Prediction and Experimental Evaluation of Soil Sorption by Natural Hormones and Hormone Mimics

Marcella L. Card,[†] Yu-Ping Chin,*,[‡] Linda S. Lee,[§] and Bushra Khan[§]

[†]Environmental Science Graduate Program and [‡]School of Earth Sciences, The Ohio State University, 125 South Oval Mall, Columbus, Ohio 43210, United States

[§]Department of Agronomy, Purdue University, 915 West State St., West Lafayette, Indiana 47907, United States

S *Supporting Information*

ABSTRACT: Surface runoff from manure-fertilized fields is a significant source of endocrine-disrupting compounds (EDCs) in the environment. Sorption by soils may play a major role in the environmental fate of manure-borne EDCs, including 17α- and 17β-estradiol (17α-E2 and 17β-E2), estrone (E1), melengestrol acetate (MGA), 17α- and 17β-trenbolone (17α-TB and 17β-TB), trendione (TND), and zeranol (α-ZAL). As a measure of sorption behavior, the organic carbon-normalized partition coefficients (K_{OC}) of 17β-E2, E1, MGA, and α-ZAL were experimentally determined for three agricultural soils with initial EDC concentrations spanning from ~0.01 to >1 μM. Sorption isotherms were linear for most solute–soil combinations. Measured K_{OC} values were compared to those predicted using a suite of single-parameter and polyparameter linear free energy relationships (sp- and pp-LFERs). Sp-LFER models were based on experimentally determined octanol–water partition coefficients (K_{OW}), whereas pp-LFER solute descriptors were calculated indirectly from experimentally determined solvent–water partition coefficients or the program ABSOLV. Log K_{OC} predictions by sp-LFERs were closest to the experimentally determined values, whereas pp-LFER predictions varied considerably due to uncertainties in both solute and sorbent descriptors determined by ABSOLV or estimates using the partition coefficient approach.

KEYWORDS: *endocrine-disrupting compound, soil sorption, growth hormone, partitioning, linear free energy relationship*

INTRODUCTION

Endocrine-disrupting compounds (EDCs), which interfere with endocrine signaling, have been identified as emerging environmental contaminants of concern.[1] EDCs may have deleterious effects such as carcinogenicity in mammary and prostate tissues[2–4] or male fish feminization, which may affect fecundity in wild fish populations.[5] Livestock waste is a significant source of EDCs in the environment, with entry through spillover from waste lagoons and surface runoff or subsurface infiltration from manure-fertilized fields.[6] Livestock waste contains naturally occurring hormones and, in the case of animals treated with such compounds, synthetic hormone mimics.

It is estimated that approximately two-thirds of the cattle slaughtered in the United States are treated with hormonally active growth promoters,[7] which are administered to accelerate livestock weight gain and improve the conversion of feed to muscle mass, with treated animals receiving one to three implants during their lifetimes.[8] Six hormonally active growth promoters are approved for use in the United States. These include three synthetic hormones zeranol (α-zearalanol, α-ZAL), melengestrol acetate (MGA), and 17β-trenbolone acetate (17β-TBA), which mimic the naturally occurring hormones 17β-estradiol (17β-E2), progesterone, and testosterone, respectively. 17β-TBA and 17β-E2 benzoate (the benzoate form is often used to administer 17β-E2) are converted in vivo to 17β-trenbolone (17β-TB) and 17β-E2, respectively. The natural hormones, along with their major metabolites, for example, estrone (E1) and 17α-estradiol (17α-E2) from 17β-E2, are produced and excreted by all mammals. Synthetic hormones and their major metabolites, for example, trendione (TND) and 17α-trenbolone (17α-TB) from 17β-TB (Figure 1), are excreted only by animals to

which the growth promoters were administered. Natural and synthetic hormones and their major metabolites are routinely detected in livestock wastes (see, e.g., refs 9–13), manure-fertilized crop fields (see, e.g., refs 12 and 14), and agriculturally affected surface waters (see, e.g., refs 15–19). Furthermore, those compounds that are not altered by livestock are converted by microbial processes in soils to their oxidized derivatives (i.e., zeranol to zeralanone and estradiol to estrone).

Sorption to soil is one primary process in which EDC mobility and bioavailability may be attenuated before reaching receiving waters. Whereas recent studies have addressed the sorption of some EDCs found in livestock wastes (see, e.g., refs 20 and 21), there have been few studies focused on the sorption behavior of MGA[22] and α-ZAL.[23] Furthermore, although 17β-E2 and E1 sorption has been extensively studied (see, e.g., refs 24–30), results have been variable, with some investigators reporting linear partitioning and others observing nonlinear adsorption. Linear sorption can be described by a solute-specific organic carbon partition coefficient (K_{OC}), which is calculated by normalizing the soil–water partition coefficient to soil organic content. Numerous efforts have been made to predict K_{OC} values using single parameter or polyparameter linear free energy relationships (sp-LFERs and pp-LFERs, respectively). In these relationships, solute properties and behavior are described by one or more descriptors.[31] Sp-LFERs describe relationships between K_{OC} and a
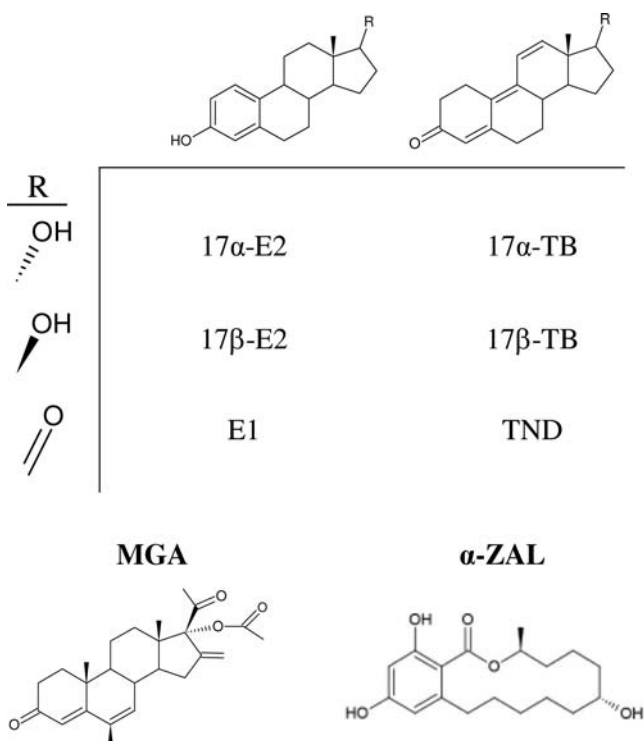
Figure 1. Structures of EDCs used in this study.

single solute property, such as the octanol−water partition coefficient (K_{OW})[32] or aqueous solubility (liquid or subcooled liquid).[33] Sp-LFERs that relate K_{OC} to K_{OW} generally take the form

$$\log K_{OC} = a \log K_{OW} + b \qquad (1)$$

Pp-LFERs, which use multiple regressions to relate solute properties (SP) such as partition coefficients to several sorbent and solute descriptors, have been developed by several groups, such as those of Abraham, Kamlet, Taft, Carr, and Poole.[34] The Abraham pp-LFER[35−37] is of the form

$$\log SP = eE + vV + sS + aA + bB + c \qquad (2)$$

where the upper case letters refer to the solute descriptors: **E** refers to the excess molar refraction, **V** is the McGowan characteristic molecular volume, **S** refers to the solute polarizability/depolarizability, **A** is the hydrogen bond acidity, and **B** is the hydrogen bond basicity [see the Supporting Information (SI) for more information]. The lower case letters represent the complementary sorbent properties, and *c* is a constant associated with the sorbent.

The aim of this work was to experimentally assess the sorption by soil of 17β-E2, E1, MGA, and α-ZAL and to evaluate the applicability of several sp- and pp-LFERs in predicting the sorption of 17α- and 17β-E2, E1, MGA, 17α- and 17β-TB, TND, and α-ZAL. These analyses will help provide insight into the environmental fate, mobility, and bioavailability of EDCs found in livestock wastes and will aid in improving their environmental fate assessment.

■ MATERIALS AND METHODS

Chemicals. Extra dry chloroform (99.9%), cyclohexane, and toluene over molecular sieves were purchased from Acros Organics (Morris Plains, NJ), and 1-octanol (99%) was purchased from Alfa Aesar (Ward Hill, MA). 17β-E2 (98%), E1 (99%), and MGA (≥97%) were obtained from Sigma-Aldrich, Inc. (St. Louis, MO). α-ZAL was extracted from

Ralgro Magnum (Schering-Plough Animal Health Corp., Union, NJ) as described in the SI. Other chemicals, including HPLC solvents, were purchased from Fisher Scientific (Pittsburgh, PA).

Soils. Sorption tests were conducted using three agricultural soils, representing a range of physical and chemical properties (Table 1).

Table 1. Soil Properties

	$f_{OC}{}^{a}$ (%)	pH^{b}	sand[c] (%)	silt (%)	clay (%)	CEC^{d}
Coloma 32	1.1	5.9	88	7	5	4.3
Drummer 36	4.0	7.4	17	47	36	15.5
Finley Control	7.7	7.0	17	46	37	20.2

[a]Analyzed by measurement of CO_2 evolved from combustion of dry, carbonate-free soils. [b]Measured potentiometrically in an aqueous slurry of soil. [c]Soil texture analyzed using hydrometer method. [d]CEC, cation exchange capacity; measured by ammonium saturation at pH 7.0 (mequiv 100 g^{-1}).

A midwestern agricultural soil (Finley Control, FC) was collected in June 2008 at The Ohio State University Finley Farm (Madison County, OH). An area unexposed to animal manure was selected, plants and surface soil were removed, and topsoil was collected to a depth of approximately 0.3 m. The soil was sieved to achieve consistent texture and to remove large debris and then stored at 4 °C. Drummer 36 (D36) and Coloma 32 (C32) are agricultural soils from Indiana, and collection of these soils has been described previously.[20,38] The soil physical and chemical properties were evaluated by A&L Great Lakes Laboratories, Inc. (Fort Wayne, IN).

Organic Solvent−Water Partition Coefficients. For 17α-E2, 17α- and 17β-TB, and TND, the log K_{OW} and toluene−water partition coefficients (log K_{TW}) were obtained from Qiao et al.[40] For all other analytes, solvent−water partition coefficients (K_{SW}) were measured in this study. 1-Octanol was purified prior to use by extraction with 0.1 M NaOH and two rinsings with Milli-Q water (Millipore, Billerica, MA).[39] Each EDC was dissolved in organic solvent (chloroform, cyclohexane, toluene, or water-saturated purified octanol), an equal volume of Milli-Q water was added (in the case of octanol, purified octanol-saturated Milli-Q water), and the mixture was equilibrated for 24 h and then centrifuged. For 17α-E2, 17β-E2, E1, MGA, and α-ZAL, the aqueous phase from each tube was analyzed directly, and aliquots of the organic phase were diluted in methanol and then analyzed by reverse-phase high-pressure liquid chromatography with UV detection (RP-HPLC/UV). For 17α- and 17β-TB and TND, the aqueous phase was extracted with dichloromethane (DCM), and then the extracts and solvent phases were gently evaporated under nitrogen, redissolved in methanol, and analyzed by liquid chromatography−mass spectrometry (triple-quad) as described in Qiao et al.[40]

Experimental Determinations of log K_{OC}. The sorption behavior of EDCs in soils was evaluated in batch sorption studies similar to those described by Li and Lee.[38] EDC sorption from aqueous solutions (5 mM $CaCl_2$) was measured as a function of initial EDC concentration, which spanned >2 orders of magnitude: 0.02−3.0, 0.01−1.3, 0.009−1.3, and 0.006−1.3 μM for 17β-E2, E1, MGA, and α-ZAL, respectively. The highest initial concentrations were near the aqueous solubilities of the EDCs (5.5, 4.8, 2.6, and 15 μM for 17β-E2, E1, MGA, and α-ZAL, respectively), and the lowest starting concentrations were selected such that equilibrium concentrations would be near the limit of quantification (LOQ) after concentration by solid phase extraction (SPE; see below). The lower initial concentration range reflects E1 concentrations that have been measured in the wastes of dairy cattle.[41] Each isotherm was based on at least nine initial concentrations.

All samples were prepared in duplicate. Soil was placed into Corex centrifuge tubes, brought to field capacity with Milli-Q water, and incubated at room temperature for 72 h. Following incubation, samples were autoclaved (Tuttnauer Brinkmann 2340M, Tuttnauer USA Co. Ltd., Hauppauge, NY) for 1 h at 121 °C and 138 kPa. Samples were then twice returned to field capacity, incubated at room temperature for 24 h, and autoclaved, for a total of three autoclave cycles. Glassware and $CaCl_2$ solutions prepared in Milli-Q water were also sterilized by autoclave. EDCs

1481

dx.doi.org/10.1021/jf203093d | J. Agric. Food Chem. 2012, 60, 1480−1487

were spiked into $CaCl_2$ solutions, solutions were added to the tubes to a 1:10 ratio of soil to solution by mass, and samples were shaken. All isotherm samples were allowed to equilibrate for 48 h, which allowed equilibrium or near-equilibrium to be achieved in most cases based on kinetic studies (see Figure SI-2 and Table SI-1 of the Supporting Information). Longer equilibration times increased the potential for losses to nonsorption processes. Blank controls (no EDCs) and glassware controls (EDCs but no soil) were included. Target EDCs were not chromatographically detectable in blank controls, and there was no significant difference between initialEDC concentrations and those in glassware controls. Furthermore, there was no observed formation of biotically or abiotically derived transformation products, although abiotic transformation of 17β-E2 in soils has previously been described.[28]

After equilibration, tubes were centrifuged for 1 h at ~1000g, and supernatants were collected and filtered through a Gelman type A/E glass fiber filter with 1 μm pore size (Pall Corp.). For low-concentration samples (initial EDC concentrations below 0.1 μM), EDCs were concentrated from the filtered supernatant by sorption on an Empore C18 SPE disk (3 M Co., St. Paul, MN) preconditioned with two 10 mL methanol aliquots followed by 100 mL of Milli-Q water. Analytes were eluted from the SPE disks with 100% methanol. The supernatants (high concentration) or methanol elutions (low concentration) were then analyzed using RP-HPLC. Sorbed concentrations were calculated by difference between initial and final aqueous EDC concentrations. A complete mass balance was assessed in a subset of experiments for all EDCs whereby soils were extracted with methanol and EDC concentrations determined by RP-HPLC/UV. Between 75 and 100% of initial EDC mass was recovered in each sample (see Table SI-2 of the SI). The partition coefficients determined by calculating sorption by difference and by extracting the soil were not statistically different. The soil partition coefficients K_D (L/kg) were calculated by the zero-intercept linear fit model

$$C_{sorb} = K_D C_{aq} \tag{3}$$

where C_{sorb} is the concentration sorbed (μmol kg^{-1}) and C_{aq} is the aqueous concentration (μM) at equilibrium. Organic carbon partition coefficients (K_{OC}, L/kg) were normalized to the soil organic carbon content (f_{OC}) using the equation

$$K_{OC} = K_D f_{OC}^{-1} \tag{4}$$

where f_{OC} refers to the fraction of soil weight that is organic carbon. In addition, we conducted separate fits at low initial analyte concentrations that appeared to be nonlinear using the Freundlich equation

$$C_{sorb} = K_F C_{aq}^N \tag{5}$$

where K_F (μmol^{1-N} L^N kg^{-1}) and N (unitless) are the respective Freundlich adsorption coefficient and measure of isotherm nonlinearity. We used the Freundlich equation to determine concentration-specific K_{OC} values using the following equation and eq 4:

$$K_D = K_F C_{aq}^{N-1} \tag{6}$$

Details regarding the application of concentration dependent K_{OC} are described in Qiao et al.[40]

RP-HPLC/UV Analysis of EDC Concentrations. EDC concentrations were quantified using RP-HPLC (Waters 1515 isocratic pump and 717 Plus autosampler, Waters Corp., Milford, MA) with UV–vis detection (Waters 2487 dual λ absorbance detector). Wavelengths of detection were 280, 265, 297, and 220 nm for 17β-E2, E1, MGA, and α-ZAL, respectively. Injection volumes were 150 μL, and the flow rate was 1 mL min^{-1}. The RP-HPLC mobile phase was 50:50 v/v acetonitrile/water for E1 and α-ZAL and 75:25 v/v methanol/water for 17β-E2 and MGA. The HPLC column was a Sunfire C18, 5 μm, 4.6 × 150 mm manufactured by Waters Corp. Under these conditions, the analyte retention times were 5.0, 7.6, 9.1, and 5.2 min and LOQs were 0.01, 0.008, 0.0007, and 0.002 μM for 17β-E2, E1, MGA, and α-ZAL, respectively.

Predicting log K_{OC} Values. EDC log K_{OC} values were predicted using two methods: (1) published sp-LFER coefficients (Table 2) with experimental log K_{OW} values; and (2) published pp-LFER sorbent

Table 2. Sp-LFER Coefficients Used in This Study, with Equations in the Form of Equation 1

compound class	a	b	ref
non-class-specific	1.03	−0.61	42
apolar			
A/CBs and PCBs[a]	0.74	0.15	43−45
halogenated HCs[b]	0.94	−0.43	46
monoaromatic HCs	0.84	−0.28	46
monopolar			
PAHs[c32]	0.98	−0.32	43−45
PAHs[46]	1.14	−1.02	46
bipolar (phenylureas)	0.49	1.05	43−45
polar chemicals			
all[46]	0.73	0.52	46
from ref 47[d]	0.85		47

[a]A/CBs and PCBs, alkylated and chlorinated benzenes and polychlorinated biphenyls. [b]HCs, hydrocarbons. [c]PAHs, polyaromatic hydrocarbons. [d]Of the form log K_{OC} = log K_{OW}^a.

descriptors (Table 3) with solute descriptors calculated using two approaches, ABSOLV software and regressions with K_{SW} values. The

Table 3. Sorbent Descriptors for Pp-LFER Models Used in This Study

	E	V	S	A	B	C	ref
coal tar	0.50	3.86	−0.35	−1.16	−4.46	0.16	48
peat (low)[a]	0.31	3.71	1.27	−0.10	−3.94	−1.04	49
peat (high)[b]	0.43	3.51	0.19	0.02	−3.83	−0.82	49
SOC (all)[c]	1.08	2.55	−0.83	0.28	−1.85	−0.12	46
SOC (av)[d]	1.10	2.28	−0.72	0.15	−1.98	0.14	46

[a]For low concentrations of solutes (near infinite dilution). [b]For high concentrations (approaching aqueous solubility) of solutes. [c]Based on all available log K_{OC} values for selected chemicals. [d]Based on average log K_{OC} values obtained from several soils and sediments for each chemical.

ABSOLV software (ACD/Laboratories, Toronto, ON, Canada) calculates solute descriptors based on properties such as structure, aqueous solubility, vapor pressure, and hexadecane–air partition coefficient. Generally, the **E** and **V** descriptors can be reliably predicted, whereas accurate prediction of the **S**, **A**, and **B** descriptors is more difficult.[50−52] Thus, we used ABSOLV to calculate all descriptors and compared them to a second approach that estimates **S**, **A**, and **B** for each EDC by applying published regression equations (eqs 7−9)[51] to our experimental log K_{SW} values (**E** and **V** were still estimated by ABSOLV).

$$\mathbf{S} = 0.049 - 0.092 \log K_{OW} + 0.229 \log K_{CHW}$$
$$- 0.713 \log K_{CYW} + 0.625 \log K_{TW} + 0.355\mathbf{E}$$
$$- 0.188\mathbf{V} \tag{7}$$

$$\mathbf{A} = 0.108 + 0.261 \log K_{OW} - 0.155 \log K_{CHW}$$
$$- 0.248 \log K_{CYW} + 0.171 \log K_{TW} - 0.049\mathbf{E}$$
$$- 0.097\mathbf{V} \tag{8}$$

$$\mathbf{B} = -0.089 - 0.033 \log K_{OW} + 0.338 \log K_{CHW}$$
$$+ 0.178 \log K_{CYW} - 0.587 \log K_{TW} + 0.136\mathbf{E}$$
$$+ 0.595\mathbf{V} \tag{9}$$

K_{OW}, K_{CHW}, K_{CYW}, and K_{TW} refer to the octanol−water, chloroform−water, cyclohexane−water, and toluene-water partition coefficients, respectively.
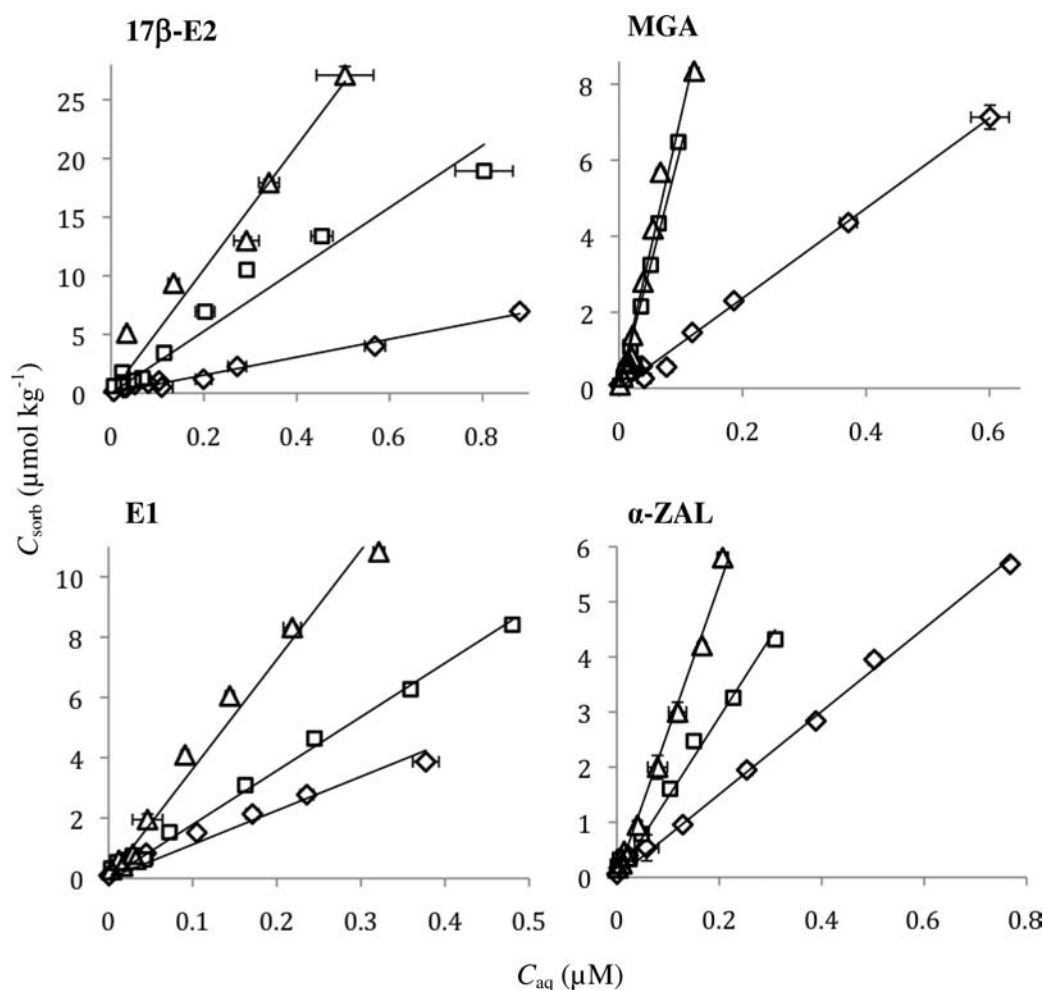
Figure 2. Soil sorption isotherms for 17β-E2, E1, MGA, and α-ZAL for three agricultural soils: ($\diamondsuit$) C32; ($\square$) D36; ($\triangle$) FC. Lines are zero-intercept linear model fits, and error bars are standard deviations.

■ RESULTS AND DISCUSSION

We have assessed the sorption behavior of several natural and synthetic manure-borne EDCs by measurement and modeling to better understand the environmental fate, mobility, and bioavailability of these compounds. Experimentally determined sorption isotherms for 17β-E2, E1, MGA, and α-ZAL were found to be generally linear with three agricultural soils over initial concentrations spanning >2 orders of magnitude. Log K_{OC} values were modeled for the target compounds using a suite of sp- and pp-LFERs, with pp-LFER solute descriptors calculated using both the regression-based log K_{SW} approach and the ABSOLV computer program. On average, the log K_{OC} values predicted by sp-LFERs were closest to experimentally determined values, and ABSOLV-based calculations outperformed those based on log K_{SW} values.

Experimentally Determined log K_{OC} Values. Linear regressions with zero intercepts reasonably fit the sorption isotherms for most combinations of soil and target EDCs over a concentration range of roughly 2 orders of magnitude ($R^2 \geq$ 0.96; Figure 2 and Table 4; Table SI-3 of the SI). Nonlinearity at low concentrations was most noticeable for α-ZAL in D36 soil (Figure SI-3 of the SI). Nonlinear sorption isotherms have previously been reported with Drummer soil[27] and may be attributable to low-abundance high-affinity sorbents in this soil.[53] For the purposes of this paper we used the linear

sorption model (eqs 3 and 4) to determine the experimental K_D and log K_{OC} (Table 4); however, at low concentrations we fitted the nonlinear data to the Freundlich equation and determined concentration-specific K_{OC} values at C_{aq} = 0.005 μM, which is in the lower range of our reported C_{aq}. The lower end of our aqueous concentration range for 17β-E2 and E1 after equilibration approaches values reported for surface waters in the United States[15] and for 17β-E2, E1, and α-ZAL are within the range of concentrations reported in Italian surface waters.[19]

Our α-ZAL sorption values are close to the range given by the manufacturer, which reports log K_{OC} values between 2.68 and 2.95.[23] MGA sorbs more readily than the other target EDCs and therefore is expected to have lower environmental mobility and bioavailability. This effect may play a role in the demonstrated persistence of MGA in agricultural soils; in one study, nearly 20% of the initial MGA remained in a cornfield soil 6 months after manure application.[12]

In some previous studies, sorption isotherms for MGA, 17β-E2, and E1 have been nonlinear. MGA sorption isotherms were highly nonlinear when tested by Schiffer et al.,[22] using aqueous MGA concentrations and soils that have (except for one soil) very low f_{OC}. Linear sorption behavior has been previously reported for 17β-E2 and E1,[21,27,29,54] but in some studies, including one with another Drummer soil, 17β-E2 and E1 isotherm data were best fit by the nonlinear Freundlich model.[25,27,54,55]

Table 4. Concentration-Specific (C_{aq} = 0.005 μM) and Linear log K_D and log K_{OC} for target EDCs

	average log K_{OC} (SD)	soil ID	linear K_D	concentration-specific $K_D{}^c$	linear log K_{OC}	concentration-specific log K_{OC}
17α-E2	2.97 (0.13)[a]					
17β-E2	2.83 (0.012)	C32	7.65	26.3	2.84	3.37
		D36	26.2	103.4	2.81	3.41
		FC	52.1	ND	2.83	ND[d]
E1	2.77 (0.20)	C32	11.2	49.9	3.00	3.65
		D36	17.8	16.5	2.64	3.06
		FC	36.2	17.0	2.67	3.05
MGA	3.06 (0.12)	C32	11.7	49.0	3.03	3.64
		D36	63.9	36.8	3.20	2.96
		FC	71.4	49.0	2.96	2.80
17α-TB	2.77 (0.12)[b]					
17β-TB	3.08 (0.10)[b]					
TND	3.38 (0.19)[b]					
α-ZAL	2.64 (0.16)	C32	7.51	74.0	2.83	3.82
		D36	14.5	80.0	2.55	3.30
		FC	26.2	56.8	2.53	2.86

[a]Reference 21. [b]Reference 20. [c]Based upon Freundlich fits at lower analyte concentrations (<0.001−0.05 μM) and a fixed C_{aq} concentration of 0.005 μM using eq 6.[40] [d]ND, not determined because low concentration isotherms were not measured.

Whereas our experimental log K_{OC} values for 17β-E2 and E1 are similar to those reported in some previous studies,[56] they were somewhat lower than were found in other studies (e.g., log K_{OC} = 3.46 and 3.22 for 17β-E2 and E1, respectively, with another Drummer soil).[27,29,55] The concentration-specific (C_{aq} = 0.005 μM) K_{OC} values, however, were higher in most cases (approaching earlier reported values) and dependent upon the magnitude of N and K_F (Table 4; Table SI-4 of the SI). For MGA with D36 and FC soils, which had the highest K_F values by far and N values close to unity (∼0.9 or higher), the concentration-specific K_{OC} values were lower than the linear K_{OC}. Thus, for sorption of MGA to these two soils, linear partitioning appears to be dominant over the entire concentration range used in this study.

In the present study, all isotherms were determined under autoclave-sterilized conditions and, based on mass balances, no significant degradation occurred. On the other hand, some previous studies were performed with unsterilized soils and degradation products were detected.[27,55] Furthermore, autoclaving our soils may have changed some of their sorption properties. Thus, the differences in protocols used in previous sorption studies may account for some of the slight disparities observed between partition coefficients.

Solvent−Water Partitioning. Solutes with more hydroxyl groups (e.g., 17α-E2, 17β-E2, and α-ZAL) interact more favorably with the aqueous phase and, therefore, generally have lower log K_{SW} values than solutes without hydroxyl groups (e.g., E1, MGA, and TND; Table 5). The exception to this pattern is with octanol, a bipolar solvent capable of accommodating a wide range of organic solutes.

Predicted log K_{OC} Values. EDC log K_{OC} values were modeled using a large matrix of potentially relevant pp- and sp-LFERs, which resulted in a broad range of predictions (Figure 3; Table SI-5 of the SI). Overall, for the EDCs in this study, log K_{OC} predictions calculated using the sp-LFER equations were

Table 5. Measured Partition Coefficients (log K_{SW}) for Target EDCs between Organic Solvent and Aqueous Phases[a]

	log K_{CHW}	log K_{CYW}	log K_{TW}	log K_{OW}
17α-E2	2.51 (0.01)	0.24 (0.02)	1.87 (0.01)[a]	3.73 (0.03)[a]
17β-E2	2.61 (0.01)	−0.43 (0.02)	1.87 (0.02)	3.70 (0.08)
E1	3.21 (0.02)	0.81 (0.02)	3.65 (0.10)	3.55 (0.01)
MGA	4.48 (0.33)	2.76 (0.02)	4.39 (0.03)	3.93 (0.18)
17α-TB	3.36 (0.01)	0.39 (0.07)	1.98 (0.01)[a]	2.72 (0.02)[a]
17β-TB	3.27 (0.01)	0.42 (0.01)	2.11 (0.02)[a]	3.08 (0.03)[a]
TND	3.63 (0.02)	1.24 (0.11)	2.75 (0.02)[a]	2.63 (0.01)[a]
α-ZAL	2.31 (0.005)	−1.95 (0.39)	2.56 (0.46)	3.88 (0.007)

[a]Standard deviation (SD) values are in parentheses. [a]Reported in ref 40.

in better agreement with our measured values, whereas the ones estimated by pp-LFERs were more variable. This finding is in contrast to previous studies, which found that pp-LFERs yielded predictions in better agreement with experimental data than sp-LFERs[46] or that predictions by sp- and pp-LFERs were similarly close to experimental results.[57] Among the sp-LFERs, the equation for bipolar phenylureas resulted in the smallest average difference between predicted and measured log K_{OC} values. However, when the EDCs are considered individually, other correlations based on different training sets (monopolar compounds, chlorinated hydrocarbons, etc.) sometimes provided better fits for specific analytes.

The range of K_{OC} values predicted by sp-LFERs was <0.6 order of magnitude for each of the target EDCs, with ranges between 0.45 log unit for E1 and 0.55 log unit for MGA. For 17α-E2, 17β-E2, E1, MGA, and 17α-TB, all of the selected sp-LFER equations predicted log K_{OC} values within 0.5 log unit above or below the average experimentally determined log K_{OC} value, whereas some sp-LFER predictions fell within that range
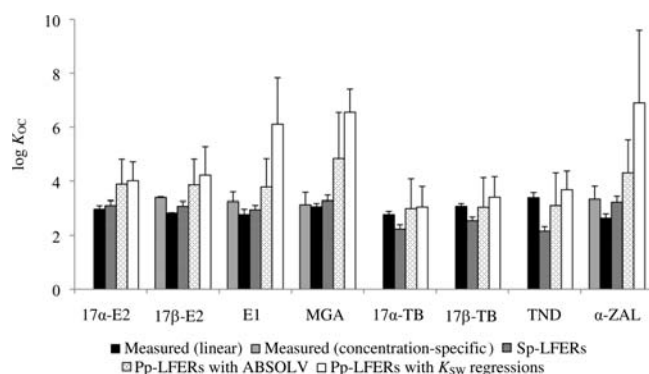
Figure 3. Average measured and predicted log K_{OC} values. Error bars represent SD.

for α-ZAL and none were within that range for 17β-TB and TND (Table SI-5 of the SI). This difference in the fit of sp-LFER predictions to experimental results may be due to errors in experimental measurements of log K_{OW} or log K_{OC}.

ABSOLV and regression equations with log K_{SW} values were both used to compute pp-LFER solute descriptors for the target EDCs (Table 6). The average difference between ABSOLV- and K_{SW}-calculated values was smallest for the hydrogen bond acidity descriptor (**A**) and greatest for the polarizability/depolarizability descriptor (**S**). Because b has the largest magnitude among the sorbent descriptors s, a (hydrogen bond acidity), and b, errors in the calculation of **B** will be magnified in the product b**B** more than errors in a**A** and s**S**. However, due to the larger range of calculated **S** values and the moderate values of s, the product s**S** had the most significant influence on the range of pp-LFER log K_{OC} predictions.

The range of log K_{OC} values predicted for each compound was similar for both ABSOLV and K_{SW} pp-LFER calculations, spanning between 2 and 7 orders of magnitude. Among the pp-LFER predictions, those based on ABSOLV were generally closer to experimental results than those based on K_{SW} regressions (Figure 3 and Table SI-5 of the SI). The coal tar sorbent descriptors combined with ABSOLV-calculated solute descriptors yielded the closest average prediction among pp-LFERs, with predicted log K_{OC} values for 17α-E2, 17β-E2, and TND that were closer to measured values than nearly all of the sp- and pp-LFER predictions for those compounds. Among the sorbent descriptors used in this study, the coal tar descriptors have previously been found to provide the best fit to experimental sorption data for several classes of organic contaminants. We cannot provide an explanation for this phenomenon because coal tar does not resemble the organic moieties present in soil organic matter

other than the presence of phenolic compounds that are similar to lignin-based soil organic matter. Finally, in a previous study with aromatic hydrocarbons, a method of K_{OC} prediction based on Raoult's law was superior to the coal tar pp-LFER in cases when the subcooled aqueous solubility values were available.[58]

The quality of any model output depends on the input data and suffers when input data must be estimated rather than measured directly. It has been found that the results of pp- and sp-LFERs are similar if the quality of solute descriptors is commensurate with that of the log K_{OW} measurements.[47] Uncertainties in sp- and pp-LFER solute descriptors (log K_{OW} and **E**, **V**, **S**, **A**, and **B**, respectively) are translated into inaccuracies in model predictions.[59] Being based on only one descriptor, sp-LFERs have a disadvantage because octanol−water partition coefficients alone cannot describe the range of chemical properties found in pp-LFER models, provided that both sorbate and sorbent descriptors accurately reflect these properties.[34]

With respect to pp-LFERs, sorbent and solute descriptors both contribute to uncertainties in predictions. The sorbents (especially those such as natural organic matter and SOM) are heterogeneous in composition and generally poorly defined.[57] Sorbent descriptors are calculated by multiple regression from experimental sorption data training sets (based upon different sorbents) and solute descriptors and thus include any biases and uncertainties in the input data, although the regression fits tend to be acceptable (R^2 > approximately 0.90).[45,46,49] Presently, the sorbent descriptors available in the literature are limited to fairly specific classes of solids (e.g., coal tar, peat) and do not cover the entire variability in soil organic matter composition.

In comparison with the sorbent phase, the solute chemicals are relatively small and easy to characterize, either through calculation or direct measurement of properties. Because the **E** and **V** solute descriptors may generally be predicted with relative accuracy,[50−52] we expect that the errors in our pp-LFER predictions are primarily the result of inaccuracies in the calculated **S**, **A**, and **B** solute descriptors. It is generally expected that predictions based on measured values will be more accurate than those based only on calculations. However, in the present study, the predictions made by ABSOLV (a largely computational approach) were superior to those based on K_{SW} values, which are reliant upon the accuracy of the training sets. Overall, pp-LFERs can be powerful predictive tools for measured solute descriptors, but they are limited with respect to solutes for which estimations must be made even with robust programs such as ABSOLV.

Although the log K_{OC} values predicted by pp-LFERs did not fit the experimental results as well as those predicted by sp-LFER equations, the pp-LFER provides insight into the mechanisms responsible for the partitioning of a solute to a given phase.

Table 6. Solute Descriptors Calculated with ABSOLV Software and Regression Equations Using K_{SW} Values

	ABSOLV[a]					K_{SW} regressions		
	E	**V**	**S**	**A**	**B**	**S**	**A**	**B**
17α-E2	1.80	2.1988	1.77	0.86	1.10	1.50	0.65	1.13
17β-E2	1.80	2.1988	1.77	0.86	1.10	2.00	0.79	1.04
E1	1.73	2.1558	2.05	0.50	1.08	2.36	0.66	0.40
MGA	1.90	3.1735	2.86	0.00	1.75	1.56	0.10	1.35
17α-TB	1.63	2.1558	2.39	0.32	1.35	1.70	0.24	1.36
17β-TB	1.63	2.1558	2.39	0.32	1.35	1.70	0.37	1.25
TND	1.56	2.1128	2.55	0.00	1.30	1.62	0.11	1.12
α-ZAL	1.50	2.5491	1.80	0.95	1.20	3.26	1.36	0.43

[a]From M. H. Abraham (personal communication). ABSOLV values for 17α-TB, 17β-TB, and TND were curved to fit log K_{OW}.

K_{SW} values have previously been used to qualitatively describe the mechanisms of soil sorption,[20] and calculation of Abraham descriptors using these data (as with the Zissimos et al.[51] regression equations used in this study) may help us better understand sorption processes. Previous studies have demonstrated these relationships between Abraham solute descriptors and the environmental fate of a compound. For example, compounds that sorb readily were generally large in size (i.e., large **V**), whereas those that remain in the aqueous phase tend to have strong dipole and specific interactions (i.e., large **S** and **B**, respectively).[47] To date, the universal utility of pp-LFERs in predicting sorption behavior is limited by the large uncertainties in the predicted solute and sorbent descriptors.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Description of pp-LFER descriptors, extraction of zeranol from Ralgro, sorption kinetics, mass balances, detailed isotherm data, and predicted log K_{OC} values. This material is available free of charge via the Internet at http://pubs.acs.org.

■ AUTHOR INFORMATION

Corresponding Author

*Phone: (614) 292-6953. Fax: (614)292-7688. E-mail: yo@geology.ohio-state.edu.

■ ACKNOWLEDGMENTS

■ ABBREVIATIONS USED

A, hydrogen bond acidity; A/CBs, alkylated and chlorinated benzenes; α-ZAL, zeranol, α-zearalanol; **B**, hydrogen bond basicity; C32, Coloma 32 soil; CEC, cation exchange capacity; CHW, chloroform−water; CYW, cyclohexane−water; D36, Drummer 36 soil; **E**, excess molar refraction; E2, estradiol; E1, estrone; EDC, endocrine disrupting compound; FC, Finley control soil; HCs, hydrocarbons; LOQ, limit of quantification; MGA, melengestrol acetate; OC, organic carbon; OW, octanol−water; PAHs, polyaromatic hydrocarbons; PCBs, polychlorinated biphenyls; pp-LFER, polyparameter linear free energy relationship; RP-HPLC, reverse-phase high pressure liquid chromatography; **S**, polarizability/depolarizability; SD, standard deviation; SOC, sool organic carbon; SP, solute property; SPE, solid-phase extraction; sp-LFER, single-parameter linear free energy relationship; SW, solvent−water; TB, trenbolone; TND, trendione; TW, toluene−water; **V**, McGowan characteristic molecular volume.

■ REFERENCES

(1) *Expert Consultation on Emerging Issues of the Great Lakes in the 21st Century*; International Joint Commission: Racine, WI, 2006; www.ijc.org/php/publications/pdf/ID1598.pdf.

(2) Liu, S. L.; Kulp, S. K.; Sugimoto, Y.; Jiang, J. H.; Chang, H. L.; Lin, Y. C. Involvement of breast epithelial-stromal interactions in the regulation of protein tyrosine phosphatase-γ (PTP γ) mRNA expression by estrogenically active agents. *Breast Cancer Res. Treat.* **2002**, *41*, 21−35.

(3) Harrenstien, L. A.; Munson, L.; Seal, U. S. Mammary cancer in captive wild felids and risk factors for its development: a retrospective study of the clinical behavior of 31 cases. *J. Zoo. Wild. Med.* **1996**, *27*, 468−476.

(4) Prins, G. S.; Birch, L.; Tang, W.-Y.; Hob, S.-M. Developmental estrogen exposures predispose to prostate carcinogenesis with aging. *Reprod. Toxicol.* **2007**, *23*, 374−382.

(5) Jobling, S.; Williams, R.; Johnson, A.; Taylor, A.; Gross-Sorokin, M.; Nolan, M.; Tyler, C. R.; van Aerle, R.; Santos, E.; Brighty, G. Predicted exposures to steroid estrogens in UK rivers correlate with widespread sexual disruption in wild fish populations. *Environ. Health Perspect.* **2006**, *114* (Suppl. 1), 32−39.

(6) Lange, I. G.; Daxenberger, A.; Schiffer, B.; Witters, H.; Ibarreta, D.; Meyer, H. H. D. Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. *Anal. Chim. Acta* **2002**, *473*, 27−37.

(7) Willingham, E. J. Trenbolone and other cattle growth promoters: need for a new risk-assessment framework. *Environ. Practice* **2006**, *8*, 58−65.

(8) ZoBell, D.; Chapman, C. K.; Heaton, K.; Birkelo, C. Beef cattle implants, 2000; http://www.thecattlesite.com/articles/744/beef-cattle-implants.

(9) Launay, F. M.; Ribeiro, L.; Alves, P.; Vozikis, V.; Tsitsamis, S.; Alfredsson, G.; Sterk, S. S.; Blokland, M.; Iitia, A.; Lövgren, T.; Tuomola, M.; Gordon, A.; Kennedy, D. G. Prevalence of zeranol, taleranol and *Fusarium* spp. toxins in urine: implications for the control of zeranol abuse in the European Union. *Food Addit. Contam., Part A* **2004**, *21*, 833−839.

(10) Hanselman, T.; Graetz, D.; Wilkie, A. Manure-borne estrogens as potential environmental contaminants: a review. *Environ. Sci. Technol.* **2003**, *37*, 5471−5478.

(11) *Environmental Assessment: Melengestrol Acetate (MGA) for Suppression of Estrus for Heifers Intended for Breeding*; Upjohn Co.: Kalamazoo, MI, 1996; www.fda.gov/downloads/AnimalVeterinary/DevelopmentApprovalProcess/EnvironmentalAssessments/UCM071896.pdf.

(12) Schiffer, B.; Daxenberger, A.; Meyer, K.; Meyer, H. H. D. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: Environmental studies. *Environ. Health Perspect.* **2001**, *109*, 1145−1151.

(13) Dixon, S. N.; Mallinson, C. B. Radioimmunoassay of the anabolic agent zeranol. 3. Zeranol concentrations in the feces of steers implanted with zeranol (Ralgro). *J. Vet. Pharmacol. Ther.* **1986**, *9*, 88−93.

(14) Kjær, J.; Olsen, P.; Bach, K.; Barlebo, H. C.; Ingerslev, F.; Hansen, M.; Halling, B. Leaching of estrogenic hormones from manure-treated structured soils. *Environ. Sci. Technol.* **2007**, *41*, 3911−3917.

(15) Kolpin, D.; Furlong, E.; Meyer, M.; Thurman, E.; Zaugg, S; Barber, L. Pharmaceuticals, hormones, and other organic wastewater contaminants in US streams, 1999−2000: a national reconnaissance. *Environ. Sci. Technol.* **2002**, *36*, 1202−1211.

(16) Kolodziej, E.; Sedlak, D. Rangeland grazing as a source of steroid hormones to surface waters. *Environ. Sci. Technol.* **2007**, *41*, 3514−3520.

(17) Zhao, S.; Zhang, P.; Melcer, M.; Molina, J. Estrogens in streams associated with a concentrated animal feeding operation in upstate New York, USA. *Chemosphere* **2010**, *79*, 420−425.

(18) Kolok, A. S.; Snow, D. D.; Kohno, S.; Sellin, M. K.; Guillette, L. J. Jr. Occurrence and biological effect of exogenous steroids in the Elkhorn River, Nebraska, USA. *Sci. Total Environ.* **2007**, *388*, 104−115.

(19) Lagana, A.; Bacaloni, A.; De Leva, I.; Faberi, A.; Fago, G.; Marino, A. Analytical methodologies for determining the occurrence of endocrine disrupting chemicals in sewage treatment plants and natural waters. *Anal. Chim. Acta* **2004**, *501*, 79−88.

(20) Khan, B.; Qiao, X.; Lee, L. S. Stereoselective sorption by agricultural soils and liquid-liquid partitioning of trenbolone (17α and 17β) and trendione. *Environ. Sci. Technol.* **2009**, *43*, 8827−8833.

(21) Mashtare, M. L.; Khan, B.; Lee, L. S. Evaluating stereoselective sorption by soils of 17α-estradiol and 17β-estradiol. *Chemosphere* **2011**, *82*, 847−852.

(22) Schiffer, B.; Totsche, K. U.; Jann, S.; Kögel-Knabner, I.; Meyer, K.; Meyer, H. H. D. Mobility of the growth promoters' trenbolone and melengestrol acetate in agricultural soil: column studies. *Sci. Total Environ.* **2004**, *326*, 225−237.

(23) *Environmental Assessment for Zeranol*; Mallinckrodt Veterinary: Mundelein, IL, 1994; www.fda.gov/downloads/AnimalVeterinary/ DevelopmentApprovalProcess/EnvironmentalAssessments/UCM071898. pdf.

(24) Casey, F. X. M.; Simunek, J.; Lee, J.; Larsen, G. L.; Hakk, H. Sorption, mobility, and transformation of estrogenic hormones in natural soil. *J. Environ. Qual.* **2005**, *34*, 1372−1380.

(25) Lai, K.; Johnson, K.; Scrimshaw, M.; Lester, J. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. *Environ. Sci. Technol.* **2000**, *34*, 3890−3894.

(26) Das, B.; Lee, L.; Rao, P.; Hultgren, R. Sorption and degradation of steroid hormones in soils during transport: column studies and model evaluation. *Environ. Sci. Technol.* **2004**, *38*, 1460−1470.

(27) Lee, L. S.; Strock, T. J.; Sarmah, A. K.; Rao, P. S. Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. *Environ. Sci. Technol.* **2003**, *37*, 4098−4105.

(28) Fan, Z.; Casey, F.; Hakk, H.; Larsen, G. Persistence and fate of 17β-estradiol and testosterone in agricultural soils. *Chemosphere* **2007**, *67*, 886−895.

(29) Stumpe, B.; Marschner, B. Long-term sewage sludge application and wastewater irrigation on the mineralization and sorption of 17β-estradiol and testosterone in soils. *Sci. Total Environ.* **2007**, *374*, 282−291.

(30) Sun, W.; Ni, J.; Xu, N.; Sun, L. Fluorescence of sediment humic substance and its effect on the sorption of selected endocrine disruptors. *Chemosphere* **2007**, *66*, 700−707.

(31) Zissimos, A.; Abraham, M.; Klamt, A.; Eckert, F.; Wood, J. A comparison between the two general sets of linear free energy descriptors of Abraham and Klamt. *J. Chem. Inf. Comput. Sci.* **2002**, *42*, 1320−1331.

(32) Schwarzenbach, R. P.; Gschwend, P. M.; Imboden, D. M. *Environmental Organic Chemistry*, 2nd ed.; Wiley: Hoboken, NJ, 2003.

(33) Razzaque, M.; Grathwohl, P. Predicting organic carbon-water partitioning of hydrophobic organic chemicals in soils and sediments based on water solubility. *Water Res.* **2008**, *42*, 3775−3780.

(34) 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.

(35) Kamlet, M.; Doherty, R.; Abboud, J.; Abraham, M.; Taft, R. Solubility − a new look. *CHEMTECH* **1986**, *16*, 566−576.

(36) Abraham, M.; Grellier, P.; Hamerton, I.; McGill, R.; Prior, D.; Whiting, G. Solvation of gaseous non-electrolytes. *Faraday Discuss.* **1988**, *85*, 107−115.

(37) Abraham, M. Scales of solute hydrogen-bonding − their construction and application to physicochemical and biochemical processes. *Chem. Soc. Rev.* **1993**, *22*, 73−83.

(38) Li, H.; Lee, L. S. Sorption and abiotic transformation of aniline and α-naphthylamine by surface soils. *Environ. Sci. Technol.* **1999**, *33*, 1864−1870.

(39) Karickhoff, S. W.; Brown, D. S. Determination of octanol/water distribution coefficients, water solubilities, and sediment/water partition coefficients for hydrophobic organic pollutants, EPA-600/4-79-032; Environmental Research Laboratory, Office of Research and Development, U.S. Environmental Protection Agency: Washington, DC, 1979.

(40) Qiao, X.; Carmosini, N.; Li, F.; Lee, L. S. Probing the primary mechanisms affecting the environmental distribution of estrogen and androgen isomers. *Environ. Sci. Technol.* **2011**, *45*, 3989−3995.

(41) Shappell, N.; Elder, K.; West, M. Estrogenicity and nutrient concentration of surface waters surrounding a large confinement dairy operation using best management practices for land application of animal wastes. *Environ. Sci. Technol.* **2010**, *44*, 2365−2371.

(42) Seth, R.; MacKay, D.; Muncke, J. Estimating the organic carbon partition coefficient and its variability for hydrophobic chemicals. *Environ. Sci. Technol.* **1999**, *33*, 2390−2394.

(43) Sabljic, A.; Güsten, H.; Verhaar, H.; Hermens, J. QSAR modeling of soil sorption. Improvements and systematics of log K_{OC} vs. log K_{OW} correlations. *Chemosphere* **1995**, *31*, 4489−4514.

(44) Chiou, C. T.; McGroddy, S. E.; Kile, D. E. Partition characteristics of polycyclic aromatic hydrocarbons on soils and sediments. *Environ. Sci. Technol.* **1998**, *32*, 264−269.

(45) Poole, S. K.; Poole, C. F. Chromatographic models for the sorption of neutral organic compounds by soil from water and air. *J. Chromatogr., A* **1999**, *845*, 381−400.

(46) Nguyen, T. H.; Goss, K. U.; Ball, W. P. Poly-parameter linear free energy relationships for estimating the equilibrium partition of organic compounds between water and the natural organic matter in soils and sediments. *Environ. Sci. Technol.* **2005**, *39*, 913−924.

(47) Brown, T. N.; Wania, F. Development and exploration of an organic contaminant fate model using poly-parameter linear free energy relationships. *Environ. Sci. Technol.* **2009**, *43*, 6676−6683.

(48) Endo, S.; Xu, W. J.; Goss, K. U.; Schmidt, T. C. Evaluating coal tar-water partitioning coefficient estimation methods and solute-solvent molecular interactions in tar phase. *Chemosphere* **2008**, *73*, 532−538.

(49) Endo, S.; Grathwohl, P.; Haderlein, S. B.; Schmidt, T. C. LFERs for soil organic carbon-water distribution coefficients (K_{OC}) at environmentally relevant sorbate concentrations. *Environ. Sci. Technol.* **2009**, *43*, 3094−3100.

(50) Abraham, M. H.; Whiting, G. S.; Doherty, R. M.; Shuely, W. J. Hydrogen bonding XVI. A new solute solvation parameter, π^{H}_{2} from gas chromatographic data. *J. Chromatogr., A* **1991**, *587*, 213−228.

(51) Zissimos, A. M.; Abraham, M. H.; Barker, M. C.; Box, K. J.; Tam, K. Y. Calculation of Abraham descriptors from solvent-water partition coefficients in four different systems; evaluation of different methods of calculation. *J. Chem. Soc., Perkin Trans. 2* **2002**, 470−477.

(52) Zissimos, A. M.; Abraham, M. H.; Du, C. M.; Valko, K.; Bevan, C.; Reynolds, D.; Wood, J.; Tam, K. Y. Calculation of Abraham descriptors from experimental data from seven HPLC systems; evaluation of five different methods of calculation. *J. Chem. Soc., Perkin Trans. 2* **2002**, *2*, 2001−2010.

(53) Chiou, C. T.; Kile, D. E.; Rutherford, D. W. Sorption of selected organic compounds from water to a peat soil and its humic-acid and humin fractions: Potential sources of the sorption nonlinearity. *Environ. Sci. Technol.* **2000**, *34*, 1254−1258.

(54) Casey, F. X. M.; Larsen, G. L.; Hakk, H.; Šimůnek, J. Fate and transport of 17β-estradiol in soil-water systems. *Environ. Sci. Technol.* **2003**, *37*, 2400−2409.

(55) Yu, Z.; Xiao, B.; Huang, W.; Peng, P. Sorption of steroid estrogens to soils and sediments. *Environ. Toxicol. Chem.* **2004**, *23*, 531−539.

(56) Ying, G.-G.; Kookana, R. S. Sorption and degradation of estrogen-like-endocrine disrupting chemicals in soil. *Environ. Toxicol. Chem.* **2005**, *24*, 2640−2645.

(57) Faria, I. R.; Young, T. Comparing linear free energy relationships for organic chemicals in soils: effects of soil and solute properties. *Environ. Sci. Technol.* **2010**, *44*, 6971−6977.

(58) Arp, H. P. H; Breedveld, G. D.; Cornelissen, G. Estimating the *in situ* sediment-porewater distribution of PAHs and chlorinated aromatic hydrocarbons in anthropogenic impacted sediments. *Environ. Sci. Technol.* **2009**, *43*, 5576−5585.

(59) Niederer, C.; Goss, K.-U.; Schwarzenbach, R. P. Sorption equilibrium of a wide spectrum of organic vapors in Leonardite humic acid: modeling of experimental data. *Environ. Sci. Technol.* **2006**, *40*, 5374−5379.