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Fate and Transport of Testosterone in Agricultural Soils

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Hormones excreted in animal waste have been measured in surface and groundwater associated with manure that is applied to the land surface. Limited studies have been done on the fate and transport of androgenic hormones in soils. In this study, batch and column experiments were used to identify the fate and transport of radiolabeled [^{14}C] testosterone in agricultural soils. The batch results indicated that aqueous-phase concentrations decreased for the first 5 h and then appeared to increase through time. The first-order sorption kinetics ranged from 0.08 to 0.640 h^{-1} for the first 5 h. Beyond 5 h the increase in aqueous ^{14}C could have been caused by desorption of testosterone back into the aqueous phase. However, metabolites were also produced beyond 5 h and would have likely resulted in the increase in aqueous ^{14}C by sorption site competition and/or by lower sorption affinity. There were weak correlations of sorption with soil particle size, organic matter, and specific surface area. Testosterone was the dominant compound present in the soil column effluents, and a fully kinetic-sorption, chemical nonequilibrium model was used to describe the data. Column experiment sorption estimates were lower than the batch, which resulted from rate-limiting sorption due to the advective transport. The column degradation coefficients (0.404−0.600 h^{-1}) were generally higher than values reported in the literature for 17β-estradiol. Although it was found that testosterone degraded more readily than 17β-estradiol, it appeared to have a greater potential to migrate in the soil because it was not as strongly sorbed. This study underlined the importance of the simultaneous transformation and sorption processes in the transport of hormones through soils.

Introduction

Documented cases of reproductive and developmental abnormalities in numerous vertebrate and invertebrate animal species have been identified over the last few decades (*1*). It has been implied that endocrine-disrupting chemicals (EDCs), such as reproductive hormones (i.e., testosterone and 17β-estradiol), are the cause of these abnormalities. A recent survey of 139 streams across 30 states in the United States has detected reproductive hormones in approximately 40% of the streams sampled (*2*). The sources of these chemicals are industrial, agricultural, and residential and can cause reproductive abnormalities in aquatic species at extremely low levels (e.g., <1 ng L^{-1}) (*3*). Some suggest that a threshold of exposure does not exist for EDCs because they mimic or antagonize the actions of an endogenous molecule (*4*). Adverse responses to EDCs in the environment are escalated if the EDC is the actual endogenous molecule, such as a hormone or synthetic hormone.

Hormonal EDCs are found and released in the environment and can result in adverse health impacts to aquatic organisms. Renner (*5*) recently reported that hormone-adulterated runoff from cattle (*Bos Taurus*) feedlots could have androgenic effects on local female fish, which have manifested male characteristics. Natural and synthetic hormones are given to cattle to enhance growth by increasing the efficiency of converting feed to meat. Manure that contain these synthetic growth hormones (concentration ranging from 6 to 10 μg kg^{-1}) are applied to soils as fertilizer and are traceable in the soil up to 8 weeks after fertilization (*6*). Testosterone is also produced naturally by poultry (*Gallus gallus* domesticu) and has been measured in their manure at average concentrations of 133 μg kg^{-1} for female broilers and 670 μg kg^{-1} for male roosters (*7*). Almost 12 million Mg of broiler litter is produced in the United States each year, which contains natural levels of testosterone, and almost all the litter is applied to soil as fertilizer. Hormonal treatments are also used in aquaculture operations to manipulate fish phenotypic sex to produce a monosex population (*8*). Synthetic androgenic hormones are provided in fish feed in doses between 0.5 and 5 mg kg^{-1} of feed. If EDCs are, as some suggest, the cause of widespread adverse health impacts, then what are the possible exposure routes to these chemicals?

Limited studies have reported on the fate and transport of androgenic hormones in the environment. Shore et al. (*9*) measured testosterone concentrations in an irrigation pond and a local stream near a field that received poultry litter (containing 34 μg kg^{-1} testosterone). The concentration of testosterone in the irrigation pond ranged from 0.5 to 5 ng L^{-1}, and the concentrations from the stream ranged from 1 to 28 ng L^{-1}. Another study by Finlay-Moore et al. (*10*) measured testosterone in soil and runoff from grasslands amended with broiler litter. Runoff concentrations of testosterone ranged between 10 and 1830 ng L^{-1} and depended on manure application rate and frequency. Following manure application, the soil testosterone concentrations increased to approximately 150−790 ng kg^{-1} from background concentrations (15−55 ng kg^{-1}). In some cases, the soil testosterone concentrations persisted or increased within (and perhaps beyond) a 14 day period after manure application. Finlay-Moore et al. (*10*) suspected possible movement of testosterone below their soil sampling depths of 0−2.4 cm and made conclusions similar to Shore et al. (*9*), who suggested that testosterone leached more readily than the principle estrogenic hormone, 17β-estradiol. 17β-Estradiol has been found at significant concentrations (ranging from 6.0 to 66.9 ng L^{-1}) in springs that are fed by aquifers underlying areas associated with poultry manure application (*10, 11*). The concentrations of the hormone in the springs closely paralleled the levels of fecal coliform associated with animal manures. In areas associated with manure application, testosterone may be of equal or greater risk to subsurface water quality compared to 17β-estradiol.

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TABLE 1. Soil Fractions Used for Experiments, Their Official Soil Series Description, Organic Matter Content, and Specific Surface Measurement

soil series	taxonomic description	organic matter content (%)	specific surface ($m^2\ g^{-1}$)
Bearden-silty clay loam	fine-silty, mixed, superactive, frigid Aeric Calciaquolls	7.5	175
Gardena-clay loam	coarse-silty, mixed, superactive, frigid Pachic Hapludolls	5.3	154
Glyndon-sandy clay loam	coarse-silty, mixed, superactive, frigid Aeric Calciaquolls	3.3	123
LaDelle-silt loam	fine-silty, mixed, superactive, frigid Cumulic Hapludolls	9.2	151
Sioux-loam	sandy-skeletal, mixed, frigid Entic Hapludolls	7.5	106
sand		0.0	

Limited studies have reported on the fate and transport of androgenic hormones in soils. Layton et al. (*12*) measured mineralization of testosterone by biosolids in a wastewater treatment system, yet little is known about degradation/transformation and mobility in soils. The objectives of this study were to investigate the sorption, transformation, and mobility of testosterone in agricultural soils. Testosterone was chosen because it is produced by all studied animal species and thus has the potential of being widespread; it is highly potent and can metabolize to produce other potent metabolites; it is a prototype for other synthetic androgenic hormones; and little is known about the fate and transport of androgenic hormones. Miscible-displacement and batch sorption experiments were used to identify the fate and transport mechanisms in soils ranging in particle size distributions and organic matter content. Inverse modeling techniques were used with the column studies to help identify transport processes. The experiments were designed using soils and application rates associated with agricultural practices that include manure fertilization.

Materials and Methods

The materials and methods that were used for the batch and column studies were nearly identical to an earlier study that identified the fate and transport of 17β-estradiol (*13*). The upper soil horizon, an A-horizon, of a Bearden-silty clay loam, Gardena-clay loam, Glyndon-sandy clay loam, LaDelle-silt loam, and Sioux-loam (Table 1) were used for the batch and miscible-displacement experiments. These soils were obtained from Ag−Vise Company (Northwood, ND) except the Glyndon. These soils are formed under prairie, predominantly found in North Central United States, and are typically high in organic matter. They also represent a broad range of soil textures. Additionally, a quartz sand (250−500 μm) was used for the batch and column experiments. All the soils were initially dried at 85 °C for 24 h. Major physical and chemical properties of each soil type were measured at the Soil and Water Environmental Laboratory at North Dakota State University. These measurements included particle size distribution (*14*) and organic matter content (*15*) (Table 1). Specific surface area was also measured using the ethylene glycol monoethylene ether method (*16*).

Equilibrium Batch Sorption Experiments. Soil and water (0.0l M $CaCl_2$) were added to 10 mL vials in a ratio of 1.6 g to 8 mL, respectively. The batch equilibrium experiments were done using concentrations of ^{14}C radiolabeled testosterone (American Radiolabeled Chemicals, St. Louis, MO), which was added to triplicate vials to create solution concentrations of 0.725, 0.399, 0.0725, and 0.00725 $\mu g\ mL^{-1}$. These concentrations were chosen because they span the range of concentrations reported for manures that are applied to fields (*7*, *9*, *10*). The soil−water slurries were agitated by rotation of the vials top to bottom (360°/5 s).

After 0.5, 1, 5, 24, 48, 96, and 168 h, the bottles were centrifuged at 1700 rpm (1000*g*), triplicate 100 μL aliquots were removed and assayed for radioactivity by liquid scintillation counting using a 1900 CA Scintillation Counter (Packard, Downers Grove, IL). Also, thin-layer chromatography (TLC) [System 2000 Imaging Scanner (Bioscan, Inc., Washington, DC)] was used to determine if transformation occurred by identifying the presence of metabolites. Thin-layer chromatography (TLC) analysis on these fractions was conducted using silica gel plates (250μm; Whatman Lab. Div., Clinton, NJ) with the following solvent system: tetrahydrofuran:ethylacetate:hexane (12.5:12.5:25). A combustion analysis assay [Packard Model 307 Oxidizer (Downers Grove, IL)] was also performed for total ^{14}C sorbed to the soil. Using these methods, the lowest calculated detection limit for testosterone was 7.3 ng L^{-1} in terms of water (8 mL) and 36 ng g^{-1} in terms of soil (1.6 g).

Freundlich isotherms were used to describe the batch experiments where the concentration of solute adsorbed on the soil, S (mg g^{-1}), is related nonlinearly to the aqueous concentration in the soil solution, C (mg L^{-1})

$$S = K_f C^n \tag{1}$$

where K_f (L g^{-1}) is the Freundlich distribution coefficient and n is an empirical constant that controls the deviation from linearity ($n = 1$ is linear). A nonlinear, least-squares approximation method (*17*) was used to obtain the best-fit of eq 1 to the observed data by optimizing the unknown parameters, K_f, and n.

Additionally first-order kinetic sorption constants, ω (h^{-1}), were estimated using the following expression

$$C(t) = C_0 e^{-\omega(t-t0)} \tag{2}$$

where t is time and the subscript 0 indicates initial values. The ω values were estimated by fitting eq 2 to measured data also using a least-squares, nonlinear curve-fitting routine (*17*). The optimized ω value was obtained by changing it iteratively until a best fit of eq 2 to the data was achieved.

Miscible-Displacement Experiments. The sand and each soil series were packed into individual columns, and both chloride anion and testosterone were passed through each column. Chloride was used as a nonsorbing tracer to identify the physical transport characteristics of the soil columns. Table 2 provides the major physical properties of each column. The soils were evenly packed in glass columns (diameter = 8.4 cm, length = 15.0 cm) with stainless steel end caps. Sandwiched between the soil and the end caps were several layers of cheesecloth and a 40-mesh stainless steel screen, which retained the soil in the column. Glass, Teflon, and stainless steel were used in the construction to minimize adsorption to the experimental apparatus.

Each column was slowly wetted from the bottom over a 24 h period using a weak salt solution (0.01 M $CaCl_2$). This reduces the amount of entrapped air and maintains soil structure. After the column was wetted, flow was established from the top down using the same 0.01 M $CaCl_2$ solution. Once steady-state pore water velocity (v (cm min^{-1})) was achieved, a pulse of chloride ion tracer (0.05 M $CaCl_2$) was applied and eluted with the 0.01 M $CaCl_2$ solution. The

TABLE 2. Soil Column Physical Properties

soil series	mass of dry soil (g)	volumetric water content ($cm^3\ cm^{-3}$)	pore water velocity ($cm\ min^{-1}$)	pore volume (mL)	pulse input relative PV
Bearden-silty clay loam	784	0.68	0.29	569	0.07
Gardena-clay loam	695	0.64	0.35	528	0.08
Glyndon-sandy clay loam	1126	0.52	0.36	435	0.09
LaDelle-silt loam	760	0.60	0.35	500	0.08
Sioux-loam	825	0.60	0.36	495	0.08
sand	1391	0.34	0.54	286	0.05

TABLE 3. First-Order Kinetic Sorption Coefficients (ω) Determined for Each Initial Aqueous Concentration (C_0)[a]

soil type	$C_0 = 0.725\ mg\ L^{-1}$ ω 0−5 h (h^{-1})	$C_0 = 0.399\ mg\ L^{-1}$ ω 0−5 h (h^{-1})	$C_0 = 0.0725\ mg\ L^{-1}$ ω 0−5 h (h^{-1})	$C_0 = 0.00725\ mg\ L^{-1}$ ω 0−5 h (h^{-1})
Bearden-silty clay loam	0.377	0.388	0.416	0.461
Gardena-clay loam	0.335	0.325	0.318	0.268
Glyndon-sandy clay loam	0.640	0.596	0.547	n.d.
LaDelle-silt loam	0.375	0.375	0.350	0.391
Sioux-loam	0.343	0.320	0.226	0.077

[a] Sorption coefficients were estimated for time interval 0−5 h.

effluent was fraction collected every 2 min, and the conductivity of each fraction was measured using a conductivity meter (Oakton PC 300,Vernon Hills, IL). Table 2 provides the volumetric water content (θ ($cm^3\ cm^{-3}$)), pore volume (PV), and v of each column experiment. The residence times in the columns ranged between 42 and 52 min. Although the velocities fell in the range of observed soil water flow, they were somewhat high. These types of velocities might be observed after a large rainfall or irrigation event.

Following the chloride ion breakthrough curve experiments, several PVs of the 0.01 M $CaCl_2$ solution were flushed through the soil column. A 40 mL pulse of ^{14}C testosterone (0.91 μCi; 0.131 $\mu g\ mL^{-1}$) was then applied to the surface of the soil column and eluted with the 0.01 M $CaCl_2$ solution for 7−12 PVs. This input testosterone concentration was relatively high but realistic. Finlay-Moore et al. (*10*) uses a broiler litter application rate of 7.05 Mg ha^{-1}, and Shore et al. (*7*) reports testosterone levels reaching 1 $\mu g\ g^{-1}$ in broiler litter. This application rate and testosterone concentration would have resulted in approximately 2.5 μg of testosterone applied compared to 5.2 μg used in this study. The column effluent was fraction collected every 2 min, and each fraction was analyzed for ^{14}C and metabolites using the liquid scintillation and TLC methods described earlier.

Additionally, the distribution of resident ^{14}C in the column was determined. The soil was extruded from each column in 1-cm increments, then dried and assayed for ^{14}C by combustion analysis as described earlier. Solution extracts were then obtained from each 1-cm increment by sequential elution with toluene, ethyl acetate, and methanol in the cell of an Accelerated Solvent Extractor (model 200; Dionex, Sunnyvale, CA). Analysis for metabolites was also done on these extracts using TLC analysis described for the batch experiments.

Miscible-Displacement Model. The miscible-displacement experiments were modeled with the computer program HYDRUS-1D, version 2.0 (*18*). This program uses an inverse modeling technique to fit the model solution to the observed data in order to estimate reaction and transport parameters. The inverse modeling approach uses a least-squares optimization routine to obtain the best-fit model solution and does this by iteratively changing model parameters until a best fit is achieved.

A fully kinetic, one-site (*19, 20*), convective-dispersive model with degradation and Freundlich kinetic sorption was considered in describing the miscible-displacement experiments. This model assumes that solutes exist in either the sorbed or aqueous phases and that degredation occurs in the sorbed phase. The following is the partial differential equation that governs the nonequilibrium chemical transport for a homogeneous system during one-dimensional, steady-state water flow

$$\theta\frac{\partial C}{\partial t} + \rho_b\frac{\partial S}{\partial t} = \theta v\lambda\frac{\partial^2 C}{\partial x^2} - \theta v\frac{\partial C}{\partial x} - \mu\rho_b S \qquad (3)$$

$$\frac{\partial S}{\partial t} = \omega[K_f C^n - S] - \mu S \qquad (4)$$

where C is the aqueous concentration ($mg\ L^{-1}$), t is time (h), ρ_b is soil bulk density ($g\ L^{-1}$), S is sorbed phase concentration ($mg\ g^{-1}$), λ(cm) is the dispersivity, v is steady-state velocity ($cm\ h^{-1}$), x is depth (cm), ω is a sorption rate coefficient (h^{-1}), μ is a first-order degradation/transformation rate constant (h^{-1}), and K_f($L\ g^{-1}$) and n (−) are Freundlich sorption coefficients (defined in eq 1). For a stable nonsorbing solute, such as the chloride anion tracer, it is assumed that $S = 0$ and $\mu = 0$.

Results and Discussion

Batch Experiments. Sorption to the solid phase generally increased from 0 to 5 h with first-order sorption kinetic coefficients that ranged from 0.08 to 0.640 h^{-1} (Table 3). After 5 h the sorbed phase concentrations appeared to steadily decrease for each soil and initial concentration (Figure 1). Note that each graph in Figure 1 uses a different vertical scale and that this scale does not start at zero. Thus, observed desorption was significantly smaller than it may seem after visual inspection of Figure 1. Lai et al. (*21*) showed similar 1−5 h kinetic sorption values (0.07−0.37 h^{-1}) for similar steroidal estrogen hormones in the presence of river and estuary sediments. Lai et al. (*21*) also found similar sorption behavior through time, where the various estrogens were first sorbed rapidly then sorption decreased. They suggest the decrease in sorption was caused by estrogen desorption back into the aqueous phase. The observed decrease in sorbed concentrations at times >5 h (Figure 1) may have resulted from the desorption of testosterone back into the aqueous phase, which was similar to estrogens (*21*, *22*) and other hydrophobic organics. Lai et al. (*21*) explains the cause of estrogen desorption back into solution as a result of organic matter in the water phase (*23*, *24*). In this study, the increase

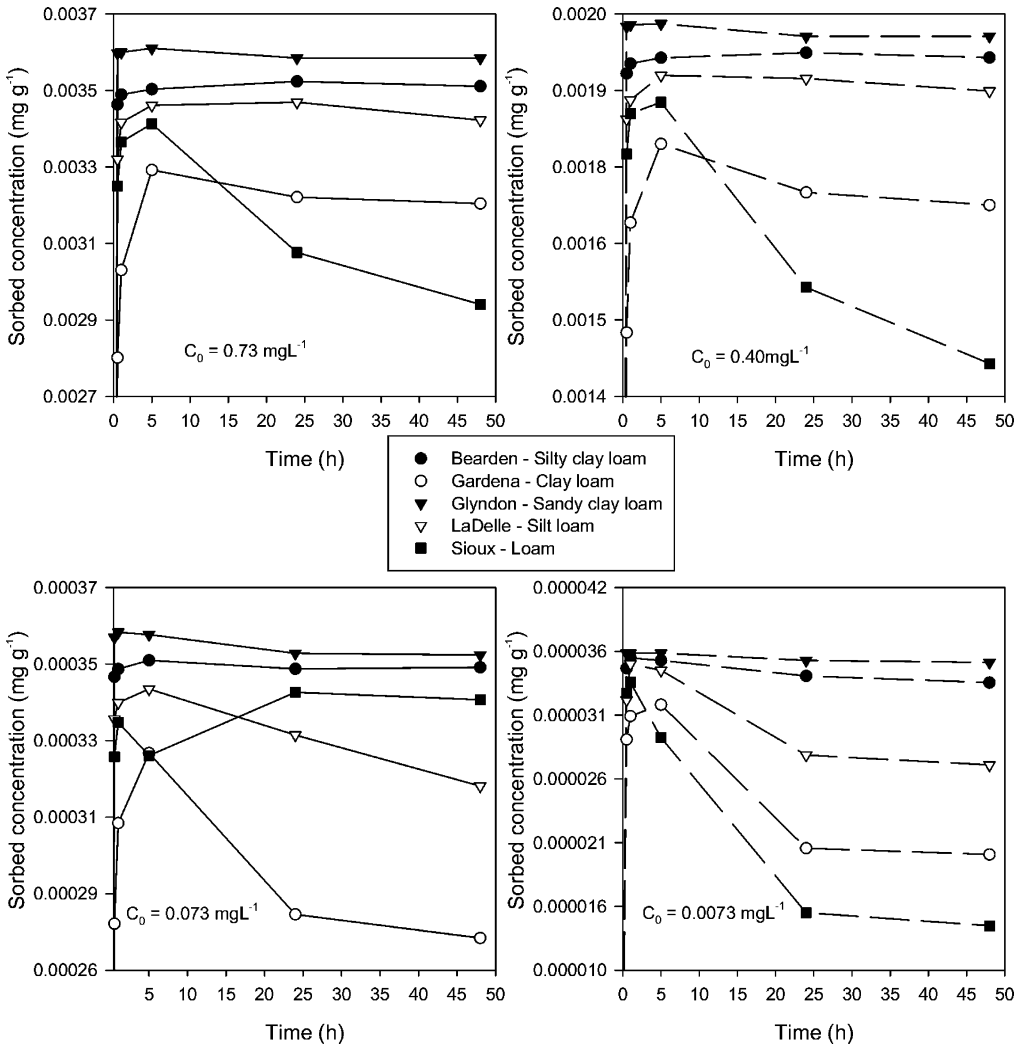

FIGURE 1. Time series plots of sorbed testosterone for each initial concentration (C_0) of 0.73, 0.40, 0.073, and 0.0073 mg L^{-1}.

of ^{14}C in the aqueous phase after 5 h also corresponded to the presence of testosterone metabolites, which were indicated by TLC analysis. Competition for sorption sites will occur if the metabolites have a greater sorption affinity than the parent molecule, which could explain the slight decrease of sorbed concentrations. Also, metabolites that have lower sorption affinities than the parent testosterone could also explain this decrease.

Solid−solution distribution ratios (K_d = S/C, L g^{-1}) were determined for total ^{14}C concentration at each measurement time (Figure 2). For each soil, the K_d values increased then tended to decrease through time (Figure 2), where maximum values occurred between 1 and 5 h. The decrease in K_d after 5 h was likely caused by the production of metabolite compounds. Xing and Pignatello (*25*) showed similar decreases in K_d values for competitive sorption between chlorinated benzenes. At t > 5 h, testosterone metabolites would compete for sorption sites and/or could have lower sorption affinity, thus decreasing the K_d through time.

The K_d values were also used to observe any correlations between sorption and various soil properties (Figure 3). In order of strength, the correlations that were observed were between K_d and sand (r^2 = 0.34), clay (r^2 = 0.26), specific surface (r^2 = 0.22), and organic matter content (r^2 = 0.21). Similar correlations, albeit stronger, are also found between various estrogens and organic and mineral fractions of soil (*13*) and river and estuary sediments (*21*). These correlations generally indicate a relation between sorption and surface

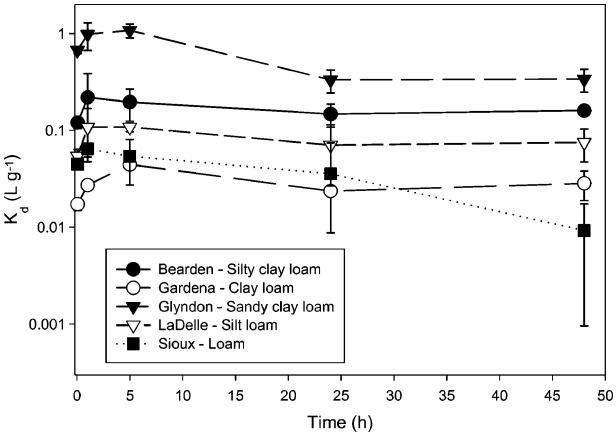

FIGURE 2. Time series plot of the solid−solution distribution ratios (K_d) with 95% confidence intervals.

area and/or organic matter content, which reflect the hydrophobic nature of the sorbate (testosterone).

The Freundlich isotherm parameters (Table 4) were determined using the 5 h data because sorption appeared to reach its maximum at this time. The Freundlich n values were essentially all equal to 1. Values of $n \approx 1$ suggest interaction between a generally hydrophobic sorbate with a hydrophobic sorbent (e.g., pesticide−organic matter interactions) *(26)*. Additionally, many of the n values were greater

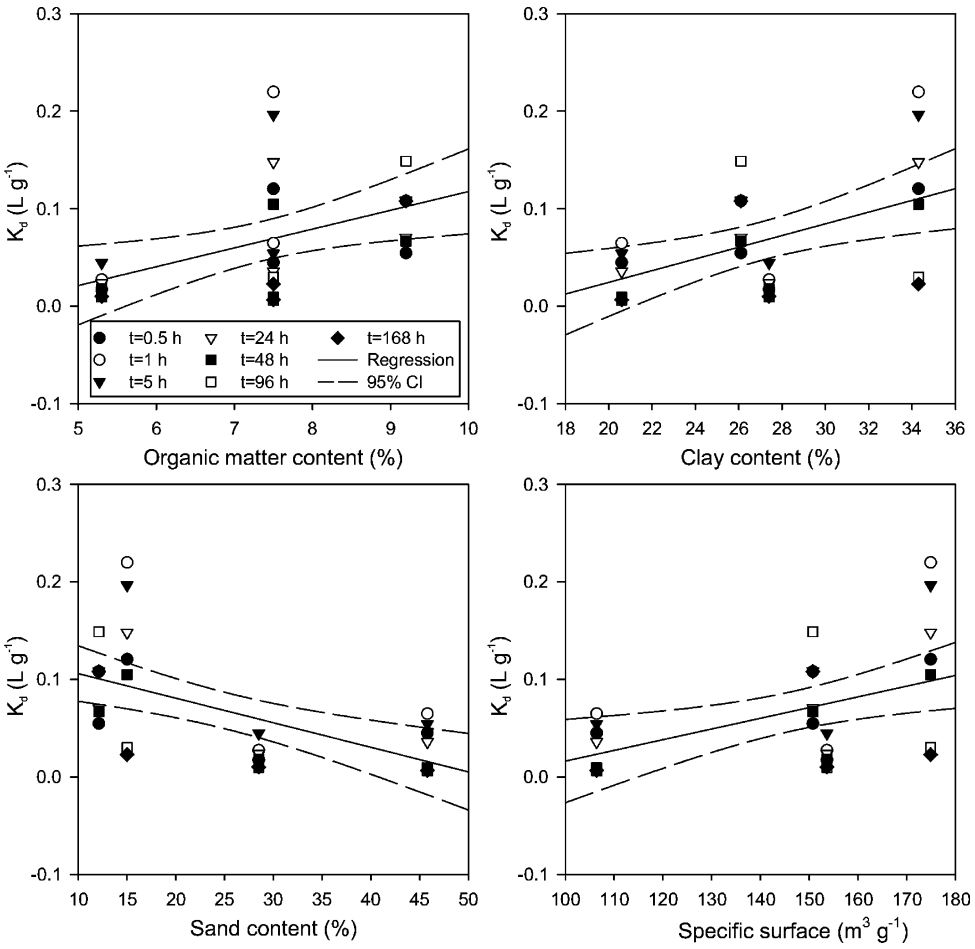

FIGURE 3. Relationships between various soil components and the solid−solution distribution ratio (K_d) calculated at the indicated experimental times. Also included are the linear regressions and 95% confidence intervals.

TABLE 4. Freundlich Coefficients (K_f, n) Determined at 5 h Time for Each Soil

soil type	K_f (L g^{-1})	n
Bearden-silty clay loam	0.15	0.98
Gardena-clay loam	0.05	1.00
Glyndon-sandy clay loam	1.20	1.00
LaDelle-silt loam	0.10	0.98
Sioux-loam	0.09	1.00
sand	0.0005	0.94

than 1 for isotherms determined at times beyond 24 h (data not presented). These results indicated that there were available sorption sites and that the decrease in the amount of sorbed phase concentrations observed in Figure 1 (t > 5 h) and the decrease in K_d values in Figure 2 (t > 5 h) were not caused by decreases in sorption site availability. Rather the decrease was likely caused by metabolite production or possibly testosterone desorption as previously discussed.

Miscible-Displacement Experiments. There was ^{14}C present in all the column effluent for each soil except the Glyndon-sandy clay loam. The total mass of ^{14}C recovered from within the column and the effluent is presented in Table 5. The mass recovery of ^{14}C ranged between 76% and 85%. Incomplete recoveries may have been the result of determining sorbed phase concentrations using solvent extractions of the soil, where complete removal of the sorbed ^{14}C may not have been achieved. Almost all ^{14}C, about 93%, was recovered for the sand column effluent, which meant little or no retention by the sand. This result indicated that testosterone sorption was likely caused by interactions with organic matter and/or particle size or clay content/type. The majority of the ^{14}C recovered from the extractions from each soil column was found in the upper 1−3 cm of the column and ranged from 14% to 69% of the total ^{14}C mass applied. Between 5% and 13% of the ^{14}C was still found in the lower 3 cm of the column, which indicated significant redistribution with depth.

Low concentrations of ^{14}C were present in the column effluent fractions. The total radioactivity present in the column effluent could be determined with confidence; however, reliable determination of the concentrations of individual metabolites was not possible. To increase the sensitivity for the TLC analysis, it was necessary to combine several effluent fractions into a single volume. This made it possible to visualize the presence of metabolites, but their quantification was difficult. Certain general conclusions could nonetheless be made from this information. Furthermore, other studies have identified possible testosterone metabolic pathways by a crustacean (*Neomysis integer* (*27*)) and a bacteria (*Comamonas testosteroni* (*28*)) and helped to speculate the identity of the metabolites. The crustacean study identifies possible testosterone metabolites using TLC, which was compared to TLC results from this study. The ^{14}C present in the effluent of the Gardena-clay loam and the sand columns was parent testosterone with no metabolite present. Very small amounts of metabolites were present in the effluent of the LaDelle-silt loam and Sioux-loam. One metabolite of greater polarity than testosterone was found in the effluent of the LaDelle-silt loam column. The polarity of this metabolite was most similar to androstenedione, but it could also be dihydrotestosterone (*27*). The Sioux-loam

TABLE 5. Column Model (eqs 3 and 4) Transport Parameter[a] Estimates

soil type	λ (cm)	K_f (L g^{-1})	n	ω (h^{-1})	μ (h^{-1})	% ^{14}C recovery	r^2
Bearden-silty clay loam	6.52 (±0.58)	0.050 (±0.053)	1.28 (±0.16)	0.613 (±0.205)	0.404 (±0.029)	76 [11][b]	0.86
Gardena-clay loam	8.10	0.012 (±0.005)	1.13 (±0.08)	0.422 (±0.102)	0.496 (±0.047)	82 [32]	1.00
Glyndon-sandy clay loam	1.68	0.054	1.30	0.057	0.600	85 [0]	0.98
LaDelle-silt loam	3.55	0.022 (±0.021)	1.27 (±0.18)	0.484 (±0.190)	0.493 (±0.190)	82 [24]	0.95
Sioux-loam	6.71 (±0.42)	0.006 (±0.010)	1.16 (±0.35)	0.877 (±0.522)	0.493 (±0.522)	77 [40]	0.94

[a] λ = dispersivity; K_f and n are Freundlich sorption coefficients; ω = first-order sorption rate coefficient, μ = first-order degradation/transformation coefficient. Values inside parentheses represent the ±95% confidence interval of the estimated parameter. [b] Values outside brackets represent total mass recovery; values within brackets represent mass recovered in the effluent.

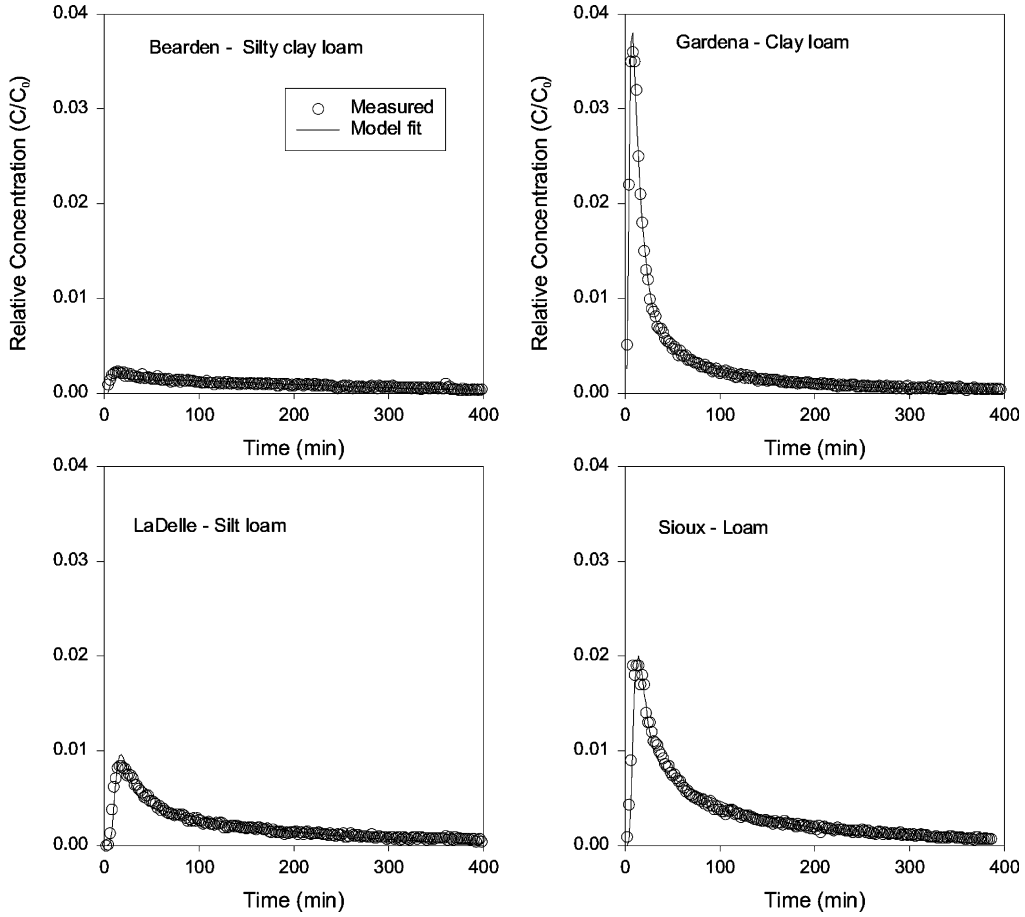

FIGURE 4. Soil column breakthrough curves of ^{14}C that show the relative effluent concentrations (C/C_0 = measure concentrated/applied concentrated) versus time.

had trace amounts of two metabolites; one metabolite was higher in polarity than testosterone, and the other was slightly lower in polarity. The metabolite of higher polarity was most similar to androstenedione (possibly dihydrotestosterone). The lower polarity metabolite was most similar to 2α-hydroxytestosterone (possibly β-boldenone or 16β-hydroxytesoterone) (*27*). All of the ^{14}C present in effluent of the Bearden-silty clay loam column was found to be a single metabolite of greater polarity than testosterone, most similar to androstenedione (possibly dihydrotestosterone) (*27*). The TLC analysis of the soil extraction from within each soil column indicated that testosterone and two other metabolites were present. One metabolite had a higher polarity than testosterone (either androstenedione or dihydrotestosterone), and the other had a lower polarity (either hydroxytestosterone, 16β-hydroxytesoterone, or β-boldenone) (*27*). In our speculation of the testosterone metabolites, it appeared that all the metabolites that were found in the effluent and within the column retained their steroidal structure. It is also possible that the metabolites could act as endocrine disruptors but at a reduced potency (*27*). Unlike 17β-estradiol (*13*), testosterone was able to elute from the soil columns intact and in some cases was the only compound present in the effluent.

Miscible-Displacement Model Analysis. The asymmetric solute breakthrough curves (Figure 4), caused by solute tailing or late arrival of solute, are characteristic of nonequilibrium transport. Physical and/or chemical processes can cause solute tailing. Physical nonequilibrium is caused by transport through structure soils, where lateral diffusion of solute in to and out of the soil matrix or immobile water causes tailing. Chemical nonequilibrium is caused by sorption/desorption kinetics. Physical and chemical nonequilibrium processes can occur simultaneously; however, each soil was repacked (i.e., the structure was disturbed during the column packing process), which decreased the likelihood of physical nonequilibrium transport. Chloride anion transport experiments further indicated that physical processes did not cause the solute tailing. Chloride is a conservative tracer and is normally transported through soil without sorption. The chloride breakthrough curves were described with an equilibrium

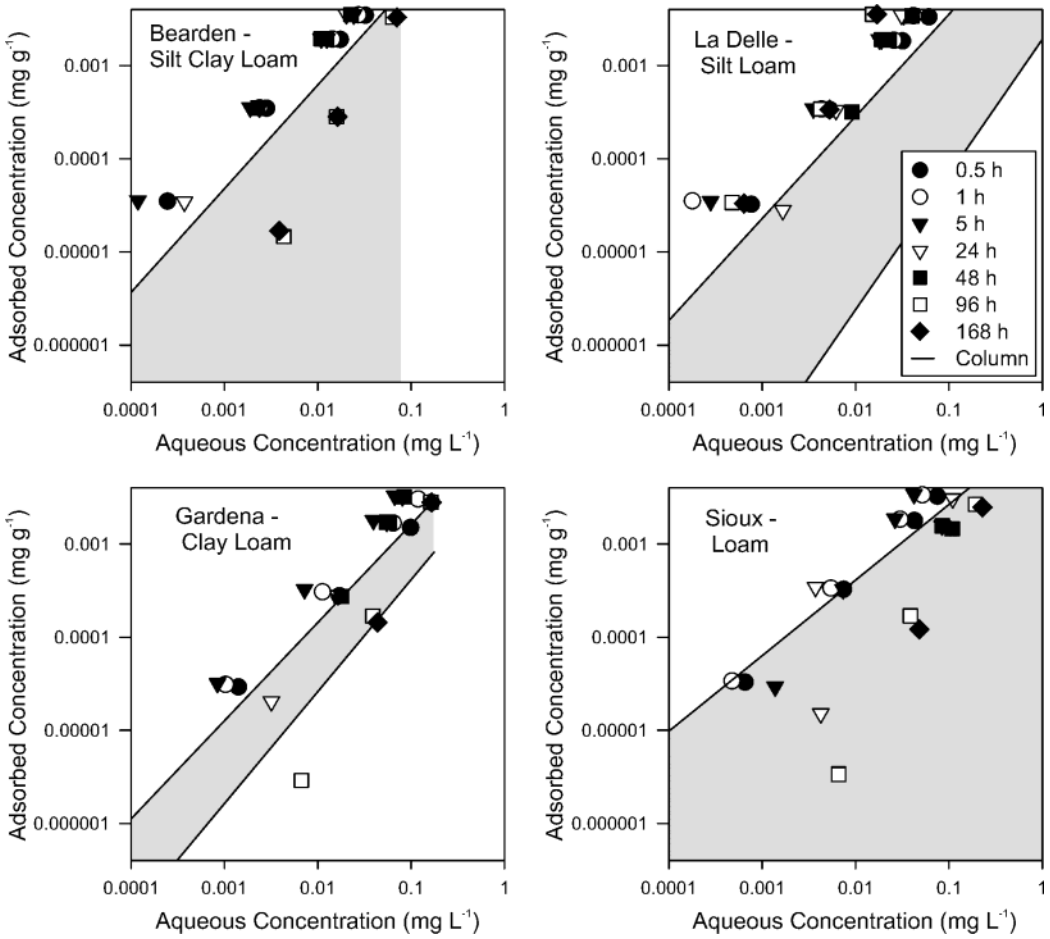

FIGURE 5. Graphical comparison between measured batch isotherm data and Freundlich parameter estimated from the column experiments. The symbols represent the measured concentrations from the batch experiments at various times. The solid lines represent the isotherms calculated from the 95% confidence values of the column Freundlich parameter estimates (Table 5). The shaded areas represent possible isotherms calculable from the combinations of column K_f and n estimates.

advective−dispersive model (i.e., eq 2 with $S = 0$) with no sorption, which indicated that the nonequilibrium transport resulted from chemical interactions (i.e., sorption kinetics).

The nonequilibrium model (eqs 3 and 4) that was used to describe the breakthrough curves was a fully kinetic sorption model with degradation/transformation occurring only on the sorbed phase. In general, the model descriptions of the column breakthrough curves were excellent (see r^2 values, Table 5) and the parameter estimates were reasonable. In most cases, especially when little or no metabolites were present (e.g., Gardena and LaDelle), the 95% confidence interval of the optimized parameters (Table 5) only spanned realistic values (e.g., the μ values spanned a range that encompassed values reported for other similar hormones and were not negative). This small 95% confidence interval indicates greater confidence in the parameter estimates (*29*). The model, eqs 3 and4, did not follow the transport of the metabolites, which may not fully represent some of the columns (i.e., Bearden) where metabolites were present. This model nonetheless was reasonable for the columns where few, if any, metabolites were present in the effluent. Also, the metabolites that were produced appeared to retain a steroidal structure, which would have similar physical−chemical characteristics as testosterone (*28*) and would likely have very similar fate and transport parameters as testosterone. Other models could have been considered and may have provided equal or better model fits; however, the number of parameters would have also increased. Increasing the number of parameters would have greatly decreased the confidence in the parameter estimates and in the processes modeled. The model that was chosen was simple but included dominant transport processes that were indicated from the batch experiments and from other column studies (*13*) that used similar hormones (i.e., 17β-estradiol). Furthermore, the model resulted in excellent descriptions of the measured data, low 95% confidence intervals of the optimized parameters, and agreement with the batch experiments and previous studies.

Sorption. The Freundlich sorption parameters that were derived from modeling the column breakthrough curves resulted in isotherms that under predicted much of the short time (t < 24 h) batch data (Figure 5). The column-derived isotherms were, however, able to describe some of the late-time batch data. Column experiment values of sorption usually result in lower sorption estimates than batch sorption studies because advective transport limits the rate of lateral diffusion of sorbate to the sorbent. Miscible-displacement experiments are also more sensitive to isotherm nonlinearity than batch isotherms (*30*), which could further decrease the accuracy at which column derived sorption parameters would predict batch data. These disparities between batch and column sorption parameters were the reasons why none of the batch parameters were used as constants in the inverse column modeling process.

The K_f values reported in Table 5 were more reliable for the columns where little or no metabolites were present in the effluent (i.e., Gardena and LaDelle). The 95% confidence intervals of the K_f values, for the columns where metabolites were present (i.e., Bearden and Sioux), spanned values that were negative. These unrealistic estimates of K_f may reflect

the inability of the model to accurately follow the fate and transport of the metabolites. Nonetheless, the other parameter estimates (e.g., n, ω, μ) for these columns had 95% confidence intervals, which provided realistic values.

Testosterone sorption isotherms obtained from soil column experiments were compared to 17β-estradiol (*13*) and indicated that testosterone had a lower sorption affinity. It was also found that testosterone was more strongly sorbed to soil than 17β-estradiol in river and estuary sediments (*21*). The lower sorption affinity of testosterone compared to 17β-estradiol would explain why field studies (*9*, *10*) have reported a deeper migration and higher mobility of testosterone compared to 17β-estradiol.

The long tail of the breakthrough curves in Figure 4 reflected the influence of sorption kinetics (ω value in Table 5). This parameter contained the greatest amount of uncertainty compared to the other parameters that were estimated. Nonetheless, the 95% confidence interval spanned values that were realistic. The column-derived ω values (Table 5) were also only slightly greater than the batch-derived values (Table 3), which further indicated reliable estimates of this parameter. The column ω values also fell within the range of values reported for estrogens in other column (*13*) and batch studies (*21*).

Degradation/Transformations. Parent testosterone was present in the column effluent in the current study, unlike the previous 17β-estradiol studies (*13*), where no 17β-estradiol escaped the column. The column μ values (Table 5) were similar for all soils, except the Glyndon, where no ^{14}C was present in the column effluent. Confidence in the Glyndon column model results was low because no effluent concentrations could be modeled. The testosterone μ values were comparable to other values reported by studies done with hormones in agricultural soils and biosolids. Mineralization rates of testosterone were reported to be 0.912 ± 0.126 h^{-1} in the presence of biosolids (*12*), which were twice as large as values found here. The degradation conditions present in biosolids may have facilitated higher rates compared to the agricultural soils used in the current study. Furthermore, the oven drying of the soil at 85 °C may have effected the soil microbial population, which in turn could have decreased the mineralization rate. Nonetheless, the testosterone μ values from the current study were almost twice as large as the 17β-estradiol ω values reported for the biosolids (0.252 ± 0.012 h^{-1}) (*12*). In agricultural soils, the testosterone μ values of the current study were approximately four times greater than 17β-estradiol values (0.06−0.12 h^{-1}) reported by Colucci et al. (*32*) and fell within the range of values reported by Casey et al. (*13*).

The transport of hormones is complicated by simultaneous nonequilibrium transport and transformations. The experimental results from this study indicated that testosterone sorption affinity was lower than 17β-estradiol (*13*) and that it transformed more readily. The present study and other field studies (*9*, *10*) have indicated that testosterone has a greater potential of migration than 17β-estradiol, even though it has a higher rate of transformation. These results underline the importance of the simultaneous transformation and sorption processes in the fate and transport of hormones.

At this time, it is not yet possible to make a definite statement regarding the potential of testosterone contamination to soil and subsurface water under natural conditions. In general though testosterone migration through the soil exists as a potential danger to subsurface water quality as long as it persists. In one case, testosterone was found to persist in the soil for 14 days and perhaps longer (*10*). In this study, it was found that testosterone could escape the upper soil horizon intact, which means it could potentially migrate to depths in the soil where biodegradation rates are reduced (i.e., beyond the root zone). If testosterone persists at these depths, where limited biological activity occurs, then the potential for subsurface water contamination increases. These benchtop laboratory experiments provided a deeper understanding of the fate and transport of testosterone; however, they used controlled boundary conditions (e.g., constant temperature, steady-state water flow) and manipulated media (e.g., oven-dried, sieved, repacked soil), which did not represent all natural field conditions. These types of experimental conditions can be observed in the field during events such as heavy rainfall or irrigation. Furthermore, the laboratory experiments did not take into consideration various soil heterogeneities (e.g., cracks, root holes, and worm holes) that can facilitate rapid transport of testosterone to the groundwater. Further field and laboratory experiments will be necessary to obtain a definite understanding of hormone fate and transport under natural conditions.

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