Environ. Sci. Technol. **2008,** *42,* 3570–3574

Degradation of Synthetic Androgens 17α- and 17β-Trenbolone and Trendione in Agricultural Soils

BUSHRA KHAN, LINDA S. LEE,* AND STEPHEN A. SASSMAN

Department of Agronomy, Purdue University, West Lafayette Indiana 47907-2054

Received October 24, 2007. Revised manuscript received February 12, 2008. Accepted February 25, 2008.

17β-trenbolone acetate (TBA) is a synthetic androgenic steroid hormone administered as a subcutaneous implant for growth promotion in beef cattle. TBA is converted metabolically to primarily 17α-trenbolone and trendione, and excreted in manure from implanted cattle. To predict the persistence of synthetic androgens once land-applied, aerobic degradation rates in two contrasting agricultural soil types (clay loam and a sandy soil) of both trenbolone isomers (17α and 17β) and their primary metabolite trendione were measured and isomer interconversion was assessed. The impact of manure application was also evaluated in the clay loam soil. A pseudo first-order exponential decay model was derived assuming irreversible transformation and no impact of sorption on availability for degradation. The model generally resulted in good fits to the data. Both isomers degraded to trendione in a similar manner with half-lives ($t_{½}$) on the order of a few hours to 0.5 days at applied concentrations of ≤1 mg/kg. Similar degradation rates were observed in the presence and absence of manure applied at rates typical for land-application of cattle manure. Trenbolone degradation was concentration-dependent with degradation rates decreasing with increasing applied concentrations. Trendione, whether applied directly or produced from trenbolone, persisted longer than trenbolone with $t_{½}$ values of 1 to 4 days. A small amount (1.5%) of conversion of trendione back to 17β-trenbolone was observed during aerobic incubation regardless of the applied concentration. A small amount of 17α-isomer also converted back to 17β-trenbolone, presumably through trendione. In autoclaved soils, no degradation of 17α- or 17β-trenbolone was observed during the first 3 days, and trendione degradation was relatively small compared to a microbially active soil.

Introduction

Anabolic agents used for growth promotion include endogenous hormones like estradiol, testosterone, or progesterone, and synthetic substances like zeranol, trenbolone acetate (17β-acetohydroxyestra-4,9,11-trien-3-one, TBA), or melengestrol acetate (MGA). TBA is a synthetic androgenic steroid hormone used in beef cattle as a growth promoter in several meat-exporting countries. It is administered as a subcutaneous implant either alone or in combination with an estrogenic compound. Over 90% of the beef cattle raised in the U.S. receive one or more implants during their growth (*1*). TBA is hydrolyzed to active 17β-trenbolone (17β-hydroxyestra-4,9,11-trien-3-one) followed by oxidation to trendione (17β-hydroxyestra-4,9,11-trien-3,17-one) and then reduction to 17α-trenbolone (17α -hydroxyestra-4,9,11-trien-3-one) with the last two steps hypothesized as reversible (*2*) (Figure 1). Cattle excrete primarily 17α-trenbolone with only small amounts of 17β-trenbolone and trendione (*2*). Using enzyme immunoassays, Schiffer et al. (*3*) reported levels up to 75 μg/kg of 17α-trenbolone and up to 5 μg/kg of 17β-trenbolone and trendione in beef dung. They also estimated half-lives ($t_{½}$) for 17α- and 17β-trenbolone of 267 and 257 days, respectively, in liquid manures where conditions are typically anaerobic.

Manure from animal production farms is typically land-applied as a fertilizer, thus one route of hormone entry into the environment. Androgenic hormones including trenbolone isomers and their metabolites have been detected in water bodies receiving cattle feedlot effluents (*4, 5*) which has given rise to concerns regarding their impact on aquatic fauna and even terrestrial organisms. Lorenzen et al. (*6*) found that fecal pats from TBA-implanted cattle exhibit significant estrogen- and androgen-receptor gene transcription activities. Although very little is known regarding the effect of androgenic hormones at the levels and mixtures likely to be found in the environment, several controlled laboratory studies have clearly shown that TBA metabolites can negatively impact various biological processes (*7*). 17β-trenbolone at exposures ≥40 ng/L caused a reduction in female plasma vitellogenin production in Japanese freshwater medaka fish (*8, 9*), and likewise, in zebrafish and fathead minnows at ≥50 ng/L (*8, 10*). Continuous exposure of 50 ng/L 17β-trenbolone to zebrafish during their early development resulted in an all male population (*9*). Similar exposures to female fathead minnows resulted in the development of dorsal nuptial tubercles that are consistent with those of breeding males (*10*). Exposure to 17β-trenbolone also caused various types of alterations in the gonads or secondary sexual characteristics of both male and female fish (*8–11*). Based on binding to mammalian androgen receptors (*12*), Jensen et al. (*13*) expected 17α-trenbolone and trendione to be less potent than 17β-trenbolone; however, they observed similar reproductive effects in fathead minnows for both isomers.They hypothesized that either 17α-trenbolone converted to 17β-trenbolone within the fish or that the androgen receptor of the fathead minnow does not have a higher affinity for the 17β isomer, unlike mammalian androgen receptors. Exposure of 17β-

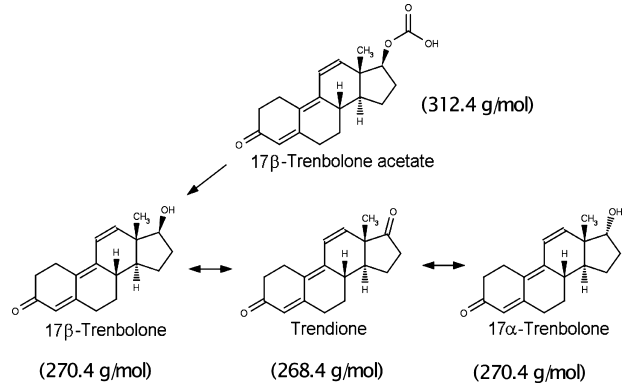

FIGURE 1. Primary path of trenbolone acetate (TBA) metabolism in bile from a 14-month old Friesian heifer collected for 24 h after intravenous dosage with ^{3}H-TBA (*2*). Figure modified from Schiffer et al. (*3*).

* Corresponding author phone: 765-494-8612; fax: 765-496-2926; e-mail: lslee@purdue.edu.

10.1021/es702690p CCC: $40.75
Published on Web 04/16/2008

trenbolone to microorganisms collected from freshwater lake sediment reduced β-glucosidase and leucine-aminopeptidase activity, which leads to a reduction in microbial substrate utilization potential (*14*).

Current trends in the United States reflect a shift from small diversified farms to large-scale intensive and confined breeding and feeding operations (*15*), which in many cases will include a concomitant increase in the rate of manure applied due to the lack of available nearby land. Very little is known on the environmental fate of synthetic androgens and associated metabolites that may be present in beef cattle manures (*7*). In the current study, aerobic degradation rates of 17β-trenbolone, 17α-trenbolone, and trendione, along with metabolite formation and reversibility (interconversion) were measured for different applied concentrations in two distinctly different agricultural soil types. In addition, abiotic versus microbial degradation was assessed for both soils by using both autoclaved-sterilized and nonsterile soils, and the effect of manure amendment was assessed for one soil.

Materials and Methods

Chemicals. 17β-Trenbolone and 17α-trenbolone were obtained from Sigma Chemical (St. Louis, MO) and Hayashi Pure Chemical IND., Ltd. (Osaka, Japan) and stored at 4 °C. Other chemicals used included acetonitrile, methanol, dichloromethane, which are all of >99% purity. Trendione was not available at the start of this study, and thus was synthesized in house (see Supporting Information for details).

Soils. Two soil types were collected at the Purdue Animal Science Research and Education Center (ASREC) (West Lafayette, IN) from the edge of two agricultural fields where effluent and manure are periodically applied. Each soil sample represented a mix of multiple sub samples taken from the surface (top 3−4 in) at least 3–4 ft apart from the sampling area, sieved moist, and stored at 4 °C prior to use. D30 and D36 are clay loams (Drummer soil series) collected from the same field at different times with 2.2 and 2.3% organic carbon (OC) content, respectively, and C32 is a sandy soil (Coloma soil series) with only 0.64% OC (see Table S1 in the Supporting Information for additional soil properties).

Aerobic Soil Microcosms. Soils (~5 g dry wt.) were placed in 40 mL or 120 mL glass vessels with Teflon-lined closures and filter-sterilized deionized water was added to adjust the soil moisture content to field capacity (29 and 6% moisture for the D30 and C32 soils, respectively). Moist soils were preincubated at 22 ± 2 °C for 72 h prior to adding the compound of interest. Hormones were added to soils via ethanol as a carrier solvent (3−5 μL) followed by manual mixing to achieve approximately 0.05, 0.1, 1.0, 7.0, or 10 mg/kg for 17β- or 17α-trenbolone and 0.04, 3.0, or 3.5 mg/kg for trendione. The initial concentration of 0.05 mg/kg was used only for 17α-trenbolone in the D36 soil both with and without the addition of manure from cattle that had been free of TBA implants for 4 months. Manure (0.1 mL, which corresponds to approximately 20 tons/acre) was added immediately prior to hormone addition. Land application rates of manure are based on crop nitrogen needs and the amount of nitrogen in the manure, and typically range from 4 to 70 tons/acre for cattle manure (*16*). The higher hormone concentrations, which are well outside the concentrations expected to be environmentally relevant, were included to optimize detection of metabolite interconversion and additional unknown metabolites. Soil microcosms were incubated at 22 ± 2 °C, and triplicate tubes were periodically sacrificed and extracted twice sequentially with 35 or 40 mL of methanol. Methanol extraction efficiencies ranged from 95 to 105% (see Supporting Information, Figure S1). To confirm that the ethanol carrier did not significantly alter hormone degradation, 17β-trenbolone degradation in soil D30 was also evaluated using dioxane as a carrier, which is a solvent resistant to microbial degradation relative to ethanol (*17*). No significant differences were observed between the two carrier solvents (Supporting Information, Figure S2).

To help differentiate between microbial and abiotic processes, a subset of soil microcosms was autoclaved for 1 h at 103.4 KPa and 121 °C after the initial 3 day preincubation similar to the procedure described by Wolf et al. (*18*). Soil microcosms were autoclaved two more times after incubation times of 2 and 1 days, respectively.

HPLC Analysis. Sample extracts were analyzed using reverse-phase high performance liquid chromatography system (HPLC) (Shimadzu, Columbia, MD) with either UV detection (λ = 350 nm) or mass spectrometry (MS) (Sciex API 3000, Applied Biosystems/MDS, Foster City, CA). The MS parameters were optimized by infusing a solution of the target hormones into the mass spectrometer along with the eluent stream from the HPLC system. Multiple reaction monitoring was used to identify and quantify the trenbolone isomers (precursor ion 271, product ion 199) and trendione (precursor ion 269, product ion 225). Matrix effects are commonly observed with HPLC/MS and are usually accounted for by using an internal standard that is structurally the same as the target analytes. However, no deuterated forms of the target analytes (17β-trenbolone, 17α-trenbolone, and trendione) were available at the time. Therefore, potential matrix effects were assessed by comparing MS responses between standard analyte solutions prepared in methanol and standards prepared in soil extract solution obtained by extracting 5 g of C32 or D30 hormone-free soils with methanol followed by the same procedure used for sample extracts (see Supporting Information). There was no significant difference in instrument response between the analyte standards with and without the soil extract matrix (Supporting Information, Figure S3). Therefore, concentrations in soil were determined using external standards of 17β-trenbolone, 17α-trenbolone, and trendione. The HPLC/MS limit of detection was 0.3 pg for each compound (with a 15 μL injection volume), which allowed detection of hormone concentrations in soil as low a 0.16 μg/kg. See the Supporting Information for additional details on how matrix effects were assessed along with the mobile phase gradients used and associated retention times.

Data Analysis

Degradation rates of the applied compound (k_a, h^{-1}) were determined using a first-order exponential decay model (1), which was derived assuming transformation is irreversible, no impact of sorption on degradation, and no significant microbial growth:

$$C_t = C_0 e^{-k_a t} \quad (1)$$

where t is time (h), C_0 and C_t are the concentrations at t = 0 and time t, respectively, of the applied compound. Likewise, degradation rates of the subsequent metabolites (k_b, h^{-1}) were estimated using a fixed k_a estimated from eq 1, assuming metabolite degradation was also first order, and optimizing for k_b:

$$C_t^* = [C_0 - (C_0 e^{-k_a t})] e^{-k_b t} \quad (2)$$

where C_t* is concentration at time t of the primary metabolite resulting from degradation of the applied compound.

Degradation rates and associated 95% confidence intervals were estimated with nonlinear regression analysis of the exponential decay model (eqs 1 and 2) using Statistical Analysis System (SAS 9.1). Multiple comparisons were conducted using ANOVA at α = 0.05 and paired t tests to assess significant difference across various treatments including comparing hormone degradation rates at the multiple

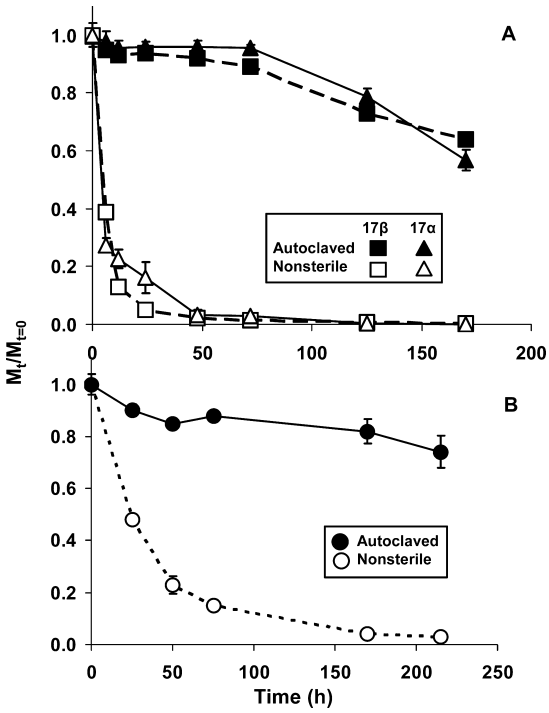

FIGURE 2. Moles over time (M_t) relative to moles applied ($M_{t=0}$) in soil D30 at 22 ± 2 °C that was either autoclaved or left microbially active (nonsterile) for (A) 17α- and 17β-trenbolone (0.1 mg/kg); and (B) trendione (0.04 mg/kg). Error bars represent the standard deviation of triplicate samples.

applied concentrations for a single soil type, across different soils at a single applied hormone concentration, between isomers, and with manure amendment.

Results and Discussion

Microbial versus Abiotic Degradation. No significant loss of 17α- and 17β- trenbolone was observed for 3 days in autoclaved systems for either soil (Figure 2A for soil D30 and Table S2 in Supporting Information for soil C32). The onset of degradation after 3 days is likely due to new microbial growth from microbes inadvertently introduced during hormone addition to the autoclaved soil microcosms. In any case, <3 and <5% of 17α- and 17β-trenbolone remained by day 3 in the nonsterile C32 and D30 soils, respectively. For trendione, concentrations decreased by almost 25% over the 9 day incubation period in the autoclaved soil microcosms (Figure 2B); however, nearly complete disappearance occurred in the nonsterile soil within the same time frame. Therefore, degradation of both trenbolone isomers, as well as trendione, is primarily microbial.

Aerobic Microbial Degradation. The degradation rates (k_a and k_b) and their 95% confidence intervals (CI) are summarized for all soil-treatment combinations in Table 1 along with half-lives ($t_{1/2}$) and coefficients of determination (R^2) reflecting goodness-of-fits. Degradation patterns for 17α- and 17β-trenbolone and trendione, when it was the applied compound, are generally well described by the pseudo first-order model (eq 1) as reflected by high R^2 values (0.85−0.99). Goodness-of-fit of eq 2 to trendione generation from trenbolone and its subsequent degradation is not as good, which is likely due to the dependence on the previously fitted k_a value (i.e., cumulative error); however, the 95% CIs are still relatively small. When trendione was applied directly to soil, some conversion back to 17β-trenbolone was observed, but at less than 1.5 mol % regardless of the initial trendione concentration; therefore, the assumption of no 17α- to 17β-trenbolone interconversion in the derivation of 2 is reasonable.

For a given soil, both trenbolone isomers degraded to trendione at similar rates (Figure 3A) with subsequent degradation of trendione being much slower. No conversion of 17β- to 17α-trenbolone was observed; however, a small amount (<1%) of isomer conversion did occur for 17α- to 17β-trenbolone, presumably through trendione (Figure 1). Although small, this isomer conversion (17α- to 17β-) offers some support to the isomer conversion hypothesized to explain the similar masculinization effects on fish and other wildlife species between the two isomers (*12*). No other quantifiable peaks were noted in the chromatograms.

Degradation rates between the two soil types for a given hormone and hormone concentration were within a factor of 2, which indicates that assuming sorption did not significantly affect degradation (eqs 1 and 2) is reasonable for the conditions of this study. When differences were statistically significant ($M_{t=0}$ = 0.1 mg/kg at the 95% confidence level), the sandy soil (C32) exhibited a slower rate than the higher OC clay loam (D30) even though sorption on D30 is three times greater. This may be due to inherently greater nutrient availability (e.g., C and N) and microbial diversity associated with the higher OC soils. Trendione, which is 5 times more sorptive than trenbolone, did degrade proportionally slower; however, whether this is due to slower desorption rates or a compound-specific microbial factor cannot be determined from this data set.

Rates of 17α- and 17β-trenbolone degradation and subsequent degradation of trendione decreased as the applied hormone concentrations increased, e.g., 10-fold decrease in rates going from 0.1 to 10 mg/kg applied 17β-trenbolone (Table 1, Figure 4). Degradation of trendione when applied directly to the soil was also retarded at higher trendione concentrations (0.04−3 mg/kg) (Figure 4). In traditional Michaelis–Menten microbial enzyme-mediated kinetics, an increase in the velocity of a reaction is expected with increasing concentration. Marcus and Talalay (*19*) also observed slower rates with increasingly higher concentrations in the degradation of testosterone and related androgens with a purified bacteria enzyme β-hydroxysteroid dehydrogenase, but not for 17β-estradiol. They proposed that this inverse trend is best explained by the formation of nonactivated bimolecular complexes (two substrate molecules per active locus of enzyme) (*19*). This concentration-dependence is not an artifact of a limitation to how many moles can be degraded at a given time, which is exemplified for 17β-trenbolone with soil D30 in Supporting Information Figure S4 where the moles lost at given time is larger for the higher applied concentration. Marcus and Talalay (*19*) also show in their pure enzyme studies that the rate of the reaction versus log concentration curve is actually parabolic such that below some critical concentration, degradation rates do follow traditional Michaelis–Menten microbial enzyme-mediated kinetics (increasing rate with increasing concentration). In any case, concentration effects on degradation become less at the lower applied concentrations such that less than a 2-fold difference was observed between applied trenbolone concentrations of 0.1 and 1 mg/kg.

Effect of Manure Amendment on 17α-Trenbolone (0.05 mg/kg) Degradation. Manure applied to a clay loam (D36) at a rate comparable to what would be done in the field did not significantly affect the degradation of 17α-trenbolone applied at 0.05 mg/kg (Table 1). For the subsequent degradation of trendione, the rate in the presence of manure is similar but significantly higher (p-value = 0.0025) than without manure. Likewise, Jacobson et al. (*20*) observed a slight increase in degradation of testosterone and 17β-estradiol in soils when amended with swine manure under optimal moisture conditions, whereas further degradation of the metabolite to CO_2 appeared somewhat retarded. Overall, rates for the lowest applied concentration of 0.05

TABLE 1. Summary of the Degradation Rates (k_a and k_b) with 95% Confidence Intervals (CI) for Trenbolone (17α and 17β) and Trendione Estimated from Fits of Eqs 1 and 2 to the Data for All Nonsterile Treatments along with Half Lives ($t_{1/2}$) and Coefficients of Determination (R^2) Reflecting Goodness-of-Fit

								trendione from trenbolone		
soil	hormone	$M_{t=0}$ (mg/kg)	incubation time (h)	k_a, h^{-1} (95% CI of k_a)	R^2	$t_{1/2}$ (h) (from k_a)	$\approx t_{1/2}$ (h) (observed)	k_b, h^{-1} (95% CI of k_b)	R^2	$t_{1/2}$ (h) (from k_b)
D30	17β	0.1	215	0.157 (0.008)	0.99	4.4	5	0.042 (0.011)	0.53	16.6
		1	168	0.074 (0.016)	0.97	9.3	8	0.0092 (0.003)	0.88	75
		1	168	0.078 (0.010)	0.96	8.9	8	0.019 (0.002)	0.87	35
		7	270	0.034 (0.002)	0.99	20	20	0.0057 (0.002)	0.88	120
		10	168	0.018 (0.004)	0.85	38	40	0.0022 (0.010)	0.82	312
	17α	0.1	215	0.166 (0.033)	0.94	4.2	5	0.053 (0.012)	0.59	13.1
		1	168	0.078 (0.008)	0.96	8.9	8	0.011 (0.002)	0.88	64
D36	17α	0.05	215	0.173 (0.024)	0.97	4	4	0.046 (0.006)	0.85	15
	17α + manure	0.05	215	0.179 (0.014)	0.99	3.8	4	0.067 (0.011)	0.75	10
C32	17β	0.1	225	0.094 (0.012)	0.96	7.4	8	0.014 (0.002)	0.86	49
		1	216	0.057 (0.004)	0.97	12	13	0.0144 (0.007)	0.68	48
		1	260	0.062 (0.010)	0.94	11	11	0.0074 (0.001)	0.91	87
	17α	0.1	225	0.086 (0.010)	0.96	8	8	0.016 (0.002)	0.50	42
		1	260	0.065 (0.014)	0.89	11	11	0.0069 (0.002)	0.52	100
D30	trendione	0.04	215	0.028 (0.002)	0.99	24	24			
		3	250	0.0069 (0.001)	0.88	100	100			
		3.5	285	0.0070 (0.001)	0.89	98	95			

mg/kg with and without manure are not significantly different from those observed for an applied concentration of 0.1 mg/kg on a soil from the same field sampled earlier (Table 1), which suggests that degradation rates may not vary significantly within the concentration range most likely to be present in manure-amended soil.

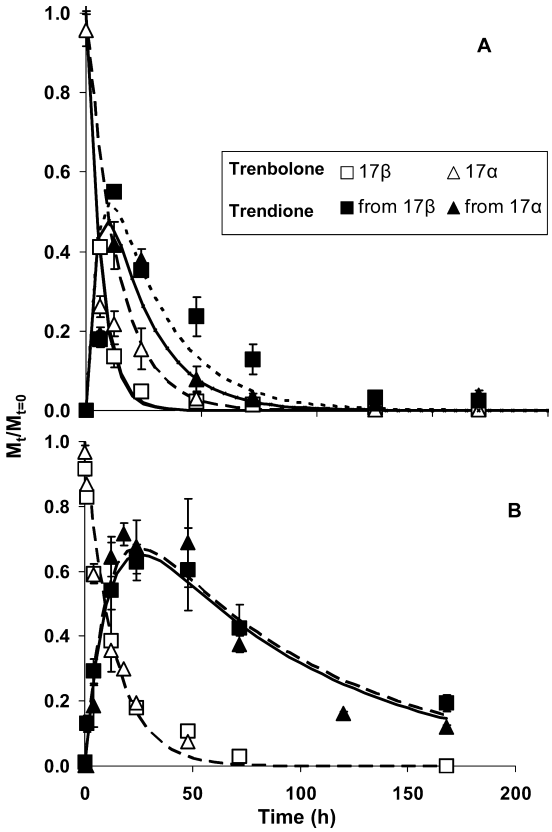

FIGURE 3. Moles over time (M_t) relative to applied moles ($M_{t=0}$) for degradation at 22 ± 2 °C of 17α- or 17β-trenbolone applied to soil D30 at (A) 0.1 mg/kg and (B) 1.0 mg/kg. Error bars represent the standard deviation of triplicate samples.

In the current study, half-lives ($t_{1/2}$) for 17α- and 17β-trenbolone at applied concentrations of ≤1 mg/kg were relatively short (≤0.5 days, Table 1) and essentially all applied trenbolone was aerobically degraded within 2 days, which is in stark contrast to the previously reported 17α- and 17β-trenbolone half-lives of over 250 days in liquid manure (*3*). The latter tends to be an anaerobic methanogenic environment unlike surface soil conditions. Therefore, it is apparent that in beef manure-applied fields under temperature and moisture conditions conducive to active microbial communities, trenbolone persistence is likely to be small. However, under dry soil conditions, such as may occur in surface soils after spring preplanting manure applications, microbial activity is likely to be reduced (*21, 22*). Likewise, longer persistence is expected during colder months typical of postharvest manure-applications; the $t_{1/2}$ for testosterone was 5 times longer at 4 °C compared to 30 °C (*20*). Therefore, cold temperatures or low moisture conditions may increase the likelihood of hormone leaching to groundwater or tile-drains from manure-applied fields. It is also plausible that under these same conditions (low microbial activity), the increased residence time in the soil may allow penetration of the hormone into soil particles, thus reducing hormone leachability and bioavailability in subsequent rain events.

The primary metabolite trendione persisted much longer than 17α- and 17β-trenbolone and was further retarded in the presence of manure (Table 1); however, the potency of trendione is likely to be at least an order of magnitude less than 17β-trenbolone (*12*). Less than 1.5% of trendione was observed to convert back to 17β-trenbolone, although the factors controlling the conversion of trendione back to 17β-trenbolone in soils are not clear. The relative importance of trendione persistence is further complicated given the hypothesis that metabolic isomer conversion within the fish (*13*), which can occur through trendione (*2*), was responsible for the similar reproductive effects observed in fish exposed to 17α- and 17β-trenbolone. Therefore, trendione's potential contribution to deleterious effects on aquatic species can

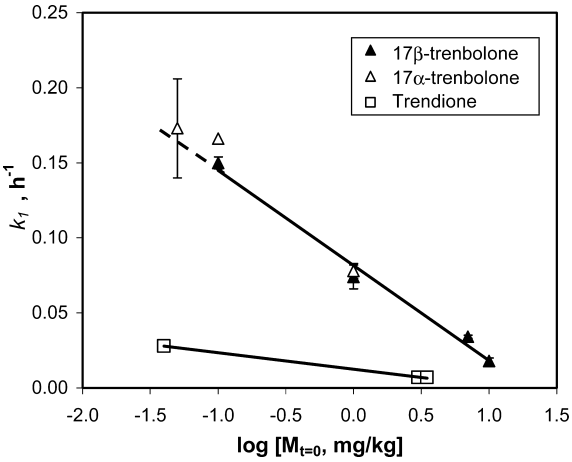

FIGURE 4. Aerobic degradation rates for 17α- and 17*β*-trenbolone, and for the subsequent degradation of the primary metabolite trendione, in a clay loam soil (D30 or D36) at 22 ± 2 °C as a function of the logarithm of the trenbolone isomer concentration applied (*t* = 0). Error bars represent the standard deviation of triplicate samples. Solid lines represent linear regression of the data points for 17*β*-trenbolone and trendione. The dashed line is extrapolated to show inclusion of all the 17α-trenbolone data points within the regression for 17*β*-trenbolone.

not be dismissed and warrants trendione-specific aquatic toxicology studies.

Acknowledgments

This work was funded in part by a U.S.EPA Science to Achieve Results (STAR) award no. RD833417.

Supporting Information Available

Additional details are provided regarding trendione synthesis, extraction efficiency, carrier solvent effects on 17*β*-trenbolone degradation rates, HPLC analysis, LC/MS analysis, LC/MS analysis matrix effects assessment, 17*β*-trenbolone degradation in autoclaved soil C32, and concentration-dependent degradation. This information is available free of charge via the Internet at http://pubs.acs.org.

Literature Cited

(1) *United States Department of Agriculture. (2000). Part I: Baseline reference of feedlot management practices, 1999*; USDA: APHIS: VS, CEAH, National Animal Health Monitoring System: Fort Collins, CO, 2000.

(2) Pottier, J.; Cousty, C. Differences in the biotransformation of a 17*β* hydroxylated steroid, trenbolone acetate, in rat and cow. *Xenobiotica.* **1981**, *11*, 489–500.

(3) Schiffer, B.; Daxenberger, A.; Meyer, K.; Meyer, H. H. D. The fate of trenbolone acetate and melengestrol acetate after application as growth promoters in cattle: Environmental studies. *Environ. Health Perspect.* **2001**, *109*, 1145–1151.

(4) Soto, A. M.; Calabro, J. M.; Prechtl, N. V.; Yau, A. Y.; Orlando, E. F.; Daxenberger, A.; Kolok, A. S.; Guillette, L. J., Jr.; Lebizec, B.; Lange, I. G.; Sonnenschein, C. Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in eastern Nebraska, USA. *Environ. Health Perspect.* **2004**, *112*, 346–352.

(5) Durhan, E. J.; Lambright, C. S.; Makynen, E. A.; Lazorchak, J.; Hartig, P. C.; Wilson, V. S.; Gray, L. E.; Ankley, G. T. Identification of metabolites of trenbolone acetate in androgenic runoff from a beef feedlot. *Environ. Health Perspect.* **2006**, *114*, 65–68.

(6) Lorenzen, A.; Hendel, J. G.; Conn, K. L.; Bittman, S.; Kwabiah, A. B.; Lazarovitz, G.; Masse, D.; McAllister, T. A.; Topp, E. Survey of hormone activities in municipal biosolids and animal manures. *Environ. Toxicol.* **2004**, *19*, 216–225.

(7) Kolok, A.; Sellin, M. The environmental impact of growth promoting compounds employed by the United States beef cattle industry: History, current knowledge and future directions. *Rev. Environ. Contam. Toxicol* **2008**, *195*, 1–30.

(8) Seki, M.; Fujishima, S.; Nozaka, T.; Maeda, M.; Kobayashi, K. Comparison of response to 17*β*-trenbolone among three small fish species. *Environ. Toxicol. Chem.* **2006**, *25*, 2742–2752.

(9) Orn, S.; Yamani, S.; Norrgren, L. Comparison of vitellogenin induction, sex ratio, and gonad morphology between zebrafish and Japanese medaka after exposure to 17α -ethinylestradiol and 17*β*-trenbolone. *Arch. Environ. Contam., Toxicol.* **2006**, *51*, 237–243.

(10) Ankley, G. T.; Jensen, K. M.; Makynen, E. A.; Kahl, M. D.; Korte, J. J.; Hornung, M. W.; Henry, T. R.; Denny, J. S.; Leino, R. L.; Wilson, V. S.; Cardon, M. C.; Hartig, P. C.; Gray, L. E. Effects of the androgenic growth promoter 17*β*-trenbolone on fecundity and reproductive endocrinology of the fathead minnow. *Environ. Toxicol. Chem.* **2003**, *22*, 1350–1360.

(11) Sone, K.; Hinago, M.; Itamoto, M.; Katsu, Y.; Watanabe, H.; Urushitani, H.; Tooi, O.; Guillette, L. J., Jr.; Iguchi, T. Effects of an androgenic growth promoter 17*β*-trenbolone on masculinization of mosquitofish (Gambusia affinis). *Gen. Comp. Endrocrinol.* **2005**, *143*, 151–160.

(12) Bauer, E. R. S.; Daxenberger, A.; Petri, T.; Sauerwein, H.; Meyer, H. H. D. Characterisation of the affinity of different anabolics and synthetic hormones to the human androgen receptor, human sex hormone binding globulin and to the bovine progestin receptor. *APMIS.* **2000**, *108*, 838–846.

(13) Jensen, K.; Makynen, E.; Kahl, M.; Ankley, G. T. Effects of the feedlot contaminant 17α-Trenbolone on reproductive endocrinology of the fathead minnow. *Environ. Sci. Technol.* **2006**, *40*, 3112–3117.

(14) Radl, V.; Pritsch, K.; Munch, J. C.; Schloter, M. Structural and functional diversity of microbial communities from a lake sediment contaminated with trenbolone, an endocrine disrupting chemical. *Environ. Pollut.* **2005**, *137*, 345–353.

(15) http://www.epa.gov/waterscience/guide/feedlots/execsumm.pdf.

(16) Iversen, K. V.; Davis, J. G. Cattle Manure Application Rates. 2000. Colorado State University Cooperative Extension Bulletin No. 0.560, http://www.ext.colostate.edu/pubs/crops/00560.pdf.

(17) Kelley, S. L.; Aitchison, E. W.; Deshpande, M.; Schnoor, J. L.; Alvarez, P. J. Biodegradation of 1,4-dioxane in planted and unplanted soil: Effect of bioaugmentation with Amycolata sp. CB1190. *Water Res.* **2001**, *35*, 3791–800.

(18) Wolf, D. C.; Dao, T. H.; Scott, H. D.; Lavy, T. L. Influence of sterilization method on selected soil microbiological, physical and chemical properties. *Environ. Qual.* **1989**, *18*, 39–44.

(19) Marcus, P. I.; Talalay, P. On the molecular specificity of steroid enzyme combinations—The kinetics of beta-hydroxysteroid dehydrogenase. *Proc. R. Soc. London, Ser. B* **1955**, *144*, 116–132.

(20) Jacobsen, A. M.; Lorensen, A.; Chapman, R.; Topp, E. Persistence of testosterone and 17*β*-estradiol in soils receiving swine manure or municipal solids. *J. Environ. Qual.* **2005**, *34*, 861–871.

(21) Lee, L. S.; Sassman, S. A.; Turco, R. F.; Bischoff, M. Degradation of *N,N′*-dibutylurea (DBU) in soils treated with only DBU and DBU fortified Benlate® fungicides. *J. Environ. Qual.* **2004**, *33*, 1771–1778.

(22) Zak, D. R.; Holmes, E. W.; MacDonald, N. W.; Pregitzer, K. S. Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Sci. Soc. Am. J.* **1999**, *63*, 575–584.

ES702690P