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Probing the Primary Mechanisms Affecting the Environmental Distribution of Estrogen and Androgen Isomers

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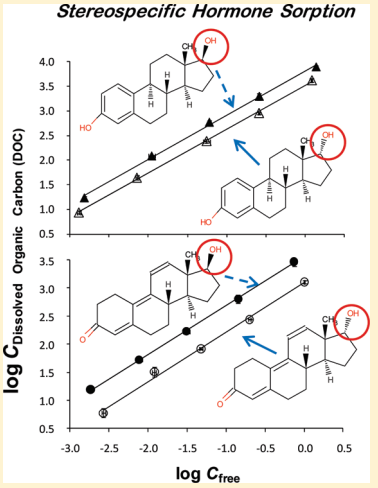
Supporting Information

ABSTRACT: Land application of animal manure has been identified as a source of natural and synthetic hormone contaminants that are frequently detected down-gradient of agricultural operations. Much research on the environmental fate of hormones has focused on the structural isomers most biologically active in mammals, e.g., the 17β-isomers of the estrogen estradiol (E2) and the synthetic androgen trenbolone (TB). However, recent work has shown that the α- and β-isomers of E2 and TB can cause comparable effects on certain aquatic species. To improve our understanding and ability to predict isomer-specific interactions with environmental sorbents, we measured the association (K_{DOC}) of the α- and β-isomers of E2 and TB as well as their primary metabolites (estrone and trendione) with two commercial dissolved organic carbon (DOC) sources by measuring both free and DOC-bound hormone concentrations. We also measured solvent−water partition coefficients partitioning (K_{SW}) for the same hormones using hexane, toluene, and octanol. Log K_{DOC}, log K_{OC} (OC-normalized sorption by soils), and K_{OW} values are all greater for the β-isomer except between the E2 isomers. Theoretical descriptors reflecting electronic character and solute−solvent interactions were calculated to elucidate isomer-specific behavior. Trends for log K_{OW} and log K_{DOC} among hormones as well as between isomers are explained reasonably well by computed electrostatic potential and H-bonding parameters.



INTRODUCTION

Several studies conducted over the past few decades have demonstrated that environmental concentrations of reproductive hormones and their metabolites can impact the sexual development of various organisms.[1,2] Both wastewater treatment discharge and land-application of animal manures are primary sources of hormone contamination. Based on approximate levels of hormones excreted and the volume of feces produced daily by livestock and poultry in the United States, it is estimated that 49 tons of estrogens and 4.4 tons of androgens are released annually by U.S. farm animals.[4] Although relatively strong sorption and rapid degradation of hormones in soils should minimize their mobility when land-applied,[5] hormones are frequently detected in runoff and leachate from manure-applied fields and in nearby receiving waters.[6−8]

Most environmental fate research on hormones has focused on the isomers known to be most biologically active in mammals, such as the 17β-isomers of the natural estrogen estradiol (E2) and the synthetic growth promoting androgen trenbolone (TB). However, in recent studies, 17α- and 17β-TB caused similar androgenic effects on fathead minnows[9,10] and 17α-E2 was a more potent estrogen to fish than hypothesized based on results from mammalian studies.[11] Likewise, most environmental fate models have assumed that both isomers have the same distribution behavior. However, recent sorption studies with soils for both the 17α- and 17β-isomers of TB[12] and E2,[13] show that the α-isomers sorbed about a factor of 2 less than the β-isomer, thus increasing their potential mobility in the soil environment.

Binding of hydrophobic organic contaminants to a wide variety of dissolved organic carbon (DOV) sources has been shown to enhance aqueous phase concentrations of otherwise poorly soluble compounds and potentially facilitate their mobility through soils.[14−16] Similar studies on steroid hormones, which are moderately hydrophobic, are limited. Using fluorescence quenching (FQ) and solubility enhancement measurements,

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Yamamoto et al.[17] reported log values of the hormone-DOC binding coefficients (log K_{DOC}, L/kg$_{OC}$) in the 2.8 to 5.7 range for 17β-E2, estriol (E3), and 17α-ethinyl estradiol for several types of commercial DOC sources. Log K_{DOC} values did not correlate well to DOC hydrophobicity, but rather DOC aromaticity and phenolic group content indicting the involvement of π-electrons and hydrogen bonding. Using similar DOC sources, Neale et al.[18] found that hormones with a keto group (monopolar strong H-acceptor) sorbed more strongly than hormones with a hydroxyl group (bipolar, weaker H-acceptor) in the same position, which also indicates hydrogen bonding. These studies have shed some light on hormone−DOC interactions, but do not include assessing isomeric differences or the androgens. This is of special importance considering the recent hormonal effects noted for both isomers, stereoselective sorption by soils,[12,13] and the fact that all animal wastes contain both E2 isomers[19] and for beef cattle wastes, both TB isomers.[20]

To address these shortcomings, the partitioning of both E2 and TB isomers were examined, along with their primary metabolites estrone (E1) and trendione (TND) as model estrogens and androgens (Figure SI-1), to two types of commercial DOC. To probe the contribution of hydrophobic partitioning versus specific interactions, solvent−water partition coefficients (K_{SW}) with hexane, toluene, and octanol were also measured. To further aid in an understanding of isomer-specific behavior, theoretical quantum chemical descriptors that reflect electronic character and solute−solvent interactions were calculated including molecular polarizability, "covalent" and "electrostatic" H-bond acceptor basicity and donor acidity, and the molecular electrostatic potential distribution.

MATERIALS AND METHODS

Chemicals. 17β-TB, 17β-TB-d3, 17β-E2, 17α-E2, 17β-E2-d3, and E1 were purchased from Sigma-Aldrich (St. Louis, MO), and 17α-TB was obtained from Hayashi Pure Chemical Industry, Ltd. (Osaka, Japan). TND was synthesized as detailed in Khan et al.[21] See Supporting Information (SI) for all other chemical information.

DOC Sources. Aldrich humic acid (AHA) was purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO) and purified prior to use (see SI). Leonardite (LEO) reference humic acid was purchased from the International Humic Substances Society (IHSS, St. Paul, MN). Assuming that log K_{DOC} values would range between 3 and 4 as previously reported,[17,18] LEO and AHA solutions were prepared to achieve a concentration $\geq$200 mg$_{OC}$/L to achieve hormone concentrations inside the dialysis bag that were >20% higher than outside the bags. LEO and purified AHA were mixed with 10 mM KH_2PO_4 (pH 7) for 24 h, and filtered through a 0.2-μm nylon membrane filter (Pall Life Sciences, East Hills, NY) followed by filter-sterilization (0.22-μm GPExpress Plus Membrane Stericup, Millipore Co., Billerica, MA). Lastly, removal of DOC $\leq$ 1000 Da from DOC solutions was done by dialyzing DOC solutions prior to measuring hormone K_{DOC} values (thus henceforth, referred to as the "preisotherm dialysis" step) as described by Carmosini et al.[22] Briefly, preisotherm dialysis was done by filling dialysis bags (1000 MWCO) with 3 mL of DOC solution, closing bags with minimal headspace using cotton/polyester sewing thread, and placing the bags in glass centrifuge tubes containing 30 mL of 10 mM KH_2PO_4 (pH 7) dialysate, which was changed 5 times in a 48-h period to remove DOC $\leq$ 1000 Da. In the preisotherm dialysis, 13.4% and 14.4% DOC was lost with LEO and AHA, respectively. Additional loss during the 48-h equilibration for measuring K_{DOC} values was less than 1.5%. Final DOC concentrations in the dialysis bags were ~200 mg/L.

DOC Characterization. Nonpurgeable DOC concentrations were measured with a TOC-V$_{CSH}$ analyzer (Shimadzu, Columbia, MD) using the high-sensitivity combustion catalytic oxidation/nondispersive infrared method. Solution pH was measured with an Accumet AR20 combined pH/EC meter (Fisher Scientific, Waltham, MA).

K_{DOC} Measurement. A dialysis technique similar to that described above for the preisotherm dialysis was used to quantify K_{DOC} values (L/kg$_{OC}$), which is defined as the ratio of the DOC-bound (C_{DOC}, mg/kg DOC) and free aqueous phase concentrations (C_w, mg/L). After the preisotherm dialysis, hormones were added to fresh dialysate by adding in a small volume of a high concentration stock solution prepared in methanol or methanol/water (final methanol content remained <0.1 vol %). Several experimental factors were assessed including equilibration time (24−96 h), electrolyte composition (10 mM KCl, 10 mM KH_2PO_4, 10 mM NaCl, and 10 mM $CaCl_2$) and pH (~5.6, 7.0, and 8.4) using 17β-TB as a representative chemical and LEO as the DOC (~ 200 mg$_{OC}$/L). Based on these results, multiple concentration isotherms for the different DOC−hormone combinations were measured using 10 mM KH_2PO_4 buffered at a near neutral pH with a 48-h equilibration time (30 rpm at room 22 °C). DOC-free control bags were filled with 3 mL of 10 mM KH_2PO_4 (pH 7.0). Triplicate tubes were prepared for each hormone concentration. After equilibration, triplicate aliquots (2 mL) from inside and outside the dialysis bags were placed into 8-mL glass centrifuge tubes, extracted with 3 mL of dichloromethane, dehydrated with anhydrous Na_2SO_4, evaporated to dryness, and resuspended in methanol.

At equilibrium, the freely dissolved hormone concentration (C_{free}, mg/L) should be equal inside and outside the dialysis bags. A higher solute concentration measured inside the bag (C_{in}, mg/L) relative to the outside (C_{out} = C_{free}, mg/L) represents hormone bound to the DOC. Hormone concentration sorbed to DOC is then calculated as follows:

$$C_{DOC} = \frac{C_{in} - C_{out}}{[DOC]}$$

where [DOC] is the DOC concentration (kg$_{OC}$/L). Isotherms were constructed by plotting C_{DOC} (mg/kg$_{OC}$) versus C_{free} (mg/L) and fit with both the linear isotherm model: K_{DOC} (L/kg$_{OC}$) = C_{DOC}/C_{free}, and the Freundlich isotherm model: C_{DOC} = $K_f C_{free}^{N}$ where K_f (mg^{1-N} L^N kg^{-1}) is the Freundlich adsorption coefficient and N (unitless) is a measure of isotherm nonlinearity.

Solvent−Water Partition Coefficients (K_{SW}). Currently, log K_{SW} values cited in the literature including K_{OW} are not experimentally determined values but calculated using various estimation techniques. Hexane−water (K_{HW}), toluene−water (K_{TW}), and octanol−water (K_{OW}) partition coefficients were quantified using a methodology based on Karickhoff and Brown.[23] Details are provided in the SI.

LC/MS/MS Analysis. Hormone quantification was performed by high-performance liquid chromatography tandem mass spectrometry using a Shimadzu LC system coupled to an Applied Biosystem API3000 mass spectrometer (MDS Sciex, Ontario, Canada). Method and quantitation details are provided in the SI.

Theoretical Quantum Chemical Descriptors. Linear Solvation Energy Relationships (LSERs), developed by Kamlet and

Table 1. Freundlich Isotherm Model Parameters, Concentration-Specific Sorption Coefficient (K_{DOC}^{*}) and Sorption Coefficients Reported in the Literature

	Leonardite humic acid (LEO)			Aldrich humic acid (AHA)			literature	soils
hormone	log K_f [a,b]	N [b]	log K_{DOC}^{*} [c]	log K_f [a,b]	N [b]	Log K_{DOC}^{*} [c]	log K_{DOC}^{*}	log K_{OC}
17α-TB	3.10 (0.10)	0.881 (0.063)	3.45	3.00 (0.06)	0.835 (0.037)	3.49		2.77 ± 0.12[g]
17β-TB	3.56 (0.08)	0.868 (0.043)	3.95	3.37 (0.07)	0.874 (0.045)	3.75		3.08 ± 0.10[g]
TND	3.50 (0.04)	0.852 (0.034)	3.94	ND[h]				3.38 ± 0.19[g]
17α-E2	3.52 (0.04)	0.889 (0.025)	3.85	3.43 (0.08)	0.888 (0.049)	3.76		2.97 ± 0.13[i]
17β-E2	3.80 (0.05)	0.894 (0.032)	4.12	3.79 (0.09)	0.951 (0.053)	3.93	3.7−5.3[d]; (4.94)[e]	3.14 ± 0.16[i]
							3.7− 4.9[f]; (4.2)[e]	3.34 ± 0.18[j]
E1	3.64 (0.09)	0.887 (0.065)	3.98	ND[h]			4.8−5.5[f]	3.2 ± 0.02[j]

[a] Freundlich sorption coefficient K_f ($mg^{1-N}\ L^N\ kg^{-1}$) and N (unitless) determined from linear regressions of the log transformed mg-based data: log [C_{DOC}, mg/kg) = N log [C_{free}, mg/L] + log K_f. [b] Parenthetical values are the average of the upper and lower 95% confidence intervals (See Table SI-4 for specific upper and lower 95% confidence intervals as well as the coefficient of determinations). [c] Concentration-specific partition coefficient (K_{DOC}^{*}, L/kg) calculated at $C_{free} = 1 \times 10^{-3}$ mg/L (1 μg/L) using the coefficients from the Freundlich model fits $K_{DOC}^{*} = K_f C_{free}^{N-1}$. [d] Range reported with 6 commercial DOC from Yamamoto et al.[17] [e] Values in parentheses are specific for AHA from each respective study. [f] Range reported at pH = 7 for 11 and 3 commercial DOC for 17β-E2 and E1, respectively, from Neale et al.[18,32] [g] Average and standard deviation (SD) of the log of OC-normalized sorption coefficients (log K_{OC}) for five soils from Khan et al.[12] [h] Not determined. [i] Average log K_{OC} and SD for seven soils from Mashtare et al.[13] [j] Average log K_{OC} and SD for two soils from Lee et al.[5]

co-workers, have been successfully applied to describe solute−solvent interactions for predicting many physical, chemical, and biological properties.[24,25] Experimental values needed in the LSERs are oftentimes not available; therefore, Theoretical Linear Solvation Energy Relationship (TLSER) descriptors were developed based on LSER parameters for related compounds. TLSER descriptors have been found to account fairly well for acidity[26] and some toxicological indices.[27] Moreover, it has been found that molecular electrostatic potential (ESP) parameters are good descriptors of octanol/water[28] and noncovalent interactions.[29] In this study, both TLSER descriptors and molecular ESP parameters were computed by the Gaussian 09 program[30] (details in the SI) to probe isomer-specific behaviors of hormone compounds. TLSER parameters included van der Waals molecular volume (V_{mc}, cm^3/mol); polarizability index (π_i, unitless); "covalent" and "electrostatic" H-bond acceptor basicity (HBAB) measures ε_b (100 eV units) and q^- (atomic charge units); and "covalent" and "electrostatic" H-bond donor acidity (HBDA) measures ε_a and q_H^+. Additionally, two ESP derived parameters were also calculated (detailed in the SI) using the approach by Brinck et al.:[28] the average deviation of the potential on the surface (Π), which reflects a local polarity or internal charge separation, and the balance parameter of surface potential (τ). These ESP descriptors were originally developed specifically for predicting log K_{ow} values for drug design as an alternative to experimentally based LSERs.[28]

RESULTS AND DISCUSSION

Dialysis Technique Optimization for K_{DOC} Determination. 17β-TB and LEO at a DOC concentration of ~200 mg_{OC}/L were used to evaluate and select an appropriate equilibration time, electrolyte composition, and pH to use for determining the K_{DOC} values for all hormone−DOC combinations. Results for 17β-TB are considered to be representative of all six hormones given their similarity in molecular size and hydrophobicity. In the DOC-free controls, TB concentrations inside and outside the dialysis bag were similar within 24 h. Changes in average log K_{DOC} for 17β-TB were <0.07 log units between 24 and 96 h (see Table SI-1 and Figure SI-2); therefore, 48 h was considered an acceptable equilibration time and minimized the degradation potential of both the DOC and hormones. Average hormone recovery across all hormone−DOC combinations is 96% ± 8% mass balance with no significant concentration-dependent trends. Mass balance for each tube is detailed in Tables SI-1 and SI-2.

Average log K_{DOC} values measured in the four 10 mM electrolyte solutions (KH_2PO_4, NaCl, KCl, and $CaCl_2$) are within 0.24 log units with the highest average obtained in KH_2PO_4 (detailed in Table SI-1). For pH effects in 10 mM KH_2PO_4, average log K_{DOC} values were within 0.22 log units and not statistically different except at the lowest pH where K_{DOC} was statistically lower (Table SI-1 and Figure SI-2). Literature generally reports either negligible change or some decrease in K_{DOC} with increasing pH depending on the DOC source.[17,18,31] Given that the pK_a values of the ionizable phenolic groups in TB and the estrogens are less than 10,[19] pH-induced changes at pH < 9 are likely due to deprotonation of DOC acid functional groups.

DOC−Hormone Sorption Isotherms. DOC−hormone isotherms measured from 10 mM KH_2PO_4 (pH 7.0) after a 48-h equilibration time were used to compare K_{DOC} values among the different hormones and DOC sources. Both the Freundlich and linear isotherm model fits are summarized in Table 1 and Table SI-3, respectively, and representative isotherms are shown in Figure 1. (All isotherm data are shown in Table SI-2.) Most isotherms exhibited some nonlinearity with Freundlich N values ranging between 0.83 and 0.95, indicating the presence of some specific interactions such as site-specific H-bonding and $\pi-\pi$ interactions, which have been hypothesized for hormone binding to DOC.[17,18,31] The Freundlich model is typically used to describe sorption to surfaces with a variety of sorption sites of varying affinities. For N < 1 isotherms, sorption at increasingly higher concentrations is often assumed to be to sites with increasingly lower free energy status.

Also shown in Table 1 are concentration-specific K_{DOC}^{*} values estimated at C_{free} = 0.001 mg/L, using the following relationship: $K_{DOC}^{*} = K_f C_{free}^{N-1}$ derived by substituting in $C_{DOC} = K_{DOC} C_{free}$ into the Freundlich model. Given isotherms with N < 1, log K_{DOC}^{*} values increase with decreasing C_{free} (Figure SI-3). To compare across hormone-DOC combinations, K_{DOC}^{*} values

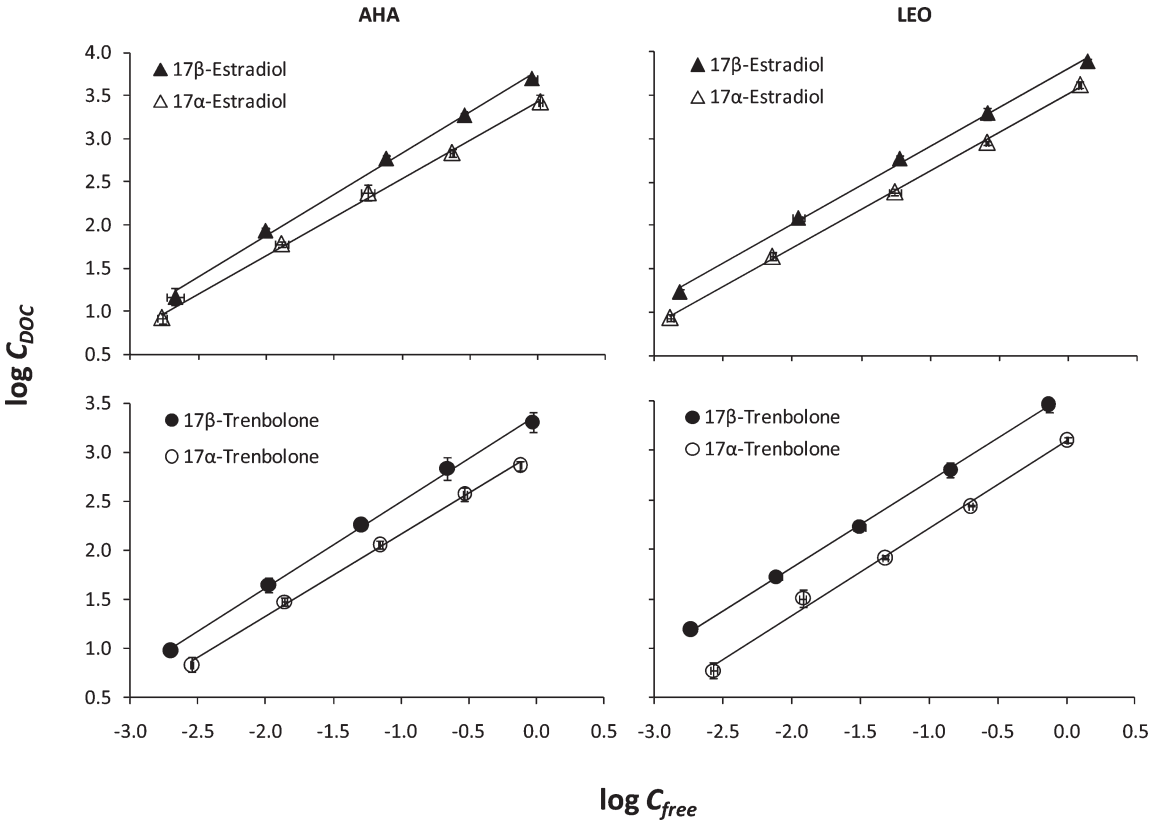

Figure 1. Hormone sorption isotherms with Aldrich humic acid (AHA, left) and Leonardite humic acid (LEO, right). Solid lines are the Freundlich isotherm model fits to the log-transformed data.

were calculated at C_{free} = 0.001 mg/L, which falls within the measured isotherm and environmentally relevant aqueous concentration range. Resulting log K_{DOC}^* values (Table 1) lie between 3.45 and 4.12 with the β-isomer consistently sorbing more than the α-isomer for both TB and E2 (Figure 1) as has been observed for sorption by soils.[12,13] For AHA, our log K_{DOC}^* value for 17β-E2 compares well with that measured by Neale et al.[31] using a solid-phase microextraction technique within a similar concentration range (0.0001−0.1 mg/L) (Table 1). In contrast, Yamamoto et al.[17] using a fluorescence quenching (FQ) technique at a considerably higher 17β-E2 concentrations (2−10 mg/L) reported a much higher log K_{DOC}^* (4.94) for the same AHA (Table 1). FQ is known to be affected by molecular oxygen[32,33] and to generate higher estimates than other approaches.

Relative to sorption by soils, the log K_{DOC}^* values for the androgens and estrogens are higher (∼ 0.6−0.8 log units) compared to average log values of OC-normalized sorption coefficients (log K_{OC}) obtained previously for soils (Table 1). However, isomer trends between log K_{DOC}^* and log K_{OC} are very good for the E2 and TB isomers (n = 4, R^2 > 0.99) (Figure SI-4A). In our HPLC analysis using hydrophobic columns with a polar mobile phase where H-bonding interactions are not expected, β isomers elute before the α isomers for both E2 and TB (see SI).

Solvent/Water Partition Coefficients (K_{SW}). To probe hormone−DOC interaction mechanisms, partition coefficients for apolar hexane and water (K_{HW}), toluene and water (K_{TW}), and bipolar n-octanol and water (K_{OW}) were measured (Table 2, detailed data presented in Tables SI-5 through SI-7). Hexane is an apolar solvent with a very low affinity for water; therefore, only aqueous activity (escaping tendency from water), and no specific hormone−hexane interactions are expected to impact K_{HW}. While solute molecules (e.g., hormones in this case) can affect solvent−solvent (e.g., hexane−hexane) interactions, this effect is expected to be minimal at low hormone concentrations in contrast to what may be expected in solubility studies. For toluene, additional $\pi-\pi$ or electron donor−acceptor (EDA) interactions will be involved.[34] Lastly, for n-octanol, H-bond donor/acceptor (HDA) interactions are expected given the different H-acceptor and H-donor sites in hormones. Thus, the general trend predicted for all hormones of $K_{OW} > K_{TW} > K_{HW}$, is in good agreement with the measured values with the exception of TND, for which $K_{OW} < K_{TW}$ (Table 2). Since TND is the only hormone investigated that does not have a −OH group,

Table 2. Measured Partition Coefficients[a] of Androgens and Estrogens

compound	Log K_{HW}[b]	Log K_{TW}[c]	Log K_{OW}[d]
17α-trenbolone (17α-TB)	−0.114 ± 0.006[e]	1.98 ± 0.01	2.72 ± 0.02[e]
17β- trenbolone (17β-TB)	−0.050 ± 0.010[e]	2.11 ± 0.02	3.08 ± 0.03[e]
trendione (TND)	1.045 ± 0.033[e]	2.75 ± 0.02	2.63 ± 0.01[e]
17α-estradiol (17α-E2)	0.051 ± 0.037	1.87 ± 0.01	3.73 ± 0.03
17β-estradiol (17β-E2)	−0.088 ± 0.037	1.95 ± 0.03	3.76 ± 0.03
estrone (E1)	0.505 ± 0.018	2.72 ± 0.09	3.53 ± 0.03

[a] Average ± SD (n = 3). [b] Hexane−water partition coefficients. [c] Toluene−water partition coefficients. [d] Octanol−water partition coefficients. [e] Khan et al.[12]

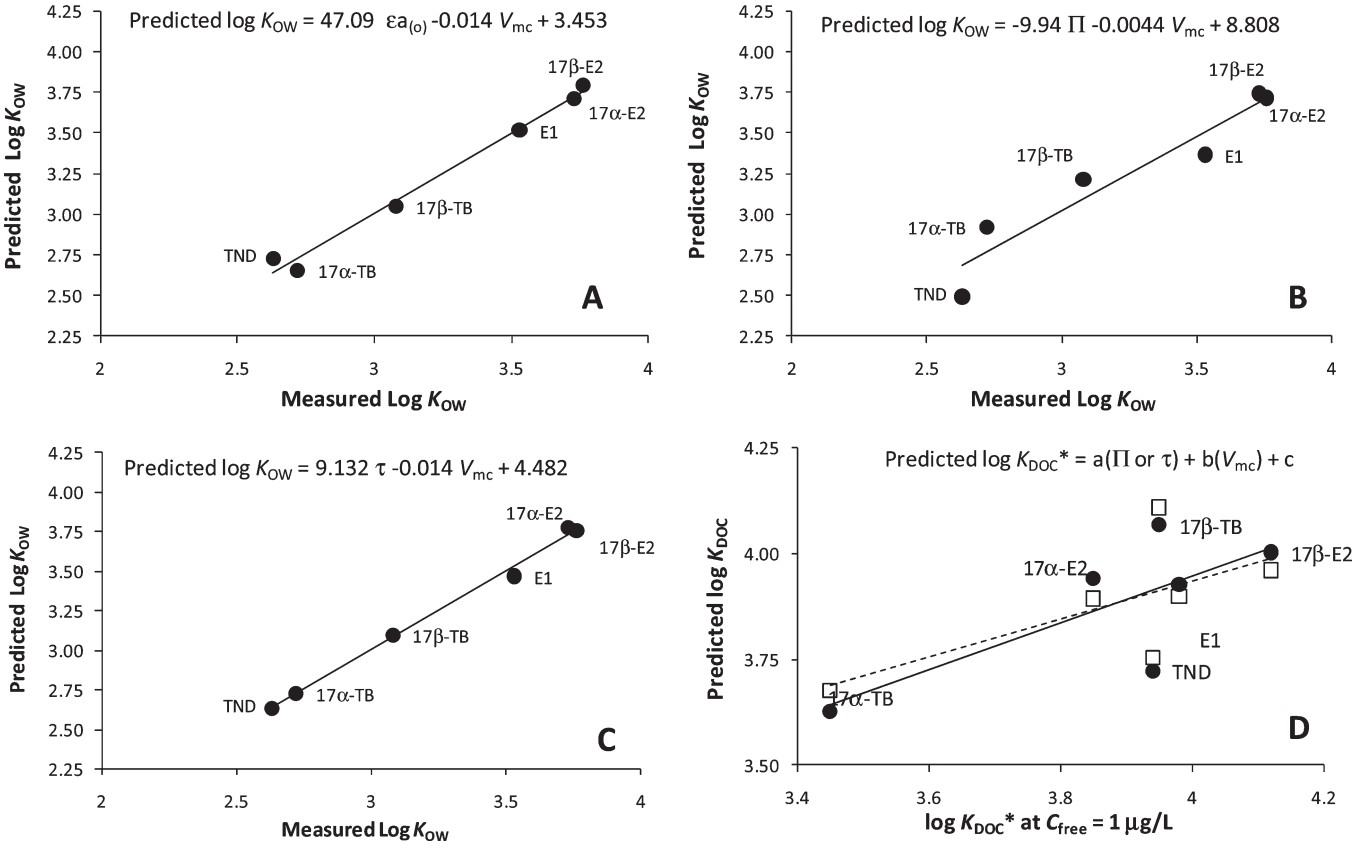

Figure 2. Correlations between measured log K_{OW} values and log K_{OW} predicted using (A) $\varepsilon a_{(o)}$ and van der Waals molecular volume (V_{mc}), (B) the ESP balance parameter (τ) and average deviation of ESP (Π), and (C) τ and V_{mc}, and (D) between measured log K_{DOC} values and those using τ (bullets, solid line) or Π (open squares, dashed line) and van der Waals molecular volume.

no H-donor interactions with water or octanol are possible. As a result, TND has the highest K_{HW} and the lowest K_{OW} of the six hormones. E2 has the highest K_{OW} value, as expected, due to the presence of two −OH groups. E1 has only one −OH group as does TB; however, the −OH in E1 is associated with an aromatic ring which possesses greater electron density, thus enhancing HDA interactions with octanol.

It is widely recognized that the log K_{OC} values of nonpolar and weakly polar hydrophobic organic chemicals typically have a high degree of positive correlation with log K_{OW}.[16] For TB, log K_{DOC}*, log K_{OC}, and log K_{OW} values are all greater for the β-isomer, with the difference between the α- and β-isomers being the greatest for K_{DOC}* (0.50 for Leo and 0.26 for AHA) and relatively similar for K_{OC} and K_{OW} (0.31 and 0.36, respectively). For E2, differences in K_{DOC}* between the two isomers are smaller (0.27 for LEO and 0.17 for HA), and for log K_{OW}, differences are not significant.

Overall, correlation of log K_{DOC}* and log K_{SW} with log K_{OW} is poor for these six hormones (Figure SI-4), with the exception of a fair positive correlation with log K_{OW} if only the E2 and TB isomers are included (n = 4) (Figure SI-4B). Yamamoto et al.[17] also did not find a correlation between log K_{DOC} and calculated log K_{OW} for 17β-E2, E3, 17α-EE2, two alkylphenols, and dibutylphthalate, even though these compounds vary greatly in size and properties. However, unlike the current study, the log K_{DOC} values reported by Yamamoto et al.[17] across compounds for a given DOC were all within ~0.1 log units. The latter seems odd and indicative of an artifact associated with the FQ technique given that log K_{DOC} values varied by two log units for a given compound across DOC sources.

Probing Isomer-Specific Differences. The spatial arrangement of the −OH group on the cyclopentane ring is the differentiating structural property of the α- and β-isomers, and characterizes the distance between the O and C_{CH3} atoms attached to the cyclopentane ring, which for 17α- and 17β-TB are 3.731 and 2.813 Å, respectively, and for 17α- and 17β-E2 are 3.767 and 2.825 Å, respectively. Although these spatial differences can result in significant differences in molecular properties, these isomeric differences are not accounted for, or reflected, in common online calculators as exemplified for log K_{OW} values estimated using SPARC, Marvinsketch, and KowWin methods where identical log K_{OW} values resulted for both α and β isomers (see Table SI-8). Similar results are obtained for other SPARC-estimated log K_{SW} values (no differences between isomers); however, a strong linear correlation between predicted and the measured log K_{SW} values (this study) results with SPARC values being consistently higher (SPARC log K_{SW} = 0.934 log K_{SW} + 0.873, R^2 = 0.97, n = 18, Figure SI-5). Isomer-specific parameters are also not currently available for effectively employing poly parameter linear free energy relationships.

TLSER descriptors that can serve to reflect isomeric differences include molecular polarizability (π_1), "'covalent" and "electrostatic" H-bond acceptor basicity (HBAB, indicated by ε_b and q^-), and "covalent" and "electrostatic" H-bond donor acidity (HBDA, indicated by ε_a and q_H^+). Table SI-9 summarizes the descriptors calculated for the hormones, and ESP distributions

are visualized in Figure SI-6. Descriptors q^- and q_H^+ represent the most negative and positive Mulliken atomic charges (Table SI-10), respectively. The O-atom on the cyclopentane ring (O_D) has the most negative ESP with its associated H-atoms having the most positive ESP (Figure SI-6). These are the most active sites in the hormone structure with one exception. For 17α-E2, the O-atom attached to the aromatic ring (O_{ar}) has a more negative Mulliken atomic charge (q^-) than O_D (Table SI-10).

To identify the primary descriptors that best reflect isomer distribution behavior, a stepwise multilinear regression between the K_{SW} and K_{DOC}^* coefficients with TLSER and ESP parameters was examined using SPSS 13.0. Good relationships result between log K_{OW} and several individual TLSER descriptors such as HBDA descriptors $\varepsilon_{a(W)}$ and $\varepsilon_{a(O)}$ (Figure SI-8A,), but fall short in accounting for the difference between the TB isomers. However, adding a molecular volume descriptor (V_{mc}), (reflecting a cavity term) is sufficient to predict the differences between log K_{OW} for the TB isomers (Figure 2A). Other single TLSER descriptors, q_H^+ and q^- (HBDA and HBAB indicators, respectively) and polarizability (π_i) reflect greater interaction potential for 17β-TB with octanol compared to 17α-TB, which is in good agreement with the isomer-specific partition coefficient trends. Conversely, for E2, q_H^+ and q^- values reflect greater interactions for the α-isomer with octanol whereas π_i (Table SI-9) and the Mulliken charges for the aromatic ring carbons (C_A values in Table SI-10) reflect greater interaction for 17β-E2. These competing interaction potentials offer an explanation as to why the differences in partition coefficients between the two E2 isomers are smaller or insignificant compared to the TB isomers. In addition, the most negative Mulliken charge (q^- a measure of HBAB) for all the isomers except 17α-E2 is for O_D, which is part of the −OH group defining the α and β isomers. For 17α-E2, the O on the aromatic ring (O_A) has a greater (more negative) potential for HBDB interactions.

The ESP descriptors τ and Π do a good job in reflecting differences in log K_{OW} values between isomers and in predicting log K_{OW} values for all six hormones (Figure SI-8B and C, Figure 2C) consistent with the fact that these descriptors were developed specifically for predicting log K_{OW} values.[29] Using both V_{mc} with τ further improved predictions (Figure 2C). These same parameters could account for differences in log K_{DOC}^* between the E2 and TB isomers (Figure 2D). The difference between the sorption of the E2 isomers for both soils and DOC, but not with partitioning to octanol may be due to additional interactions with other functional groups present in soil OC and DOC or additional steric hindrances not present with octanol.

Environmental Implications. Previous studies using commercial DOC materials reported log K_{DOC} values in the 3 to 5 range for a 17β-E2, E1, E3, and EE2[17,18,31] comparable to some nonsteroidal hydrophobic organic pollutants. Recent work has shown that sorption by soil is greater for the β-isomers of both estradiol and the synthetic androgen trenbolone.[12,13] In the current study, K_{DOC} values were also greater for the β-isomers with the overall log K_{DOC} range of ∼3.5 to 4. Based on K_{SW} values and theoretical quantum descriptors, it is apparent that hormone sorption to both DOC and soil OC include site-specific electrostatic and H-bonding interactions, which have been hypothesized by others but not previously quantified,[17,18,31] and reasonably explain isomer-specific sorption behavior. Given the similar trends between log K_{OC} and log K_{DOC}, predicted DOC-enhanced transport would be greater for the β-isomers. For example, assuming DOC from animal wastes behave similarly to the commercial sources used in this study, E2 mobility in typical agricultural soils (1−3% OC) may increase 59% and 73% for the α and β isomers, respectively. However, based on some initial work in our lab with beef manure-derived DOC and 17β-TB, K_{DOC} values for manure-derived DOC may be approximately a log unit lower leading to much smaller effects in mobility of 12−20%. Future studies need to focus on quantifying K_{DOC} for the various animal and human waste-derived DOC sources to better assess the role of DOC in the transport of hormones from fields and in streams.

■ ASSOCIATED CONTENT

Supporting Information. Additional experimental details, all partitioning and sorption data, additional figures highlighting trends, and additional details on the Theoretical Chemical Quantum Descriptors. This material is available free of charge via the Internet at http://pubs.acs.org.

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