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Sorption and Mineral-Promoted Transformation of Synthetic Hormone Growth Promoters in Soil Systems

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Ⓢ *Supporting Information*

ABSTRACT: This work examines the fate of synthetic growth promoters (trenbolone acetate, melengestrol acetate, and zeranol) in sterilized soil systems, focusing on their sorption to organic matter and propensity for mineral-promoted reactions. In organic-rich soil matrices (e.g., Pahokee Peat), the extent and reversibility of sorption did not generally correlate with compound hydrophobicity (e.g., K_{ow} values), suggesting that specific binding interactions (e.g., potentially hydrogen bonding through C17 hydroxyl groups for the trenbolone and melengestrol families) can also contribute to uptake. In soils with lower organic carbon contents (1−5.9% OC), evidence supports sorption occurring in parallel with surface reaction on inorganic mineral phases. Subsequent experiments with pure mineral phases representative of those naturally abundant in soil (e.g., iron, silica, and manganese oxides) suggest that growth promoters are prone to mineral-promoted oxidation, hydrolysis, and/or nucleophilic (e.g., H_2O or OH^-) addition reactions. Although reaction products remain unidentified, this study shows that synthetic growth promoters can undergo abiotic transformation in soil systems, a previously unidentified fate pathway with implications for their persistence and ecosystem effects in the subsurface.

KEYWORDS: *animal agriculture, endocrine disruptors, environmental fate, adsorption, transformation products*

■ INTRODUCTION

The widespread use of the endocrine-active compounds trenbolone acetate (TBA), melengestrol acetate (MGA), and zeranol (Table 1) as FDA-approved growth promoters in animal agriculture[1−3] has resulted in their detection in soil at levels on the order of 1−100 ng/kg.[2,4−6] There, biotransformation is believed to be the major sink of these synthetic growth promoters and their metabolites (hereafter simply SGPMs). For example, Khan et al.[7] reported half-lives of 17α-trenbolone (17α-TBOH), 17β-trenbolone (17β-TBOH), and trendione (TBO) between 4 and 100 h in different aerobic soil microcosms, observing some variation in transformation rates with TBA metabolite concentration.

Sorption represents a critical determinant not only for the rate of SGPM biotransformation in soils (i.e., sorbed phases are generally assumed less bioavailable) but also for their transport potential in groundwater or other receiving waters. To date, sorption of SGPMs has often been attributed to hydrophobic partitioning, with experimental metrics of sorption [e.g., equilibrium coefficients from linear (K_d values) or Freundlich (K_f values) isotherms] generally scaling with measures of soil organic carbon content (OC).[8,9] However, because many SGPMs and related steroid hormones exhibit logK_{ow} values suggesting only moderate hydrophobicity (e.g., the TBA family; see Table 1), nonhydrophobic interactions [e.g., specific interactions such as hydrogen bonding via hydroxyl (H-bond donor) and/or keto (H-bond acceptor) moieties,[8,10] electron donor−acceptor interactions,[11] and aromatic-type interactions[12,13]] may also contribute to their uptake in some soil systems. For instance, Qiao et al.[14] measured the association of 17α-TBOH, 17β-TBOH, and TBO with two commercial humic acids, observing nonlinear isotherms consistent with some contribution of site-specific electrostatic and H-bonding interactions during partitioning. Furthermore, greater uptake of 17β-TBOH relative to 17α-TBOH occurred, a trend notably inconsistent with their relative retention times on a C18 liquid chromatography column when using a polar mobile phase. This provided yet another line of evidence that specific binding interactions were likely important, at least for the TBA family.

Ultimately, questions persist as to what characteristics of SGPMs and soil are most critical for sorption, particularly for species not yet the focus of experimental investigation including metabolites of zeranol and derivatives of melengestrol acetate (MGA). Notably, another factor rarely, if at all, addressed in prior studies with SGPMs[8,9,14,15] is the degree to which sorption exhibits thermodynamic reversibility, a necessary detail for predicting SGPM transport through soil and subsurface systems.[16] As different interactions of various strengths have previously been proposed to drive their sorption, it is reasonable to expect that the extent of reversibility will differ across SGPMs and even within families of SGPMs.

Finally, another poorly understood factor to date is the influence of inorganic mineral phases (e.g., metal oxides and clays) on SGPM fate in soils. Such phases may function as sorbents, as has been reported for estrogens on clays and metal

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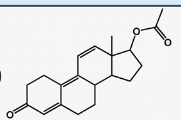
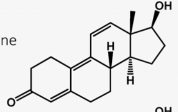
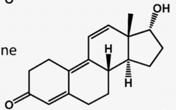
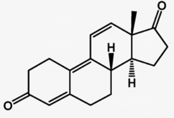
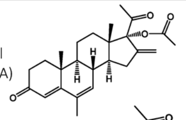
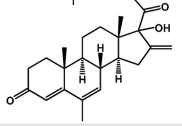
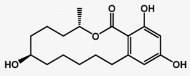
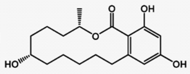
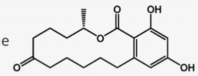
Table 1. Structures and Properties of SGPMs Used in Sorption Studies

Name	Structure	CAS Number	MW (g/mol)	logK_{ow}	Estimated[a] Solubility (mg/L)
Trenbolone Acetate (TBA)		10161349	312.4	4.21[b]	7.9
17β-trenbolone (17β-TBOH)		10161338	270.4	3.08[c]/3.09[b]	320
17α-trenbolone (17α-TBOH)		80657176	270.4	2.72[c]	320
Trendione (TBO)		4642959	268.4	2.63[c]	280
Melengestrol Acetate (MGA)		2919666	396.5	3.93[c]	0.55
Melengestrol		5633181	354.5	3.39[b]	10.2
Zeranol		26538443	322.4	3.88	2.9
β-zearalanol		42422684	322.4	3.29	2.9
Zearalanone		5975780	320.4	4.10	2.5

[a] Aqueous solubilities were estimated via the U.S. EPA EPISuite using WSKOW (v. 1.41).[36] [b] Experimentally measured in this study. [c] Value reported by Card et al.[9]

oxides,[17] via mechanisms including electrostatic interactions and surface complexation. Alternatively, they may represent reactive substrates able to promote transformation reactions. For example, MGA and the zeranol family have hydrolyzable functionalities, whereas the 4,9,11-triene structure of trenbolone includes several electron-deficient carbon centers possibly susceptible to nucleophilic attack. Although hydrolysis is reportedly slow at near-neutral pH values for SGPMs,[18−20] metal oxides common to soil can catalyze hydrolysis, and presumably nucleophilic addition, via the formation of surface complexes that leave carbon centers more favorable to attack by H_2O or OH^- than the freely dissolved molecule.[21] Furthermore, manganese oxides and some iron oxides can oxidize species structurally analogous to SGPMs[22,23] including estrone and 17β-estradiol. Although such mineral-promoted transformation pathways remain uncharacterized, we suspect some SGPMs are highly reactive toward inorganic phases routinely encountered in soil.

Here, we explore the abiotic pathways influencing SGPM fate in soils. The rate and extent of SGPM sorption were considered in sterilized (with azide) systems using a model, organic-rich soil (Pahokee Peat, 43% organic carbon) and several commercially available model soils exhibiting a range of soil organic carbon (1.0−5.9% OC). Sorption kinetics and isotherms were used to examine trends both within (e.g., 17*α*-TBOH, 17*β*-TBOH, and TBO) and across SGPM families (e.g., comparing TBA metabolites, MGA and melengestrol, and metabolites), explore the nature of chemical interactions (e.g., specific or hydrophobic) contributing to uptake, and establish the extent to which SGPM sorption is reversible. We emphasize that in all model soil systems, solvent (i.e., methanol) extraction of equilibrated soils was used to recover the mass of sorbed SGPM, and loss of mass over time in our experimental systems was interpreted as evidence for abiotic transformation processes (i.e., reaction with inorganic mineral phases). To further explore the potential for such abiotic transformations, experiments also monitored the stability of select SGPMs (17*β*-TBOH, MGA, and zeranol) in suspensions of metal oxides (e.g., manganese, iron, and silica oxides) representative of those naturally occurring in soil. Results from this study will help to characterize more fully the environmental fate and mobility of SGPMs in subsurface environments, thereby improving our ability to predict the risk posed to ecosystems by this emerging pollutant class.

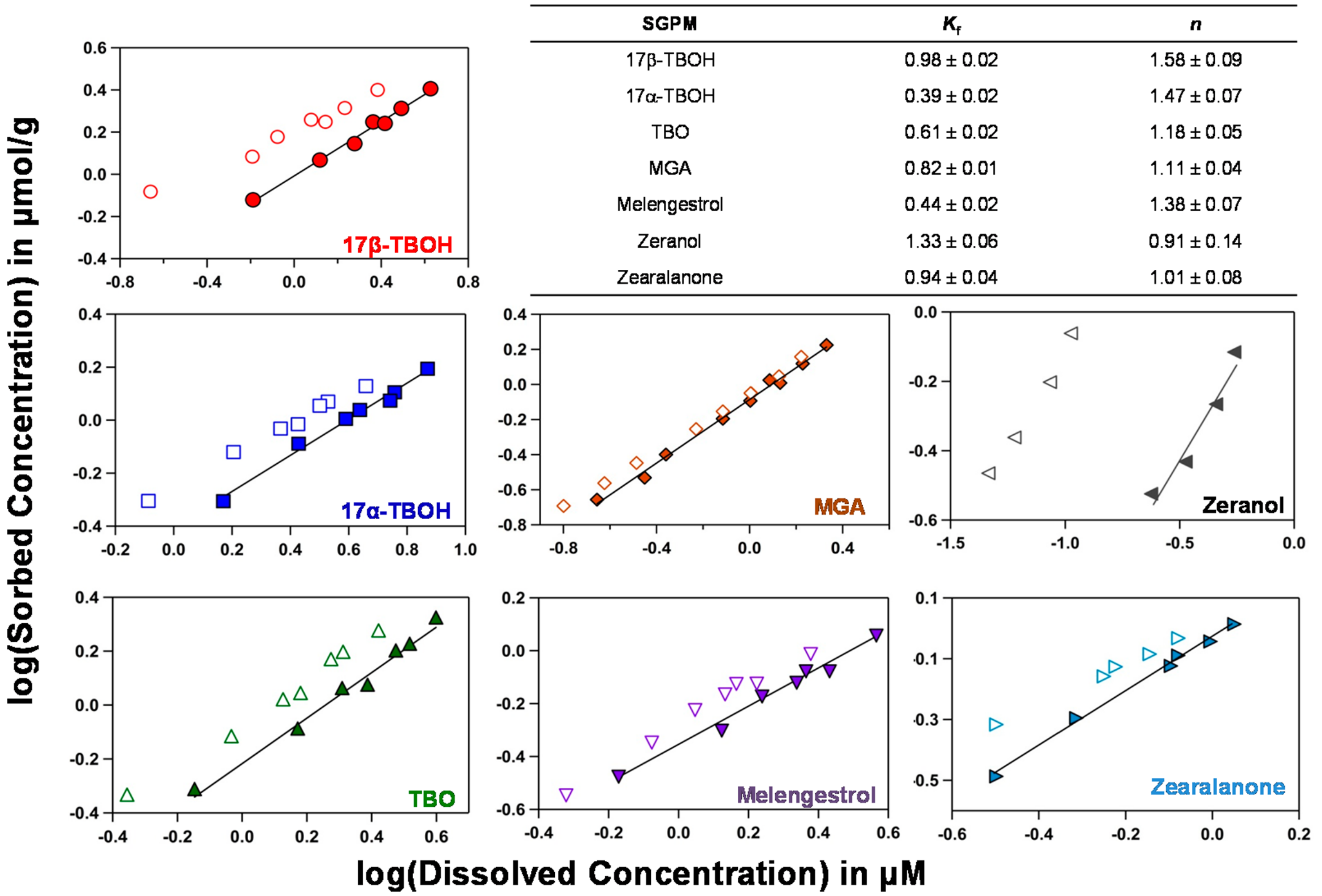
■ MATERIALS AND METHODS

Reagents. A complete reagent list is provided in the Supporting Information (SI).

Measurement of K_{ow} Values for Select SGPMs. Octanol−water partitioning coefficients (K_{ow} values) were measured for SGPMs without published values available in the literature. These were trenbolone acetate, melengestrol, zearalanone, and *β*-zeralanol. Details of our method, which was adapted from that of Khan et al.,[8] are provided in the SI. To assess the accuracy of our approach, we also measured the K_{ow} value for 17*β*-trenbolone so that our experimental value could be compared to a reference value available in the literature.

Model Soils and Mineral Oxides. Seven commercial, model soils exhibiting a range of soil OC content were used in sorption studies. Pahokee Peat (43% OC) was purchased from the International Humic Substances Society (IHSS), whereas other soils with various textures and OC contents were acquired from AGVISE Laboratories (Benson, MN, USA). These include two sandy loams (referred to as PVPF and LBLS with OC contents of 5 and 3%, respectively), two loams (PVLPF and EL-7 with OC contents of 5.9 and 2.8%, respectively), a clay loam (DUL; 3.3% OC content), and a loamy sand (RMN; 1% OC content). The OC content of Pahokee Peat was determined by Test America via the Lloyd Kahn method,[24] whereas the OC values of all other soils were provided by AGVISE Laboratories and quantified by loss-on-ignition methods.[25] Also explored were several mineral phases representative of naturally occurring metal oxides including a commercially available silica dioxide (SiO_2; Sigma-Aldrich, 99%), ferrihydrite synthesized via the method of Burleson and Penn,[26] and an amorphous manganese dioxide (MnO_2) synthesized according to the method of Murray.[27] Synthesis details are provided in the SI.

Sorption Experiments. Soils were first sterilized by adding sodium azide (NaN_3) as described by Wolf et al.,[28] with the exception that the dose was increased to 30.8 mmol NaN_3/kg soil.[29] Additional details and our rationale for sterilizing with azide as opposed to other methods are provided in the SI. Batch sorption experiments were then conducted at pH 7.0 in a 5 mM phosphate buffer. The rate of SGPM sorption was explored using initial concentrations between 2 and 5 μM and a soil loading between 2 and 10 g/L. Sorption isotherms were collected over a range of initial concentrations (1−10 μM) using a constant sorbent (i.e., soil sample or metal oxide) loading of 2 g/L at room temperature. Although these initial concentrations are higher than environmental relevance, they were necessary to facilitate SGPM analysis via high-pressure liquid chromatography with diode array detection (HPLC-DAD), which was the preferred method for processing the large number of samples generated experimentally. In



SGPM	K_f	*n*
17β-TBOH	0.98 ± 0.02	1.58 ± 0.09
17α-TBOH	0.39 ± 0.02	1.47 ± 0.07
TBO	0.61 ± 0.02	1.18 ± 0.05
MGA	0.82 ± 0.01	1.11 ± 0.04
Melengestrol	0.44 ± 0.02	1.38 ± 0.07
Zeranol	1.33 ± 0.06	0.91 ± 0.14
Zearalanone	0.94 ± 0.04	1.01 ± 0.08

Figure 1. Linearized Freundlich isotherms for SGPMs on Pahokee Peat (please note the difference in the *x*- and *y*-axes scales for each species). Solid symbols indicate measured concentrations after 3 days (sufficient to achieve sorption equilibrium), whereas open symbols correspond to data collected to test the reversibility of sorption according to the protocol described in the text. Linear regression and resulting best-fit parameters for values of K_f (in $\mu\text{mol}^{(1-(1/n))}\ L^{(1/n)}\ \text{g}^{-1}$) and *n* (unitless) are provided, along with 95% confidence intervals associated with the regression analysis. Experimental details are provided in the text.

all cases, the highest initial concentration used for each SGPM was below aqueous solubility (based upon reported values of 1330, 2.6, and 15 μM for 17α-trenbolone, MGA, and zeranol, respectively;[18−20] estimated aqueous solubilities are provided for all SGPMs in Table 1). Nevertheless, control experiments were conducted in the absence of soil to ensure the aqueous phase stability of the SGPMs at these concentrations.

To initiate sorption experiments, a small volume (2−18 μL) of SGPM methanolic stock solution was added to a suspension of sterilized soil via syringe to achieve the desired initial concentration. In all cases, this approach resulted in no more than 0.1% of methanol by volume in our reactors, below the threshold likely to induce cosolute effects.[30] To avoid sorption losses to vial septa (which was observed for some species in preliminary experiments), all experiments were conducted in 20 mL glass beakers, and the contents were well-mixed via a glass-coated, magnetic stir bar and electronic stir plate. We note that initial experiments using Teflon-coated stir bars revealed losses of some SGPMs in controls, presumably due to sorption on the stir bar. Over the duration of the experiments, reactors were sealed with Parafilm to slow evaporation and covered in Al foil to prevent photoreactions.

Sorption rate studies were typically conducted over 1 week, with sample collection at predetermined time intervals to monitor the change in SGPM solution concentration over time. For sorption isotherms, equilibrium was typically established after 3 days, at which time samples were collected to monitor the mass of SGPM remaining in solution. The extent to which sorption was reversible was considered as follows. After 3 days, the entire equilibrated suspension (18 mL) was centrifuged (3000 rpm for 5 min), and a fixed volume (typically 8 mL) of supernatant was replaced by fresh buffer to perturb the sorption equilibrium. The altered suspension was then allowed to re-equilibrate for another 3 days, at which time the aqueous phase SGPM concentration was once again measured.

Reactor samples were centrifuged, and the supernatant was analyzed immediately on HPLC-DAD to quantify dissolved phase concentrations. Centrifugation was chosen for solid removal rather than filtration because most commercially available filters (PTFE or nylon) resulted in some loss of SGPMs during application. Using this approach, the amount of SGPM sorbed to the soil was quantified via mass balance from the difference in the initial SGPM concentration in the reactor and the aqueous phase concentration of SGPM present in solution at equilibrium.

At the conclusion of sorption experiments, the mass of SGPM sorbed on the soil sample was quantified via extraction with organic solvent.[8] The entire suspension was centrifuged, the supernatant was removed, and 3 mL of methanol was added to the soil. The resulting suspension was sonicated for 5 min, which was found to be sufficient for recovery of the sorbed mass. After sonication, the suspension was centrifuged once again to separate the methanol from the solid, and the solvent phase was analyzed by HPLC-DAD.

SGPM Reactivity toward Metal Oxides. Reactions with metal oxides SiO_2 and ferrihydrite were conducted at pH 7.0 in 5 mM phosphate buffer at room temperature. For MnO_2, we observed reactivity inhibition in phosphate buffer; thus, experiments were conducted in 5 mM $NaClO_4$ at either pH 6 or 7. Experiments with metal oxides were conducted over a range of solid loadings (1−10 g/

L) and SGPM initial concentrations (1−5 μM). The oxide suspensions were allowed to equilibrate with the buffer/electrolyte solution for 20 min, after which the pH of the system was adjusted (if necessary) back to the desired initial value using 1 M HCl or NaOH. Once the pH was stabilized, a small volume of methanolic stock solution delivered the SGPM to the suspension. The suspension was well-mixed using a glass-coated magnetic stir bar and stir plate, and reactors were covered by Al foil to prevent photoreaction. Over time, 1 mL aliquots of suspension were collected and centrifuged to separate the metal oxide from the aqueous phase, and the supernatant then was transferred to an amber autosampler vial for immediate HPLC-DAD analysis.

Analytical Methods. Aqueous samples were analyzed on a 1200 series Agilent HPLC-DAD equipped with an Eclipse XBD-C18 column (4.6 × 150 mm, 5 μm particle size) according to the methods presented in our earlier work[31] and also detailed in the SI. All soils and metal oxides were characterized via powder X-ray diffraction using a Rigaku MiniFlex II with a Co Kα source.

RESULTS AND DISCUSSION

SGPM Sorption Isotherms on Organic-Rich Pahokee Peat. Sorption rate experiments suggested that most SGPMs, with the exception of β-zearalanol (see SI), achieved steady state (i.e., no further decrease in solution phase concentration over time) in Pahokee Peat suspensions within 3 days (at 2 g/L Pahokee Peat and ∼5 μM initial SGPM; Figure S1). Accordingly, sorption isotherms collected after 3 days are shown in Figure 1, in which data are presented on a log−log scale. Also shown are fits in which sorption is assumed to follow the Freundlich isotherm model (eqs 1 and 2).

$$C_s = K_f C_w^{\,1/n} \tag{1}$$

$$\log C_s = \log K_f + 1/n \log C_w \tag{2}$$

In eqs 1 and 2, C_s is the sorbed SGPM concentration and C_w is the aqueous phase concentration at equilibrium (after 3 days). The Freundlich adsorption capacity parameter, K_f, and the Freundlich adsorption intensity parameter, $1/n$, were obtained from linear regression analysis of experimental data (according to eq 2), as summarized in Figure 1 for each SGPM.

These model fit parameters were used to explore trends in SGPM sorption on Pahokee Peat. For example, K_f values indicate the magnitude of sorption,[32] and correlations between K_f values and metrics of sorbate hydrophobicity (e.g., K_{ow} values) have occasionally been suggested as evidence for hydrophobic interactions driving sorption.[33] Here, the largest K_f values (with units of $\mu\text{mol}^{(1-(1/n))}\ L^{(1/n)}\ \text{g}^{-1}$) were measured for zeranol (1.33 ± 0.06) and its metabolite zearalanone (0.94 ± 0.04), 17β-TBOH (0.98 ± 0.02), and MGA (0.82 ± 0.01). However, across families of growth promoters, octanol−water partitioning coefficients (K_{ow} values) were a weak predictor for sorption on Pahokee Peat (Figure S2a). Moreover, we failed even to observe clear trends in K_f values within SGPM families; trendione exhibited greater sorption than 17α-TBOH despite its lower K_{ow} value.

A notable and important distinction across SGPMs in Pahokee Peat systems was that some isotherms were essentially linear. The greatest degrees of nonlinearity, based on model fit values of n, were observed for 17β-TBOH, 17α-TBOH, and melengestrol, whereas the slopes of isotherms for zeranol and zearalanone were statistically equivalent to unity (i.e., their isotherms were linear). Linear isotherms are most often observed when hydrophobic interactions drive uptake and an excess of such hydrophobic sorption sites exists.[30] In contrast, nonlinear isotherms are typically observed for more polar compounds, for which specific interactions will also contribute to sorption.[30] SGPM sorption appears consistent with these scenarios, with linear isotherms most frequently observed for more hydrophobic SGPMs (based on reported or measured K_{ow} values; Figure S2b). Thus, the linear isotherms for zeranol and zearalanone suggest an excess of hydrophobic sites on Pahokee Peat suitable for partitioning, behavior likely linked to the aromatic functionalities of zeranol and it metabolites. In contrast, nonlinear isotherms for 17β-TBOH, 17α-TBOH, and melengestrol are evidence that more limited, specific sites on Pahokee Peat also contribute to sorption.

We also interpret trends in n values within a family of compounds as evidence of the functional groups involved in such specific interactions. Specifically, isotherms for 17β-TBOH and 17α-TBOH exhibited greater nonlinearity than TBO, whereas the isotherm for melengestrol exhibited greater nonlinearity than that of MGA. In each of these cases, therefore, the presence of an −OH group at the C17 position increased the degree of specific interactions. We attribute this observation to the ability of this hydroxyl group to promote sorption via hydrogen bonding with functionalities of Pahokee Peat.

Reversibility of SGPM Sorption on Organic-Rich Pahokee Peat. A final consideration for SGPM sorption in Pahokee Peat systems is the extent to which the process is reversible. Data pertaining to sorption reversibility are also presented in Figure 1. Open symbols represent data collected after system equilibrium was first disrupted after 3 days and then once again allowed to achieve steady state over an additional 3 days. In Figure 1, therefore, overlap between open and solid symbols indicates a sorption process that is entirely reversible.

MGA was the only SGPM to sorb onto Pahokee Peat reversibly (see overlap in Figure 1 of open and solid symbols), whereas sorption of 17α-TBOH, TBO, melengestrol, and zearalanone was partially reversible. Sorption of zeranol and 17β-TBOH was essentially irreversible, as evidenced by the nearly identical concentration of adsorbed species after system equilibrium was perturbed (i.e., open symbols are horizontally, but not vertically, offset from their solid counterparts). Notably, we were able to recover completely all of the sorbed mass via extraction with methanol. Measured percent recoveries in Pahokee Peat systems were 110 (±8), 99 (±7), 165 (±10), 125 (±0.12), 105 (±9), and 124 (±11) for 17β-TBOH, 17α-TBOH, MGA, melengestrol, zeranol, and zearalanone, respectively. Values represent the mean and standard deviation for at least $n = 3$ extractions (we note that only one instance of trendione extraction was completed, resulting in 115% recovery). Thus, concentration changes in Pahokee Peat systems occur via sorption and not from processes such as abiotic transformation reactions or biodegradation.

Several trends in the reversibility of SGPM sorption are noteworthy, if not surprising. MGA and zeranol have comparable logK_{ow} values, and both produced linear isotherms, collectively suggesting that hydrophobic interactions govern their sorption. However, the nature of these hydrophobic interactions must be fundamentally different because MGA uptake was entirely reversible, whereas zeranol sorption was essentially irreversible. Likewise, 17β-TBOH is among the more polar of SGPMs considered herein, and the presumed specific interactions (e.g., hydrogen bonding) associated with its sorption are typically considered relatively weak. Nevertheless, the irreversible nature of 17β-TBOH sorption implies rather

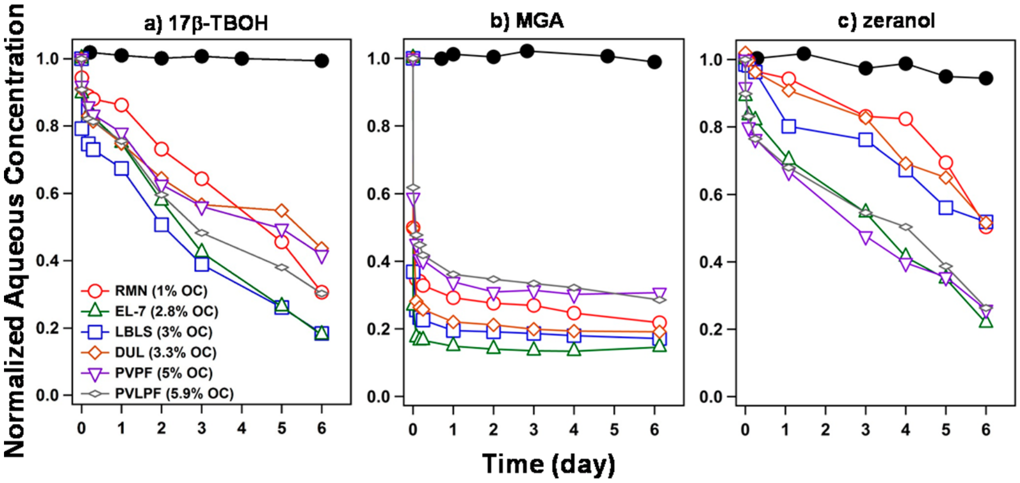


Figure 2. Change in aqueous phase concentration over time for (a) 17*β*-TBOH, (b) MGA, and (c) zeranol in 4 g/L suspensions of azide-treated model soils. Results from soil-free controls are shown as solid black symbols. All experiments were conducted in 5 mM phosphate buffer at pH 7. The initial concentrations were 4, 2, and 2 *μ*M for 17*β*-TBOH, MGA, and zeranol, respectively.

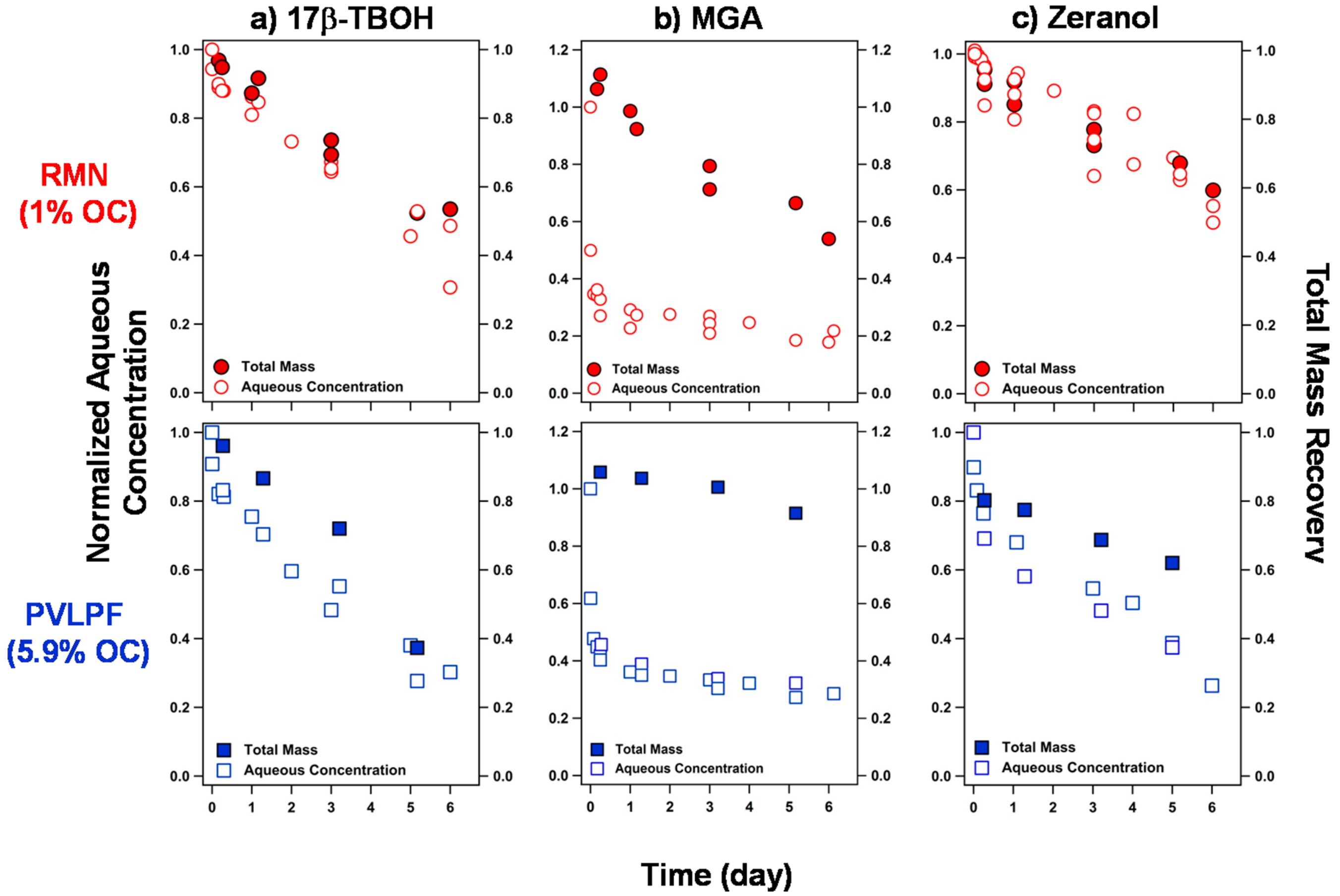


Figure 3. Aqueous phase concentration change over time for (a) 17*β*-TBOH, (b) MGA, and (c) zeranol in suspensions (4 g/L at pH 7) of two model soils, RMN (red; top) and PVLPF (blue; bottom), which represent those with the lowest (1%) and highest (5.9%) organic carbon content. Also presented (solid symbols) are data pertaining to the total mass of each species in the systems over time, which also includes the mass that was recoverable from extraction of solids with methanol.

strong binding with Pahokee Peat, perhaps suggesting multiple sorption mechanisms occurring in parallel (e.g., hydrogen bonding in combination with electron donor−acceptor interactions) to strengthen the overall degree of interaction. Ultimately, these findings complicate prediction of SGPM mobility in soils and the subsurface; not only do logK_{ow} values fail to reasonably predict the extent of sorption onto soil organic matter either within or across SGPM families, but they also appear to have little correlation with the extent to which sorption is reversible.

SGPM Sorption on Model Soils. Initial experiments with 17*β*-TBOH, MGA, and zeranol explored the time scales

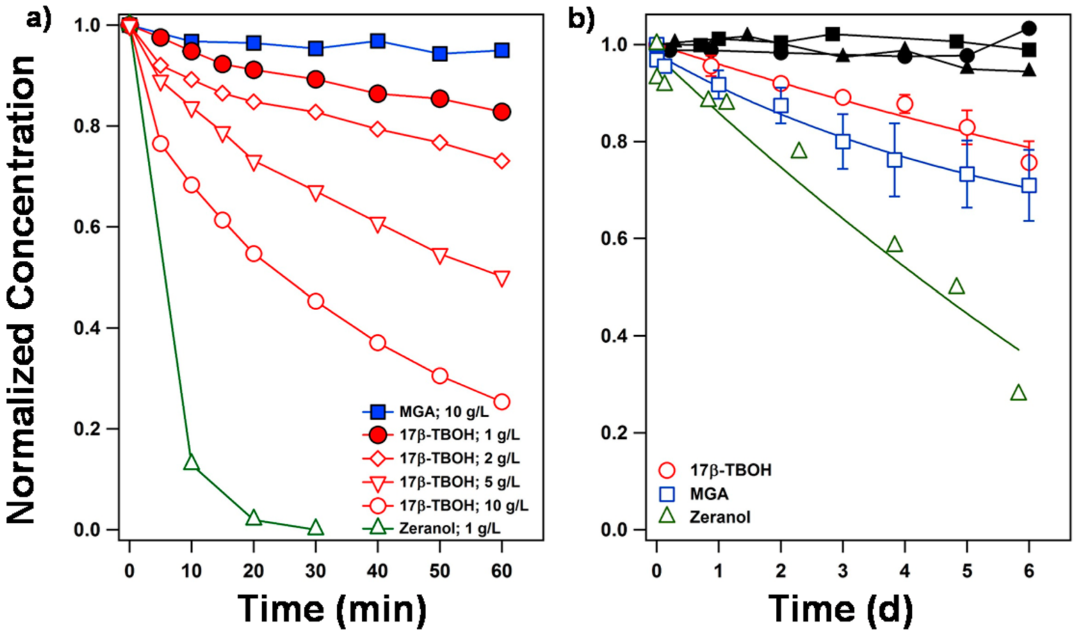


Figure 4. (a) Aqueous phase concentration change over time of MGA, 17*β*-TBOH, and zeranol in suspensions of MnO_2. For 17*β*-TBOH, data are shown for several MnO_2 solid loadings. All experiments were conducted in 5 mM $NaClO_4$ at pH 6. The initial concentrations of 17*β*-TBOH, MGA, and zeranol were 10, 2, and 2 *μ*M, respectively. All SGPMs were stable in MnO_2-free controls (data not shown). (b) Aqueous phase concentration change of 17*β*-TBOH, MGA, and zeranol in ferrihydrite suspensions (5 g/L). Reactions were conducted in 5 mM phosphate buffer at pH 7. Lines are exponential decay model fits from which half-lives were estimated. Data are also presented from control systems conducted in the absence of solid (shown as corresponding solid black symbols).

necessary to achieve sorption equilibrium in suspensions of azide-sterilized model soils (Figure 2). Over 6 days, the MGA appeared to reach steady state; after an initially sharp, nearly immediate drop, aqueous phase MGA concentration was essentially stable over the remainder of the 6 day period for all soils. However, the amount of MGA lost from solution did not scale with soil organic carbon content. Rather, if anything, we observed an approximately inverse relationship, in which soils with the highest OC content (PVPF with 5.0% and PVLPF with 5.9%) tended to produce the smallest amount of MGA loss from solution.

Unlike MGA, the solution phase concentrations of 17*β*-TBOH and zeranol failed to achieve steady state, even after 6 days of mixing with soils (whereas soil-free controls were stable over the same time scale). For 17*β*-TBOH, there was no apparent relationship between the rate of disappearance and soil OC content, whereas zeranol loosely exhibited behavior expected if hydrophobic interactions were driving sorption in these suspensions (i.e., rates and extent of loss were generally greatest in higher OC soils). However, zeranol's inability to achieve sorption equilibrium in these model soil suspensions was notable; whereas equilibrium was readily achieved with high OC Pahokee Peat, the lack of equilibrium in lower OC content soils implies that other, nonconservative, processes may be contributing to the loss of solution phase zeranol.

Additional experiments with select model soils suggest that sorption occurs concurrently with abiotic chemical transformation of SGPMs. Figure 3 shows dissolved concentration versus time plots for 17*β*-TBOH, MGA, and zeranol for low (RMN; 1% OC) and high (PVLPF; 5.9% OC) OC content soils, which are identical to the data previously shown in Figure 2 for these soils. However, also provided for comparison are data corresponding to the total mass in these systems over time (i.e., dissolved + sorbed concentration), in which sorbed concentration was determined via methanol extraction of soils at each sampling point (recall that this extraction technique successfully recovered all sorbed mass from Pahokee Peat during our isotherm experiments).

In nearly all systems, we observed loss in total SGPM mass, and the extent of this loss generally increased over time. For 17*β*-TBOH and zeranol, there was little, if any, difference in the measured aqueous concentration and the total recoverable mass, and equivalent behavior was observed for both soil systems considered. The agreement in aqueous concentration and total mass profiles is consistent with all 17*β*-TBOH and zeranol loss in these soil systems being attributable to some nonconservative process (e.g., reaction) rather than sorption. Because systems were sterilized, we propose that this loss is due to mineral-promoted surface reactions such as hydrolysis or oxidation occurring on underlying inorganic phases in these soils. We note, however, that no products were detected using LC-DAD methods, suggesting that such products either are too polar to be separated on C-18 columns with our existing analytical method or are no longer chromophoric.

Results with MGA may elucidate factors controlling the rate at which SGPMs are degraded in soil systems via such nonconservative processes (i.e., transformation reactions). For both soils in Figure 3, the aqueous phase concentration of MGA showed nearly identical profiles; about 60−70% of the solution concentration disappeared within the first day, after which it remained relatively constant over the experiment's 6 day duration. In contrast, the total mass profiles were distinct for each soil system. For high OC PVLPF, the total mass of MGA in suspension was relatively stable and ~90% remained after 5 days, consistent with most if not all of the MGA loss from solution being due to sorption. In contrast, total MGA mass decreased steadily over time in the low OC soil RMN, with only 50% remaining after 6 days. However, the rapid,

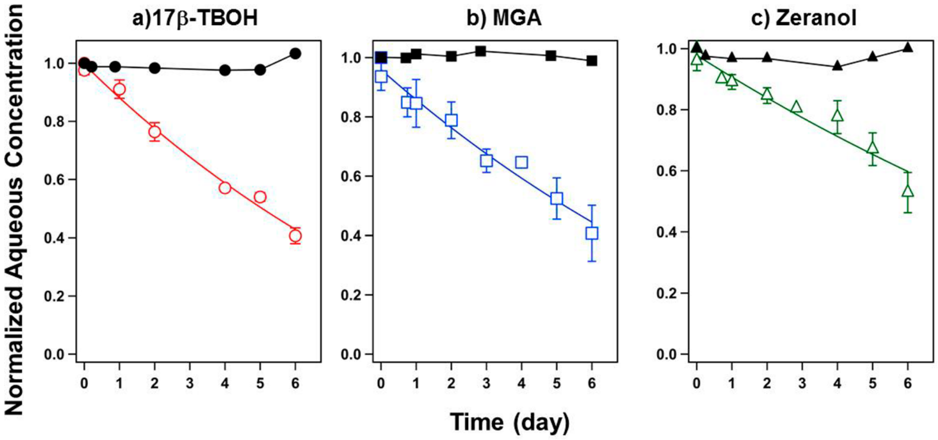


Figure 5. Reaction of 17β-TBOH, MGA, and zeranol with 10 g/L SiO_2 at pH 7. Lines represent exponential decay model fits from which half-lives were estimated. Experiments were conducted in 5 mM phosphate buffer at pH 7 and used initial concentrations of 4, 2, and 2 μM for 17β-TBOH, MGA, and zeranol, respectively.

initial MGA loss from solution appears to result entirely from sorption, as the total mass recovery for the RMN system was nearly 100% over the short time scales associated with this initial, steep decrease in aqueous concentration. Then, although there was relatively little change in the solution phase concentration of MGA between days 2 and 6, the total MGA mass in the system decreased by ~50%. This suggests that transformation processes in RMN suspensions involve the initially sorbed MGA mass.

One potential explanation for our MGA results is a two-step process for its mineral-promoted transformation in low-to-moderate OC-containing soils. The MGA first sorbs to the organic layer coating the particle, through which it must then diffuse to react at the surface of the underlying inorganic phase, which we assume is the same reactive mineral in both RMN and PVLPF systems. Because MGA has a relatively high K_{ow} value and its isotherm with Pahokee Peat is consistent with hydrophobic partitioning, it is not surprising that the extent of its mass loss from reaction is highly dependent upon soil OC content. Specifically, for high OC content PVLPF (5.9%) MGA is more tightly and/or frequently bound to the organic phase, ultimately making it less available for transformation at the underlying mineral interface. In contrast, the smaller reservoir for hydrophobic partitioning on lower OC RMN (1%) allows MGA to more readily access reactive sites on the surface of the inorganic phase.

When this scenario is extended to 17β-TBOH and zeranol, their relative rates of organic partitioning (sorption) versus surface transformation (reaction) must be considered. Notably, instances when losses in total mass and solution phase concentration are equivalent (e.g., as we observed for 17β-TBOH and zeranol) imply that the rate of surface reaction is much faster than that of organic partitioning, thereby limiting the amount of sorbed mass that can accumulate in the system. Unfortunately, without greater understanding of the differences in OC composition and/or the nature of the reacting species in these two soil systems, it is difficult to more fully explain why 17β-TBOH and zeranol behave so distinctly from MGA.

Reactivity of SGPMs toward Inorganic Mineral Phases. The preceding discussion is predicated on the reactivity of SGPMs toward naturally occurring mineral phases. To assess the potential for such mineral-promoted transformations, additional experiments were conducted with several metal oxides representative of those naturally occurring in soil. Figure 4a shows the reactivity of amorphous manganese oxide (hereafter MnO_2), a known oxidant of certain steroids,[22] toward 17β-TBOH, MGA, and zeranol at pH 6. Zeranol was most reactive, undergoing nearly complete transformation over the course of 0.5 h at a relatively low MnO_2 loading (1 g/L). Although not as reactive as zeranol, the rate of 17β-TBOH decay increased with MnO_2 solid loading (from 1 to 10 g/L), behavior that is consistent with loss via surface reaction. Also, HPLC-DAD analysis of samples from these 17β-TBOH suspensions provided evidence of product formation. We note that these products, although not yet structurally identified, are considerably more polar than the monohydroxylated photoproducts of 17β-TBOH we have elucidated,[31,34,35] which is consistent with highly oxidized products resulting from 17β-TBOH oxidation by MnO_2. Interestingly, MGA is essentially unreactive toward MnO_2, exhibiting stability in 10 g/L at pH 6. In fact, we observed evidence of only limited MGA transformation under far more extreme suspension conditions (20 g/L at pH 4).

Iron oxides such as amorphous ferrihydrite are weaker oxidants than MnO_2 but may also promote other processes including hydrolysis[21] or, similarly, nucleophilic (e.g., H_2O or OH^-) addition. Indeed, a modest amount of 17β-TBOH and MGA loss (~25%) was observed over approximately 1 week in ferrihydrite suspensions (5 g/L, pH 7) (Figure 4b), whereas zeranol was most reactive. These losses in ferrihydrite suspensions correspond to half-lives of 5 days for zeranol and roughly 15 days for 17β-TBOH and MGA. Furthermore, attempts to recover any SGPM mass sorbed to the ferrihydrite surface via methanol extraction were unsuccessful, consistent with a nonconservative process being responsible for SGPM loss. However, the relative reactivity of MGA and17β-TBOH is far different from the trend observed for MnO_2, which is reasonably assumed as an oxidant. Thus, we believe the data from ferrihydrite suspensions are evidence of a nonredox, surface-promoted reaction for SGPMs, although reaction products were not detected. This is particularly noteworthy for MGA, as melengestrol, a plausible hydrolysis product easily detected via our analytical methods, was not observed.

Finally, the stability of 17β-TBOH, MGA, and zeranol was examined in suspensions (10 g/L, pH 7) of SiO_2 (Figure 5). SiO_2 is not a viable oxidant, so it would be expected to promote only nonredox pathways (e.g., hydrolysis or nucleophilic addition). Solution phase concentrations of 17β-TBOH, MGA, and zeranol decreased over time in these suspensions, and once again we were unable to recover any of this lost mass via methanol extraction of the SiO_2 at the conclusion of the experiment. Similar to observations with ferrihydrite, 17β-TBOH and MGA exhibited comparable rates of decay in SiO_2 (half-lives of 4.9 and 5.3 days, respectively), seemingly supporting a shared transformation pathway between iron and silica oxide systems. In fact, because 17β-TBOH and MGA share identical structures in the steroid A ring, we propose these similar time scales indicate A-ring susceptibility to hydroxylation via surface-promoted nucleophilic addition rather than hydrolysis. Zeranol, which has a hydrolyzable ester group, decays at a slightly slower rate compared to the other SGPMs ($t_{1/2}$ of ~8 days).

Although work is ongoing to better characterize SGPM reactivity and fate in these metal oxide systems, these data clearly support the surface-promoted transformation of SGPMs in model soils. Oxidation by manganese oxide appears viable for the TBA and zeranol families, whereas all SGPMs are susceptible to hydrolysis or nucleophilic addition on iron and silica oxides. As for the primary reactive inorganic phase in our model soil systems, our data appear to implicate SiO_2. Assuming exponential decay in our model SiO_2 systems, the relative rate of transformation for 17β-TBOH, MGA, and zeranol is 1:1.1:1.6. Notably, if we apply a similar exponential decay model to the total SGPM mass data obtained in our low OC model soil (i.e., RMN; see Figure 3), we find an equivalent trend in relative reactivity for 17β-TBOH, MGA, and zeranol (1:1.1:1.5). Consistent with these identical trends in relative reactivity, silica was the only mineral phase detected during X-ray diffraction analysis of model soils, although we cannot rule out that iron and manganese oxides were present at levels below the limit of analytical detection. We note that reactivity differences observed in the high OC content soil (PVLPF; 5.9% OC) may indicate a combination of reactive oxides (e.g., SiO_2 and MnO_2) at play or simply reflect an influence of the organic matter coating on transformation rates by concentrating the SGPMs at the interface of the reactive mineral phase.

Environmental Implications. Consistent with initial expectations, our results indicate that the fate of SGPMs in soil is likely far more complex than currently appreciated. Although sorption and biodegradation have long been thought to dominate SGPM fate in soil, we provide converging lines of evidence that abiotic transformation reactions promoted by naturally abundant mineral surfaces, including quartz (i.e., SiO_2), can occur over time scales comparable to those previously reported for biotransformation.[7] These transformation pathways likely will influence the persistence of SGPMs in soil while also producing novel, yet uncharacterized, transformation products.

Collectively, our findings highlight the challenges in accurately predicting SGPM fate in soil as a result of their many competing fate pathways (e.g., sorption, biotransformation, mineral-promoted transformation). Generally, we can conclude that SGPM sorption will dominate only in soil systems with very high OC content. For example, the Pahokee Peat systems used herein may indeed be a reasonable approximation of organic-rich agricultural topsoil or land-applied manure, both of which would then be expected to effectively retain SGPMs. However, the extent and reversibility of SGPM uptake do not appear easily predictable from traditional metrics of compound hydrophobicity (e.g., K_{ow} values), which are typically used as a starting point to assess soil partitioning. In particular, more polar species (i.e., those with functionalities capable of specific binding interactions such as hydrogen bonding) will be most difficult to model, as their interactions with organic-rich soil will be influenced heavily by soil organic carbon properties responsible for such specific binding interactions.

A clear need arising from this work is a closer examination of mineral-promoted transformation pathways of SGPMs. This includes identifying the key properties and constituents of soils most likely to promote such reactions, especially because prior studies with these compound classes in model soils (e.g., Card et al.[9]) have failed to observe similar evidence of transformation. Another priority is the structural identification of products generated via mineral-promoted reactions. Whereas the structure of some products may be inferred on the basis of the presence of reactive moieties in the parent structure (e.g., the hydrolyzable ester functionality in zeranol), such mechanistic details remain less obvious in other cases. For example, there are several electron-deficient carbon centers within the trienone structure of trenbolone that could be prone to surface-promoted hydroxylation, although our initial assessment suggests this reaction may proceed through the A ring.

We contend that identification of trenbolone transformation products is a particular priority on the basis of our prior findings regarding trenbolone photolysis. Recently, we reported[35] on the formation of metastable photoproducts of trenbolone, C5 and C12 hydroxylated species, that unexpectedly decompose, or revert, into the parent metabolite from which they were generated. Mineral-promoted nucleophilic addition, as we have proposed, will also likely yield hydroxylated products. We believe that if such hydroxlyation occurs within or adjacent to trenbolone's unique trienone moiety, such products may also be prone to similar transformations to yield novel steroidal byproducts with unknown implications for ecosystem health. We reiterate, however, that all evidence to date suggests that products of mineral-promoted reactions with trenbolone are different from those generated photolytically. Furthermore, changes in solution conditions that promote product-to-parent reversion in photoproduct mixtures (e.g., lowering solution pH to promote acid-catalyzed reversion) have thus far failed to regenerate parent trenbolone metabolites in reacted mineral suspensions.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Additional experimental details including the reagent list, sterilization protocol for model soil systems, methodology for measurement of octanol−water partitioning coefficients, and synthesis methods of ferrihydrite and manganese oxide; additional results and discussion regarding the fate of synthetic growth promoters and their metabolites (SGPMs) in model soil and metal oxide systems. 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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