Trenbolone Acetate Metabolite Transport in Rangelands and Irrigated Pasture: Observations and Conceptual Approaches for Agro-Ecosystems

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Supporting Information

ABSTRACT: To assess the relative ecological risks of trenbolone acetate (TBA) use in agro-ecosystems, we evaluated the spatiotemporal dynamics of TBA metabolite transport during irrigation and rainfall events. Within a pasture, TBA-implanted heifers (40 mg TBA, 8 mg estradiol) were briefly penned (24 h) at high stocking densities (500 animal units (AU)/ha), prior to irrigation. Irrigation runoff concentrations of 17α-trenbolone (17α-TBOH) 0.3 m downslope were 11 ng/L in the wetting front, but quickly decreased to ~0.5 ng/L, suggesting mass transfer limitations to transport. At 3 and 30 m downslope, efficient attenuation of 17α-TBOH concentrations is best explained by infiltration and surface partitioning. At plot scales, transport through vegetated filter strips resulted in <0.5–7 ng/L 17α-TBOH concentrations in rainfall-induced runoff with partial subsequent attenuation. Thus, even under intense grazing scenarios, TBA-metabolite transport potential is expected to be low in rangelands, with ecological risks primarily arising from uncontrolled animal access to receiving waters. However, 17α-TBOH concentrations in initial runoff were predicted to exceed threshold levels (i.e., no observed adverse effect levels [NOAELs]) for manure concentrations exceeding 2.0 ng/g-dw, which occurs throughout most of the implant life. For comparison, estrone and 17β-estradiol were modeled and are likely capable of exceeding NOAELs by a factor of ~2–5 in irrigation runoff, suggesting that both endogenous and exogenous steroids contribute to endocrine disruption potential in agro-ecosystems.

INTRODUCTION

Some observations of endocrine disruption in aquatic organisms have been linked to animal agriculture.1–7 While causative agents responsible for explaining these observations remain unclear, endogenous androgens and estrogens (e.g., 17β-estradiol, testosterone) as well as potent synthetic anabolic steroids (e.g., trenbolone acetate (TBA) metabolites) are excreted in animal wastes and can elicit endocrine responses in exposed organisms.8–12 Agricultural discharges of steroids are potentially pervasive given that TBA is likely administered to as many as 20 million beef cattle annually in the U.S., usually at confined animal feeding operations (CAFOs), and >30% of U.S. land area (i.e., 3.2 million km²) is used for grazing.13–15 In laboratory studies, exposure to trace concentrations (e.g., 5–100 ng/L) of 17α-trenbolone (17α-TBOH) and 17β-trenbolone (17β-TBOH) results in phenotypic sex reversal and fecundity reductions in fish.11,16–18 Although no observed adverse effects levels (NOAELs) are not reported for TBA metabolites (i.e., 17α-TBOH, 17β-TBOH, and trendione [TBO]), a reasonable estimate is 1 ng/L, similar to other synthetic steroids.11,19,20 Concentrations of 17α-TBOH and 17β-TBOH in runoff from TBA-implanted cattle can be as high as 350 and 270 ng/L, respectively,21,22 although detections in agricultural runoff, including from manure-fertilized fields, and receiving waters are typically sporadic (i.e., 0–15% of all samples) with variable concentrations (e.g., no detect—160 ng/L).21,26 While these values may represent concentration upper bounds in agricultural runoff, little data currently link steroid occurrence and concentration to system characteristics and operational practices (e.g., stocking density, implant mass, etc.). Most studies to date have focused on TBA metabolite occurrence in runoff from CAFOs or manure fertilized fields.21–26 On less studied rangelands and irrigated pastures, the lower doses (typically 40 mg doses versus 80–200+ mg implants on CAFOs) and stocking densities might suggest reduced transport risks. However, despite the concentrated

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source, CAFO runoff is generally strictly controlled with concerns only for incidental or permitted discharges. On rangelands, steroids can leach from manure into irrigation water, which is applied approximately every 7–14 days during dry months, or rainwater for subsequent transport, often with limited treatment.27 Similarly, cattle often congregate near and even have direct access to receiving waters, increasing the potential for direct discharge of manure-derived constituents (e.g., steroids, ammonia, orthophosphate, pathogens) to surface waters. Thus, while the available mass is substantially lower on rangelands, the transport potential is potentially higher.

The transport potential, or the capability for a contaminant to reach nearby receiving waters, is a function of mass leaching upon water contact and attenuation processes during transport, while the overall ecological risk is defined by integration of these processes at watershed and system scales. Previously, we decoupled leaching and attenuation processes to investigate their relative contributions on TBA metabolite fate and transport in bench and plot-scale experiments.27,28 Because pollution issues associated with agriculture runoff are so widespread and challenging for control, our aim here was to extend these experimental results to plot and field-scale systems and validate leaching and attenuation predictions to better understand these issues. Specific goals were to (1) quantify the transport potential during irrigation and rainfall events at plot and pasture-scales; (2) utilize leaching and attenuation characteristics to predict concentrations in agricultural runoff; and (3) assess the relative ecological risks of TBA metabolite transport from various types of animal agriculture operations (e.g., rangelands, CAFOs, etc.) and scenarios to prioritize management strategies.

■ MATERIALS AND METHODS

Site Characteristics. Field studies were conducted at the University of California Sierra Foothills Research and Extension Center (SFREC) near Browns Valley, CA on three plot-scale (i.e., 6, 8, and 10 m²) experimental vegetative filter strips (VFSs), which are grass strips used for nonpoint source pollution control downslope of agricultural fields, and a field-scale irrigated pasture (0.6 ha; NAD 83 UTM 10S-645527E, 4346006N (Figure 1, see Supporting Information [SI] for further experimental details).28 A 4.9 m wide × 9.8 m long enclosure (48 m²) was constructed near the top of the pasture to allow applied irrigation water to flow through the enclosure, contact manure and generate runoff, with off-field runoff measured by a Parshall flume (7.62 cm throat width). Aluminum surface-runoff collectors collected samples 1 m upslope and 0.3, 3, and 30 m downslope from the enclosure. While a VFS was not constructed within the irrigated pasture, the area downslope of the enclosure was considered a de facto VFS given its isolation from the animals and location from the enclosure.

Four 18-month old Hereford/Angus cross heifers (∼350 kg) were implanted with Revalor G (40 mg TBA and 8 mg estradiol, for use on rangeland cattle)27 and penned within the enclosure for field-scale irrigation experiments or penned in a covered barn to collect manure for plot-scale rainfall experiments. Animals were handled in accordance with UC Davis Animal Care and Use Committee guidelines.

Irrigation Transport. To evaluate the irrigation transport of TBA metabolites, nutrients, TOC, and pathogens, we flood irrigated the pasture on October 6, 2012 (background samples) and again on October 11, 2012 (runoff samples). The irrigation rate was 0.031 m³/s/ha (0.44 ft³/s/acre), and the total water volume applied was 27.5 m³ (970 ft³). Therefore, the bulk application rate was 1.1 cm/h, similar to typical irrigation rates used at SFREC.20 On October 10, four heifers were penned in the enclosure for 24 h, which was the time period needed to consume the available forage. While the absolute stocking density was ∼500 animal units (AU)/ha (yearlings = 0.6 AU), the time-weighted stocking density, which accounts for grazing intensity, was 500 animal unit days (AUD)/ha or 17 animal unit months (AUM)/ha (6.7 AUM/acre), near typical for pastures regionally.30 Typical values range from 0.05 to 2.5 AUM/ha for rangelands and 2–30 AUM/ha for irrigated pastures.30–35 For comparison, equivalent stocking densities for CAFOs range from 100 to 1000 AUM/ha (40–400 AUM/acre).22 While the AUM quantifies pasture carrying capacity for management purposes, the grazing intensity determines the time animals spend on a single pasture. Under the highest grazing intensities, cattle consume all available forage in 1–5 days and must be rotated to different pastures.30–35

In relation to manure-derived contaminants, the average manure age on the land surface decreases as grazing intensity increases, which results in higher TBA metabolite concentrations available for leaching and increased transport in irrigation runoff.27 To evaluate a high risk transport scenario (i.e., irrigation immediately following high intensity (∼500 AUD/ha) grazing), the pasture was irrigated immediately after the heifers were removed, also a typical management practice. Irrigation water was applied at sunrise to minimize direct sunlight exposure and potential phototransformation.15 As the wetting front moved down slope, we collected the “first flush” (i.e., initial runoff, t = 0) and subsequent samples at t = 15, 30, 60, and 90 min for each of the four runoff-collector trays (i.e., upslope and 0.3, 3, and 30 m downslope) and opportunistically collected water samples of off-field runoff at the Parshall flume. The 4-L samples were collected and processed in the field/laboratory for TBA metabolites, nutrients (i.e., total ammonia, dx.doi.org/10.1021/es503406h | Environ. Sci. Technol. 2014, 48, 12569–12576

Figure 1. (a) Schematic diagram of vegetative filter strips (VFSs) of 2 m width × 3, 4, and 5 m length. On 16 March, 2012, 4 kg-ww of manure (depicted by dark gray rectangle, not drawn to scale) was placed at the top or bottom of 3 VFS whereas on 2 December, 2012, manure was only placed at the top (specific VFS ID number in parentheses). Rainfall runoff was collected in aluminum troughs at the bottom of each strip. (b) Irrigation water was applied to a 0.1 ha pasture (wetted area, defined by outline) within the Scott-l watershed of SFREC. A ∼48 m² enclosure was subjected to irrigation flow. The water flow path is estimated by the dotted line. Water was collected at five sampling locations (circles): 1 m upslope of the enclosure and 0.3, 3, and 30 m downslope within the flowpath and within a drainage ditch outside of the wetted area.
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nitrate, nitrite, and orthophosphate), dissolved organic carbon (DOC), total coliforms, and *E. coli* as previously described. All coliform and most nutrient and DOC samples were analyzed within 24 h (see SI for details). TBA metabolite samples (1 L) were pressure filtered (0.7 μm AP40 filters, Millipore, Billerica, MA), spiked with 1 mL of 100 μg/L (i.e., 100 ng) of 17β-TBOH-d3 internal standard in methanol, and immediately loaded onto 6 mL C-18 solid phase extraction (SPE) cartridges (Ristek, Bellefonte, PA, U.S.A.; Q < 10 mL/min) within 4 h of sample collection. SPE cartridges were stored at 1 °C prior to elution. Subsequent analytical methods are described elsewhere. Samples were not analyzed for TBA.

**Rainfall Transport.** Prior to rainfall events (12–24 h), groundwater was used to saturate VFSs to promote runoff. We attempted to evaluate TBA metabolite transport for 6 rainfall events, but runoff was only generated during two events (March 16 and December 2, 2012), during which, 4 and 19 kg-wet weight (ww) of manure, respectively, was applied to each of 3 VFSs (Figure 1). The manure density on these plots ranged from 0.4 to 3.2 kg-ww/m2 corresponding to an average 24-h stocking density of 110–620 AU/ha (see SI for calculation).

Previously, we reported on the rainfall leachable mass of TBA metabolites from 1 kg-ww samples (estimated interfacial area = 590 cm2) from these same events. We used this information to linearly estimate the total TBA metabolite mass leached from the 4–19 kg-ww of manure applied to each VFS (see SI for estimates). For March 16, manure was placed at the top and bottom of different VFSs (Figure 1); therefore, assuming no treatment occurred when manure was placed at the bottom of the VFS, we estimated the treatment efficiency by comparing runoff concentrations when manure was placed at the bottom and top of each VFS. For December 2, manure was only placed at the top of each VFS (Figure 1), so no direct estimate of treatment efficiency was made. At runoff inception, 4 L water samples were collected from each VFS at 30 min intervals throughout each event. Depending on the intensity, bottles were filled in 1–5 min. Runoff samples were immediately processed onsite as previously described, although nutrients, DOC, and pathogens were not analyzed for rainfall events.

**Quality Control.** QA/QC measures included field blanks (i.e., irrigation and rainwater) and laboratory spikes (1 mL of 100 μg/L [i.e., 100 ng] of 17α-TBOH, 17β-TBOH, and TBO), and all collected samples were analyzed in triplicate. Confidence intervals throughout represent 95% confidence intervals unless otherwise noted. All field blanks were below the limit of detection. TBO was not detected in irrigation or rainwater, but analysis was complicated by a coeluting interference precluding TBO quantification in surface runoff samples. Therefore, TBO data are not discussed within. In all samples, the average 17β-TBOH-d3 recovery was 91 ± 13% (n = 109), and reported concentrations were corrected using 17β-TBOH-d3 recoveries, but not spike recoveries. Spike recoveries of 17α-TBOH, 17β-TBOH, and TBO were 109 ± 17%, 83 ± 14%, and 103 ± 38%, respectively (n = 5).

**RESULTS AND DISCUSSION**

**Irrigation Transport.** Upon flood irrigation, at 4 and 19 min respectively, irrigation water entered and exited the enclosure (wetting front velocity ≈ 0.67 m/min). During this initial period, constituents leached from manure were quickly infiltrated into the subsurface with the wetting front, thus downslope transport occurred as the wetting front left the enclosure. At 0.3, 3, and 30 m down slope of the enclosure, runoff began at t = 19, 41, and 70 min, respectively. Irrigation ceased after 150 min, and after 210 min, the drainage-ditch flows returned to baseline levels. The total runoff volume from the pasture was 17.9 m3. Therefore, the bulk runoff rate was 0.8 cm/h and the bulk infiltration rate was estimated by difference as 0.3 cm/h, suggesting that ~27% of the applied water infiltrated. At the end of the experiment, the estimated wetted area in the pasture was 0.1 ha.

In background samples, irrigation water, and upslope samples, 17α-TBOH and 17β-TBOH were not detected. After cattle were removed from the enclosure but prior to irrigation, manure samples were collected and analyzed as previously described. Concentrations of 17α-TBOH were 14 ± 3 ng/g-dry weight (dw), while 17β-TBOH was not detected. Concentrations of 17α-TBOH within the wetting front at 0.3 m downslope of the enclosure were 11.0 ± 0.3 ng/L (Figure 2).

**Figure 2.** Manure-derived constituents in runoff (a) 1 m upslope of the enclosure, and (b) 0.3 m, (c) 3.0 m, and (d) 30 m down slope of the enclosure. All concentrations were normalized to the concentrations measured within the first flush (values shown in parentheses in legend) at 0.3 m down slope of the enclosure. Error bars represent 95% confidence intervals (n = 3), and the vertical line indicates the temporal movement of the wetting front. Abbreviations include dissolved organic carbon (DOC) and total coliforms (TC).

Concentrations here subsequently decreased with time, falling below 1 ng/L within 60 min of the first flush. At 3 m downslope, 17α-TBOH concentrations within the wetting front dropped by 82% to 2.0 ± 0.1 ng/L, indicating the importance of downslope attenuation processes during ~3 m of overland flow. After 30 min of runoff, concentrations dropped to <1.0 ng/L. At 30 m downslope, 17α-TBOH was not detected in any sample.

At all pasture locations, 17α-TBOH concentrations exceeded the likely NOAEL (1 ng/L) for less than 60 min, indicating that the “first flush” contains the highest TBA metabolite concentrations and likely presents the greatest threat to receiving waters. Despite no-detects in manure samples, 17β-TBOH was detected within the wetting front at 0.3 m at 10 ± 4 ng/L and at 3 m at 1.0 ± 0.1 ng/L. Because manure was collected from only the most intact, recently excreted samples, we speculate that sampling bias may have limited 17β-TBOH detections in manure resulting from interconversion of 17α-TBOH in older, physically dispersed samples.  

metabolites were not detected in the drainage ditch, suggesting complete attenuation of TBA metabolites during 60 m of transport. Mechanistically, these concentration reductions are consistent with differential leaching, surface partitioning, and infiltration, but we cannot completely exclude dilution as a factor in these detections (see SI for further discussion).

**Leaching Predictions.** To investigate leaching dynamics, we utilized the following equation to predict the irrigation runoff concentration ($C_f$) of 17α-TBOH (modified from eqs 5–8 in Jones et al.):

$$C_f = \frac{AMS V^{-1}}{4\pi f} \left( \frac{64D}{\alpha f} \right)^{1/2} \varphi \epsilon_m \left( \frac{1}{1 - \varphi + K_D \times 1} \right)^{1/2}$$

where $A$ is the interfacial manure/water surface area (cm$^2$/kg-dw), $M$ is the manure mass excreted onto the land surface (kg-dw/AU), $S$ is the stocking density (AU/ha), $V$ is the applied irrigation volume (L/ha), $C_{m}$ is the total mass of 17α-TBOH in manure (ng/g-dw), $t$ is the total manure/water contact time (s), and physical/chemical constants associated with 17α-TBOH and manure include $D$ (diffusivity; cm$^2$/s), $f$ (dissolved fraction of TBA metabolites; unitless), $K_D$ (manure/water equilibrium partitioning coefficient; cm$^3$/g) and $\varphi$ (porosity; unitless) (see SI for derivation and Jones et al. for typical constant values). Using eq 1, we estimated the 17α-TBOH concentration within the wetting front immediately downslope of the enclosure to be $\sim$3 ng/L, which is $\sim$4 fold less than what was measured (11 ng/L). Though similar, the disparity between observed and modeled results is primarily due to uncertainty in manure/water interfacial area estimates. Within the enclosure, it was readily apparent that the manure was physically disturbed by the cattle, thus substantially increasing the interfacial area. This effect may have implications for manure fertilized fields where soil incorporation substantially increases interfacial areas and likely leaching potentials. To characterize relative leaching predictions for manure-derived constituents, accurate interfacial surface area estimates are necessary and are likely an important aspect to understanding contaminant dynamics in agricultural systems.

Although this analysis is sensitive to estimates of interfacial surface area, eq 1 is mechanistically based and is appropriate for estimating runoff concentrations of constituents based solely on physical/chemical properties and agricultural practices. As a first approximation, we assumed the disparity between observed and estimated leaching arose from errors in the interfacial area, so we scaled eq 1 by a factor of 4 and rearranged it to predict the threshold manure concentration required to exceed NOAELs (substituted for $C_f$) in runoff:

$$C_m = \frac{C_f V}{AMS} \left( \frac{64D}{\alpha f} \right)^{-1/2} \varphi^{-1} \left( \frac{1}{1 - \varphi + K_D \times 1} \right)^{-1} t^{-1/2}$$

Using a Monte Carlo simulation ($n = 1000$ iterations) and using a range of values for each parameter, 17α-TBOH concentrations in manure exceeding 2.04 ± 0.04 ng/g-dw should result in runoff concentrations $>1$ ng/L within the first 15 min of runoff. At 113 days postimplantation, measured concentrations of 17α-TBOH in manure were 10 ± 1 ng/g-dw, indicating that runoff concentrations have the potential to exceed NOAELs during irrigation through a significant portion of the implant life. For 17α-TBOH, 17β-TBOH, and TBO, concentrations are only expected to drop below 2.0 ng/g-dw threshold values after 129, 38, and 4 days, respectively. This analysis suggests that 17α-TBOH and 17β-TBOH can contribute to the endocrine disrupting potential of irrigation runoff for $\sim$95 and 30% of the implant life, respectively, while TBO has little risk of exceeding NOAELs in runoff.

For comparison, these approaches can easily be extended to estimate mass leaching and risk potentials for other steroids excreted by livestock. For estrone (E1), 17β-estradiol (E2), and estriol (E3), predicted NOAELs are 6, 2, and 60 ng/L, respectively. Therefore, we estimate that NOAEL thresholds will be exceeded when E1, E2, and E3 manure concentrations exceed 13.8 ± 0.4, 4.6 ± 0.2, and 460 ± 15 ng/g-dw, respectively. While manure concentrations are infrequently reported, reported concentrations for E1 (<26 ng/g-dw) and E2 (<20 ng/g-dw) were 2–5 times higher than threshold concentrations, indicating that these endogenous estrogens also contribute to the endocrine disrupting potential in first flush runoff samples.

We emphasize that this methodology is mechanistically based and can be applied to other contaminant sources (e.g., biosolids) in addition to any compound (e.g., antibiotics) with available values for the diffusion coefficient and $K_{oc}$ (see SI Table S1 for typical values) given that their fate and transport is dominated by similar mechanisms. Therefore, eq 2 is a potentially powerful tool for generalizing contaminant occurrence in runoff given accurate estimates of the manure/water interfacial area. For example, for a steroid with $K_{oc} = 2.0$ and NOAEL = 25 ng/L, runoff concentrations will exceed NOAELs once manure concentrations exceed $\sim$11 ng/g-dw. Conversely, for a steroid with a similar NOAEL but $K_{oc} = 4.5$, NOAELs will not be exceeded until manure concentrations exceed $\sim$350 ng/g-dw (Figure 3). This result is expected; as sorption affinity increases, the ecological risks associated with transport decrease. Given their moderate affinities for organic carbon ($K_{oc} \approx 2.5–4$, SI Table S1)

![Figure 3. Modeled (eq 2) relationship between steroid manure concentrations, organic carbon partitioning coefficients ($K_{oc}$), and resulting initial runoff concentration. Manure concentrations were estimated (see text and SI ) using typical values for physical/chemical constants as well as the management parameters implemented during the irrigation experiment. The interfacial area was assumed at 20 240 cm$^2$/kg-dw manure, which is 4 times higher than the idealized cylinder case (see text). These figure outputs can be scaled linearly using different management conditions (see SI ).](dx.doi.org/10.1021/es503406h)
and reported manure concentrations generally range from \(<1\)–20 ng/g-dw \(^{21–23,26–28}\) first flush runoff concentrations for steroids are expected to range from \(<1\)–25 ng/L in most scenarios. Some steroids like 17\(\alpha\)-TBOH and progesterone can attain 50–150 ng/g-dw \(^{21,27}\) suggesting that leachate concentrations can reach \(<100\) ng/L in irrigation runoff. However, for dairy cattle, reported manure concentrations can exceed 1000 ng/g-dw, particularly for estrogens \(^{9,10,40}\) yielding substantially higher runoff concentrations (100–1000 ng/L) at similar stocking densities. Because the risk associated with steroids in runoff scales linearly with manure concentration, this analysis would suggest that dairy cattle pastures may exhibit higher potentials for discharges of certain steroids relative to pastures with beef cattle.

While these data suggest that their transport potential is low on rangelands and irrigated pastures, this does not necessarily imply that the risk associated with TBA use on these systems is always minimal. Runoff concentrations are a function of both leaching and attenuation potentials. When leaching increases and attenuation decreases, runoff concentrations would increase. This is expected when cattle have direct access to water (e.g., ponds, lakes, streams, etc.), which can be common, or when standing water exists on fields. In these scenarios, the risks associated with TBA use increase substantially because mass transfer limitations to leaching cease. Furthermore, some attenuation processes (e.g., sorption, transformation, and infiltration) also may be substantially reduced or bypassed entirely during long-term direct manure-water contact.\(^{22,27,28,37,41,44}\) Upon the basis of previous data,\(^{27,28}\) we predict that the mass of TBA metabolites and other steroids that can impact surface waters is \(<40\) times higher when cattle have direct water access compared with access-restricted scenarios (see SI for calculation). Therefore, for a range of contaminant issues, management practices should focus on preventing direct access to receiving waters.

We compared steroid data to other manure-derived constituents. Compared to 17\(\alpha\)-TBOH, the data for most of these constituents, particularly ammonia, exhibited similar leaching and attenuation patterns (Figure 2), consistent with previous data.\(^ {28}\) Background concentrations for total ammonia were 0.07 ± 0.03 mg NH\(_3\)-N/L, while concentrations in the first flush at 0.3 m downslope increased to 10.70 ± 0.01 mg NH\(_3\)-N/L, then dropped to 4.0 mg NH\(_3\)-N/L and 1.3 mg NH\(_3\)-N/L after 15 and 90 min. At 30 m downslope, ammonia was not detected, suggesting attenuation by sorption or subsurface infiltration. Concentrations of nitrate, orthophosphate, DOC, and total coliforms peaked in the first flush at 0.3 m downslope (0.85 ± 0.05 mg NO\(_3\)-N/L, 1.48 ± 0.01 mg PO\(_4\)-P, 85 ± 1 mg C/L, 2.0 × 10\(^8\) CFU/100 mL, see SI Figure S1) and decreased with subsequent transport. Unlike 17\(\alpha\)-TBOH and ammonia, each were present in the wetting front at 30 m (0.17 ± 0.05 mg NO\(_3\)-N/L, 0.10 ± 0.07 mg PO\(_4\)-P/L, 24.4 ± 0.3 mg C/L, and 1.2 × 10\(^7\) CFU/100 mL), but were quite similar to background concentrations at the same location (0.30 ± 0.05 mg NO\(_3\)-N/L, 0.21 ± 0.06 mg PO\(_4\)-P/L, 25.4 ± 0.1 mg-C/L, 1.0 × 10\(^7\) CFU/100 mL). This suggests that the pasture was a source for these constituents, and the data also are consistent with published observations for irrigated pasture runoff.\(^ {29,38,43–46}\) Most management practices have focused on minimizing nutrients and sediments transport from agro-ecosystems. Because the fate and transport of 17\(\alpha\)-TBOH is reasonably well correlated with these constituents, these data indicate that management strategies used to control ammonia, orthophosphate, and DOC also should control TBA metabolite transport with similar efficacy.

**Rainfall Transport.** We placed manure from TBA-implanted heifers on VFSs and collected runoff during storms to evaluate the rainfall-mediated transport potential. On March 16, when manure was placed at the bottom of the VFS, measured runoff concentrations of 17\(\alpha\)-TBOH were 9 ± 2 ng/L (Figure 4; 17\(\beta\)-TBOH was not detected) while predicted concentrations based on the ratio of the estimated mass leached and the bulk rainfall volume were 6 ng/L. When manure was placed at the top of the VFSs, no TBA metabolites were detected suggesting that the VFS treatment efficiency likely exceeded 95% in order to reduce concentrations below the limit of detection (i.e., <0.5 ng/L; Figure 4). This is consistent with observed ∼70–90% removal of 17\(\alpha\)-TBOH removal from irrigation experiments on these VFSs.\(^ {28}\) On December 2, measured rainfall runoff concentrations from all VFSs ranged from no detects -7 ng/L while predicted concentrations were 1–3 ng/L (Figure 4, see SI). Within a single sample, 17\(\beta\)-TBOH was detected at 2 ng/L. Despite high equivalent 24-h stocking densities during these events (∼110–620 AU/ha), average measured runoff concentrations following VFS treatment were <2 ng/L. Because the likely NOAEL of 17\(\alpha\)-TBOH is 1 ng/L, we expect little risk associated with TBA metabolites transport under similar rainfall scenarios, especially considering likely watershed-scale dilution capacities.

For our data, the mass leached divided by the bulk rainfall volume that fell onto each VFS was a surprisingly accurate method for predicting runoff concentrations. Therefore, using a previously developed rainfall leaching model\(^ {27}\) and the volume of water than can fall on 1 ha, we estimated rainfall runoff concentrations normalized to 1 AU/ha stocking density (see SI). For a 40 mg TBA implant, at any day post implantation, the average 17\(\alpha\)-TBOH mass that can leach from manure accumulated on the land surface during a hypothetical 1 or 10 cm rainfall event is ∼2100 and 30 000 ng/AU, respectively (see SI Figure S2). When these estimates are normalized to rainfall volumes of rain per hectare (10\(^8\) and 10\(^9\) L), the estimated 17\(\alpha\)-
TBOH concentration in runoff from a 1 ha pasture is 0.021 and 0.030 ng/L. Despite the wide range in the mass leached, the estimated concentrations are surprisingly insensitive to rainfall depth because of observed near-linear relationships between rainfall and mass leaching, implying that additional dilution nearly balances increased mass transfer.27 This suggests that unlike irrigated pastures where the risk was primarily associated with the first flush, rainfall runoff concentrations are more independent of hydrologic dynamics and should be more constant during storms, which is supported by our observations (Figure 4).

Because these functions are normalized to 1 AU, these estimates can be linearly scaled based on the stocking density to predict runoff concentrations for other animal agriculture operations. The above runoff estimate (0.021–0.030 ng/L) was based on the mass that can leach from a single animal unit. At 30 AUM, near the maximum carrying capacity for continuously grazed pastures, and assuming a first-order transformation rate constant for pastures of 0.17/d,28 the maximum runoff concentration of 17α-TBOH from a 1 ha pasture under normal operating conditions during a 1–10 cm rainfall event is estimated at ∼0.6–0.9 ng/L (Figure 5). Applying 80% attenuation in runoff concentration using VFS techniques, it is reasonable to expect off-field discharge concentrations <0.2 ng/L in rainfall runoff. However, this analysis does not account for system specific heterogeneity, for example if cattle use pastures in a nonrandom manner and congregate near water sources, shade, salt blocks, etc.

Extended these results conceptually to other animal agriculture operations and TBA doses can be accomplished by assuming 17α-TBOH concentrations in manure scale linearly with TBA dosage. For a 200 mg TBA implant, typical for CAFOs, and assuming a first-order transformation rate constant for CAFOs of 0.028/d,22 yields the average 17α-TBOH mass leached during a 1–10 cm rainfall event of ∼74 000–1 000 000 ng/AU (Figure S2, see SI for calculations). Similarly, normalizing by rainfall volume per hectare, the average estimated 17α-TBOH concentration in CAFO runoff from a stocking density of 100–1000 AU/ha is ∼74–1000 ng/L (Figure S). For an 80 mg TBA implant, the average expected runoff concentration from a CAFO would range from 26 to 370 ng/L. Generally, these numbers are consistent with reported TBA metabolite concentrations in rainfall-induced runoff from CAFOs.21,22,25

For comparison, and again as a first approximation, we also estimated potential 17α-TBOH mass leaching from manure-fertilized fields based on manure mass typically applied for fertilization. Upon the basis of crop nutrient requirements (thus fixing manure application rates), equivalent stocking densities for manure-fertilized fields are 50–100 AU/ha (see SI for calculation). Assuming 0–6 months of manure storage and a typical first order transformation rate constant of 0.0026/d,36 the expected 17α-TBOH manure concentration is 11.5 ng/g-dw for 40 mg TBA implants. Scaling to 80, 140, and 200 mg TBA implants, for 1–10 cm rainfall, 17α-TBOH runoff concentrations are expected to range from 42 to 340 ng/L. We note that these estimates do not account for subsequent attenuation, but merely approximate the relative upper bounds for potential discharges. This analysis, based upon relative areal intensities of animal agriculture operations, suggests that in terms of initial risk to receiving waters, the potential for significant discharges are likely greatest for CAFOs, followed by fertilized fields, and then rangelands, although more accurate estimates of discharges obviously depend on system-specific characteristics and better accounting for some of the uncertainties like interfacial area inherent in the analysis.

Agro-Ecosystem Risk. Within this study, the concentrations of TBA metabolites, predominately 17α-TBOH, were no-detect to 11 ng/L in irrigation and rainfall runoff, generally lower relative to concentrations reported for other animal agriculture systems.3,21–24 Thus, this analysis suggests that for well-managed pastures (i.e., grazing intensity below carrying capacities, VFS management, no direct access to surface waters), negligible transport of TBA metabolites from rangelands and pastures is expected. Although we expect that average runoff concentrations for TBA metabolites (and other steroids) to be below NOAELs, concentrations can exceed threshold levels within the first flush for short periods of time (30–60 min) during irrigation (Figure 2). For example, 17α-TBOH first flush concentrations could exceed NOAELs during any irrigation event for 127 days postimplantation. Given that irrigation typically lasts 6–12 h, we predict that as long as the first flush is managed well or is subject to enough dilution, any ecosystem risks associated with these steroids is probably transient at best for most systems. Restricting direct access to water and maximizing the transport distances to receiving waters are likely the best management practices to eliminate any risk from TBA metabolites or other steroids from rangelands and irrigated pastures. For manure fertilized fields, which have intermediate stocking densities, or incidental discharges from CAFOs, some management strategies or dilution capacities to attain threshold values are likely more important.

Figure 5. (a) Modeled concentrations of 17α-TBOH in runoff from a 1, 5, and 10 cm rainfall events on a 1 ha pasture for various agricultural scenarios. Concentrations were calculated based on the ratio of the average mass leached from manure accumulated on the land surface at any day post implantation to the rainfall volume per hectare. Leaching estimates were made for different TBA implant doses (40, 80, 140, and 200 mg TBA), which are bracketed in (a) for illustration. Brackets were left off in (b) but the same relative grouping applies. Under all pasture/rangeland scenarios, 17α-TBOH concentrations were below the likely NOAEL (1 ng/L) and do not appear on this figure. (b) Rainfall runoff concentrations were scaled by assuming 80% treatment effectiveness to demonstrate the impact of runoff management (see SI Figure S3 for English units).
Finally, we believe that potential retained bioactivity in transformation products may be one remaining uncertainty that is currently unaddressed for endocrine active steroids in agroecosystems. For example, on the basis of our studies and published literature, we can only account for ~10% of the total TBA implant mass that is excreted as the known metabolites 17α-TBOH, 17β-TBOH, or TBO. Thus, over 90% of the TBA implant mass remains uncharacterized with respect to potential endocrine activity.27,30 The selective application of bioanalytical tools to address residual bioactivity of nontarget constituents in these complex leachate mixtures is likely merited.

**ASSOCIATED CONTENT**

* Supporting Information

Experimental data, including site characteristics, chemicals, contaminant analysis, carrying capacity and stocking densities; discussion and supporting text for calculations, including attenuation mechanisms, irrigation leaching: model development, parameter estimates, Monte Carlo simulations, leaching in fully submerged conditions, and rainfall leaching; total coliform (TC) and *E. coli* pollutagenics in runoff (Figure S1); estimated mass leached from a 1–10 cm rainfall event from manure accumulated on the land surface that was excreted from a single animal unit at any day post implantation (Figure S2); modeled concentrations of 17α-TBOH in runoff (Figure S3); steroid hormone parameters used in eq 3 (Table S1); and additional references. This material is available free of charge via the Internet at http://pubs.acs.org.

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**Notes**

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