17β-Estradiol and Testosterone Sorption in Soil with and without Poultry Litter


17β-estradiol and testosterone are naturally occurring steroids that co-occur in poultry litter. The effects of litter on sorption of these hormones to soil are not known. Sorption isotherms were developed for 14C-labeled testosterone and 3H-labeled estradiol in a Cecil sandy clay loam with and without poultry litter addition. The effect of applying the hormones alone (single-sorbate) or together (multisorbate) was also investigated. 14C-testosterone sorption in soil increased from 2 to 48 h and remained relatively constant thereafter. 3H-estradiol sorption in soil was relatively constant from 2 to 24 h and then decreased to 72 h. These differences may reflect transformation of the parent hormones to products with different solid-phase affinity. The maximum sorption coefficient ($K_d$) in soil for 14C-testosterone (20.2 mL g$^{-1}$) is similar to that for 3H-estradiol (19.6 mL g$^{-1}$) in single-sorbate experiments. When hormones were applied together, sorption of both hormones in soil decreased, but the 14C-testosterone $K_d$ (12.5 mL g$^{-1}$) was nearly twice as large as the 3H-estradiol $K_d$ (7.4 mL g$^{-1}$). We propose this resulted from competition between the hormones and their transformation products for sorption sites, with 14C-testosterone and its expected transformation product (androstenedione) being better competitors than 3H-estradiol and its expected transformation product (estrone). When poultry litter was mixed with soil, sorption increased for 3H-estradiol but decreased for 14C-testosterone. This may have been because poultry litter slowed the transformation of parent hormones. Our results show that poultry litter could have important effects on the mobility of estradiol and testosterone.

The presence of estrogens (estradiol, estrone, and estriol) and androgens (testosterone) in land-applied manures and domestic and industrial waste, even in very low concentrations, has become a growing concern due to their adverse effect on endocrine systems of humans and wildlife (Ying et al., 2002). Although estradiol and testosterone help to regulate secondary sex characteristics under normal conditions, chronic exposure to these steroids has been associated with abnormal physiological processes and reproductive abnormalities in birds (Moccia et al., 1986), turtles (Bishop et al., 1991), and mammals (Martineau et al., 1988). Land-applied manures and wastes have the potential to reach surface and subsurface waters, and a number of studies have reported endocrine disruption in aquatic species (Jafari et al., 2009; Routledge et al., 1998).

According to the USDA National Agricultural Statistics Service, the United States produces more chickens (Gallus gallus domesticus) than any other country (USDA–NASS, 2011). The production of broilers (chickens raised specifically for meat consumption) in the United States increased from 4.1 billion kg in 1968 to 22.3 billion kg in 2010. Georgia produced 1.3 billion broilers in 2010, more than any other state in the nation. This rapid growth of broiler production has increased the production of poultry litter (a combination of bedding material and manure), which is applied to agricultural land as a source of nutrients and a means of disposal.

In addition to nitrogen, phosphorus, and potassium, poultry litter contains 17β-estradiol and testosterone. Lorenzen et al. (2004) estimated the contents of estradiol and testosterone to be 55 and 30 ng g$^{-1}$ in broiler litter and 70 and 25 ng g$^{-1}$ in breeder (chickens raised for egg production) litter, respectively. In an Arkansas rainfall simulation study, Nichols et al. (1997) sampled runoff immediately after application of poultry litter at different rates. Estradiol concentrations increased linearly with application rate from about 300 to 1300 ng L$^{-1}$. In a Georgia natural rainfall study, Finlay-Moore et al. (2000) measured hormone concentrations in runoff from 0.8-ha paddocks fertilized with poultry litter. Flow-weighted concentrations ranged from 120 to 820 ng L$^{-1}$ for estradiol and from 50 to 920 ng L$^{-1}$ for testosterone. Hormones pose a potential hazard to humans and wildlife, even in concentrations at the nanogram per liter level (Routledge et al., 1998).

The degree of surface and subsurface water contamination depends in part on the sorption of estrogen and testosterone to...
Most studies have shown that estrogen and testosterone sorption isotherms are linear or nearly linear (Casey et al., 2003, 2005; Fan et al., 2008; Hildebrand et al., 2006; Kozarek et al., 2008; Lee et al., 2003; Sangsapan et al., 2006; Sarmah et al., 2008). However, two studies found that estradiol isotherms were highly nonlinear (Bonin and Simpson, 2007; Yu et al., 2004). Sorption coefficients for estradiol generally increase with organic C content of soil or sediment, but the reported coefficients vary widely. Some of this variability can be reduced by expressing the sorption coefficient in terms of the soil organic carbon content ($K_{oc}$), but the log values still range from 2.27 to 4.64 for estradiol. According to Lai et al. (2000), the estimated value for log $K_{oc}$ based on the octanol-water partition coefficient ($K_{oc}$) is 3.5, which is within this range. However, it is clear that other factors in addition to organic C content affect sorption. Lai et al. (2000) showed that estrogen sorption occurred on iron oxides in the absence of organic matter. Other studies found more sorption in soils or river sediments with high specific surface and cation exchange capacity (Bonin and Simpson, 2007; Holthaus et al., 2002).

Other factors that can affect sorption include the method of measuring the estradiol. Some studies have used $^{14}$C-labeled estradiol and were consequently unable to distinguish between 17β-estradiol and its metabolites (Casey et al., 2003; Fan et al., 2008; Sangsapan et al., 2006). The method of calculating the sorption coefficients has varied, as well. Most studies estimate sorbed concentrations by calculating the difference between the initial solution concentration and the solution concentration after shaking (Casey et al., 2003; Kozarek et al., 2008; Lee et al., 2003; Sangsapan et al., 2006; Sarmah et al., 2008). In this case, the sorbed phase includes any fraction that is irreversibly sorbed. Several studies have calculated sorption coefficients indirectly, using a model that includes transformation to an irreversibly sorbed form (Casey et al., 2005; Fan et al., 2008). With this method, the coefficient is for reversible sorption only.

Fewer studies have reported sorption coefficients for testosterone, but the results are similar to estradiol, with log $K_{oc}$ in a slightly higher range, 2.97 to 5.06 (Casey et al., 2004; Fan et al., 2007; Lee et al., 2003). Only two studies have examined desorption of estradiol from soil, and no studies have looked at desorption of testosterone from soil. Hildebrand et al. (2006) found that 82 to 97% of different forms of estradiol were irreversibly sorbed in four soils. At low initial concentrations (10 μg L$^{-1}$), however, 0% of estrone, 20% of estradiol, and 80% of ethynilestradiol did not desorb. Loffredo and Senesi (2002) also found that ethynilestradiol did not desorb from surface and deep horizons of two soils.

No studies have examined the effect of poultry litter on the soil sorption of 17β-estradiol and testosterone. Organic matter in litter could retard the movement of the hormones due to strong sorption, or it could enhance movement due to cotransport with fine particulate matter. Also, because both hormones are present in poultry litter, they have the potential to compete for sorption sites. Bonin and Simpson (2007) showed the competitive effect of different forms of estrogen on sorption. No studies have examined the competitive effects of estrogen and testosterone.

The objectives of our research were (i) to evaluate the effect of poultry litter on the sorption of 17β-estradiol and testosterone in soil and (ii) to evaluate the effect of applying the hormones alone or together. This work was performed in conjunction with a transport study using soil columns and breakthrough curves to be presented in a later paper.

**Materials and Methods**

**Soil and Poultry Litter Samples**

Bulk soil samples for sorption experiments were collected from the 0- to 30-cm soil depth in paddocks located at the Central Georgia Research and Education Center near Eatonton, GA. These paddocks had received broiler litter for approximately 12 yr at the time of sample collection. The soil at the site was a Cecil sandy clay loam (fine, kaolinitic, thermic Typic Kanhapludult), which is a common soil in the Southern Piedmont physiographic region. Soil samples were air-dried, ground, passed through a 2-mm sieve, and stored at 20°C. Poultry litter was obtained from a poultry farm located in northeast Georgia. Litter was air-dried, ground, and stored at 20°C.

**Soil and Poultry Litter Analyses**

Soil samples were analyzed for pH (1:5 soil:water ratio), particle size distribution (Gee and Or, 2002), total carbon (C) by dry combustion (Nelson and Sommers, 1982), and cation exchange capacity (CEC) and base saturation. To determine CEC, the soil was extracted with Mehlich-1 solution (Mehlich, 1953), the bases (Ca, Mg, K, and Na where applicable) were determined by inductively coupled plasma mass spectrometry and then added to the amount of exchangeable hydrogen. Base saturation was calculated by dividing bases by the CEC and expressing the result as percentage. Poultry litter samples were analyzed for total C by dry combustion (Nelson and Sommers, 1982).

To extract hormones from the litter, 1 g of litter was placed in narrow mesh Nytex bags and extracted with 15-mL of dichloromethane (CH$_2$Cl$_2$; high-pressure liquid chromatography [HPLC] grade) in 40-mL amber-colored glass tubes. The tubes were sonicated for 1 h before being centrifuged (2000 rpm for 20 min) and the supernatants decanted into 50-mL evaporation tubes. The bagged litter was reextracted twice in 10 mL of dichloromethane for 30 min, centrifuged, and the resulting supernatants combined. The CH$_2$Cl$_2$ extracts were evaporated (TurboVap II, LV; Zymark Corp., Hopkinton, MA) to near dryness under N$_2$. A final drying step using two successive 1-mL aliquots of acetonitrile under N$_2$ removed the remaining CH$_2$Cl$_2$. Temperature in the Turbovap never exceeded 70°C. The dry residues containing the hormones were derivatized to the trimethylsilyl derivatives and made up to suitable volume with acetonitrile before gas chromatography analysis.

The concentrations of 17β-estradiol and testosterone were measured in the poultry litter using a gas chromatograph tandem mass spectrometer (GC–MS–MS; Agilent Technologies, Palo Alto, CA) operated in selected ion storage mode following derivatization of the hormones to trimethylsilyl forms with N,O-bis(trimethylsilyl)-trifluoroacetamide containing 1% (w/w) of trimethylchlorosilane. Chromatographic
separation was conducted on a J&W capillary column (30 m by 0.25 mm i.d. with 0.25-mm film thickness; Agilent Technologies, Wilmington DE) following splitless injection using 1 mL min⁻¹ He carrier gas. The column temperature profile was increased from 90 to 250°C (12°C min⁻¹ ramp) followed by a second increase to 270°C (20°C min⁻¹ ramp) and held for 5 min. Injection port and transfer lines were held at 260 and 70°C, respectively, while the ion trap and source were held at 210 and 270°C, respectively. Quality assessment and control procedures included running one blank and one spiked sample every 10 samples and a duplicate sample every 20 samples. Limit of quantification was 0.7 ng g⁻¹ for 17β-estradiol, 1.7 ng g⁻¹ for estrone, and 0.50 ng g⁻¹ for testosterone. Average recovery was 86% for 17β-estradiol, 83% for estrone, and 95% for testosterone.

The concentrations of hormones in the soil samples in our study were not measured. However, monthly measurements were made in the paddocks from which our soil samples were collected. The procedure for extracting and measuring hormones in soil were the same as described above for the poultry litter samples, except mesh bags were not used and 5 g of soil was used instead of 1 g of litter.

**Hormones**

Unlabeled estradiol and testosterone (both >98% purity) were obtained from Sigma-Aldrich (St. Louis, MO). Solutions were stored in a refrigerator at 4°C. Radio-labeled 6,7-³H-estradiol (specific activity 50 Ci mmol⁻¹) and 4-¹⁴C-testosterone (specific activity 55 mCi mmol⁻¹) were obtained from American Radiolabeled Chemicals (St. Louis, MO). In this study, we refer to the ³H label as “³H-estradiol” and to the ¹⁴C label as “¹⁴C-testosterone” but recognize that the label may actually be in a metabolite of these hormones. Where appropriate, we discuss the transformation products.

**Equilibrium Sorption Isotherms**

Batch equilibrium sorption experiments were performed for soil, soil mixed with litter (soil+litter), and litter alone. Sorption experiments were conducted for ³H-estradiol and ¹⁴C-testosterone separately as well as with both hormones together for soil to determine the interaction of these hormones. Unlabeled hormones were diluted to 100 µg mL⁻¹ with HPLC-grade methanol to avoid precipitation due to the inorganic solvent 0.01 M CaCl₂. Then they were diluted to 0.001, 0.01, 0.1, 0.5, and 1 µg mL⁻¹ with 0.01 M CaCl₂ solution for sorption isotherms following the procedure used by Sangsupan et al. (2006). Ten milliliters of unlabeled estradiol or testosterone of each concentration were added to 1 g air-dried soil or litter in a 15-mL glass centrifuge tube. For soil mixed with litter, 0.07 g of litter was added to each g of soil. Test tubes were sealed by Teflon-lined caps. In each test tube, 5 µL of labeled estradiol and estradiol (0.059 KBq 6,7-³H-estradiol and 0.015 KBq 4-¹⁴C-testosterone) were added. The test tubes were shaken in a reciprocating shaker at 300 oscillations per minute for 2, 24, and 48 h at 20°C.

The experiment was repeated for soil (not for soil-litter and litter alone) with the hormones applied together. One gram of air-dried soil was shaken with 10 mL of unlabeled solution (5 mL unlabeled estradiol and 5 mL unlabeled testosterone of corresponding concentrations). Five microliters of labeled ³H-estradiol and ¹⁴C-testosterone were added to each test tube and shaken for 2, 24, 48, 72, 120, and 144 h at 20°C.

Samples were centrifuged for 15 min at 3500 rpm at each time interval. The centrifugal force was 4400 times the force of gravity. One milliliter of supernatant was then taken for analysis of radioactivity by a Beckman LS6500 liquid scintillation counter (Beckman Coulter, Inc., Brea, CA). The efficiency of the counter was routinely monitored. Counting efficiency for ³H was 88% and for ¹⁴C was 98%. The instrument was regularly calibrated using standard ³H and ¹⁴C quench standards provided by Beckman Coulter, Inc. (Brea, CA). An external quench monitor (cesium-137) was used to compensate for quench. The instrument software converted raw counts per minute data to disintegrations per minute based on quench correction.

Three replications of each experimental unit were performed. The difference in initial and final radioactivity was considered as that adsorbed by 1 g of soil, litter, or the mixture. Sorption experiments were performed in sterile (0.12 mg mL⁻¹ HgCl₂) and nonsterile medium. Mercuric chloride (HgCl₂) effectively destroys the microbial population without changing soil physical and chemical properties (Wolf et al., 1989).

Batch equilibrium sorption data were fit with the linear Freundlich (1909) sorption equation:

\[ s = K_f c \]

where \( s \) is the concentration of hormone in the sorbed phase (µg g⁻¹), \( c \) is the concentration of hormone in solution (µg mL⁻¹), and \( K_f \) is the sorption coefficient (mL g⁻¹). The sorption data were also fit with the nonlinear Freundlich (1909) sorption equation:

\[ s = K_n c^n \]

where \( K_n \) is the nonlinear Freundlich sorption coefficient (µg⁻¹ mL⁴ g⁻¹) and \( n \) is the Freundlich exponent.

Desorption isotherms were also developed. Ten milliliters of unlabeled estradiol or testosterone (1 µg mL⁻¹) were added to 1 g air-dried soil or litter in a 15-mL glass centrifuge tube. For soil mixed with litter, 0.07 g of litter was added to each g of soil. Test tubes were sealed by Teflon-lined caps. In each test tube, 5 µL of labeled testosterone and estradiol (0.082 KBq 6,7-³H-estradiol and 0.019 KBq 4-¹⁴C-testosterone) were added. Samples were shaken in a reciprocating shaker for 24 h, centrifuged for 15 min at 3500 rpm, and then 1 mL of supernatant was taken for analysis of radioactivity using the liquid scintillation counter. The supernatant was discarded and 10 mL of hormone-free 0.01 M CaCl₂ solution was added to the same test tube and it was shaken for 24 more hours. This process was repeated four times at 24-h intervals.

**Statistical Analysis**

Results for sorption coefficients were analyzed statistically using the General Linear Model (PROC GLM) procedure in SAS (SAS Institute, Inc., Cary, NC). Differences between means were tested using Fisher’s protected least significance difference (LSD) with \( p = 0.05 \) or 0.01, except as noted.
Results and Discussion
Soil and Litter Properties
The sand, silt, and clay contents of the soil from the 0- to 30-cm depth were 40, 28, and 32% by weight, respectively (sandy clay loam textural class). Other properties were pH = 6.15, base saturation = 87.3%, total C = 16.8 g kg⁻¹, and CEC = 9.19 cmol kg⁻¹. The background concentrations of 17β-estradiol and testosterone in the litter were 60 and 303 ng g⁻¹, respectively, similar to the values reported by Lorenzen et al. (2004). The background soil concentrations of hormones in the plot where we took the samples for our study were measured on 14 May 2008 (we collected our study samples in mid-June). The concentration in the top 0- to 2.5-cm depth increments were 52, 61, and 110 ng g⁻¹ for 17β-estradiol, estrone, and testosterone, respectively.

Sorption Kinetics
The sorption kinetics and equilibrium isotherm data were not significantly different in nonsterile and sterile medium, indicating that biotic processes such as mineralization were not important in the time frame of our study. Therefore, only the sterile data are presented.

Sorption of both hormones varied with contact time in our soil and soil+litter treatments, whereas sorption to litter was equal within error across all reaction times (Fig. 1 and 2). 14C-testosterone sorption to soil and soil+litter increased from 2 to 48 h before reaching pseudo-equilibrium (Fig. 1), whereas 3H-estradiol exhibited a sorption maximum to soil and soil+litter between 2 and 24 h followed by a subsequent decrease in the amount retained on the solid after 24 h (Fig. 2).

In a prior study of 14C-testosterone and 3H-estradiol sorption to surface samples from a similar Cecil series soil, Sangsupan et al. (2006) found comparable time-dependent sorption trends with a 14C-testosterone sorption maximum at 72 h and a decrease in 3H-estradiol sorption after 48 h. However, Casey et al. (2004) found that maximum sorption of testosterone occurred much more rapidly (1 to 5 h) for an assortment of Mollisols. Rapid 14C-testosterone sorption (<2 h to apparent equilibrium) was also observed by Sangsupan et al. (2006) when examining subsurface samples of the Cecil series soil. Because each of these studies—ours included—monitored sorption by loss of the 14C-label from solution, this heterogeneity in the time to maximum sorption across different soils may result from variation in the rates of testosterone transformation and subsequent transformation-product sorption. When Lee et al. (2003) used HPLC to monitor testosterone transformation during sorption reactions, they found the terminal hydroxyl group in testosterone to be rapidly transformed into a ketone group forming the daughter product androstenedione. Ketone groups are stronger hydrogen acceptors than hydroxyl groups but cannot serve as hydrogen donors (Neale et al., 2009). Thus, depending on the H-bonding environment in the soil, the rate of testosterone transformation could alter the apparent sorption kinetics (Lee et al., 2003).

Transformation of the parent hormone may also explain the decrease in 3H-estradiol sorption with increasing reaction time (Fig. 2), as again our methodology could not distinguish between estradiol and its daughter transformation products (Lai et al., 2000) if they retained the 3H label. Similar to the transformation reaction described above for testosterone, Lee et al. (2003) found that within 11 min after reacting estradiol with soils, the phenolic hydroxyl group at position 17 on estradiol was oxidized to a ketone, forming the daughter product estrone. This transformation reaction is found to occur even in sterile soil, suggesting an abiotic mechanism (Colucci et al., 2001). In addition to estrone, Casey et al. (2003) found trace concentrations of estriol as a transformation product of 17β-estradiol. The presence of the ketone group on estrone compared with the hydroxyl group on estradiol has important bearing on their relative affinity for soil sorbents. Ketones evidently react much more strongly with phenolic hydrogen donors than they do with carboxylic acid or alcohol hydrogen donors (Neale et al., 2009). Thus, estrone has a lower Kow value than estradiol because the hydrogen donor in octanol is an alcohol group, whereas estrone exhibits higher Kow values than estradiol for sorbents dominated by phenolic hydrogen donors such as humic acid, tannic acid, and toluene (Neale et al., 2009; Qiao et al., 2011). This seems to be reflected in
the spread of $K_d$ values for sorption of these compounds to soil across a range of studies. Most often, estradiol exhibits higher $K_d$ values than estrone for sorption to soils and sediments (Lee et al., 2003; Bonin and Simpson, 2007; Fan et al., 2008; Sarmah et al., 2008), but this is less consistent when sorption is particularly nonlinear (Yu et al., 2004), or involves sandy soils with low organic matter content where Hildebrand et al. (2006) measured a higher $K_d$ for estrone than estradiol, although Lee et al. (2003) did not.

**Equilibrium Sorption Isotherms**

All $^{14}$C-testosterone and $^3$H-estradiol equilibrium isotherms were strongly linear across the concentration range examined (0.001 to 1 µg mL$^{-1}$) (Fig. 3 and 4), yielding Freundlich exponent values ($n$) between 0.92 to 0.98 for all treatments, with the majority of $n$ values greater than 0.95 (data not shown). These values are comparable to values reported previously (Casey et al., 2003), although nonlinear isotherms for sorption of estradiol to soils are also common (Yu et al., 2004). Because the linear model adequately fit the isotherm data, it will be used in the remainder of our discussion. The background concentrations of hormones in the soil (52, 61, and 110 µg kg$^{-1}$ for 17β-estradiol, estrone, and testosterone, respectively) were small compared with the concentrations of unlabeled 17β-estradiol and testosterone added (10, 100, 1000, 5000, and 10000 µg kg$^{-1}$), except at the lowest levels. The fact that the isotherms were linear indicated that the background concentrations did not have an effect.

The maximum linear sorption coefficients to soil were similar for both hormones (Table 1), although $K_d$ values for $^3$H-estradiol were larger at early reaction time and smaller at long reaction time than $^{14}$C-testosterone, reflecting our kinetic sorption data (Fig. 1 and 2). Estradiol typically exhibits higher affinity for soils, sediment (Lee et al., 2003; Casey et al., 2004), and organic (Neale et al., 2009) sorbents than testosterone, although Lee et al. (2003) reported higher testosterone linear sorption coefficients compared with estradiol for the sandy, low-organic matter soil, EPA-1. We used total C as a surrogate for organic C in our soil because of its noncalcareous nature and lack of liming history. Using total C, we calculated monosorbate log $K_w$ values for $^3$H-estradiol and $^{14}$C-testosterone from the 24-h isotherm to be 3.04 and 3.07, respectively. These are within the range of log $K_w$ values for similar Cecil topsoils (Sangsupan et al., 2006; Kozarek et al., 2008) and comparable to literature values reported for a wide range of soil series (Fig. 5 and 6).

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<th>$^{3}$H-Estradiol $K_d$</th>
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<td>Hormones applied separately</td>
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<td>Hormones applied together</td>
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<td>Soil</td>
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<td>$r^2$</td>
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The Influence of Poultry Litter

Sorption coefficients were much higher in litter without soil than in soil alone for both $^3$H-estradiol and $^{14}$C-testosterone (Table 1); this was expected given the attraction of these hormones to organic matter (Casey et al., 2003, 2004). We measured higher $K_d$ values for sorption of $^3$H-estradiol (max. 59.8 mL g$^{-1}$) to litter compared with $^{14}$C-testosterone (max. 38.0 mL g$^{-1}$). When poultry litter was mixed with soil, the affinity of $^3$H-estradiol for the resulting solid phase materials was higher than the soil-only treatment ($K_d$ soil+litter > $K_d$ soil; Table 1) for all reaction times. Similar to the soil-only treatment, we observed a trend of rapid initial sorption ($K_d$ = 23.1 mL g$^{-1}$) followed by subsequent desorption leading to a significant decrease in $K_d$ values after 48 h of reaction (19.6 mL g$^{-1}$). This desorption step was not detected in the litter-only treatment. If we interpret the desorption as transformation to a lower affinity sorbate, as discussed above, then our data suggest either (i) the affinity of the transformation product for litter is equal to or within error of that of estradiol, or (ii) that the transformation reactions were suppressed in the litter-only treatment.

In contrast, sorption of $^{14}$C-testosterone in the soil+litter treatment was significantly lower than in the soil-only treatment in both our sorption isotherm experiments after 2, 24, and 48 h of reaction (Table 1 and Fig. 4) and in our single-concentration sorption experiments that extended to 140 h of reaction (Fig. 1). These results suggest that leaching of testosterone from soil may be greater in the presence of poultry litter than when testosterone is applied alone. Sorption of testosterone to dissolved organic matter (DOM) released from the litter might explain these results as the centrifugation rates used in our experiments were unlikely to sediment most DOM. However, we would expect similar artifacts to reduce our measurements of estradiol sorption, but they did not.

Instead, we propose the presence of litter inhibited the oxidation of the phenolic hydroxyl on both hormones and delayed the transformation of 17β-estradiol and testosterone to estrone and androsterone, respectively. Because 17β-estradiol has a higher $K_d$ than estrone (Lee et al., 2003; Bonin and Simpson, 2007; Fan et al., 2008; Sarmah et al., 2008), this would increase apparent sorption of $^3$H-estradiol in the soil+litter treatment (Table 1, Fig. 3). Delaying the transformation of testosterone to androsterone—which has a higher $K_d$ than testosterone (Lee et al., 2003)—would decrease sorption (Table 1, Fig. 4). Supporting this hypothesis, equilibrium concentrations of $^3$H-estradiol and $^{14}$C-testosterone were constant in our litter-only treatments (Fig. 1 and 2). In addition, the desorption of $^3$H-estradiol, which became readily apparent between 24 and 48 h in our soil-only experiment, was much more gradual in the soil+litter treatment, extending from 24 to 120 h (Fig. 2). $^{14}$C-testosterone increased slowly from 2 to 48 h in both the soil and soil+litter treatments (Fig. 1).

To explore the effect of delayed transformation, we modeled the sorption coefficient of the soil+litter treatments after 24 and 48 h of reaction using a mass-weighted average (1 g of soil and 0.07 g of litter) of the soil-only and litter-only sorption coefficients after 2 h of reaction and compared them to measured $K_d$ values for 24 and 48 h of reaction (Table 1). For $^{14}$C-testosterone, we calculated an estimated $^{14}$C-testosterone $K_d$ for soil+litter of 16.2 mL g$^{-1}$, which compared well with the measured $K_d$ for soil+litter of 16.0 and 17.0 after 24 and 48 h of reaction, respectively (Table 1). Thus, the reduction in sorption in the 24-h soil+litter treatment with $^{14}$C-testosterone is consistent with our hypothesis that the addition of litter slowed the transformation process. For $^3$H-estradiol, the calculated $K_d$ for the soil+litter treatment was 21.9 mL g$^{-1}$, which was intermediate between the measured values of 24.3 and 19.6 mL g$^{-1}$ after 24 and 48 h of reaction, respectively (Table 1). Further investigation involving direct measurements of both the hormones and their transformation products would be necessary to confirm this hypothesis.

As discussed above in regard to the soil samples, the background concentrations of 17β-estradiol and testosterone in the litter (60 and 303 μg kg$^{-1}$, respectively) were small compared with the concentrations of unlabeled 17β-estradiol and testosterone calculated from previous studies and our study of estradiol as a function of reaction time. Some studies used radio-labeled estradiol (our study; Casey et al., 2003, 2005; Fan et al., 2008; Sangsaran et al., 2006). Other studies measured 17β-estradiol, estrone, or 17α-ethynylestradiol directly (Bonin and Simpson, 2007; Hildebrand et al., 2006; Kozarek et al., 2006; Lee et al., 2003; Sarmah et al., 2008; and Yu et al., 2004).
vals, more of the 14C-testosterone was retained on the soil than single-sorbate experiment. After four washings at 24-h intervals, testosterone or its transformation products. A likely explanation for the higher affinity for soil of 3H-estradiol sorption to poultry litter and soil+litter than for 14C-testosterone and its transformation products, with 14C-testosterone being better competitors than estradiol transformation products, estrone and androstenedione from estradiol and testosterone, respectively. Estrone has a lower reported affinity for soil than estradiol, whereas androstenedione has a higher reported affinity for soil than testosterone. When the hormones were applied together to soil, K_d for both hormones decreased and the 14C-testosterone K_d was significantly greater than the value for 3H-estradiol. We suggest this is due to competition for sorption sites between hormones and their transformation products, with 14C-testosterone and its transformation products being better competitors than estradiol and its transformation products. Desorption experiments also showed more 14C-testosterone was retained on the soil than 3H-estradiol. When poultry litter was mixed with soil, sorption in soil increased for 3H-estradiol but decreased for 14C-testosterone. This may have been due to poultry litter slowing the transformation of parent hormones. These results suggest that leaching of testosterone from soil may be greater in the presence of poultry litter than when testosterone is applied alone.

Acknowledgments
This work was supported by EPA STAR grant R833419.

References

Combined Effect of Testosterone and Estradiol
Multisorbate experiments were also performed to evaluate the affinity of 14C-testosterone and 3H-estradiol for soil when both hormones were present (as they always are in poultry litter). After 24 h of reaction, both K_d values were significantly lower in the multisorbate system (7.4 and 12.5 mL g⁻¹) for 3H-estradiol and 14C-testosterone, respectively) than in the single-sorbate system (18.6 and 19.7 mL g⁻¹ for 3H-estradiol and 14C-testosterone, respectively) (Table 1). The affinity of estradiol for soils and sediment is commonly observed to decrease in the presence of competing estrogen sorptives (Lai et al., 2000; Yu et al., 2004; Bonin and Simpson, 2007). This effect has not been reported previously for testosterone but could be expected given its structural similarities to estradiol.

Unexpectedly, the testosterone K_d was significantly higher (12.5 mL g⁻¹) than the estradiol K_d (7.4 mL g⁻¹) (Table 1). Estradiol typically exhibits higher affinity for soil (Lee et al., 2003; Casey et al., 2004), sediment (Lee et al., 2003), and organic (Neale et al., 2009) sorbents than testosterone, although there may be exceptions in sandy soils with low organic matter (Lee et al., 2003). Indeed, we found much larger K_d values for 3H-estradiol sorption to poultry litter and soil+litter than for 14C-testosterone (Table 1). However, as we note above, there is increasing evidence that the hydroxyl group at position 17 of estradiol and testosterone undergoes rapid oxidation to ketone moieties during sorption to soil (Lee et al., 2003), leading to the transformation products estrone and androstenedione. After confirming substantial hormone transformation in 17β-estradiol-soil and testosterone-soil experiments, Lee et al. (2003) calculated higher soil K_d values for androstenedione than for estrone. Furthermore, the observed desorption of 3H-estradiol with increasing reaction time suggests estradiol or its transformation products are less tightly bound than testosterone or its transformation products.

To investigate this, we tracked 3H or 14C desorption following sorption of 3H-estradiol or 14C-testosterone to soil in a single-sorbate experiment. After four washings at 24-h intervals, more of the 14C-testosterone was retained on the soil than 3H-estradiol (Fig. 7). The differences in coefficients of the fitted polynomials were statistically significant at the p = 0.059 level. This suggests testosterone or its transformation products bind more strongly to the soil than estradiol or its transformation products. A likely explanation for the higher affinity for soil of 14C-testosterone than 3H-estradiol in our multisorbate experiments is the combined effects of a lower desorption potential (Fig. 7) and greater affinity of its transformation product for soil (Lee et al., 2003).

Conclusions
Our results suggest the environmental fate of estradiol and testosterone in land-applied poultry litter is influenced by the co-occurrence of the hormones and the influence of the litter on sorption to soil. We found when the hormones were applied separately to soil, the maximum K_d values for 14C-testosterone and 3H-estradiol were similar, with log K_oc values that were comparable to prior studies. Kinetic experiments showed that 14C-testosterone sorption to soil increased steadily to 48 h before reaching pseudo-equilibrium, whereas 3H-estradiol sorption peaked between 2 and 24 h followed by desorption of the 3H label. These trends for both hormones are consistent with the oxidation of the phenolic hydroxyl group to a ketone group forming the transformation products, estrone and androstenedione from estradiol and testosterone, respectively. Estrone has a lower reported affinity for soil than estradiol, whereas androstenedione has a higher reported affinity for soil than testosterone. When the hormones were applied together to soil, K_d for both hormones decreased and the 14C-testosterone K_d was significantly greater than the value for 3H-estradiol. We suggest this is due to competition for sorption sites between hormones and their transformation products, with 14C-testosterone and its transformation products being better competitors than estradiol and its transformation products. Desorption experiments also showed more 14C-testosterone was retained on the soil than 3H-estradiol. When poultry litter was mixed with soil, sorption in soil increased for 3H-estradiol but decreased for 14C-testosterone. This may have been due to poultry litter slowing the transformation of parent hormones. These results suggest that leaching of testosterone from soil may be greater in the presence of poultry litter than when testosterone is applied alone.

Fig. 7. Percentage of 3H-estradiol and testosterone sorbed as a function of four daily washings with hormone-free solution. All data points shown without averaging. The lines through the data points are fitted second-order polynomials.


