0038-075X/08/17307-456–467
Soil Science
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July 2008
Vol. 173, No. 7
Printed in U.S.A.

TRANSPORT OF 17β-ESTRADIOL AND TESTOSTERONE IN A FIELD LYSIMETER

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17β-Estradiol (E2) and testosterone (T) are present in sources such as waste treatment effluent and manures, and can potentially disrupt aquatic organisms at low concentrations. Laboratory studies consistently indicate limited mobility and rapid attenuation of E2 and T in soils; however, these hormones are regularly detected in the environment. A steady-state field lysimeter (2.4 m in length × 2.4 m in width × 2.3 m in depth) study was done to identify the significant fate and transport factors controlling E2 and T disposition in the field. The transport of E2 and T were compared with the transport of a conservative nonsorbing tracer, pentafluorobenzoic acid (PFBA). Concentration redistributions of water-extractable E2, T, and PFBA through depth were determined. In addition, lysimeter effluent drainage concentrations of water-dissolved E2, T, and PFBA were determined. Effluent PFBA was successfully modeled with the convective-dispersive equation assuming no sorption or preferential transport. Effluent mass recovery of PFBA was 100%. Resident profile mass recoveries of E2 and T were 0.46% (±0.01%) and 0.02% (±0.01%), respectively. Statistical analyses indicated that soil water status and organic matter were the predominant factors effective in explaining variations of E2 and T in the lysimeter profile. Lysimeter effluent mass recoveries of E2 and T were 1.3% (±0.15%) and 0.2% (±0.02%), respectively. 17β-Estradiol and T were detected before the PFBA peak in the effluent, which may have indicated the antecedent presence of E2 and T, analytical nonspecificity, and/or facilitated transport (likely colloidal). In addition, E2 and T concentrations were correlated to lysimeter drainage, perhaps indicating significant colloidal facilitated transport. This is one of the first field studies that gives evidence for likely processes that explain greater-than-expected mobility and concentrations of E2 and T in the environment. (Soil Science 2008;173:456–467)

Key words: 17β-Estradiol, testosterone, fate and transport, solute transport, lysimeter, soil.

ALL animals eliminate estrogenic and androgenic hormones from their bodies through their feces and urine. The principal steroidal hormones eliminated are 17β-estradiol (E2) and testosterone (T). Once these hormones are introduced into the environment, it is possible that they can bind to and activate hormone-responsive organs (Melnick, 1999). Fish are especially sensitive to exogenous hormones because of their unique reproductive strategies that allow for physiological responses (e.g., sex differentiation, gametogenesis), sexually adapting them to match a diversity of natural habitats (Jalabert, 2005). For example, intermittent exposures (alternate days, 1 day in 4, or 3 days in 6) of male fathead minnows (*Pimephales promelas*) to 0.120 μg L^{-1} of E2 significantly induced vitellogenin production (Panter et al., 2000). Vitellogenin is an egg yolk precursor protein and is normally produced by female fish. In

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Received Oct. 9, 2007; accepted Mar. 27, 2008.
DOI: 10.1097/SSL.0b013e318178e71f

addition, when newly hatched medaka (*Oryzias latipes*) were exposed to 100 μg L^{-1} of T for 6 days, they displayed intersex gonads (Koger et al., 2000). Very limited information about hormone toxicology for other species exists. Shore et al. (1993) measured average T concentrations in poultry (*Gallus gallus domesticus*) manure (133 μg kg^{-1} for broilers and 670 μg kg^{-1} for roosters) that was used for cattle (*Bos taurus*) feed, and have suggested that the hormone concentrations approached or surpassed levels that delay puberty, enlarge clitorises (Shemesh and Shore, 1993), disrupt estrous cycles, and lead to premature udder development in heifers (Shore et al., 1988).

When hormones enter the environment from manures, wastewater, or other sources, they undergo various fate and transport processes. Natural hormone degradation rates are rapid. For example, Fan et al. (2007b) reported that the first-order degradation rates of E2 and T in soil were about 0.001 h^{-1} and 0.01 h^{-1}, respectively. In addition, degradation of hormones readily occurs under aerobic but not anaerobic conditions (Fan et al., 2007b; Joss et al., 2004), is facilitated under warmer temperatures (Hemmings and Hartel, 2006; Jacobsen et al., 2005), and is correlated to soil water content (Colucci et al., 2001). Bioavailability also plays a large role in the rate of hormone degradation (Fan et al., 2007b; Jacobsen et al., 2005). Abiotic mechanisms, such as photodegradation, can also degrade steroidal hormones but are insignificant in soils (Fan et al., 2007a; Jürgens et al., 2002; Mansell et al., 2004).

The primary sorption domain of steroidal hormones is organic, and partitioning is consistent with the hydrophobic process; that is, there is a linear relation between the Log_{10} octanol-water partitioning coefficient (Log K_{ow}) and Log K_{oc} (Log K_{oc} = Log_{10} [K_d /{(organic carbon fraction)/100}]; where K_d is the linear partitioning coefficient [L kg^{-1}]) (Lee et al., 2003). Sorption has also been shown to have a kinetic component that is related to the soil organic content (Casey et al., 2005; Das et al., 2004; Holthaus et al., 2002). Nonhydrophobic sorption interactions of E2 may also occur, where it is hypothesized that the phenolic group of E2 can interact with humic acids or mineral surfaces via hydrogen and covalent bonding (Yu et al., 2004). In addition, Fan et al. (2007a; 2007b) used extraction methods (Kaplan and Kaplan, 1982) and demonstrated that ^{14}C-labeled E2 and T will become irreversibly sorbed into various organic matter (OM) fractions (e.g., humic acid, fulvic acid, and humin).

These aforementioned studies indicate that there should be limited hormone detections in the environment caused by the labile nature of these compounds; nonetheless, natural hormones are consistently detected at parts-per-trillion levels (Barber et al., 2000; Kolpin et al., 2002). 17β-Estradiol (0.1-3.5 μg L^{-1}) and T (0.01–1.8 μg L^{-1}) have been detected in runoff and receiving surface waters as a result of field manure application or other agricultural practices (Busheé et al., 1998; Finlay-Moore et al., 2000; Nichols et al., 1997; Nichols et al., 1998). Natural hormones (E2, 0.006–0.160 μg L^{-1}; estrone, 0.004–0.600 μg L^{-1}; T, 0.002–0.090 μg L^{-1}) have also been measured in subsurface waters in proximity to intensive livestock production (Fine et al., 2003; Herman and Mills, 2003; Kolodziej et al., 2004; Peterson et al., 2005; Wicks et al., 2004). In the vadose zone beneath a manure-fertilized corn (*Zea mays*) field, E2 detections in lysimeter leachate (installed at 15-, 46-, and 91-cm depths) were frequent, for whenever leachate was collected, there were detections (min. detect., 0.023 μg L^{-1} at 91-cm depth; max detect., 0.160 μg L^{-1} at 46-cm depth) (Herman and Mills, 2003).

There exists a bifurcation in our understanding of the fate and transport of natural hormones, where laboratory results indicate very limited persistence and mobility, and field observations suggest moderate persistence and transport. Extended persistence of hormones in the field and processes such as facilitated transport (Schiffer et al., 2004) can result in greater-than-expected detections. The objective of this study was to bridge an understanding between field and laboratory studies on hormones using a controlled large-scale field lysimeter experiment. We hypothesize that the significant factors that explain E2 and T distributions in a field soil can be used to identify fate and transport process discerned from laboratory studies.

MATERIALS AND METHODS

The transport of pentafluorobenzoic acid (PFBA, a conservative nonsorbing tracer [Jaynes, 1994]), E2, and T were simultaneously measured in a lysimeter described by Montgomery et al. (1987). This box-shaped lysimeter was constructed in 1980. The lysimeter has a cross-sectional area of 2.4 m × 2.4 m, where the sides

of the lysimeter start 0.3 m below the soil surface and extend for a total of 2.3 m, which allows for surface cultivation. A slotted polyvinyl chloride drain was installed horizontally in a gravel envelope at the bottom of the lysimeter to allow drainage. The lysimeter drainage can be collected from a polyvinyl chloride pipe in an access manhole belowground. This lysimeter contains a reconstructed profile of the surrounding soil, a Hecla loamy fine sand (frigid Oxyaquic Hapludoll). The profile was reconstructed in less than or equal to 0.5-m increments and was packed to densities similar to the profile's undisturbed condition (Table 1). The soil profile is relatively uniform in texture, dominated by sands. The upper 1.02 m of the profile was 84.5% to 91.8% sand, 4.6% to 7.8% silt, and 3.3% to 7.7% clay. The lower 1.27 m of the profile was less variable and had 91.7% sand, 5.3% silt, and 3.0% clay. Although this reconstructed soil has a dissimilar structure compared with the undisturbed soil adjacent to the lysimeter, structure development was evident after 20 years.

Some measured properties of the lysimeter are presented in Table 1. This lysimeter is located at the Oakes Irrigation Field Trials, 7 km south of Oakes, ND. Numerous studies have been conducted on this particular lysimeter, including a study of ^{15}N-nitrogen fate and transport (Prunty and Greenland, 1997; Prunty et al., 2000) and a crop-growth curve determination study (Steele et al., 1996). Since construction, no domestic animal manures have been applied to this lysimeter.

For this study, soil was carefully excavated from above the top edges of the lysimeters, and a plywood frame covered with plastic sheeting

TABLE 1

Some soil physicochemical characteristics of the soil cores taken from the lysimeter and the concentrations of the measured chemicals through depth

Depth, m	Water content, $m^3\ m^{-3}$	Bulk density, $Mg\ m^{-3}$	Porosity, $m^3\ m^{-3}$	CEC, $cmol^{(c)}\ kg^{-1}$	OM, %	E2 concentration, $\mu g\ kg^{-1}$	T concentration, $\mu g\ kg^{-1}$	PFBA concentration, mM
Core 1								
0.00–0.08	0.18	1.43	0.46	13.00	2.41	78.71	17.24	0.00000
0.08–0.23	0.18	1.39	0.48	13.00	2.41	164.57	21.94	0.00022
0.23–0.38	0.16	1.34	0.49	9.10	1.14	80.42	10.91	0.00042
0.38–0.53	0.17	1.34	0.49	9.10	1.14	115.01	15.13	0.00019
0.53–0.69	0.19	1.40	0.47	6.00	0.33	187.69	39.51	0.00000
0.69–0.84	0.22	1.46	0.45	6.00	0.33	227.29	37.88	0.00030
0.84–0.99	0.18	1.54	0.42	5.00	0.13	75.83	35.00	0.00005
0.99–1.14	0.14	1.61	0.39	5.00	0.13	88.17	9.70	0.00023
1.14–1.30	0.14	1.61	0.39	4.80	0.16	22.93	30.95	0.00016
1.30–1.45	0.15	1.61	0.39	4.80	0.16	34.29	34.29	0.00006
1.45–1.60	0.12	1.61	0.39	4.60	0.33	20.79	6.16	0.00011
1.60–1.75	0.16	1.61	0.39	4.60	0.33	19.09	4.03	0.00041
1.75–1.91	0.30	1.61	0.39	4.60	0.33	45.58	11.39	0.00011
1.91–2.06	0.37	1.61	0.39	4.60	0.33	45.50	16.25	0.00032
Core 2								
0.00–0.08	0.20	1.43	0.46	13.00	2.41	19.70	9.85	0.00007
0.08–0.23	0.18	1.39	0.48	13.00	2.41	18.73	10.64	0.00006
0.23–0.38	0.16	1.34	0.49	9.10	1.14	13.88	10.14	0.00020
0.38–0.53	0.16	1.34	0.49	9.10	1.14	15.48	8.93	0.00019
0.53–0.69	0.18	1.40	0.47	6.00	0.33	42.71	20.20	0.00035
0.69–0.84	0.21	1.46	0.45	6.00	0.33	20.40	5.40	0.00054
0.84–0.99	0.13	1.54	0.42	5.00	0.13	24.05	9.02	0.00004
0.99–1.14	0.13	1.61	0.39	5.00	0.13	31.70	6.85	0.00058
1.14–1.30	0.14	1.61	0.39	4.80	0.16	25.39	6.62	0.00011
1.30–1.45	0.16	1.61	0.39	4.80	0.16	–	–	–
1.45–1.60	0.17	1.61	0.39	4.60	0.33	9.77	4.46	0.00037
1.60–1.75	0.17	1.61	0.39	4.60	0.33	6.37	3.54	0.00013
1.75–1.91	0.19	1.61	0.39	4.60	0.33	19.86	7.32	0.00017
1.91–2.06	0.33	1.61	0.39	4.60	0.33	10.45	6.62	0.00005

was built and attached. This extended the sides of the lysimeter above the soil surface and enabled the ponding of water. Initially, a water tank was connected to the outlet drain at the bottom of the lysimeter to saturate the gravel envelope and facilitate near steady-state conditions. Approximately 1000 L of water (from a local surface water irrigation supply) was introduced into the bottom of the lysimeter in this manner in 1 day (September 5, 2001), but after 5 days, very little additional water entered the lysimeter. On September 11, 2001, the outlet drain was closed, and water was applied at the surface of the lysimeter. The water supply hose was attached to a float valve so that once the lysimeter was ponded, a constant hydraulic head of 7.5 cm was maintained. Water was applied in this fashion until September 14, 2001, when the bottom drain outlet valve was opened, and flow through the lysimeter was established. The drainage outlet valve was adjusted so that ponding of 7.5 cm could be maintained at the surface.

Drainage was measured in a vessel that automatically emptied when 20.6 L of drainage had been collected. The level of water in the drainage vessel was measured and logged every minute using a pressure transducer and a Campbell Scientific CR23X datalogger (Campbell Scientific, Inc, Logan, UT). The transducer in the drainage collection vessel was calibrated so that the volume of drainage could be calculated from the logged transducer voltage.

After 3 days of steady-state water transfer, the E2, T, and PFBA solutions were applied to the lysimeter surface. Before application, most of the ponded water at the surface was allowed to infiltrate. When 1 cm of water was at the soil surface, the PFBA and hormone solutions were applied using a galvanized metal sprinkler watering can to achieve a uniform application. The PFBA (77.47 g) was applied in a 5-L solution, and the E2 (0.2 g) and T (1.25 g) were applied in a 20-L solution with an ethanol cosolvent. The resulting concentrations of PFBA, E2, and T in the ponded water were 0.94 g L^{-1}, 2.42 mg L^{-1}, and 15.13 mg L^{-1}, respectively, and the ethanol cosolvent was approximately 0.13% of the total solution. Less than 0.5% ethanol cosolvent has been shown not to affect sorption of organic pollutants in soil (Wauchope and Koskinen, 1983). After the tracer and hormone solution application, which took about 2 min, the surface ponding of 7.5 cm and steady water flow were reestablished.

Samples for chemical analysis were taken from the lysimeter leachate with an ISCO (Teledyne ISCO, Inc; Lincoln, NE) automated wastewater sampler. Sample collection intervals ranged from 70 min (for the first 44 samples) to 120 min (for samples 45–138). A 0.75-L water collection device was built and attached to the lysimeter drainage pipe. At specified time increments, the water in the sampling device was extracted by the ISCO sampler and placed into a sample bottle. After the sample cycle was complete, all samples in the ISCO fraction collector were subsampled and stored in a refrigerator and analyzed within 48 h for hormones and 15 days for PFBA.

The total duration of the transport portion of the experiment was 13 days. At the end of the transport experiment, water flow was stopped, two soil cores were taken from the lysimeter using a 2.54-cm diameter soil probe. Soil cores were taken to a depth of 2.3 m and in 0.15-m increments. The soil water was extracted from the probe samples to determine the tracer and the water-extractable hormone distribution through the soil profile. In addition, water contents were determined from subsamples of the probe cores. Each of the 0.15-m core increments was homogenized, and a 20-g subsample was placed in a 0.25-L Erlenmeyer flask. Distilled water was added to this flask in a ratio of 1:2 of soil–distilled water and then shaken for 5 min on a wrist action shaker. After shaking, the slurry was allowed to settle for 5 min, after which the supernatant was decanted through no. 40 filter paper into a scintillation vial. This liquid extract and lysimeter effluent samples were also centrifuged at 10,000*g* for 5 min to remove any suspended particulates.

Both the soil extract solution and the lysimeter effluent samples were analyzed for PFBA. The method to determine the PFBA was identical to the high-performance liquid chromatography (HPLC) method described by Lee and Casey (2005). A Beckman (Fullerton, CA) 110B HPLC pump was used with a 210 A injector with a 50-μL sample loop. The separation column was an Allsphere SAX 5-μm packing (250 × 4.6 mm; Alltech, Deerfield, IL), and the detector was a Hewlett-Packard 1050 (Palo Alto, CA) ultraviolet detector. The mobile phase was a 1:1 mixture of acetonitrile and a solution of 20 mmol L^{-1} KH_2PO_4, adjusted to a pH of 3.5 using dilute H_3PO_4. The flow rate was 2 mL min^{-1}, and the detection wavelength was 205 nm.

The fraction-collected effluent samples and soil core extracts were also analyzed for the water-soluble forms of free E2 and T using Cayman Chemical enzyme immunoassay (EIA) kits (Ann Arbor, MI). The EIA analysis is a spectrophotometric measure of a sample vial in which free E2 or T (i.e., the E2 or T to be detected in the lysimeter samples) competes with tracer E2 or T for limited binding sites on a hormone-specific antiserum. The spectrophotometric measure of absorbance is then inversely proportional to free E2 or T in solution, which is then related to a concentration using a standard curve. The intra-assay coefficient of variation (CV) for the E2 and T standard curves increases for the lower concentrations. This CV was calculated using the following log-linear relationship based on the manufacturer's reported precision:

$$CV = -m \times \ln[C] + b \quad (1)$$

where C is concentration (ng L^{-1}), $-m$ was 36.40 and 4.77, and b was 29.39 and 28.88 for E2 and T, respectively. Eq.(1) was used to calculate the error bars of the measured hormone concentrations. The EIA detection limits for E2 and T were 0.019 μg L^{-1} and 0.006 μg L^{-1}, respectively, according to the manufacturer's specifications. In addition, the hormone-specific antiserum may bind hormone conjugates (e.g., glucuronides) and metabolites. For the E2 kit, specificities were reported for E2 (100%), estradiol-3-glucuronide (17%), estrone (4%), estriol (0.47%), and other minor compounds (<0.1%). For the T kit, specificities were reported for T (100%), 5α-dihydrotestosterone (21%), 11-ketotestosterone (12.4%), 5β-dihydrotestosterone (10%), androstenedione (3.6%), 11-hydroxytestosterone (1.2%), and other compounds (<0.4%).

Transport of PFBA tracer was assumed to be conservative with no sorption, and a steady-state convective-dispersive equation was used to model its transport:

$$\frac{\partial C}{\partial t} = D\frac{\partial^2 C}{\partial x^2} - v\frac{\partial C}{\partial x} \quad (2)$$

where t is time (h), D is dispersion ($m^2\ h^{-1}$), x is depth (m), and v is pore water velocity (m h^{-1}). The model, CXTFIT (Toride et al., 1999), provides an analytical solution to Eq.(2) and can be used inversely to estimate transport parameters. This model was used to estimate the parameter D.

The resident and effluent hormone and tracer data were first evaluated using a stepwise regression analysis, the results of which were used to develop a standard least-squares regression model. The statistical program JMP (JMP, 2004) was used for this analysis. For the stepwise regression, all measured variables (e.g., depth, water content, texture) and all interactions (e.g., cation exchange capacity [CEC] × OM) were included as possible factors that may explain concentration variations. The stepwise regression process iteratively selected only the most significant factors that explained concentration variations at $P = 0.25$. Iterative selection of factors continued until there were no more improvements in the model's explanation of the concentration variations. After the most significant factors were found using this stepwise analysis, a standard least-squares regression model was then constructed and fit to the data using these most significant factors. This standard least-squares model would produce a whole model account of the variation around the mean for the compound of interest and an analysis of variance. In addition, distributions of the residual errors were determined to verify that there were no violations of normality assumptions.

RESULTS AND DISCUSSION

The average drainage rate established through the lysimeter was 0.021 m h^{-1}. The CV of the drainage rate for the duration of this experiment (about 13 days) was 12.9%, which was low given the size and time span of this experiment. Variations in volumetric water content (θ_v) and porosity (ϕ) through depth are shown in Table 1 for the two soil cores. Porosity was calculated assuming a particle density of 2.65 Mg m^{-3}. The soil at the bottom of the lysimeter was near saturation as a result of saturating from the bottom up and from differences in the textures between the soil and gravel pack, that is, the finer textured soil needs to be near saturation before entering the coarser gravel pack. Integrating θ_v through depth and multiplying by cross-sectional area of the lysimeter resulted in the calculated lysimeter pore volume of 3096 L. This calculated pore volume corresponded to an average θ_v of 0.23 $m^3\ m^{-3}$, and an average pore water velocity of 0.093 m h^{-1}. This information was used to normalize the PFBA breakthrough curve (Fig. 1). Equation (2) provided an excellent fit to the data (Fig. 1; coefficient of determination, 0.98), and the

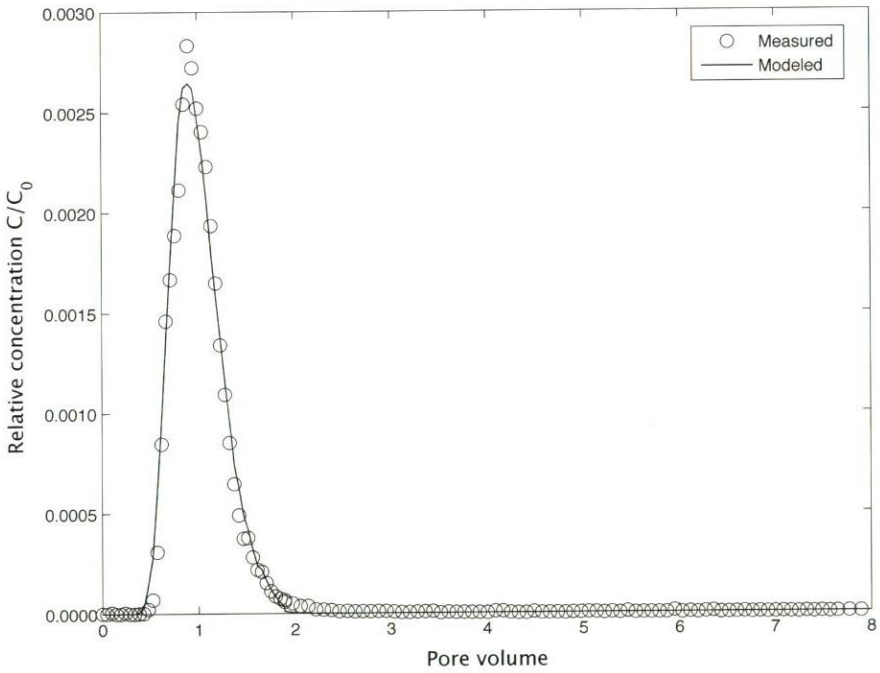

Fig. 1. Pentafluorobenzoic acid effluent concentrations and modeled concentrations versus lysimeter pore volume.

inverse estimate of D was 0.0078 m^2 h^{-1} (dispersivity [$\lambda = D/v$] = 0.84 m; Peclet number [$P = v \times L/D = 27.4$). The total mass balance of the PFBA collected in the effluent was calculated to be 100.6%. There was no evidence of significant preferential transport, retardation, or degradation of PFBA.

The concentrations of E2 and T applied to the lysimeter were high, but relevant based on manure application rates and reported hormone activities in manures. The University of Minnesota's manure management guidelines (Schmitt, 1999) would recommend a raw manure application rate of 2.4 kg m^{-2} to supply the nutrient needs for the lysimeter plot for corn. In addition, a survey by Lorenzen et al. (2004) found estrogenic activity levels of 5965 μg kg^{-1} (E2 equivalents per dry weight) in manure from finishing pigs and androgenic activity levels of 1737 μg kg^{-1} (T equivalents per dry weight) in manure from pregnant dairy cows. Based on the previously mentioned recommended manure application rates and E2 and T activities, it was calculated that a total mass of 82,500 μg E2 and 24,000 μg T could have been applied to this lysimeter in 13.8 kg of manure. The rate of E2 and T applied in this study was 2.43 times and 52.06 times greater, respectively.

RESIDENT CONCENTRATIONS

Using the mean water-extractable concentrations of E2 and T at each sample-depth interval, the mass resident recovery for E2 and T was calculated to be 0.46% (±0.01%) and 0.02% (±0.01%), respectively. The low mass recoveries were likely a result of nonextractable hormone still bound to soil and/or hormone transformations into metabolites or mineralization into carbon dioxide. Fan et al. (2007b) demonstrated that after 5 days of incubation in a similar sandy soil, 70% to 73% of E2 and 19% to 25% of T were nonextractable and were associated with humic substances. In addition, only parent hormones, E2 and T, could be determined using the EIA kits, therefore, the transformation products of E2 and T could not be determined, which would exasperate the mass recovery error. Several first-order degradation/transformation rate constants (μ) for E2 (0.0006 h^{-1} [Fan et al., 2007b]–0.252 h^{-1} [Layton et al., 2000]) and T (0.012 h^{-1} [Fan et al., 2007b]–0.912 h^{-1} [Layton et al., 2000]) were used to calculate predicted hormone concentrations

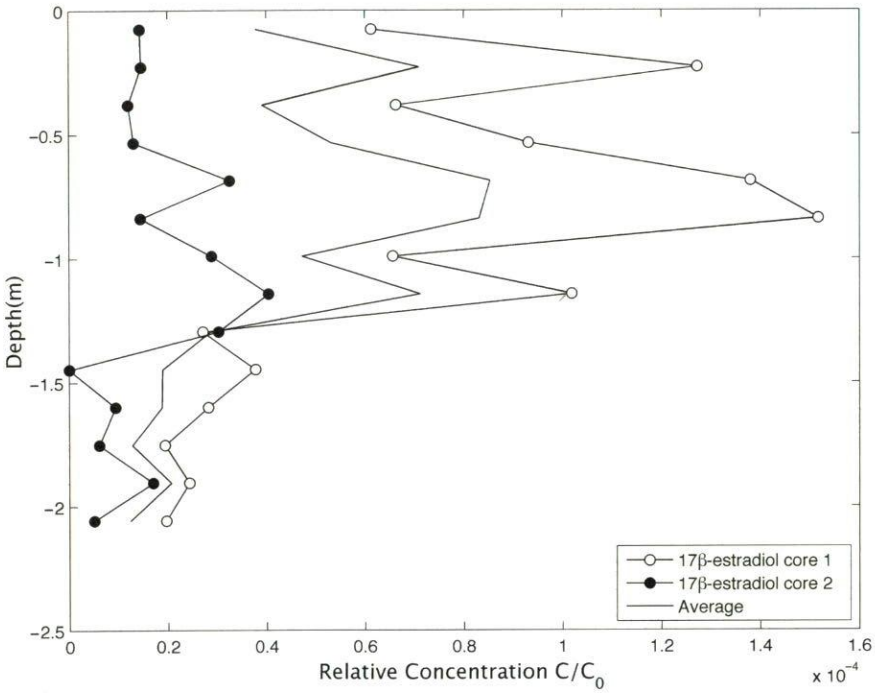

Fig. 2. Measured relative resident concentrations of the water-extractable E2 through depth from the two soil cores and the mean of the two cores.

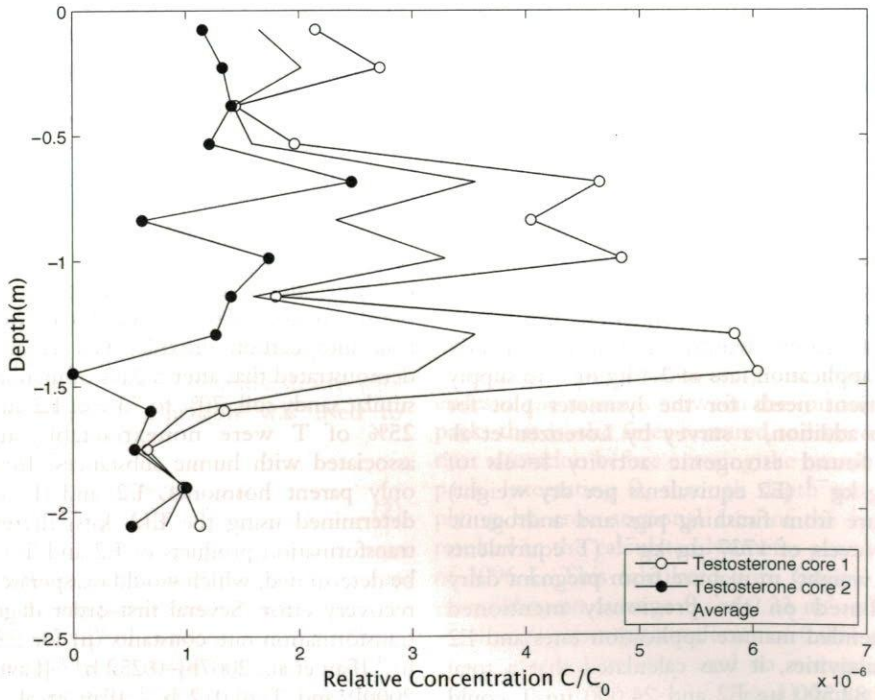

Fig. 3. Measured relative resident concentrations of the water-extractable T through depth from the two soil cores and the mean of the two cores.

TABLE 2

Standard least-squares regression model estimates and summary statistics for the factors used to predict variation in E2 and T concentrations (n = 28)

Factors	Estimate	SE	*t* ratio	*P*
E2				
Intercept	−3579.63	1592.88	−2.25	0.0361
Depth	3.29	1.93	1.70	0.1041
Water content	10,859.30	2193.97	4.95	<.0001
Porosity	8998.95	3082.61	2.92	0.0085
%Sat	−4265.68	711.35	−6.00	<.0001
Porosity × Depth	140.69	44.55	3.16	0.0049
%Sat × Depth	46.81	9.33	5.02	<.0001
Porosity × water content	284,278.87	70,963.08	4.01	0.0007
Testosterone				
Intercept	−51.21	177.11	−0.29	0.7752
Water content	1686.46	630.67	2.67	0.0139
%Sat	−692.15	233.68	−2.96	0.0072
CEC	60.81	34.91	1.74	0.0955
%OM	−397.57	159.20	−2.50	0.0205
CEC × %OM	30.41	12.51	2.43	0.0237

assuming no sorption. The higher μ values that were considered overpredicted the E2 and T concentrations, whereas the lower μ values underpredicted concentrations. In addition, sorption occurs at the same time as degradation and would contribute to the lower concentrations.

The redistribution of water-extractable E2 (Fig. 2) and T (Fig. 3) varied through the soil profile and seemed to correspond to different soil/water factors. For E2, the following standard least-squares regression model was constructed, and coefficients were determined:

$$\begin{aligned} E2_predicted = & -3579.63 \\ & + 3.29 \times Depth \\ & + 10,859.3 \times \theta_v + 8998.95 \\ & \times \phi - 4265.68 \times \%\,Sat \\ & + (\phi - 0.43) \times [(Depth \\ & - 106.68) \times 140.69] \\ & + (\%\,Sat - 0.49) \times [(Depth \\ & - 106.68) \times 46.81] + (\phi \\ & - 0.43) \times [(\theta_v - 0.18) \times 28] \quad (3) \end{aligned}$$

Eq.(3) accounted for 80% of the variation around the mean (R^2) of resident E2 concentrations. The remaining residual error was estimated to have an S.D. (root mean square error) of 222.98. The computed *F* ratio (model mean square divided by the mean square for error) from the analysis of variance of the whole model was 11.07, which was highly significant ($P < 0.0001$). In addition, distributions of the residual errors indicated that normality assumptions were not violated. For T profile distributions, the following standard least-squares model was constructed, and coefficients were determined:

$$\begin{aligned} T_predicted = & -51.21 + 1686.46 \times \theta_v \\ & - 692.15 \times \%Sat + 60.81 \\ & \times CEC - 397.57 \times OM \\ & + (CEC - 6.73) \times [(OM \\ & - 0.69) \times 30.41] \quad (4) \end{aligned}$$

The R^2 of Eq.(4) to predict T concentrations was 47%, and the root mean square error was 81.88. The computed *F* ratio from the analysis of variance was 3.94, which was significant ($P < 0.0105$). The distributions of the residual errors indicated that normality assumptions were appropriate. For the PFBA, stepwise regression analysis indicated that there were no significant factors that could explain its resident concentrations, and therefore least-squares regression analysis was not pursued.

The mean water-extractable resident concentration of T in the soil profile was much lower compared with E2. The difference in T and E2 concentrations may reflect T's higher degradation rate under both aerobic and anaerobic conditions compared with E2 (Fan et al., 2007b). Table 2 shows the summary statistics for the factors that contributed to the standard least-squares regressions, Eq.(3) and Eq.(4). Higher E2 and T concentrations corresponded significantly with higher θ_v, which may indicate greater persistence of E2 and T with higher water contents. Several studies have shown that oxygen status in soils is related to hormone persistence, where low oxygen or anaerobic conditions increase persistence (D'Ascenzo et al., 2003; Fan et al., 2007b; Hemmings and Hartel, 2006; Shappell et al., 2007).

Both E2 and T were significantly related in an inverse manner to %saturation (%Sat) (Table 1), which was likely a result of the high θ_v at the bottom of the lysimeter, corresponding to low E2 and T concentrations. It was expected for profile concentrations of the hydrophobic E2 and T to decrease with depth, which also corresponded to increasing θ_v. The %Sat × Depth interaction indicated a significant proportional relationship for E2 but not T (Table 2). This significant factor (%Sat × Depth) would further indicate the greater persistence of E2 under low oxygen conditions. This factor may also indicate that T continued to degrade under

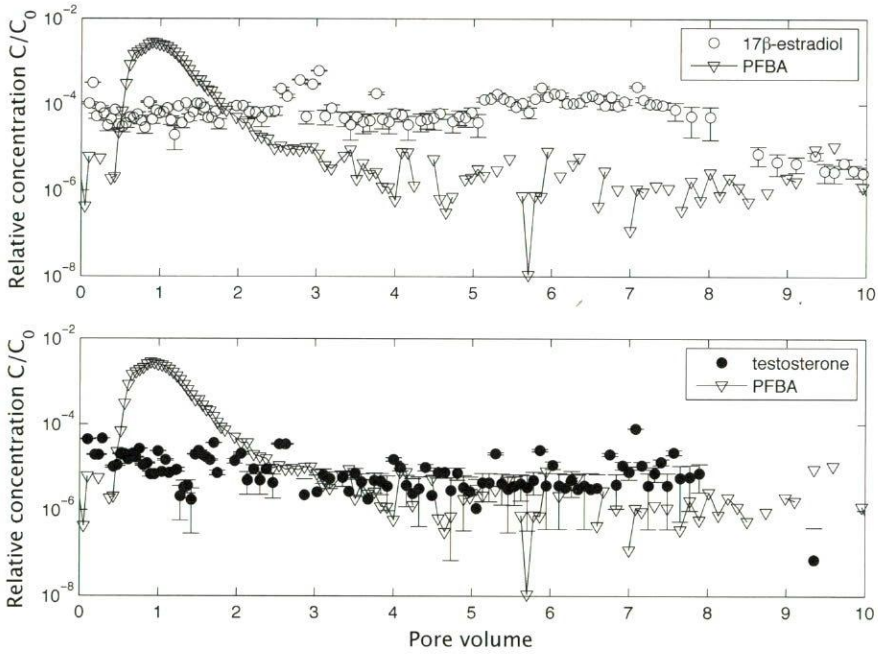

Fig. 4. Measured relative lysimeter drainage effluent concentrations of PFBA, E2, and T versus lysimeter pore volume. The error bars were calculated from the coefficient of variation in the concentration measurement.

the low oxygen levels. Fan et al. (2007b) studied the degradation of E2 and T under aerobic and anaerobic conditions and found T degraded at higher rates under aerobic conditions and continued to degrade under anaerobic conditions. The continued degradation of T under low oxygen conditions may explain why %Sat × Depth was not significant for T.

The OM content was significant in explaining distributions of water-extractable T but not E2 in the lysimeter profile (Table 2). This result may indicate that the predominant factor that controls water-extractable T was sorption rather than persistence because the hydrophobic T would bind to OM.

None of the factors (e.g., θ_v, OM) were significant in explaining the resident concentrations of PFBA. This result was expected because PFBA was nonsorbing and conservative, and θ_v or OM would not affect PFBA fate under steady-state transport. This result also supported the statistical interpretation of the E2 and T because these factors (θ_v and OM) were significant in explaining the distribution of these labile compounds.

EFFLUENT CONCENTRATIONS

In the lysimeter effluent, both E2 and T were detected immediately at the onset of the experiment and throughout the duration of the experiment (Fig. 4). Both E2 and T concentrations in the effluent were determined to be log-normally distributed. The log-transformed mean and S.D. for E2 were 0.066 μg L^{-1} and 0.002 μg L^{-1}, respectively. The range of detections for E2 was 0.012 to 0.525 μg L^{-1}. For T, the log-transformed mean and S.D. were 0.045 μg L^{-1} and 0.003 μg L^{-1}, respectively. The range of detections for T was 0 to 0.050 μg L^{-1}. The mass of E2 and T recovered in the effluent was 1.3% (±0.15%) and 0.2% (±0.02%), respectively. Relative to the input concentration, the T detection concentrations were much lower compared with the E2, which would reflect T's greater degradation rate and perhaps E2's persistence under low oxygen, as explained previously (Fan et al., 2007b; Jacobsen et al., 2005; Layton et al., 2000).

Travel times of E2 and T through the lysimeter (t_r) were calculated using measured fluxes and K_d values from literature, assuming advective transport, no dispersion, and no degradation [$t_r = (1 + \rho_b \times K_d/\theta_v) \times L \times \theta_v/J_l$; where L is the travel length, θ_v is water content, and J_l is the Darcian flux]. The K_d value of 3.5 L kg^{-1} for E2 in an EPA1 sand sediment (Lee et al., 2003) and a K_d value of 0.5 L kg^{-1} for T in sand (Casey et al., 2004) resulted in the

shortest calculated breakthrough times of 620 h and 108 h, respectively. The actual E2 and T effluent detections were earlier than these calculated times (Fig. 4), which could have resulted from (i) the presence of antecedent hormones, (ii) E2 and T analytical nonspecificity, and/or (iii) facilitated transport.

Although no domestic animal manure had been applied to these lysimeters, wildlife or other unknown sources may have contributed hormones to the soil. The antecedent hormones would have had to be persistent and at large quantities, which was not likely from wildlife. Finlay-Moore et al. (2000) measured background E2 concentrations of 0.055 $\mu g\ kg^{-1}$ in the surface 2.5-cm of soil, which was 116 times lower than the lowest resident E2 concentration (6.37 $\mu g\ kg^{-1}$) and 4127 times lower than the maximum resident concentration (227.29 $\mu g\ kg^{-1}$) measured in this study. The background T concentration Finlay-Moore et al. (2000) measured was 0.03 $\mu g\ kg^{-1}$, which was 118 times lower than the lowest resident T concentration (3.54 $\mu g\ kg^{-1}$) and 1317 times lower than the highest T concentration (39.51 $\mu g\ kg^{-1}$) measured in this study. In addition, the plot that Finlay-Moore et al. (2000) used had received manure historically. The effluent concentrations from the current study were similar to lysimeter concentrations from fields where manure had been applied (Herman and Mills, 2003), and also similar to runoff concentrations from field where manure was applied (Busheé et al., 1998). Although no manure had been applied to the lysimeter and other reported values would indicate minimal antecedent hormones, it nonetheless cannot be eliminated as an explanation because no *a priori* measurements were made.

The EIA kits used to measure E2 and T can also cross-react with estrogenic and androgenic conjugates or metabolites. These metabolites and conjugates may be more polar than the parent hormones and may transport through the soil more readily. However, arrival of the metabolites or conjugates in the effluent would still not be expected before PFBA (Fig. 4).

A more likely explanation for the earlier arrival of E2 and T was facilitated transport. Although the PFBA breakthrough curve indicated that there was no physical preferential transport (Fig. 1), a closer examination of Figure 4 indicated that small amounts of PFBA arrived early in the column effluent, and at the same time of E2 and T. The E2 and T may have been transport with soil colloids (Bradford et al., 2003), where E2 and T applied to the soil surface would partition rapidly to soluble colloidal OM, which would be transported rapidly through depth (Schiffer et al., 2004). Effluent samples were centrifuged before HPLC analysis for PFBA to remove suspended particulates that would clog the HPLC column. These particulates may have acted as the colloidal particles that could facilitate E2 and T transport. In addition, least-squares regression analysis indicated that drainage was significant in explaining E2 ($P < 0.0001$) and T ($P < 0.0192$) effluent concentrations. This result may indicate that greater amounts of water flowing through the lysimeter could mobilize hormone-bound colloids and result in more effluent detections.

CONCLUSIONS

This large-scale field lysimeter study helped to bridge field and laboratory hormone fate and transport studies in that the significant field processes were discerned based on laboratory results. During this study, the steady-state transport in the lysimeter may have been exaggerated compared with average vadose zone transport conditions. Nonetheless, this experiment revealed significant information about the dominant fate and transport processes of E2 and T in the field especially when extreme natural events occur (e.g., flooding, focused recharge events). Results from this study indicated that the most significant factors contributing to the fate and transport of hormones in the field were soil-water status, OM content, and colloidal facilitated transport. High soil saturation percentages increased the persistence of hormones, thus resulting in higher hormone concentrations. In addition, the observed rapid transport of hormones through soil and the effect of drainage on hormone detections indicated that some type of facilitated transport was significant in explaining the E2 and T concentrations. This study also indicated that there is a possibility of antecedent hormones naturally present in the soil, which should be further investigated. For pristine areas and areas that have not received obvious sources of hormones, background concentrations are largely unknown. The fact that we measured appreciable amounts of

hormones in the lysimeter that had not received manure is very significant, even if we did not measure antecedent concentrations.

ACKNOWLEDGMENTS

This research was based upon work supported by the National Science Foundation under Grant no. 0730492. Any opinions, findings, and conclusions or recommendations expressed in this article are those of the authors and do not necessarily reflect the views of the National Science Foundation. The authors thank Mr Nathan E. Derby, Mrs Barbara K. Magelky, and Mrs Colleen M. Pfaff for their tremendous contributions.

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