoëfficiënten te bepalen. Daarnaast kan deze techniek ook worden gebruikt om vrije concentraties in

vervuilde bodems, sedimenten of andere complexe matrices te bepalen voor locatiespecifieke risicoanalyse.

Op dit moment is er nog relatief weinig bekend over de bodemsorptie van hydrofiele ioniseerbare organische verbindingen zoals diergeneesmiddelen. Meer onderzoek is daarvoor nodig. Dit proefschrift laat zien dat de pH en het zoutgehalte van het poriewater en allerlei bestanddelen van de bodem de sorptie van deze stoffen beïnvloedt. De complexiteit van de interacties met de bodem en de vele bodembestanddelen, die van invloed zijn op het sorptieproces, maakt dit moeilijk. Experimentele gegevens zijn daarom van grotere waarde voor het beoordelen van het milieurisico dan schattingsmodellen. Voorspelde sorptiecoëfficiënten moeten op dit moment meer als een ruwe kwalitatieve schatting gezien worden, op basis waarvan kan worden besloten of het nodig is verder onderzoek naar de sorptie van dit soort stoffen te doen. Daarnaast zal er meer gedetailleerd onderzoek nodig zijn om de verschillende sorptieprocessen in bodem te begrijpen.

Literature

(1) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental organic chemistry*; Second ed.; John Wiley & Sons: Hoboken, New Jersey, 2003.

(2) Semple, K. T.; Morriss, W. J.; Paton, G. I. Bioavailability of hydrophobic organic contaminants in soils: fundamental concepts and techniques for analysis. *Eur. J. Soil Sci.* **2003**, *54*, 809-818.

(3) Alexander, M. How toxic are toxic chemicals in soil. *Environ. Sci. Technol.* **1995**, *29*, 2713-2717.

(4) McBride, M. B. *Environmental chemistry of soils*; Oxford University Press, Inc.: New York, 1994.

(5) Sposito, G. *The chemistry of soils*; Oxford University Press: New York, 1989.

(6) Goss, K.-U.; Schwarzenbach, R. P. Rules of thumb for assessing equilibrium partitioning of organic compounds: successes and pitfalls. *Sci. Educat.* **2003**, *80*, 450-455.

(7) Luthy, R. G.; Aiken, G. R.; Brusseau, M. L.; Cunningham, S. D.; Gschwend, P. M.; Pignatello, J. J.; Reinhard, M.; Traina, S. J.; Weber, W. J.; Westall, J. C. Sequestration of hydrophobic organic contaminants by geosorbents. *Environ. Sci. Technol.* **1997**, *31*, 3341-3347.

(8) Weber, W. J.; DiGiano, F. A. *Process dynamics in environmental systems*; Wiley: New York, 1996.

(9) Westall, J. C.; Leuenberger, C.; Schwarzenbach, R. P. Influence of pH and ionic strength on the aqueous-nonaqueous distribution of chlorinated phenols. *Environ. Sci. Technol.* **1985**, *19*, 193-198.

(10) Brownawell, B. J.; Chen, H.; Collier, J. M.; Westall, J. C. Adsorption of organic cations to natural materials. *Environ. Sci. Technol.* **1990**, *24*, 1234-1241.

(11) De Jonge, H.; Wollesen De Jonge, L. Influence of pH and solution composition on the sorption of Glyphosate and Prochloraz to a sandy loam soil. *Chemosphere* **1999**, *39*, 753-763.

(12) Turner, A. Salting out of chemicals in estuaries: implications for contaminant partitioning modeling. *Sci. Tot. Envir.* **2003**, *314-316*, 599-612.

(13) Doucette, W. C. In *Handbook of property estimation methods for chemicals: environmental and health sciences*; Boethling, R. S., Mackay, D., Eds.; CRC press LLC: Boca Ralton, Florida, 2000; pp 141-188.

(14) Schrap, S. M.; de Vries, P. J.; Opperhuizen, A. Experimental problems in determining sorption coefficients of organic chemicals; an example for chlorobenzenes. *Chemosphere* **1994**, *28*, 931-945.

(15) Koelmans, A. A. Correction of experimental sorption coefficients using DOC measurements and apparent solubility enhancements. *Environ. Toxicol. Chem.* **1995**, *14*, 2015-2016.

(16) Laor, Y.; Rebjun, M. Complexation-flocculation: a new method to determine binding coefficients of organic contaminants to dissolved humic substances. *Environ. Sci. Technol.* **1997**, *31*, 3558-3564.

(17) Doucette, W. J. Quantitative structure-activity relationships for predicting soil-sediment sorption coefficients for organic chemicals. *Environ. Toxicol. Chem.* **2003**, *22*, 1771-1788.

(18) Gauthier, T. D.; Shane, E. C.; Guerin, W. F.; Seitz, W. R.; Grant, C. L. Fluorescence quenching method for determining equilibrium constants for polycyclic aromatic hydrocarbons binding to dissolved humic materials. *Environ. Sci. Technol.* **1986**, *20*, 1162 - 1166.

(19) Landrum, P. F.; Nihart, S. R.; Eadle, B. J.; Gardner, W. S. Reverse-Phase separation method for determining pollutant binding to Aldrich humic acid and dissolved organic carbon of natural waters. *Environ. Sci. Technol.* **1984**, *18*, 187-192.

(20) Södergren, A. Solvent-filled Dialysis membranes simulate uptake of pollutants by aquatic organisms. *Environ. Sci. Technol.* **1987**, *21*, 855-859.
(21) Arthur, C. L.; Pawliszyn, J. Solid phase microextraction with thermal desorption using fused silica optical fibers. *Anal. Chem.* **1990**, *62*, 2145-2148.
(22) Huckins, J. N.; Tubergen, M. W.; Manuweera, G. K. Semipermeable membrane devices containing model lipid: A new approach to monitoring the bioavailability of lipophilic contaminants and estimating their bioconcentration potential. *Chemosphere* **1990**, *20*, 533-552.
(23) Koelmans, A. A.; De Lange, H. J.; Lijklema, L. Desorption of chlorobenzenes from natural suspended solids and sediments. *Water Sci. Technol.* **1993**, *28*, 171-180.
(24) Lüers, F.; Ten Hulscher, D. E. M. Temperature effect on the partitioning of polycyclic aromatic hydrocarbons between natural organic carbon and water. *Chemosphere* **1996**, *33*, 643-657.
(25) Pawliszyn, J. *Solid phase microextraction: theory and practice*; Wiley-VCH, Inc.: New York, 1997.
(26) Booij, K.; Sleiderink, H. M.; Smedes, F. Calibrating the uptake kinetics of semipermeable membrane devices using exposure standards. *Environ. Toxicol. Chem.* **1998**, *17*, 1236-1245.
(27) 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*, 5177-5183.
(28) Jonker, M. T. O.; Koelmans, A. A. Polymethylene solid phase extraction as a partitioning method for hydrophobic organic chemicals in sediment and soot. *Environ. Sci. Technol.* **2001**, *35*, 3742-3749.
(29) Wilcockson, J. B.; Gobas, F. A. P. C. Thin-film solid-phase extraction to measure fugacities of organic chemicals with low volatility in biological samples. *Environ. Sci. Technol.* **2001**, *35*, 1425-1431.
(30) Van Der Wal, L.; Jager, T.; Fleuren, R. H. L. J.; Barendregt, A.; Sinnige, T. L.; Van Gestel, C. A. M.; Hermens, J. L. M. Solid Phase microextraction to predict bioavailability and accumulation of organic micropollutants in terrestrial organisms after exposure to a field-contaminated soil. *Environ. Sci. Technol.* **2004**, *38*, 4842-4848.
(31) Ozretich, R. J.; Smith, L. M.; Roberts, F. A. Reversed-phase separation of estuarine interstitial water fractions and the consequences of C18 retention of organic matter. *Environ. Toxicol. Chem.* **1995**, *14*, 1231-1272.
(32) Maxin, C. R.; Kogel-Knabner, I. Partitioning of polycyclic aromatic hydrocarbons (PAH) to water-soluble soil organic matter. *Eur. J. Soil Sci.* **1995**, *46*, 193-204.
(33) Mayer, P.; Tolls, J.; Hermens, J. L. M.; Mackay, D. Equilibrium sampling devices. *Environ. Sci. Technol.* **2003**, *37*, 184A-191A.
(34) 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*, 575-587.
(35) Karickhoff, S. W.; Brown, D. S.; Scott, T. A. Sorption of hydrophobic pollutants on natural sediments. *Water Res.* **1979**, *13*, 241-248.
(36) Sabljic, A.; Piver, W. T. Quantitative modeling of environmental fate and impact of commercial chemicals. *Environ. Toxicol. Chem.* **1992**, *11*, 961-972.
(37) Brusseau, M. L. Using QSAR to evaluate phenomenological models for sorption of organic compounds by soil. *Environ. Toxicol. Chem.* **1993**, *12*, 1835-1846.
(38) Bintein, S.; Devillers, J. QSAR for organic sorption in soils and sediments. *Chemosphere* **1994**, *28*, 1171-1187.
(39) Goss, K.-U.; Schwarzenbach, R. P. Linear free energy relationships used to evaluate equilibrium partitioning of organic compounds. *Environ. Sci. Technol.* **2001**, *35*, 1-9.

(40) Burkhard, L. P. Estimating dissolved organic carbon partition coefficients for nonionic organic chemicals. *Environ. Sci. Technol.* **2000**, *34*, 4663-4668.
(41) Tolls, J. Sorption of veterinary pharmaceuticals in soils - A review. *Environ. Sci. Technol.* **2001**, *35*, 3397-3406.
(42) Karickhoff, S. W. Semi-empirical estimation of sorption of hydrophobic pollutants on natural sediments and soils. *Chemosphere* **1981**, *10*, 833-836.
(43) Jafvert, C. T. Sorption of organic acid compounds to sediments: initial model development. *Environ. Toxicol. Chem.* **1990**, *9*, 959-968.
(44) Spadotto, C. A.; Honrsby, A. G. Soil sorption of acidic pesticides: modeling pH effects. *J Environ. Qual.* **2003**, *32*, 949-956.
(45) Leversee, G. J.; Landrum, P. F.; Giesy, J. P.; Fannin, T. Humic acids reduce bioaccumulation of some polycyclic aromatic hydrocarbons. *Can. J. Fisheries Aq. Sci.* **1983**, *40*, 63-69.
(46) McCarthy, J. F.; Jimenez, B. D. Reduction in bioavailability to bluegills of polycyclic aromatic hydrocarbons bound to dissolved humic material. *Environ. Toxicol. Chem.* **1985**, *4*, 511-521.
(47) Suffet, H. I.; Jafvert, C. T.; Kukkonen, J.; Servos, M.; Spacie, A.; Williams, L. L.; Noblet, J. A. In *Bioavailability, Physical, Chemical, and Biological Interactions*; Hamelink, J. L., Landrum, P. F., Bergman, H. L., Benson, W. H., Eds.; Lewis Publishers: Ann Arbor, 1994.
(48) Urrestarazu Ramos, E.; Meijer, S. N.; Vaes, W. H. J.; Verhaar, H. J. M.; Hermens, J. L. M. Using solid phase microextraction (SPME) to determine partition coefficients to humic acids and bioavailable concentrations of hydrophobic chemicals. *Environ. Sci. Technol.* **1998**, *32*, 3430-3435.
(49) Freidig, A. P.; Artola Garicano, E.; Busser, F. J. M.; Hermens, J. L. M. Estimating the impact of humic acid on the bioavailability and bioaccumulation of hydrophobic chemicals in guppies using a kinetic solid phase extraction. *Environ. Toxicol. Chem.* **1998**, *17*, 998-1004.
(50) Haitzer, M.; Burnison, B. K.; Hoess, S.; Traunspurger, W.; Steinberg, C. E. W. Effects of quantity, quality, and contact time of dissolved organic matter on bioconcentration of benzo[a]pyrene in the nematode *Caenorhabditis elegans*. *Environ. Toxicol. Chem.* **1999**, *18*, 459-465.
(51) Krop, H. B.; Van Noort, P. C. M.; Govers, H. A. J. Determination and theoretical aspects of the equilibrium between dissolved organic matter and hydrophobic organic micropollutants in water (K_{DOC}). *Rev. Environ. Contamin. Toxicol.* **2001**, *169*, 1-122.
(52) Zeng, E. Y.; Noblet, J. A. Theoretical considerations on the use of solid-phase microextraction with complex environmental samples. *Environ. Sci. Technol.* **2002**, *36*, 3385-3392.
(53) Mackay, D.; Shiu, W.-Y.; Ma, K.-C. *Physical-chemical properties and environmental fate Handbook*; Chapman & Hall: Boca Raton, 1999.
(54) McCarthy, J. F.; Jimenez, B. D. Interactions between polycyclic aromatic hydrocarbons and dissolved humic material: binding and dissociation. *Environ. Sci. Technol.* **1985**, *19*, 1072-1076.
(55) Backhus, D. A.; Gschwend, P. M. Fluorescent polycyclic aromatic hydrocarbons as probes for studying the impact of colloids on pollutant transport in groundwater. *Environ. Sci. Technol.* **1990**, *24*, 1214-1223.
(56) Schlautmann, M. A.; Morgan, J. J. Effects of aqueous chemistry on the binding of polycyclic aromatic hydrocarbons by dissolved humic materials. *Environ. Sci. Technol.* **1993**, *27*, 961-969.
(57) 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*, 4463-4467.
(58) 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*, 597-600.

(59) Poerschmann, J.; Kopinke, F.-D. Sorption of very hydrophobic organic compounds (VHOCs) on dissolved humic organic matter (DOM). 2. Measurement of sorption and application of a Flory-Huggins concept to interpret the data. *Environ. Sci. Technol.* **2001**, *35*, 1142-1148.
(60) Haftka, J. *Analysis of freely dissolved PAHs with negligible depletion-SPME in the presence of DOC,* SETAC Europe, Prague, 2004
(61) Pörschmann, J.; Kopinke, F.-D.; Pawliszyn, J. Solid-phase microextraction for determining the binding state of organic pollutants in contaminated water rich in humic organic matter. *J. Chromatogr. A* **1998**, *816*, 159-167.
(62) Woodburn, K. B.; Doucette, W. J.; Andren, A. W. Generator column determination of octanol/water partition coefficients for selected polychlorinated biphenyl congeners. *Environ. Sci. Technol.* **1984**, *18*, 457-459.
(63) Van Haelst, A. G.; Zhao, Q.; van der Wielen, F. W. M.; Govers, H. A. J. Determination of aqueous solubilities of tetrachlorobenzyltoluenes individually and in a mixture by a modified generator column technique. *Chemosphere* **1996**, *33*, 257-264.
(64) Mayer, P.; Wernsing, J.; Tolls, J.; de Maagd, P. G. J.; Sijm, D. T. H. M. Establishing and controlling dissolved concentrations of hydrophobic organics by partitioning from a solid phase. *Environ. Sci. Technol.* **1999**, *33*, 2284-2290.
(65) Brown, R. S.; Akhatar, P.; Akerman, J.; Hampel, L.; Kozin, I. S.; Villerius, L. A.; Klamer, H. J. C. Partition controlled delivery of hydrophobic substances in toxicity tests using poly(dimethylsiloxane) (PDMS) films. *Environ. Sci. Technol.* **2001**, *35*, 4097-4102.
(66) Lee, S.; Gan, J.; Liu, W. P.; Anderson, M. A. Evaluation of K_D underestimation using solid phase microextraction. *Environ. Sci. Technol.* **2003**, 5597-5602.
(67) Dulfer, W. J.; Bakker, M. W. C.; Govers, H. A. J. Micellar solubility and micelle/water partitioning of polychlorinated biphenyls in solutions of sodium dodecyl sulfate. *Environ. Sci. Technol.* **1995**, *29*, 985-992.
(68) Fan, C.; Jafvert, C. T. Margules equations applied to PAH solubilities in alcohol-water mixtures. *Environ. Sci. Technol.* **1997**, *31*, 3516-3522.
(69) Graphpad; Graphpad Prism for windows 3.00, San Diego, USA, 1999.
(70) Mayer, P.; Vaes, W. H. J.; Hermens, J. L. M. Absorption of hydrophobic compounds into the poly(dimethylsiloxane) coating of solid-phase microextraction fibers: high partition coefficients and fluorescence microscopy images. *Anal. Chem.* **2000**, *72*, 459-464.
(71) Vaes, W. H. J.; Mayer, P.; Oomen, A. G.; Hermens, J. L. M.; Tolls, J. Comments on "adsorption versus absorption of polychlorinated biphenyls onto solid-phase microextraction coatings". *Anal. Chem.* **2000**, *72*, 639-641.
(72) Baltussen, E.; Sandra, P.; David, F.; Janssen, H.-G.; Cramers, C. Study into the equilibrium mechanism between water and poly(dimethylsiloxane) for very apolar solutes: adsorption or absorption. *Anal. Chem.* **1999**, *71*, 5213-5216.
(73) Poerschmann, J.; Gorecki, T.; Kopinke, F.-D. Sorption of very hydrophobic organic compounds onto polydimethylsiloxane and dissolved humic organic matter. 1. Adsorption or partitioning of VHOC on PDMS-coated solid-phase microextraction fibers - a never ending story? *Environ. Sci. Technol.* **2000**, *34*, 3824-3830.
(74) Georgi, A. Ph.D.Thesis, UFZ, Leipzig University, 1998.
(75) Langenfeld, J. J.; Hawthorne, S. B.; Miller, D. J. Quantitative analysis of fuel-related hydrocarbons in surface water and wastewater samples by solid phase microextraction. *Anal. Chem.* **1996**, *68*, 144-155.
(76) Paschke, A.; Popp, P. Solid-phase microextraction fibre-water distribution constants of more hydrophobic organic compounds and their correlations with octanol-water partition coefficients. *J. Chromatogr. A* **2003**, *999*, 35-42.
(77) Doong, R.; Chang, C.-P. Determination of distribution coefficients of priority polycyclic aromatic hydrocarbons using solid-phase microextraction. *Anal. Chem.* **2000**, *72*, 3647-3652.

(78) Potter, D. W.; Pawliszyn, J. Rapid determination of polyaromatic hydrocarbons and polychlorinated biphenyls in water using solid-phase microextraction and GC/MS. *Environ. Sci. Technol.* **1994**, *28*, 298-305.
(79) Kopinke, F.-D.; Georgi, A.; Mackenzie, K. Sorption and chemical reactions of PAHs with dissolved humic substances and related model polymers. *Acta Hydrochim. Hydrobiol.* **2000**, *7*, 385-399.
(80) Poerschmann, J.; Kopinke, F.-D.; Pawliszyn, J. Solid phase microextraction to study sorption of organotin compounds onto particulate and dissolved humic organic matter. *Environ. Sci. Technol.* **1997**, *31*, 3629-3636.
(81) Lassen, P.; Carlsen, L. Solubilization of phenanthrene by humic acids. *Chemosphere* **1997**, *34*, 817-825.
(82) Raber, B.; Kogel-Knabner, I.; Stein, C.; Klem, D. Partitioning of polycyclic aromatic hydrocarbons to dissolved organic mater from different soils. *Chemosphere* **1998**, *36*, 79-97.
(83) Laor, Y.; Farmer, W. J.; Aochi, Y.; Strom, P. F. Phenanthrene binding and sorption to dissolved and to mineral-associated humic acid. *Water Res.* **1998**, *32*, 1923-1931.
(84) Nielsen, T.; Siigur, K.; Helweg, C.; Jorgensen, O.; Hansen, P. E.; Kirso, U. Sorption of polycyclic aromatic compounds to humic acids as studied by high-performance liquid chromatography. *Environ. Sci. Technol.* **1997**, *31*, 1102-1108.
(85) Lee, C.-L.; Kuo, L.-J.; Wang, H.-L.; Hsieh, P.-C. Effects of ionic strength on the binding of phenanthrene and pyrene to humic substances: Three-stage variation model. *Water Res.* **2003**, *37*, 4250-4258.
(86) Morehead, N. R.; Eadie, B. J.; Lake, B.; Landrum, P. F.; Berner, D. The sorption of PAH onto dissolved organic matter in lake Michigan waters. *Chemosphere* **1986**, *15*, 403-412.
(87) Li, N.; Lee, H. K. Tandem-cartridge solid phase extraction followed by GC-MS analysis for measuring partition coefficients of association of polycyclic aromatic hydrocarbons to humic acid. *Anal. Chem.* **2000**, *72*, 5272-5279.
(88) Rav-Acha, C.; Rebhun, M. Binding of organic solutes to dissolved humic substances and its effects on adsorption and transport in the aquatic environment. *Water Res.* **1992**, *26*, 1645-1654.
(89) Perminova, I. V.; Grechishcheva, N. Y. U.; Petrosyan, V. S. Relationships between structure and binding affinity of humic substances for polycyclic aromatic hydrocarbons: relevance of molecular descriptors. *Environ. Sci. Technol.* **1999**, *33*, 3781-3787.
(90) Gauthier, T. D.; Seitz, W. R.; Grant, C. L. Effects of structural and compositional variations of dissolved humic materials on pyrene K_{OC} values. *Environ. Sci. Technol.* **1987**, *21*, 243-248.
(91) Chin, Y.-P.; Aiken, G. R.; Danielsen, K. M. Binding of pyrene to aquatic and commercial humic substances: The role of molecular weight and aromaticity. *Environ. Sci. Technol.* **1997**, *31*, 1630-1635.
(92) Kopinke, F.-D.; Poerschmann, J.; Stottmeister, U. Sorption of organic pollutants on anthropogenic humic matter. *Environ. Sci. Technol.* **1995**, *29*, 941-950.
(93) Kumke, M. U.; Lohmannsroben, H.-G.; Roch, T. Fluorescence quenching of polycyclic aromatic compounds by humic-acid. *Analyst* **1994**, *119*, 997-1001.
(94) Carter, C. W.; Suffet, H. I. Binding of DDT to dissolved humic materials. *Environ. Sci. Technol.* **1982**, *16*, 735-740.
(95) Kopinke, F.-D.; Georgi, A.; Mackenzie, K. Sorption of pyrene to dissolved humic substances and related model polymers. 1. structure-property correlation. *Environ. Sci. Technol.* **2001**, *35*, 2536-2542.
(96) Oomen, A. G.; Mayer, P.; Tolls, J. Nonequilibrium solid-phase microextraction for determination of the freely dissolved concentration of hydrophobic organic compounds: matrix effects and limitations. *Anal. Chem.* **2000**, *72*, 2802-2808.

(97) Shor, L. M.; Rockne, K. J.; Taghon, G. L.; Young, L. Y.; Kosson, D. S. Desorption kinetics for field-aged polycyclic aromatic hydrocarbons from sediments. *Environ. Toxicol. Chem.* **2003**, *37*, 1535-1544.
(98) Rozycki, M.; Bartha, R. Problems associated with the use of azide as an inhibitor of microbial activity in soil. *Appl. Environ. Microbiol.* **1981**, *41*, 833-836.
(99) De Maagd, P. G. J.; Ten Hulscher, T. E. M.; Van Den Heuvel, H.; Opperhuizen, A.; Sijm, D. T. H. M. Physicochemical properties of polycyclic aromatic hydrocarbons: aqueous solubilities, n-octanol/water partition coefficients, and Henry's law constants. *Environ. Toxicol. Chem.* **1998**, *17*, 251-257.
(100) Szolar, H. J.; Rost, H.; Braun, R.; Loibner, A. P. Analysis of polycyclic aromatic hydrocarbons in soil: minimizing sample pretreatment using automated soxhlet with ethyl acetate as extraction solvent. *Anal. Chem.* **2002**, *74*, 2379-2385.
(101) Chiou, C. T.; Malcolm, R. L.; Brinton, T. I.; Kile, D. E. Water solubility enhancement of some organic pollutants and pesticides by dissolved humic and fulvic acids. *Environ. Sci. Technol.* **1986**, *20*, 502-508.
(102) Robbins, G. A.; Wang, S.; Stuart, J. D. Using the static headspace method to determine Henry's law constants. *Anal. Chem.* **1993**, *65*, 3113-3118.
(103) Kraaij, R. Ph.D.Thesis, Environ. Toxicol. Chem., University of Utrecht, 2001.
(104) De Bruijn, J.; Busser, F.; Seinen, W.; Hermens, J. L. M. Determination of octanol/water partition coefficients for hydrophobic organic chemicals with the 'slow-stirring' method. *Environ. Toxicol. Chem.* **1989**, *8*, 499-512.
(105) Leslie, H. A.; Ter Laak, T. L.; Busser, F. J. M.; Kraak, M. H. S.; Hermens, J. L. M. Bioconcentration of organic chemicals: is a solid phase microextraction fiber a good surrogate for biota? *Environ. Sci. Technol.* **2002**, *36*, 5399-5404.
(106) Kopinke, F.-D.; Poerschmann, J.; Remmler, M. Sorption behavior of anthropogenic humic matter. *Naturwissenschaften* **1995**, *82*, 28-30.
(107) Verhaar, H. J. M.; Busser, F.; Hermens, J. L. M. Surrogate parameter for the baseline toxicity content of contaminated water: simulating bioconcentration and counting molecules. *Environ. Sci. Technol.* **1995**, *29*, 726-734.
(108) Cornelissen, G.; Van Noort, P. C. M.; Govers, H. A. J. Desorption kinetics of chlorobenzenens, polycyclic aromatic hydrocarbons, and polychlorinated biphenyls: sediment extraction with Tenax(r) and effects of contact time and solute hydrophobicity. *Environ. Sci. Technol.* **1997**, *16*, 1351-1357.
(109) Schrap, S. M. Ph.D.Thesis, Ritox (IRAS), University of Utrecht, 1992.
(110) Sabljic, A.; Gusten, H.; Verhaar, H.; Hermens, J. L. M. QSAR modeling of soil sorption improvements and systematics of log K_{OC} vs. log K_{OW} correlations. *Chemosphere* **1995**, *31*, 4489-4514.
(111) Jonker, M. T. O.; Smedes, F. Preferential sorption of planar contaminants in sediments from lake Ketelmeer, The Netherlands. *Environ. Sci. Technol.* **2000**, *34*, 1620-1626.
(112) Allen-King, R. M.; Grathwohl, P.; Ball, W. P. New modeling paradigms for the sorption of hydrophobic organic chemicals to heterogeneous carbonaceous matter in soils, sediments, and rocks. *Adv. Water Resour.* **2002**, *25*, 985-1016.
(113) Huang, W. L.; Ping, P. A.; Yu, Z. Q.; Fu, H. M. Effects of organic matter heterogeneity on sorption and desorption of organic contaminants by soils and sediments. *Appl. Geochem.* **2003**, *18*, 955-972.
(114) Cornelissen, G.; Kukulska, Z.; Kalaitzidis, S.; Christianis, K.; Gustafsson, O. Relations between environmental black carbon sorption and geochemical sorbent characteristics. *Environ. Sci. Technol.* **2004**, *38*, 3632-3640.
(115) Wilcke, W. Polycyclic aromatic hydrocarbons (PAHs) in soil - a review. *J. Plant Nutrition Soil Sci.* **2000**, *163*, 229-248.
(116) Nguyen, Q. T.; Goss, K.-U.; Ball, W. P. Polyparameter linear free energy relationships for estimating the equilibrium partitioning of organic compounds between water and the natural organic matter in soils and sediments. *Environ. Sci. Technol.* **2005**, *39*, 913-924.

(117) Tang, J.; Carroquino, M.; Robertson, B.; Alexander, M. Combined effect of sequestration and bioremediation in reducing the bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ. Sci. Technol.* **1998**, *32*, 3586-3590.
(118) Alexander, M. Aging, bioavailability, and overestimation of risk from environmental pollutants. *Environ. Sci. Technol.* **2000**, *34*, 4259-4265.
(119) Kraaij, R.; Seinen, W.; Tolls, J.; Cornelissen, G.; Belfroid, A. C. Direct evidence of sequestration in sediments affecting the bioavailability of hydrophobic organic chemicals to bentic deposit-feeders. *Environ. Sci. Technol.* **2002**, *36*, 3525-3529.
(120) Gustafsson, O.; Haghseta, F.; Chan, C.; Macfarlane, J. K.; Gschwend, P. M. Quantification of the dilute sedimentary soot phase: implications for PAH speciation and bioavailability. *Environ. Sci. Technol.* **1997**, *31*, 203-209.
(121) Ghosh, U.; Gillette, J. S.; Luthy, R. G.; Zare, R. N. Microscale location characterization, and association of polycyclic aromatic hydrocarbons on harbour sediment particles. *Environ. Sci. Technol.* **2000**, *34*, 1729-1736.
(122) Jonker, M. T. O.; Koelmans, A. A. Sorption of polycyclic aromatic hydrocarbons and polychlorinated biphenyls to soot and soot-like materials in aqueous environment: Mechanistic considerations. *Environ. Sci. Technol.* **2002**, *36*, 3725-3734.
(123) Hawthorne, S. B.; Poppendieck, D. G.; Grabanski, C. B.; Loehr, R. C. Comparing PAH bioavailability from manufactured gas plant soils and sediments with chemical and biological tests. 1. PAH release during water desorption and supercritical carbon dioxide extraction. *Environ. Sci. Technol.* **2002**, *36*, 4795-4803.
(124) Cornelissen, G.; Elmquist, M.; Groth, I.; Gustafsson, O. Effect of sorbate planarity on environmental black carbon sorption. *Environ. Sci. Technol.* **2004**, *38*, 3574-3580.
(125) Tang, J.; Alexander, M. Mild extractability and bioavailability of polycyclic aromatic hydrocarbons in soil. *Environ. Toxicol. Chem.* **1999**, *18*, 2711-2714.
(126) Kelsey, J. W.; Kottler, B. D.; Alexander, M. Selective chemical extractants to predict bioavailability of soil-aged organic chemicals. *Environ. Sci. Technol.* **1997**, *31*, 214-217.
(127) Pignatello, J. J. Desorption of tetrachloroethene and 1,2-dibromo-3-chloropropane from aquifer sediments. *Environ. Toxicol. Chem.* **1991**, *10*, 1399-1404.
(128) Yeom, L.-T.; Ghosh, M. M.; Cox, C. D.; Ahn, K.-H. Dissolution of poycyclic aromatic hydrocarbons from weathered contaminated soil. *Water Sci. Technol.* **1996**, *34*, 335-342.
(129) Ten Hulscher, T. E. M.; Postma, J.; den Besten, P. J.; Stroomberg, G. J.; Belfroid, A. C.; Wegener, J. W.; Faber, J. H.; van der Pol, J. J. C.; Hendriks, A. J.; Van Noort, P. C. M. Tenax extraction mimics benthic and terrestrial bioavailability of organic compounds. *Environ. Toxicol. Chem.* **2003**, *22*, 2258-2265.
(130) Carroll, K. M.; Harkness, M. R.; Bracco, A. A.; Balcarcel, R. R. Application of a permeant/polymer diffusional model to the desorption of polychlorinated biphenyls from Hudson River sediments. *Environ. Sci. Technol.* **1994**, *28*, 253-258.
(131) Björklund, E.; Nilsson, T.; Bowadt, S.; Pilorz, K.; Mathiasson, L.; Hawthorne, S. B. Introducing selective supercritical fluid extraction as a new tool for determining sorption / desorption behavior and bioavailability of persistent organic pollutants in sediment. *J. Biochem. Biophys. Methods* **2000**, *43*, 295-311.
(132) Resendes, J.; Shiu, W. Y.; Mackay, D. Sensing the fugasity of hydrophobic orgainic chemicals in aqueous systems. *Environ. Sci. Technol.* **1992**, *26*, 2381-2387.
(133) Tolls, J.; Mclachlan, M. S. Partitioning of semivolatile organic compounds between air and *Lolium multiflorum* (welsh ray grass). *Environ. Sci. Technol.* **1994**, *28*, 159-166.

(134) Sproule, J. W.; Shiu, W. Y.; Mackay, D.; Schroeder, W. H.; Russell, R. W.; Gobas, F. A. P. Direct in situ sensing of the fugacity of hydrophobic chemicals in natural waters. *Environ. Toxicol. Chem.* **1991**, *10*, 9-20.
(135) Jensen, J. *Liberation progress report, Year 3,* NERI, 2005.
(136) Droge, S. T. J.; Ter Laak, T. L.; Hermens, J. L. M.; Van Gestel, C. A. M. *Use of Bioassays and SPME to determine the toxicity of PAHs in polluted field soils,* SETAC, Portland, USA, 2004
(137) Ter Laak, T. L.; Durjava, M.; Struijs, J.; Hermens, J. L. M. Solid phase dosing and sampling technique to determine partition coefficients of hydrophobic chemicals in complex matrixes. *Environ. Sci. Technol.* **2005**, *39*, 3736-3742.
(138) Brinch, U. C.; Ekelund, F.; Jakobsen, C. S. Method for spiking soil samples with organic compounds. *Applied and Environmental Micribiology* **2002**, *68, no 4*, 1808-1816.
(139) Yalkowsky, S. H.; Valvani, S. C.; Roseman, T. J. Solubility and partitioning. VI: octanol solubility and octanol water partition coefficients. *J. Pharm. Sci.* **1983**, *72*, 866-870.
(140) Ma, K. C.; Shiu, W. Y.; Mackay, D. *A critically reviewed compilation of physical and chemical and persistence data for 110 selected EMPPL substances,* A report prepared for the Ontario Ministry of Environment, Water Resources Branch, 1990.
(141) Jager, T. Ph.D.Thesis, Utrecht University, 2003.
(142) Flynn, G. L.; Yalkowsky, S. H. Correlation and prediction of mass transport across membranes I: Influence of alkyl chain length on flux-determining properties of barrier and diffusant. *J. Pharm. Sci.* **1972**, *61*, 838-852.
(143) Vaes, W. H. J.; Hamwijk, C.; Urrestarazu Ramos, E.; Verhaar, H. J. M.; Hermens, J. L. M. Partitioning of Organic Chemicals to Polyacrylate-Coated Solid Phase Microextraction Fibers: Kinetic Behavior and Quantitative Structure-Property Relationships. *Anal.Chem.* **1996**, *68*, 4458-4462.
(144) Verbruggen, E. M. J.; Vaes, W. H.; Parkerton, T. F.; Hermens, J. L. M. Polyacrylate-coated SPME fibers as a tool to simulate body residues and target concentrations of complex organic mixtures for estimation of baseline toxicity. *Environ. Sci. Technol.* **2000**, *34*, 324-331.
(145) Van Eijkeren, J. C. H.; Heringa, M. B.; Hermens, J. L. M. Modelling SPME data from kinetic measurements in complex samples. *Analyst* **2004**, *129*, 1137-1142.
(146) Mayer, P.; Christensen, P. S.; Jonsen, A. R.; Karlson, U. *A micro-scale technique to determine the diffusive mass transfer of hydrophobic organic substances through diffusive boundary layers (DBLs),* SETAC Europe, 2004
(147) Luthy, R. G.; Ramaswami, A.; Ghoshal, S.; Merkel, W. Interfacial films in coal tar nonaqueous-phase liquid-water systems. *Environ. Sci. Technol.* **1993**, *27*, 2914-2918.
(148) Jonker, M. T. O.; Hawthorne, S. B.; koelmans, A. A. Extremely Slowly Desorbing Polycyclic Aromatic Hydrocarbons from Soot and Soot-like Materials: Evidence by Supercritial Fluid Extraction. **submitted**.
(149) Ter Laak, T. L.; Agbo, S. O.; Barendregt, A.; Hermens, J. L. M. Freely dissolved concentrations of PAHs in soil pore water: measurements via solid phase extractions and consequences for soil tests. **submitted**.
(150) Northcott, G. L.; Jones, K. C. Partitioning, extractability, and formation of nonextractable PAH residues in soil. 1. compound differences in aging and sequestration. *Environ. Sci. Technol.* **2001**, *35*, 1103-1110.
(151) Cousins, I. T.; S, M. M.; Jones, K. C. Lack of an aging effect on the soil-air partitioning of polychlorinated biphenyls. *Environ. Sci. Technol.* **1998**, *32*, 2734-2740.
(152) Palomo, M.; Bhandari, A. Time-dependent sorption-desorption behavior of 2,4-dichlorophenol and its polymerization products in surface soils. *Environ. Sci. Technol.* **2005**, *39*, 2143-2151.

(153) Kraaij, R.; Tolls, J.; Sijm, D. T. H. M.; Cornelissen, G.; Heikens, A.; Belfroid, A. C. Effects of contact time on the sequestration and bioavailability of different classes of hydrophobic organic chemicals to benthic oligochaetes (tubificidae). *Environ. Toxicol. Chem.* **2002**, *21*, 752-759.
(154) Smedes, F.; Luszezanec, A. *Methodological concept to estimate bio-availability parameters for hydrophobic contaminants in sediments using solid phase samplers made from silicone rubber,* Impact bioavailability and assessment of pollutants in sediments and dredged materials under extreme hydrological conditions, Berlin, 2003
(155) Mulder, H.; Breure, A. M.; Rulkens, W. H. Application of a mechanistic desorption-biodegradation model to describe the behavior of polycyclic aromatic hydrocarbons in peat soil aggregates. *Chemosphere* **2001**, *42*, 285-299.
(156) Volkering, F.; Breure, A. M. In *PAHs: An Ecotoxicological Perspective*; Doube, P. E. T., Ed.; John Wiley & Sons ltd.: New York, 2003; pp 81-96.
(157) Artola-Garicano, E.; Borkent, I.; Damen, K.; Jager, T.; Vaes, W. H. J. Sorption kinetics and microbial biodegradation activity of hydrophobic chemicals in sewage sludge: model measurements based on free concentrations. *Environ. Sci. Technol.* **2003**, *37*, 116-122.
(158) Hwang, S.; Cutright, T. J. Preliminary exploration of the relationships between soil characteristics and PAH desorption and biodegradation. *Environment International* **2003**, *29*, 887-894.
(159) Huesemann, M. H.; Hausmann, T. S.; Fortman, T. J. Does bioavailability limit biodegradation? A comparison of hydrocarbon biodegradation and desorption rates in aged soils. *Biodegradation* **2004**, *15*, 261-274.
(160) Namiesnik, J.; Zabiegala, B.; Kot-Wasik, A.; Partyka, M.; Wasik, A. Passive sampling and/or extraction techniques in environmental analysis: a review. *Anal. Bioanal. Chem.* **2005**, *381*, 279-301.
(161) White, J. C.; Hunter, M.; Nam, K.; Pignatello, J. J.; Alexander, M. Correlation between biological and physical availabilities of phenanthrene in soils and soil humin in aging experiments. *Environ. Toxicol. Chem.* **1999**, *18*, 1720-1727.
(162) Londo, M.; Bakker, M. I.; Schrap, M. S. Measurements on water containing dissolved and suspended matter from natural sediments. *Chemosphere* **1996**, *32*, 1699-1708.
(163) Sijm, D.; Kraaij, R.; Belfroid, A. Bioavailability in soil or sediment: exposure of different organisms and approaches to study it. *Environ. Poll.* **2000**, *108*, 113-119.
(164) Lohmann, R.; Nelson, E.; Eisenreich, S. J.; Jones, K. C. Evidence for dynamic Air-water exchange of PCDD/Fs: a study in the raritan bay/hudson river estuary. *Environ. Sci. Technol.* **2000**, *34*, 3086-30293.
(165) Ter Laak, T. L.; Durjava, M.; Struijs, J.; Hermens, J. L. M. Development of a solid phase dosing and sampling technique to determine partition coefficients of hydrophobic chemicals in complex matrices. *Environmental Science and Technology* **in press**.
(166) Ter Laak, T. L.; Barendregt, A.; Droge, S. T. J.; Hermens, J. L. M. *Total and free porewater concentrations of PAHs in spiked and field contaminated soils,* SETAC, Portland, USA, 2004
(167) Yang, Y.; Miller, D. J.; Hawthorne, S. B. Solid-phase microextraction of polychlorinated biphenyls. *J. Chromatogr. A* **1998**, *800*, 257-266.
(168) Hawthorne, S. B.; Grabanski, C. B.; Miller, D. J.; Kreitinger, J. P. Solid phase microextraction measurement of parent and alkyl polycyclic aromatic hydrocarbons in milliliter sediment pore water samples and determination of K_{DOC} values. *Environ. Sci. Technol.* **2005**, *39*, 2795-2803.
(169) De Maagd, P. G. J.; Sinnige, T. L.; Schrap, S. M.; Opperhuizen, A.; Sijm, D. Sorption coefficients of polycyclic aromatic hydrocarbons for two lake sediments: Influence of the bactericide sodium azide. *Environ. Toxicol. Chem.* **1998**, *17*, 1899-1907.

(170) Kraaij, R.; Mayer, P.; Busser, F.; Van Het Bolscher, M.; Seinen, W.; Tolls, J. Measured pore-water concentrations make equilibrium partitioning work - a data analysis. *Environ. Sci. Technol.* **2003**, *37*, 268-274.
(171) Poon, K.-F.; Lam, P. K. S.; Lam, M. H. W. Determination of polychlorinated biphenyls in human blood serum by SPME. *Chemosphere* **1999**, *39*, 905-912.
(172) Ohlenbusch, G.; Kumke, M. U.; Frimmel, F. H. Sorption of phenols to dissolved organic matter investigated by solid phase microextraction. *Sci. Tot. Envir.* **2000**, *253*, 63-74.
(173) Dixon-Garrett, S. V.; Nagai, K.; Feeman, B. D. Ethylbenzene solubility, diffusivity, and permeability in poly(dimethylsiloxane). *Journal of Polymer Science* **2000**, *38*, 1461-1473.
(174) Burgess, R. M.; R, L. Role of black carbon in the partitioning and bioavailability of organic pollutants. *Environ. Toxicol. Chem.* **2004**, *23*, 2531-2533.
(175) Mulder, H.; Breure, A. M.; Van Andel, J. G.; Grotenhuis, T. C.; Rulkens, W. H. Effect of mass-ransfer limitations on bioavailability of sorbed naphthalene in synthetic soil matrices. *Environ. Toxicol. Chem.* **2000**, *19*, 2224-2234.
(176) Van Gestel, C. A. M.; Wei-chun Toxicity and bioaccumulation of Chlorophenols en Earthworms, in relation to Bioavailability in soil. *Ecotox. Environ. Saf.* **1988**, *15*, 289-297.
(177) Connell, D.; Markwell, R. Bioaccumulation in the soil to earthworm system. *Chemosphere* **1990**, *20*, 91-100.
(178) Schirmer, K.; Dixon, D. G.; Greenberg, B. M.; Bols, N. C. Ability of priority PAHs to be directly cytotoxic to a cell line from the rainbow trout gill. *Toxicology* **1998**, *127*, 129-141.
(179) Sverdrup, L. E.; Nielsen, T.; Krogh, P. H. Soil Ecotoxicity of Polycyclic Aromatic Hydrocarbons in Relation to Soil Sorption, Lipophilicity, and Water Solubility. *Environ. Sci. Technol.* **2003**, *36*, 2429-2435.
(180) Bleeker, E. J. A.; Wiegman, S.; Droge, S. T. J.; Kraak, M. H. S.; Van Gestel, C. A. M. *Towards an improvement of the risk assessment of Polycyclic (Hetero) Aromatoc Hydrocarbons,* UvA/VU, 2003.
(181) FEDESA. *Antibiotic use in farm animals does not threaten human health*, 2001.
(182) Thiele-Bruhn, S. Pharmaceutical antibiotic compounds in soils - a review. *Journal of Plant Nutrition and Soil Science* **2003**, *166*, 145-167.
(183) Halling-Sorensen, B.; Nors Nielsen, S.; Lanzky, P. F.; Ingerslev, F.; Holten Lutzhoft, H. C.; Jorgensen, S. E. Occurrence Fate and effects of pharmaceutical substances in the environment - a review. *Chemosphere* **1998**, *36*, 357-393.
(184) Sixma, J. J. *Milieurisico's van geneesmiddelen (Environmental risks of medicines),* Health council of the Netherlands, 2001.
(185) Jongbloed, R. H.; Kan, C. A.; Blankendaal, V. G.; Bernhard, R. *Milieurisico's van diergeneesmiddelen en veevoeradditieven in Nederlandse oppervlaktewateren,* TNO-MEP, 2001.
(186) Veterinary International Conference on Harmonization. *Pharmaceuticals in the European Union. Office for official publications of the European communities,* DG Enterprise, 2000.
(187) European Medicines Evaluation Agency. *Note for guidance: Environmental risk assessment for veterinary medicinal products other than GMO-containing and immunological products.*, 1997.
(188) Winckler, C.; Grafe, A. *Charakterisierung und Verwertung von Abfällen aus der Massentierhaltung unter Berücksichtigung verschiedener Böden,* Umweltbundesamt, 2000.
(189) Winckler, C.; Grafe, A. Use of veterinary drugs in intensive animal production. *Journal of Soil and Sediment* **2001**, *1*, 66-70.
(190) Kroker, R. Aspekte zur Ausscheidung antimikrobiell wirksamer Substanzen nach de chemotherapeutischen Behandlung von Nutztieren. *Wissenschaft und Umwelt* **1983**, *4*, 305-308.
(191) Aiello, S. E. *The Merck veterinary manual*; 8 ed.; Whitehouse Station, NJ, 1998.

(192) Halling-Sorensen, B.; Jensen, J.; Tjornelund, J.; Montforts, M. H. M. M. In *Pharmaceuticals in the environment*; veterinary antibiotics and residues in Danish agriculture, Ch 13 ed.; Kümmerer, K., Ed.; Springer Verlag: Germany, 2001; pp 1-21.
(193) Van Gool, S. Mogelijke effecten van antibiotica residuen in dierlijke mest of het milieu. *Tijdschrift Diergeneeskunde* **1993**, *118*, 8-10.
(194) Ingerslev, F.; Halling-Sorensen, B. Biodegradability of Metrobidazole, Olaquindox and Tylosin and formation of tylosin degradation products in aerobic soil-manure slurries. *Ecotox. Environ. Saf.* **2001**, *48*, 311-320.
(195) Kolpin, D. W.; Furlong, E. T.; Meyer, M. T.; Thurman, E. M.; Zaugg, S. D.; Barber, L. B.; Buxton, H. T. Pharmaceuticals, hormones, and other organic wastewater contaminants in U.S. streams, 1999-2000: a national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202-1211.
(196) Hamscher, G.; Sczesny, S.; Hopner, H.; Nau, H. Determination of persistent tetracycline residues in soil fertilized with liquid chromatography with electrospray ionization tandem mass spectrometry. *Anal. Chem.* **2002**, *74*, 1509-1518.
(197) Holm, J. V.; Rugge, K.; Bjerg, P. L.; Christensen, T. H. Occurrence and distribution of pharmaceutical organic compounds in the groundwater downgradient of a landfill (Grindsted, Denmark). *Environ. Sci. Technol.* **1995**, *29*, 1415-1420.
(198) Meyer, M. T.; Bumgarner, J. E.; Varns, J. L.; Daughtridge, J. V.; Thurman, E. M.; Hostetler, K. A. Use of radioimmunoassay as a screen for antibiotics in confined animal feeding operations and confirmation by liquid chromatography/mass spectrometry. *Sci. Tot. Envir.* **2000**, *248*, 181-187.
(199) Jafvert, C. T.; Westall, J. C.; Grieder, E.; Schwarzenbach, R. P. Distribution of hydrophobic ionogenic compounds between octanol and water: organic acids. *Environ. Sci. Technol.* **1990**, *24*, 1795-1803.
(200) Johnson, A. C.; Westall, J. C. Effect of pH and KCl concentration on the octanol-water distribution of methylanilines. *Environ. Sci. Technol.* **1990**, *24*, 1869-1875.
(201) Escher, B. I.; Schwarzenbach, R. P. Partitioning of substituted Phenols in liposome-water biomembrane-water and Octanol-water Systems. *Environ. Sci. Technol.* **1996**, *30*, 260-270.
(202) Doluisio, J. T. Ph.D.Thesis, Purdue University, 1962.
(203) Westall, J. C.; Chen, H.; Zhang, W.; Brownawell, B. J. Sorption of linear alkylbenzenesulfonates on sediment materials. *Environ. Sci. Technol.* **1999**, *33*, 3110-3118.
(204) German-Heins, J.; Flury, M. Sorption of Brilliant Bleu FCF in soils as affected by pH and Ionic strength. *Geoderma* **2000**, *97*, 87-101.
(205) Boxall, B. A.; Blackwell, P.; Cavallo, R.; Kay, P.; Tolls, J. The sorption and transport of a sulphonamide antibiotic in soil systems. *Toxicology Letters* **2002**, *131*, 19-28.
(206) Ghandour, M. A.; Azab, H. A.; Hassan, A.; Ali, A. M. Potentiometric studies on the complexes of tetracycline (TC) and oxytetracycline (OTC) with some metal ions. *Monatsh. Chem.* **1992**, *123*, 51-58.
(207) Martin, S. R. Equilibrium and kinetic studies on the interaction of tetracyclins with calcium and magnesium. *Biophys. Chem.* **1979**, *10*, 319-326.
(208) Doluisio, J. T.; Martin, A. N. Metal complexation of the tetracycline hydrochlorides. *J. Med. Chem.* **1963**, *6*, 16-20.
(209) Baker, W. A.; Brown, P. M. Metal binding in tetracyclines. Cobalt(II) and Nickel(II) complexes. *J. Am. Chem. Soc.* **1966**, *88*, 1314-1317.
(210) Essington, M. E. *Soil and water chemistry*; CRC press LLC: Boca Raton, Florida, USA, 2004.
(211) OECD. *OECD guideline for the testing of chemicals (106): Adsorption - Desorption using a batch equilibrium method,* OECD, 2000.
(212) SRC *SRC PhysProp Database* http://esc.syrres.com/interkow/database.htm,

(213) Meylan, W. M.; Howard, P. H. Estimation log P with atom/fragments and water solubility with log P. *Perspectives in Drug Discovery and Design* **2000**, *19*, 67-84.
(214) Lin, C. E.; Lin, W.-C.; Chen, Y. C.; Wang, S.-W. Migration behavior and selectivity of sulfonamides in capillary electrophoresis. *J. Chromatogr. A* **1997**, *792*, 37-47.
(215) Budavari, S. *The Merck Index*; 11 ed.; Rahway: New York, 1989.
(216) EPI-suite; KOWWIN 1.66, Toronto University, 2000.
(217) Mitscher, L. A. *The chemistry of the tetracycline antibiotics*; Marcel Dekker: New York, 1978.
(218) Ter Laak, T. L.; Gebbink, W. A.; Tolls, J. The effect of pH and ionic strength on the sorption of Oxytetracyclin, Tylosin and Sulfachloropyridazin to soil. **submitted**.
(219) Holten Lützhøft, H. C.; Vaes, W. H. J.; Freidig, A. P.; Halling-Sørensen, B.; Hermens, J. L. M. Influence of pH and other modifying factors on the distribution behavior of 4-quinolones to solid phases and humic acids studied by "negligible-depletion" SPME-HPLC. *Environ. Sci. Technol.* **2000**, *34*, 4989-4994.
(220) Wessels, J. M.; Ford, W. E.; Szymczak, W.; Schneider, S. The complexation of tetracycline and Anhydrotetracycline with Mg^{2+} and Ca^{2+}: A spectroscopic study. *J Physic. Chem. B* **1998**, *102*, 9323-9331.
(221) Billo, E. J. In *Excel (r) for chemists: A comprehensive guide*; 2nd ed.; Wiley-VCH: New York, 2001; pp 349-367.
(222) Langhammer, J.-P. Ph.D.Thesis, Food Chemistry, Rheinische Friedrich-Wilhelms-Universität Bonn, 1989.
(223) Fontaine, D. D.; G, L. R.; R, M. J. Soil Adsorption of neutral and anionic forms of a sulfonamide herbicide, Flumetsulan. *J Environ. Qual.* **1991**, *20*, 759-762.
(224) Thurman, E. M.; Lidsey, M. E. *Transport of antibiotics in soil and their potential for ground-water contamination,* The 11th SETAC Europe meeting, Madrid, 2001
(225) Thiele-Bruhn, S.; Aust, M.-O. Effects of pig slurry on the sorption of sulfonamide antibiotics in soil. *Environ. Contam. Toxicol.* **2004**, *47*, 31-39.
(226) Rabolle, M.; Spliid, N. H. Sorption and mobility of metronidzole, olaquindox, oxytetracycline and tylosin in soil. *Chemosphere* **2000**, *40*, 715-722.
(227) Tolls, J.; Gebbink, W. A.; Cavallo, R. E. *pH-dependence of sulfonamode antibiotic sorption: Data and model evaluation,* SETAC Europe, Vienna, 2002
(228) Sithole, B. B.; Guy, R. D. Models for tetracycline in aquatic environments. I. Interaction with bentonite clay systems. *Water, Air Soil Poll.* **1987**, *32*, 303-314.
(229) Figueroa, R. A.; Leonard, A.; MacKay, A. A. Modeling tetracycline antibiotic sorption to clays. *Environ. Sci. Technol.* **2004**, *38*, 476-483.
(230) Sithole, B. B.; Guy, R. D. Models for tetracycline in aquatic environments. II. Interaction with humic substances. *Water, Air Soil Poll.* **1987**, *32*, 315-321.
(231) Lee, L. S.; Rao, P. S. C.; Nkedi-Kizza, P.; Delfino, J. J. Influence of solvent and sorbent characteristics on distribution of pentachlorophenol in octanol-water and soil-water systems. *Environ. Sci. Technol.* **1990**, *24*, 654-661.
(232) Rocha, W. S. D.; Regitano, J. B.; Alleoni, L. R. F.; Tornisielo, V. L. Sorption of imazaquin in soils with positive balance charges. *Chemosphere* **2002**, *49*, 263-270.
(233) McCarthy, J. F.; Howard, P. H.; McKay, L. D. Effect of pH on sorption and transport of fluobenzoic acid ground water tracers. *J Environ. Qual.* **2000**, *29*, 1806-1813.
(234) Seremet, D. E.; Mackay, D. *Sorption of ciprofloxacin and its substructures to pure clays,* American Chemical Society, New York, 2003
(235) Figueroa, R. A.; MacKay, A. A. *Sorption oxytetracycline to iron oxide particles,* American Chemical Society, New York, 2003

(236) Rubio, J. A.; Gonzalez-Mazo, E.; Gomez-Parra, A. Sorption of linear alkylbenzenesulfonate (LAS) on marine sediment. *Mar. Chem.* **1996**, *54*, 171-177.
(237) MacKay, A. A.; Figueroa, R. A.; Leonard, A.; Seremet, D. E. *Antibiotic sorption to soils: Consideration of multiple physico-chemical mechanisms,* American Chemical Society, New York, 2003
(238) EPA. *Fate, transport and transformation test guidlines,* Environmental protection agency, 1998.
(239) Salloum, M. J.; Dudas, M. J.; McGill, W. B.; Murphy, S. M. Surfactant sorption to soil and geologic samples with varying mineralogical and chemical properties. *Environ. Toxicol. Chem.* **2000**, *19*, 2436-2442.
(240) Vasudevan, D.; Cooper, E. M. 2,4-D sorption in iron oxide-rich soils: role of soil phosphate and exchangeable Al. *Environ. Sci. Technol.* **2004**, *38*, 163-170.
(241) De Groot, A. C.; Peijnenburg, W. J. G. M.; Van Den Hoop, M. A. G. T.; Ritsema, R.; Van Veen, R. P. M. *Heavy metals in Dutch field soils: an experimental and theoretical study on equilibrium partitioning,* RIVM, 1998.
(242) Minitab-Inc.; SCAN 1.1, 1995.
(243) Nowara, A.; Burhenne, J.; Spiteller, M. Binding of fluoroquinolone carboxylic acid derivatives to clay minerals. *J. Agric. Food Chem.* **1997**, *45*, 1459-1463.
(244) Ter Laak, T. L.; Gebbink, W. A.; Tolls, J. *Influence of pH and ionic strenght on the sorption of veterinary pharmaceuticals to soil,* ACS, New York, 2003
(245) Chung, N.; Alexander, M. Differences in sequestration and bioavailability of organic compounds aged in dissimilar soils. *Environ. Sci. Technol.* **1998**, *32*, 855-860.
(246) Chung, N.; Alexander, M. Effect of concentration on sequestration and bioavailability of two polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.* **1999**, *33*, 3605-3608.
(247) Tang, J.; Robertson, B.; Alexander, M. Chemical extraction methods to estimate bioavailability of DDT, DDE, and DDD in soil. *Environ. Sci. Technol.* **2000**, *33*, 4346-4351.
(248) Cornelissen, G.; Gustafsson, O. Sorption of phenanthrene to environmental black carbon in sediment with and without organic matter and native sorbates. *Environ. Sci. Technol.* **2004**, *38*, 148-155.
(249) Xiao, B.; Yu, Z.; Huang, W.; Song, J.; Peng, P. Black carbon and kerogen in soils and sediments. 2. their roles equilibrium sorption of less-polar organic pollutants. *Environ. Sci. Technol.* **2004**, *38*, 5842-5852.
(250) Bucheli, T. D.; Gustafsson, O. Quantification of the soot-water distribution coefficient of PAHs provides mechanistic basis for enhanced sorption observations. *Environ. Sci. Technol.* **2000**, *34*, 5144-5151.
(251) Cornelissen, G.; Gustafsson, O. Importance of unburnt coal carbon, black carbon and amorphous organic carbon to phenantrene sorption in sediments. *Environ. Sci. Technol.* **2005**, *39*, 764-769.
(252) Cornelissen, G.; Gustafsson, O.; Bucheli, T. D.; Jonker, M. T. O.; Koelmans, A. A.; Van Noort, P. C. M. Extensive sorption of organic compounds to black carbon, coal and kerogen in sediments and soils: Mechanisms and consequences for distribution, bioaccumulation and biodegradation. *Environ. Sci. Technol.* **2005**, Accepted.
(253) Reid, B. J.; Stokes, J. D.; Jones, K. C.; Semple, K. T. Nonexhaustive cyclodextrin based extraction technique for the evaluation of PAH bioavailability. *Environ. Sci. Technol.* **2000**, *34*, 3174-3179.
(254) Cornelissen, G.; Rigterink, H.; Ferdinandy, M. M. A.; Van Noort, P. C. M. Rapidly desorbing fractions of PAHs in contaminated sediments as a predictor of the extent of bioremediation. *Environ. Sci. Technol.* **1998**, *32*, 966-970.

(255) Holten Lützhøft, H.-C.; Vaes, W. H. J.; Freidig, A. P.; Halling-Sørensen, B.; Hermens, J. L. M. 1-Octanol/water distribution coefficient of oxolinic acid: influence of pH and its relation to the interaction with dissolved organic carbon. *Chemosphere* **2000**, *40*, 711-714.
(256) Myers, P. A.; Quinn, J. G. Association of hydrocarbons and mineral particles in saline solution. *Nature* **1973**, *244*, 23-24.
(257) Vasudevan, D.; Cooper, E. M.; Exem, O. L. Sorption-Desorption of ionogenic compounds at mineral-water Interface: Study of metal oxide-rich soils and pure-phase minerals. *Environ. Sci. Technol.* **2002**, *36*, 501-511.
(258) Xu, S.; Boyd, S. A. Cationic surfactant adsorption by swelling and nonswelling layer silicates. *Langmuir* **1995**, *11*, 2508-2514.
(259) Ingerslev, F.; Halling-Sorensen, B. Biodegradability of metrobidazole, olaquindox and tylosin and formation of tylosin degradation products in aerobic soil-manure slurries. *Ecotoxicol. Environ. Safety* **2001**, *48*, 311-320.
(260) Haller, M. Y.; Müller, S. R.; McArdell, C. S.; Alder, A.; Suter, M. J. F. Quantification of veterinary antibiotics (sulfonamides and trimethoprim) in animal manure by liquid chromatography-mass spectrometry. *J. Chromatogr. A* **2002**, *952*, 111-120.

Appendix I

Table A: Partitioning of 7 PAHs between PDMS and a 1:1 methanol-water mixture at 20 ±1°C. The 30 μm PDMS fibers were loaded for at least 24 hours in the methanol water mixture. Fiber concentrations (n= 5) were divided by the concentrations in the methanol water mixture (n= 4) in order to obtain the partition coefficient, and the relative standard deviation.

Compound	$K_{PDMS/MEOH-WATER}$	*RSD%*
Phenanthrene	36.4	2.8
Fluoranthene	50.7	2.9
Pyrene	54.0	1.9
Benz[a]antracene	86.8	2.3
Benzo[b]fluoranthene	109.2	2.4
Benzo[k]fluoranthene	140.0	5.0
Benzo[ghi]perylene	194.9	4.5

Table B: pH and conductivity were monitored with increasing DOC concentrations. It can be observed that the conductivity is stable, but pH increases 0.8 units with increasing DOC.

DOC concentration mg/L (nominal)	*pH*	*conductivity μS/cm*
0	7.03	736
0.2	7.08	778
0.4	7.10	776
1.9	7.31	784
3.8	7.21	782
9.6	7.39	782
19.1	7.62	784
37.4	7.82	802

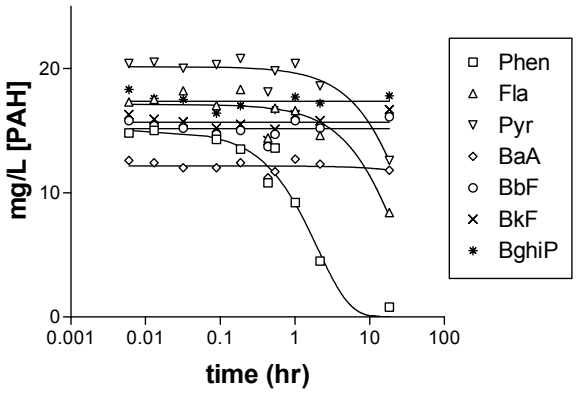


Figure A: The evaporation of PAHs from a 30 μm PDMS fiber under a gentle stream of air. Loaded fibers were exposed to a gentle stream of air in the fume hood. Fibers were sampled at different time intervals, concentrations were measured, and a one-phase exponential decay curve (Equation 2) was fitted on the concentrations (except for BbF, BkF and BghiP, where no decrease was observed in one day).

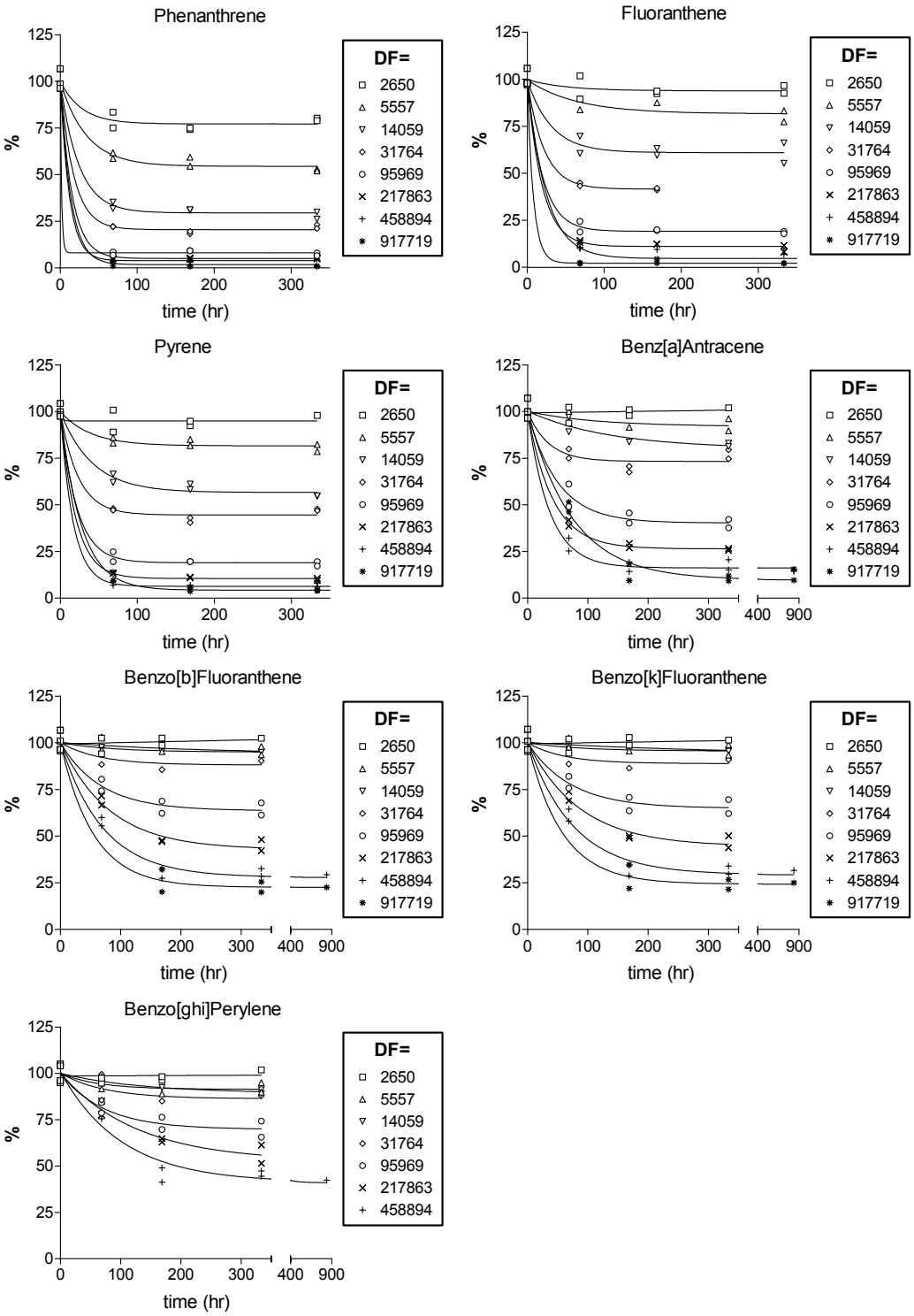


Figure B: The depletion of 7 PAHs from the SPME plotted against time, at 8 different water-PDMS ratios.

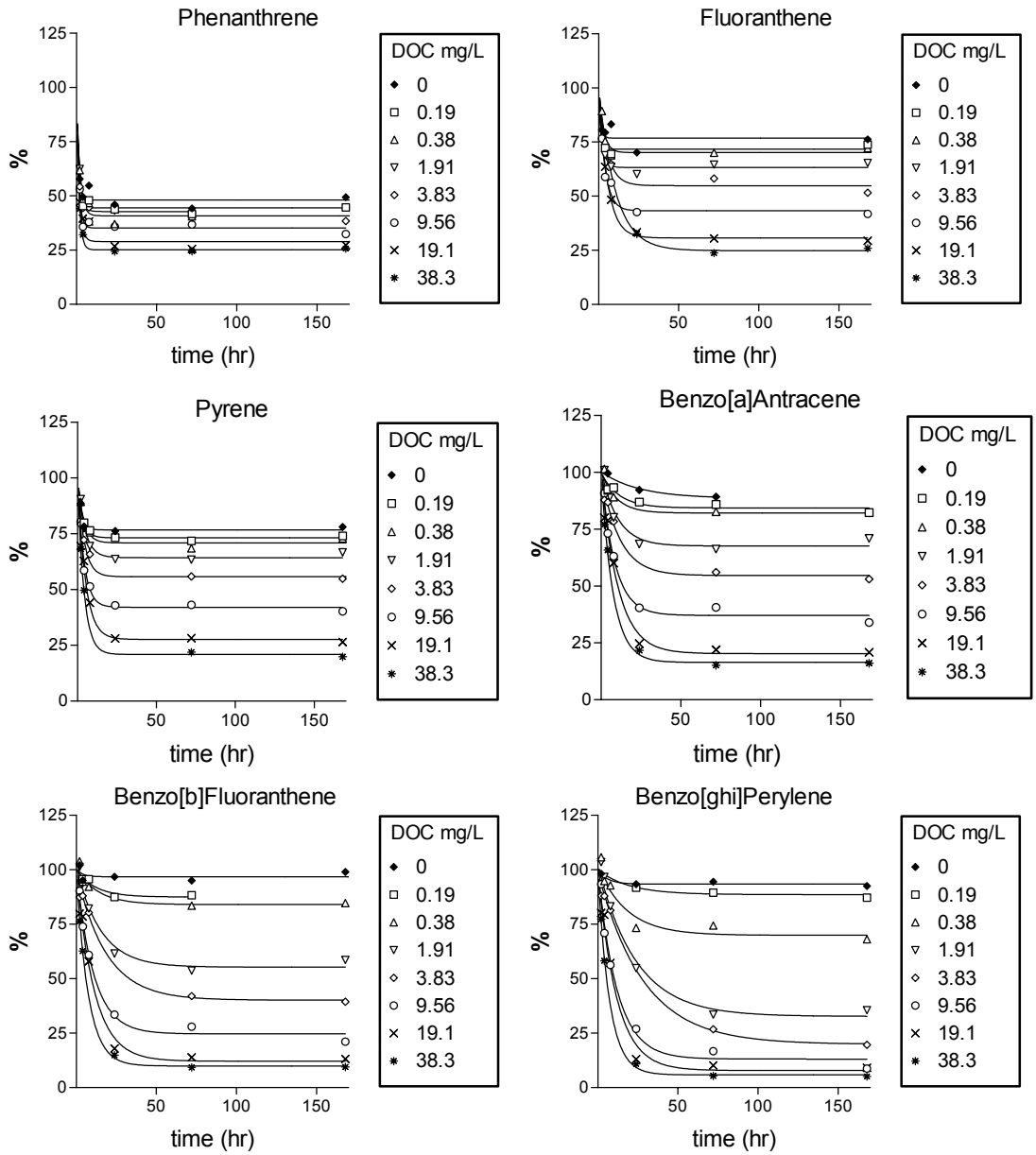


Figure C: The depletion of 6 PAHs from the SPME fibers against time, at 8 different DOC (Aldrich humic acid) concentrations.

Derivation of Equation 3

V_{aq} = Volume of the aqueous phase (L)
V_f = Volume of the fiber-coating (L)
DF = Dilution factor = V_{aq}/V_f (L/L)
$C_{f(initial)}$ = Initial concentration of the chemical in the freshly charged fiber (mg/L)
$C_{f(DF)}$ = Equilibrium concentration of the chemical in the fiber at a certain DF (mg/L)
$C_{aq(DF)}$ = Equilibrium concentration of the chemical in aqueous phase at a certain DF (mg/L)
K_f = Partition coefficient between fiber (PDMS) and water = C_f / C_{aq} (L/L)

A mass balance of the fiber-water system at equilibrium (a)

$$V_f * C_{f(initial)} = V_f * C_{f(DF)} + V_{aq} * C_{aq(DF)}$$

dividing by V_f and substituting V_{aq}/V_f by DF (b)

$$C_{f(initial)} = C_{f(DF)} + DF * C_{aq(DF)}$$

substituting $C_{aq(DF)}$ with $C_{f(DF)}/C_{aq(DF)} = K_f$ (c)

$$C_{f(initial)} = C_{f(DF)} + DF * \frac{C_{f(DF)}}{K_f}$$

dividing by $C_{f(DF)}$ (d)

$$\frac{C_{f(initial)}}{C_{f(DF)}} = 1 + \frac{DF}{K_f}$$

inverting and expressing the ratio $C_{f(DF)} / C_{f(initial)}$ as a percentage gives (e)

$$\frac{C_{f(DF)}}{C_{f(initial)}}(\%) = \frac{100\%}{1 + \frac{DF}{K_f}}$$

Derivation of Equation 4

V_{aq} = Volume of the aqueous phase (L)
V_f = Volume of the fiber-coating (L)
DF = Dilution factor = V_{aq}/V_f (L/L)
[DOC] = Concentration of DOC in the water (mg/L)
$C_{f(initial)}$ = Concentration of the chemical in the freshly charged fiber (mg/L)
$C_{f(DOC)}$ = Equilibrium concentration of the chemical in the fiber at a certain DOC concentration (mg/L)
$C_{aq(DOC)}$ = Equilibrium concentration of the chemical in aqueous phase at a certain DOC concentration (mg/L)
$C_{DOC(DOC)}$ = Concentration of PAHs in /on the DOC (mg/kg) at a certain DOC concentration (mg/L)
K_f = Partition coefficient between fiber (PDMS) and water = $C_f / C_{aq} = C_{f(DOC)} / C_{aq(DOC)}$ (L/L)
K_{DOC} = Partition coefficient between DOC and water = $C_{DOC} / C_{aq(DOC)}$ (L/kg)

A mass balance of the fiber-water-DOC system at equilibrium (a)

$$V_f * C_{f(initial)} = V_f * C_{f(DOC)} + V_{aq} * C_{aq(DOC)} + [DOC] * C_{DOC(DOC)} * V_{aq}$$

dividing by V_f and substituting V_{aq}/V_f by DF (b)

$$C_{f(initial)} = C_{f(DOC)} + DF * C_{aq(DOC)} + [DOC] * C_{DOC(DOC)} * DF$$

substituting $C_{DOC}/C_{aq(DOC)}$ with K_{DOC} (c)

$$C_{f(initial)} = C_{f(DOC)} + DF * C_{aq(DOC)} + [DOC] * K_{DOC} * C_{aq(DOC)} * DF$$

substituting $C_{aq(DOC}$ with $C_{f(DOC)}/C_{aq(DOC)} = K_f$ (d)

$$C_{f(initial)} = C_{f(DOC)} + DF * C_{f(DOC)} / K_f + [DOC] * K_{DOC} * C_{f(DOC)} / K_f * DF$$

dividing by $D_{f(DF)}$ (e)

$$\frac{C_{f(initial)}}{C_{f(DOC)}} = 1 + \frac{DF}{K_f} + [DOC] * K_{DOC} * \frac{DF}{K_f}$$

inverting and expressing the ratio $C_{f(DOC)} / C_{f(initial)}$ as a percentage gives (f)

$$\frac{C_{f(DOC)}}{C_{f(initial)}}(\%) = \frac{100\%}{1 + \frac{DF}{K_f} * (1 + [DOC] * K_{DOC})}$$

So the percentage recovered from the fiber ($100\% * C_{f(DOC)} / C_{f(initial)}$) can be described as a function of the initial concentration on the fiber ($C_{f(initial)}$) and the different properties of the system (DF, [DOC]) and the hydrophobic phases (K_f, K_{DOC}). Since, only [DOC] was varied, the K_f was known from the previous experiment, and the DF was constant (8064), the K_{DOC} was the only variable that was fit from the data.

Appendix II

Table A: Initial concentrations of the PAHs spiked to the clean field soils (mg/kg dry weight).

Compound	*Askov*	*Borris-2*	*Kettering*	*Waschbach*	*Norway*
Phe	28.9	28.8	28.8	28.8	28.8
Fla	21.6	20.9	20.9	20.9	20.9
Pyr	22.9	20.1	20.1	20.1	20.1
BaA	11.5	9.5	9.5	9.5	9.5
BbF	9.4	9.6	9.6	9.6	9.6
BkF	10.0	9.4	9.4	9.4	9.4
BghiP	9.63	7.15	7.15	7.15	7.15
ΣPAH	113.9	105.4	105.4	105.4	105.4

Table B: Concentrations of the PAHs in the field contaminated soils (mg/kg dry weight).

Compound	*Andujar-B2*	*B101*	*E6068-K*	*K3840*	*Olst-J*	*Skaegen*	*TP44*
Naph	592	- [a]	9.5	- [a]	- [a]	- [b]	- [b]
Flu	- [a]	- [a]	3.8	- [a]	- [a]	- [b]	- [b]
Phe	1450	5.02	46.4	0.47	- [a]	49.2	46.1
Anth	309	1.32	14.0	0.17	0.18	8.53	19.1
Fla	1418	13.2	114.3	3.82	1.43	101.5	218.2
Pyr	395	6.21	35.0	1.46	1.29	95.4	159.8
BaA	115	4.15	19.2	1.18	1.21	43.9	49.7
Chr	197	7.69	36.1	2.61	1.09	79.6	90.1
BbF	31.6	2.92	14.6	1.44	3.11	37.5	93.2
BkF	17.7	2.05	9.0	0.85	1.28	19.3	55.4
BaP	25.9	4.66	25.0	2.21	3.02	51.4	145.3
DahA	- [a]	0.38	1.91	0.21	0.35	3.25	13.6
BghiP	9.52	5.87	16.7	2.25	3.05	32.6	130
ΣPAH	4560	53.5	345	16.7	16.0	522	1021

[a] Peaks could not be quantified.
[b] Compounds were not analyzed.

Table C: Recovery of initial spiked concentrations after different periods of aging (%).

Comp.	*Askov*			*Borris-2*			*Kettering*			*Waschbach*			*Norway*		
	21 d	*240 d*	*553 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*
Phe	89.4	6.3	2.3	89.7	91.2	80.5	93.2	96.1	28.1	94.3	94.3	13.2	91.6	89.8	2.5
Fla	85.7	56.2	6.1	81.6	78.2	71.7	87.7	85.4	70.6	87.2	80.5	68.1	80.1	75.4	32.9
Pyr	86.7	76.1	8.9	88.8	86.1	83.5	92.8	90.5	83.6	95.1	89.8	86.7	88.5	79.7	56.2
BaA	86.7	59.2	20.0	87.9	80.9	70.4	89.9	87.8	81.0	93.8	87.8	80.3	87.4	76.6	57.0
BbF	91.8	72.7	48.2	84.0	80.6	71.8	85.2	86.3	81.3	90.2	85.5	80.6	83.5	75.4	65.4
BkF	82.0	73.9	58.3	84.7	80.3	71.4	85.0	86.4	80.3	89.3	85.1	79.3	85.3	77.3	67.6
BghiP	92.5	79.3	71.4	84.3	77.8	71.5	88.3	87.0	83.1	90.7	83.4	81.0	87.9	74.2	69.5

Note that recoveries are calculated from nominal spiking concentrations, the spiking procedure using air-dried soil sub-sample and evaporating the carrier solvent (acetone) overnight already led to some losses by volatilization.

Table D: Organic carbon normalized sorption coefficients (log K_{OC}) of spiked soils.

Comp.	*Askov*			*Borris-2*			*Kettering*			*Waschbach*			*Norway*		
	21 d	*240 d*	*553 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*	*19 d*	*71 d*	*177 d*
Phe	4.31	-[a]	-[a]	4.38	4.38	4.49	4.03	4.07	5.17	4.05	4.05	4.98	4.45	4.50	5.07
(SE)	(0.05)	-[a]	-[a]	(0.03)	(0.02)	(0.02)	(0.05)	(0.01)	0.12	(0.02)	(0.01)	(0.10)	(0.02)	(0.01)	(0.06)
Fla	4.89	5.35	-[a]	5.00	5.01	5.10	4.63	4.68	4.90	4.62	4.65	4.90	5.07	5.10	5.31
(SE)	(0.05)	(0.10)	-[a]	(0.04)	(0.01)	(0.02)	(0.07)	(0.02)	(0.05)	(0.03)	(0.02)	(0.05)	(0.02)	(0.02)	(0.04)
Pyr	5.03	5.54	-[a]	5.10	5.11	5.21	4.72	4.76	4.98	4.73	4.73	4.98	5.16	5.20	5.39
(SE)	(0.05)	(0.10)	-[a]	(0.03)	(0.01)	(0.02)	(0.06)	(0.01)	(0.04)	(0.02)	(0.02)	(0.03)	(0.02)	(0.01)	(0.02)
BaA	5.73	5.88	5.94	5.83	5.82	5.94	5.42	5.46	5.70	5.44	5.42	5.64	5.89	5.93	6.15
(SE)	(0.05)	(0.02)	(0.03)	(0.02)	(0.01)	(0.02)	(0.06)	(0.02)	(0.04)	(0.02)	(0.02)	(0.04)	(0.02)	(0.01)	(0.01)
BbF	6.43	6.56	6.70	6.56	6.57	6.71	6.10	6.16	6.40	6.09	6.11	6.33	6.59	6.61	6.83
(SE)	(0.05)	(0.02)	(0.02)	(0.02)	(0.01)	(0.02)	(0.07)	(0.02)	(0.04)	(0.02)	(0.03)	(0.04)	(0.02)	(0.02)	(0.01)
BkF	6.39	6.54	6.59	6.58	6.58	6.72	6.11	6.17	6.41	6.13	6.16	6.40	6.59	6.62	6.83
(SE)	(0.05)	(0.01)	(0.01)	(0.02)	(0.01)	(0.02)	(0.07)	(0.02)	(0.05)	(0.02)	(0.03)	(0.04)	(0.02)	(0.02)	(0.01)
BghiP	6.94	7.12	7.22	7.27	7.27	7.43	6.73	6.77	7.00	6.73	6.73	7.04	7.19	7.22	7.45
(SE)	(0.05)	(0.01)	(0.02)	(0.03)	(0.02)	(0.03)	(0.07)	(0.02)	(0.05)	(0.02)	(0.03)	(0.04)	(0.02)	(0.01)	(0.01)

[a] Severe degradation of the compounds made the quantification of the concentrations in the fibers impossible.

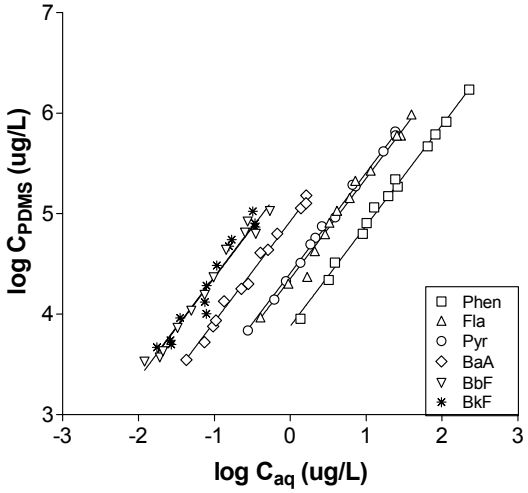


Figure A: The concentrations in the fiber coating (log C_f) vs. measured aqueous concentration (log C_{aq}).

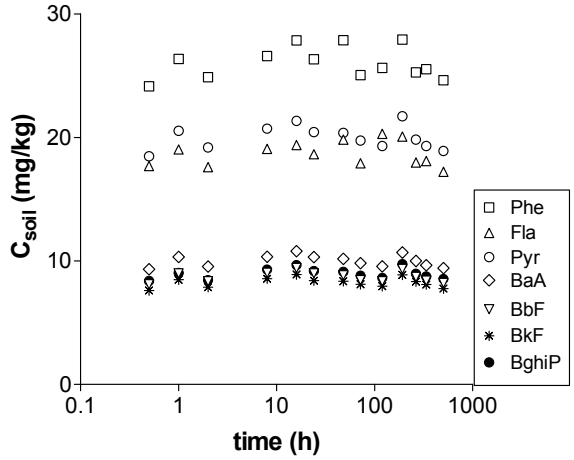


Figure B: The total soil concentrations in the spiked Askov soil (21 d aging) after different periods of fiber-exposure. Total concentrations vary due to the heterogeneously spread PAHs (and organic carbon), but no trend can be observed over the 504 hours of fiber-exposure.

Dankwoord

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Thomas

Curriculum Vitae

Thomas ter Laak was born on July the 29th, 1978 in Leiden, the Netherlands. He graduated from the Montessori Lyceum Herman Jordan in 1996, and started Biology studies at the University of Amsterdam the same year. During the final part of his studies he did two research projects. In 1999, he studied the development of toxicity tests for benthic Cladocerans at the department of Aquatic Ecology and Ecotoxicology (AEE) of the University of Amsterdam under supervision of Gerdit Greve and Tineke Dekker. One year later he studied biomimetic SPME techniques to predict accumulation into macro invertebrates at the Institute for Risk Assessment Sciences (IRAS) of the Utrecht University under the supervision of Heather Leslie and Leon van der Wal. After graduation in 2001, he started his PhD-research at the IRAS that resulted in this thesis.