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Stereoselective Sorption by Agricultural Soils and Liquid−Liquid Partitioning of Trenbolone (17α and 17β) and Trendione

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Trenbolone acetate (TBA) is a synthetic anabolic hormone used for growth promotion in beef cattle, which excrete primarily 17α-trenbolone along with small amounts of 17β-trenbolone and trendione. To aid in predicting transport of manure-borne TBA metabolites, multiconcentration sorption isotherms for 17α- and 17β-trenbolone and trendione were generated with five autoclaved-sterilized soils that represented a range in soil properties. Hormone concentrations were measured independently in solution and soil phases, and quantified using liquid chromatography with electrospray mass spectrometry. In addition, partition coefficients between apolar hexane and water (K_{hw}) and bipolar octanol and water (K_{ow}) were measured for the three androgens to better ascertain the mechanisms that may be responsible for the sorption differences observed between isomers. In all five soils, trendione sorbed the most, and 17α- and 17β-trenbolone isomers exhibited different sorption magnitudes. 17β- trenbolone consistently sorbed a factor of 2 more than 17α-trenbolone. For all three androgens, sorption is proportional to the soil organic carbon (OC) content with average log OC-normalized distribution coefficients (log K_{oc}, L/kg OC) of 2.77 ± 0.12 for 17α-trenbolone, 3.08 ± 0.1 for 17β-trenbolone and 3.38 ± 0.19 for trendione, which suggests the dominance of hydrophobic partitioning. However, differences in K_{hw} values between 17α- and 17β-trenbolone were small indicating differences are not simply due to differences in aqueous activity. In contrast, similarly different K_{ow} and K_{oc} values for the two isomers indicate the likely contribution of H-bonding to stereoselective sorption.

Introduction

Regulatory decisions regarding the land application of manure and the use of growth-promoting hormone implants in beef require quantitative information on the environmental behavior of steroid hormones. There are several primary growth promoters approved for use in the U.S including the androgens testosterone and trenbolone acetate (TBA), the estrogens 17β-estradiol and zeranol, and the progesterone melengestrol acetate (MGA). Estrogen-containing implants were first used in the 1950s with the synthetic anabolic agent 17β-trenbolone acetate (17β-acetohydroxyestra-4,9,11-trien-3-one, TBA) entering the U.S. market in 1987 (*1*). Trenbolone implants typically include the synthetic androgen 17β-trenbolone acetate in combination with a natural estrogen such as 17β- estradiol. It is marketed with trade names *Revalor* and *Finaplix* which are sold in various combinations of trenbolone acetate and estradiol ratios. TBA is hydrolyzed in the bloodstream of cattle to active 17β-trenbolone (17β-hydroxyestra-4, 9, 11-trien-3-one) followed by oxidation to trendione (17β-hydroxyestra-4,9,11-trien-3,17-one) and a stereoselective reduction to 17α-trenbolone (17α -hydroxyestra-4,9,11-trien-3-one) (*2*). Although all three metabolites (17α- and 17β-trenbolone and trendione, Figure 1) are detected in the manure of implanted cattle, 17α- trenbolone comprises about 95% of the excreted product (*3*). Schiffer et al. (*3*) reported levels up to 75 μg/kg of 17α-trenbolone and up to 5 μg/kg of 17β-trenbolone and trendione in beef dung. Under aerobic soil conditions, the first microbial transformation step for both isomers is to trendione (*4*).

A few studies have looked at TBA metabolites in effluent and waters potentially impacted by beef cattle. Using human androgen receptor bioassays to assess total androgen activity in aqueous samples after removal of suspended solids, Soto et al. (*5*) found that up to 1.1% of the total androgenic activity was due to trenbolone and its metabolites in a beef effluent pond in Nebraska with lower levels observed in streams considered to be partially impacted by livestock operations. Attempts to quantify specific synthetic androgens in the water yielded low pg/L levels with the highest level of 35 pg/L for 17α-trenbolone. In a similar study monitoring discharge drains from feedlots in Ohio, Durhan et al. (*6*) detected 17α-trenbolone more frequently and at higher concentrations (0.01−0.12 μg/L) than 17β-trenbolone (0.01−0.02 μg/L). Only small amounts of both isomers were detected further downstream.

The extent to which hormones may be transported from a field treated with manure or irrigated with livestock effluent into a water body is highly dependent on hormone−soil interactions. Laboratory studies with estrogens and natural androgens have shown that hydrophobic mechanisms and organic matter are the primary factors contributing to their sorption (*7*−*11*). The only sorption data published to date on TBA metabolites present in beef wastes are for 17β-trenbolone (*12*). Schiffer et al. (*12*) measured sorption of 17β-trenbolone by two agricultural soils with organic carbon (OC) contents of 1.6% and 0.3%. They observed a positive correlation between sorption magnitudes and soil OC as well as substantial sorption nonlinearity (*12*). The K_{oc} values estimated from their data appeared high relative to other hormones for similar soils (*13*). However, Schiffer et al. (*12*) used unsterile soils, measured only solution concentrations, had poor recoveries in their validation tests (33−64%), and used an enzyme immunoassay for analysis. Based on work by Khan et al. (*4*), half-lives for 17β-trenbolone under optimal moisture and temperature conditions in agricultural soils

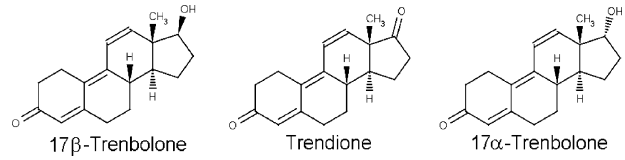

FIGURE 1. Structures for of 17β-trenbolone (17β-hydroxyestra-4, 9, 11-trien-3-one), trendione (17β-hydroxyestra-4,9,11trien-3,17-one), and 17α-trenbolone (17α-hydroxyestra-4,9,11-trien-3-one).

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TABLE 1. Selected Soil Properties[a]

soil (ID)	soil taxonomy[b]	pH[c] in water 1:2 g:mL	OC[d] (%)	clay (%) [e]	sand (%) [e]	silt (%) [e]	CEC[f] ($cmol_c$/kg)	dominant clay type
EPA14	Ultic Hapludalfs	4.5	0.48	64	2	34	18.8	Kaolinite
Coloma-32 (C32)	Lamellic Udipsamments	5.9	0.64	8	81	11	4.3	Illite
Toronto-4 (T4)	Udollic Epiaqualfs	4.4	1.30	20	12	68	11.0	Smectite
Drummer-36 (D36)	Typic Endoaquolls	7.4	2.30	36	17	47	15.5	Smectite
7CB	Mollic Gleysol[g]	6.3	7.50	7	24	69	41.0	ND[h]

[a] Properties determined by A & L Great Lakes Laboratories, Inc., Fort Wayne, IN except for 7CB, which was analyzed by MDS Harris Laboratories in Lincoln, NE. [b] U.S. Taxonomy System. [c] pH of a 1:1 soil (g):water (mL) slurry. [d] % organic carbon determined by loss on ignition at 360 °C (*21*). [e] Hydrometer method (*21*). [f] Cation exchange capacity, $cmol_c$/kg (1 M NH_4OAc (ammonium acetate) buffered at pH 7.0) (*21*). [g] Using mapping units and the World Reference Base Taxonomic System. [h] Not determined.

with similar %OC ranged from 4 to 12 h. Therefore, estimating sorption by difference assuming 17β-trenbolone is conserved in an unsterile soil is not a valid approach, and yields artificially high K_{oc} values. Indeed, Schiffer et al. (*12*) observed conversion of 17β-trenbolone to 17α-trenbolone in their continuous flow column studies reported within the same publication.

17α-trenbolone (the dominant metabolite excreted) is considered less potent than the 17β-isomer based on competitive binding assays using mammalian androgen receptor (*14*). However, recent studies with fathead minnows (*Pimephales promelas*) found similar reproductive effects on fecundity and masculinization of adult females for both isomers relative to controls (zero trenbolone exposure) in a 21-day exposure assessment. The effective concentration (EC_{50}) for fecundity inhibition of α- and β-isomers were estimated to be 0.011 μg/L (95% confidence interval: 0.007−0.017 μg/L) and 0.018 μg/L (95% confidence interval: 0.007−0.037 μg/L), respectively (*15, 16*). In α-isomer exposure studies, substantial amounts of the β-isomer were found in the fish tissue with no β-isomer detected in the tank water leading Jensen et al. (*5*) to hypothesize that conversion of 17α-trenbolone to 17β-trenbolone occurred within the fish. Conversion of 17α-trenbolone to 17β-trenbolone ($\approx$ 1.5%), presumably through trendione, was also observed by Khan et al. (*4*) during aerobic microbial transformation of 17α-trenbolone in agricultural soils.

In the current study, our goal was to quantify the sorption affinities of the metabolic products of the synthetic androgens present in manure of TBA-implanted cattle and their correlation to soil properties, and to assess if the two isomers exhibited the same sorption affinities. Sorption of 17α-trenbolone, 17β-trenbolone, and trendione was measured by independently quantifying solution and sorbed phase concentrations in five autoclaved-sterilized soils varying in pH, CEC, % organic matter, % clay and clay type. In addition, octanol−water (K_{ow}) and hexane−water (K_{hw}) partition coefficients were measured for the three androgens to better ascertain the mechanisms that may be responsible for the sorption differences observed between isomers. Hexane is apolar, thus the androgens will have no specific interactions with hexane; therefore, any differences observed in K_{hw} between androgens will be due to differences in aqueous phase activity (*17*). For the androgens partitioned into octanol, additional H-bonding type interactions may occur, which are likely to be impacted by the spatial arrangement in trenbolone of the −OH group.

Materials and Experimental Methods

Chemicals. 17β-trenbolone and 17α-trenbolone were obtained from Sigma Chemical, St. Louis MO and Hayashi Pure Chemical IND., LTD, Japan. Reagent grade *n*-octanol was purchased from EM Scientific (Gibbstown, NJ). Reagent grade *n*-hexane (>99% purity) was purchased from Alfa Aesar (Ward Hill, MA). Other chemicals used included acetonitrile, methanol, dichloromethane, Na_2SO_4 and $CaCl_2 \cdot 2H_2O$ (all of >99% purity). Trendione was not available commercially, thus was synthesized by oxidation of the alcohol functional group to a ketone using Dess-Martin periodinane as detailed in the Supporting Information (SI).

Soils. Five soils representing a range in soil properties were selected for the sorption studies (Table 1). EPA14, Toronto-4 (T4), and 7CB were previously characterized in other studies (*18*−*20*). EPA14 is a soil from an eroded hillside in southeast Ohio (*18*); 7CB was collected in northern Costa Rica (*20*); and T4 is an agricultural soil from the Purdue Agronomy Research Farm (West Lafayette, IN) (*19*). Coloma-32 (C32) and Drummer-36 (D36) soils were newly collected from the same Agronomy Research Farm. All soils had been air-dried, gently crushed to pass a 2 mm sieve, thoroughly mixed, and stored in closed containers at room temperature prior to use for the sorption studies.

Batch Sorption. A batch-equilibration method was used to measure sorption of 17α- and 17β-trenbolone and trendione by soils from aqueous 0.005 M $CaCl_2$ solutions. A 0.005 M $CaCl_2$ matrix was selected because it is representative of typical soil-solution ionic strengths and facilitates a good separation of solution and soil phases during centrifugation. Sorption isotherms of the parent compound were constructed using five concentrations ($\sim$ 4, 9, 50, 95, 500 μg/L) in duplicate plus a blank (zero androgen concentration). Hormone concentrations and soil mass (g) to solution volume (mL) ratios of 1:10−2:35 (see SI Table S-1) were selected to achieve a concentration range well above the method limit of quantitation (MLOQ) in both solution and sorbed phase extracts for all compounds.

Soils were sterilized by autoclaving using the method described by Wolf et al. (*22*). Soil (1−2 g air-dried) were added to sterile 40 mL glass tubes, adjusted to field capacity using sterile water, and incubated for 72 h at room temperature. At the end of the incubation period, samples were autoclaved at 103.4 KPa and 121 °C for 1 h, readjusted to field capacity, incubated again for 24 h, and autoclaved again for 1 h. All glassware and deionized water were also sterilized by autoclaving. Sterilization methods may impact the properties of some soils, but minimizing artifacts arising from degradation are often more important. We commonly use both moist autoclaving and cobalt radiation in our lab since both of these methods are effective at killing microbes and generally have a minimal impact on soil properties affecting sorption (*22*). Recently in assessing the biotransformation potential of other compounds in our lab, it appeared that cobalt radiation did not effectively deactivate all residual microbial enzymes allowing compound transformation in the absence of live microbes. Similar results were not observed with autoclaving, which is known to deactivate residual soil enzymes (*23*).

TABLE 2. Summary of the Sorption Coefficients Estimated from Linear Sorption Model Fits to Multiple-Concentration Sorption Isotherms for 17α-, 17β-trenbolone, and Trendione from Autoclaved-Sterilized Soils[a]

hormone	soil ID	% mass recovery ± SD[b]	K_d (L/kg)	R^2 [c]	log K_{oc}[d]	average log K_{oc} ± SD[b]
17α-trenbolone	EPA14	94 ± 7	2.2	0.97	2.65	2.77 ± 0.12
	C32	92 ± 6	5.3	0.99	2.92	
	T4	97 ± 4	6.3	0.99	2.68	
	D36	96 ± 5	17.0	0.98	2.87	
	7CB	101 ± 3	41.1	0.99	2.74	
17β -trenbolone	EPA14	87 ± 6	4.7	0.99	2.99	3.08 ± 0.10
	C32	94 ± 3	10.6	0.99	3.22	
	T4	99 ± 7	14.5	0.99	3.05	
	D36	93 ± 4	32.6	0.99	3.15	
	7CB	91 ± 3	73.5	0.99	2.99	
trendione	EPA14	96 ± 5	20.6	0.99	3.63	3.38 ± 0.19
	C32	91 ± 6	20.1	0.98	3.50	
	T4	80 ± 3	30.0	0.99	3.36	
	D36	86 ± 11	44.7	0.99	3.29	
	7CB	95 ± 5	100.7	0.99	3.13	

[a] Each isotherm is represented by five concentrations plus zero in duplicate. [b] Standard deviation. [c] Goodness of fit. [d] Organic-carbon normalized sorption coefficient.

After soil sterilization, hormone solutions were added, tubes capped with Teflon-lined screw caps, samples equilibrated on an end-over-end rotary shaker (40 rpm) for 20 h at room temperature (22 ± 2 °C), and centrifuged at 630*g* for 20 min. A preliminary sorption study with two contrasting soils (D36 and EPA14) conducted over a 120 h period using a single hormone concentration of 50 μg/L for each of the three androgens indicated that a 20 h equilibration time was sufficient (see SI Figure S1 for time profile). The aqueous supernatant was removed, and a 9 mL aliquot was extracted using 4 mL of dichloromethane (DCM). In preliminary experiments, a second sequential extraction with DCM yielded negligible to no additional solute mass similar to what was observed in studies with natural hormones (*8*). Soto et al. (*5*) also found DCM to be an excellent solvent in liquid−liquid extraction of aqueous samples for hormones with 100% recovery of trenbolone isomers. The soil plug was then extracted with methanol (30 mL). In both cases, a solvent exchange and concentration step was performed by taking known aliquots of extract, evaporating off solvent, and redissolving the residual precipitates in methanol. Extraction efficiencies of these androgens from soil using methanol was previously shown to be 95−100% for both sandy and clay loam soils at a soil mass to methanol volume ratio of 5 g to 35 mL (*4*).

Sorption data were fit with the linear sorption model; $C_s = K_d C_w$, where C_s (μg/kg) is the extractable sorbed concentration and C_w (μg/L) is the solution concentration, and K_d (L/kg) is the linear distribution coefficient.

LC/MS Analysis. Separation of hormones was performed using a Shimadzu liquid chromatography system with a methanol/water gradient on a Phenomenex Hyperclone ODS column (150 = 2.0 mm, dp = 3 μm) followed by detection with a Sciex API3000 mass spectrometer with electrospray ionization in the multiple reaction monitoring (MRM) mode and quantification using external standards. Details were previously reported in pp S5−S6 of the Supporting Information from Khan et al. (*4*). Androgens were quantified using external calibration curves with selected standards run in between every 5−10 samples. Matrix effects are commonly observed with LC/MS and usually accounted for by using an internal standard that is most like the target analytes. However, the preferred internal standards of choice (deuterated forms of the target analytes) were not available for 17α-trenbolone or trendione; therefore, deuterated 17β-trenbolone was used as a confirmation, but external standards were used to quantify all three androgens. The potential matrix effects were previously assessed in detail by Khan et al. (2008) where no significant difference in MS response between the analyte standards with and without the soil extract matrix were observed (see pp S6−S7 in the Supporting Information of Khan et al. (*4*)). The HPLC/MS on column limit of detection was 0.3 μg (a 15 μL injection volume of 0.02 μg/L), which allowed a MLOQ for soil concentrations of 0.9 μg/kg and solution concentrations of 0.06 μg/L. Chromatographic retention times under these conditions were 3.8, 4.5, and 5.5 min for 17β-trenbolone, 17α-trenbolone, and trendione, respectively.

Octanol−Water (K_{ow}) and Hexane−Water Partition (K_{hw}) Coefficients. K_{ow} and K_{hw} values were quantified using procedures modified from Karickhoff and Brown (*24*). Octanol was extracted with 0.1 M NaOH, extracted twice with deionized water, and then distilled at 195 °C to eliminate residual water. The purified octanol or hexane was saturated with ultrapure water, and ultrapure water was saturated with either purified octanol or hexane. Solutions of 17α-, 17β-trenbolone, and trendione of approximately 120, 180, and 20 mg/L, respectively, were prepared by dissolving pure compounds in the water-saturated purified octanol. For K_{hw}, 17α- and 17β-trenbolone and trendione were dissolved in water-saturated purified hexane solutions of approximately 10, 130, and 6 mg/L. An octanol:water ratio of 1:6 was used for all hormones. For K_{hw}, hexane:water ratios of 1:3 and 1:5 were used for the trenbolone isomers and trendione, respectively. All samples were done with 3−4 replicates, rotated for 24 h, and centrifuged (1750*g*) for 20 min. Octanol phase samples were diluted in methanol prior to analysis. For hexane, a solvent exchange to methanol was done first and then diluted if needed. The water phase was extracted by DCM, dehydrated with anhydrate sodium sulfate, DCM evaporated, residues dissolved in methanol, and samples analyzed with LC-MS analysis as previously described.

Results and Discussion

Sorption by Soils. During the 20 h equilibration, no degradation or isomer interconversion was observed. The average % mass balance and associated standard deviations across all soils for 17α-trenbolone, 17β-trenbolone, and trendione are 95 ± 6%, 91 ± 7%, and 88 ± 9%, respectively (see Table 2 for averages for each soil-hormone specific isotherm and SI Table S1 for % recoveries for individual replicates). Sorption isotherms for both trenbolone isomers

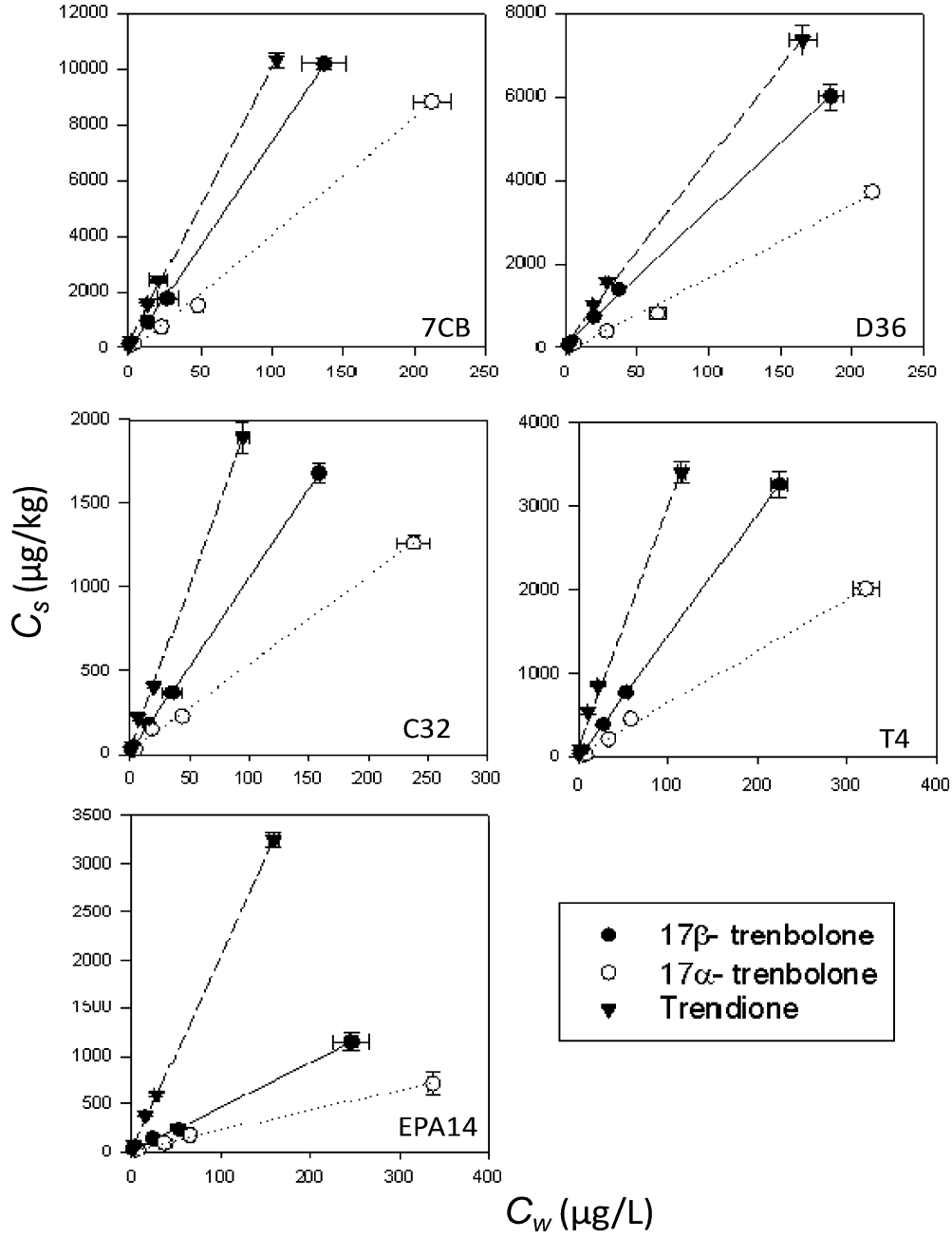

FIGURE 2. Sorption isotherms of 17α- and 17β- trenbolone, and trendione for five soils. Error bars are for standard deviations (small errors are hidden by symbol) and lines are the linear isotherm model fits.

(17α-and 17β) and trendione were constructed from measured C_w and C_s values (Figure 2) and fit well with the linear sorption model (Table 2). In all five soils, trendione sorbed the most, and 17α- and 17β-trenbolone isomers exhibited different sorption magnitudes. 17β- trenbolone consistently sorbed approximately a factor of 2 more (0.31 log units) than 17α-trenbolone (Table 2, Figure 2). For all three androgens, sorption is proportional to the soil OC content with average log OC-normalized distribution coefficients (log K_{oc}, L/kg OC) of 2.77 ± 0.12 for 17α-trenbolone, 3.08 ± 0.1 for 17β-trenbolone, and 3.38 ± 0.19 for trendione (Table 2). The C_w values ranged from ~1−50 μg/L for the four lowest applied concentrations with C_w values approaching ~340 μg/L for the fifth and highest applied concentration, which is well outside the concentrations likely to be observed in the environment. However, excluding this highest concentration from the linear isotherm model fits resulted in similar sorption coefficients for most androgen-soil combinations (see SI Table S3) with no effect on the average log K_{oc} values for the trenbolone isomers and no significant effect on the log K_{oc} for trendione (within standard deviations). Also shown in SI Table S3 for reference are the Freundlich nonlinear sorption model fits to the entire concentration range, which reflects minimal nonlinearity for most androgen−soil combinations.

Correlation coefficients resulting from the linear regressions between K_d (L/kg) and various soil properties (Table 3) show that the best correlation by far is between K_d (L/kg) and % OC (R^2 values >0.97) as indicated by the small standard deviations about the average K_{oc} values (Table 2). High correlations also resulted between K_d and CEC (R^2 range ~0.76−0.84 for the three androgens, Table 3); however, this is a direct result of the high positive correlation between %OC and CEC ($R^2 = 0.83$). Organic matter (OM) is the greatest source of CEC in most soils (*25*). Hormones (e.g., estradiol, trenbolone, testosterone, ethynl estradiol, etc.) have no basic functional groups, thus do not exist as cations, and therefore, a direct correlation with CEC should not be expected. Also the pK_a for the most ionizable phenolic group is greater than

TABLE 3. Summary of Correlation Coefficients from Linear Regressions between K_d (L/kg) and Various Soil Properties. Also Shown Are Correlations between % OC, % Clay, and CEC

	goodness of fit values (R^2)[a]			
correlation	17α-trenbolone	17β-trenbolone	trendione	between soil properties
K_d versus % OC	0.982	0.978	0.996	
K_d versus pH	0.294	0.298	0.202	
K_d versus % Clay	0.233	0.242	0.203	
K_d versus CEC[b]	0. 773	0.757	0.836	
% OC versus CEC				0.832
% OC versus % clay				0.229
% Clay versus CEC				0.012

[a] Goodness of fit values (R^2) from a linear regression. [b] Correlation is high due to high correlation between the soil properties of % OC and CEC and the high correlation between K_d and % OC.

TABLE 4. Summary of Measured Log K_{ow} and Log K_{hw} Values, Predicted Log K_{ow} from Two Online Software Calculators, and the RPLC Retention Time from a Methanol/Water Gradient and a C-18 (10% carbon loading) Hyperclone Chromatographic Column for 17α-Trenbolone, 17β-Trenbolone, and Trendione

	average ± SD[a]					
hormone	log K_{oc}[b]	log K_{ow}[c]	log K_{hw}[d]	SPARC[e] log K_{ow}	ALOGPS[f] log K_{ow}	RPLC[g] t_r(min)
17α-trenbolone	2.77 ± 0.12	2.72 ± 0.02	−0.114 ±0.006	3.49	2.65	4.5
17β-trenbolone	3.08 ± 0.10	3.08 ± 0.03	−0.050 ± 0.010	3.49	2.65	3.8
Trendione	3.38 ± 0.19	2.63 ± 0.05	1.045 ± 0.033	3.13	2.13	5.5

[a] Standard deviation. [b] Organic-carbon normalized sorption coefficient. [c] Octanol−water partition coefficient. [d] Hexane−water partition coefficient. [e] Calculated using SPARC online calculator http://ibmlc2.chem.uga.edu/sparc/ (*31*). [f] Calculated using KOWWIN method in ALOGPS 2.1 from online model software, Virtual Computational Chemistry Laboratory Web site; http://www.vcclab.org/lab/alogps/ (*32*). [g] Reverse-phase liquid chromatography retention time.

10 for common estrogens (*26*) and approaches 20 for common androgens like testosterone and trenbolone (*27*). Therefore, hormones will not ionize at environmentally relevant pH values (*4*−*8*), thus anion exchange to iron oxides is not expected. In previous work for similar hormones, sorption to a pure clay and iron oxides appeared high (*7, 28*), which led to inferences of ion exchange mechanisms. However, these inferences assumed that loss from solution was due to sorption, which is likely in err due to the high reactivity of pure clay surfaces and metal oxides to abiotic transformation. The EPA14 soil in the current study was intentionally included because of its relatively high clay content and low %OC. EPA14 is the one soil reducing the coincidental goodness of fit between K_d and CEC, otherwise it would mirror the correlation between K_d (L/kg) and % OC, which exemplifies the need to examine correlations between soil properties when assessing sorption trends across soils.

The high correlation between K_d and % OC and the consistency of K_{oc} values across soils suggests the dominance of hydrophobic-type partitioning into soil organic matter for these androgens, as previously reported for natural hormones and estrogen ethynyl estradiol (*8, 10, 29*). However, other interactions such as H-bonding involving the phenol or ketone groups may also contribute to hormone sorption by soils. If only hydrophobic-type partitioning is occurring, then differences in sorption should be similar to differences in aqueous phase activities. Aqueous phase activity is best estimated by calculating hypothetical liquid solubility data (S_l) from accurate aqueous solubility and melting point data (*17*). In the absence of such data, as is the case with these androgens, other types of data such as liquid−liquid partition coefficients (*17*) and retention order in reverse-phase liquid chromatography (RPLC) can help to elucidate the differences. Typically, RPLC retention is well correlated to K_{oc} when solute hydrophobic partitioning prevails (*30*) suggesting that the greater sorption of the β-isomer is not due to just aqueous activity.

Octanol−Water (K_{ow}) and Hexane−Water Partition (K_{hw}) Coefficients. The estimated K_{hw} and K_{ow} values for both isomers and trendione are summarized in Table 4 along with the average log K_{oc} values and RPLC retention times for easy reference. Also shown are two sets of log K_{ow} values computed for reference using online software (e.g., the SPARC online calculator http://ibmlc2.chem.uga.edu/sparc/ (*31*) or KOWWIN method in ALOGPS 2.1 from Virtual Computational Chemistry Laboratory Web site, http://www.vcclab.org/lab/alogps/) (*32*).

The K_{hw} for monopolar trendione is more than 10× greater than the K_{hw} for bipolar trenbolone as expected, given that hexane is apolar and the absence of a polar −OH group in trendione decreases its propensity for the water phase relative to trenbolone. The average log K_{hw} values of 17α- and 17β-trenbolone are significantly different at the 95% confidence interval; however, the difference is small (0.06 units) with log K_{hw} of 17β-trenbolone being greater. This indicates that 17β-trenbolone has a slightly higher escaping tendency from water (lower S_l) (*17*), than that of the α-isomer. Interestingly, 17β-trenbolone eluted before 17α-trenbolone in our RPLC analysis with a C-18 end-capped column with a 10% carbon loading (the Hyperclone chromatographic column), which is inconsistent with a pure hydrophobic-partitioning process (*30*) (Table 4). However, given the rigidity of C-18 column material compared to liquid hexane, steric-specific hindrances could be controlling the observed RPLC retention order as have been observed for other steriods on some HPLC colums (*33*).

K_{ow} values are 2−3 orders of magnitude higher than K_{hw} consistent with the bipolar nature of octanol. The K_{ow} for monopolar trendione is smaller than the bipolar trenbolone isomers, which is consistent with computed reference values, although the actual values differ (Table 4). Note the online calculators do not include specific parameters for differentiating isomer behavior. What is most significant for the purpose of the current study is that the log K_{ow} of 17β-trenbolone is 0.36 units greater than its α-isomer, and

interestingly, the measured log K_{ow} values for the trenbolone isomers are almost identical to the corresponding log K_{oc} values (Table 3). Although the K_{oc}-concept seems to work well for these androgens, the differences in K_{oc} and K_{ow} values between 17α- and 17β-trenbolone, relative to the small difference in aqueous activity estimated from the K_{hw} values, indicates that additional nonhydrophobic interactions such as H-bonding, which can be influenced by differences in the orientation of the —OH group, does contribute to sorption by soils.

Environmental Implications

Sorption of trenbolone and trendione appeared well correlated to soil OC; however, clear differences in sorption magnitude were observed for the two trenbolone isomers. Sorption affinity of 17α-trenbolone is consistently a factor of 2 lower than that of 17β-trenbolone in all five soils (Table 2), which represented a range in soil pH, soil OC, % clay, and clay type (Table 1). The K_{oc} values for 17β-trenbolone and trendione fall within the range of K_{oc} values (10^3 to 10^4 L/kg OC) published for other hormones including 17β-estradiol, 17α-ethynyl estradiol, testosterone, and androstendione whereas 17α-trenbolone's K_{oc} value falls below this range. The lower sorption affinity of the α-isomer increases the likelihood of it being leached from agricultural fields to streams and rivers relative to other hormones. This is noteworthy given that 17α-trenbolone is the most common metabolite excreted by TBA-implanted cattle (*3*), is more frequently detected and at higher concentrations than 17β-trenbolone in waters impacted by livestock operations (*6*), and has been observed to have similar reproductive effects as 17β-trenbolone on fathead minnows (*15*). Even with 17α-trenbolone's greater mobility, androgens tend to degrade rapidly under moisture and temperature conditions optimal for microbial activity (*4*), which are also conditions conducive to healthy crop growth. There is some evidence that trendione degradation, which is slower than trenbolone, may indeed be limited by association with the soil phase, thus potentially prolonging its persistence. In addition, hormones present in manure applied in the late fall when temperatures are relatively cool may persist into the winter and early spring snowmelt periods. During this time, hormones will be prone to enter ditches and streams through runoff events. Once these hormones get into a stream, they may become associated with anaerobic sediments, which likely provide less favorable conditions for degradation. Studies are needed to assess the latter as well as systematic field-scale studies to better evaluate the contributions of land-applied beef-manure to hormone discharges to the aquatic environment.

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Supporting Information Available

Additional details on the sorption time study. This material is available free of charge via the Internet at http://pubs.acs.org.

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