Occurrence of Trenbolone Acetate Metabolites in Simulated Confined Animal Feeding Operation (CAFO) Runoff

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Supporting Information

ABSTRACT: Metabolites of androgenic synthetic growth promoters used at confined animal feeding operations (CAFOs) pose a demonstrated ecological risk. To evaluate the transport of trenbolone acetate (TBA) metabolites from beef cattle CAFOs, rainfall simulation experiments were conducted at the University of California, Davis, research CAFO. Steroid concentrations in solid and aqueous samples from the research CAFO and solids samples from a commercial CAFO were analyzed by gas chromatography-tandem mass spectrometry. The data indicate that 17α-trenbolone (17α-TBOH), 17β-trenbolone (17β-TBOH), and trendione (TBO), the three primary TBA metabolites, occur in soils and runoff. Soils at the research CAFO contained up to 8.2 (±1.1) ng/g-dw of 17α-TBOH and 1.2 (±0.1) ng/g-dw of 17β-TBOH, with slightly higher (~20 ng/g-dw) 17α-TBOH concentrations observed in commercial CAFO soils. In simulated runoff, 17α-TBOH concentrations of 1–350 ng/L and TBO concentrations from 1–170 ng/L were observed. The metabolite 17β-TBOH intermittently occurred in runoff samples at 5–26 ng/L and may be correlated to anaerobic soils. Metabolite concentrations observed in CAFO runoff correspond to 5–15% of potential maximum steroid concentrations predicted by mass balances. First order transformation rates of 0.028/day (25 day half-life) were estimated for 17α-TBOH in CAFO soils. Results suggest that ecologically relevant concentrations of TBA metabolites can be mobilized from CAFO surfaces in storm runoff and may lead to receiving water concentrations at or above ecological effects thresholds for a very limited number of discharge scenarios.

INTRODUCTION

Over the past decade, many studies have documented elevated steroid hormone concentrations in receiving waters.1–3 In some cases, these steroids are believed to induce endocrine disruption in sensitive species of fish with profound biological consequences.4–10 Effects of steroid-induced endocrine disruption include decreased egg production, delayed onset of sexual maturity, spermatogenesis inhibition, gonadal growth reductions, and even localized population collapse.7,8 Municipal wastewater effluent and agricultural runoff are likely important sources of steroids to surface waters. In particular, confined animal feeding operations (CAFOs) are potentially the most significant source of steroids to aquatic ecosystems, accounting for up to 90% of the endogenous steroid mass discharge into U.S. waters.9

One steroid class of special interest is metabolites of the synthetic growth promoter trenbolone acetate (TBA). TBA-containing implants are widely used in U.S. beef cattle production,5,16 and primary metabolites include 17α-trenbolone (17α-TBOH), 17β-trenbolone (17β-TBOH), and trendione (TBO).10 Of these, 17α-TBOH is the most abundant metabolite. Approximately 8% of the applied TBA dose is excreted as 17α-TBOH, and it accounts for 95% of excreted metabolite mass.10 Low concentrations (10–50 ng/L) of 17α-TBOH and 17β-TBOH cause physiological masculinization such as development of malelike nuptial tubercles in females, inhibited embryonic development, and irreversibly skewed sex ratios in fish.11–13 Morthorst et al. (2010) reported that zebrafish (Danio rerio) exposed to 16 ng/L 17β-TBOH exhibited 100% male populations at 60 days posthatch.13 More importantly, adverse effects on reproductive output are demonstrated for 17α-TBOH and 17β-TBOH, with fecundity reductions occurring in fathead minnow (Pimephales promelas).
at 11 and 18 ng/L exposures, respectively, although it is likely that the no-effects concentrations for these compounds are even lower.\textsuperscript{5,11}

Due to their widespread use, high excretion rates, and limited sorption, the presence of TBA metabolites in beef cattle CAFO runoff is likely. In cases of direct or incidental runoff discharge to receiving waters, even substantial dilution could result in concentrations exceeding effects thresholds for endocrine disruption. For example, using a conservative estimate of 8\% dose excretion, a single 160 mg TBA implant could yield 11 ng/L 17α-TBOH in 1.2 × 10\(^{-4}\) L of receiving water. As many CAFOs contain over 1,000 animals, each typically receiving several TBA implants over their life, potential ecological risks are a substantial concern for incidences of TBA metabolite transport to receiving waters.

Occurrence studies of TBA metabolites demonstrate transport from agricultural soils, persistence in manure and soil, and presence in CAFO-impacted waters.\textsuperscript{10,14–19} However, these studies are limited in number and have not often accounted for specific CAFO characteristics. For example, no studies to date have correlated TBA metabolite occurrence and transport with variables such as implant dose, time postimplantation, stocking density, soil and manure aging, or rainfall rates. These uncertainties complicate the assessment of ecological risks arising from the use of TBA implants in CAFOs.

The objectives of this study were to quantify TBA metabolite occurrence in beef cattle CAFO soils, manure, and runoff while identifying environmental variables governing steroid release and transport. For improved control over runoff production and sampling, rainfall simulators were used to generate runoff on a well characterized research feedlot. Variables such as soil and manure aging, stocking density, and rainfall rate were assessed using concurrent solids and runoff sampling, while soil transformation was assessed by soil analysis at a commercial CAFO with implanted cattle. Finally, results were used to develop a predictive framework that correlates ecological risk in nearby receiving waters with CAFO characteristics, rainfall rate, and metabolite transformation rates. A published companion manuscript reports the results of this study approach for the occurrence, fate, and transport of endogenous steroid hormones from these CAFOs, including ancillary water quality and experimental data.\textsuperscript{24}

\section*{MATERIALS AND METHODS}

\textbf{Research Sites.} Rainfall simulation studies were conducted at University of California, Davis (Davis, CA), research feedlot pens that held 28 TBA-implanted steers weighing 340–450 kg each.\textsuperscript{24} The feedlot surface consisted of a dense Yolo silt loam clay-pan covered with a surface duff-layer composed of animal manure, soil, and organic matter. The steers were divided into two random groups of fourteen following subcutaneous implantation (120 mg TBA and 24 mg estradiol) and placed on two identical 190 m\(^2\) pens (stocking density of 13 m\(^2\)/animal unit (AU), typical of commercial CAFOs). Background runoff samples were collected once prior to animal introduction, and four sequential rainfall simulation trials were performed on the pens postimplantation.\textsuperscript{24} Spatially composited solid samples (manure and soil, top 5 cm of surface) were collected pre- and postrainfall simulation, then homogenized, and frozen (−20 °C) prior to triplicate analysis.

To validate results from rainfall simulation trials and assess additional CAFO characteristics such as implantation period and soil degradation, a commercial cattle finishing CAFO was sampled. Steers at the commercial CAFO (stocking density of 10–100 m\(^2\)/AU, mixed steer breeds) received 100 mg TBA/14 mg estradiol implants. Preimplantation, cattle averaged 425 kg and gained an average of 177 kg over 163 days while implanted. Soils were collected randomly at three locations along pen transects from each of 18 pens that contained cattle implanted at various dates 20–160 days prior to sample collection, then composited, and frozen.

\textbf{Rainfall Simulation.} CAFO runoff was generated using a programmable valve-electric solenoid rainfall simulator design.\textsuperscript{20,21} The pressurized sprinkler valve was suspended 3 m above the feedlot. Rainfall rates (15 mm/h (low rate) and 25 mm/h (high rate)) were controlled by varying the period that the sprinkler valve was open and calibrated by direct measurement. Eight 3.0 m\(^2\) plots on each CAFO pen were enclosed by aluminum sheeting to isolate plots and collect runoff. During trials, runoff volume was measured and correlated to the runoff hydrograph by collecting samples at 5, 20, 40, and 60 min after observation of runoff to capture first flush and washout characteristics (Supporting Information, Table S1). For each rainfall trial, samples collected from the 4 plots per pen were composited to account for spatial heterogeneity. Runoff samples were collected in 1 L amber glass bottles and transported on ice to the laboratory for immediate processing. After each trial, feedlot pens were scraped to the clay-pan layer to prepare for successive trials. All trials were conducted after implanted cattle had used the pens for at least 14 days, with two trials sampling immediately after 14 days and two trials including an additional 7 day aging period with cattle removed from the pens (day 14) prior to sampling (day 21).

\textbf{Chemicals.} 17α-TBOH (17α-hydroxyestra-4,9,11-trien-3-one) was obtained through BDG Synthesis (Lower Hut, NZ). 17β-TBOH (17β-hydroxyestra-4,9,11-trien-3-one) was obtained from Steraloids (Newport, RI). Trendione was synthesized and purified at the University of Nevada, Reno using published methods.\textsuperscript{16} Deuterated 17β-trenbolone (d3-17β-hydroxyestra-4,9,11-trien-3-one) was obtained from the RIVM Bank of Reference Standards (Netherlands). As this was the only deuterated TBA metabolite standard available, this compound was used for isotopic dilution recovery correction for all TBA metabolites. HPLC-grade solvents were obtained from Fisher (Pittsburgh, PA). Derivatization grade N-methyl-N-trimethylsilyl-trifluoroacetimide (MSTFA) and I\(_2\) (99.9999\% purity) were obtained from Sigma Aldrich (Milwaukee, WI).

\textbf{Steroid Analysis.} Steroid analysis employed gas chromatography-tandem mass spectrometry.\textsuperscript{25} Briefly, solids samples (5 g wet weight) were solvent-extracted via sonication (10 min) with three successive 25 mL methanol aliquots and centrifuged (3500 rpm, 6 min), and the supernatant was collected. The combined extracts were diluted with deionized water to 1 L, spiked with 1 mL of 100 ppb d\(_3\)-17β-TBOH internal standard in methanol, and processed as for aqueous samples. To measure water content, samples were oven-dried at 105 °C, and organic carbon content was measured by comparing sample mass pre- and postoven drying at 550 °C (1 h).

To remove suspended solids and particulate organic carbon, whose concentrations usually exceeded 1000 mg/L in runoff,\textsuperscript{22} aqueous samples were first centrifuged (3500 rpm, 6 min) and then pressure filtered (0.45 μm AP40 filters, Millipore, Billerica, MA). Filtered samples were spiked with d\(_3\)-17β-TBOH (100 ng/L final concentration) and extracted on 6 mL C18 solid phase extraction cartridges (Restek, Bellefonte, PA). If
necessary (e.g., instrument maintenance), cartridges were stored at −20 °C for a period of up to 3 months prior to extract elution. Following elution (95.5 v/v methanol/water), samples were dried, resuspended (95.5 v/v dichloromethane/methanol), and passed through 6 mL Florasil cartridges (Restek) to further reduce organic matrix interferences. Next, extracts were dried and resuspended using 50 μL of MSTFA-I2 (2 mg I2/mL MSTFA). Samples were immediately dried again to remove residual iodine, resuspended in 100 μL of MSTFA, heated to 60 °C (40 min.), and then transferred to GC vials for analysis.

A Waters (Milford, MA) Quattro-Micro triple quadrupole mass spectrometer (ionization energy = 70 eV) coupled to an Agilent 6890N gas chromatograph was used in conjunction with a Supelco (Bellefonte, PA,) SLB-5 ms fused silica capillary column (30 m × 0.25 mm × 0.25 μm). UHP-grade helium (1 mL/min) was used as a carrier gas. The temperature ramp was as follows: Isothermal 120 °C (2 min), ramped at 45 °C/min to 260 °C (1 min), 5 °C/min to 270 °C (8 min), 45 °C/min to 285 °C (6 min), 45 °C/min to 300 °C (2 min). The injection port and MS interface were held at 250 °C, and the MS source temperature was 180 °C. Method limits of detection were approximately 0.2 ng/g for extraction of 5 g solids samples and <1 ng/L in aqueous samples, based upon SPE extraction of approximately 0.2 ng/g for extraction of 5 g solids samples and temperature was 180 °C 

<table>
<thead>
<tr>
<th>Sample Set</th>
<th>Initial Soil Concentration (ng/g-dw)</th>
<th>Final Soil Concentration (ng/g-dw)</th>
<th>Difference (ng/g-dw)</th>
<th>Sum of Steroid Mass (ng/g-dw)</th>
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<tbody>
<tr>
<td>Feedlot Surface</td>
<td>17α/17β/TBO-13 (Standard Deviation)</td>
<td>17α/17β/TBO-13 (Standard Deviation)</td>
<td>17α TBO</td>
<td>17β TBO</td>
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<td>Not collected</td>
<td>*NA</td>
<td>NA</td>
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<td>9.5/13.1/1 (1.3/0.5/0.7)</td>
<td>-2.3</td>
<td>+0.6 +1.1</td>
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<td>-4.5</td>
<td>+0.2 +0.8</td>
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<tr>
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<td>-1.9</td>
<td>+0.5 +0.7</td>
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<td>2.3/1.2/0.6 (0.5/0.4/0.7)</td>
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<td>+1.1 +2.6</td>
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<td>Series 4 Pens B</td>
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<td>+0.6</td>
<td>0 +0.7 +1.3</td>
</tr>
</tbody>
</table>

*NA: not applicable.

### RESULTS AND DISCUSSION

Fresh manure samples (n = 8, 28 days postimplant) were analyzed from implanted cattle at the research CAFO. In these samples, 17α-TBOH (21 ± 2.7 ng/g-dw) and 17β-TBOH (3.1 ± 0.4 ng/g-dw) were present, while TBO was not detected in the manure. Schiffer et al. (2001) reported 17α-TBOH concentrations of 13.8 and 75.4 ng/g in two manure samples, although it is unclear if the reported data account for moisture content. Also, applied implant doses of 470 mg of TBA (4X the dose used in this study) were used, likely explaining the higher concentrations. The manure 17β-TBOH:17α-TBOH ratio (15%) observed in this study is slightly higher than the ratio (6%) reported by Schiffer et al. (2001).

In contrast to the results of Bartelt-Hunt et al. (2012), all soil samples collected from pens with implanted cattle (research and commercial CAFOs, n = 27) contained 17α-TBOH and 17β-TBOH. Similar to manure measurements, TBO was rarely detected in research CAFO soils prior to rainfall (Table 1). TBA metabolite ratios in CAFO soils were consistent with measured manure ratios. 17α-TBOH was again the dominant metabolite (0.6–11.8 ng/g-dw), compared to 0.5–0.9 ng/g-dw for 17β-TBOH, which averaged 8% of estimated 17α-TBOH concentrations. However, unlike this study, Schiffer et al. (2001) reported similar concentrations of TBO and 17β-TBOH in CAFO soils. The lower TBA metabolite concentration in soils relative to fresh manure likely reflects both dilution of fresh manure with low steroid concentration soil matrix and also in situ metabolite transformations.

TBA metabolites also were detected in surface soils from a series of commercial CAFO pens containing cattle with...
different time periods postimplantation (100 mg TBA implants). With one exception, 17α-TBOH soil concentrations were highest (14 ng/g-dw) in samples with the shortest elapsed time (20 d) postimplantation. Through a series of pens that reflect the 100 day expected implant lifetime (per manufacturer specifications), 17α-TBOH soil concentrations decreased to 2 ng/g-dw in pens at 110 days postimplantation, a decrease which cannot be fully explained by increased manure production due to animal growth. Increased manure production can only account for 20% of the observed decrease, suggesting that substantial transformation of TBA metabolites is occurring on CAFO surfaces. Additionally, a pen containing 29 implanted cattle at 160 days postimplantation yielded no detectable steroids in soil, indicating that TBA metabolites are not excreted in substantial quantity and may not persist in soils long past the expected implant lifetime. Alternatively, TBA metabolite excretion also varies with time over the expected 100 day implant lifetime, although implants are specifically designed to maintain near constant blood concentrations of TBA metabolites over the implant lifetime. The only available study on TBA implant metabolism in cattle suggests that TBA excretion occurs at a near constant rate over most of the implant lifetime. If TBA excretion actually declines during later stages of the implant lifetime, some decrease in soil steroid concentrations in CAFO soils are likely stocking density and steroid transformation. Stocking density may play an additional role in the implant lifetime, some decrease in soil concentrations would naturally result from reduced excretion.  

These data suggest that the critical variables controlling steroid concentrations in CAFO soils are likely stocking density and steroid transformation. Stocking density may play an especially critical role in determining steroid runoff concentrations after rainfall. Stocking density determines the average land area on which contaminant mass is “diluted” via spreading, thereby defining the bulk rate of steroid accumulation and soil concentration on CAFO surfaces. Stocking density also dictates the rainfall collection area that governs further dilution of contaminants in runoff, in much the same way that receiving water volume controls the exposure concentrations for aquatic organisms.  

These factors can be incorporated into a mass balance approach that can be used to predict steroid concentration and potential ecological risk in receiving waters. First, soil concentrations can be linked to steroid excretion by estimating the amount of manure produced from the animals over the implant lifetime. If TBA metabolite accumulation on feedlot surfaces is solely a function of manure production and constant metabolite excretion (no transformation), soil mass accumulation would be predicted as linear with respect to time and stocking density (Figure 1). For example, most commercial TBA implants have an estimated in vivo lifetime of 100 days and should have a near constant steroid release throughout this period. Cattle with a 120 mg of TBA implant (e.g. research CAFO implant) would metabolize 1200 μg of TBA per day and excrete approximately 8% of this dose as primary metabolites. Therefore, approximately 96 μg/AU of TBA metabolites should reach the feedlot surface each day, resulting in linear accumulation in the absence of transformation, with specific soil concentrations largely depending on stocking density (Figure 1). Typical stocking densities used on commercial CAFOs range from 10 to 20 m²/AU.  

In practice though, attenuation processes (e.g., transformation, irreversible sorption) need to be accounted for. After estimating the total mass accumulation in soils, transformation models can be applied to accumulated mass to improve predicted soil concentrations with time. To quantify TBA metabolite attenuation in CAFO surface soils, data from the commercial CAFO (square data, Figure 2) were fit to a first order decay model that accounts for continuous metabolite excretion using the following equation:

\[ C_t = \frac{1}{M(t)} \sum_{0}^{t} S_t \exp\left(-kt^0\right) \]

In eq 1, \( C_t \) is the TBA metabolite soil concentration at time \( t \), \( S_t \) is the predicted metabolite mass excreted at time \( t \) (e.g. 80 μg/d for a 100 mg, 100 day lifetime implant, with 8% 17α-TBOH excretion), \( M(t) \) is the cumulative manure mass excreted by time \( t \) (e.g. 5–8 kg dw/d), and \( k \) is the first order rate constant. Using eq 1 to fit the observed soil concentration data by minimizing the sum of the squared error yields a decay constant of 0.028/day (25 day half-life) for 17α-TBOH in surface soils at the commercial CAFO (Figure 2). Although this approach does not include the effect of soil matrix dilution on concentration, the observed rate constant would be independent of dilution effects as long as dilution is constant over the study period. In comparison to this data, TBA metabolites have reported half-lives of hours—days in aerobic agricultural soils. For example, transformation rates for TBA metabolites in aerobic conditions suggest half-lives ranging from 4 to 50 h for 17α-TBOH and 17β-TBOH and up to 225 h for TBO. In anaerobic conditions, half-lives of 260 and 270 days have been reported for 17α-TBOH and 17β-TBOH, respectively. The CAFO soil data from this study suggest intermediate transformation rates, thus increased metabolite persistence, at these CAFOs relative to results for aerobic agricultural soils. For example, 6.2 ng/g-dw 17α-TBOH was still present in research CAFO soils seven days after cattle were removed, which is only slightly lower than predicted initial soil concentrations of 10–20 ng/g-dw. This observation suggests that CAFO soils may contain microenvironments not conducive to steroid attenuation under aerobic conditions. Khan et al. (2010) suggested that high temperatures and low moisture, typical conditions for these CAFOs (e.g. 3–6% moisture content of surface soils), inhibit degradation of TBA metabolites, also potentially explaining observed metabolite persistence in the surface soils.
Both research and commercial CAFO soils had consistently higher initial 17α-TBOH concentrations compared to 17β-TBOH and TBO, while 17β-TBOH and TBO were present at higher concentrations in saturated soils after rainfall trials on the research CAFO, although the differences are not statistically significant (Table 1). For example, 17β-TBOH increased from 0.2 to 1.1 ng/g-dw and TBO increased from 0.7 to 1.1 ng/g-dw after saturation. These increases might suggest that 17β-TBOH and TBO form from 17α-TBOH transformation in anaerobic microenvironments that develop after soil saturation. However, the transformations observed by Khan et al. (2008) suggest 1.5% conversion of 17α-TBOH, while these CAFO data suggest 10% conversion, though sample numbers are inadequate to prove statistical significance. Also, saturated soil metabolite ratios are consistent with Schiffer et al. (2001), who reported 17β-TBOH and TBO at 7% of 17α-TBOH concentration.

Rainfall simulation trials, conducted between July and October, were somewhat complicated by an unexpected, early season rainstorm (∼6 cm over 24 h) at the research CAFO prior to the final trial (Table 1, sampling series 4). This event resulted in decreased prerainfall soil concentrations and altered metabolite ratios, consistent with some TBA metabolite leaching and transformation during the rainstorm, although no runoff left the pens during this storm. Increased metabolite concentrations were detected in deeper clay pan layers after the storm, suggesting that the storm transported steroid from surface soils to deeper soils, something not observed during other simulator trials. Surface soils collected in series 4 had 0.6–2.1 ng/g-dw 17α-TBOH, with 7.7 ng/g-dw 17α-TBOH in the clay pan, compared to 1.3 and 1.6 ng/g-dw in previous clay pan measurements. In all other rainfall trials, substantial decreases in TBA metabolites occur in the clay pan with corresponding increases of the metabolites in surface soils, suggesting that anaerobic environments of deeper CAFO soils are potentially a persistent reservoir of steroid metabolites.

To investigate the water-extractable fraction of soil-associated steroids on CAFO surfaces, some soil samples were concurrently extracted with methanol and water. Approximately 60% of methanol-extractable steroids also were water-extractable, suggesting the existence of a strongly bound or otherwise unavailable fraction of steroids in soils. Steroids deeply partitioned in organic matter phases and on soil particles away from water-soil interfaces are likely not available for leaching. Also, equilibrium partitioning on high organic matter content soils such as those present on CAFO surfaces favors steroid sorption to solids. Organic matter contents (fom) of soils were 52% (commercial CAFO) and 40% (research CAFO). TBA metabolite partitioning has been correlated with organic carbon content in agricultural soils (fom = 7.5%). Khan et al. (2009) reported log Koc values for 17α-TBOH, 17β-TBOH, and TBO of 2.77, 3.08, and 3.38, respectively. Using the reported Koc values with measured organic matter content, estimated Kd values (235, 480, and 960 L/kg for 17α-TBOH, 17β-TBOH, and TBO, respectively) for these CAFO soils are 5–7 times higher in feedlot surface soils than soils typical of agricultural fields. Assuming 5,000 mg/L TSS, this calculation suggests that at least 40% of total steroid mass is solids/colloid associated in CAFO runoff, with higher solids-associated fractions in soil environments. Therefore, sequestering particles in CAFO runoff by settling or similar means (e.g., treatment lagoons, bioswales, constructed wetlands) may be somewhat effective for controlling steroid transport to receiving waters.

Estimates of TBA concentration in runoff samples collected from the rainfall simulator trials are complicated by the inconsistent QA/QC data (see Table S1, Supporting Information). Without applying isotopic recovery correction, 17α-TBOH was detected in every research CAFO runoff sample (n = 63) at estimated concentrations of 1–390 ng/L (34 ng/L median concentration). Without any recovery correction, these estimates likely underestimate actual concentrations in runoff; however, the estimates are quite similar to the <10–120 ng/L for 17α-TBOH and <10–20 ng/L 17β-TBOH measurements reported for CAFO runoff by Durhan et al. (2006). Sample time series did not demonstrate a “wash out” trend or any concentration trends that correlated with the

Figure 2. Observed 17α-TBOH soil concentrations as a function of implantation durations at the research and commercial CAFOs. First order transformation model estimate (solid line) is fit to commercial CAFO soil data only (square boxes). For comparison, diamonds show soil concentration data from the research CAFO. The upper dashed line demonstrates the predicted conservative (no degradation) CAFO soil concentration based on a 100 mg TBA implant with a 100 day metabolism and estimated 8% total excretion of metabolized TBA.
runoff hydrograph. Similarly, estimated concentrations were statistically independent of the rainfall rates used in the trials. 17β-TBOH was detected in 23 of 63 runoff samples, and detects were correlated to specific sampling series or pens. For example, in one trial, 17β-TBOH was detected in every sample from one pen, with no detects in the replicate pen. This heterogeneity may suggest the uneven spatial distribution or development of microenvironments conducive to enhanced metabolite transformations, although no factors measured in this study can account for this heterogeneity. 17β-TBOH concentrations ranged from 5 to 26 ng/L, with a median concentration of 16 ng/L when detected. TBO was present in 41 runoff samples but was below the limit of quantification in 17 of these. TBO concentrations ranged from 5 to 180 ng/L when detected, with a median concentration of 19 ng/L in detects.

Pre- and postrainfall measurements of soil concentrations suggest that approximately 10% of the steroid mass was leached during rainfall simulation. However, estimated runoff concentrations account for only 30% of the predicted mass leached. The remainder of the mass balance is likely due to particle-associated steroids that were filtered out during sample processing and are not measured as “dissolved” phase steroids, although solvent extraction of the filtered particles likely would quantify any residual mass. Equilibrium partitioning suggests that particle-associated steroids should account for approximately 40% of the predicted mass leached. Also, these data are not isotopic standard recovery-corrected, implying concentrations are potentially underestimated by a further 20–40%. Considering these two factors, estimated runoff concentrations seem consistent with soil measurements. Also, these soil and runoff estimates agree with soil (13.8 ng/g) and runoff (227 ng/L) concentrations reported by Schiffer et al. for 17α-TBOH.10

Most importantly, the application of first order transformation models to steroids on CAFO surfaces results in a “plateau” effect for accumulated TBA metabolite mass on feedlot surfaces in all cases where attenuation dominates steroid fate (Figure 3). This plateau prediction suggests that logical upper bounds exist for the steroid mass available for subsequent transport in runoff. For example, starting with a linear accumulation prediction for the research CAFO (13 m²/AU), the maximum accumulated mass per square meter of CAFO surface ranges from 6, 25, 210, or 544 μg/m² when applying transformation rates reported by Khan et al. (2010)16 (k = 4.3/day; 0.288/day), this study (k = 0.028/day), or the anaerobic rate (k = 0.0027/day) of Schiffer et al. (2001),10 respectively. If the entire soil steroid mass was leached during a 25 mm rainstorm at each of those individual plateaus, the resulting runoff concentrations would be 240, 1000, 8,400, and 22,000 ng/L, respectively.

However, soil concentration measurements suggest that complete mass leaching during rain events is unlikely. Instead, the data suggest 5–10% leaching of total steroid mass (Table 1), implying that the runoff concentrations listed above are likely overestimated by at least an order of magnitude. The observed low leachable mass fraction may be due to steroid partitioning to solids regions distant from soil–water interfaces, and the moderately hydrophobic character of TBA metabolites suggests substantial affinity for sorption to organic carbon rich CAFO surface soils. Comparisons of estimated 17α-TBOH concentration in runoff to initial surface soil concentrations (Figure 4) reveal that metabolite concentration in runoff is strongly correlated to soil concentrations. Convergence of runoff concentration estimates with the “10% leaching” predictions (straight lines, Figure 4) at the highest measured soil concentrations may suggest that TBA metabolites are easier to leach (e.g., closer to soil–water interfaces) from higher TBA metabolite concentration soils. By contrast, residual TBA metabolites in the lowest concentration soils may represent steroid not easily available for leaching and transformation, thus leaching efficiency is well below 10%. However, estimated 17α-TBOH runoff concentrations are all within 1 order of magnitude of the predicted 10% leaching value, despite uncertainty arising from the lack of recovery correction and...
loss of steroid mass during filtration. Additional studies would be necessary to further resolve these issues.

Results from this study suggest that low levels of TBA metabolite accumulation in CAFO soils limit the mass available for transport from the feedlot surface during runoff events. After accounting for excretion, transformation, and leaching losses, it is likely that <1% of the applied implant dose can occur in CAFO runoff. Depending on the stocking density and size of precipitation events, there is potential for significant additional mass dilution in runoff. CAFO rainfall events may be likely to quickly leach or mobilize soil-associated steroids at the soil–water interface but also quickly dilute the dissolved steroids, suggesting that the relationship between steroid leaching rate and rainfall rate is an important predictor of final runoff steroid concentration.

Concentration estimates based upon stocking density, steroid excretion, soil transformation, and limited leaching suggest that it is likely very difficult to ever exceed 1,000–3,000 ng/L TBA metabolite concentrations in CAFO runoff. These concentrations may only be expected for cases of very high stocking density with anaerobic CAFO surface soils, possibly only occurring after long periods without runoff that would reduce soil concentrations. Instead, maximum TBA metabolite concentrations in the low-mid hundreds of ng/L, and potentially lower, in direct CAFO runoff seem to be more likely for the majority of CAFO runoff scenarios. Therefore, to assess the ecological risk that incidental or direct CAFO runoff discharges pose to aquatic organisms, it may be sufficient to understand the stocking density and transformation rate characteristics of the CAFO to derive upper-bound runoff concentration estimates. Once runoff concentration estimates are available, comparisons to the dilution capacity of nearby receiving waters will drive expected exposure concentrations for risk assessments for aquatic organisms (Figure 5). Similar to observations of endocrine disruption in wastewater effluent dominated systems, Figure 5 suggests that dilution of CAFO runoff by receiving waters is likely the greatest factor determining when environmental concentrations could result in endocrine disruption in fish, as runoff concentrations typically exceed ecological effects thresholds. Only in cases of high runoff concentration (low rainfall, high stocking density and soil concentration), and low dilution factors (<100), would steroid concentrations exceed the 11–18 ng/L EC50 values for endocrine disruption. However, if substantially lower (≤1 ng/L) no-effects concentrations are utilized in ecological risk assessments, a much wider range of exposure scenarios would likely exceed acceptable risk thresholds for trenbolone metabolites. Additional factors increasing ecological risk include colocation of multiple CAFOs in a watershed and mixture effects arising from co-occurrence of endogenous and exogenous steroids in runoff.24 Careful comparisons of CAFO system characteristics with volumes of nearby receiving waters is likely a good risk assessment strategy to identify particularly problematic CAFOs in need of especially strong management actions to prevent or control incidental stormwater runoff.

ASSOCIATED CONTENT

Supporting Information
Additional information, including discussion of the QA/QC results and data for TBA metabolite occurrence in simulated CAFO runoff. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes
The authors declare no competing financial interest.

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