Impact of sediment particle size on biotransformation of 17 β -estradiol and 17 β -trenbolone

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ABSTRACT: Soil/sediment particle size has been reported to influence the sorption and bioavailability of steroid hormones in the environment. However, the impact of particle size on biotransformation has not been well elucidated. The present study investigated the dissipation of 17 β -estradiol and 17 β -trenbolone and the formation and degradation of the subsequent transformation products in different size fractions of a sandy and a silt loam sediment. The results showed that the decay of 17 β -estradiol and 17 β -trenbolone associated with fine particles followed a biphasic pattern with more rapid decay in the initial phase followed by a second phase with slower decay of the residues compared to their decay rates in the sand fraction. Estrone and trendione were detected as a primary biotransformation product for 17 β -estradiol and 17 β -trenbolone, respectively. The parent-to-product conversion ratios and the degradation rates of estrone and trendione varied among different size fractions, but no consistent correlation was observed between decay rates and sediment particle size. Estrone and trendione decayed in the whole sediments at rates not statistically different from those associated with the fine fractions. These results indicate that fine particles may play an important role in influencing the persistence of and the potential risk posed by steroid hormones in the aquatic systems.

KEY WORDS: estrogen, androgen, biotransformation, sand fraction, fine fraction

1. Introduction

Steroid hormones are widely used as active components of contraceptive pills, hormone therapy drugs, and growth promoters for animal production. They have been detected in watersheds in the proximity of sewage treatment plants (Kolok et al., 2007; Labadie and Budzinski, 2005; Vajda et al., 2008), animal feeding operations (Bartelt-Hunt et al., 2012; Kolok et al., 2007), and manure-amended fields (Finlay-Moore et al., 2000; Gall et al., 2011). Exogenous steroid hormones and their metabolites disrupt the endocrine system of aquatic organisms and cause hormone-responsive gene alteration, sex reversal, and population decreases at environmentally relevant concentrations (Baumann et al., 2014; Dorts et al., 2009; Filby et al., 2007; Jenkins et al., 2003; Kidd et al., 2014; Kidd et al., 2007; Parks et al., 2001; Sellin et al., 2009; Vajda et al., 2011). Given the increasing evidence for endocrine disrupting effects caused by exogenous steroid hormones and the subsequent formation of metabolites, knowledge of their partitioning, transport, and attenuation processes is of great importance to assess and control the risk posed by these compounds in aquatic systems.

Steroid hormones tend to associate with sediment due to their hydrophobicity after entering the aquatic environment, and the interaction with sediment has great impact on the fate and bioavailability of steroid hormones. Prior studies have evaluated the sorption capacity of soils and sediments and reported partition coefficients for estrogens, androgens, and progestogens. Sorption coefficients are reported to be strongly correlated with the organic carbon content of soil/sediment (Holthaus et al., 2002; Khan et al., 2009; Sun et al., 2012). Recently, Qi et al. (Qi et al., 2014) showed that fine particles exhibited higher sorption capacity and lower desorption potential for testosterone compared to larger particles, likely due to an increase in surface area and/or organic carbon content as particle size decreased. Sangster et al. (Sangster et al., 2015)

reported that 17 β -estradiol (17 β -E2), estrone (E1), progesterone and testosterone were preferentially sorbed to fine size fractions (clay and colloids) within whole sediments. These results indicated that fine sediment particles may facilitate the transport of steroid hormones in the environment. Particle size also affected the bioavailability of steroid hormones to aquatic organisms. Duong et al. (Duong et al., 2009) observed that 17 β -E2 bound with certain particle size fractions (450-800 nm) significantly induced hepatic vitellogenin production in male Japanese medaka while 17 β -E2 associated with particles of 1-50 μ m and larger than 50 μ m did not.

The impact of soil/sediment particle size on the attenuation rates of steroid hormones has not been well elucidated. Transformation and degradation processes are critical for determining the persistence of steroid hormones (Khan et al., 2008; Mashtare et al., 2013a; Stumpe and Marschner, 2009). Biotransformation is a dominant transformation process in the environment where light penetration is limited, for instance in deep or turbid water, as well as in soil and alluvial sediment. Due to the complexity and heterogeneity of the environment, a wide range of half-lives of steroid hormones has been reported. Temperature, pH, redox potential, and organic content have been found to affect the attenuation rates of steroid hormones (Bradley et al., 2009; Lee et al., 2011; Mashtare et al., 2013b; Sarmah and Northcott, 2008; Stumpe and Marschner, 2009; Stumpe and Marschner, 2010; Yang et al., 2010; Zheng et al., 2012). However, little attention has been paid to the particle size effects on the biotransformation of steroid hormones. Although slower degradation of 17 β -E2, 17 α -ethynyl estradiol, and testosterone was observed in a soil with higher sand content (Khan et al., 2008; Lee et al., 2003), the role of distinct size fractions in the persistence of steroid compounds is still unclear.

The objective of this study was to investigate the biotransformation kinetics of steroid hormones and their primary metabolites associated with different size fractions of alluvial sediment via microcosm experiments. 17β -E2 and 17β -trenbolone (17β -TB) were chosen as models of potent estrogens and androgens, respectively. 17β -E2 is a natural estrogen which is excreted by humans and livestock. 17β -TB is a synthetic androgen typically occurring as a major metabolite of trenbolone acetate, an active component of growth promotion formulae and usually administered in combination with 17β -E2. The lowest observable effect levels (LOELs) of 17β -E2 and 17β -TB reported in fish are 1- 28 ng/L (Gross-Sorokin et al., 2006; Seki et al., 2006; Young et al., 2002) and 10-50 ng/L (Ankley et al., 2003), respectively. As a first study evaluating the particle size effect on the biotransformation kinetics of steroid hormones, the present results will provide some insights into the fate of sediment associated endocrine disrupting compounds.

2. Materials and methods

2.1 Chemicals

17β -E2 (purity $\geq 98\%$), 17α -E2 (99.8%), Estriol (E3, 99.3%), and 17β -TB (purity $\geq 95\%$) were purchased from Sigma-Aldrich Chemicals (St. Louis, MO). Estrone (E1, purity $\geq 99\%$) was obtained from MP Biomedicals (Aurora, OH). 17α -TB was purchased from Australian Government National Measurement Institute (Lindfield, NSW, Australia). Trendione (TD, purity $\geq 95\%$) was synthesized as described in Supplementary Information. Selected chemical properties are shown in Table S1 (Supplementary Information). Methanol (OptimaTM grade) was obtained from Fisher Scientific (Pittsburgh, PA).

2.2 Sediment collection and fractionation

84 Two types of sediment, a sand and a silt loam, were collected from the Elkhorn River near
85 Winslow, Nebraska (41°37'15"N, 96°30'07"W) and Plum Creek at Seward, Nebraska
86 (40°53'54"N, 97°05'26"W), respectively. These sediments have been used in prior laboratory
87 studies investigating the sorption and biological effects of steroid hormones (Sangster et al.,
88 2014; Sangster et al., 2015; Sangster et al., 2016). No background steroids of interest were
89 detected in either sediment. The sand consisted of 92% sand, 2% silt and 6% clay with illite as
90 the dominant clay type and contained only 0.10% organic carbon (OC); the silt loam consisted of
91 16% sand, 60% silt and 24% clay with interlayer smectite as the dominant clay type and OC
92 content was 1.45% (Table 1).

93 **Table 1**

94 Properties of fractions separated from two sediments.

Sediment Classification ^a	Fraction	Particle Size (μm) ^b	Surface Area (m ² /g) ^c	OC ^d (g/kg dw)	TP ^e (g/kg dw)	TN ^f (g/kg dw)	Water content ^g (%)	pH ^h
Sandy	Whole (100%)		1.36	1.01	0.07	6.19	26.8 ± 2.4	7.55
	Sand (92 %)	63-2000	1.38 ± 0.06	0.74	0.09	8.24	23.8 ± 0.5	7.46
	Fines (8 %)	0.75-17.7	29.6 ± 0.36	16.1	0.95	10.7	40.4 ± 2.3	7.56
Silt loam	Whole (100%)		19.9	14.5	0.99	9.66	37.7 ± 0.4	7.49
	Sand (16 %)	63-2000	1.50 ± 0.27	0.33	0.16	10.0	28.7 ± 0.6	7.11
	Silt (60 %)	8.1-17.7	20.78 ± 0.68	9.49	0.78	13.9	46.7 ± 1.9	7.16
	Clay (24 %)	0.81-1.74	47.66 ± 8.83	36.4	4.99	21.9	67.9 ± 0.1	6.69

95 ^a Determined by Midwest Laboratory Inc., Omaha, NE, using hydrometer analysis.96 ^b Fines of sandy sediment and silt and clay of silt loam sediment were measured using zetasizer.97 ^c BET analysis by Department of Chemistry, University of Nebraska-Lincoln.98 ^d Organic carbon: hot-plate digestion (HEANES, 1984).99 ^e Total phosphorus (EPA Method 365.3, 1978).100 ^f Total nitrogen (EPA Method 351.2, 1978).101 ^g The water content (by weight) in the sediment at saturation.102 ^h Solid was mixed with deionized water at a ratio of 1:2 (g dw: mL) except clay fraction in silt loam sediment. The ratio used for the clay fraction
103 was 1:5 in order to get enough liquid for pH measurement.

The sediments were fractionated using modified sieve and deposition methods (Qi et al., 2014; Sangster et al., 2015) and details are provided in Figure S1 (Supplementary Information). Briefly, the whole sediment was first wet sieved through ASTM Sieve No. 10 (mesh size 2 mm) and No. 230 (mesh size 63 μm). The fraction passing Sieve No. 10 and retained by Sieve No. 230 was gently rinsed three times to remove the remaining small particles and then preserved as the “sand fraction” (63 μm - 2 mm). Two liters of the slurry passing Sieve No. 230 was collected in 20-liter buckets, mixed completely with another 18 L of pure water, and allowed to settle for 1 hr. After the supernatant was decanted, the sediment settled at the bottom of the buckets was resuspended in pure water and settled for another 1 hr. This step was repeated three times and the settled sediment was centrifuged at 2000 rpm for 20 min to remove free water. The settled portion was designated as the “silt fraction” (2 - 63 μm). All the slurry decanted from the previous step was collected in 1-L graduated cylinders. After 6 hr sedimentation, the supernatant was siphoned out and centrifuged at 3000 rpm for 40 min. The pellet was collected and preserved as the “clay fraction” (< 2 μm). The “sand”, “silt” and “clay” fractions in this study are defined according to ISO 14688-1: 2002 (ISO, 2002). Since the percentages of silt and clay in the sandy sediment are 2% and 6%, respectively, and it was not feasible to generate large enough quantities for the microcosm degradation experiments, the sandy sediment was separated into two fractions, sand and fines. Each fraction separated using the sedimentation procedure were saturated with water but did not contain freely flowing water. All whole sediments and sediment fractions were stored in the dark at 4°C immediately until use. Selected properties are shown in Table 1. Although the moisture content varied between fractions due to differences in water holding capacity, it was kept at 100% of maximum water holding capacity of each fraction

through the degradation period. Notably, for both sandy and silt loam sediments, specific surface area, organic carbon, total phosphorus and nitrogen increased as particle size decreased.

2.3 Biotransformation microcosms

Ten milligrams of 17 β -E2 or 17 β -TB were dissolved in 10 mL methanol and diluted to 10 mg/L using methanol. 150 μ L of the spike solution (10 mg/L) was spiked to 20-mL glass vials and methanol was completely evaporated under a gentle stream of nitrogen gas. 3 g (dry weight) of whole sediments or sediment fractions conditioned at $21 \pm 1^\circ\text{C}$ overnight were then weighed into the glass vials. As a result, the nominal initial concentration of 17 β -E2 or 17 β -TB in each microcosm was 0.5 mg/kg dw. No extra water was added. All the microcosms were wrapped with parafilm M® to allow for oxygen exchange and to maintain moisture content. Moisture content was measured before weighing sediments and fractions into the reactors but was not monitored during the reaction. They were incubated in a $21 \pm 1^\circ\text{C}$ shaker with a lightproof cover. Triplicate microcosms were periodically sacrificed and preserved at -20°C until analysis. Another subset of microcosms was prepared as abiotic controls. They were autoclaved for 30 min at 121°C and 103.4 kPa (repeated three times over 3 consecutive days) prior to addition of 17 β -E2 or 17 β -TB.

2.4 Sample extraction and derivatization

Sediment-associated steroids and their metabolites were extracted using microwave-assisted solvent extraction (Snow et al., 2013). Thawed sediment in a microcosm was completely transferred to a 10-mL Teflon™ extraction vessel and spiked with 1 mg of butylatedhydroxytoluene (antioxidant) as well as 1000 ng of diethylstilbestrol as surrogate. After adding 6 mL of methanol, the vessels were capped, mixed thoroughly, and placed in a MARS-Xpress microwave system (CEM Microwave Technology, Buckingham, UK). The samples were

irradiated at 800 W with the temperature ramped to 90°C within 10 min and held for 5 min. After cooling down, the extract was carefully pipetted out and another 6 mL of methanol was mixed with sediment to rinse. Both portions of the methanol extract were combined and evaporated to approximately 0.5 mL under a steady nitrogen gas. The remaining extract was then transferred to a 1-mL conical reaction vial and spiked with 1000 ng internal standards (¹³C₃-17β-E2 for estrogens and d₅-17β-TB for androgens) prior to evaporation to dryness. Estrogens were derivatized at 75°C for 30 min using 100 μL of *N,N*-dimethylformamide and 100 μL of *N,O*-bis(trimethylsilyl)trifluoroacetamide with 1% trimethylchlorosilane (BSTFA+1% TMCS) (Shareef et al., 2006). Derivatization of androgens followed a two-step procedure. Fifty microliter of *N*-methyl-*N*-(trimethylsilyl)trifluoroacetamide (MSTFA) containing iodine (8.8 mg I₂/mL MSTFA) was added into the reaction vial and evaporated to dryness under nitrogen gas. Superfluous I₂ was removed by continuing evaporation for another 1 hr. Then another 100 μL of MSTFA was added to the vial, vortexed for 1 min and placed in 60°C for 40 min. After cooling down, the derivatization extracts were transferred to inserts placed in autosampler vials for gas chromatography mass spectrometry (GC/MS) analysis.

2.5 GC/MS analysis

The derivatized extracts were analyzed on Agilent 5972 GC/MS operated in the selective ion monitoring (SIM) mode. A DB-1MS column (30 m × 0.25 mm I.D., 0.25 μm film) was used for gradient separation, and helium (He) was used as carrier gas with a column head pressure of 7.5 psi. One microliter of each sample was injected at 280°C. The oven ramp started at 80°C, held for 2 min, ramped at 15°C/min to 150°C, 5°C/min to 250°C, 15°C/min to 275°C, and then held for 10 min (38 min in total). The detector transfer line temperature was 280°C. Identification of target steroids was accomplished by comparing the retention times to those of standards analyzed

under the same conditions, and quantification was done by internal standard calibration. Method detection limits of all analytes ranged between 10.8 ng/g and 32.0 ng/g, and recovery ranged between 87.4% and 132.5% (Table S1, Supplementary Information). Additional instrumental details and the quality assurance/quality control information are provided in Table S2 and Table S3 (Supplementary Information).

2.6 Data analysis

Statistical analysis, curve fitting and parameter estimation were performed using GraphPad Prism v6.0 (GraphPad Software, La Jolla, CA). The attenuation kinetics of parent compounds (17 β -E2 and 17 β -TB) was modeled with one phase exponential decay model (Khan et al., 2008; Mashtare et al., 2013a; Scherr et al., 2008) or double exponential decay model (Scherr et al., 2008), assuming there is no significant microbial growth and initial substrate concentration is relatively low compared to a half-saturation constant.

One phase exponential decay model for parent compound:

$$C_t/C_0 = e^{-k_1 t} \quad (1)$$

Double exponential decay model for parent compound:

$$C_t/C_0 = g e^{-k_1 t} + (1 - g) e^{-k_2 t} \quad (2)$$

where t is time (day), C_t and C_0 are molar concentrations of parent compound at day t and day 0, respectively, and k_1 and k_2 are the parent degradation rate constants (day⁻¹). Double exponential decay model consists of a fast degradation compartment and a slow degradation compartment. Thus usually $k_1 > k_2$. g is the fraction of C_0 applied to the fast degradation compartment.

The formation and dissipation kinetics of biotransformation products was also fitted with one phase or double exponential decay models and incorporated best-fitted parent decay model.

One phase exponential decay model for a product:

$$C_t'/C_0 = f(1 - C_t/C_0)e^{-k_1't} \quad (3)$$

Double exponential decay model for a product:

$$C_t'/C_0 = f(1 - C_t/C_0)[g'e^{-k_1't} + (1 - g')e^{-k_2't}] \quad (4)$$

where C_t' is molar concentration of metabolite generated from the biodegradation of parent compound at day t , C_t and C_0 are molar concentrations of parent compound at day t and day 0, f is the fraction of degraded parent that is converted to the metabolite, k_1' and k_2' are the metabolite degradation rate constants (day^{-1}) with usually $k_1' > k_2'$, and g' is the fraction of the metabolite applied to the fast degradation compartment.

Mass concentrations (ng/g) of parent and daughter compounds, were converted to molar concentrations and then normalized by dividing C_t or C_t' by C_0 (C_0 was the average of the replicates at $t = 0$). The mean of replicates at each time interval and standard error of the mean were plotted versus time to generate transformation profiles. Nonlinear least-squares regression was used to fit the normalized data considering each replicate as an individual data point. A preliminary analysis showed negligible variation in predicted parameters when curve fitting was performed with the normalized data compared with the original data. Akaike's Information Criteria (AIC_c) was used to compare the candidate models and select the best-fit model (Scherr et al., 2008). The rate constants (k) and the time required for 50% and 90% dissipation of the initial concentration ($t_{1/2}$ and $t_{1/10}$) were estimated based on the best-fit model. For the one phase exponential decay models, $t_{1/2} = \ln 2 / k$ and $t_{1/10} = \ln 10 / k$. For the double exponential decay models, $t_{1/2}$ and $t_{1/10}$ were interpolated from the best fitted model by setting $C_t/C_0 = 0.5$ and 0.1, respectively, in equation (2) or (4) and solving the equation through an iterative process. Analysis of variance (ANOVA) followed by Tukey's multiple comparison test or t test was

performed to test if the degradation rate constants were statistically different (significant when $p < 0.05$).

3. Results and discussion

3.1 Biotransformation of 17 β -E2 and 17 β -TB associated with different size fractions

Normalized concentrations and the best-fit models of the parent compounds 17 β -E2 and 17 β -TB are presented in Figure 1. Predicted decay rate constants and dissipation times ($t_{1/2}$ and $t_{1/10}$) are summarized in Table 2. Generally, 17 β -E2 and 17 β -TB decayed rapidly in both sediments and different fractions with half-lives ranging from 1.5 to 16 hours which are consistent with the ranges reported in prior studies (Khan et al., 2008; Mashtare et al., 2013a; Sarmah and Northcott, 2008; Stumpe and Marschner, 2009). In both whole sediments, 17 β -E2 decayed faster than 17 β -TB with rate constants 3-4 times greater than those of 17 β -TB. Sediment type did not make a significant difference in the dissipation of either parent compound ($p = 0.2018$ for 17 β -E2 and $p = 0.0820$ for 17 β -TB), whereas Lee et al. (Khan et al., 2008; Lee et al., 2003) has observed that the degradation of 17 β -E2, 17 α -ethynyl estradiol, and testosterone was slower in sediment with higher sand content. This discrepancy indicates soil/sediment type and the sand content may not be directly correlated to the degradation rates of steroid hormones although they may exert an indirect impact by providing distinct microhabitats and living conditions for microbial communities.

Figure 1. Biotransformation of (A) 17β-E2 in the sandy sediment, (B) 17β-E2 in the silt loam sediment, (C) 17β-TB in the sandy sediment, and (D) 17β-TB in the silt loam sediment. Error bars represent standard error of the means of replicates ($n = 3$ except for clay where $n_{\text{clay}} = 2$). The vertical red lines separate the two phases of degradation with different rates.

242 **Table 2**243 Selected models, estimated parameters, and the dissipation time of 17 β -E2 and 17 β -TB in the fractions of two sediments.

Sediment	Fraction	Model selection ^a		Estimated parameters ^b			Dissipation Time ^c	
		Model	Adj <i>R</i> ²	<i>g</i> (SE) (%)	<i>k</i> ₁ (SE) (day ⁻¹)	<i>k</i> ₂ (SE) (day ⁻¹)	<i>t</i> _{1/2} (95%CI) (hour)	<i>t</i> _{1/10} (95%CI) (day)
Parent compound 17β-E2								
Sandy	Whole	One	0.997		8.05 (0.303)		2.07 (1.92-2.24)	0.29 (0.26-0.31)
	Sand	One	0.976		1.86 (0.098)		8.92 (8.04-10.0)	1.24 (1.10-1.37)
	Fines	Double	0.975	86.1 (4.33)	3.18 (0.365)	0.142 (0.095)	6.45 (5.58-7.43)	2.34 (1.15-6.89)
Silt loam	Whole	Double	0.997	79.9 (1.65)	9.11 (0.837)	0.338 (0.055)	2.53 (2.18-2.92)	2.07 (1.67-2.63)
	Sand	Double	0.991	91.1 (3.50)	7.53 (0.910)	0.396 (0.283)	2.51 (2.10-2.99)	0.47 (0.39-0.77)
	Silt	Double	0.998	82.0 (1.18)	10.4 (0.860)	0.285 (0.041)	2.15 (1.86-2.47)	2.07 (1.68-2.57)
	Clay	Double	0.999	86.1 (0.64)	7.03 (0.271)	0.104 (0.015)	2.95 (2.74-3.18)	3.14 (2.43-3.99)
Parent compound 17β-TB								
Sandy	Whole	One	0.995		2.19 (0.078)		7.59 (7.05-8.22)	1.05 (0.97-1.13)
	Sand	One	0.958		1.04 (0.082)		16.0 (13.7-19.1)	2.21 (1.84-2.56)
	Fines	Double	0.986	78.8 (3.37)	7.30 (1.301)	0.195 (0.057)	3.25 (2.44-4.24)	3.86 (2.28-6.28)
Silt loam	Whole	One	0.955		2.67 (0.218)		6.24 (5.33-7.53)	0.86 (0.72-1.01)
	Sand	One	0.985		1.14 (0.056)		14.6 (13.2-16.2)	2.02 (1.81-2.22)
	Silt	Double	0.984	73.3 (1.80)	18.0 (14.42)	0.160 (0.027)	1.51 (0.46-3.74)	6.14 (4.68-8.22)
	Clay	Double	0.994	76.7 (2.12)	8.00 (1.010)	0.166 (0.038)	3.12 (2.50-3.84)	5.12 (3.64-7.71)

244 ^a “One” and “Double” refer to one phase and double exponential decay models, respectively. Only the best-fit results are reported in this table. Adjusted R^2 is
 245 reported instead of R^2 because it includes penalty for adding more parameters (see Supplementary Information).

246 ^b g and k_2 are reported only when double exponential decay model is the best fit model.

247 ^c $t_{1/2}$ = half-life; $t_{1/10}$ = the time needed for 90% of initial 17 β -E2 or 17 β -TB concentration to dissipate; “CI” means confidence interval.

Different decay patterns were observed between the sand fraction and the fine fractions (fines of the sandy sediment; silt and clay of the silt loam sediment) for both 17 β -E2 and 17 β -TB. The dissipation of 17 β -E2 and 17 β -TB in the sand fraction followed the one phase exponential decay kinetics except for 17 β -E2 in the sand fraction of the silt loam sediment. Double exponential decay model fits the profiles associated with the fine fractions better. In the first phase, the decay of 17 β -E2 did not exhibit significant differences among fractions of either sediment, while 17 β -TB decayed faster in the fine fractions than in the sand fraction. In the second phase, the 17 β -E2 and 17 β -TB residue in the fine fractions decayed much more slowly relative to the first phase and appeared more persistent than in the sand fraction. The dividing points for the four scenarios (Figure 1A to 1D) occurred roughly when $C/C_0 = 0.2$, therefore $t_{1/2}$ (when $C/C_0 = 0.5$) and $t_{1/10}$ (when $C/C_0 = 0.1$) occurred in the first and the second phases, respectively. A consequence from this phenomenon was that $t_{1/2}$ of 17 β -E2 and 17 β -TB in the fine fractions were similar to or smaller than that in the sand fraction while $t_{1/10}$ in the fine fractions were 1-4 days longer than that in the sand fraction (Table 2), prolonging the potential exposure time for aquatic organisms. The hormone residues associated with fine particles are of concern since fine particles are more likely consumed by aquatic organisms such as fish (Duong et al., 2009).

Sorption by sediment organic matter and diffusion into the glassy phase of organic matter and the micropores of sediment particles can shelter organic contaminants from further microbial degradation (Duong et al., 2010; Harms and Bosma, 1997; Pignatello and Xing, 1995; Stumpe and Marschner, 2010; Xing and Pignatello, 1997), which may explain the observed persistence of 17 β -E2 and 17 β -TB residues in the fine fractions in the second phase of the biphasic decay pattern. The fine particles have greater specific surface area and higher level of organic matter compared to the coarse particles (the sand fraction), which further supports this explanation.

However, in the first phase, 17 β -E2 and 17 β -TB decayed at a similar or faster rate in the fine fractions, which is not consistent with the second phase. In fact, in the early stage, the initial instantaneous sorption occurs on the exterior surface of the particles and the contaminants are still available to the microbes. The fine particles have been reported to contain more abundant and diverse microbial communities (Kanazawa and Filip, 1986; Kandeler et al., 2000; Monrozier et al., 1991; Sessitsch et al., 2001; Van Gestel et al., 1996) and higher enzymatic activities (Kandeler et al., 2000; Kandeler et al., 1999; Stemmer et al., 1998), which may contribute to the initial faster dissipation of 17 β -TB than in the sand fraction. The readily available fraction of a contaminant decreases and the recalcitrant fraction increases as contact time increases due to sorption and diffusion processes, resulting in the biphasic degradation pattern of contaminants in soil/sediment (Reid et al., 2000; Semple et al., 2003).

Differences in the decay rates and kinetic models were observed between 17 β -E2 and 17 β -TB. Generally, 17 β -E2 decayed faster than 17 β -TB with decay rate constants of 17 β -E2 larger than those of 17 β -TB. In the whole sediment and the sand fraction of silt loam, double exponential decay model fits 17 β -E2 better while one phase decay model fits 17 β -TB better. Although 17 β -E2 and 17 β -TB are similar in the steroidal structure of four carbon rings, a hydroxyl group is attached on the third carbon of the first ring in 17 β -E2 while in 17 β -TB, it is a carbonyl group in this position. In comparing the two compounds, the number and location of double bonds on the first and the second rings are also different. These functional groups react differently with microbes, resulting in differences in biotransformation kinetics. From the microbial aspects, the species, abundance and degrading ability of 17 β -E2 and 17 β -TB degraders could also be different, which directly affect the biotransformation route and rate (Iasur-Kruh et al., 2011; Yu et al., 2007).

3.2 Formation and degradation of the biotransformation products of 17 β -E2 and 17 β -TB

E1 and TD were detected as a primary biotransformation product for 17 β -E2 and 17 β -TB, respectively, in all fractions of both sediments. Normalized concentrations and the best-fit models of E1 and TD are presented in Figure 2. Predicted decay rate constants (k') and dissipation times ($t_{1/2}$ and $t_{1/10}$) are summarized in Table 3. Generally, one phase exponential decay model fits E1 and TD profiles in all fractions of either sediment. The estimated f values in Table 3 show that 64%-108% of the dissipated 17 β -E2 was converted to E1, and 53%-142% of 17 β -TB was converted to TD prior to further biotransformation or mineralization. Mass balances (Figure S2 in the Supplementary Information) show that the recoveries of total molar mass (17 β -E2+E1 and 17 β -TB+TD) exceed 100% at several time points. These errors may come from experimental variations among replicates as well as matrix effect on the analytical recoveries. Increased recoveries of TD (122-141%, see Table S3) contribute to the high TB-to-TD conversion rate (up to 142%) reported here, but do not change the shape of TD curves and thus the decay rate constants. E1 and TD are more persistent than their parent compounds with half-lives ranging from 0.9 to 6 days and from 0.8 to 24 days, respectively. This is consistent with the results of prior studies evaluating the biotransformation of 17 β -E2 and 17 β -TB (Bradley et al., 2009; Cole et al., 2015; Khan et al., 2008; Lee et al., 2003; Mashtare et al., 2013a; Sangster et al., 2014; Sarmah et al., 2008). Although E1 and TD are less potent than their parent compounds (Bauer et al., 2000; Thorpe et al., 2003), they are of extensive concern due to higher concentration in the environment and more persistence compared to 17 β -E2 and 17 β -TB.

Figure 2 Formation and dissipation of (A) E1 in the sandy sediment, (B) E1 in the silt loam sediment, (C) TD in the sandy sediment, and (D) TD in the silt loam sediment. Error bars represent standard error of the means of replicates ($n = 3$ except for clay where $n_{\text{clay}} = 2$).

319 **Table 3**

320 Selected models, estimated parameters, and dissipation times of E1 and TD in the fractions of two sediments

Sediment	Fraction	Model selection ^a		Estimated parameters		Dissipation Time ^c	
		Model	Adj R^2	k' (SE) (day ⁻¹)	f (SE) ^b (%)	$t_{1/2}$ (95%CI) (hour)	$t_{1/10}$ (95%CI) (day)
Metabolite of 17β-E2: E1							
Sandy	Whole	One	0.980	0.248 (0.013)	93.5 (2.01)	2.80 (2.52-3.14)	9.29 (8.37-10.4)
	Sand	One	0.869	0.130 (0.022)	108 (6.39)	5.35 (3.95-8.28)	17.8 (13.12-27.5)
	Fines	One	0.886	0.211 (0.028)	79.6 (4.06)	3.28 (2.56-4.57)	10.9 (8.52-15.2)
Silt loam	Whole	One	0.978	0.235 (0.014)	107 (2.58)	2.95 (2.63-3.37)	9.81 (8.72-11.2)
	Sand	One	0.969	0.792 (0.082)	63.7 (3.23)	0.88 (0.72-1.12)	2.91 (2.39-3.71)
	Silt	One	0.957	0.128 (0.010)	101 (2.94)	5.40 (4.64-6.46)	17.9 (15.4-21.5)
	Clay	One	0.907	0.116 (0.023)	76.1 (4.21)	5.96 (4.12-10.8)	19.8 (13.7-35.8)
Metabolite of 17β-TB: TD							
Sandy	Whole	One	0.913	0.087 (0.014)	111 (6.75)	8.01 (5.96-12.2)	26.6 (19.8-40.6)
	Sand	One	0.904	0.029 (0.012)	52.9 (3.95)	23.8 (12.9-157)	79.0 (42.7-523)
	Fines	One	0.840	0.081 (0.018)	102 (9.30)	8.59 (5.76-16.9)	28.5 (19.1-56.1)
Silt loam	Whole	One	0.950	0.283 (0.029)	122 (6.34)	2.45 (2.00-3.15)	8.13 (6.65-10.5)
	Sand	One	0.887	0.062 (0.011)	77.2 (4.62)	11.3 (8.27-17.6)	37.4 (27.5-58.5)
	Silt	One	0.833	0.101 (0.017)	77.8 (5.10)	6.85 (5.02-10.8)	22.8 (16.7-35.7)
	Clay	One	0.934	0.828 (0.219)	142 (17.4)	0.84 (0.51-2.38)	2.78 (1.69-7.92)

321 ^a “One” refers to one phase exponential decay model. Adjusted R^2 is reported instead of R^2 because it includes penalty for adding more parameters (see
322 Supplementary Information).

323 ^b f is the fraction of degraded parent that is converted to the product.

324 ^c $t_{1/2}$ = half-life; $t_{1/10}$ = the time needed for 90% of initial E1 or TD concentration to dissipate; “CI” means confidence interval.

The concentration profiles of E1 and TD are quite different among sediment fractions, and no consistent correlation can be established between the decay rates and the particle size. The impact of particle size on E1 dissipation was sediment type dependent. E1 decayed faster in the fine fraction ($k' = 0.211 \pm 0.028 \text{ day}^{-1}$) relative to the sand fraction ($k' = 0.130 \pm 0.022 \text{ day}^{-1}$) in the sandy sediment while the trend was reversed in the silt loam sediment. In both sediments, faster dissipation of TD was observed in the fine fractions: in the sandy sediment, $k'_{sand} = 0.029 \pm 0.012 \text{ day}^{-1}$ and $k'_{Fines} = 0.081 \pm 0.018 \text{ day}^{-1}$; in the silt loam sediment, k increased from $0.062 \pm 0.011 \text{ day}^{-1}$ to $0.828 \pm 0.219 \text{ day}^{-1}$ with decreasing particle size. Lee et al. (Lee et al., 2003) has reported that 17 β -E2, ethinylestradiol, and testosterone were most persistent in a soil containing the highest sand content and lowest organic carbon among five soils investigated, indicating the coarse particles may contribute to the slow degradation of steroid hormones. This is consistent with what we observed for E1 in the sandy sediment and TD in both sediments. Faster dissipation of the parent compound 17 β -TB was also observed in the first phase of degradation in the fine fractions. As discussed earlier, more abundant biomass and more diverse microbial communities associated with the fine particles can facilitate the degradation process (Kanazawa and Filip, 1986; Kandeler et al., 2000; Monrozier et al., 1991; Sessitsch et al., 2001; Van Gestel et al., 1996), and higher level of organic matter and nutrients (Table 1) also provided favorable conditions. Sorption and diffusion into the interior microstructure might reduce the bioavailability of a portion of steroid hormones and this portion can be greater in the fine particles due to higher level of organic matter and specific surface area compared to the coarse particles. However, the impact of sorption and diffusion on the degradation of E1 and TD seems limited, at least weaker than the impact of microbial aspects according to our results.

The abundance and degrading ability of the degraders of a particular steroid exert a direct influence on the decay rates as well as the formation of biotransformation products. It has been reported that most 17 β -E2 degraders convert it to E1 prior to further degradation while some species are capable of mineralizing 17 β -E2 directly without E1 formation (Yu et al., 2007). Less bacterial species are able to degrade E1 compared to the number of 17 β -E2-degrading species, and majority of 17 β -E2-degrading bacteria are not able to further degrade E1 (Iasur-Kruh et al., 2011; Yu et al., 2007). This could be one reason for faster degradation of 17 β -E2 than E1. It is unclear to date if the degraders of a particular steroid hormone and the producers of a particular metabolite are evenly distributed among different size fractions. They may have specific preferences for the microhabitats associated with different particle sizes. For example, bacteria belonging to the *Holophaga/ Acidobacterium* division has been found to be most abundant in smaller size fractions, whereas *α -Proteobacteria* and fungi are dominant in coarse particles (Kandeler et al., 2000; Sessitsch et al., 2001). This helps to explain why a consistent correlation between degradation rates and particle sizes could not be established and why the parent-to-compound conversion ratios (*f* values) varied among different size fractions.

Although the correlation between the degradation rates and the sediment particle size was not consistent, the fine particles seem to control the persistence of E1 and TD in the whole sediments. *ANOVA* followed by Tukey's multiple comparison tests (Figure 3) show that the decay rate constants of E1 or TD in the whole sediments are not significantly different from those in the fine fractions (*p* values = 0.307 - 0.960, details are provided in the Supplementary Information). This result can be attributed to the greater sorption capacity of and preferential sorption of steroid hormones by fine particles in the whole sediment. The sorption capacity of finer fractions (silt and clay) for 17 β -E2, E1, progesterone and testosterone is 2-20 times the

capacity of the coarser sand particles (Qi et al., 2014; Sangster et al., 2015). A preferential sorption study used the same sand and silt loam sediments as used in the present study and demonstrated that 60% - 90% of 17β -E2 and 80% - 98% of E1 were preferentially associated with fine particles (Sangster et al., 2015). In addition, Duong et al. (2009) indicated that steroid hormones bound with fine particles caused endocrine disruption in fish more easily than those bound with coarse particles. All these knowledges together with our results justify the importance of fine particles in the fate and toxicity of steroid hormones.

Figure 3 ANOVA followed by Tukey's multiple comparison of the decay rate constants of (A) E1 in the sandy sediment, (B) E1 in the silt loam sediment, (C) TD in the sandy sediment, and (D) TD in the silt loam sediment. The same lower case letter indicates decay rate constants not statistically different ($p > 0.05$).

No other transformation products of 17β -E2 were detected in either sediment or fraction, while low concentrations of 17α -TB ($< 2.42\%$ of the initial 17β -TB concentration) was detected

in the silt fraction of the silt loam sediment amended with 17 β -TB in our study. In prior studies, estriol (E3) was found as another biotransformation product of 17 β -E2 in addition to E1 typically in sewage and activated sludge (Layton et al., 2000; Lee and Liu, 2002; Ternes et al., 1999). Interconversion between 17 α -E2 and 17 β -E2 via E1 has been observed in dairy lagoon water and sediment (Mashtare et al., 2013b; Zheng et al., 2012) as well as between 17 α -TB and 17 β -TB via TD in agricultural soils and mixed culture suspensions (Cole et al., 2015; Khan et al., 2008). E3, 17 α -E2 and 17 α -TB did not occur in our microcosms at a significant level probably due to the absence of particular reaction conditions, microbial species or enough parent compounds.

4. Conclusions

Given the heterogeneity of alluvial sediment, evaluation of particle size effect on the biotransformation of steroid hormones may give us a better understanding of their persistence and potential risk in the aquatic environment. The results of this study show that 17 β -E2 and 17 β -TB dissipated quickly within one day regardless of size fractions but their residues are persistent in the fine fractions. Although the residues occur at low concentrations, they deserve close attention due to their potency in terms of endocrine disruption in aquatic organisms. The one phase exponential decay model has been used in many prior studies, but it fails to accurately simulate the low concentrations observed at later stages in the attenuation process and use of this model may underestimate the exposure risk to aquatic organisms. The majority of the dissipated 17 β -E2 and 17 β -TB is converted to E1 and TD, respectively. In most cases, E1 and TD decay faster when associated with silt and clay compared to the sand fraction. The fine fractions determine the overall persistence of E1 and TD in the whole sediment probably due to larger sorption capacity and preferential sorption by fine particles. Additional microbiological assays, such as the quantitation of steroid hormone-degrading microbes associated with different size

409 particles, will provide insight into the mechanisms for the phenomena we observed in this study.
410 This is a challenging but interesting research direction for our future efforts.

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415 **Appendix A. Supplementary Information**

416 Supplementary Information to this article can be found online.

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