Sorption of Testosterone on Partially-Dispersed Soil Particles of Different Size Fractions: Methodology and Implications

Yong Qi^a and Tian C. Zhang^b

^aPost-doc research assistant and corresponding author, 136 PKI, email: yqi01@unomaha.edu; ^bProfessor, 205D PKI, Civil Engineering Dept., University of Nebraska-Lincoln, Omaha, NE 68182, USA.

Abstract. Sorption of hormones to soil particles of different size fractions (DSFs) has been studied to understand their fate and transport (F/T) in soils. Conventional studies fractionated the soil particles into DSFs by using the high speed stirring method and/or adding surfactants to fully disperse the bulk soil. However, the natural processes (e.g., soil erosion, irrigation) often are relatively mild, and many soil particles may be still in the aggregate form. In this study, a method was developed for conducting the sorption test of a representative hormone (i.e., testosterone) to bulk soils first and then analyzing the results against DSFs. Results indicated the particle size distribution (PSD) of the two representative soils tested with partially-dispersed and fully-dispersed methods was significantly different due to the attachment of clay particles on sand and silt. Testosterone was sorbed mainly by the dominant aggregates even though they might have relatively lower sorption affinity than that of clays. However, the small particles (< 2000 nm), even with ~5% mass of the bulk soil, contributed more than 30% of sorbed testosterone in the “whole” soils. The partially-dispersed soil particles of DSFs should be used to understand the transport of hormone in runoff, because using the fully-dispersed soil particles will overestimate while the whole soil method will underestimate the transport potential. With the methodology developed in this study, the sorption tests will not compromise soil’s original properties (e.g., aggregates) or the competition (e.g., sorption) among soil particles, and the contribution of DSFs (particularly the partially-dispersed aggregates) to the sorption of the “whole” soil can be determined.

Keywords: Sorption; Soil; Partially-dispersed; Particle size fraction; Testosterone

1 Introduction

The increasing natural and synthetic steroidal hormones found in environments worldwide have caused wide public concern because of their potential of adverse effects on aquatic lives and human beings at very low concentrations (Kolpin et al., 2002; Orlando et al., 2004; Martinovic et al., 2007; Young et al., 2014). Currently, one of the critical research areas is to investigate the fate and transport (F/T) of hormones in the soil environment (Das et al., 2004; Salvia et al., 2014), with sorption properties of hormones to soils of different textures and organic contents being a major research focus. Soils with finer texture and higher organic content were reported to have a higher sorption affinity (Lee et al., 2003; Casey et al., 2004; Ma 2009).

Soils contain a wide range of particles with different size fractions (DSFs). These particles have different mobility during rainfall events (Mouri et al., 2014). Over 80% of the total soil particles found in storm water runoff were less than 20 μm (Randall, 1982; Furumai et al., 2002). It has been reported that the sorption and desorption properties of pesticides (e.g., atrazine and diuron) associated with soils exhibited significant particle-size dependence (Wang and Keller 2008). A slight change in clay content in the sediment can greatly change the sediment's potential for transporting chemicals sorbed on the solid phase (Foster et al., 1985). Therefore, it is necessary to determine the sorption-desorption properties of hormones associated with soil particles of DSFs. Qi et al. (2014) used wet-sieve-washing and repeated-sedimentation methods to fractionate soils into five soil particles of DSFs and used each of the DSFs for conducting sorption-desorption tests of testosterone. They found that the sorption and desorption properties of soil particles of DSFs associated with hormones are very different; clay particles have relatively high sorption capacity, mainly due to their high specific surface area. These previous studies fractionated the soil particles into DSFs by using the high speed stirring method and/or

adding surfactants (ASTM 2004), and then conducted the sorption tests with soil particles of each size fraction independently (Wang and Keller 2008; Qi et al., 2014). These methods can be used to determine the ultimate texture of the soil with respect to certain chemical or physical properties and the sorption-desorption behavior of these soil particles of DSFs.

One concern is that these sorption tests may compromise the soil's original properties (e.g., aggregates, attached organic matter) and the competition among soil particles of DSFs. It is well-known that the fundamental difference between fully-dispersed and partially-dispersed soil particles is very significant (Fig. 1). A soil aggregate is defined as a group of primary soil particles that cohere to each other more strongly than to other surrounding particles (Le Bissonnais 1996). Many soil aggregates are stable under the forces of erosion (Young 1980). In nature, the dispersion processes (e.g., irrigation, soil erosion during storm events) often are relatively mild, and these soil particles of DSFs are mixed together in the form of aggregates (i.e., partially-dispersed soil particles). Therefore, one should conduct the sorption tests with the 'bulk' soil first, and then fractionate the soil into particles (including soil colloids) of DSFs using partially-dispersed methods (e.g., no chemical, mild stirring). However, the existing knowledge gap is: *after the sorption test with the bulk soil, how to fractionate the soil particles of DSFs and then evaluate their contributions to sorption of hormones?*

The goal of this study is to fill this knowledge gap. The specific objectives of this study were to: 1) develop the procedures to conduct sorption test with bulk soil first, and then fractionate soil particles into DSFs under series sedimentation and centrifugation conditions; 2) compare the properties [e.g., total organic carbon (TOC), particle size distribution (PSD)] of partially-dispersed and fully-dispersed soil particles of DSFs; and 3) investigate the sorption distribution of testosterone among partially-dispersed soil particles of DSFs in the bulk soils.

2 Materials and Methods

Figs. 2 and 3 shows the major procedures developed in this study. Details are as follows.

2.1 *Selected Hormones and Soils*

Testosterone was selected as the representative hormone because 1) it is a prototype for other synthetic androgenic hormones (Kanayama and Pope 2012); 2) little is known about the F/T of androgenic hormones (Casey et al., 2004); and 3) it was used previously (Qi et al., 2014; Qi and Zhang 2015a; Ma et al., 2015). In the present study, sorption tests were conducted with testosterone at environmentally relevant low concentration levels (20–150 ng/L) (Finlay-Moore et al., 2000).

Two representative soils were selected to evaluate the effects of soil properties on methodology establishment and sorption properties of hormones; they were HAL (Haskell Agriculture Laboratory, Concord, NE, USA) soil and Iowa Black (IA, USA). These two soils were selected because they were sampled from the sites where the concentrated animal feeding operations (CAFOs) were nearby, and they were used in the previous studies (Ma et al., 2015; Qi et al., 2014). The HAL soil (Nora silty clay loam, fine-silty, mixed, mesic Udic Haplustolls) contains 0.95% total organic carbon (TOC). The total Fe, Cu, Ca, Mg are 21, 4.7, 2131, 318 mg/kg, respectively. The cation exchange capacity (CEC) of HAL soil is 13.9 meq/100 g. The Iowa Black (Dickinson sandy loam soil, coarse-loamy, mixed, superactive, mesic Typic Hapludolls) contains 0.68% TOC. The CEC of Iowa Black is 13.8 meq/100 g. No residual testosterone was detected in the soil samples (Ma et al., 2015). After air drying and sieving with a 2-mm sieve to remove larger particles, the soils (called original soils) were ready for use.

2.2 Chemicals and Solution

^{14}C -labeled testosterone came from the American Radio Chemicals Society (St. Louis, MO, USA). Low activity stock solution ($= 1 \mu\text{g/L}$, directly used in the experiment) was made by adding $1 \mu\text{L}$ of high activity ($= 50 \mu\text{Ci}$) stock solution of ^{14}C -labeled testosterone to 10 mL deionized (DI) water (electrical resistivity $= 18.2 \text{ M}\Omega \text{ cm}$). Unlabeled testosterone stock solution ($= 10 \mu\text{g/mL}$) was made by dissolving 0.001 g testosterone into 100 mL methanol, and preserved at 4°C in a brown glass container under the dark condition. The concentration of methanol was less than 1% by volume. Under this concentration, no effect of methanol on sorption of soils was observed by other researchers (Wauchope and Koskinen 1983; Fan et al., 2007). CaCl_2 (96% purity) and NaN_3 (99% purity) were purchased from Fisher Scientific Inc.

2.3 Fully-dispersed and Partially-dispersed Particle Size Distribution of Original Soils

In this study, six diameter size ranges were studied for partially-dispersed soil particles (Table 1): sand-sized aggregates (SDA = primary sand + large aggregates) ($2\text{--}0.075 \text{ mm}$), silt-sized aggregates (STA = primary silt + small aggregates) ($0.075\text{--}0.002 \text{ mm}$), clay 1 ($2\text{--}0.9 \mu\text{m}$), Colloids #1 ($0.9\text{--}0.6 \mu\text{m}$), #2 ($0.6\text{--}0.4 \mu\text{m}$), and #3 ($0.4\text{--}0.2 \mu\text{m}$). For fully-dispersed ones, corresponding size ranges of primary particles were investigated by Qi et al. (2014).

The PSD of HAL and Iowa Black soils were determined with both fully- and partially-dispersed methods. The fully-dispersed (conventional method) PSD was evaluated according to the standard methods (ASTM D422) (ASTM 2004) of both mechanical and hydrometer analyses. While for the partially-dispersed PSD, the soils were evaluated with the similar procedure as standard method except that $(\text{NaPO}_3)_6$ was not added (Fig. 2), and the soil slurry was rotated (top to bottom, one cycle per 5 second ($360^\circ/5 \text{ s}$), NSI-34RH, Gearmotor, Bodine Electric Co.) for 24 hours instead of using high speed stirring as suggested by ADTM D422 (Fig. 2). The fully-

dispersed and partially-dispersed clay mass distributions were measured with a particle size analyzer (NanoBrook 90Plus/Bi-MAS, Brookhaven Instrument Corp).

The mass distribution of the particles of six DSFs in the original soil was calculated by integrating the area below the PSD curve of the bulk soil. After sorption tests (see Section 2.5), the contribution of the particles of each size fraction to sorption of testosterone could be calculated by multiplying the results of their sorption concentrations to their corresponding weights in the original soil.

2.4 Characterization of Partially-dispersed Soil Particles

The SDA of HAL and Iowa Black soils was collected with the following steps: 1) place 5 g of original soil with 250 mL DI water in each of plastic bottles; 2) put these bottles in a rotator (top to bottom, 360°/5 s) for 24 hours; 3) wet sieving these soil slurry with 200# (75 μ m) sieve with another 1000 mL DI water; 4) oven dry the residue (SDA) in the sieve at 105 °C for 24 hours; and 5) test the TOC of the SDA and original soils in a commercial lab (Mid-West lab, Omaha, NE) with the standard method of ASTM D 5373.

The STA and clays from the original soils were fractionated with the sedimentation and centrifugation methods. First, the original HAL (or Iowa Black) soil (1.0 g) was placed into each of 24 plastic centrifuge tubes (#220-3550-080, 50-mL, Evergreen Scientific, Los Angeles, CA, USA). After adding 50 mL DI water, these 24 tubes were sealed with caps. All these tubes were placed in a rotator (top to bottom, 360°/5 s) for 24 hours. Then, four tubes were taken randomly for analyzing the properties of STA: 1) each of the two tubes settled for 30 min and 240 min (Fig. 3), respectively; 2) the soil particle weight (SPW) of the supernatant of 30 min and 240 min sedimentation was detected by a UV-spectrophotometer (GENESYS 10 UV, Thermo Electron Co. Madison, WI, USA) at the wavelength of 288 nm (Qi and Zhang 2015b) of MC₁ and MC₂:

$$W_{1-2} = (MC_1 - MC_2) \times V_c \times 10^{-6} \quad (1)$$

where W_{1-2} = weight of STA (between sedimentation conditions 1 and 2) (g); MC_1 and MC_2 represent the total particle mass concentration of supernatant of separation conditions 1 and 2 (mg/L), respectively; V_c = volume of solution in centrifuge tube (= 50 mL); and 3) the TOC of the supernatants of conditions 1 and 2 was tested with Fusion TOC machine (Model #14-9600-100, Teledyne Tekmar, Mason, OH, USA). The TOC of STA was calculated with Eq. 2:

$$f_{oc} (\%) = \frac{TOC_1 - TOC_2}{MC_1 - MC_2} \times 100\% \quad (2)$$

where f_{oc} = soil particle organic carbon fraction (%); TOC_1 and TOC_2 represent the concentration of total organic carbon in the supernatant of conditions 1 and 2 (mg/L), respectively; MC_1 and MC_2 are the same as Eq. 1. The supernatant of condition 2 contains smaller and less weight of particles than that of condition 1 due to longer sedimentation time.

For Clay 1 and Colloids #1 to #3, the centrifugation method was used instead of sedimentation to accelerate the separation process for the other 20 tubes (each 4 tubes for 5 conditions). The operation conditions (e.g., centrifuge force and time) for HAL and Iowa black soils are shown in Fig. 3 according to the previous study (Qi and Zhang 2015b). The TOC and SPW of the supernatants collected under these separation conditions were detected with the same methods as that of STA described above. Similarly, the weight and TOC of these clay particles were calculated with Eq. 1 and Eq. 2. The principle of the methodology for evaluating the property of the STA or clay particles was that the supernatant of condition 2 contains smaller particles and less weight of particles than that of condition 1 due to the stronger centrifugal force and/or longer centrifugal/sedimentation time. Therefore, the properties (e.g., TOC, weight, sorption concentration) of particles of interest between conditions 1 and 2 could be calculated

based on the properties' difference of the corresponding supernatants (Detailed calculation examples are shown in Section 3.2 below).

2.5 Sorption Tests with Original Soil and Analysis of Sorption Properties of DSFs

Fig. 3 shows the procedure of how to fractionate the soil particles of DSFs and then evaluate their hormone sorption concentration after the sorption tests of the bulk soil (collectively). All sorption tests described below were conducted with triplicates at room temperature ($23 \pm 1^\circ\text{C}$). Original HAL (or Iowa black) soil (1.0 g) was placed into each of 50-mL glass centrifuge tubes (Corning Screw Thread Finish, Fisher Scientific). Then, 5 mL of CaCl_2 stock solution (0.1 M) and 5 mL NaN_3 (2.0 g/L) were added to reach 0.01 M CaCl_2 (for controlling the ion strength) (Casey et al., 2004) and 200 mg/L NaN_3 (for eliminating the microbial influence). Previous studies showed the NaN_3 could be used to eliminate the microbial influence on the study of hormone sorption to soils, and no influence had been reported (Kozarek et al., 2008; Li et al., 2013). Three centrifuge tubes with the same situation of solution and soil without testosterone were treated as blank. 100, 200, 350, 500, 750 μL low stock ^{14}C labeled testosterone were added to the corresponding tubes to reach initial testosterone concentration of 20, 40, 70, 100, 150 ng/L, respectively. Then, a corresponding volume of DI water was added into each tube to reach 50 mL solution. All these tubes were rotated in the rotator (top to bottom, $360^\circ/5$ s) for 24 hours to reach sorption equilibrium (Ma et al., 2015). Then, the centrifuge tubes with each initial hormone concentration were then settled or centrifuged with the selected separation conditions (Fig. 3) to allow the particles with desired and smaller diameters to be left in the supernatant. Then, 500 μL supernatant (including particles of interest and smaller ones) from the centrifuge tube was pipetted into a 20-mL counting vial (Wheaton Millville, New Jersey, USA) with 10 mL cocktail (Perkin Elmer, Waltham, MA, USA). The radioactivity of the

sample was measured with 2500 TR liquid Scintillation Counter (Packard, Downers Grove, IL, USA), and the hormone concentration was determined.

Therefore, the distributions of the testosterone in the liquid phase, soil particles (settled) and soil particles of each diameter size range were determined based on mass balance as shown in Fig. 3. Similarly, the testosterone concentration difference of the two supernatants obtained from the two selected groups of separation conditions is due to the testosterone sorbed by the particles, which would settle down due to stronger centrifugal force and/or longer centrifuge/sedimentation time. Combining with the weight of particles calculated above (Eq. 1), the sorbed concentration of testosterone to the soil particles of each size fraction (except SDA; see Fig. 3) can be calculated:

$$S = \frac{C_1 - C_2}{MC_1 - MC_2} \times 10^3 \quad (3)$$

where S = sorbed concentration of testosterone to soil particles of the diameter range between separation conditions 1 and 2 (ng/g); C_1 and C_2 represent the testosterone concentration of supernatant (including both dissolved phase in the liquid and residual hormone in suspended particles) of separation conditions 1 and 2 (ng/L), respectively. MC_1 and MC_2 are the same as Eq. 1. The sorption concentration of SDA was calculated based on mass balance by submitting all the sorption of STA, clay 1, and Colloids #1 to 3 from the sorption mass of original soil (S_{SDA} of Fig. 3).

For the sorption isotherm of testosterone to soil particles of each size fraction, the linear model was used to fit the sorption data in order to compare with the previous studies (Lee et al., 2003; Qi et al., 2014; Ma et al., 2015):

$$S = K_d C \quad (4)$$

where S is the same as Eq. 3; K_d = linear sorption coefficient (liquid and solid phase partition coefficient) (L/kg); and C = equilibrium aqueous testosterone concentration (ng/L). The normalized sorption coefficient for organic carbon (K_{oc}) was calculated as:

$$K_{oc} = \frac{K_d}{f_{oc}} \quad (5)$$

where f_{oc} = soil particles' organic carbon fraction (%).

2.6 Other Analytical Methods

The TOC fractions of SDA and bulk soils were determined in a commercial lab (Mid-West lab, Omaha, NE) with the standard method of ASTM D 5373. The PSD of supernatant was analyzed with particle size analyzer (NanoBrook 90Plus/Bi-MAS, Brookhaven Instrument Corp). In order to eliminate the influence of testosterone sorbed to Teflon caps and glass tubes, centrifuge tubes ($n = 3$) with the same amount of testosterone and solution without soil particles were used as control. All the radioactivity detections were based on three blank samples (without hormone) of centrifuge tubes that had the same situation (e.g., CaCl_2 and NaN_3 concentration, soil-water ratio) as testing tubes. A microscope (LR33310Mod E, Fisher Scientific, Micro Master Inc.) was used to check the diameter size of SDA and STA.

3 Results and Discussion

3.1 Weight-based Particle Size Distribution of the Bulk Soils

The PSD tested with fully-dispersed and partially-dispersed methods are significantly different. The percentage of clay particles tested with the partially-dispersed method (5.9% for HAL soil in Fig. 4a and 4.9% for Iowa Black soil in Fig. 5a) is much lower than that with the fully-dispersed method (44.6% for HAL soil in Fig. 4a and 23.4% for Iowa Black soil in Fig. 5a).

Most of the clay particles are associated with the SDA (Figs. 4c & 5c) and STA (Figs. 4d & 5d) under the partially-dispersed condition. For HAL soil, the STA percentage is 85.95% for partially-dispersed soil, while that of silt is 49.52% for fully-dispersed soil (Fig. 4a). Therefore, using the ASTM fully-dispersion methods [i.e., adding sodium hexametaphosphate (NaPO_3)₆, high-speed stirring] would definitely separate clay from silt particles, which otherwise would be associated with silt to form STA (Fig. 4d). Similarly, for Iowa Black soil, the percentage of SDA obtained from the partially-dispersed method is 74.19% (Fig. 5a), while that of sand obtained from the fully-dispersed methods is 65% (Fig. 5a). Moreover, the PSD of HAL and Iowa Black soils are different (Fig. 4a and Fig. 5a): Iowa Black soil has more sand, and thus, more SDA, while HAL soil has more silt and thus, more STA. In addition, the discrepancy between partially-dispersed and fully-dispersed PSD of HAL soil is larger than that of Iowa Black soil, indicating that the adhesion strength between the particles of HAL soil is much stronger than that of Iowa Black soil. These results indicate that the new dispersion method (i.e., rotating the mixture of soil and water without high speed stirring or adding surfactant) would mainly disperse the soils into soil aggregates of DSFs rather than into primary particles.

3.2 Total Organic Carbon

For STA, clay 1 and Colloids #1 to #3, the TOC fractions were determined with Eqs. 1 and 2. Take the weight of HAL Colloid #1 as an example, $\text{MC}_1 = 386.1 \text{ mg/L}$ for the supernatant with an effective diameter size of 900 nm at 500 rpm and 5 min (condition 1), $\text{MC}_2 = 98.3 \text{ mg/L}$ for that of 600 nm at 2000 rpm and 5 min (condition 2), the difference of MC_1 and MC_2 is caused by the settled colloids between 600–900 nm due to a stronger centrifugal force. The weight of analyzed soil colloids between 600–900 nm is $W_{1-2} = (\text{MC}_1 - \text{MC}_2) \times 50 \text{ mL} = (386.1 - 98.3) \text{ mg/L} \times 0.05 \text{ L} = 14.39 \text{ mg}$. Similarly, TOC_1 and TOC_2 represent the concentration of TOC

in the supernatant of conditions 1 and 2 (mg/L), respectively. Therefore, the f_{oc} of these particles can be calculated with Eq. 2.

As shown in Table 1, for HAL soil, the f_{oc} of SDA and STA are 0.57% and 0.60%, respectively, which is much higher than that of the f_{oc} of primary sand (0.12%) and silt (0.19%) as reported by Qi et al. (2014). This is because the corresponding aggregates have a higher TOC content. The TOC distribution among particles of each size fraction could be calculated by multiplying their TOC concentration (mg/g) (Table 1) with the corresponding weight (g) distribution (Figs. 4 & 5). For HAL soil, STA has the highest TOC distribution (51%) among the partially-dispersed soil particles of DSFs (Table 4 and Fig. 6). While for Iowa Black soil, Colloid #1 has the highest TOC distribution (26.9%) among the DSFs (Fig. 6). These results are important as TOC is one of the most important parameters to evaluate the F/T of hormones in soils. It was reported that the sorption behavior of 17 α -ethinylestradiol (EE2) to soils depended greatly on the soil organic matter content and characteristics (Lima et al., 2012). Qi and Zhang (2015a) studied the influence of humic acid on the sorption of testosterone to soil particles; they found that the sorption capacity of soil particles increased significantly when the humic acid was added. Furthermore, the weight and TOC results indicate that it is feasible to use sedimentation and centrifugation methods to fractionate soil particles of DSFs with their properties being successfully evaluated.

3.3 Sorption Isotherm

The K_d value can be used to represent the sorption affinity of micro-pollutants to soil particles (Conkle et al., 2010). K_d is 79.9 L/kg for the HAL bulk soil and 36.7 L/kg for Iowa Black bulk soil (Table 1), which are the lumped result of all soil particles (i.e., SDA, STA, Clay 1, Colloids #1 to #3). For HAL soil, the K_d of SDA, STA and Clay 1 are 47.2, 63.5 and 189.4

L/kg, respectively (Table 1) when the sorption tests are conducted collectively. However, the K_d values of primary sand, silt and clay were 16.4, 60.1 and 87.8 L/kg, respectively when the sorption tests were conducted with individually fully-dispersed soil particle fractions (Qi and Zhang 2015a). Therefore, due to the attachment of clay and silt particles to sand particles (Fig. 4c), SDA has much higher sorption affinity than that of primary sand. For STA of HAL soil, the sorption affinity is only slightly higher than that of primary silt; this might be because when the sorption tests were conducted collectively, the clay particles, which have stronger sorption affinity, competed with STA to sorb testosterone. On the contrary, the K_d of clay particles is much higher in collective situation (= 189.4 L/kg) than that of primary clay (= 87.8 L/kg) (i.e., when the sorption tests were conducted with fully-dispersed clay particles), which might be caused from the reserved organic matter in the partially-dispersed clay particles.

Normalizing TOC of these particles would show how organic matter would affect the sorption affinity of testosterone to the partially-dispersed soil particles. The Log K_{oc} (calculated by Eq. 5) are 3.30 to 4.02 for HAL soil particles and 2.84 to 3.98 for Iowa Black (Table 1). The results indicated that smaller soil particles have higher organic matter content, and thus, have a larger sorption affinity.

3.4 Sorption distribution of testosterone among soil particles of DSFs

Based on mass balance, the sorption distribution of testosterone among soil particles of each size fraction was calculated by multiplying their sorption concentration (ng/g) (Fig. 7) and their weight (g) distribution in the original soil (Figs. 4 & 5). For HAL soil, at the initial testosterone concentration of 150 ng/L, the STA contributed ~73% of the testosterone sorbed by all of the particles together (Table 2). When the initial concentration was 20 ng/L, the STA only contributed ~39% (Table 2). While for clay 1, the contributions were 13.42% and 7.79% at the

initial testosterone concentration of 20 and 150 ng/L, respectively. This might be because the smaller particles had higher sorption affinity than that of bigger ones (Fig. 7). When the testosterone concentration was low, the bigger particles could not compete with the smaller ones, leading to a relatively low sorption contribution. When the testosterone concentration was higher, a large portion of the strong sorption sites of the smaller particles might be fulfilled, and thus, the sorption contribution of STA and SDA became relatively larger.

As shown in Table 2, for HAL soil, the average sorption contribution is 63.13% for STA, and 10.2, 14.1, 6.1 and 1.3% for Clay #1, Colloids #1, #2, and #3, respectively. Even though it has a relatively low sorption affinity (K_d), STA still sorbed most of the testosterone due to its high percentage of mass (Fig. 4a). On the other hand, even though the total mass of clays was 5.94% (Fig. 4b), clay sorbed more than 30% of the testosterone sorbed by all of the particles (Table 2). For sandy loam Iowa Black soil, the SDA contributed 41.94% (Table 2) of the total sorbed testosterone even though its mass percentage was 74.2% (Fig. 5a). Similar as the clay of HAL soil, even though the mass added up of clay was 4.9% (Fig. 5b), 34% of sorbed testosterone were contributed by clays (Table 2). Therefore, the small particles (< 2000 nm) with a higher sorption affinity will play critical roles in determining the F/T of hormones in soils due to their high sorption potential and mobility.

3.5 Analysis of Relative Error Based on Different Particles to Evaluate Testosterone Transport Potential

Being lipophilic (log K_{ow} being 2.6–4.0), most steroidal hormones are expected to be sorbed on soils and/or organic matter and are unlikely to be very mobile (Lai et al., 2000; Schiffer et al., 2004; Khanal et al., 2006). However, this conclusion cannot explain the frequent detections of hormones in ground and surface water. For example, it was reported that

reproductive hormones were found in approximately 40% of the 139 streams sampled across 30 states in the United States (Kolpin et al., 2002). Testosterone has been detected in the soils sampled from 45 m below a dairy-farm wastewater lagoon (Shai et al., 2008). In the past, it was believed that particles- or colloids-facilitated transport of hormones to surface water through runoff and leachate is the major mechanism for the aforementioned phenomena. However, *it is unknown how much difference there will be if one estimates the transport of testosterone based on fully-dispersed soil particles, partially-dispersed soil particles or whole soil.*

To do a preliminary estimation, additional tests were conducted with a soil slab reactor (Fig. S1 in Supplementary Materials) under one artificial rainfall event, and determined the mass distribution of SDA, STA, Clay 1, Colloids #1 to #3 of partially-dispersed soils particles and that of fully-dispersed ones in the soil slurry collected through runoff (Table S1). Based on the combined results shown in Table S1 and Table 1, Table 3 is created, which indicates that if using the results of partially-dispersed soil particles as the datum, the transport of testosterone by surface runoff will be either overestimated (by the method based on fully-dispersed soil particles, +80.55%) or underestimated (by that based-on the whole soil, -44.92%). Therefore, the sorption parameter of partially-dispersed soil particles should be used to study the F/T of hormones rather than based on fully-dispersed soil particles or whole soil.

3.6 Relative Contribution of TOC and Specific Surface Area to Hormone Sorption

Results of this study indicate that the sorption affinity of the soil particles was mainly determined by the specific surface area and TOC content, which is consistent with previous studies (Fan et al., 2007; Wang et al., 2008; Gineys et al., 2012; Li, et al., 2013; Qi et al., 2014). Sarmah et al. (2008) conducted the sorption of 17 β -estradiol (E2) and 17 α -ethynylestradiol (EE2) on six selected soils with batch reactors. They found that the sorption capacity of the soil was

attributed to the surface area; the sorption difference was attributed by the soil organic carbon content. However, the contribution of these two factors to hormones' sorption capacity has not been compared because of the inconsistency of the soil properties (e.g., different soil mineral components). Because TOC and particles sizes are the two main factors of the particles studied in this research, the contribution of the two factors to the sorption capacity of these particles is compared with the following equation:

$$W = aT + bF \quad (6)$$

where W = sorption contribution of soil particles of each size fraction to the “whole” soil (% , Column 5 in Table 4). T and F = TOC and surface area contribution of soil particles of each size fraction to the “whole” soils (% , Columns 3 and 4 of Table 4, respectively). The a and b parameters represent the contribution weighting factors of TOC and surface area, respectively, with $a + b = 1$. The regression of Eq. 6 with the HAL and Iowa Black data shown in Table 4 was conducted with Matlab R 2003a. The results showed that a and b were 0.589 and 0.411, respectively, with the R^2 being 0.9795 for HAL soil, and were 0.373 and 0.562, respectively, with the R^2 being 0.7318 for Iowa Black. Therefore, for these two soils, both TOC and surface area of the soil particles determined their sorption contributions. For HAL soil, TOC weights higher than surface area, while for Iowa Black soil, the surface area of the soil particles plays a more important role in their sorption contributions, which might be because HAL soil is a silty clay loam and has a higher organic matter content (Table 1).

3.7 Discussion

In the past, the testing materials used by most previous studies for interaction of soil and micro-pollutants were either whole soils (Lee et al. 2003; Kim et al. 2007; D'Alessio et al. 2014) or fully-dispersed soil particles (Wang and Keller 2008; Qi et al. 2014; Qi and Zhang 2015a).

However, in the real world, the transport of micro-pollutants is not through the whole soil due to the mobility differences among soil particles in DSFs; more often than not, bulk soils are dispersed in a partially-dispersed way due to the mild dispersion strength in nature. In this study, we used sedimentation and centrifugation methods to fractionate two representative soils into partially-dispersed soil particles of six DSFs, and, *for the first time*, investigated the sorption distribution of testosterone among these particles under their natural conditions (i.e., with all particles and aggregates being mixed together). The results indicated that the PSD of the two soils tested with fully-dispersed and partially-dispersed methods are significantly different, which was attributed by the attachment of clay particles on the sand and silt particles. Testosterone was sorbed mainly by the dominant aggregates even though they might have relatively lower sorption affinity than that of clays. However, the small particles (< 2000 nm), even with ~5% mass only, contributed more than 30% of the sorbed testosterone in the “whole” soil, leading to a high potential to facilitate the transport of hormones to surface and ground water due to their high mobility.

Results of this study indicate that many factors may affect the potential for particles of DSFs to facilitate the transport of testosterone to water bodies, such as PSD, particles’ sorption affinity, their relationship and mobility related to other particles. When studying the transport of hormone with the particle-facilitated mechanism, the results of the sorption distribution of testosterone among partially-dispersed soil particles of DSFs can be used to understand the transport of testosterone in the soil environments in a more realistic way. Therefore, this study provides a new perspective and methodology to better understand the interaction of soils and hormones in soils. However, one still cannot predict the F/T of hormones in soils even with the information of hormones’ distribution among soil particles of DSFs. Roberts et al. (2014)

reported that, in order to model the F/T of a pollutant comprehensively, one needs to incorporate, other than sorption parameters, solute transport with other mechanisms (e.g., advection, dispersion) as well as the transformation of the pollutant, let alone the variety of rainfall events. Therefore, this study is only one step forward toward understanding and predicting the F/T of hormones in soils. In the future, a new framework concerning the effects of partially-dispersed soil particles on the F/T of micro-pollutants should be set up with the corresponding procedure being standardized. Further studies considering other parameters and processes are needed to obtain comprehensive results.

4 Conclusion

In light of the above analysis, the following conclusions could be made:

- 1) The PSD evaluated through partially-dispersed and fully-dispersed process is significantly different for the two soils tested; Testosterone sorbed mainly to STA for HAL soil even though STA has a relatively lower sorption affinity. However, the small particles (< 2000 nm), even with only ~5% mass, contributed > 30% of sorbed testosterone by the whole soil;
- 2) The procedure developed in this study can be used to fractionate the soil particles of DSFs and then evaluate their contributions to sorption of hormones after the sorption test with the bulk soil, without compromising soil's original properties (e.g., aggregates) or the competition among soil particles and aggregates;
- 3) The partially-dispersed soil particles of DSFs should be used to understand the transport of hormone in runoff because the fully-dispersed method will overestimate while the whole soil method will underestimate the transport potential; and

- 4) Both TOC content and surface area of the soil particles will affect particles' sorption contribution to the "whole" soil with the weight factors being different of different soils.

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Table 1. Parameters of bulk soil and partially-dispersed soil particles of six different size fractions (DSFs) of HAL and Iowa Black soils.

Soil particles		Diameter range (μm)	f_{oc} (%) ^a	K_d (L/kg)	$\log K_{oc}$	1	Specific surface area (m^2/g) ^b
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)
AL	Bulk soil	< 2000	.95	9.9	.92	3	NA ^c
	DA	75	.57	7.2	.92	3	0.59
	TA	75–2	.6	3.5	.02	4	1.63
	Clay 1	2–0.9	.97	89.4	.98	3	2.59
	C	0.9–	1	2		3	4.14
	Colloid #1	0.6	0.5	07.8	.30	3	6.20
	C	0.6–	1	4		3	6.20
	Colloid #2	0.4	1	93.6	.65	3	10.34
	C	0.4–	1	6		3	10.34
	Colloid #3	0.2	6.4	91	.62		
Iowa Black	Bulk soil	< 2000	.68	6.7	.73	3	NA ^c
	DA	75	.21	0.1	.98	3	0.35
	TA	75–2	.86	3.2	.70	3	2.58
	Clay 1	2–0.9	.36	06.2	.65	3	2.59
	C	0.9–	1	1		2	4.14
	Colloid #1	0.6	3.3	14.5	.93	3	6.20
	C	0.6–	1	2		3	6.20
	Colloid #2	0.4	8.5	08	.05	2	10.34
	C	0.4–	2	1		2	10.34
	Colloid #3	0.2	6.3	81.2	.84		

^aWeight percentage of TOC (calculated with Eq. 2); ^bSpecific surface area of SDA and STA of these two soils was tested by a commercial laboratory (Micrometrics analytical services, Norcross, GA, USA) with multipoint surface area using Nitrogen gas (ISO 9277). ^cNA = Not available

539 **Table 2.** Contribution percent of soil particles of each size fraction to the accumulated
540 sorption of particles of all size fractions.

Soil particles		Initial testosterone concentration (ng/L)					Average sorption (%) ^c
		2	4	7	1	1	
		0	0	0	00	50	
(1)		(	(	(	(	(	(7)
		2)	3)	4)	5)	6)	
All		1	1	1	1	1	100
particles ^a		00 ^b	00	00	00	00	
SDA		5	5	4	5	5	5.26
		.01	.51	.25	.80	.75	
STA		3	6	7	6	7	63.13
		8.81	8.20	0.95	4.77	2.93	
F	Clay	1	1	8	1	7	10.15
AL	1	3.42	0.94	.09	0.51	.79	
	Collo	3	7	9	1	8	14.12
	id #1	3.67	.86	.07	1.56	.46	
	Collo	8	5	5	6	3	6.07
	id #2	.33	.92	.77	.37	.99	
	Collo	0	1	1	0	1	1.26
	id #3	.77	.57	.88	.99	.09	
I	All	1	1	1	1	1	100
owa	particles ^a	00	00	00	00	00	
Black	SDA	3	3	4	4	4	41.94
		6.73	6.77	3.99	8.10	4.13	

		2	2	1	2	2	
	STA						24.00
		5.42	2.93	8.00	5.94	7.70	
	Clay	1	1	1	1	1	
							1.40
1		.40	.47	.71	.27	.15	
	Collo	1	1	1	8	1	
							13.78
id #1		6.48	7.00	6.51	.02	0.88	
	Collo	1	1	1	1	1	
							12.41
id #2		2.13	3.94	4.02	0.73	1.26	
	Collo	7	7	5	5	4	
							6.47
id #3		.84	.90	.77	.95	.88	

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^aAll particles = SDA + STA + Clay 1+ Colloids #1, #2 and #3 based on data shown in Fig.

8; ^bThe total testosterone sorbed by all of the particles is treated as 100%; ^cAverage value of

Column (2) to (6).

Table 3. Error caused by using fully-dispersed soil particles and whole soils of HAL as testing materials to predict the transport of testosterone in runoff.

Methodology	Particles	Sorption capacity (ng/g) ^a	Particle mass (g) ^b	Testosterone taken away (ng)		Error (%)
				Individual	Sum	
Partially-dispersed	DA	4.72	0	0.00		
	TA	6.35	5	34.65		
	Clay 1	18.94	2	64.03	14	
	Colloid #1	20.78	1	3	50.50	0.00 ^c
	Colloid #2	49.36	4	2		
	Colloid #3	69.1	0	6		
			.93	4.26		
Fully-dispersed	Sand	1.64	0	0.00		
	Silt	6.01	3	2		
	Clay 1	18.94	1	1		
	Colloid #1	20.78	2	5	26	0.55
	Colloid #2	49.36	1	8		
	Colloid #3	69.1	1	8		
			1.92	23.67		
Whole soil		7.99	100	799.00	79.00	44.92

^aData calculated from Column (4) of Table 1 at the initial testosterone concentration of

100 ng/L; ^bData from Table S1 by assuming the total soil particles taken away by runoff is 100 g;

^cUsing the result of partially-dispersed soil particles as the datum for other two estimations.

Table 4. Particle-based contribution percent to the properties of the “whole” HAL and Iowa Black soils.

Soil particles		Weight (g) ^a	TOC (%) ^b	Surface area (%) ^c	Average sorption (%) ^d	
(1)		(2)	(3)	(4)	(5)	
AL	H	SDA	9.1	5.2	3.25	5.26
		1				
		84.	51.0	83.53	63.13	
		95				
		2.8	5.5	4.39	10.15	
		2				
	d #1	Colloi	2.4	26.1	6.19	14.12
		9				
	d #2	Colloi	0.5	5.9	2.01	6.07
		4				
	d #3	Colloi	0.1	1.7	0.63	1.26
		0				
Iowa Black	I	SDA	74.	13.73	24.14	41.94
		19				
		20.	15.82	50.07	24.00	
		87				
		0.3	0.67	0.77	1.40	
		2				
	d #1	Colloi	2.2	26.84	8.82	13.78
		9				
	d #2	Colloi	1.6	26.25	9.28	12.41
		1				
	d #3	Colloi	0.7	16.69	6.92	6.47
		2				

^aData from Fig. 4 for HAL, Fig. 5 for Iowa Black, assuming the “whole” soil is 100 g; ^b=
{[Column (2) x Table 1’s Column (3)]/[Total organic carbon = Σ [Column (2) x Table 1’s
Column (3)]]} × 100%; ^c= {[Column (2) x Table 1’s Column (6)]/[Total surface area = Σ
[Column (2) x Table 1’s Column (6)]]} × 100%; ^dData from Table 2’s Column (7).

Figure 1. Relationship between the fully-dispersed and partially-dispersed soil particle size distribution (Foster et al., 1985; Di Stefano et al., 2002).

Figure 2. Procedures for determining the mass distribution of particle size fractions in the bulk soil, based on ASTM D 422 but without steps: a) adding $(\text{NaPO}_3)_6$ or b) high-speed stirring. Steps a) and b) would result in a fully-dispersed size distribution, but soil with a partially-dispersed size distribution is more appropriated for F/T studies (Table 4). SDA = primary sand and large aggregates; STA = primary silt and small aggregates (see Fig. 1 for definitions).

Figure 3. Procedures for conducting sorption tests *collectively* (with the bulk soil) and then determining *individually* the hormone sorption to the particles of different size fractions. Other parameters (e.g., f_{oc}) of different size fractions can be determined by the same method as long as C_i can be detected. Here, we assume solids (diameter < 200 nm) in the supernatant after centrifugation at 4000 rpm for 45 minutes to be in the dissolved phase. The units of the equations must be adjusted accordingly. SPW = soil particle weight; D_{eff} = effective diameter; STA, SDA are shown in Fig. 2. The tests were conducted with triplicated and three blanks, total 27 parallel samples.

Figure 4. Fully-dispersed and partially-dispersed particle size distribution of HAL soil. Error bars indicate the standard deviation of duplicated results.

Figure 5. Fully-dispersed and partially-dispersed particle size distribution of Iowa Black soils.

Error bars indicate the standard deviation of duplicated results.

Figure 6. TOC distribution (%) among partially-dispersed soil particles of six DSFs of HAL and Iowa Black soils.

Figure 7. Sorption isotherm of HAL and Iowa Black bulk soils and their partially-dispersed soil particles of six DSFs on testosterone at the initial testosterone concentration of 20–150 ng/L. Error bars indicate the standard deviation of triplicated results.

Figure 8. Sorption mass distribution of testosterone to soil particles of six DSFs of HAL (a) and Iowa Black (b) at the initial testosterone concentration of 20–150 ng/L. Data from Figs. 4, 5 & 7.

Graphic Abstract: