Bioavailability and Fate of Sediment-Associated Progesterone in Aquatic Systems

Article *in* Environmental Science & Technology · March 2016

DOI: 10.1021/acs.est.5b06082

CITATIONS
3

READS
66

5 authors, including:

Jonathan Ali
University of Nebraska Medical Center
10 PUBLICATIONS **19** CITATIONS
SEE PROFILE

Daniel D Snow
University of Nebraska at Lincoln
111 PUBLICATIONS **2,202** CITATIONS
SEE PROFILE

Alan S Kolok
University of Idaho
103 PUBLICATIONS **1,792** CITATIONS
SEE PROFILE

Shannon L Bartelt-Hunt
University of Nebraska at Lincoln
118 PUBLICATIONS **1,724** CITATIONS
SEE PROFILE

Environmental Science & Technology

Article

pubs.acs.org/est

Bioavailability and Fate of Sediment-Associated Progesterone in Aquatic Systems

Jodi L. Sangster,[†] Jonathan M. Ali,[‡] Daniel D. Snow,[§] Alan S. Kolok,[‡,∥] and Shannon L. Bartelt-Hunt*[,†]

[†]Department of Civil Engineering, University of Nebraska-Lincoln, Peter Kiewit Institute, Omaha, Nebraska 68182-0178, United States

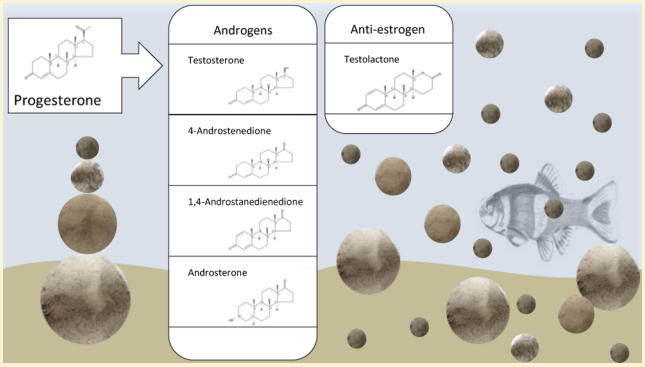
[‡]Department of Environmental, Agricultural and Occupational Health, University of Nebraska - Medical Center, 986805 Nebraska Medical Center, Omaha, Nebraska 68198-6805, United States

[§]Nebraska Water Center and School of Natural Resources, University of Nebraska-Lincoln, Lincoln, Nebraska 68583-0844, United States

[∥]Department of Biology, 6001 Dodge Street, University of Nebraska at Omaha, Omaha, Nebraska 68182-0040, United States

Supporting Information

ABSTRACT: The environmental fate and bioavailability of progesterone, a steroid hormone known to cause endocrine-disrupting effects in aquatic organisms, is of growing concern due to its occurrence in the environment in water and sediment influenced by wastewater treatment plant and paper mill effluents, as well as livestock production. The objective of this study was to evaluate the fate of progesterone in two natural sediments and the corresponding alteration of gene expression in three steroid-responsive genes; vitellogenin, androgen receptor and estrogen receptor alpha. When exposed to progesterone-spiked sand, fathead minnows (*Pimephales promelas*) exhibited significant reductions in the expression of vitellogenin and androgen receptor expression. In contrast, fish exposed to progesterone associated with the silty loam sediment did not show a biological response at 7 days and only realized a significant reduction in vitellogenin. In both sediments, progesterone degradation resulted in the production of androgens including androsteinedione, testosterone, and androstadienedione, as well as the antiestrogen, testolactone. Differences in compound fate resulted in organism exposure to different suites of metabolites either in water or associated with the sediment. Results from this study suggest that environmental progestagens will lead to defeminization at environmentally relevant concentrations, and that exposure is influenced by sediment properties.



INTRODUCTION

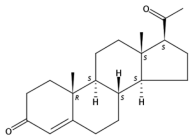
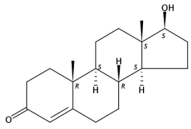
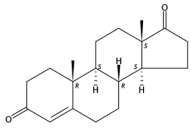
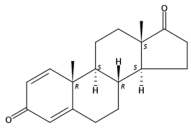
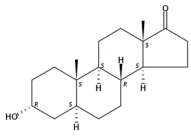
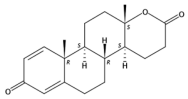
There is growing concern about potential endocrine disrupting effects stemming from the occurrence of progesterone (P4) in aquatic systems. Several recent studies have suggested P4 may be responsible for the increased presence of androgens in soil and water adjacent to agricultural areas[1−3] and may explain the masculinization of female fish downstream of paper mills, an industry known to release significant amounts of plant-derived progesterone into aquatic systems.[1] A recent review reported average P4 concentrations below 5 ng/L in surface waters receiving wastewater treatment plant effluent, although concentrations as high as 199 ng/L were also reported.[4] Studies evaluating paper mill effluents detected aqueous P4 in the low μg/L range[1] and studies of runoff from beef cattle feedlots found aqueous P4 at concentrations as high as 1070 ng/L[2,5] with average feedlot soil concentrations of 148 ng/g.[5] Exposure of adult female fathead minnows (*Pimephales promelas*) to aqueous P4 at concentrations as low as 10 ng/L resulted in reduced reproductive success and reduced expression of vitellogenin (Vtg), an egg yolk precursor protein.[6] Additionally, studies have documented endocrine effects in zebra fish (*Danio rerio*) at aqueous P4 concentrations as low as 3.5 ng/L in adult females[7] and after embryonic exposure to concentrations as low as 2 ng/L.[8]

Although P4 has been shown to be released in runoff from areas treated with manure from animal production facilities,[2,3,5] there are limited studies evaluating P4 occurrence in surface waters adjacent to agricultural areas. This may be due in part to the spatially and temporally diffuse nature of agricultural runoff, which results in episodic loading of contaminants during periods of snowmelt, precipitation, and irrigation.[2,3] In aquatic

Received: December 14, 2015
Revised: February 28, 2016
Accepted: March 3, 2016
Published: March 3, 2016

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

Table 1. Properties of Steroid Hormones

Compound	MW (g/mol)	Solubility (mg/L)[A]	Log K_{oc}
Progesterone 	314.5	9.4	4.16, 3.91[B]
Testosterone 	288.4	20	3.18, 3.23[B]
4-Androstenedione 	286.4	34	2.85[A]
1,4-Androstanedienedione 	284.4	34	2.80[A]
Androsterone 	290.4	7.3	3.52[A]
Testolactone 	300.4	28	2.59[A]

[a]Presented in SciFinder as calculated using ACD/Laboratories v11.02. [b]Determined using sediments from this study for sand and silty loam, respectively.[12]

systems susceptible to episodic loading of steroid hormones, there is growing evidence of the importance of sediment interactions on both contaminant fate[9−12] and the subsequent bioavailability to aquatic organisms.[13−17] In a previous study, we determined fathead minnows exposed to sediment-associated trenbolone exhibited significant differences in Vtg expression that could not be explained solely by measured concentrations of aqueous steroids.[13] More recently, Zhang et al.[18] evaluated agrichemical fate and bioavailability in field-based mesocosms within an agriculturally influenced watershed and determined that exposure to river sediment was necessary for the observed biologic response in fathead minnows.

There is a growing body of work indicating sediment composition can affect the bioavailability of steroids in aquatic systems[13,15,18] by influencing compound fate as it relates to distribution[12,19,20] and degradation[10,11,21] in sediment−water systems. It is unclear if biological effects stemming from sediment-associated steroids are due to desorption from the sediments and/or by direct contact between fish and sediment-associated steroid. Some studies have shown limited detection of aqueous phase steroid[13,17,22] suggesting direct contact may at least be partially responsible for the observed biologic effects while one study found that steroid interactions with finer particle size fractions actually enhanced biological effects of the steroid.[15] Recently, Jessick et al.[14] attempted to evaluate the mechanism by which sediment-associated Tb is bioavailable to female fathead minnows. It was determined that direct contact with whole sediments is not necessary for defeminization of fish to occur. It was not possible to determine conclusively if effects seen in the fish were caused by Tb associated with a small quantity of fine particles in the water column or by free steroid that had desorbed from sediments. A few studies have shown increased sorption capacity[12,19,20] and preferential sorption[12] of steroids to finer particle size fractions within whole sediments with a higher degree of desorption from coarser fractions which may help to explain the effects observed in the fish. Additionally, sediment texture may affect the degradation rates and metabolites formed during microbial degradation of the parent compound[10,11,21] which will affect biological response in aquatic vertebrates, such as fish. Several studies have evaluated aqueous progesterone degradation by pure species of bacteria including *Aspergillus*, *Anthrobacter*, *Chlorella*, *Penicillium*, *Bacillus*, and *Streptomyces*[23−28] with intermediate products reported, including the androgens testosterone acetate, testosterone (T), hydroxytestosterone, androstenedione (AD), and testolactone, an antiestrogen. To date, the

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

transformation and bioavailability of P4 have not been evaluated in more complex sediment-water systems.

The present study was designed to evaluate how differences in sediment composition affect P4 fate as well as the biologic response of fathead minnows to P4 under conditions that are consistent with agriculturally impacted aquatic systems. Two natural aquatic sediments with marked differences in texture and organic carbon content were used. Biological effects stemming from exposure to sediment-associated P4 were determined by evaluating differences in hepatic expression of Vtg, androgen receptor (AR), and estrogen receptor alpha (ERα) in an environmental sentinel species, the fathead minnow. Compound fate was determined by measuring aqueous and sediment-associated steroid hormones in both the fish exposure aquaria and in controlled microcosms. Results of the biological analysis were compared with analytical chemistry data obtained from controlled microcosms and periodic grab samples of sediment and water from exposure aquaria to correlate contaminant fate with bioavailability in the target organism.

MATERIALS AND METHODS

Chemicals and Reagents. All reagents used in this project were purchased from Fisher Scientific and used the highest purity available (Optima, Thermofisher Scientific, St. Louis, MO). Pure steroids (Table 1), including progesterone, testosterone, 4-androstenedione, androsterone, androstanedienedione, and testolactone were purchased from Sigma-Aldrich (St. Louis, MO) or Acros Chemicals with ^{14}C radiolabeled progesterone from American Radiolabeled Chemicals (St. Louis, MO). Internal standards included testosterone-d3, obtained from Sigma-Aldrich (St. Louis, MO), and $^{13}C_6$-estradiol purchased from Cambridge Isotopes (Andover, MA).

Sediment Collection and Storage. Natural aquatic sediments were collected from two sites representing differences in physical properties and include (1) a silty loam collected from Plum Creek in Seward, Nebraska; and (2) a sandy sediment from the Elkhorn River near Winslow, Nebraska. These locations were selected as steroid hormone contamination had not previously been detected at these sites,[16] nor were the untreated sediments found to induce endocrine disrupting effects in previous laboratory studies.[13,14] Sediments from these locations have been used frequently in laboratory and field studies either directly[12−14] or in close proximity to the collection sites used in this study.[16,17,29] To ensure natural microbial activity would be present, sediment was collected from the top 10 cm of the streambed within 36 h of the beginning of experiments and kept in a cool dark location until use. Select properties of each sediment are presented (Table 2) with additional information about specific mineralogy in Supporting Information (Table S1).

Fish Exposure Experiments. Laboratory experiments were conducted in 45 L glass aquaria using sexually mature fathead minnows from an on-site culture using previously described protocols.[13,14] Nine groups consisting of 40 fish each (20 male and 20 female) were used with four groups exposed to each sediment (sand and silty loam) at sediment concentrations of 0, 5, 50, and 500 ng/g (dry weight) P4. The final group consisted of fish unexposed to sediment or hormone (i.e., lab water only) to ensure that any observed change in gene expression could not be attributed to pre-existing contaminants in the sediments or to solvents used in hormone stock solutions (data not shown). In the experimental tanks using sediment, a layer of sediment approximately 6 cm deep (8 kg dry weight) was pre-equilibrated with P4 and was not replenished during the experiment. All aquaria contained 45 L aerated and dechlorinated tap water at 26 ± 0.2 °C with a photo period of 16:8 h light:dark. Static renewal of the water was performed at a rate of 0.3 d^{-1}. Fish were fed twice daily with a commercially available food, Tetramin (Melle, Germany), and frozen *Artemia salina*.

Table 2. Selected Sediment Properties

sediment classification[a]	surface area[b] m^2/g	organic carbon[c] %	cation exchange capacity[d] m-eq/100g	dominant clay type[e]
silty loam	19.9	2.55	16.6	smectite
sand	1.36	0.23	4.8	Illite

[a]Determined by Midwest Laboratory Inc., Omaha, NE. [b]BET analysis performed by Particle Technology Laboratories, Downers Grove, IL. [c]Microwave digestion. [d]Compulsive exchange method. [e]XRD analysis performed by KT-Geo, Gunnison, CO.

To simulate progesterone-contaminated sediment, a solution containing P4 dissolved in methanol and deionized water was added to six of the eight 45 L aquaria containing sediment. Each solution and sediment was combined in separate aquaria and mixed thoroughly every few hours over a 12 h period to ensure a homogeneous substrate. The final methanol concentration was kept to a minimum (<0.1 μL/L) to avoid solvent effects in the fish.[13] After mixing, all tanks were allowed to further equilibrate with water for 12 h before the addition of fish. P4 has been shown to reach equilibrium within 24 h for these sediments.[12]

Target concentrations of 5, 50, 500 ng/g were chosen based on previously published research detailing P4 occurrence in agriculturally impacted waterways.[1] Sediment concentrations of P4 have been detected at or below 50 ng/g in aquatic sediments[1] and represent known environmentally relevant concentrations; however, many studies evaluating occurrence of steroids in the environment assess only aqueous steroid and do not measure steroid in sediments. Therefore, the 500 ng/g exposure groups serve as a possible worst case scenario of environmental contamination while allowing for detection of both parent steroid and metabolites in both the aqueous and sediment phases using analytical chemistry techniques.

Sediment and water samples were collected from each aquaria after 24 h of equilibration but immediately before the introduction of fish (e.g., 0 days); and daily for the duration of the exposure period. At each sampling event, a 250 mL aqueous grab sample was collected and stored in an amber glass bottle at −20 °C until analysis. A composite sediment sample was obtained by collecting three to four 10 g randomly selected sediment subsamples within each aquaria and stored −20 °C until analysis.

Biological Analysis. Since steroid hormones are subject to biotransformation and excretion within organisms, the effective bioavailability of compounds was inferred based on molecular changes in estrogen responsive, antiestrogen responsive, and androgen responsive genes. To evaluate bioavailability, 10 male and 10 female fish were randomly collected from each aquaria at 7 days with the remaining harvested at 14 days. After collection, fish were euthanized and weighed. Livers and gonads were collected from each individual and weighed. These were then flash frozen in liquid nitrogen and stored at −80 °C until

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

analysis. Hepatosomatic index (HSI) and gonadosomatic index (GSI) were calculated for each fish as the mass of the tissue divided by the total mass of the fish multiplied by 100 (Table S6).

Hepatic Vtg, AR, and ERα mRNA expression was evaluated using ribosomal L8 as a normalization standard[30] and expressed in relative terms as outlined in Schmittgen and Livak.[31] Briefly, RNA was extracted from liver tissue using the SV Total RNA Isolation System (Promega) in accordance to the manufacturer's recommendations (Promega, Madison, WI). Extracted RNA was then converted to cDNA using iScript cDNA synthesis kit (Bio-Rad).[30] All RT-qPCRs were performed in duplicate using iQ SYBRGreen Supermix (Bio-Rad) with primers obtained from EurofinsGenomics (Huntsville, AL). Amplification efficiencies were determined using a standard curve prepared from a serially diluted pool of cDNA and were between 85.2 and 119.9% ($r^2 > 0.980$ for all reactions). Normalized expression of target genes were quantified using the $2^{-\Delta Ct}$ method based on mean cycle thresholds (Ct).[31] Comparison of relative gene expression, body mass, and organ indices between treatment groups was conducted using one-way ANOVA, or Welch's test when variances significantly differed.[32] Observed differences from ANOVA results were followed by Dunnett's comparison test relative to control. Statistical significance was assumed at $p < 0.05$.

Sample Extraction and LC-MS/MS Analysis. Steroids were extracted from 100 mL water samples using online solid phase extraction (SPE) using a Spark Holland Symbiosys Environ automated extraction system with 500 ng/L testosterone-d_3, $^{13}C_6$-estradiol, and 17α-methyltestosterone as a surrogate. Microwave-assisted solvent extraction (MASE) was used for all sediment samples using 25 ng of the same surogates. Briefly, 2−3 g of sample was weighed into a 10 mL Teflon microwave digestion vessel, mixed with 1 mg of butylated hydroxytoluene and 5 mL of high purity methanol prior to microwaving in a CEM MARS Xpress microwave at 1000 W for 10 min.

All extracts were analyzed for steroids using liquid chromatography tandem mass spectrometry with atmospheric pressure photoionization (APPI) utilizing multiple reaction monitoring (MRM) with argon collision gas as detailed in Snow et al.[33] Briefly, a Thermo HyPurity C18 column (250 × 2 mm, 5 um, 50 °C) was used for gradient separation at a flow rate of 0.35 mL/min. The gradient consisted of solvent A (0.1% formic acid in water) and solvent B (0.1% formic acid in methanol), with 0 to 3 min at 50% B, 3−14 min at 65% B, and 14−20 min at 95% B, with a return to initial solvent conditions for the last 10 min of the gradient (30 min total). Instrument control, data acquisition and evaluation used MassLynx 4.0 software (Waters Corporation, Milford, MA). Identification of target compounds was accomplished by comparing the retention times for the respective MRM transition in a sample to that of a standard analyzed under the same conditions (Supporting Information Table S2). Retention times were considered to match if they were within ±5% of the standards. Testosterone-d_3 was used for quantification of androgens, melengesterol acetate and progesterone, whereas $^{13}C_6$-estradiol served as the internal standard for steroid estrogens in both the online SPE and MASE LC-tandem MS methods. Recovery of the synthetic androgen (17α-methyltestosterone) surrogate was calculated to evaluate individual sample extraction efficiency and possible matrix effects, but analyte concentrations were not corrected for surrogate recovery. Recovery from laboratory fortified sediment blanks was 74% for P4, 58% for T, 86% for androstenedienedione, and 73% for 4-androstenedione. Recovery of laboratory fortified blanks for aqueous compounds ranged between 80 and 106% for all compounds detected. Please refer to Supporting Information for additional information on the method and QA/QC samples (Tables S4 and S5).

Steroid Degradation Microcosms. Batch experiments using a combination of nonlabeled and ^{14}C- radiolabeled P4 were conducted to determine detailed degradation profiles and metabolite generation within the sediment/water systems. The ^{14}C radiolabel was used as a tracer to identify the metabolites formed. Microcosms were designed such that the sediment to water ratio (approximately 1g:5.6 mL), lighting regime (16:8 light:dark), and temperature (26 ± 0.2 °C) would be the similar to that used in the fish exposure experiments. Each steroid was delivered to experimental microcosms using a stock solution of 250 mg/L P4 in ethanol. Solvent was evaporated from each microcosm under a steady stream of nitrogen before the addition of water and sediment to eliminate the possibility of solvent effects. Each microcosm consisted of 3 g of sediment spiked with 500 ng/g dw P4 in 17 mL of distilled deionized water in 50 mL glass centrifuge tubes. Microcosms were opened for 30 min each day to ensure aerobic conditions. This was verified using two additional reactors containing resazurin dye. Microcosms were sacrificed at 0 h, 6 h, 12 h, 18 h, 24 h, 36 h, and daily from day 2 to day 14. Sediment and aqueous phases of each microcosm were separated by centrifugation at 2000 rpm for 15 min. Steroids were extracted from aqueous samples upon collection using the previously described methods with resulting extracts along with sediment phase samples stored at −20 °C until further analysis. The same experimental design was used for both sediments (silty loam and sand) with both aqueous and sediment phase of microcosm assessed for concentrations of parent steroid and metabolites.

Characterization of Steroid Hormone Transformation Products Using LC/MS and Radioactivity Detection. Extracted steroids from the microcosm experiments were separated by a water/methanol gradient (0.4 mL/min) on a 2695 HPLC (Waters, Milford, MA) equipped with a HyPurity C18 column (Thermo Scientific, Waltham, MA) at 50 °C and using an injection volume of 50 μL.

Both UV (230 nm, 486 MS, Waters, Milford, MA) and β-particle (β-RAM, IN/US Systems, Tampa, FL) ^{14}C radioactivity detectors were used to produce chromatograms for each extract injection and peaks compared to retention times measured using an ion trap mass spectrometer. The mobile phase gradient was optimized for steroid hormone separation. A Thermo LCQ ion trap mass spectrometer (San Jose, CA) was used to characterize degradation products in positive full scan mode from 150 to 450 amu with atmospheric pressure chemical ionization (APCI) source. Source conditions were Vaporizer temperature: 450 °C; Sheath gas: 80 (arbitrary units); Auxiliary gas: 1 (arbitrary units); Discharge current: 9.00 μA; Capillary temperature: 200 °C; Capillary voltage: 6.00 V; and Tube Lens offset: −15.00 V. The β-Ram radioactivity detector was used to identify regions of each chromatogram likely to contain transformation products. Peaks in the radioactivity chromatogram for each sample were compared to the MS spectrum taken at approximately the same time to determine the identity of the radio-labeled compound eluting during that time. We did not detect peaks in radioactivity that would correspond to any other unknown transformation products. Steroid concentra-

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

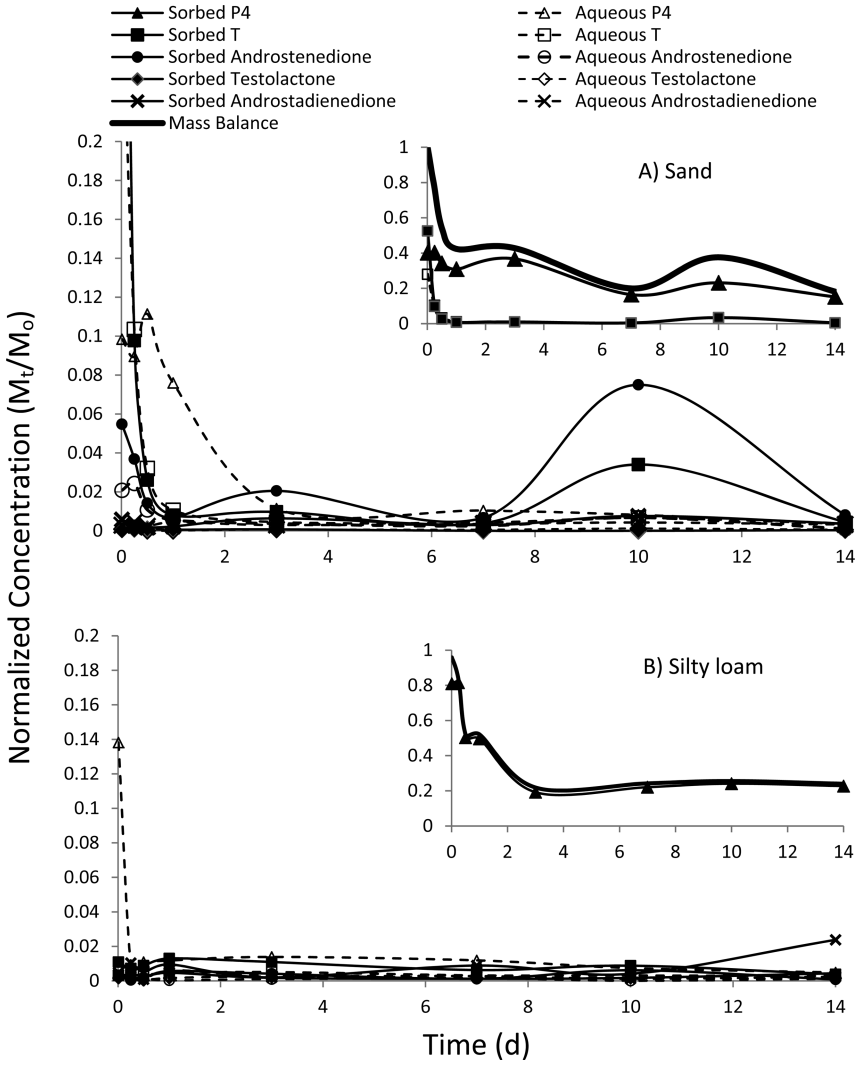


Figure 1. Normalized concentrations expressed as Mt/Mo from microcosms spiked at 500 ng/g progesterone containing (A) sand and (B) silty loam sediments. For each sediment, normalized concentrations of steroids ≤0.2 are presented with mass balance and steroids with normalized concentrations exceeding 0.2 as insets. Solid lines represent sediment-associated steroids. Dashed lines indicate steroids measured in the aqueous phase.

tions were quantified by internal standards from full scan (150−440 amu) APCI MS data (LCQ, Thermo Finnigan, San Jose, CA) with the Xcalibur software (version 1.3, Thermo Finnigan, San Jose, CA) using a five-point linear regression (50−2000 μg/L).

RESULTS AND DISCUSSION

The goal of this study was to conduct an integrated evaluation of the fate of P4 in sediment and water to understand the corresponding bioavailability. This was accomplished by using two natural aquatic sediments to determine if fathead minnows exposed to sediment contaminated with P4 would exhibit altered endocrine function, while measuring degradation and metabolite formation of P4 over the course of the experiment in both the exposure aquaria and in controlled microcosms. Results from this study indicate that P4 degradation will result in known androgens and an antiestrogen and confirms P4 exposure will result in defeminization of female fathead minnows. While the current study was not designed to evaluate the mechanism of steroid exposure, the effects measured in the fish coupled with analytical chemistry data from both the exposure aquaria and microcosms suggests that fish exposed to P4 associated with sand exhibit a biological response in response to aqueous steroids; while fish exposed to P4 associated with silty loam sediment exhibit biological effects consistent with exposure to the dominant sediment associated compound.

Progesterone Degradation in Controlled Microcosms Results in Androgens and an Antiestrogen. In the microcosms (Figure 1), steroids were predominantly detected in the sediment phase with sediment-associated P4 as the dominant compound in both the sand and silty loam. In the sand microcosms, there was rapid transformation of P4 to T within the first 6 h in both the aqueous and sediment phases with sediment-associated androsteinedione, T, and androstadienedione appearing around 10 days. In the silty loam microcosms, there were measurable albeit low levels of androgen throughout the 14 day period with an increase in androstadienedione measured at 14 days (Figure 1B). The silty loam microcosms also had a greater conservation of mass after 14 days (24%) compared to the sand microcosms (18%) after 14 days (Figure 1). Additionally, testolactone, an antiestrogen,

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

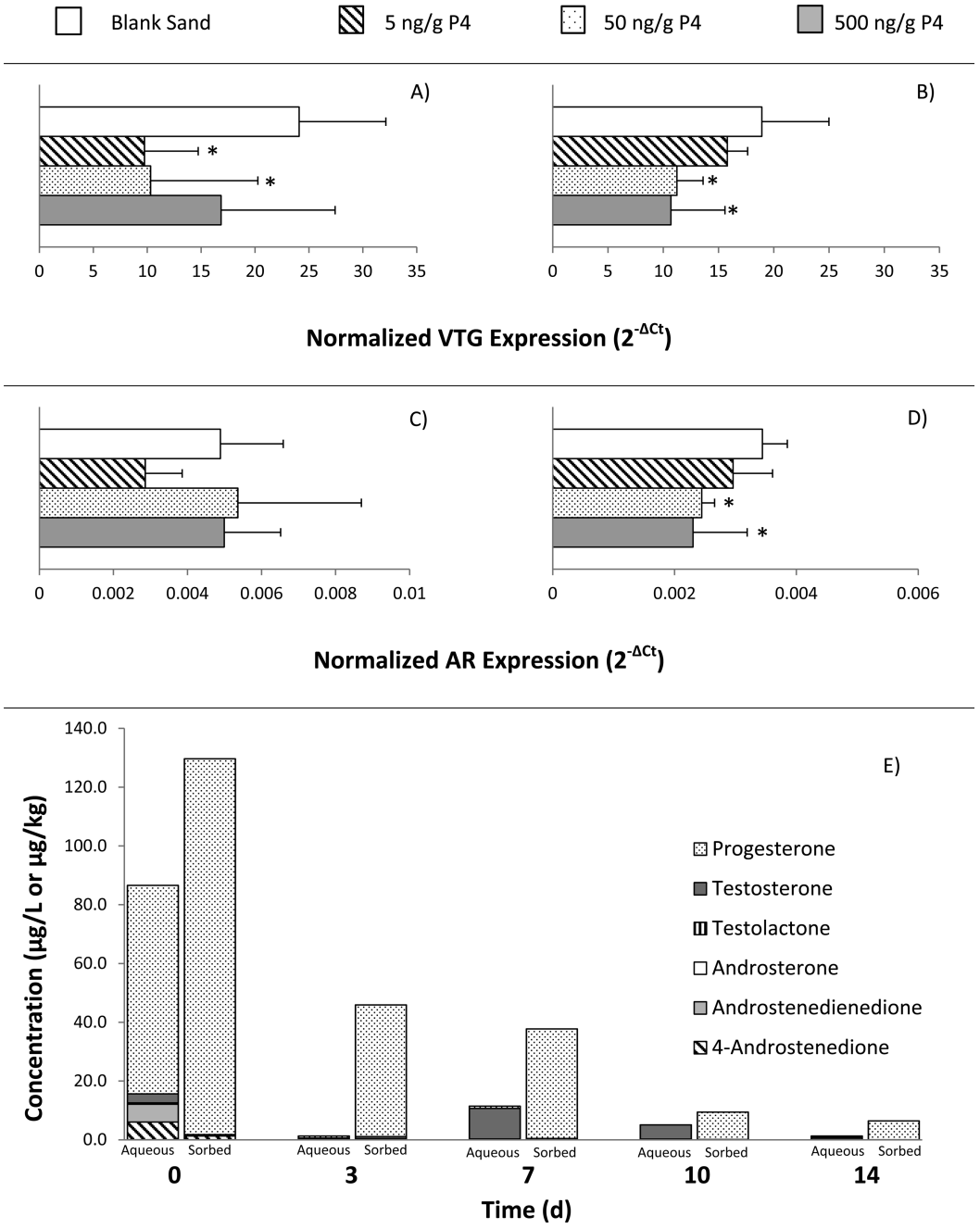


Figure 2. Normalized hepatic expression ($X \pm$ sd) of vitellogenin (VTG) and androgen receptor (AR) of female fathead minnows exposed to sand-associated progesterone after 7 (panels A and C) and 14 (panels B and D) day exposures with measured sorbed and aqueous phase concentrations (panel E) obtained during periodic grab samples. * Denotes significant differences compared to blank sediment group.

was frequently detected in both the water and sediment phases within the microcosms at concentrations ranging from 0.05–0.11 μg/L and 0.6–2.3 ng/g in the sand and 0.10–0.15 μg/L and 0.59–2.3 ng/g in the silty loam microcosms, respectively. While previous studies have evaluated P4 degradation using pure cultures in enriched matrices,[23−27] this confirms that P4 degradation will result in the production of several known androgens and at least one antiestrogen in a more complex system.

Effects Measured in Female Fatheads Minnows Exposed to Progesterone Associated with Sandy Sediment Are Likely Due to Desorption and Transformation. Fish exposed to P4 associated with the sandy sediment exhibited significant decreases (p = 0.0159) in the expression of hepatic Vtg in the 5 and 50 ng/g treatments groups when compared to the control sediment after 7 days of exposure (Figure 2A) with no significant differences in the expression of AR (Figure 2C) or ER-α (Figure S1, panel C). By 14 days, fish exposed to P4 associated with the sandy sediment exhibited significant decreases in the expression of Vtg (p = 0.003) in the 50 and 500 ng/g treatment group (Figure 2B) with a corresponding decrease in the expression of AR (p = 0.006) (Figure 2D). It is common for exposure to endocrine disruptors including steroids to cause a nonmonotonic dose response in organisms. When evaluating fathead minnows exposed to aqueous progesterone for 21 days, a similar trend

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

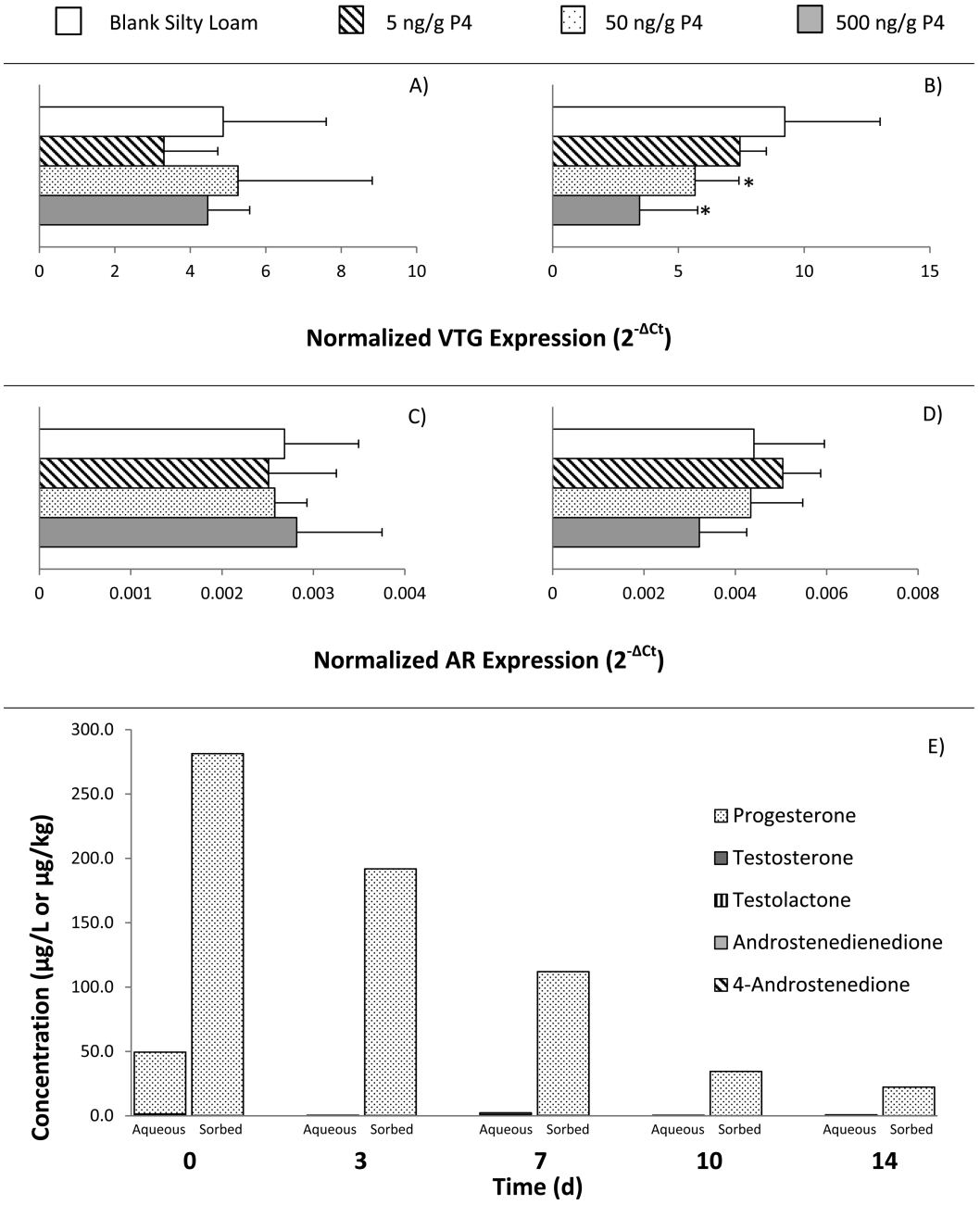


Figure 3. Normalized hepatic expression ($X \pm$ sd) of vitellogenin (VTG) and androgen receptor (AR) of female fathead minnows exposed to silty loam-associated progesterone after 7 (panels A and C) and 14 (panels B and D) day exposures with measured sorbed and aqueous phase concentrations (panel E) obtained during periodic grab samples. * Denotes significant differences compared to blank sediment group.

was observed where the female fish exposed to 10 and 100 ng/L P4 had significant reductions in the expression of Vtg; while the Vtg measured in fish exposed to 1000 ng/L was not significantly different from the negative control.[6] There were no significant differences found in morphometric data for either male or female fish regardless of the exposure regime (Table S6). The reduction in Vtg expression is consistent with P4 exposure as previous studies using female fathead minnows exposed to aqueous P4 documented reduced expression of Vtg;[6] however, exposure to androgens would lower Vtg expression in female fish as well. While prior studies evaluating aqueous exposures of fish to progesterone did not assess AR response,[6] ex vivo studies evaluating fat head minnow ovaries[34] and in vitro studies evaluating nuclear AR[35] have shown P4 acts as a weak AR agonist.

Measured concentrations of sorbed and aqueous phase steroids within the exposure aquaria spiked at 500 ng/g P4 are shown in Figure 2E with additional details in Table S7. Through the duration of the 14 day exposure, sediment-associated P4 persisted and, with exception of day 10, was also measured in the aqueous phase within the aquaria. Interestingly, androsterone was not detected in the microcosms for either sediment (Figure 1), but was found in the aqueous phase at day 10 in the sand exposure aquaria (Figure 2E). Steroids were detected in the aqueous phase for longer time periods compared with our previous laboratory studies assessing sediment-associated steroids,[13,17] however, this may be due in

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

part to the higher initial P4 concentration evaluated in the present study. It is also important to note that coextracted matrix in sediment extracts from the exposure aquaria produced high levels of background noise that may have limited the detection of several compounds (Table S7). Steroids were detected in aquaria containing the 5 ng/g and 50 ng/g-amended sediments (data not shown); however rapid degradation was observed with steroid concentrations approaching the detection limits by 3 days with minimal detection of metabolites over the exposure period.

Based on equilibrium partitioning calculations using log K_{oc} values either determined experimentally using the sediments in this study[12] or reported previously (Table 1), desorption of P4 from the sand is expected between days 3 and 10 (Table S7). Interestingly, there is an initial peak of aqueous P4 and the androgens, T, androstenedienedione, and 4-androstenedione, that disappears rapidly (Figure 2E) with another increase in aqueous concentrations of T and 4-androstenedione beginning at 7 days coinciding with the predicted movement of P4 from the sediment to the aqueous phase. This suggests that the association between the sediment and parent compound, P4, acts to preserve the compound with transformation occurring in the aqueous phase. Additionally, we would expect movement of steroids, P4, 4-androstenedione, androstenedienedione, T, and testolactone from the aqueous phase to the sediment at day 0 and 14 (Table S7). However, the aquaria data show that with the exception of androsteinedienedione, sediment concentrations continue to decrease relative to the aqueous phase.

Taken together, it is likely that the effects measured in female fish exposed to P4 associated with the sandy sediment are predominantly in response to aqueous steroid in the system. The initial concentration of aqueous P4 coupled with the presence of androgenic and antiestrogenic compounds is such that Vtg is significantly reduced; however, the androgens are not present either at high enough concentrations or for a long enough period to cause significant differences in AR response. After 14 days of exposure, the female fish respond to the increase in aqueous T evidenced by both significant differences in Vtg and AR (Figure 2B and D).

Effects Measured in Female Fatheads Minnows Exposed to Progesterone Associated with Silty Loam Sediment Are Likely Due to Contact with Sediment-Associated Steroid. Female fish exposed to P4 associated with the silty loam sediment showed alteration in gene expression after 14 days of exposure in the 50 and 500 ng/g P4 treatment groups. These fish exhibited significant reductions (p = 0.0039) in the expression of hepatic Vtg (Figure 3B), but not AR (Figure 3C and D) or ER-α (Figure S1, panel F). This is in contrast to the fish exposed to P4 in sand where significant reductions in the expression of Vtg were found at both 7 and 14 day, and were also accompanied by reductions in AR expression at 14 days (Figure 2). While the reduction in Vtg is consistent with exposure to P4[6], the differential response measured between the two sediment types suggests differences may exist between mechanism of exposure and P4 fate influenced by differences in sediment characteristics.

Measured concentrations of sorbed and aqueous phase steroids within the silty loam exposure aquaria spiked at 500 ng/g P4 are shown in Figure 3E with additional details in Table S7. P4 was detected in both the aqueous and sediment-associated phase consistently during the 14 day exposure. The only other sediment-associated steroid detected in the exposure aquaria was 4-androstenedione during days 0, 3, and 7. The androgens, 4-androstenedione, androstenedienedione, and T, along with the antiestrogen, testolactone, were detected in the aqueous phase of the exposure aquaria at low (<0.731 μg/L) but measurable concentrations (Table S7). Androsterone was not detected in the silty loam exposure aquaria at any time point evaluated (Table S7).

Sediment-associated P4 persisted over the 14 days period (Figure 3E) with conservation of mass (above 26% in the first 7 days) well above that from the sand aquaria (Table S7). This is not surprising as P4 sorbs more readily to the silty loam sediment (K_d = 207.2 L/kg) as compared to the sand (K_d = 33.3 L/kg).[12] Desorption of P4 from the silty loam is expected based on equilibrium partitioning calculations at days 3 and 10 (Table S7) and was followed by an increase in the aqueous concentration of T and 4-androstenedione on days 7 and 14. Interestingly, aqueous concentrations of T peak at 7 days in both the sand and silty loam sediments at 10.39 μg/L and 0.646 μg/L, respectively. In both instances, this was proceeded by expected of desorption of P4 from the sediments at day 3 (Table S7).

For both sediments, over 60% of sediment-associated P4 may be found in the finer particles (approximately 24% by weight in the silty loam and 8% by weight in the sand) within the whole sediment.[12] As finer particles are more likely to be suspended in the water column, contact with fish would be greater in the silty loam sediments. Even with higher concentrations of sediment-associated P4 measured in the silty loam exposure aquaria (Figure 3E), biological effects were not seen until 14 days of exposure (Figure 3B). The lack of significant AR response coupled with the limited detection of aqueous steroids suggest the significant reduction of Vtg after 14 days of exposure (Figure 3B) is due to low dose exposure either from sediment-associated steroid or small amounts of aqueous steroid desorbed from the sediments over the 14 day period.

Environmental Implications of Sediment-Associated Progesterone in Aquatic Systems. In systems subject to episodic loading of steroid hormones, the importance of sediment/contaminant interactions on both contaminant fate and the subsequent bioavailability to aquatic organisms needs to be considered when determining environmental risk associated with a contaminant. The results of this study, as well as those reported in Sangster et al.,[13] illustrate the effect sediment type has on compound fate and bioavailability in aquatic systems. In both sediments evaluated in this study, P4 degradation resulted in the androgens, androsteinedione, T, and androstadienedione, and the antiestrogen testolactone confirming results of previous work using pure cultures is applicable in more complex environmentally relevant systems. The finer texture silty loam sediment allows for greater persistence of the compounds and may provide for increased transport and contact by providing greater quantities of suspended particles; however, biological response appeared to be mitigated by steroid interactions with the silty loam sediment. The coarser sand sediment composition allowed for persistence of P4 while providing an immediate source for biologically active compounds to the aqueous phase. This suggests the occurrence of steroid hormones in sediment rather than water alone should be assessed to determine the environmental risks in impacted systems.

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.est.5b06082.

Sediment mineralogy (Table S1), compound retention times (Table S2) and instrument detection limits (Table S3) related to analytical methods, QA/QC information for sediment (Table S4) and water (Table S5) samples, morphometric data for male and female fathead minnows (Table S6), measured aqueous and sorbed steroids from exposure aquaria (Table S7), and detailed hepatic gene expression data (Figure S1) (PDF)

■ AUTHOR INFORMATION

Corresponding Author

*Phone (402) 554-3868; fax (402) 554-3288; e-mail: sbartelt2@unl.edu.

Notes

The authors declare no competing financial interest.

■ ACKNOWLEDGMENTS

We acknowledge Sarah Skolness, Yasmine Farhat, Yun Zhang, and Ryan Krysl for their assistance in collection of sediment samples and during dissections. Additionally, the authors would like to thank Dave Cassada and Sathaporn Onanong for their assistance during the analysis of sediment and water samples. Funding for this project was provided by National Science Foundation grants CBET-1040895 and CBET-0966850, the Robert G. Daugherty Water for Food Institute Graduate Assistantship, University of Nebraska (J. Ali), and the National Science Foundation Graduate Fellowship Program (J. Sangster).

■ REFERENCES

(1) Jenkins, R. L.; Wilson, E. M.; Angus, R. A.; Howell, W. M.; Kirk, M. Androstenedione and progesterone in the sediment of a river receiving paper mill effluent. *Toxicol. Sci.* **2003**, *73* (1), 53−59.

(2) Scott Mansell, D.; Bryson, R. J.; Harter, T.; Webster, J. P.; Kolodziej, E. P.; Sedlak, D. L. Fate of Endogenous Steroid Hormones in Steer Feedlots Under Simulated Rainfall-Induced Runoff. *Environ. Sci. Technol.* **2011**, *45* (20), 8811−8818.

(3) Gall, H. E.; Sassman, S. A.; Lee, L. S.; Jafvert, C. T. Hormone discharges from a midwest tile-drained agrecosystem receiving animal wastes. *Environ. Sci. Technol.* **2011**, *45*, 8755−8764.

(4) Kumar, V.; Johnson, A. C.; Trubiroha, A.; Tumova, J.; Ihara, M.; Grabic, R.; Kloas, W.; Tanaka, H.; Kroupova, H. K. The Challenge Presented by Progestins in Ecotoxicological Research: A Critical Review. *Environ. Sci. Technol.* **2015**, *49* (5), 2625−2638.

(5) Bartelt-Hunt, S. L.; Snow, D. D.; Kranz, W. L.; Mader, T. L.; Shapiro, C. A.; van Donk, S. J.; Shelton, D. P.; Tarkalson, D. D.; Zhang, T. C. Effect of Growth Promotants on the Occurrence of Endogenous and Synthetic Steroid Hormones on Feedlot Soils and in Runoff from Beef Cattle Feeding Operations. *Environ. Sci. Technol.* **2012**, *46* (3), 1352−1360.

(6) DeQuattro, Z. A.; Peissig, E. J.; Antkiewicz, D. S.; Lundgren, E. J.; Hedman, C. J.; Hemming, J. D. C.; Barry, T. P. Effects of progesterone on reproduction and embryonic development in the fathead minnow (Pimephales promelas). *Environ. Toxicol. Chem.* **2012**, *31* (4), 851−856.

(7) Zucchi, S.; Castiglioni, S.; Fent, K. Progesterone Alters Global Transcription Profiles at Environmental Concentrations in Brain and Ovary of Female Zebrafish (Danio rerio). *Environ. Sci. Technol.* **2013**, *47* (21), 12548−12556.

(8) Zucchi, S.; Castiglioni, S.; Fent, K. Progestins and Antiprogestins Affect Gene Expression in Early Development in Zebrafish (Danio rerio) at Environmental Concentrations. *Environ. Sci. Technol.* **2012**, *46* (9), 5183−5192.

(9) Stumpe, B.; Marschner, B. Dissolved organic carbon from sewage sludge and manure can affect estrogen sorption and mineralization in soils. *Environ. Pollut.* **2010**, *158* (1), 148−54.

(10) Mashtare, M. L.; Green, D. A.; Lee, L. S. Biotransformation of 17 alpha- and 17 beta-estradiol in aerobic soils. *Chemosphere* **2013**, *90* (2), 647−652.

(11) Khan, B.; Lee, L. S. Soil temperature and moisture effects on the persistence of synthetic androgen 17α-trenbolone, 17β-trenbolone and trendione. *Chemosphere* **2010**, *79* (8), 873−879.

(12) Sangster, J. L.; Oke, H.; Zhang, Y.; Bartelt-Hunt, S. L. The effect of particle size on sorption of estrogens, androgens and progestagens in aquatic sediment. *J. Hazard. Mater.* **2015**, *299*, 112−121.

(13) Sangster, J. L.; Zhang, Y.; Hernandez, R.; Garcia, Y. A.; Sivils, J. C.; Cox, M. B.; Snow, D. D.; Kolok, A. S.; Bartelt-Hunt, S. L. Bioavailability and fate of sediment-associated trenbolone and estradiol in aquatic systems. *Sci. Total Environ.* **2014**, *496*, 576−584.

(14) Jessick, A. M.; Skolness, S.; Kolok, A. S. Sandy sediment and the bioavailability of 17B-trenbolone to adult female fathead minnows. *Aquat. Toxicol.* **2014**, *148*, 48−54.

(15) Duong, C. N.; Schlenk, D.; Chang, N. I.; Kim, S. D. The effect of particle size on the bioavailability of estrogenic chemicals from sediments. *Chemosphere* **2009**, *76* (3), 395−401.

(16) Sellin, M. K.; Snow, D. D.; Schwarz, M.; Carter, B. J.; Kolok, A. S. Agrichemicals in Nebraska, Usa, Watersheds: Occurrence and Endocrine Effects. *Environ. Toxicol. Chem.* **2009**, *28* (11), 2443−2448.

(17) Sellin, M. K.; Snow, D. D.; Kolok, A. S. Reductions in hepatic vitellogenin and estrogen receptor alpha expression by sediments from an agriculturally impacted waterway. *Aquat. Toxicol.* **2010**, *96* (2), 103−108.

(18) Zhang, Y.; Krysl, R. G.; Ali, J. M.; Snow, D. D.; Bartelt-Hunt, S. L.; Kolok, A. S. Impact of Sediment on Agrichemical Fate and Bioavailability to Adult Female Fathead Minnows: A Field Study. *Environ. Sci. Technol.* **2015**, *49* (15), 9037−9047.

(19) Qi, Y.; Zhang, T. C.; Ren, Y. Testosterone sorption and desorption: Effects of soil particle size. *J. Hazard. Mater.* **2014**, *279*, 493−501.

(20) Sun, K.; Jin, J.; Gao, B.; Zhang, Z.; Wang, Z.; Pan, Z.; Xu, D.; Zhao, Y. Sorption of 17 alpha-ethinyl estradiol, bisphenol A and phenanthrene to different size fractions of soil and sediment. *Chemosphere* **2012**, *88* (5), 577−583.

(21) Khan, B.; Lee, L. S.; Sassman, S. A. Degradation of Synthetic Androgens 17α- and 17β-Trenholone and Trendione in Agricultural Soils. *Environ. Sci. Technol.* **2008**, *42* (10), 3570−3574.

(22) Sellin Jeffries, M. K.; Conoan, N. H.; Cox, M. B.; Sangster, J. L.; Balsiger, H. A.; Bridges, A. A.; Cowman, T.; Knight, L. A.; Bartelt-Hunt, S. L.; Kolok, A. S. The anti-estrogenic activity of sediments from agriculturally intense watersheds: Assessment using in vivo and in vitro assays. *Aquat. Toxicol.* **2011**, *105* (1), 189−198.

(23) Hunter, A. C.; Coyle, E.; Morse, F.; Dedi, C.; Dodd, H. T.; Koussoroplis, S. Transformation of 5-ene steroids by the fungus Aspergillus tamarii KITA: Mixed molecular fate in lactonization and hydroxylation pathways with identification of a putative 3 beta-hydroxy-steroid dehydrogenase/Delta(5)-Delta(4) isomerase pathway. *Biochim. Biophys. Acta, Mol. Cell Biol. Lipids* **2009**, *1791* (2), 110−117.

(24) Hunter, A. C.; Carragher, N. E. Flexibility of the endogenous progesterone lactonisation pathway in Aspergillus tamarii KITA: transformation of a series of cortical steroid analogues. *J. Steroid Biochem. Mol. Biol.* **2003**, *87* (4−5), 301−308.

(25) Bartmanska, A.; Dmochowska-Gladysz, J.; Huszcza, E. Steroids' transformations in Penicillium notatum culture. *Steroids* **2005**, *70* (3), 193−198.

(26) Mostafa, M. E.; Zohri, A. A. Progesterone side-chain degradation by some species of Aspergillus flavus group. *Folia Microbiol. (Dordrecht, Neth.)* **2000**, *45* (3), 243−247.

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036

(27) Yildirim, K.; Uzuner, A.; Gulcuoglu, E. Y. Baeyer-Villiger. Oxidation of some steroids by Aspergillus tamarii MRC 72400. *Collect. Czech. Chem. Commun.* **2011**, *76* (6), 743−754.
(28) Pollio, A.; Pinto, G.; DellaGreca, M.; Fiorentino, A.; Previtera, L. Biotransformations of progesterone by Chlorella spp. *Phytochemistry* **1996**, *42* (3), 685−688.
(29) Jeffries, M. K. S.; Conoan, N. H.; Cox, M. B.; Sangster, J. L.; Balsiger, H. A.; Bridges, A. A.; Cowman, T.; Knight, L. A.; Bartelt-Hunt, S. L.; Kolok, A. S. The anti-estrogenic activity of sediments from agriculturally intense watersheds: Assessment using in vivo and in vitro assays. *Aquat. Toxicol.* **2011**, *105* (1−2), 189−198.
(30) Kolok, A. S.; Snow, D. D.; Kohno, S.; Sellin, M. K.; Guillette, L. J. Occurrence and biological effect of exogenous steroids in the Elkhorn River, Nebraska, USA. *Sci. Total Environ.* **2007**, *388* (1−3), 104−115.
(31) Schmittgen, T. D.; Livak, K. J. Analyzing real-time PCR data by the comparative C-T method. *Nat. Protoc.* **2008**, *3* (6), 1101−1108.
(32) DeBeuckelaer, A. A closer examination on some parametric alternatives to the ANOVA F-test. *Stat. Pap.* **1996**, *37* (4), 291−305.
(33) Snow, D. D.; Damon-Powell, T.; Onanong, S.; Cassada, D. A. Sensitive and simplified analysis of natural and synthetic steroids in water and solids using on-line solid-phase extraction and microwave-assisted solvent extraction coupled to liquid chromatography tandem mass spectrometry atmospheric pressure photoionization. *Anal. Bioanal. Chem.* **2013**, *405* (5), 1759−1771.
(34) Chishti, Y. Z.; Feswick, A.; Martyniuk, C. J. Progesterone increases ex vivo testosterone production and decreases the expression of progestin receptors and steroidogenic enzymes in the fathead minnow (Pimephales promelas) ovary. *Gen. Comp. Endocrinol.* **2014**, *199*, 16−25.
(35) Ellestad, L. E.; Cardon, M.; Chambers, I. G.; Farmer, J. L.; Hartig, P.; Stevens, K.; Villeneuve, D. L.; Wilson, V.; Orlando, E. F. Environmental Gestagens Activate Fathead Minnow (Pimephales promelas) Nuclear Progesterone and Androgen Receptors in Vitro. *Environ. Sci. Technol.* **2014**, *48* (14), 8179−8187.

DOI: 10.1021/acs.est.5b06082
Environ. Sci. Technol. 2016, 50, 4027−4036