THE SORPTION, BIOTRANSFORMATION, AND DETECTION OF HORMONES IN THE ENVIRONMENT

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ABSTRACT OF THE DISSERTATION

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In this dissertation, the sorption, biotransformation, and presence in the environment of five hormones, 17β-estradiol, 17α-ethinylestradiol, estrone, androstenedione, and testosterone, were chosen for study. Sorption to various soils and sediments appears to assume non-linear characteristics, with *n* values in the Freundlich isotherm model falling below unity as well as there being a tendency for log K_{OC} values to increase as the amount of sorbate decreases. As for inter-soil sorption comparisons, there appeared to be no obvious correlation between the sorption capacity of the hormones and the quantity of organic carbon of the soil, which suggests site-specific interactions between the functional groups of the hormones and the complex surfaces of the soils/sediments employed.

Biotransformation studies of three of the hormones to river sediments reveal that the rate of reaction increased in the order of 17α-ethinylestradiol < 17β-estradiol < testosterone. The synthetic hormone used in the birth control pills, 17α-ethinylestradiol, was relatively recalcitrant compared to the two natural hormones. When the hormone

biotransformation data was compared to the sorbent characteristics of the same select hormones on the same sediments, it was found in general that sediments with lower organic carbon content yielded longer lag times for both female and male hormones.

The field samples of various sewage treatment plant effluent and river waters of central and northern New Jersey for hormones yielded frequent detections. At least one hormone was detected at all 9 sampling locations in central and northern New Jersey. Androstenedione and estrone were the most frequently detected and found at the highest concentrations. Hormones were detected at levels known to either induce vitellogenin production or have pheromonal effects in fish. The low levels of unconjugated hormone at the combined sewer overflow were most likely due to the lack of deconjugation in the freshly discharged sewage/rain water mixture.

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LIST OF SYMBOLS AND ABBREVIATIONS

$[A]_0$	Initial concentration of species A (mass/volume)
$[A]$	Concentration of species A (mass/volume)
AD	Androstenedione
BC	Black Carbon
BE	Base Extracted
C	Aqueous phase concentration of a compound
C	Carbon
C_0	Initial aqueous phase solute concentration (μg/L)
C_e	Aqueous phase solute concentration at equilibrium (μg/L)
C_e/S_W	Ratio of aqueous phase solute concentration to solute solubility (used for K_{OC} calculations)
CSO	Combined Sewer Overflow
DRM	Distributed Reactivity Model
DWTP	Drinking Water Treatment Plant
EE2	17α-Ethinylestradiol
E2	17β-Estradiol
E1	Estrone
FA	Fulvic Acid
f_{OC}	Fraction of organic carbon
HA	Humic Acid
HM	Humin
k	Biotransformation rate constant (1/time)

KB	Kerogen and Black Carbon
K_D	single point distribution coefficient for the solute with the soil (L/kg)
K_F	Aqueous phase solute concentration at equilibrium ($\mu g^{1-n} kg^{-1} L^{-n}$)
K_{OC}	Organic carbon normalized distribution coefficient (mL/kg-OC)
K_{OW}	Octanol-Water partitioning coefficient
LOD	Limit of detection (ng/ml)
MS	Matrix Spike
q_e	Solid phase solute concentration at equilibrium (µg/kg)
n	Freundlich isotherm linearity parameter (dimensionless)
O	Oxygen
POM	Particulate Organic Matter
PSD	Particle Size Distribution
R	Recovery of surrogate compound (%)
R	Retardation factor
ρ_b	bulk density of soil (g/cm^3)
SOM	Soil Organic Matter
SPE	Solid Phase Extraction
STP	Sewage Treatment Plant
S_w	Solubility in the aqueous species (µg/L)
t	time (min, hr, d)
$t_{1/2}$	half life (time)
θ	volumetric moisture content of the soil (unitless)
TOC	Total Organic Carbon

TT	Testosterone

CHAPTER 1 – INTRODUCTION

1.1 Importance

Over the past two decades, the effects of xenoestrogens (manufactured compounds that mimic the action of natural estrogens) have become increasingly important in society. Strangely, the study of actual hormones has only become of interest in recent years despite the fact that natural hormones are generally much more potent from an endocrine disruption point of view than xenoestrogens such as PCBs and the well-studied bisphenol-A. Hormones can come from both point and non-point source in the environment. Humans discharge hormones on a daily basis, which are transported to municipal sewage treatment plants (STPs) and subsequently to surface water, creating a point source. For those not connected to the public sewer system, the hormones released from their bodies are discharged from septic systems and pollute aquifers and ground water. Animal husbandry practices and the land application of biosolids and animal litter are a prime example of non-point source discharges of hormones to the environment. These surface deposits of fecal matter and urine to the land, in addition to the deliberate placement of hormone containing waste onto surface soils for fertilizer, will naturally be exposed to the elements and potentially washed away into surface water (lakes and streams) during precipitation events.

The ubiquitous nature of hormones presents a potentially serious problem to the health of both humans and wildlife, especially in densely populated regions such as New Jersey and the Philadelphia area of Pennsylvania. In order to understand the extent of this problem, three major issues must be addressed: 1) the nature of hormone mobility

(sorption); 2) biotransformation of hormones (biotransformation studies); and 3) prevalence of the hormones and their concentrations (field studies). Understanding these three aspects of fate and transport will create a clearer picture of the fate of hormones in the environment.

1.2 Objectives

Given the mildly hydrophobic nature of steroid hormones and the variety of functional groups amongst the female and male variety, it is expected that the extent of sorption will vary from compound to compound. Further, the diverse nature of soils and sediments will add an additional factor to take into consideration: all soil organic matter (the primary constituent of soils and sediment responsible for sorption) is not created equal and consists of numerous fractions that play different roles in the uptake of contaminants. These two crucial factors, molecular structure and soil/sediment composition, will also have an impact on the second issue to be tackled, the biotransformation rates of hormones in the environment. In the end, the combination of results obtained from the sorption studies (including further study of hormone sorption to soil fractions) in conjunction with the biotransformation rate data obtained from the biotransformation studies is expected to provide a solid framework from which to evaluate the data obtained from field studies of STP effluent and river water. The objectives of this dissertation were the following:

- Evaluate the sorption (to soils and sediment) of testosterone and its biotransformation product, the frequently overlooked androstenedione, independently from each other

- Compare the nature of sorption between a natural male, natural female, and synthetic female hormone (testosterone, 17β-estradiol, and 17α-ethinylestradiol) on river sediments collected from the Philadelphia area
- Compare the nature of biotransformation between a natural male, natural female, and synthetic female hormone (testosterone, 17β-estradiol, and 17α-ethinylestradiol) on river sediments collected from the Philadelphia area
- Determine the contribution of individual soil fractions to the sorption of a male and female hormone (androstenedione and 17α-ethinylestradiol)
- Determine if sorption capacity and/or organic carbon content of soil are related to biotransformation
- Determine the concentrations of five hormones, androstenedione, testosterone, 17β-estradiol, 17α-ethinylestradiol, and estrone in the effluents of various municipal STPs, in CSOs, and in the waters of the Passaic and Raritan Rivers of central and northern New Jersey.

1.3 Overview

This dissertation consists of five additional chapters. Chapter 2 provides background information about the endocrine functions, prevalence, and ecological importance of hormones. Chapter 3 discusses the nature of sorption of both male and female hormones to various soils and sediments. Sorption to various soil fractions is also discussed. In addition, sorption rates of two male hormones are determined. Chapter 4 focuses on the biotransformation of male and female hormones in river sediment. Chapter 5 covers the detection of male and female hormones in sewage treatment plant

(STP) effluent and rivers. Chapter 6 provides conclusions, discusses implications, and makes suggestions for future work.

CHAPTER 2 – LITERATURE REVIEW

Overview

Contaminant mobility and biotransformation are two of the most important fate and transport characteristics of a pollutant, especially for emerging compounds such as hormones, where even low ng/L concentrations can have deleterious effects on wildlife (Kolodziej et al., 2003; Purdom et al., 1994). The rest of this chapter will summarize the chemical background of male and female hormones, including their functions in the endocrine system, molecular structure, and ecological effects, occurrences in the environment, and their point and non-point sources.

Sorption isotherms, which describe the distribution of a solute between the aqueous and solid phase, will be the method by which this study will evaluate the mobility of hormones in soils and sediments. In addition to the study of bulk soils, background information will be provided on sorption to individual soil fractions, which is a more mechanistically detailed sub-topic of sorption modeling and may prove important for the suite of hormones chosen for this study since their chemical structures are so similar. The biotransformation work in this study will be based on batch reactor biotransformation, thus this chapter will also present the equations used to determine the biotransformation rates and half lives of the hormones in sediments.

In order to put the aforementioned sorption and biotransformation work into perspective, field studies need to be carried out to determine what the actual environmental concentrations are. Therefore information regarding the choice of sample locations for the field study will be discussed as well.

2.1 Hormones

Hormones are a relatively new class of environmental contaminants that have risen in the public eye over the last few years (Kolpin et al., 2002). Due to the ubiquitous nature of these compounds in everyday life and their potency at low concentrations (Kolodziej et al., 2003; Purdom et al., 1994), it is crucial to understand their chemical nature, source, effects on wildlife, and concentrations in the environment. The hormones that form the basis of this study are 17β-estradiol, 17α-ethinylestradiol, estrone, androstenedione, and testosterone. From here on, they will be referred to as E2, EE2, E1, AD, and TT, respectively (Table 3.1). Despite the fact that EE2 is technically a xenoestrogen, because it was designed to act like a natural estrogen, EE2 will also be referred to as a "hormone" herein.

2.1.1 Characteristics

TT and AD are endogeneous anabolic steroid hormones, and are the major circulating androgens required for normal sexual differentiation in vertebrates (Das et al., 2004). TT is the principal male sex hormone and is the precursor to the estrogenic estrone and estradiol. AD is a 19-carbon steroid derived from cholesterol and produced as an intermediate step in the biochemical pathway that produces TT (Brook and Marshall, 2001). In addition to being secreted naturally, both TT and AD are manufactured as dietary supplements and are used to enhance growth. Androgens promote protein synthesis and growth of those tissues with androgen receptors (Brook et al., 2001).

E2 is one of the two main ovarian hormones (Brook and Marshall, 2001). The synthetic EE2 is structurally similar to E2 and used in oral contraceptives (Arcand-Hoy et

al., 1998). E1 is one of the most common metabolites of E2 (Lee et al., 2003). These three female hormones contain an aromatic A-ring whereas the male hormones do not exhibit any aromaticity.

2.1.2 Ecological Effects

Male and female hormones (androgens and estrogens) have been found in the environment in recent years and pose a threat to ecosystems worldwide. Their deleterious effects have been documented (Pelley et al., 2003; Purdom et al., 1994, Renner et al. 2002, Barel-Cohen et al., 2006, Kolodziej et al., 2003, Kolodziej et al., 2007, Shore et al., 2004). The effects of male hormones on living organisms can be quite substantial. While the detected concentration levels of androgens in surface water appear to be very low (i.e., sub – parts per billion level), it should be noted that female hormone concentrations of less than 1 ng/L (< 1 ppt) were shown to be able to induce reproductive abnormalities in aquatic species (Purdom et al., 1994). TT, AD, and E1 are known to have pheromonal effects at concentrations of 3, 300, and 30 ppt, respectively (Adams et al., 1987; Poling et al., 2001; Murphy et al., 2001).

In northwestern Ontario, a whole lake experiment demonstrated that estrogens at a concentration of 5-6 ng/L resulted in the kill off of entire fish populations within three years due to the feminization of male fish (Pelley et al., 2003). In addition, extremely low concentrations of 17α-EE2 (EE2) (i.e., 1.0 ng/L) have been shown to induce feminization in wild male fish as well (Purdom et al., 1994).

When the potency of E1, E2, and EE2 were compared to each other, E1 displayed 30% of the potency of E2 whereas EE2 has 20 times the potency of E2 (Thorpe et al., 2003; Sumpter et al., 2005). There is a large difference in the potency amongst

chemicals, including compounds beyond the hormones, having estrogenic activity. The compounds with the highest potency are the actual estrogens such as E2 and EE2, as opposed to other xenoestrogens used for non-hormonal functions (Sumpter et al., 2005).

2.1.3 Occurrence

The ubiquity of these hormones in ecosystems is partly attributed to less than optimal treatment plant systems, as prior studies have shown that conventional wastewater treatment is unable to completely remove them. For example, EE2 concentrations as high as 7.0 ng/L were detected in wastewater treatment plant (WWTP) effluents dominated by domestic input (Desbrow et al., 1998).

It should be noted that there are hormone sinks other than soil and surface water. The female hormone E2 has been shown to be present in ground water as well (Peterson et al., 2000). Agricultural practices are also a source of hormones in the environment (Finlay-Moore et al., 2000; Shore et al., 2004). A study on the aerobic biotransformation of a suite of hormones in soil and sediment concluded that TT had the shortest half-life. This same study also confirmed that AD is a transformation product of TT (Lee et al., 2003). As well as being discharged alongside TT, the breakdown of TT can contribute to AD's presence as well.

2.1.4 Sources

The presence of steroid hormones in the environment has mainly been associated with anthropogenic sources. For instance, up to 6.1 ng/L and 4.5 ng/L of TT and AD , respectively, have been measured in WWTP effluent (Kolodziej et al., 2003). One study has shown that the mineralization of TT in municipal biosolids is only 55% to 65%

(Layton et al., 2000). Incomplete removal of androgens in treatment systems will inevitably result in their discharge to surface water bodies.

Humans are not the only source of TT and AD in the environment. A study of 15 sites in the Upper Jordan Valley after a major rain event revealed TT concentrations up to 6 ng/L. The area included areas of small farms and cattle pasture which strongly suggests that surface runoff from agricultural locations are a source of androgens (Shore et al., 2004). Similarly, runoff derived from grasslands amended with broiler litter had TT concentrations ranging from 10 to 1830 ng/L (Finlay-Moore et al., 2000). Usually poultry litter is land-applied to pasture for both disposal and for fertilizer for forage crops (Nichols et al., 1997). TT and AD have been detected in the raceways and effluents of three fish hatcheries at concentrations near 1 ng/L (Kolodziej et al., 2004).

The aforementioned detections of androgens in WWTP effluent are not surprising since the more widely studied female hormones have been detected in WWTP effluent as well (Andersen et al, 2003; Baronti et al., 2000; Holbrook et al., 2002; Holbrook et al., 2004; Holthaus et al., 2002; Johnson et al., 2000). For instance, in a study conducted in the Netherlands, the following maximum concentrations of E2, EE2, and E1, respectively, were found in WWTP effluent: 12, 7.5, and 47 ng/L (Belfroid et al.,1999). As for concentration of E2, EE2, and E1 in surface waters, Kolpin et al. (2002) detected levels up to 93, 831, and 112 ng/L, respectively, in the U.S.A. It is interesting to note that these values are higher than those found in WWTP effluent. The reason for this could be due to the aforementioned non-point sources, varying WWTP removal efficiencies, or different analytical methods.

2.2 Sorption

Sorption is described as the distribution of a solute between the liquid and solid phase. Several types of sorption isotherms have been developed and implemented over the past few decades such as the Linear, Freundlich, Langmuir, and Distributed Reactivity Model (DRM) (Schwarzenbach, 2003) Based on preliminary studies, the Freundlich model was found to be the most suitable isotherm for our purposes and was chosen to model all sorption data obtained in this study.

2.2.1 Sorption Isotherm Model

The equilibrium sorption data obtained for all sorbates and all sorbents were statistically fit to the Freundlich sorption model:

$$\log q_e = \log K_F + n \log C_e \qquad (1)$$

where q_e is the equilibrium solid-phase solute concentration (μg/kg), C_e is the equilibrium aqueous-phase solute concentration (μg/L), K_F is the Freundlich sorption coefficient (μg/kg)/(μg/L)^n, and n is the isotherm nonlinearity index (unitless).

2.2.2 Sorption of Hormones to Bulk Soils and Sediments

Sorption plays an important role in the fate of androgens in the aquatic environment, but there is currently no detailed information regarding the true sorptive nature of TT and AD that were measured under biologically controlled conditions. Unfortunately, the only related studies determined soil-water distribution coefficients (K_D) under uncontrolled experimental conditions. Lee et al. (2003) combined sorption and degradation experiments which obviously could not result in accurate sorption

isotherms for both TT and AD. Das et al. (2004) determined K_D values under continuous flow conditions, which did not account for the effect of isotherm nonlinearity. The batch sorption experiments of Casey et al., (2004) had no biological control and involved both AD and TT, which could compete with each other, lowering the overall sorption capacity at a given concentration level.

2.2.3 Sorption of Hormones to Soil Fractions

Sorption to soils and sediments may reduce the speed of pollutant transport in both ground and surface water systems. Several studies have been conducted on the sorption of male and female hormones to bulk soils and sediments (Lee et al., 2003; Yu et al., 2004; Casey et al., 2004; Das et al., 2004; Kim et al., 2007). Equilibrium sorption coefficients and sorption rates were determined under various experimental conditions. While the study of hormone sorption to bulk soils and sediments is useful in assessing their transport in the environment, it would be helpful to know which individual components of the soil contribute most to the sorption process. Commonly studied organic soil/sediment fractions include humic acid and black carbon. Humic acids are heterogeneous mixtures of macromolecules that are base soluble and can weigh between a few thousand to several thousand Daltons (Stevenson et al., 1994). Black carbon (BC) is created by the incomplete combustion of vegetation and fossil fuels. It is a relatively inert material and, due to atmospheric and fluvial transport, found globally (Schmidt et al., 2000). Previous studies have been done involving sorption of polyaromatic hydrocarbons (PAHs) and herbicides on these aforementioned organic matter constituents (Xiao et al., 2004, Yu et al., 2006). Their results revealed that important environmental

parameters such as sorption and degradation potential are often times correlated with the nature of organic matter in soil. So far, no research has been conducted on the sorption of male and female hormones to individual soil fractions.

2.3 Biotransformation of Hormones

To date, very little research has been conducted on the biotransformation of hormones in soils and sediments. A few studies have attempted to quantify the rates of biotransformation in various batch and column experiments. The estimated biotransformation half-life for TT in soil-water slurries ranged from 0.3 to 7.3 d (Lee et al., 2003). The biotransformation characteristics of TT obtained from a flow-interruption column study revealed a pseudo first order biotransformation rate constant (k) of 0.002 to 0.015 h^{-1}. Further, the biotransformation rate constants for the primary metabolites were one to two orders of magnitude larger than those of the parent chemical (Das et al., 2004). Another column study reported the biotransformation rate coefficients ranged from 0.404 to 0.600 h^{-1} for TT, indicating that TT was more readily degradable than the more studied E2 (Casey et al., 2004).

As for batch reactor biotransformation studies, examples of some of the limited studies carried out thus far for hormones include Australian marine sediment (Ying et al., 2003), temperate oceanic agricultural Welsh soils (Lucas et al., 2006), and Australian aquifer material (Ying et al., 2003). The few batch biotransformation studies of hormones with soils and sediments that have been carried out so far by others have assumed pseudo-first order reaction kinetics, thus the same estimation will be used in the biotransformation data contained in this study. In addition to fitting the data to first order kinetics, the data sets were also plotted according to zero order kinetics.

2.3.1 First Order Reactions

First order reactions only depend on the concentration of one reactant. A reaction that is first order with respect to a reactant A can be represented by the following:

$$r = -\frac{d[A]}{dt} = k[A] \tag{2}$$

The integrated form of the above equation is:

$$\ln[A] = -kt + \ln[A]_0 \tag{3}$$

where a plot of ln[A] vs time t will give a straight line with a slope of $-k$.

The half life ($t_{1/2}$)can be determined by:

$$t_{1/2} = \frac{\ln(2)}{k} \tag{4}$$

2.3.2 Zero Order Reactions

A zero order reaction possesses a rate that is independent of the concentration of the reactant(s). Increasing the concentration of the reacting species will not lead to an increase in reaction rate. The rate law for a zero order reaction is the following:

$$r = -\frac{d[A]}{dt} = k \tag{5}$$

With an integrated form of:

$$[A]_t = -kt + [A]_0 \quad (6)$$

where $[A]_t$ is the concentration of the species of interest at a particular time and $[A]_0$ is the initial concentration. A reaction is considered zero order if the concentration plotted against time results in a straight line. The slope of said line is the negative of the rate constant for a zero order reaction.

2.4 Detection of Hormones in STP Effluent and River Water

Two rivers and one bay were chosen to be the subject of field study for the detection of hormones in STP effluent and surface water: the Passaic River, the Raritan River, and Raritan Bay. To our knowledge no studies have been conducted on the presence of hormones in rivers, STP effluents, or CSOs in New Jersey.

2.4.1 Passaic River Basin

The Passaic River Basin contains some of the most densely populated land in the U.S. with severe environmental contamination along the lower reaches of the river. The drainage basin covers approximately 2,400 square kilometers and is characterized by a complex web of major and minor tributaries. The river system drains much or part of eight New Jersey counties. A main point of interest along this river for this study was near the confluence of the Passaic and Pompton rivers. It is here that the North Jersey District Water Supply Commission withdraws water from both rivers and pumps it to the Wanaque Reservoir. In addition, the Passaic Valley Water Commission withdraws water

for drinking water purposes and operates a water treatment plant near one of this study's sampling points (http://pages.csam.montclair.edu/pri/basin.html).

2.4.2 Raritan River Basin

The Raritan Basin is the home of 1.2 million New Jersey residents in addition to thousands of species of plants and animals. Water provided by the Basin is treated for drinking and used for agriculture and industrial processes. Various point and non-point sources affect the quality of the Raritan River Basin. Examples of point sources include discharges from municipal sewage treatment plants (STPs) while examples of non-point sources include soil erosion, fertilizer runoff, and various pollutants deposited on the land surface and washed off by storm water (Shallcross et al., 2002)

2.4.3 Combined Sewer Overflows

Combined sewer overflows (CSO) are part of a type of sewage collection system that consists of single piping that also collects urban runoff from streets and roofs. They are typically found in older cities where, at a time when most cities did not have sewage treatment plants, the populace did not see any health advantage to constructing a separate storm sewer system. When cities added STPs, they constructed relief structures in the collection system that would allow for bypass of the STP during excessive flow during large storm events. The excess water is discharged (overflows) to a nearby water body to prevent backup into streets or homes.

A CSO in Perth Amboy, NJ, was found to be in a relatively safe and convenient location for sampling during a rain event and chosen for this study. It was anticipated

that the CSO discharge flowing to the Raritan Bay would have an exceptionally high chance of carrying steroid hormones due to the raw sewage it contains.

CHAPTER 3 - SORPTION

Overview

This chapter consists of three subchapters about different aspects of hormone sorption to soils and sediments. Section 3.1 deals with differentiating the sorption characteristics of TT and AD separately. separate isotherms have not previously been developed for these two male hormones. In addition, and to our knowledge, AD's mobility has only been analyzed as a daughter product of TT within the same reactor and not isolated. Because of the potential for male hormones to interfere with pheromone response, it is necessary to work with them separately as well. In addition to sorption equilibrium, the male hormones were also used to estimate the time for steroid hormones to reach equilibrium (i.e., sorption rates).

Section 3.2 is a location-specific sorption study based specifically on sediments collected recently by our group from rivers in the Philadelphia area. It elaborates on the work done in Section 3.1 by including E2, EE2, and TT, allowing comparisons between the two genders and the synthetic hormone. This data is also used in Chapter 4 in an attempt to find a correlation between sorption and biotransformation, if any.

Section 3.3 is focused on the differences in sorption capacity and linearity found amongst hormones and sediments in Sections 3.1 and 3.2. By studying the hormones' sorption to individual soil fractions, such as HA and POM, it is hoped that the wide ranging sorption parameters obtained for such similarly structured compounds can be better understood.

3.1 Sorption of Male Hormones to Soils and Sediments

Sorption of male hormones were approached first because they have received less study than the female hormones. In addition, in order to determine how long it takes for equilibrium to be reached, rate studies were done with the male hormones. The rate information obtained from this part of the study was applied to all future sorption equilibria experiments.

3.1.2 Rationale and Objectives

This study was set out to measure sorption properties for individual androgen compounds with a batch technique under biologically controlled conditions. The goal of the current study was to systematically investigate the equilibria and rates of sorption of both TT and AD. Sorption rates were measured for single-solute systems at two different initial concentrations and the potential relationship between rate and concentration was discussed. A relatively wide range of concentrations was used for the single-solute sorption isotherms and nonlinearity served as an explanation for the concentration-dependent sorption capacities. The experimental results were extrapolated for estimating the sorptive properties of the androgens at concentrations typically detected in aquatic systems.

3.1.3 Materials and Methods

Sorbents

The four sorbents used in this study included Chelsea topsoil (Chelsea, MI, USA), Pahokee peat (Everglades, FL, USA), a pond sediment (Guangzhou, China) and Environmental Protection Agency – 5 (EPA-5) sediment (Beaver Creek, ND, USA). The total organic carbon (TOC) contents of the Chelsea topsoil, Pahokee peat, pond sediment,

and EPA-5 sediment were 5.45, 45.7, 2.10, and 2.28 weight %, respectively (Huang et al., 1997; Li et al., 2003; Song et al., 2002; Means et al., 1980). These sorbents were chosen because a wide range of TOC contents was desired for experimental purposes and because they were used in experiments to assess the equilibria and rates of natural and synthetic female hormones in a prior study by our research group (Yu et al., 2004). The major physical and chemical properties of the four sorbents have been documented previously (Huang et al., 1997; Song et al., 2002; Means et al., 1980; Xiao et al., 2004).

Sorbates

Two androgens, AD and TT, were selected as the sorbates in this study. Both chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used as received. The purities of both chemicals checked in our lab by High Performance Liquid Chromatography (HPLC) were > 99%. Molecular structures and major physicochemical properties of the two steroid hormones are summarized in Table 3.1.

Solutions

Three primary stock solutions of each target compound at 1000, 2000, and 5000 mg/L were prepared with volumetric flasks and HPLC-grade methanol. Stock solutions were stored at 4°C. A background aqueous solution was prepared from Milli-Q® water (Millipore, Bedford, MA, USA), and contained $CaCl_2$ (0.005 *M*) and $NaHCO_3$ (100 mg/L). NaN_3 (100 mg/L) was added to inhibit biological activity. The initial aqueous solutions for each sorption experiment were prepared by mixing a desired volume of a specific stock solution with the background aqueous solution in a volumetric flask. The volumetric fraction of methanol in each initial solution was < 0.5%, a level at which no

measurable effect of the co solvent was found for sorption of organic pollutants (Wauchope et al., 1983).

3.1.4 Experiments

Solubility

The aqueous solubilities of the two compounds in background aqueous solution were determined in this study for better interpretation of the sorption data. In these tests, two sets of triplicate glass ampules containing the background solution at pH 6.8 and excess solid of the steroid hormones were flame-sealed and mixed at 22 ± 0.1°C on a shaker. After mixing for 14 d, the ampules were set in the upright position for 24 h to allow residual organic solids to accumulate at the air-water interface. They were broken open, and a syringe was used to carefully transfer an aliquot of subnatant (2 mL) from each ampule into a 5-mL glass vial, which contained a predetermined amount of HPLC-grade methanol. The mixtures were analyzed for the target chemicals with the technique described below.

The aqueous solubility limits (S_w) measured for AD and TT are 50.5 and 32.2 mg/L, respectively (Table 3.1). The reported aqueous solubilities are 18 to 25 mg/L at ambient temperature for TT, and 37 to 41 mg/L at 37 °C for AD (Nuez et al. 1997; Suzuki et al. 2001). Apparently, the large variations in the reported solubility data may result from the differences in temperature and solution chemistry conditions at which they were measured. Since our data were collected at the same conditions as for the sorption experiments, they are used in the following discussion.

Sorption Rates

The rates of sorption were measured for AD and two sorbents, Chelsea soil and Pahokee peat, over a time period of almost 1 month (672 h) and at initial aqueous concentrations of 300 and 10,000 μg/L. The soil-to-water ratios used for the rate study were selected based on 80% of reduction in initial aqueous concentrations at the equilibrium sorption conditions. At designated times, replicate ampules were taken out of the shaker, broken open, and set upright for 5 min to allow large particles to settle. A disposable glass pipette was then used to transfer approximately 5 ml of the liquid sample from the top of the ampule to a precleaned and pre-labeled centrifuge vial. Solid-solution separation was achieved by centrifugation at 285 G for 7 min. After centrifugation, an aliquot of 3 mL of the supernatant was withdrawn and diluted with methanol in the same manner as the equilibrium study.

Sorption

Completely mixed batch reactor (CMBR) systems were used for the sorption experiments under both rate-limiting and equilibrium conditions. Preliminary tests were conducted first to determine the optimum ratio of aqueous and solid phase to achieve between 30 to 60 % reduction of the initial aqueous phase concentrations at the end of the sorption experiment. Three initial solute concentrations at 500, 1000, and 5000 μg/L and a reaction time of 14 d were chosen for the preliminary equilibrium sorption tests. The optimal soil-to-water ratios were calculated from the preliminary test data for the final rate and equilibrium experiments reported in this study.

For both preliminary and final tests, flame-sealed glass ampules (10 or 20 mL) were utilized as CMBRs. All reactors contained a predetermined amount of sorbent and were filled with the initial aqueous solution up to the shoulder of the ampule. Each reactor was flame-sealed immediately and placed horizontally on a rotary shaker set at 125 rpm and 22 ± 1°C. For the sorption equilibrium study, all of the reactors were set upright for 24 h at the end of the experiment to allow for solid suspensions to settle. Each reactor was then opened and a disposable glass pipette was used to carefully transfer approximately 3 mL of the supernatant to a pre-weighed glass vial (5-mL) containing approximately 2 mL of methanol. A prior study showed that this method for solid-water separation had no difference from the centrifugation method (Huang et al., 1998). The vials were sealed with Teflon®-topped caps and kept at 4°C before chemical analysis of the sorbate with the HPLC method detailed below. Methanol added was to minimize the potential loss of solutes to components of gas chromatography (GC) vials used during analysis. In case of HPLC failures, the 5-mL mixture was sufficient for multiple analyses.

In all experiments, control reactors prepared similarly but containing no sorbent materials were run simultaneously for assessing loss of solutes to reactor components during sorption tests. Results of triplicate reactors at each C_o level showed that the average solution phase concentrations of each solute were consistently within 98 to 102% of the respective initial concentration of the same solution analyzed similarly. Hence, no correction was made during reduction of the sorption data.

3.1.5 Chemical Analysis

A reverse-phase HPLC (model 1100, octadecyl silane, 5 μm, 2.1 X 250 mm C_{18} column) with the diode array ultraviolet detector (Hewlett-Packard, Avondale, PA, USA) was used. A mixture of HPLC-grade acetonitrile and Milli-Q water was used as the mobile phase for both compounds. For AD-containing samples, the ultraviolet (UV) detector was set at a wavelength of 244 nm. Samples with low AD concentrations (25−5,000 μg/L) were analyzed with a mobile phase flow rate of 0.3 mL/min, injection volume of 10 μL and acetonitrile-Milli-Q water ratio at 40/60 (v/v), and samples with high concentrations (5,000−10,000 μg/L) were analyzed with a flow rate of 0.27 ml/min, injection volume of 2 μl, and acetonitrile-Milli-Q water ratio at 35/65 (v/v). For TT-containing samples, the UV detector was set at a wavelength of 242 nm and the flow rate of the mobile phase at 0.28 mL/min. The injection volumes were 25 and 10 μL for samples having low (25−100 μg/L) and high (100−10,000 μg/L) TT concentrations, respectively. Ten external methanol solution standards with concentrations of 25 to 10,000 μg/L were used to establish calibration curves for both solutes. The retention time for both AD and TT was 5.1 minutes.

3.1.6 Data Reduction

The concentrations in the sampled supernatants were calculated from the HPLC analyses and the dilution factors, which were computed based on the density data of the water-methanol mixture (Weber et al., 1996). The solid-phase sorbate concentrations were computed based on a mass balance calculation for the sorbate between the two phases. The underlying assumptions of the mass balance calculation are that no sorbate was lost to the headspace or compartments of CMBRs or glass vials and that no chemical

transformation nor biotransformation of the sorbate occurred during the sorption process and storage and analysis of the methanol-supernatant mixtures.

3.1.7 Modeling

Sorption

The equilibrium sorption data obtained for both sorbates and four sorbents were statistically fit to the Freundlich sorption model described in Equation 1, Section 2.2.1. The Freundlich model parameters were obtained with a linear regression using statistics software (Systat, Ver 10.0, SYSTAT, Chicago, IL, USA).

3.1.8 Results and Discussion

Sorption Rates

The rates of AD sorption by Chelsea soil and Pahokee peat are shown in Figures 3.1a and b, respectively. In all four datasets, the sorbate uptake by the soils was very rapid within the first hour of reaction time and eventually became slower to approach equilibrium. At low C_o (300 μg/L), the sorption had attained apparent equilibrium at approximately 336 to 504 h (14−21 d) for both Chelsea soil and Pahokee peat. At high C_o (= 10,000 μg/L), the times required for attainment of apparent equilibrium were similar to the low C_o scenario, with equilibrium being attained at 168 to 336 h (7−14 d) for both sorbents. The data shown here are comparable to the rate data reported by Yu et al. (2003) for E2, which showed sorption by Chelsea soil had attained apparent equilibrium within 160 h (~7 d) at low E2 C_o = 160 μg/L. However, the measured rate of sorption was much slower than the data presented in Casey et al. (2004). Due to no control of biological activity in their reactor systems, Casey et al. (2004) observed

gradual decline of $q(t)$ after 5 h of solution-sorbent contact. They tentatively defined that 5 h was the time for their reactor systems to approach sorption "equilibrium". The relatively faster rates of uptake at higher $C(t)$ values were also consistent with previously conducted rate experiments for phenanthrene (Weber et al., 1996; Huang et al., 1998). This concentration dependent uptake rate may be related to the deformation or reconfiguration of condensed soil organic matter (SOM) matrixes due to solute sorption (Huang et al., 1998).

In each concentration situation, Chelsea soil appeared to attain equilibrium at a faster rate than Pahokee peat. One explanation is because of a large difference in sorbent mass and hence the total surface areas of sorbent for pollutant uptake between the two CMBR systems. Due to the difference in overall sorption capacity, a CMBR needed a mass of Chelsea soil six times greater than the peat to achieve an equal 80% of uptake. The specific surface areas of Chelsea soil and the peat are 3.92 and 0.97 m^2/g, respectively. As a result, the CMBRs with Chelsea soil had 27 times more total surface areas for sorption than the ones containing the peat sample, causing faster apparent rates of sorption by Chelsea soil.

The relatively slow rates of sorption indicate that the K_D values measured in a short time period (e.g., several hours) with batch systems underestimate the uptake capacity of these chemicals by soils. For instance, the K_D values for the AD sorption by Chelsea at C_o = 300 μg/L for 1, 2, and 4 d were 75,000, 93,000, and 98,000 L/kg which are 68, 85, and 89%, respectively, of the equilibrium K_D at 14 d (110,000 L/kg). Similar results can be found for the same system initiated with C_o = 10,000 μg/L and for the Pahokee peat.

Sorption Equilibria

Table 3.2 lists the Freundlich sorption isotherm parameters K_F and n values and their standard deviations, along with the total number of observations for each isotherm. The organic carbon (OC) normalized single-point distribution coefficients (K_{OC}) were calculated at C_e/S_W = 0.1, 0.01, 0.003 from the TOC data and the fitted isotherm parameters listed in Table 3.2 with the following equation,

$$K_{OC} = \frac{K_F C_e^{\,n-1}}{f_{OC}} \tag{8}$$

where K_{OC} has units of L/kg and f_{OC} is the mass fraction of the OC in soil. The calculated results are summarized in Table 3.2. The sorption isotherm data and the Freundlich model fits for the two sorbates and the four sorbents are also presented in Figure 3.2.

Table 3.2 and Figure 3.2 show that the Freundlich sorption model is adequate for quantifying the equilibrium sorption of the two androgens on the four natural sorbents tested in this study. The n values for the eight sorption isotherms range from 0.698 to 0.899. The sorbent EPA-5 has the most linear isotherms for both hormones with n values of 0.812 and 0.899 for AD and TT, respectively. The most nonlinear isotherm for AD was Pahokee peat (n = 0.720) and for TT it was Pond sediment (n = 0.698). Among the four sorbents, EPA-5 has the largest K_{OC} values for both AD and TT (log K_{OC} = 6.87 and log K_{OC} = 6.81 at C_e/S_w= 0.01) whereas Chelsea soil has the lowest K_{OC} value for both AD and TT (log K_{OC} = 6.09 and log K_{OC} = 6.25 at C_e/S_w= 0.01). The K_{OC} values tended to increase at lower C_e/S_w ratios, which is consistent with previous studies on isotherm nonlinearity. Values of the organic-carbon normalized distribution coefficient vary with the aqueous-phase solute concentration (Huang et al., 1997).

Comparison of the sorption isotherms shown in Figure 3.2 indicates that each of the two soils (Chelsea soil and Pahokee) exhibits slightly greater sorption capacity for TT than AD, but the two sediments have the opposite trend, each having higher sorption capacity for AD than TT.

The equilibrium sorption of AD and TT shown in this study is comparable to that of the sorption study by Yu et al. (2004) for E2, E1, and 17α-EE2. The isotherm nonlinearity parameters reported for the three female hormones on Chelsea soil ranged from 0.593 to 0.860. A comparison between male and female hormone log K_{OC} values with Chelsea soil at similar C_e/S_w ratios (C_e/S_w = 0.02) reveals that the two groups of related compounds exhibit different sorption characteristics. The log K_{OC} values for the three female hormones ranged from 6.55 and 6.81, whereas for the male hormones, log K_{OC} ranged from 5.88 to 6.16. Such a difference is likely due to the lower aqueous solubilities of female hormones. It should be noted that both this study and that by Yu et al. (2004) were conducted under similar experimental conditions and that the compounds examined all share a similar molecular structure with the female hormones being slightly less soluble in water.

Literature sorption data on these chemicals differ greatly due to varied experimental procedures. Casey et al. (2004) reported Freundlich isotherm parameters for TT based on 5-h sorption data collected with batch systems without biological control. Their n parameters were close to or equal to unity, indicating nearly-linear distribution of the chemical between soil and water. The log K_{OC} values calculated from their sorption data were 6.56, 6.28, 7.86, 6.30, and 6.38 in all solution phase TT concentrations. Our TT log K_{OC} values at C_e/S_w = 0.003 exhibited relatively little

variability, varying less than one order of magnitude (6.26-6.70). As discussed above, a time period of 5 h was likely not adequate for attainment of sorption equilibrium, and the sorption capacity and single-point K_{OC} values measured at 5 h are expected to be lower than those measured at equilibrium condition (i.e., 2 weeks). Such a difference is larger at lower concentrations. For example, at C_e = 5 ng/L, our log K_{OC} values fell in a range of 7.24 to 7.73.

Lee et al. (2003) reported sorption isotherms for TT and AD as well. Similar to the study by Casey et al. (2004), microbial activities were not controlled in Lee's study. In fact, AD measured in Lee's systems was the metabolite of TT, not a solute introduced at the beginning of the experiment. Based on 24 to 31 h sorption data, their K_F value was 59.1 L/kg and their n parameter for TT was 0.62, indicating non-linear isotherms in accordance with our study. Their Drummer (7) soil had TOC comparable to the EPA-5 sediment tested here. Using their published sorption parameters, the log K_{OC} value calculated at C_e/S_w = 0.003 was 6.77 which was comparable to our data (6.70). In addition, they quantified AD concentrations in both phases and reported the sorption parameters of K_F = 27.9 L/kg and n = 0.51 for this daughter product of TT. Based on these reported values, the log K_{OC} value calculated at C_e/S_w = 0.003 was 6.44 for AD which was lower than our data (6.92). It is likely that our data was more accurate because sorption data for the daughter product was measured under biologically controlled and single-solute conditions.

Retardation Potential

While attention has been paid to the fate and transport of steroid hormones in surface water systems, the findings of Peterson et al. (2000) have proven that the

transport of these chemicals in groundwater can not be overlooked. Solute retardation (R) will play a significant role in the environmental fate of steroid hormones such as TT and AD. R is usually calculated as:

$$R = 1 + (\rho_b / \theta)(K_D) \qquad (9)$$

where ρ_b is the dry bulk mass density of the soil (g/cm^3), θ is the volumetric moisture content of the soil (unitless), and K_D is the single point distribution coefficient for the solute with the soil (ml/kg). For comparison purposes, ρ_b was assumed to be 1.68 g/cm^3 and a value of 0.33 was assumed for θ. The log K_F and n values from our experiments, Casey et al. (2004) Gardena soil and Lee et al. (2003) Drummer soil were used to determine K_D at an aqueous phase concentration of 5 ng/L. Three different R values of TT calculated for three hypothetical soils having TOC values of 0.1, 0.5, and 2.0 % were 125, 620, and 2,477, respectively, based on our Chelsea soil data, compared respectively to 11, 49, and 194 based on Casey's Gardena soil sorption data and 1,302, 6,507, and 26,027 based on Lee's Drummer (7) sorption data. The difference of the calculated retardation factor for a given soil apparently resulted from the nonlinearity of sorption isotherms measured in different studies. The use of non-equilibrium sorption data, which tend to exhibit a more linear sorption isotherm (Weber et al., 1996), could dramatically underestimate sorption-related retardation of estrogen compounds in groundwater.

Similar effects of isotherm nonlinearity also can be expected for transport of male hormones in surface water systems. We calculated colloid-bound mass fractions of TT in the bulk water phase of the Schuylkill River and Delaware River (Philadelphia, PA,

USA) using the colloid data of Mannino et al. (1999). They reported an average suspended colloid concentration of 10 mg/L for both rivers and an average total organic carbon content of 10 weight percent. Using the Chelsea sorption isotherm data from this study it was determined that under experimental conditions (500 μg/L) and field conditions (5 ng/L), the mass fraction of TT on the colloid phase was 0.14% and 2.37%, respectively. Similar calculations were performed based on the TT sorption isotherms for the Gardena-clay loam of Casey et al. (2004) and Drummer (7) soil of Lee et al. (2003). The distributions of the hormone under the same proposed experimental and field conditions were 0.19% and 0.19% using the Gardena-clay loam data and 0.32 % and 20.36 % using the Drummer data.

3.1.9 Summary

This study reports the sorption of two male hormones, TT and AD by four soil and sediment samples at both equilibrium and rate-limiting conditions. Unlike prior studies, AD was studied independently of TT. Apparent sorption equilibrium is achieved in one to two weeks when the initial aqueous hormone concentrations (C_o) was 10,000 μg/L (~ 15% of their solubility limits S_w) and two to three weeks when the C_o was 300 μg/L (less than 1% of S_w). The Freundlich model parameter n ranged from 0.698 to 0.899 for all soil-solute systems indicating nonlinear sorption isotherms. Isotherm nonlinearity leads to an inverse correlation between single-point organic carbon-normalized sorption distribution coefficients (K_{OC}) and equilibrium androgen concentration (C_e). When $C_e/S_w = 0.10$, the log K_{OC} values for TT and AD on the various sorbents ranged from 5.92 to 6.62 and 5.81 to 5.92, respectively, compared to 6.26 to 6.70 and 6.17 to 6.92 when $C_e/S_w = 0.003$. This study suggests that soils and sediments

may have greater sorption distribution coefficients (K_D) when concentrations fall into the ng/L range.

3.2 Sorption of Male and Female Hormones to Philadelphia Area River Sediments

In this subchapter the work in Chapter 3.1 was expanded to include female hormones as well, including the synthetic hormone EE2. There is a lack of information regarding the mobility of hormones in river sediments in the Philadelphia area, so the results obtained in this study will help to fill this void.

The naming system for the samples was based on the naming system used by the Philadelphia Water Department. Thus, samples named Wissahickon Creek 135, Wissahickon Creek Bank, Tacony 265, Belmont DWTP, and Queen's Lane DWTP will be referred to as Wiss135, WissRB, Taco, 4901, and 5903, respectively.

3.2.1 Rationale and Objectives

The first purpose of this study was to assess any differences in sorption characteristics of three hormones, E2, EE2, and TT in the presence of river sediments collected from various sites in the Philadelphia area. In addition, the experimental results were extrapolated for estimating the sorptive properties of these hormones at concentrations typically detected in aquatic systems. The second purpose of this study was to collect data in anticipation of biotransformation work conducted shortly after the completion of this study. We expected to find correlations between sorption and the biotransformation characteristics of hormones in the presence of the very same Philadelphia sediments (biotransformation work to be discussed in Chapter 6). It was anticipated that the sediment-hormone systems with a higher degree of aqueous phase

sorbate would be more likely to exhibit biotransformation (due to higher bioavailability in the solution phase). While the aforementioned sorption studies of hormones in surface soils and sediment were focused on only male hormones (Chapter 3.1), in this study, female hormones were included as well in order to gain a broader perspective on this suite of endocrine disrupting chemicals.

3.2.2 Materials and Methods

Sorbent Location

Sediment samples were collected from four locations in the Philadelphia area. Sediment was collected from the intake of the Belmont Drinking Water Treatment Plant (DWTP) (4903) along the Delaware River, from near the intake of the Queen's Lane DWTP along the Schuylkill River (5903), from Tacony Creek (Taco), and from the Wissahickon Creek (Wiss135). A single river bank sediment (WissRB) was collected right from the edge of the shore adjacent to the Wissahickon Creek river sediment sample for comparison purposes. It was anticipated that the sporadically submersed shore sample would have different microbial behavior compared to the constantly submersed river bed sample adjacent to it. These rivers were chosen because they are frequently used for recreational purposes and also serve as a point of discharge for numerous WWTP's with effluent potentially containing hormones. The choices of sediments near the intake of DWTPs also were of interest for drinking water quality reasons. The locations of all sites can be found in Figure 3.3.

Sorbent Collection and Preparation

Sediments were collected from the top 10 cm of the river bed and placed in glass jars. Upon arrival in the laboratory, the samples were freeze dried, then stored at room temperature until needed. The sediments were analyzed for particle size distribution (PSD) and sieved down to 1 mm for experimental use.

Sorbent Characteristics

The total organic carbon (TOC) contents of 5903, 4901, WissRB, Wiss135, and Taco were 7.65, 4.43, 3.36, 1.81, and 1.99 weight %, respectively. The PSD of the samples is shown graphically in Figure 3.4.

Sorbates

TT, E2, and EE2 were selected to compare the sorption characteristics of male, female, and synthetic female hormones. All three chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used as received. Molecular structures and major physicochemical properties of the two steroid hormones are summarized in Table 3.1.

Solutions

For additional details on solution preparation for each compound, see Section 3.1.2.3. Briefly, three primary stock solutions of EE2, E2, and TT were prepared in HPLC-grade methanol. A background aqueous solution was prepared from Milli-Q® water and contained $CaCl_2$ (0.005 *M*) and $NaHCO_3$ (100 mg/L). NaN_3 (100 mg/L) was added to inhibit biological activity.

3.2.3 Experiments

Sorption

The sorption experiments carried out for the three hormones with the five river bed sediments/river bank sediment were done in the same manner as those conducted for the sorption of the two male hormones (AD and TT) in Chapter 3.1.2. In brief, CMBR systems were used for the sorption experiments under equilibrium conditions. Preliminary tests were conducted first to determine the optimum ratio of aqueous and solid phase. A reaction time of 14 d was chosen for the preliminary equilibrium and final sorption tests (based on the rate study done earlier in Chapter 3.1).

For both preliminary and final tests, flame-sealed glass ampules (10 or 20 mL) were utilized as CMBRs. All reactors contained a predetermined mount of sorbent and were filled with the initial aqueous solution up to the shoulder of the ampule. Each reactor was flame-sealed immediately and placed horizontally on a rotary shaker set at 125 rpm and 22 ± 1°C. After two weeks, an aliquot of the supernantant was methanol-diluted. In all experiments, control reactors prepared similarly but containing no sorbent materials were run simultaneously for assessing loss of solutes to reactor components during sorption tests.

3.2.4 Chemical analysis

For the TT containing solutions, the method is the same as that detailed in Chapter 3.1.4. For the female compounds, a reverse-phase HPLC equipped with a 5 μm, 2.1 X 250 mm C_{18} column was used once again. A mixture of HPLC-grade acetonitrile

and Milli-Q water was also used as the mobile phase for both female hormones, E2 and EE2. For EE2-containing samples, the fluorescence detector was set at an excitation wavelength of 250 nm and an emission wavelength of 312 nm and the flow rate of the mobile phase at 0.28 ml/min. For E2-containing samples, the fluorescence detector was set at an excitation wavelength of 285 nm and an emission wavelength of 315 nm and the flow rate of the mobile phase at 0.30 ml/min. The injection volumes were 10 and 2 µl for samples having low (10−75 µg/L) and high (75−3,000 µg/L) EE2 and E2 concentrations, respectively. The ratio of acetonitrile to Milli-Q water was 40/60 (v/v) for both chemicals as well. Ten external methanol solution standards with concentrations of 10-3000 and were used to establish calibration curves for EE2 and E2.

3.2.5 Data Reduction

See Chapter 3.1.6 for the method of data reduction. Briefly, the concentrations in the sampled supernatants were calculated from the HPLC analyses and the dilution factors, which were computed based on the density data of the water-methanol mixture (Weber et al., 1996). The solid-phase sorbate concentrations were computed based on a mass balance calculation for the sorbate between the two phases.

3.2.6 Modeling

Sorption

The equilibrium sorption data obtained for the three sorbates and five sorbents were statistically fit to the Freundlich sorption model (Equation 1), which was employed successfully in Chapter 3.1. The Freundlich model parameters were obtained with a linear regression approach using Systat statistics software.

3.2.7 Results and Discussion

Table 3.3 lists the sorption isotherm parameters K_F and n values and their standard deviations, along with the total number of observations for each isotherm. The organic carbon (OC) normalized single-point distribution coefficients (K_{OC}) were calculated at C_e/S_W = 0.17, 0.14, and 0.11 from the TOC data and the fitted isotherm parameters listed in Table 3 with Equation 8, used previously in Chapter 3.1.5 for the surface soils data analysis. The calculated results are summarized in Table 3.3. The sorption isotherm data and the Freundlich model fits for the three sorbates and the five sorbents are also presented in Figure 3.5. Table 3.3 and Figure 3.5 show that the Freundlich sorption model is adequate for quantifying the equilibrium sorption of E2, EE2, and TT on the five Philadelphia area sorbents.

Sorption Equilibria

The diverse physicochemical nature of the 5 selected sorbents in the Philadelphia area is reflected in their wide ranging sorption capacities and linearity. Overall, amongst the fifteen experiments run, minimum and maximum log K_F values were 1.44 and 3.83 for the TT-Wissahickon Creek (Wiss135) system and E2-Wissahickon Creek Bank (WissRB) systems, respectively, whereas the minimum and maximum n values were 0.505 and 0.943 for the TT-Wissahickon Creek Bank (WissRB) and EE2-Taconey River (Taco) systems. As for the inter-compound comparison of the three EDC's used in this study, E2 exhibited the highest sorption capacity when the five sorbents' log K_F values were averaged for each compound. In contrast, its synthetic counterpart EE2 had the

lowest average sorption capacity for the five sorbents. Regarding inter-sorbent comparisons, it appears that the river bank sample collected along Wissahickon Creek Bank (WissRB) possessed the highest sorption capacity when the log K_F values for the three compounds were averaged together, whereas the bed sediment sample collected from Tacony Creek (Taco) had the lowest average sorption capacity. Interestingly, the trend observed for average log K_F during the inter-sorbent data analysis conforms to theoretical sorption "rules," when one takes into consideration the average linearity coefficients for each sorbent. The lowest average linearity was exhibited by Wissahickon Creek river bank (WissRB) sample whereas the highest average linearity was shown by the Tacony Creek (Taco) sample. Thus the sediment with the minimum K_F exhibited the lowest linearity, and vice versa. This makes sense because a sorbent that has low sorption capacity is often associated with an n value close to 1 (i.e., more linear), while a sorbent that exhibits high sorption capacity is usually associated with an n value <1 (i.e., more non-linear) (Weber et al., 1992; Huang et al., 1997; Xiao et al., 2004; Huang et al., 2003; Weber et al., 1998). Thus the average min/max sorption coefficient trends for the inter-sorbent comparison compliment each other in a predictable fashion.

Concentration Specific Comparisons

The parameter log K_F is a representation of the bulk sorbent and can be useful on its own, but due to the highly non-linear nature of many of the sediments and compounds in this study, it is beneficial to do inter-sorbent and inter-chemical sorption comparisons on a concentration specific level. When isotherms exhibit n values less than one, the sorption capacity will vary according to the liquid phase concentration, thus three different C_e/S_W (0.17, 0.14, and 0.11) were chosen and the corresponding sorption

capacities at each level were compared (the concentration-specific Log K_F values were also normalized to organic content, hence the use of the term Log K_{OC} for these new values). It was also of interest to re-confirm the non-linear nature of the isotherms by noting the increase in K_{OC} as the ratio of C_e/S_W decreased (see Table 3.3). The difference in sorption capacity at two different C_e/S_W's for a single sorbate-sorbent system was a function of the system's linearity. The lower the *n* value, the more dramatic a leap in sorption capacity a sorbent had as the liquid phase concentration decreased. For systems where the *n* value is close to one, the change in sorption capacity at different concentrations will be smaller.

TOC and PSD

In contrast to other studies (Holthaus et al., 2002; Lai et al., 2000), no correlation was found between Log K_F of the EDC's and the organic content of the sorbents. When Log K_F and f_{oc} were plotted against each other for the 15 systems, it was found that they are not interrelated in this particular study ($R^2 = 0.01$). This may come as a surprise, but the inherently complex nature of organic carbon may explain the lack of correlation between this group of mildly hydrophobic compounds and f_{oc}. Using a larger data set in the future would help to confirm this. Xiao et al. (2004) and Yu et al. (2006) revealed that sorption of PAHs and herbicides to isolated organic matter fractions gave way to isotherm parameters that diverged greatly from the original bulk sorbent. Soon to be published data of hormone sorption to isolated soil fractions further supports their conclusions (Section 3.3) Older and harder organic matter such as kerogen and black carbon have relatively high sorption capacity while softer and younger materials such as humic acid have lower sorption capacity (Weber et al., 1992; Huang et al., 1997; Xiao et

al., 2004; Huang et al., 2003; Weber et al., 1998). Because the sorbents used in this chapter were not separated into individual fractions, the makeup of the organic matter is unknown and is most likely a complex mixture.

Again, unlike other studies (Holthaus et al., 2002), there did not appear to be a correlation between PSD and sorption capacity. The PSD data collected for these sorbents indicate that the two DWTP intakes (5903 and 4901) had the highest proportion of fine particles, yet these two sorbents did not always yield the highest Log K_F values. As mentioned earlier, the River Bank (WissRB) sample from along side Wissahickon Creek (Wiss135) yielded the highest bulk sorption capacity. Although PSD analysis was not performed on this river bank sediment sample, visual and manual inspection of the material suggests that it was not composed of particles smaller than the obviously very fine-textured drinking water treatment plant intake samples.

Comparison to other Studies

Although few studies have assessed sorption of hormones, especially under proper experimental conditions, it is still useful to compare the data from this study to others. Several groups have delved into the task of understanding the nature of hormone sorption to various sorbents, many internationally. Samples have been collected from a dairy farming region of New Zealand and agricultural fields across southern Manitoba in Canada (Sarmah et al., 2008 ; Hildebrand et al., 2003). In Southern Australia, samples were collected from agricultural land as well as aquifer material from a well in Bolivar (Ying et al., 2003; Ying et al., 2005). No-till and conventionally tilled bulk soil samples were studied and compared from Georgia, USA (; Sangsupan et al., 2006). Sediments from various rivers, near river banks, and estuaries in the UK and various parts of the

USA were also examined. (Holthaus et al., 2002; Lai et al., 2000; Bowman et al., 2002; Yu et al., 2004; Kim et al., 2007). In order to better compare the sorption parameters of other research groups to the ones obtained in this study, their Freundlich coefficients were converted to our units (when necessary) and used to calculate Log K_{OC} at the C_e/S_W ratio of 0.11, one of three ratios used in this study. The *n* values could be compared directly, of course.

Working with E2 and EE2, Sarmah et al. (2008) determined that most isotherms were linear and attributed the few non-linear isotherms to finer textured particles and/or the high surface area resulting from allophonic soil characteristics. It should be noted that no sterilization attempt was made in their study and that the incubation times employed were 42 hours for EE2 and 72 hours for E2. The short reaction time is expected to result in much more linear isotherms than ours.

Although Hildebrand et al. (2003) autoclaved their sorbents, their reaction time was short as well - usually 24 hours. As with Sarmah et al. (2008), their *n* values for E2 and EE2 were much closer to unity than the Philadelphia sediments. Unlike our data, they found that sorption of EE2 was greater for fine-textured than course-textured soils.

Ying et al. (2003) apparently achieved equilibrium in 1 hour with the aquifer material during their preliminary experiments and yet their *n* values do indeed reflect non-linearity (0.4 for E2 and 0.46 for EE2). They did document low K_F values, which they attributed to low organic content in the aquifer. We hypothesize that these low values are more likely due to the extremely short incubation time. In a second study by Ying et al. (2005), again using E2 and EE2, this time using soil instead of aquifer material, linear sorption was assumed across the board. They transposed the preliminary

sorption kinetic data from their previous aquifer study to their 2005 work and assumed 1 hour equilibrium with the new sorbents. Regardless of the accuracy in isotherm parameters, they noted a snapshot correlation between sorption capacity and organic carbon for E2 and especially for EE2. They also noted that EE2 was the more sorptive of the two compounds, opposite of our findings (Ying et al., 2005).

Sangsupan et al. (2006) implemented a shaking time of 3 days for E2 and TT. E2 tended to have a higher sorption capacity compared to TT in their work, which coincides with this study's observations regarding the natural female hormone and the male hormone. Their research compared sorption at two different soil depths and they pointed out that the sorption of TT decreased more sharply with soil depth compared to the E2.

Lai et al. (2000) stated that the synthetic estrogens were more likely to be removed from the liquid phase than natural estrogens. That was not the case in our study. In fact, not only was the synthetic hormone EE2 less sorptive compared to its natural counterpart E2, it even had a lower average log K_F value than the more-polar TT. In addition, their strong correlation between organic carbon content and sorption capacity deviated from our results. However their comment regarding the decrease in estrogen removal at higher estrogen concentrations does agree with our observations. Lai et al. did not implement sterilization measures and decided upon a 1 hour incubation time based on a decrease in sorption noticed after that amount of time. Given the ease with which hormones are biotransformd, the decrease they observed was most likely biotransformation, not desorption as Lai et al. suggested. Further, the hormone research of Lai et al. involved estrogen mixtures, not single solutes, which may further obscure the true sorption parameters.

Despite being aware that sorption equilibrium was not reached after 2 days, Holthaus et al. (2002) carried out their experiments (using anaerobic conditions to inhibit biotransformation) with E2 and EE2 in 1 day, achieving what they claim to be 80-90% of equilibrium. In their bed sediments they observed higher sorption with smaller particle size and higher organic content, phenomena that this study did not encounter. They also noticed a trend opposite to ours where, for their study, EE2 exceeded E2's sorption by up to a factor of three. They stated that most of the bed sediments used in their work were collected from near river banks, where fine particles usually abound. Our study also found the WissRB sample from along the bank of the Wissahickon Creek to be highly sorptive. The results in our study confirm their speculation that sorption for samples collected near the river banks may be higher than from the river cross section. Like various other research groups mentioned here, they too found that higher sorption was generally associated with smaller particle sizes. They also found some weaker positive correlations for clay content and organic carbon content.

Lee et al. (2003) investigated the same three compounds as in this study but with surface soils from Indiana. Their combination of short retention time (24 to 31 hours) and absence of biocide yielded results that may underestimate sorption capacity and be subject to competitive sorption from the resulting daughter products. The competitive sorption work of Yu et al. (2004) illustrates the competitive nature of sorption amongst hormones when mixed together simultaneously. Casey et al. (2003 and 2004) evaluated E2 and TT after 48 hours of shaking due to the larger amounts of metabolites detected at later times. Once again, the short contact time and absence of biological control led to results very different from the data collected in the current study after a full two weeks of

incubation. Most of the systems in Casey's two studies yielded *n* values of 1 or greater. Although the Log K_{oc} values we calculated from their data at a C_e/S_w of 0.11 were in the range of ours, their linear results will lead to results very different from equilibrium based and biological-controlled based values if extrapolated to lower concentrations.

Yu et al. (2004) and Kim et al. (2007) carried out sorption experiments with E2, EE2, and TT on a variety of EPA sediments, a top soil, peat, and pond sediment. The experimental protocol was nearly identical to the one used in this study and so results can be compared directly to our data. In Kim et al. (2007), the range of *n* for TT ranged from 0.7-0.9 and the Log K_F values ranged from 2.36-3.59. In Yu et al. (2004), the range of *n* and Log K_F values for E2 were 0.48-0.73 and 2.75-3.18, while those for EE2 were 0.61-0.89 and 1.84-2.54. In the case of Yu et al. (2004), 17β-estradiol had higher sorption than EE2, as in the current study.

Sorption at Low ng/L Levels

The diverse range of sorbents and experimental conditions used by others provide an interesting setting in which to evaluate our data. Despite the short incubation times that nearly everyone used (except for Yu et al. (2004) and Kim et al. (2007), whose experimental work was conducted in the same laboratory as this study), the isotherm parameters they obtained can still be useful in an intra-study sense rather than an inter-study one. Sorption capacity and linearity of one compound/soil can, to some extent, be compared to another within the same experiment. The main shortcoming of short shaking times is the acquisition of inaccurate *n* values, which might underestimate sorption when extrapolating down to lower concentrations. We took the other researcher's parameters and our own parameters and extrapolated both sets down to 5

ng/L in order to obtain and compare the resulting Log K_{oc} values. The sorption capacity for the hormones was in general much higher with our sediments than the others, mostly due to their higher *n* values, which lead to an underestimation of sorption capacity at lower aqueous concentrations. Our Log K_{oc} values at 5 ng/L went up to 9.57 whereas none of the other groups exceed 8.41 (again, with the exception of Yu et al. (2004), whose experimental protocol was similar to ours). Because the isotherms "cross" each other, it is interesting to note within our own study that the ranking of Log K_{oc} values from least to most sorbent within each chemical shifts as Log K_{oc} values are calculated at higher or lower concentrations. For instance, when the sediments are ranked from low to high with respect to E2, the order when $C_e/S_w = 0.17$ goes as follows: Taco, 5903, Wiss135, 4901, and finally WissRB. On the other hand when Log K_{oc} values are calculated for $C_e = 5$ ng/L, the ranking of sediments for the same compound is 5903, Taco, 4901, Wiss135, and finally WissRB. So, aside from the last sediment, the other four sorbents fell onto a different rung on the sorption capacity ladder. Thus in addition to having higher sorption at lower aqueous concentrations, the relative potential to sorb amongst a suite of compounds may vary – a location which, compared to another, appears to sorb more of a particular hormone at one concentration might actually sorb less of the compound compared to the other site given a different concentration.

Log K_F and n

Plotting log K_F versus *n* for the 15 systems yielded a strong negative correlation ($R^2 = 0.66$). Figure 3.6 represents the tendency for log K_F to decrease as *n* increases for the three hormones on the five Philadelphia area rivers sediments. This is typical and classic behavior for a group of non-linear isotherms. A sorbent-sorbate system exhibiting

n values substantially less than 1 (towards the left of the plot) will experience a limit to sorbate uptake while systems with *n* values close to 1 (towards the right of the plot) or even greater will behave as if sorption potential is infinite.

3.2.8 Summary

The order in which sorption increases for the hormones is EE2 < TT < E2. The order in which the Philadelphia area river sediments are able to take up hormones is Tacony < Queen's Lane DWTP Intake < Belmont DWTP Intake < Wissahickon < Wissahickon River Bank. It is unusual that EE2 has a lower tendency to sorb compared to TT which is more soluble. This may be due to the fact that EE2 is a slightly bulkier molecule (ethinyl group) compared to the other two compounds and might have trouble penetrating further into the sediment matrix.

Sorption parameters aside, two important aspects of our work that seemed to deviate from that of others is the lack of correlation between sorption capacity and particle size distribution and/or organic carbon content. In addition to the aforementioned issues regarding organic matter heterogeneity, there could be other inorganic factors playing a role in what we observed (aside from potential sample sieving and handling factors). For instance, Lai et al. (2000) observed in their research that although sorption of estrogen to sediments was correlated with organic carbon, they noted that organic carbon was not a requirement for sorption. Iron oxide by itself was capable of achieving 40% of the sorption capacity of a sediment possessing 1.1% total organic carbon (Lai et al., 2000). The nature of sorption to iron oxide consists partially of ion exchange between a charged or polar solute and a surface hydroxyl group on the

oxide surface (Stumm et al. 1998). Holthaus et al. (2002) investigated clay mineralogy in their work and noted that the clay fraction of the illite group was a stronger hormone sorbent than kaolinite and smectite. The clay mineralogy of the Philadelphia sediments was not determined, so we can not be certain if the phenomena noted by Holthaus applies to this study.

The sorption of the E2, EE2, and TT to the five Philadelphia area river/riverbank sediments clearly follows the general rules of isotherm linearity as Figure 3.6 indicates. Thus, it is peculiar that correlations of the sorption parameters to the two most common soil sorption characteristics, TOC and PSD, were not observed. Due to the complex nature of sediment, site specific interactions between the sorbents and the functional groups of the hormones may be taking place. In the next section, regarding the sorption of hormones to soil fractions, the lack of correlation between sorption capacity and TOC or PSD in this chapter dealing with the Philadelphia area sediments and three hormones is further explored.

3.3 Sorption of Hormones to Soil Fractions

This subchapter delves into more detail regarding the sorption of hormones to bulk soils by determining their sorption towards individual soil fractions. As was seen in Section 3.2, E2, EE2, and TT did not always have a preference for the Philadelphia sediments with higher TOC levels. This may be attributed to the structure and age of organic matter within each of the sediment samples. By chemically separating the individual constituents of bulk sorbent and conducting sorption experiments with them, it is anticipated that preference for the hormones for a particular sediment will be elucidated.

3.3.1 Rationale and Objectives

To date, no research has been conducted for hormones on various soil fractions. One male and one female hormone were chosen for this study, EE2 and AD. The sorption of hormones such as AD and EE2 to individual humic acid, particulate organic matter (POM), and humin fractions had yet to be reported and was the focus of this study. Although hormones are only mildly hydrophobic, it was still expected that they would behave differently towards each of the individual soil fractions and the bulk soil. The goal of this study was two-fold, to determine the extent of sorption difference of the mildly hydrophobic hormones towards the three soil fractions and, if differences were found, the contribution of each fraction towards the overall sorption. The information obtained in this study will help explain the lack of correlation between sorption capacity and TOC contents of the sorbents used in the previous chapter (Philadelphia area sediments).

3.3.2 Materials and Methods

Sorbents

A soil and three samples derived from the soil were used as the sorbents in this study. The soil sample was a topsoil collected from Chelsea, Michigan (USA). A humic acid (HA) and a base-extracted soil (HM) were obtained after the soil had been base-extracted following a procedure recommended by the International Humic Substances Society (http://ihss.gatech.edu). A concentrated POM sample was obtained after demineralization of the base-extracted soil by the procedure of Song et al. (2002). The four sorbents were characterized with an elemental analyzer to determine their organic carbon, hydrogen, oxygen, and nitrogen contents. The results are summarized in Table

3.4. Relative HA, HM and POM contents were calculated based on the mass of these fractions recovered; their TOC values are reported in Table 4 as well. The HA, HM and POM fractions extracted had TOC contents of 51.5 %, 3.33 %, and 30.4 %, respectively. Approximately 41.6% of Chelsea SOM was not base extractable and may consist of fulvic acids, bound HAs, solvent-extractable organic acids, and fine particles of POM.

Sorbates

AD and EE2 were selected to compare the sorption characteristics of male and female hormones towards the soil fractions used as sorbents in this study . As mentioned earlier, both chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used as received. Molecular structures and major physicochemical properties of the two steroid hormones are summarized in Table 3.1.

Solutions

For additional details on solution preparation for each compound, see Section 3.1.2.3. Briefly, three primary stock solutions each of EE2 and AD were prepared in HPLC-grade methanol. A background aqueous solution was prepared from Milli-Q® water and contained $CaCl_2$ (0.005 *M*) and $NaHCO_3$ (100 mg/L). NaN_3 (100 mg/L) was added to inhibit biological activity.

3.3.3 Experiments

The sorption experiments carried out for the three hormones with the five river bed sediments/river bank sediment were done in the same manner as those conducted for the sorption of the two male hormones (AD and TT) in Chapter 3.1.2. In brief, CMBR

systems were used for the sorption experiments under equilibrium conditions. Preliminary tests were conducted first to determine the optimum ratio of aqueous and solid phase. A reaction time of 14 d was chosen for the preliminary equilibrium and final sorption tests.

For both preliminary and final tests, flame-sealed glass ampules (10 or 20 mL) were utilized as CMBRs. All reactors contained a predetermined mount of sorbent and were filled with the initial aqueous solution up to the shoulder of the ampule. Each reactor was flame-sealed immediately and placed horizontally on a rotary shaker set at 125 rpm and 22 ± 1°C. After two weeks, an aliquot of the supernantant was methanol-diluted. In all experiments, control reactors prepared similarly but containing no sorbent materials were run simultaneously for assessing loss of solutes to reactor components during sorption tests.

3.3.4 Chemical Analysis

The chemical analysis of AD is described in section Section 3.1.4 and the chemical analysis of EE2 is described in Section 3.2.4. Once again, a reverse-phase HPLC equipped with a 5 μm, 2.1 X 250 mm C_{18} column was used for both. A mixture of HPLC-grade acetonitrile and Milli-Q water was used as the mobile phase again for both hormones.

3.3.5 Data Reduction

Data reduction was carried out in the same manner as for the AD versus TT sorption study in Chapter 3.1.5. In brief, the concentrations of the supernatants obtained from the HPLC were converted to actual concentrations based on the solution densities.

The solid-phase sorbate concentrations were computed based on a mass balance calculation for the sorbate between the two phases.

3.3.6 Modeling

The equilibrium sorption data obtained for the three sorbates and five sorbents were statistically fit to the Freundlich sorption model (Equation 1) successfully employed in Section 3.1.6. The Freundlich model parameters were obtained with a linear regression approach using Systat statistics software.

It was of interest to see how the individually measured fractions compared to the bulk soil when added together in different combinations. Three C_e values (corresponding to the Ce/Sw values selected in Table 3.5) were used to calculate a raw q_e value (not yet taking into account the mass contribution of each fraction to the total bulk mass) for each individual fraction. This raw q_e value was then corrected for that particular fraction's percentage in the original soil resulting in a mass fraction-corrected K_{oc} value. The mass fraction-corrected HA and HM nominally might be expected to add up to the bulk soil, and their sums for the three C_e's are listed alongside that of the original soil in Table 3.6. The total organic component is important for mildly non-polar compounds such as EE2 and AD, so the sum of the mass fraction-corrected POM and HA are listed in Table 3.6 as well. Comparisons between the summed, individual fraction-based values and the bulk soil will be discussed in more detail below.

3.3.7 Results and Discussion

Table 3.5 lists the sorption isotherm parameters for each isotherm. The organic carbon (OC) normalized single-point distribution coefficients (K_{OC}) were calculated at

C_e/S_W = 0.02, 0.04, 0.08 from the TOC data and the fitted isotherm parameters with Equation 8. The calculated results are summarized in Table 3.5. The sorption isotherm data and the Freundlich model fits for the two sorbates and the four sorbents are also presented in Figure 3.7.

All eight of the isotherms yielded r^2 values of nearly 1, indicating that the Freundlich equation is an appropriate method to model sorption to soil fractions as well as to the bulk soil.

Sorption Data

The data summarized in Figure 3.7 and Table 3.5 show the heterogeneous nature of soil organic matter that is responsible for the wide range of sorption capacities and linearities of bulk soil. When the bulk soil was compared to its respective fractions, it was found that n values ranged from 0.639 to 0.899 and 0.692 to 1.043 for AD and EE2, respectively. As for log K_F, the corresponding ranges were 2.531 to 3.947 and 2.528 to 3.626.

Humic acid

For both AD and EE2, the extracted humic acid component of the SOM exhibited the most linear isotherm (n = 0.899 and n = 1.043) compared to the other two fractions and the bulk Chelsea soil. When compared to the results of Sharma et al. (yet to be published) for pesticide sorption on identical sorbents, a similar trend was found – atrazine, metolachlor, and napropamide all displayed the most linearity with humic acid. Similar results were also noted for sorption of phenanthrene and naphthalene on humic acids extracted from a pond sediment and sandy soil (Xiao et al., 2004).

Given the widely accepted theory of SOM being a multi-domain phase, the aforementioned linearity of humic acid supports the assumption that this particular SOM fraction is indeed an amorphous/rubbery domain. According to this conceptual model, sorption in the amorphous organic matter, in this case humic acid, follows a partitioning process (Huang et al., 1997; Weber et al., 1998). According to the distributed reactivity model (DRM), humic acid falls into the Domain II category and can be described as an expanded and highly swollen organic matter, generally exhibiting absorptive rather than adsorptive behavior (Weber et al., 2001; Huang et al., 1997). In the case of the EE2 system, which had an *n* value greater than one, the humic acid behaves similarly to a rubbery polymer, a sorbent that can yield isotherms with not only linear, but slight upward curvature (Weber et al., 2001). None of the aforementioned PAHs and pesticides had *n* values greater than one, so the chemical structure of the synthetic female hormone may lend itself to absorptive, rather than adsorptive behavior. The reason for the similarly structured androstenedione's lack of upward curvature can only be speculated, but functional group-specific interactions may play a role in the sorption of the two hormones on the humic acid.

It has been postulated that non-polar domains of humic substances are the sites of hydrophobic organic carbon uptake with, for example, aromatic moieties considered important (Gauthier et al., 1987). The phenolic groups of humic acid may interact preferentially with slightly more polar solutes. It is not surprising, therefore, that EE2 has the greater carbon normalized K_D. In contrast to AD, EE2 has a phenolic group that may explain why the sorption capacity of humic acid for EE2 is higher than that of the

male hormone. Furthermore, of the two compounds, EE2 has a lower aqueous solubility and a higher log K_{ow}, which would indicate a higher sorption potential than AD.

Particulate Organic Matter

POM was the second organic component to be extracted from the Chelsea soil. Compared to humic acid, both AD and EE2 exhibited more nonlinear sorption isotherms (n = 0.685 and 0.825, respectively) on the POM than on the HAs. Other studies with herbicides and PAHs on soil fractions also exhibited less linearity on POM compared to humic acid (Xiao et al., 2004; Yu et al, 2006). The constituents of black carbon and kerogen (POM) are considered to be "hard" or "condensed" carbon. This particular domain of SOM is characterized by surface adsorption processes and is considered responsible for isotherm non-linearity and slow sorption rates (Weber et al., 1992; Huang et al., 1997; Xiao et al., 2004; Huang et al., 2003). The POM fraction of Chelsea soil had a greater sorption capacity (K_f) not only compared to humic acid, but it had the highest sorption capacity of all the SOM fractions. This is not surprising considering that the sorption of hydrophobic organic compounds to soils and sediments has been found to be dominated by adsorption to "hard carbon" materials when present (Allen-King et al., 2002; Cornelissen et al., 2005). In fact, PAH sorption to sediments with a high percentage of BC, unburned coal, and kerogen was 1-2 orders of magnitude higher than predictions based only on partitioning to amorphous organic carbon (Cornelissen et al., 2006). The results presented in this study were once again consistent with the PAH and pesticide results obtained by Xiao et al. (2004),Yu et al. (2006) and Sharma et al. (2008) - the POM fraction of soils seems to have a large capacity for a wide range of chemicals.

A look at the molecular composition of the fractions may explain why POM behaves the way it does. The O:C ratio for the POM fraction was lower than for the other 3 sorbents. A lower O:C ratio indicates a carbon rich (non-polar) material with a higher propensity for sorption of non-polar/semi-polar compounds, which may explain the greater sorption of the two hormones on this fraction. According to the DRM, POM falls into the Domain III category, which includes a wide range of heterogeneous sites and is responsible for different types of adsorption processes (Weber et al., 2001).

Humin (Base-extracted)

Although POM is generally considered the component most responsible for the nonlinear nature of sorption to bulk soil, the present study suggests that this may not be necessarily true – the humin fraction appears to exhibit the most non-linearity. Humin has been historically hard to define and may include kerogen and black carbon (Song et al., 2002). The base-extracted humin may have exhibited the most non-linearity of all 4 sorbents, but this may be due to the fact that it is actually a mixture of POM plus the mineral component of the bulk soil. Previously reported work has shown that sorption isotherm nonlinearity decreased in the following order: humin > soil > humic acid (Xing and Pignatello, 1997; Gunasekara and Xing, 2003). The data in the present study follow this trend for both compounds. The reason why the base extracted soil displayed less linearity than the original soil may have to do with the absence of humic acid in the fraction. In contrast, the POM fraction was more linear than the HM fraction in both cases. This may be due to the fact that mineral constituents within the HM lent a "rigid" character to the POM associated with it.

As for capacity, the humin consistently exhibits higher sorption capacity compared to the bulk soil but lower sorption compared to POM – comparison of Log K_{oc} for various Ce/Cw ratios confirms this. Factors similar to the ones that explain variations in linearity can be utilized to explain differences in sorption capacity as well. The base-extraction process removes HAs thus enabling the sorption sites of the POM within the HM to be more exposed. One reason for the highly sorptive nature of humin could be the micro- and mesopores that dominate humin's surface (Malekani et al., 1997). A study by Nam et al. (2002) revealed that a substantial quantity of 0.1-1 μm pores were present on the humin-mineral surface and that upon removal of HA and FA from the bulk soil, pore volume originating from said pores increased by 43%. Thus, this first extraction step caused the HM to exceed the bulk soil capacity-wise. Furthermore, the de-mineralization process (2^{nd} step) removed the mineral components (whose contribution to the sorption process is minimal), which results in the POM fraction having higher sorption capacity than HM.

Despite the ambiguous nature of humin, other studies have also arrived at the same conclusions regarding its degree of nonlinearity – an analysis of phenanthrene and pyrene sorption to a fulvic/humic acid combination and humin revealed that that amount of solute sorbed by the FAs/HAs did not change substantially after 2 days whereas sorption by the HM fraction continued to increase slowly to the end of the experimental period (720 h) (Pan et al., 2006). It appears that the nonlinear nature of HM contributes importantly to the overall general nonlinearity of the bulk soil and may outweigh the linear effects of the humic acid fraction.

Contribution of SOM Fractions to Overall Sorption

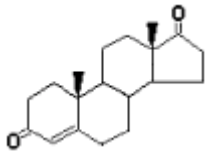
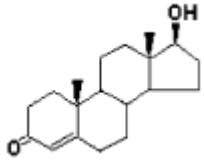
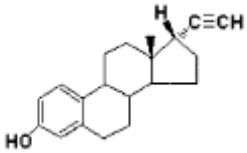
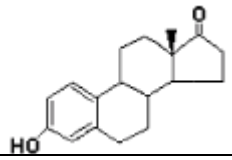
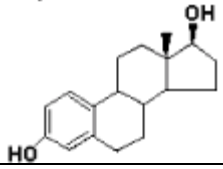
Table 3.4 reveals the sorption potential of the fractions when their percent contributions in the original bulk soil are accounted for. Both hormones displayed greater sorption when the HM and HA contributions were summed compared to the bulk soil. Technically, the sum of HM and HA should be approximately equal to the original soil, but due to the absence of aggregation and mineral protection, the sorption potential of the isolated HA and HM fractions may be slightly enhanced. When the sum of the two organic fractions, HA and POM, is compared to the original soil, we find that the male and female hormone behave differently. The sum of the K_D for the HA and POM fractions for AD was slightly greater than that of the bulk soil. For EE2 the K_D of the bulk soil was actually greater than the sum of the K_D of the isolated organic fractions. Both organic fractions (HA and POM) were present in low quantities in the bulk soil (less than 5 % each) and the aforementioned protective issues (aggregation and mineral protection of sorption sites) may mean that EE2 has a greater affinity than AD for the mineral fraction of the soil. Thus, the reduced role of HA and POM in bulk soil only appears to apply to the male hormone in this particular study. Other factors that need to be taken into consideration are the potential loss of sorptive fulvic acids and lipids during the fractionation process. The ratio of humic acid to fulvic acid is an important characteristic of organic matter that has been correlated to the base extractable portion of soil organic carbon (You et al., 2006). Although the data presented in this study indicates that the two isolated organic fractions, humic acid and POM, have high sorption capacities, it is obvious from the bulk soil experiments that their full sorption potential is not realized in the bulk soil.

3.3.8 Summary

Soil organic matter (SOM) consists of different fractions such as humic acid (HA), humin (HM), and particulate organic matter (POM). When these SOM constituents are obtained from bulk soil via chemical extraction processes, they exhibit physical and chemical properties that vary substantially from the original soil. In this study, EE2 and AD have been chosen to illustrate the different sorption properties of individual SOM fractions. The sorption with base-extracted HA appeared to be more linear than with the bulk soils for both the hormones. In contrast, the POM, which was obtained after demineralization of HM, exhibited isotherms that were less linear than the bulk soil for each compound. This phenomenon is consistent with the opposing absorption versus adsorption properties of HA and POM, respectively. Thus, due to aggregation, it was logical for the bulk soil's linearity to fall somewhere in between the two extremes. As for sorption capacity, POM appeared to have the greatest sorption potential at various values of C_e/S_w. However, despite the presence of such a strongly sorbing organic material, the lower sorption capacity of the bulk soil does not reflect the contribution potential of POM. This is most likely due to entrapment of POM within the complex soil matrix, resulting in a pollutant's inability to fully access all sorption sites.

Regarding the outcome from the experiments with river sediment and hormones in Chapter 3.1 (i.e. the genuine non-linear nature of all experimental systems despite the lack of correlation to TOC and PSD), the vast differences in sorption capacity and linearity between various soil fractions in this study may serve to provide an explanation for such phenomena. Site specific interactions may dominate the sorption behavior of hormones to river sediments.

Table 3.1 Physicochemical properties of the 5 hormones

Hormone	Molecular formula	Molecular weight (g/mol)	*Aqueous solubility (mg/L)	log K_{OW}
Androstenedione (AD) 	$C_{19}H_{26}O_2$	286.4	50.5 ± 2.1	2.75
Testosterone (TT) 	$C_{19}H_{28}O_2$	288.4	32.2 ± 1.6	3.22
17α-Ethinylestradiol (EE2) 	$C_{20}H_{24}O_2$	296.4	3.1 ± 0.03	4.15
Estrone (E1) 	$C_{18}H_{22}O_2$	270.4	2.1 ± 0.03	3.43
17β-Estradiol (E2) 	$C_{18}H_{24}O_2$	272.3	3.1 ± 0.02	3.94

* at 23ºC

Table 3.2 Freundlich sorption isotherm parameters and single point K_{OC} values for androstenedione and testosterone on bulk soils and sediments

Chemical	Sample	n	SE[a]	log K_F[b]	SE[c]	N[d]	r^2	log K_{OC}[e]			
								(C_e/S_w= 0.012)	(C_e/S_w= 0.007)	(C_e/S_w= 0.004)	(C_e = 5 ng/L)[f]
Androstenedione	Chelsea soil	0.73	0.011	2.53	0.031	9	0.997	6.06	6.12	6.19	7.41
	Pahokee Peat	0.72	0.010	3.65	0.031	9	0.997	6.22	6.28	6.35	7.63
	Pond sediment	0.75	0.017	2.25	0.050	10	0.990	6.23	6.29	6.35	7.50
	EPA-sediment 5	0.81	0.015	2.72	0.031	9	0.995	6.84	6.89	6.93	7.79
Testosterone	Chelsea soil	0.75	0.018	2.55	0.052	10	0.990	6.24	6.30	6.36	7.39
	Pahokee Peat	0.77	0.014	3.59	0.042	10	0.994	6.39	6.45	6.50	7.45
	Pond sediment	0.70	0.017	2.360	0.049	10	0.995	6.33	6.40	6.47	7.73
	EPA-sediment 5	0.90	0.022	2.37	0.063	10	0.990	6.77	6.80	6.82	7.24

[a] Standard error.

[b] K_F = Freundlich capacity parameter with units of (µg/kg)/(µg/L)^n.

[c] Standard error of log K_F.

[d] Number of observations.

[e] K_{OC} = organic carbon-normalized sorption distribution coefficient; with units of (µg/kg)/(µg/ml) calculated by using the best-fit Freundlich isotherm parameters and total organic carbon of the sorbents.

[f] Extrapolated to frequently reported androgen concentrations in surface water.

Table 3.3 Freundlich sorption isotherm parameters and single point K_{OC} values for E2, EE2, and TT on Philadelphia river sediments

Chemicals	Sample	n	SE[a]	log K_F[b]	SE[c]	N[d]	r^2	log K_{OC}[e]			
								(Ce/Sw= 0.17)	(C_e/S_w= 0.14)	(C_e/S_w= 0.11)	(C =5 ng/L)[f]
17β-Estradiol											
	4901	0.67	0.029	3.32	0.081	9	0.972	6.78	6.81	6.84	8.42
	5903	0.88	0.023	2.19	0.062	5	0.995	5.98	5.99	6.00	6.59
	WissRB	0.45	0.036	3.83	0.092	5	0.950	6.80	6.85	6.90	9.57
	Wiss135	0.56	0.047	2.97	0.138	10	0.894	6.50	6.54	6.59	8.73
	Taco	0.83	0.069	2.01	0.191	5	0.948	5.93	5.95	5.98	6.84
Testosterone											
	4901	0.81	0.027	2.23	0.090	5	0.991	5.90	5.92	5.94	7.02
	5903	0.51	0.026	3.03	0.078	10	0.956	5.39	5.43	5.48	8.28
	WissRB	0.51	0.032	3.29	0.098	9	0.941	5.98	6.03	6.08	8.90
	Wiss135	0.71	0.047	1.44	0.149	10	0.928	5.14	5.17	5.20	6.84
	Taco	0.75	0.039	1.90	0.123	10	0.953	5.72	5.74	5.76	6.84
17α - Ethinylestradiol											
	4901	0.80	0.019	2.51	0.058	10	0.990	6.32	6.33	6.36	7.33
	5903	0.94	0.042	1.76	0.128	10	0.966	5.71	5.72	5.72	7.09
	WissRB	0.75	0.049	2.56	0.153	9	0.935	6.35	6.38	6.40	7.45
	Wiss135	0.63	0.028	2.50	0.085	5	0.985	6.24	6.27	6.31	7.72
	Taco	0.94	0.047	1.55	0.145	10	0.958	6.10	6.11	6.11	7.68

[a] Standard error. [b] K_F = Freundlich capacity parameter with units of (μg/kg)/(μg/L)^n. [c] Standard error of log K_F. [d] Number of observations.

[e] K_{OC} = organic carbon-normalized sorption distribution coefficient; with units of (μg/kg)/(μg/L) calculated by using the best-fit Freundlich isotherm parameters and TOC of the sorbents.

[f] Extrapolated to frequently reported androgen concentrations in surface water.

Table 3.4 Elemental composition of the individual soil fractions

Sorbent	C [ac]	O[a]	H[a]	O/C[b] Ratio	H/C[b] Ratio	Relative contents in bulk soil
Bulk Soil	5.29	6.76	0.90	0.96	2.04	-
Base Extracted (HM)	3.33	3.53	0.68	0.79	2.44	90.0
Particulate Organic Matter (POM)	30.4	14.02	2.91	0.35	1.15	3.57
Humic Acids (HA)	51.5	30.62	5.04	0.45	1.17	3.89

[a] Weight %

[b] Atomic ratio

[c] Total Organic Carbon (% TOC)

Table 3.5 Freundlich sorption isotherm parameters and single point K_{OC} values for AD and EE2 to isolated soil fractions

Chemical	Sample	n	SE[a]	log K_F[b]	SE[c]	N[d]	r^2	log K_{OC}[e]		
								(C_e/S_w= 0.08)	(C_e/S_w= 0.04)	(C_e/S_w= 0.02)
Androstenedione										
	Original Chelsea soil	0.73	0.011	2.53	0.031	9	0.997	5.83	5.91	6.00
	Base – extracted humin	0.64	0.015	2.82	0.050	10	0.990	6.00	6.11	6.22
	Particulate organic matter	0.69	0.006	3.95	0.019	10	0.998	6.33	6.42	6.52
	Humic acids	0.90	0.005	3.22	0.014	10	1.000	6.14	6.17	6.20
17α - Ethinylestradiol										
	Original Chelsea soil	0.83	0.012	2.53	0.029	10	0.996	6.41	6.46	6.51
	Base – extracted humin	0.69	0.012	2.77	0.029	10	0.995	6.51	6.60	6.69
	Particulate organic matter	0.83	0.011	3.63	0.026	10	0.997	6.72	6.78	6.83
	Humic acids	1.04	0.017	3.00	0.040	10	0.997	6.39	6.38	6.36

[a] Standard error.

[b] K_F = Freundlich capacity parameter with units of (µg/kg)/(µg/L)^n.

[c] Standard error of log K_F.

[d] Number of observations.

[e] K_{OC} = organic carbon-normalized sorption distribution coefficient; with units of (µg/kg)/(µg/ml) calculated by using the best-fit Freundlich isotherm parameters and total organic carbon of the sorbents.

Table 3.6. Contribution of SOM fractions towards sorption of AD and EE2 in soil

Hormone	C_e/S_w	K_D (L/kg) Bulk Soil	HM + HA	HA + POM
Androstenedione	0.08	37	58	51
	0.04	45	68	59
	0.02	54	81	68
17α - Ethinylestradiol	0.08	135	146	107
	0.04	152	167	113
	0.02	170	194	120

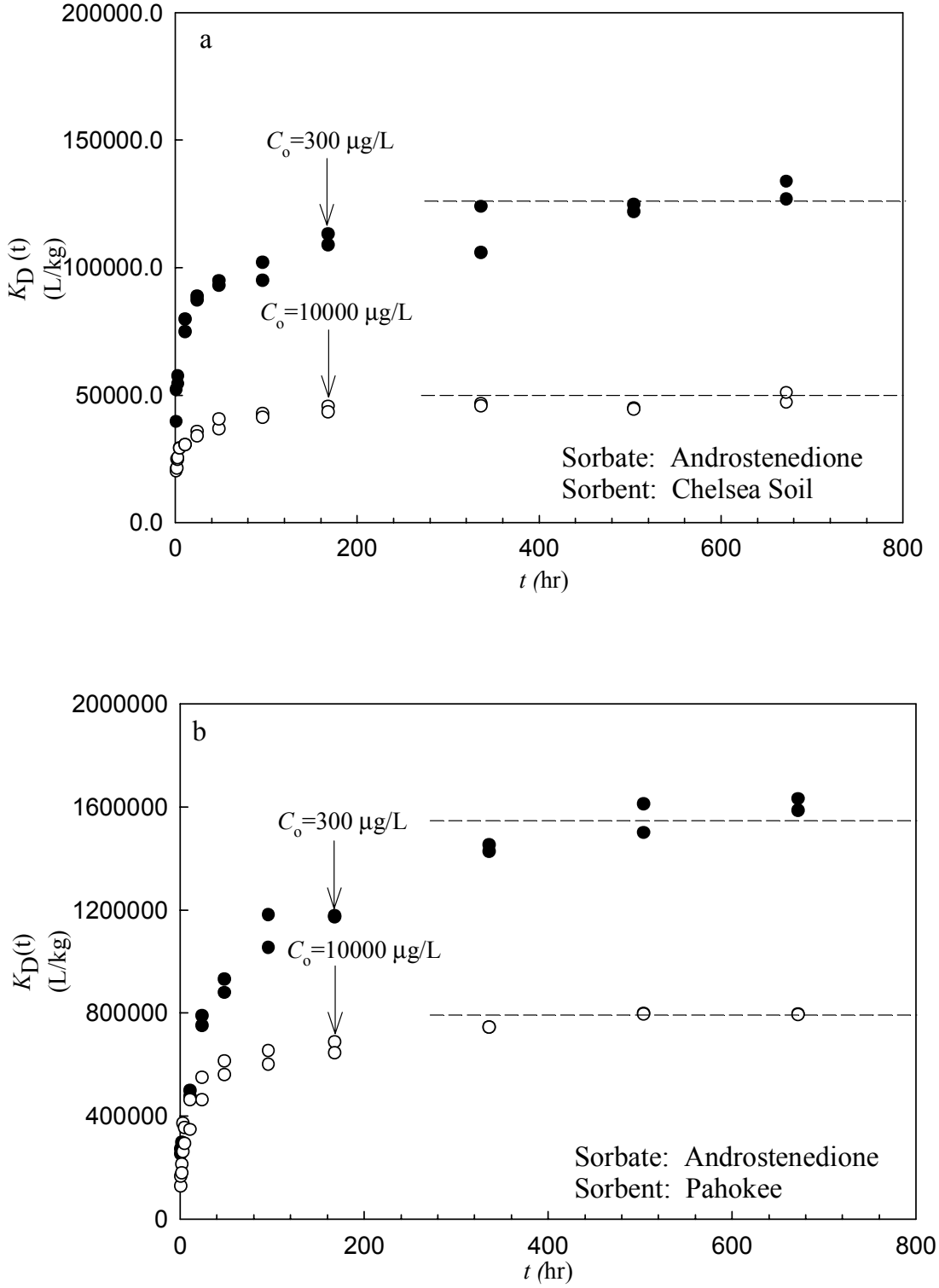


Figure 3.1 Sorption rates of AD by a) Chelsea soil and b) Pahokee Peat. Dashed line represents approximate sorption equilibrium.

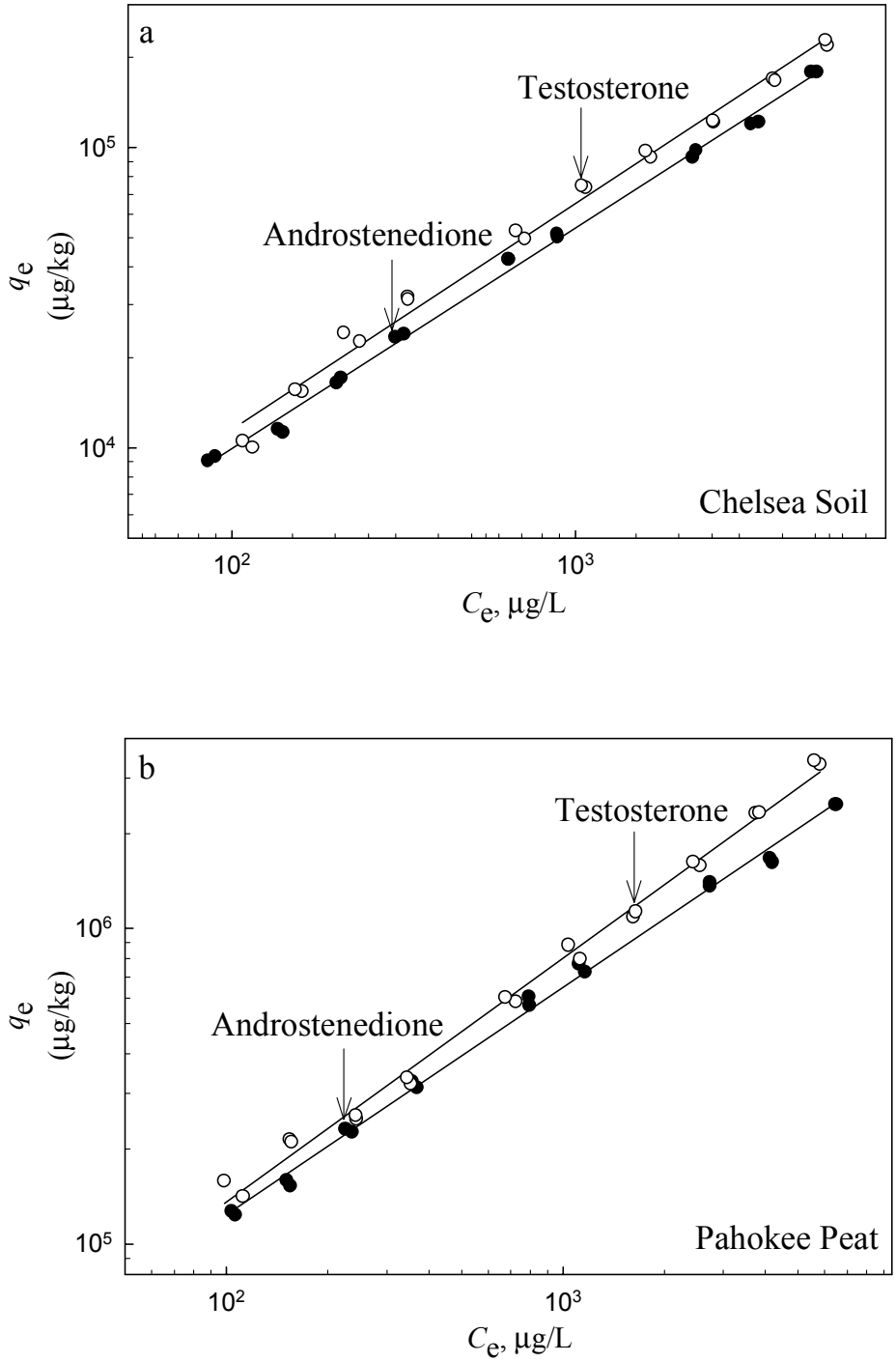


Figure 3.2. Sorption isotherms for AD and TT on a) Chelsea Soil and b) Pahokee Peat
(continued on next page)

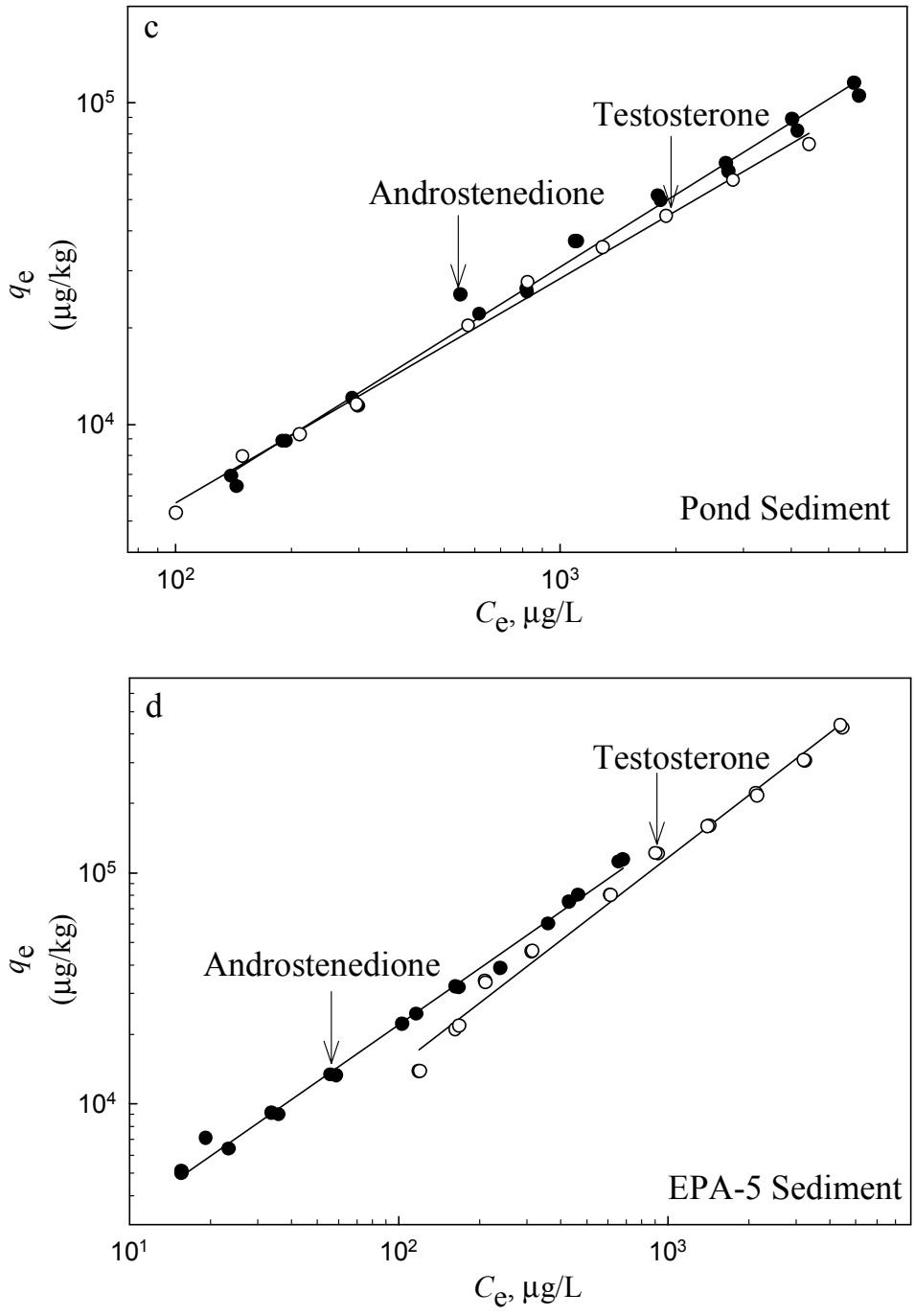


Figure 3.2 (contd) Sorption isotherms for AD and TT on c) Pond Sediment and d) EPA-5 Sediment

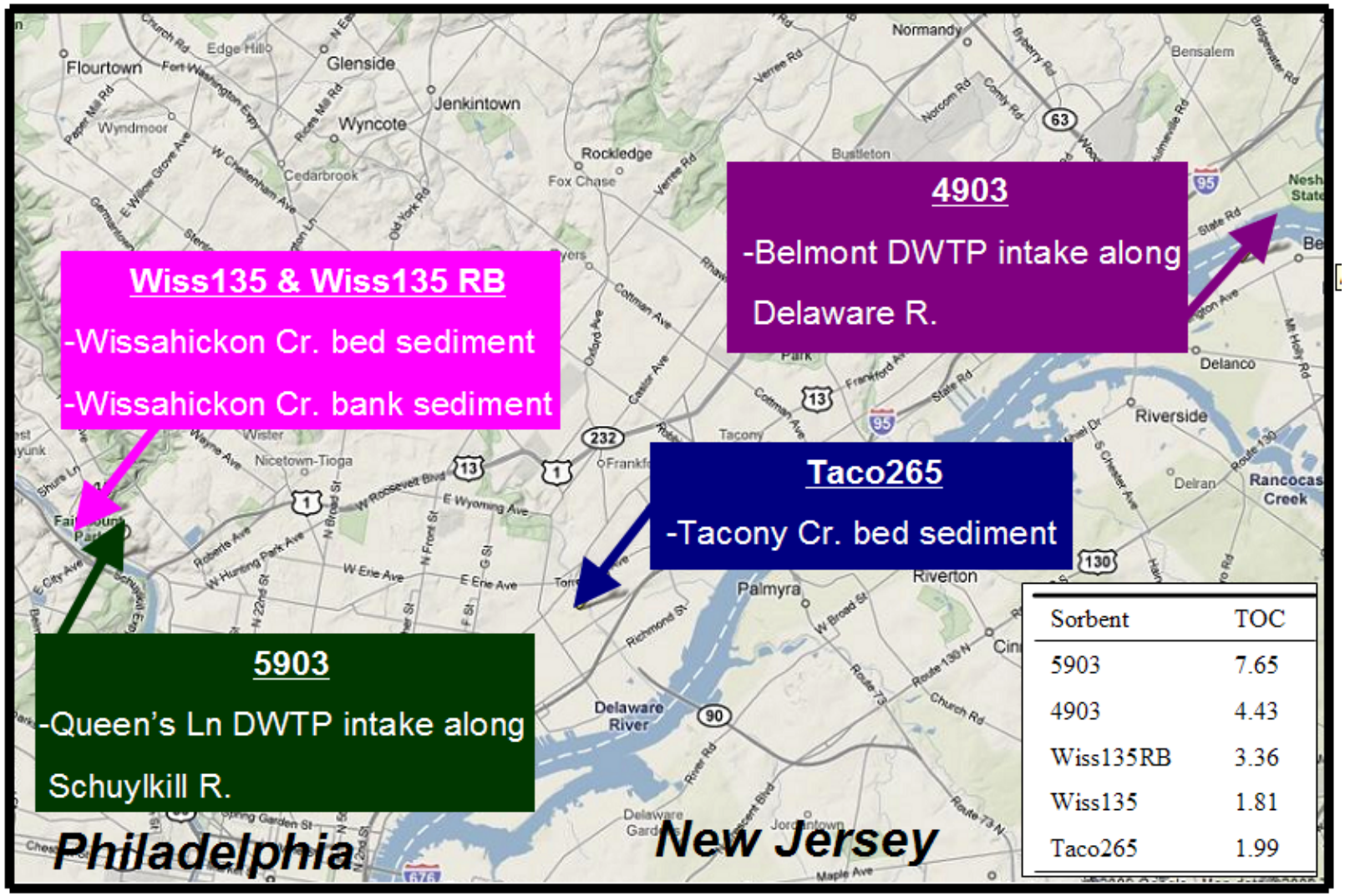
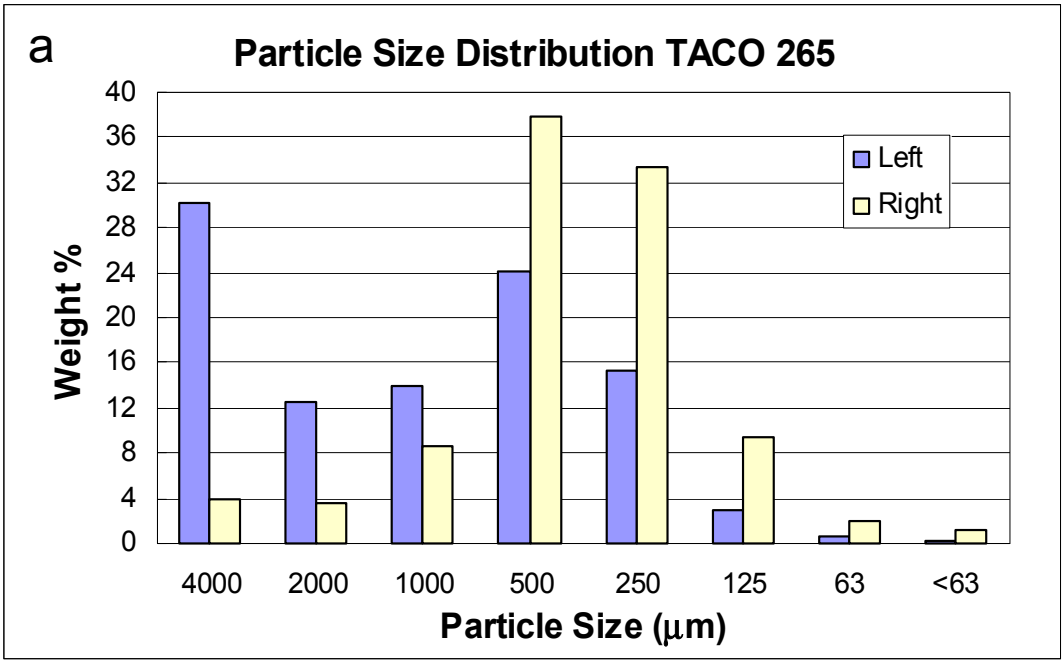


Figure 3.3 Sampling location map of Philadelphia area river sediments



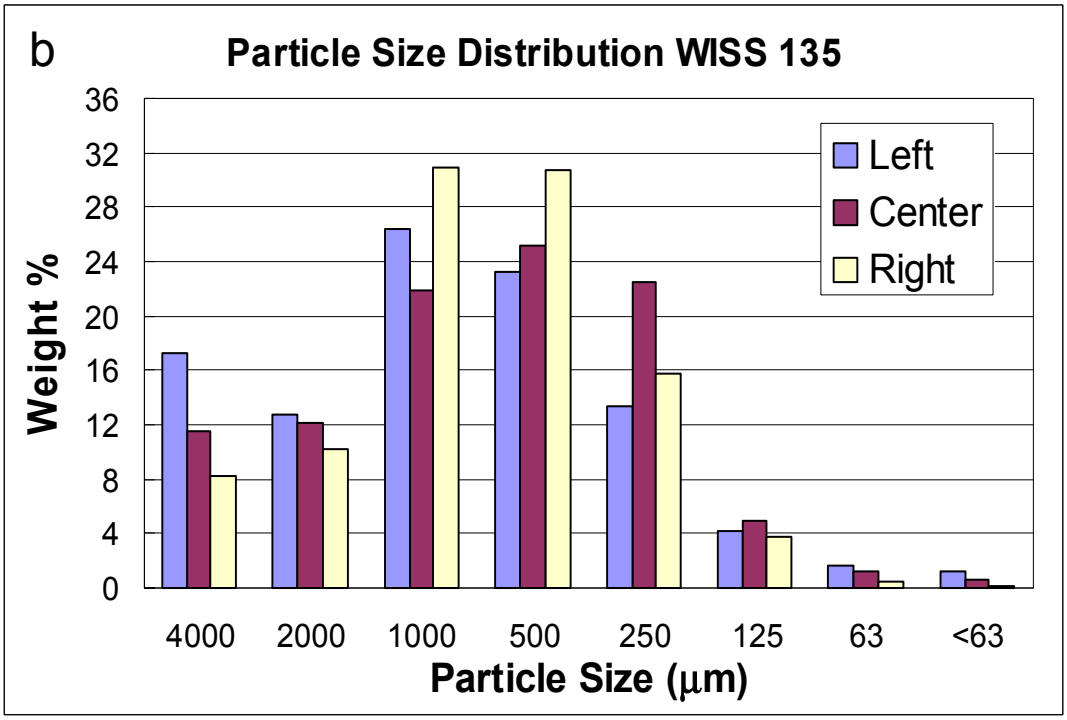
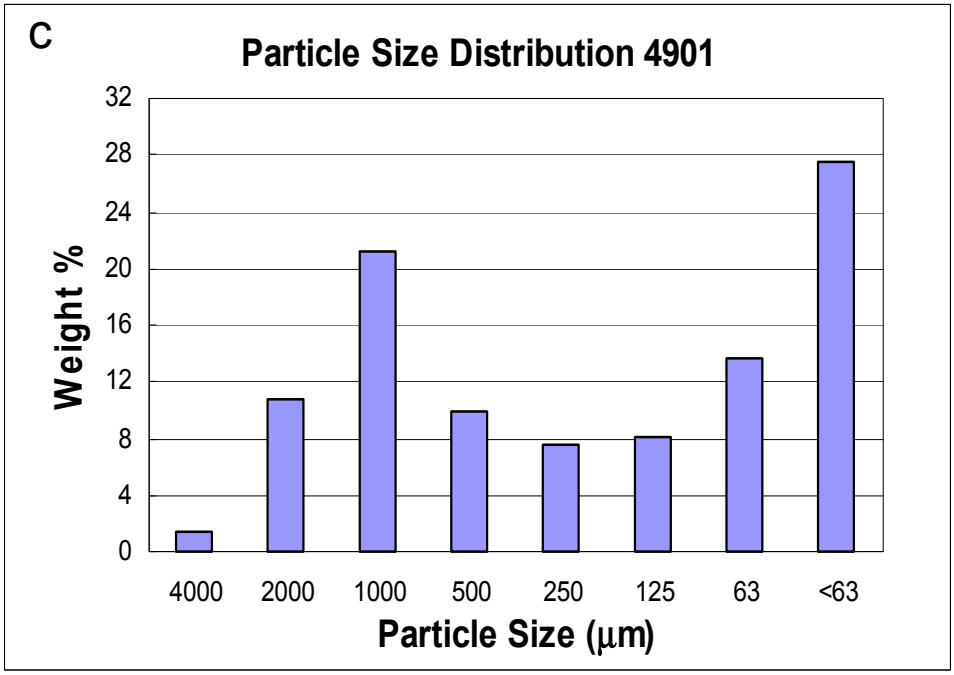


Figure 3.4 Particle size distribution of Philadelphia area sediments a) Taco and b) Wiss135. Left, Center, and Right denotes sample location facing downstream.
(continued on next page)



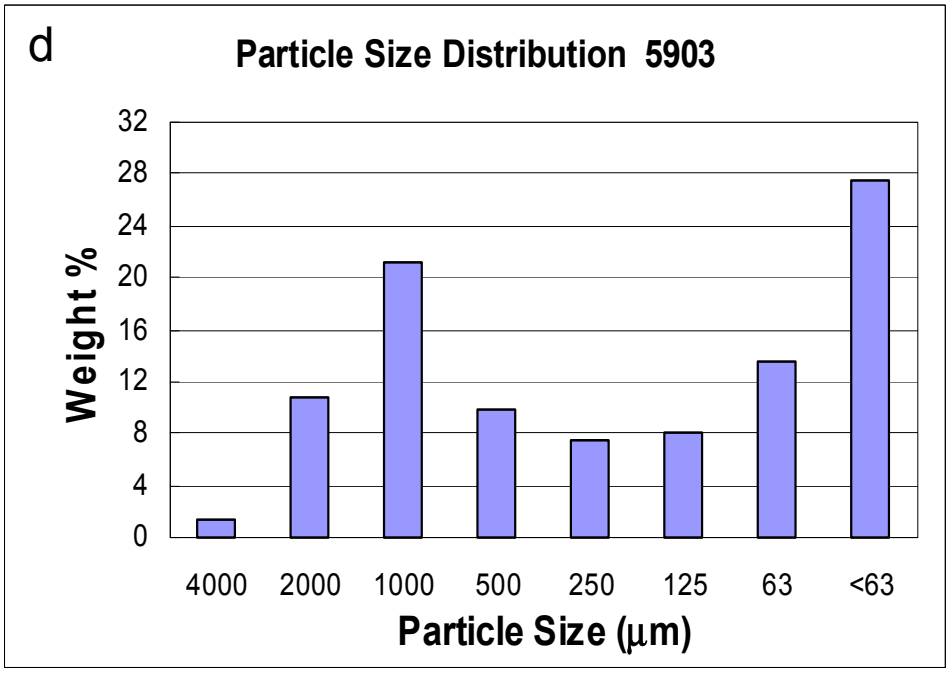
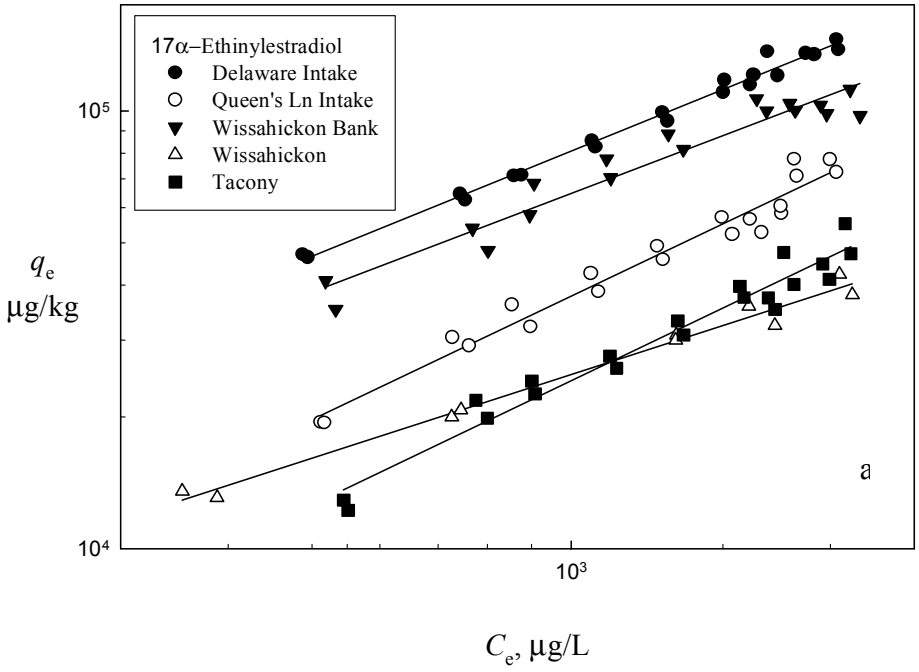


Figure 3.4 (cont.) Particle size distribution of Philadelphia area sediments c) 4901 and d) 5903



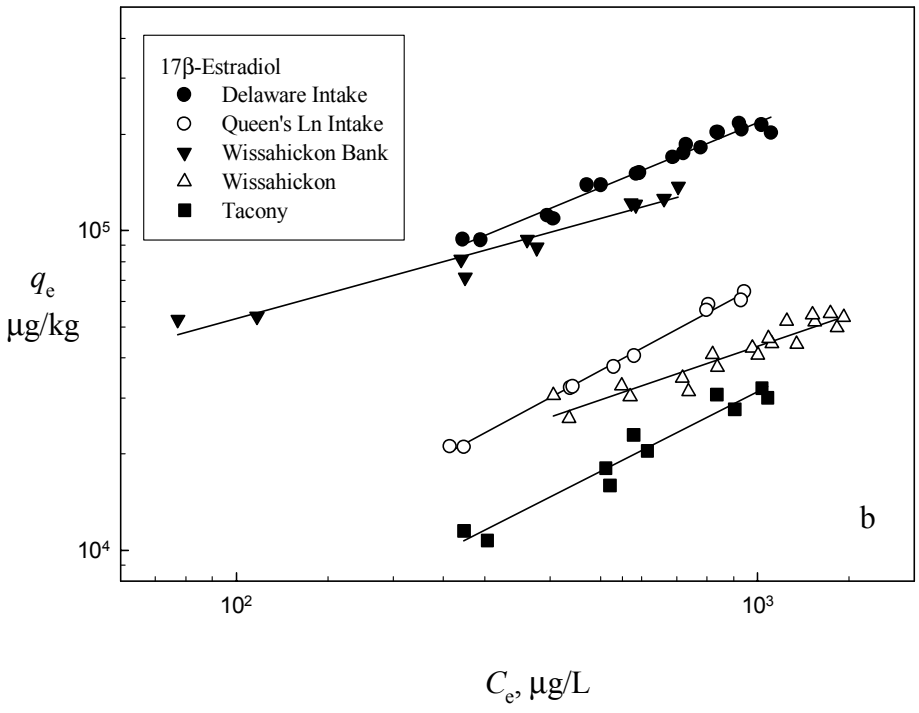


Figure 3.5 Sorption isotherms for 4903, 5903, WissRB, Wiss135, and Taco265 with a) EE2 and b) E2 (continued on next page)

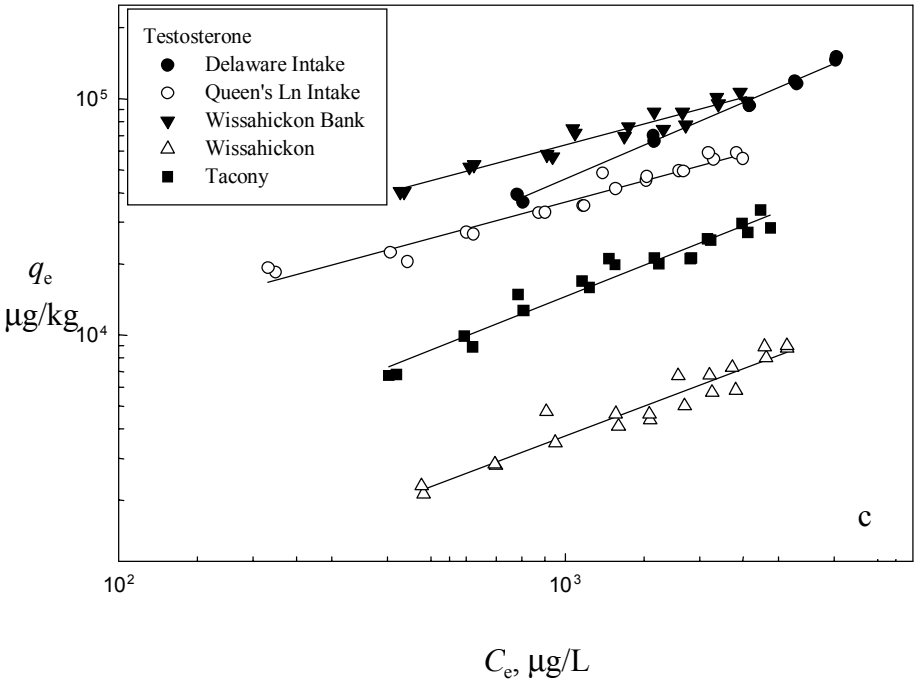


Figure 3.5 (cont.) Sorption isotherms for 4903, 5903, WissRB, Wiss135, and Taco265 with c) TT

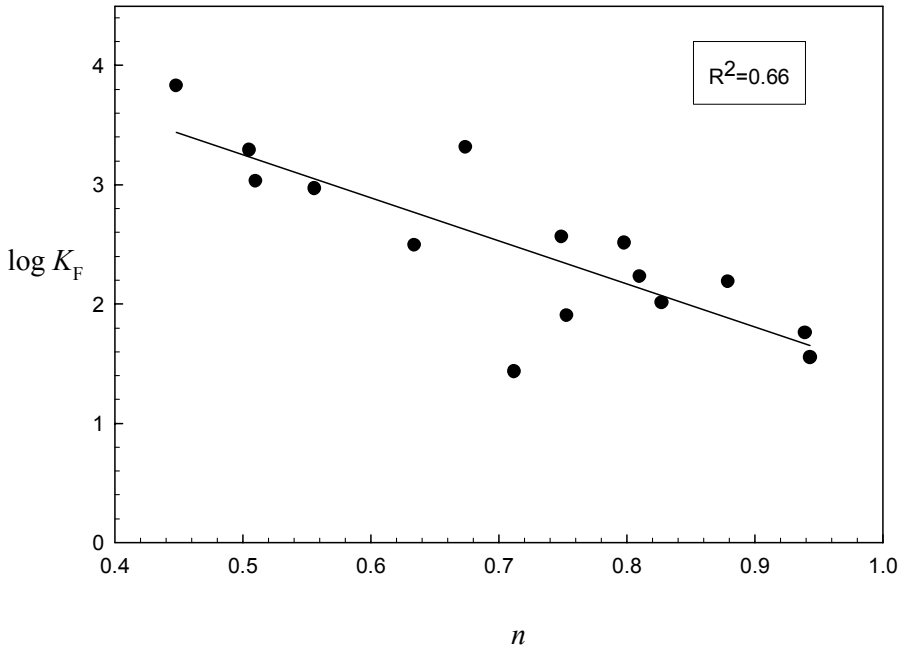


Figure 3.6. Log K_F versus n for all Philadelphia sorption isotherms

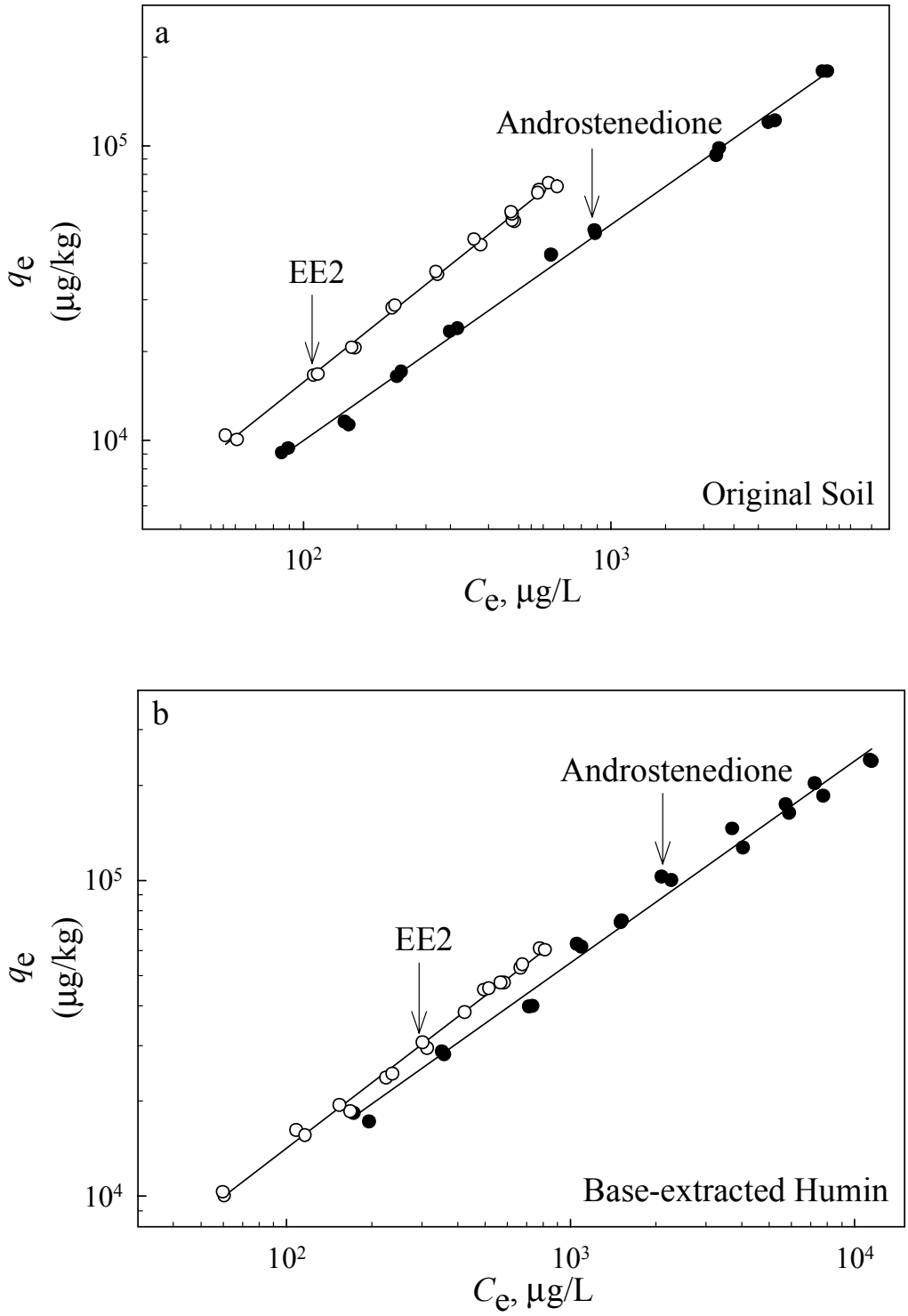


Figure 3.7 Sorption isotherms for AD and EE2 on a) Original Soil and b) HM (continued on next page)

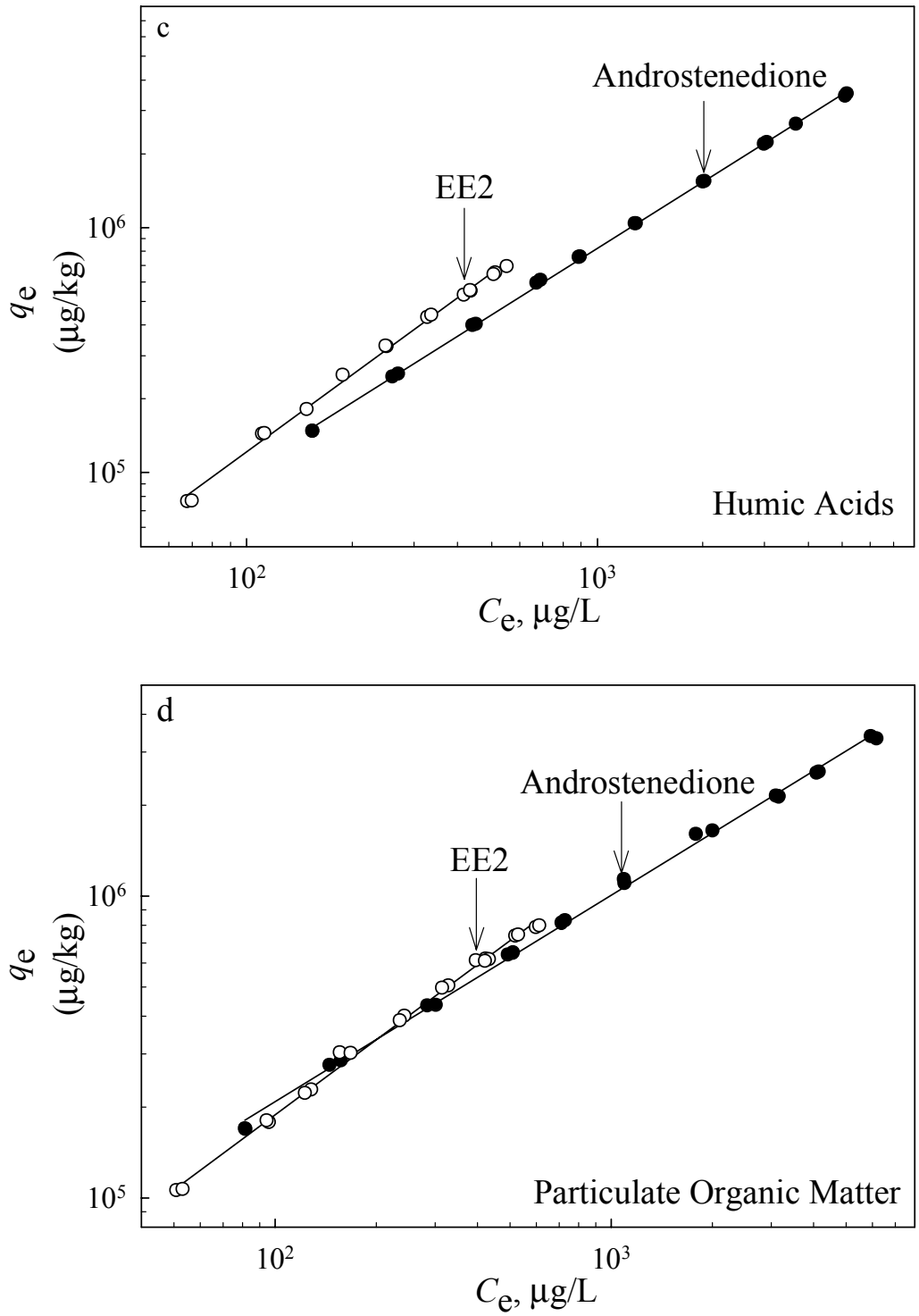


Figure 3.7 (cont.) Sorption isotherms for AD and EE2 on c) HA and d) POM

CHAPTER 4 – BIOTRANSFORMATION

Overview

The chapter is focused on the biotransformation of E2, EE2, and TT in five Philadelphia area river sediments, WissRB, Wiss135, Taco, 4901, and 5903. The hormones and sediments are identical to those examined in section 3.2, which deals with their sorption. The biotransformation information obtained from the research in this chapter augments the previously discussed sorption data and provides a second dimension to the fate of hormones in the river sediments in the Philadelphia area.

4.1 Rationale and Objectives

The purpose of this study was to gain a general sense about the biotransformation potential of hormones in river sediments near urban sites in the Philadelphia area. In addition, and most importantly, the biotransformation rate data obtained from the experiments were compared directly to their corresponding sorption equilibria data obtained in an earlier study (discussed in Chapter 3.2) using sediment obtained from the same sites and using the same selection of compounds (TT, E2, and EE2). It was anticipated that compounds with the lowest potential to sorb to the solid phase would be most bioavailable to microorganisms in the water-soil system and thus, have the shortest half-lives.

4.2 Chemicals

TT, E2, and EE2 were selected to compare the biotransformation characteristics of a male, female, and synthetic female hormone. As mentioned earlier (Chapter 3), all

three chemicals were purchased from Sigma-Aldrich (St. Louis, MO, USA), and used as received. Molecular structures and major physicochemical properties of the two steroid hormones are summarized in Table 3.1.

4.3 Sediments

4.3.1 Location

See section 3.2.2.1 for details on the sources of sediment samples that were collected from four locations in the Philadelphia area. Briefly, sediment was collected from the intake of the Belmont Drinking Water Treatment Plant (DWTP) (4901) along the Delaware River, from near the intake of the Queen's Lane DWTP along the Schuylkill River (5903), from Tacony Creek (Taco), and from the Wissahickon Creek (Wiss135). A single river bank sediment (WissRB) was collected right from the edge of the shore adjacent to the Wissahickon Creek river sediment sample for comparison purposes. The locations of all sites can be found on Figure 3.3.

4.3.2 Collection and Preparation

Sediments were collected from the top 10 cm of the river bed and placed in glass jars. Upon arrival back to the laboratory, the jars were kept under refrigeration until the drying stage. Unlike the sediments collected from the sorption study in Chapter 3, a portion of the sediment sample was set aside and spread evenly over aluminum foil and air dried for over one week prior to being disaggregated and sieved down to 1 mm for experimental use. Air drying was expected to better maintain the microbial communities than freeze-drying of the sediment samples.

4.3.3 Sediment Characteristics

See section 3.2.2.3 for the TOC and PSD information of these river sediments. While the TOC and PSD analyses for these river sediments were performed on the freeze-dried portion of the samples, it was assumed that the results would be the same for the air-dried portion of the sample utilized in this study.

4.4 Experiments

4.4.1 Reactor Setup

A total of 15 systems were set up for the 5 sorbents and 3 hormones utilized in this study. As mentioned earlier these are the same sorbents and sorbates used in the aforementioned section about the sorption of hormones to river sediments. However, in this case, the sediments were not freeze-dried, but rather air dried. Experiments were conducted in 20 mL glass vials with Teflon-lined lids. Preliminary biotransformation work was conducted to assess the time scale and logistics of the biotransformation experiments and incubation periods of 8-14 days were used depending on the system and how recalcitrant the compound was. Due to the large number of reactors operating simultaneously, constant introduction/measurement of oxygen was infeasible. Thus, the reactors were initially aerobic, but it remains uncertain whether or not aerobic conditions were maintained over the course of the incubation period. Microbial community studies were not carried out, so accurate oxygen demand calculations for the system were not possible.

The aqueous solution was comprised of 1 mg/L hormone (in all cases), 0.005M $CaCl_2$, 5 mg/L $NaHCO_3$, and 100 mg/L NaN_3 or $HgCl_2$ for biological control in the control reactors. It was found that analytical complications occurred for EE2 when

$HgCl_2$ was used as a biocide, so while E2 and TT used $HgCl_2$, EE2 used NaN_3. One gram of air-dried mass of sediment/soil was used in all vials for all systems.

Duplicate reactors were employed for each point as well as duplicate control reactors. In addition to biocide, the control soil-containing vials were autoclaved three times on three separate days before being filled with the reaction solution. After the reactors were filled with 20 ml of solution, they were placed horizontally on a shaker at room temperature and at 125 rpm.

4.4.2 Liquid and Solid Phase Extraction

At predetermined intervals, one set of duplicate reactors and controls was centrifuged. Afterwards, both the liquid phase and solid phase underwent liquid-liquid extraction. First 10 mL of the supernatant was extracted with 5 mL of DCM. The rest of the supernatant was carefully removed from the reactor and replaced with 8 mL of DCM. The remaining soil pellet was extracted for 2 days with the DCM. At the end of the experiment, the DCM from both the liquid-liquid extraction aliquot and the solid phase extraction were evaporated. The collection vials were then refilled with 1-2 ml of methanol to resuspend the hormones for HPLC analysis.

4.5 Chemical Analysis

The chemical analysis of E2, TT, and EE2 has been discussed in detail in section 3.2.4. The HPLC method parameters remained the same with the exception of the ratio of ACN/H_2O. Due to the biotransformation products present in the samples, an adjustment of approximately 5% was made to the solvent ratios for each of the methods

to enhance separation of the parent peak. The new longer retention times for E2, TT, and EE2 were 4.0, 5.5, and 3.4 minutes, respectively.

4.6 Results and Discussion

4.6.1 Half-lives

Half-lives for all systems were determined both graphically and numerically using pseudo first order kinetics. The half-lives obtained from the latter were compared to the former in order to get a general sense of the nature of biotransformation kinetics order. See Table 4.2 for a comparison of the graphically determined half-lives versus the half-lives determined by plotting the data based on pseudo first-order kinetics (using Equation 3). The graphically estimation of half-life was not based on the *k* value, rather it based upon visual inspection of the plotted data. It was apparent that obtaining half-life values from the two methods did not yield identical results in most cases. While the graphical and numerical half-life for WissRB with E2 were identical, the calculated pseudo first order rate constants led to half-lives that differed from the graphically values by a factor of 12-59% for the other systems. A reason for this may be due to insufficient data points to properly model the systems. Due to these modeling issues, the graphical determinations of half-life for all ten systems had precedence over the numerically derived half-life values derived from the linear plots.

The half life of E2 ranged from 12.5 to 73 hrs while the half life of TT ranged from 3.5 to 13 hrs. Thus, TT biotransformed at a faster rate compared to the female hormone. The half-live values obtained from modeling reflected faster TT biotransformation as well despite the time underestimations. See Figures 4.1, 4.2 and 4.3

for the biotransformation of E2, TT, and EE2 hormone over time and Table 4.1 for a summary of the half-life data of E2 and TT (absence of EE2 half-life data is explained below).

The synthetic female hormone EE2 was examined with the same suite of soils/sediments, but the incubation time was not long enough to obtain data beyond the lag period. Preliminary experiments suggested that incubation times between 213 and 310 hrs would be ample for EE2 biotransformation, but this was not the case. No biotransformation was witnessed under the incubation times used in this experiment (up to 310 hours).

4.6.2 Lag Period

Lag times were observed in four out of the five soils. The exception was WissRB, which displayed obvious biotransformation from the second data point. The systems that displayed the longest lag time (aside from all EE2 systems) was E2 with Wiss135 and TT with Wiss135 (48 and 99 hours, respectively). Due to the lag times for four out of five soils, "time zero" for the linear plot was taken to be from the point at which biotransformation began to occur, thus half life was corrected for this time differential in the data set.

4.6.3 Biotransformation Rates

The aqueous phase used in this study was neither nutrient enriched, nor did we utilize the corresponding river water obtained from the sediment sampling sites, which means the biotransformation potential of the soils/sediments were isolated. It also means that the experimental conditions used may potentially underestimate the

biotransformation kinetics of hormones in actual river sediments. Our 20:1 ratio of water to solids is not unrealistic given the fact that only the surface bed sediments (top 10 cm) were collected from the rivers.

With the exception of TT with Wiss135, the recovery of hormone from the control reactor decreased with time. Because the controls were autoclaved and the liquid phase contained biocide, the decrease in recovery as time progressed was most likely due to the inability of dichloromethane to extract E2 and TT sorbed deeply into the soil/sediment matrix. The system which experienced the most difficulty with extraction was E2 with the Delaware R. DWTP intake sediment (4903) while the system which presented no problems with hormone recovery was TT with Wiss135. The low organic content of Wiss135 combined with the fact that TT has a higher solubility compared to E2 is most likely the reason for its consistently high recovery over time in this particular system. Fan et al. (2007) also noticed incomplete extraction from their soils and performed post-incubation soil fractionation work. It was revealed that the non-extractable hormones after solvent extraction were mostly associated with humic substances. Further they noted that most of the non-extractable ^{14}C for E2 was associated with humic acids while TT was mostly associated with the humin fraction. This agrees with previous work (section 3.3) on the preferential sorption of hormones to different soil fractions. There was indeed a tendency for different hormones to sorb to certain soil fractions. This preference for various soil fractions may have an impact on bioavailability.

4.6.4 Sorption and Biotransformation

Prior work (section 3.2) studied the sorption of hormones to the same 5 Philadelphia soils/sediments used herein and an attempt was made to find a correlation between the biotransformation results of this study and the previous sorption work. No connection was found between Log K_F or n to biotransformation; however, in the case of Wiss135, the sample with the lowest organic carbon content, E2 and TT experienced the longest lag times. We were not the only ones to notice the connection between low organic carbon content and biotransformation time. In Lee et al.'s (2003) work, all the hormones took the longest to biotransform on the sediment with the highest sand and lowest organic carbon content.

4.6.5 Comparisons to other Studies

Limited studies have been conducted in order to evaluate the biotransformation of hormones in soils/sediments. The following is a comparison of our results to those obtained from other studies about male and female hormone biotransformation on various media.

A study evaluating the biotransformation of E2 and EE2 in marine sediment and seawater from South Australia was conducted by Ying et al. (2003). The biotransformation of the two hormones at a concentration of 1 μg/g were investigated in a mixture of marine sediment and seawater. It was found that E2 biotransformd faster than EE2 and that there was no lag period for either compound. Their seawater-marine sediment experiment yielded a E2 half-life of 4.4 days (first-order reaction kinetics), which was similar to our biotransformation on W135L1, while their EE2 had a half-life

greater than 20 days (greater than the incubation time allowed for in this study, which subsequently did not yield any biotransformation).

In addition to marine and river environments, the fate and transport of hormones in aquifer environments is important as well. Ying et al. (2003) evaluated E2 and EE2 in aquifer material and native groundwater from South Australia at a concentration of 1 μg/g. They found that E2 biotransformed very quickly whereas the synthetic female hormone biotransformed very slowly. They determined that the half-life for E2 was 2 days and for EE2 it was 81 days. The organic carbon content of the aquifer material was 0.5%, so although their OC content was much less than the Philadelphia river sediments/soils, their biotransformation rates were still comparable to the ones obtained in this study.

Lucas et al. (2006) determined the biotransformation potential of E2 (C_0 = 0.81 μg/g) on soils obtained from temperate oceanic agricultural grasslands in Wales. Experiments were done on both soil-solvent (water and urine) and soil-manure mixtures. The half-lives of E2 for various soil-solvent were predictably longer than in the soil-manure mixtures. The half-lives for E2 in their soil-manure mixtures ranged from 1.3 to 8.0 days. It is interesting to see that our systems with non-nutrient enriched water with plain sediment exhibited half-lives that were similar to if not faster than Lucas' manure-enriched soil systems.

Not many biotransformation studies have been done to compare male hormones against female hormones. Stumpe et al. (2007) realized, in a study dealing with agricultural top soils comparing mineralization of TT and E2, that although the compounds are structurally similar, the mineralization rate of the female hormone was

approximately an order of magnitude less than the male hormone in all soils. While there was not an order of magnitude difference between removal of E2 and TT in our study, TT was similarly removed at a consistently faster rate than the female compound.

In another study (Lee et al., 2003) utilizing soils and sediment, the range of half-lives of E2 and TT on various soils were 0.8-9.7 days and 0.3-7.3 days, respectively. As with Stumpe et al. (2007), their results agreed with ours - they too witnessed faster kinetics with TT. For Fan et al.'s (2007) study of TT and E2 in soil, they observed first-order mineralization rate constants of 0.012 h^{-1} and 0.0006 h^{-1}, respectively. In a packed soil column study, Das et al. (2004) observed TT biotransforming faster than E2 as well, once again supporting the idea that the male hormone is more prone to biotransformation than the natural female hormone.

There appears to be a consensus that TT is more readily biotransformed than E2. E2 has an aromatic A-ring whereas TT's A-ring only has a single double-bond, which might result in the latter being more prone to biotransformation by microbes.

Although this study did not notice any biotransformation at all of EE2 after 213 to 310 hrs, it was interesting to see that other authors found that the contraceptive could occasionally biotransform in a similar manner to E2. For instance Ying et al. (2003), in contrast to their aforementioned marine slurry work, also performed experiments with the hormones in only seawater, and noticed that E2 biotransformd at nearly the same rate as EE2. In addition, in the Lee et al. (2003) study, for one of their soils, the rate of biotransformation was similar for both E2 and the synthetic contraceptive. In fact, Lee et al. (2003) actually observed rather quick kinetics for EE2 – it biotransformed in various soils with half-lives of 3.1-9.6 days. Further, in another study by Colucci et al. (2001),

EE2 biotransformed rapidly in loam, sandy loam, and silt lam soils. It was still slower than their E2 biotransformation rates, but the rates (0.22-0.33 d^{-1} = half-lives of 3.2-2.1 days) were much faster than what were experienced in this work. Thus although many others (especially those studies dealing with WWTP sludge) seem to agree that EE2 is persistent compared to the natural hormones, it is clear that there are exceptions.

4.6.6 EE2 Persistence

While the fate of EE2 may vary in aerobic experiments using soil and sediments, aerobic studies using sludge from WWTPs show that this synthetic compound is generally the most persistent of the three compounds discussed here. For instance, a diluted slurry of activated sludge from a German sewage treatment plant was used to investigate the persistence of hormones under aerobic conditions (Ternes et al., 1999). They noticed that EE2 was usually persistent under their aerobic conditions while E2 was immediately removed at both 1 μg/mL and 1 ng/mL concentrations. Layton et al. (2000), during work involving biosolids, also observed the order of mineralization from slowest to fastest was EE2, E2, and TT, just as in our study. According to Vader et al. (2002) the biotransformation of EE2 was correlated with nitrifying activity. Nitrifying conditions were not created during this current study, so if EE2 is associated with nitrifying activity in a WWTP situation, then perhaps the fairly rapid biotransformation that other authors witnessed in experiments simulating natural environments were due to either cometabolism or abiotic activity.

Our studies did not use water that was nutrient enriched, which may lead to an underestimation of biotransformation potential. Despite this, we still experienced rapid biotransformation of hormones without the use of actual river water. However, in the

case of experiments carried out by other research groups, the contribution to biotransformation kinetics of sediment versus the native water varied. For instance, in Ying et al.'s (2003) report, the sediment's presence obviously played a strong role in the biotransformation of the two hormones given the faster rates in the seawater-marine sediment slurry compared to the seawater only experiment. In the seawater-only experiment both compounds experienced a fairly lengthy lag time of approximately 30 days. It should be noted that the marine sediment had 0.1% OC, so although the marine sediment had very low OC content, its presence in the seawater still managed to make a tremendous difference in the removal of hormones compared to the seawater only study. Due to this lag time in only seawater, their work may seem to suggest that the use of non-native water in the present study would not improve upon biotransformation rates for the Philadelphia sediments. However, it should be noted that marine environments do not receive the same volume of WWTP effluent as rivers, which might create an entirely different microbial environment.

Jürgens et al. (2002) studied E2 and EE2 in water collected from English rivers. They observed that E2 at concentrations of 100-500 µg/L could be biotransformd under aerobic conditions with half-lives between 0.2 to 9 days, and that even at much lower concentrations of 100 µg/L to 20-100 ng/L, the biotransformation rates were similar. In addition, they also found a half-life of EE2 to be 17d. It appears that the removal of hormones in only river water in Jurgens et al.'s study is much greater than that in the marine water used in Ying et al.'s (2003) study. Jurgens et al.'s river water results are comparable to the results found in this study using river sediment only, leading us to

believe that the already rapid kinetics witnessed for the Philadelphia sediments may be even greater when combined with native water.

4.6.7 River Bank Versus River Sediment

Two samples were collected from the Wissahickon Creek in order to compare the biotransformation potential in the sediment and a nearby riverbank sample. The difference between WissRB (river bank sample) and corresponding Wiss135 (river sediment sample at same location as WissRB) may have to do with pre-incubation issues. The direct river sediment sample (Wiss135) would likely have more contact with hormones that may be present in the river water compared to the adjacent surface river bank sample (WissRB), with the exception of during high flow events. One would assume that W135 would be more able to biotransform the hormones. According to Layton et al. (2000), in their studies with biosolids from municipal (which would receive more estrogens in their waste) and industrial plants, the difference in mineralization of E2 was 84% and 4%, respectively, which makes sense given the lack of human waste entering the industrial plants. However, as mentioned earlier, Wiss135 had either the longest half life or the longest lag time for our hormones, so other factors may be the reason why the WissRB soil was more adept at biotransforming the hormones. For instance, the very low OC content combined with the coarse texture (based on particle size distribution) of the Wiss135 sample may have offset any potential hormone-specific microbial advantage it may have had over the WissRB sample that had a finer texture. Further, A sediment that undergoes many dry/wet cycles (WissRB) is likely to have a

more diverse microbial population, and therefore more likely to contain at least one strain that can biotransform the hormone.

Others have made observations along the same lines. According to Stumpe et al. (2007), and in contrast to Layton et al. (2000), they observed that long-term application of sewage sludge to one of their soils had no effects on hormone mineralization despite the higher OC content. They also noticed that pre-incubating the soils with unlabeled hormones or the application of hormones within a wastewater matrix only had minor effects on rates of mineralization. Thus nature of pre-incubation in the natural environment versus WWTPs may vary greatly and may not even apply to the Philadelphia soils/sediments collected for this study.

4.6.8 Potential Abiotic Reactions

Due to the aromatic nature of one of the hormones, E2, the potential for abiotic transformation must be addressed and discussed. The thin-layer chromatography (TLC) work of Fan et al. (2007) on the autoclaved soil suggests that the TT mineralization was biological. On the other hand, TLC work on autoclaved soil with E2 showed that an unidentified polar compound was found to be the female hormone's major metabolite in autoclaved soil under anaerobic conditions. In the end their work suggested that E2 could be transformed with processes that do not involve microbial activities and that TT can only be transformed by microbial activities. This means that what we had perceived as incomplete recovery from the control samples in our E2 study could possibly be abiotic transformation. In addition, Colucci et al. (2001) also conjectured that abiotic transformation could be playing a role in the disappearance of hormones. Colucci et al. (2001) observed that E2 was oxidized to E1 in both autoclaved and nonsterile loam, silt

loam, and sandy loam soils. This suggested abiotic transformations. According to Stevenson et al. (1996), the hydroxyl functional groups could potentially react with phenolic compounds.

4.7 Summary

The natural hormones E2 and TT biotransformed extremely rapidly in the presence of river sediment, while EE2 was relatively recalcitrant. As mentioned earlier, EE2 has the strongest potency of the female hormones studied here (Sumpter et al., 2005) so its persistence in river sediments may be problematic, especially for the DWTPs whose inlet river sediment samples were employed in this study. According to Kuch et al. (2001), hormones have actually been found in drinking water. The rapid biotransformation of the two natural hormones is good news; however, the biotransformation of the daughter products of these two parent compounds needs to be addressed as well. Finally, sediments with low organic carbon content may lead to long lag times for hormone biotransformation.

Table 4.1 Summary of half life data for E2 and TT

Sediment	E2 $t_{1/2}$ (h)	TT $t_{1/2}$ (h)
WissRB	14	13
5903	12.5	6.5
4901	19	3.5
Taco	32.5	13
Wiss135	73	8

Table 4.2 Comparison of half lives obtained graphically and numerically

	Graphically	1st Order	R^2
E2			
WissRB	14	14	0.96
5903 (Queen's Ln)	12.5	18	0.96
4901 (Belmont)	19	14	0.94
Taco	32.5	16	0.84
Wiss135	73	50	0.91
TT			
WissRB	13	11	0.87
5903 (Queen's Ln)	6.5	11	0.98
4901 (Belmont)	3.5	8	0.95
Taco	13	8	0.86
Wiss135	8	3	1.00

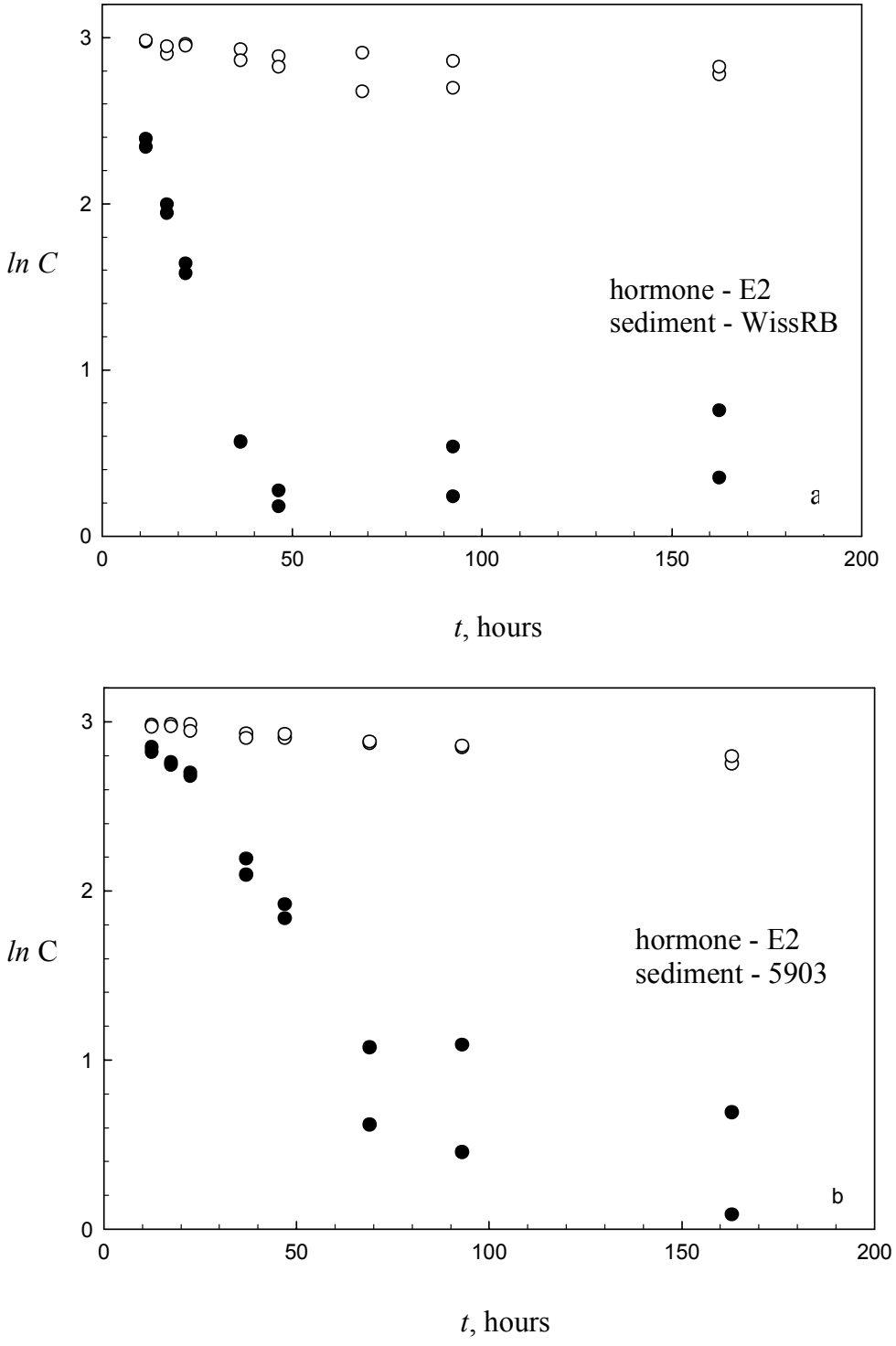


Figure 4.1 Biotransformation of E2 by a) WissRB and b) 5903. Open circles are killed controls. (continued on next page)

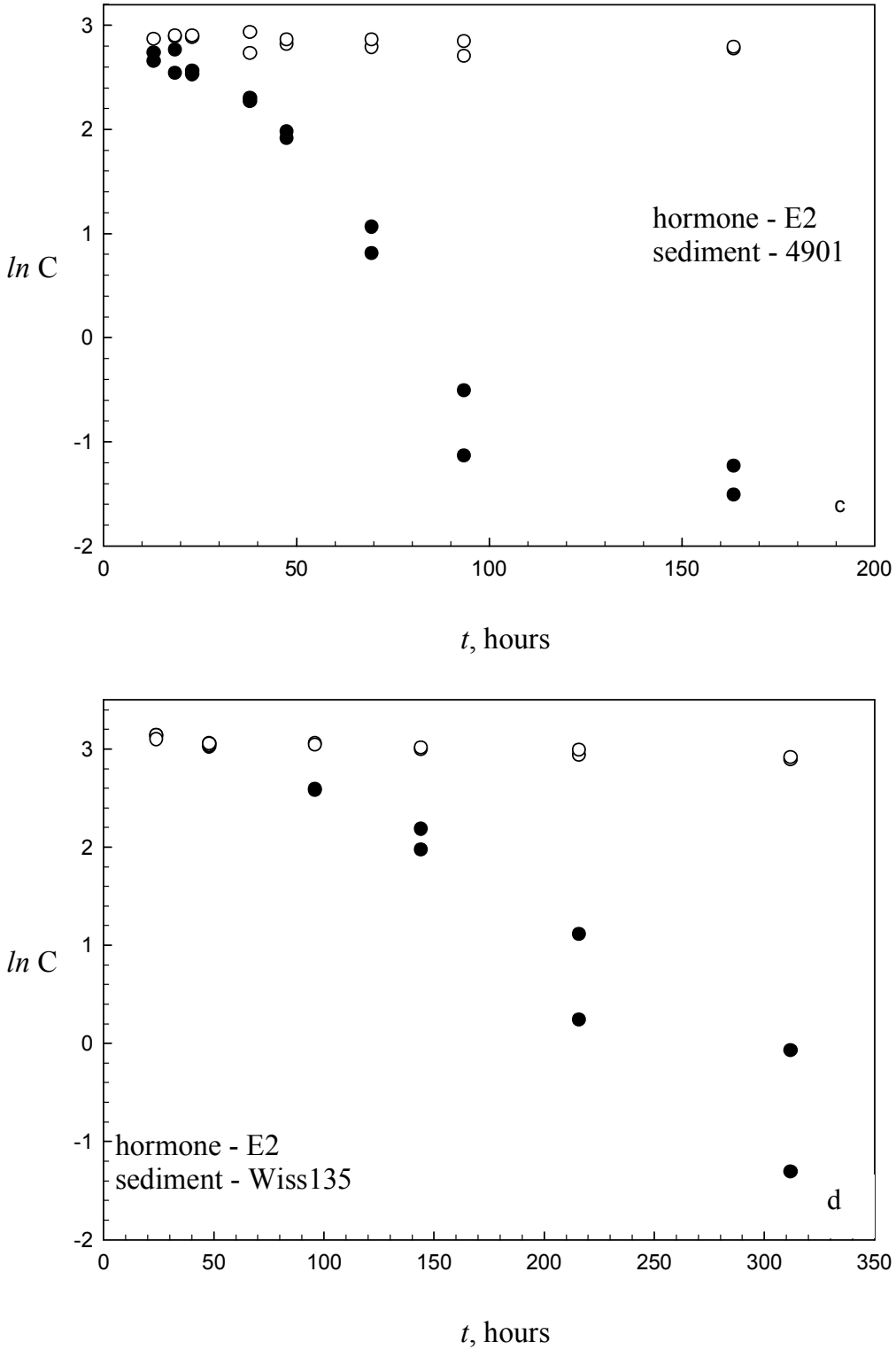


Figure 4.1 (cont.) Biotransformation of E2 by c) 4901 and d) Wiss135. Open circles are killed controls. (continued on next page)

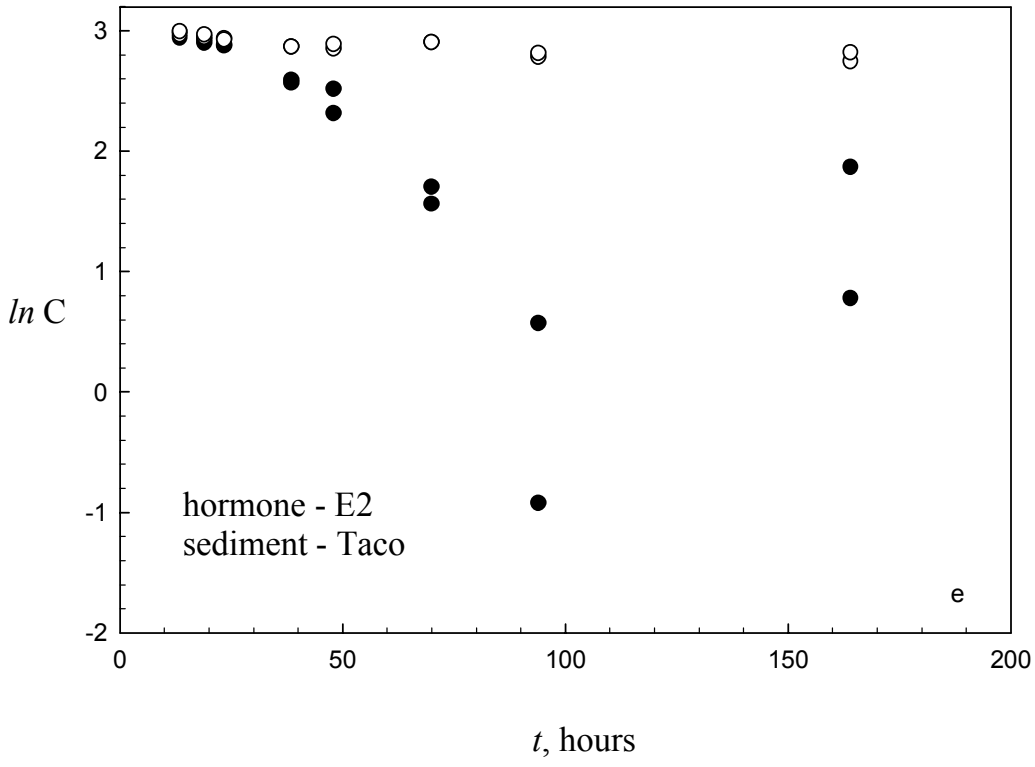


Figure 4.1 (cont.) Biotransformation of E2 by e) Taco. Open circles are killed controls.

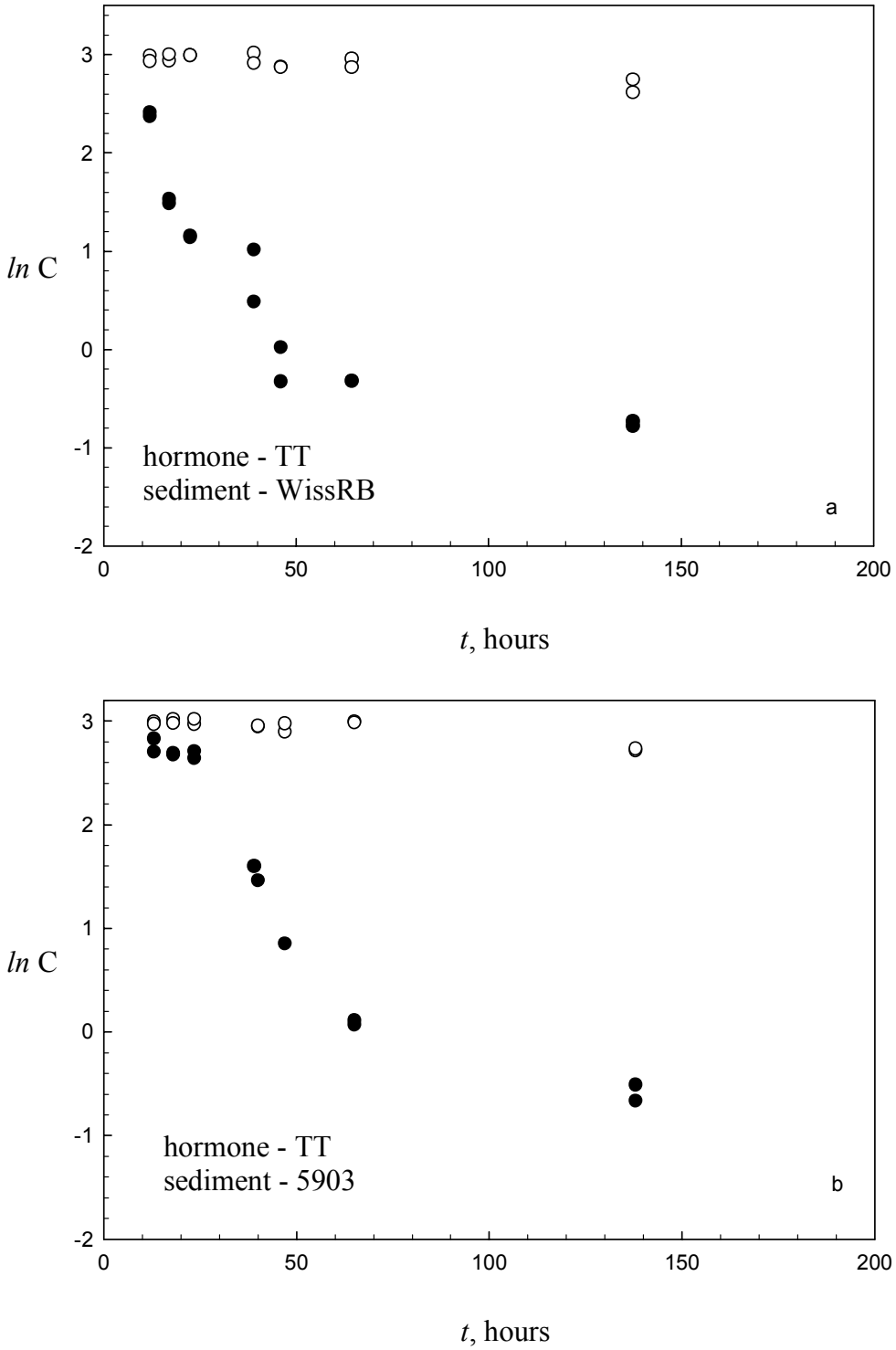
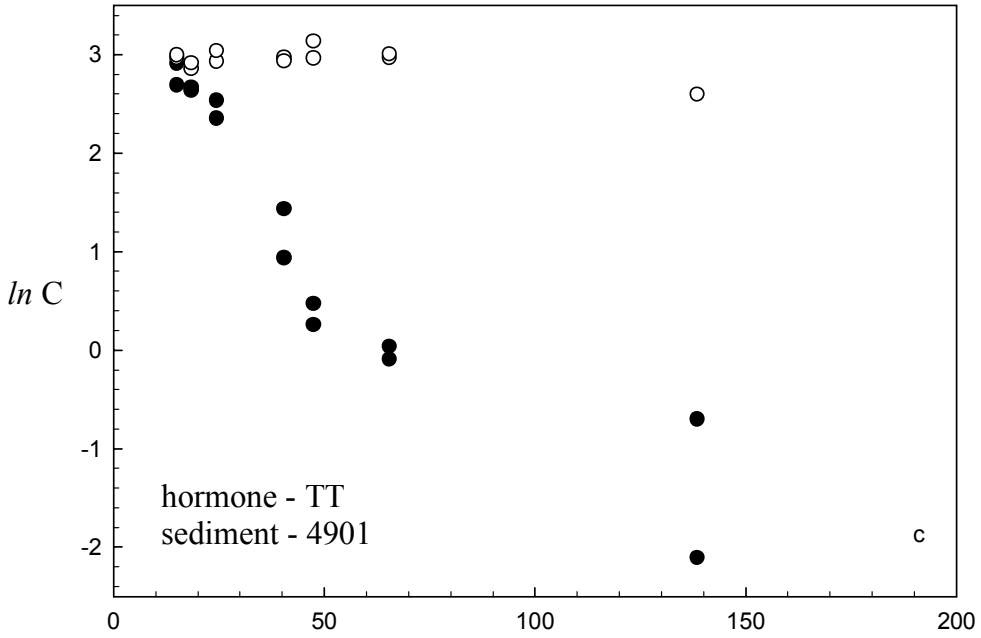


Figure 4.2 Biotransformation of TT by a) WissRB and b) 5903. Open circles are killed controls. (continued on next page)



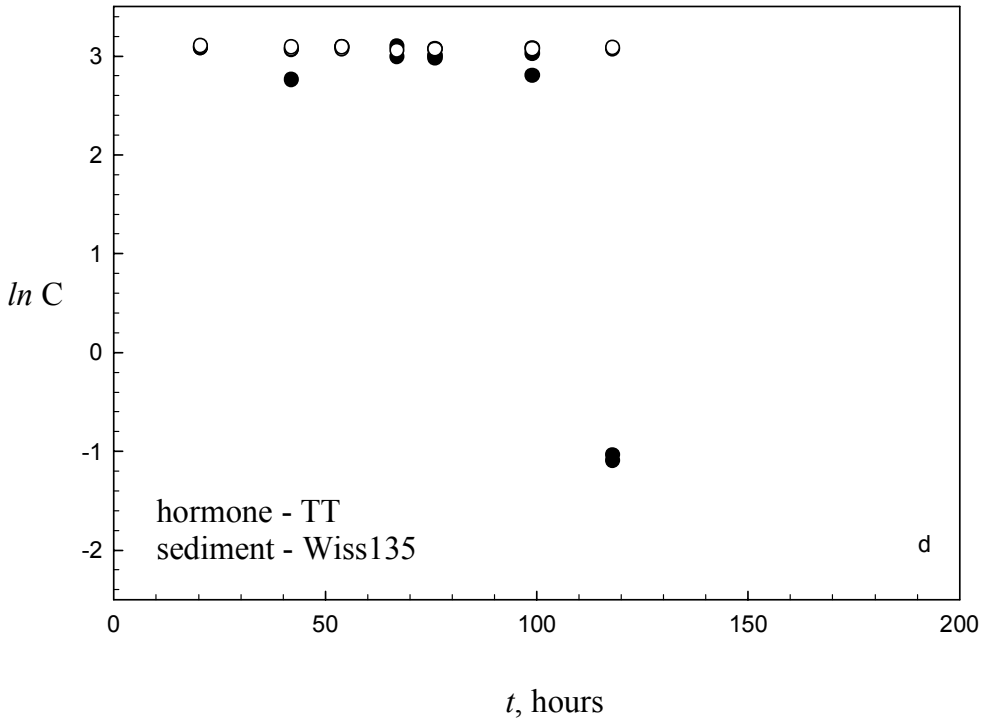


Figure 4.2 (cont.) Biotransformation of TT by c) 4901 and d) Wiss135. Open circles are killed controls. (continued on next page)

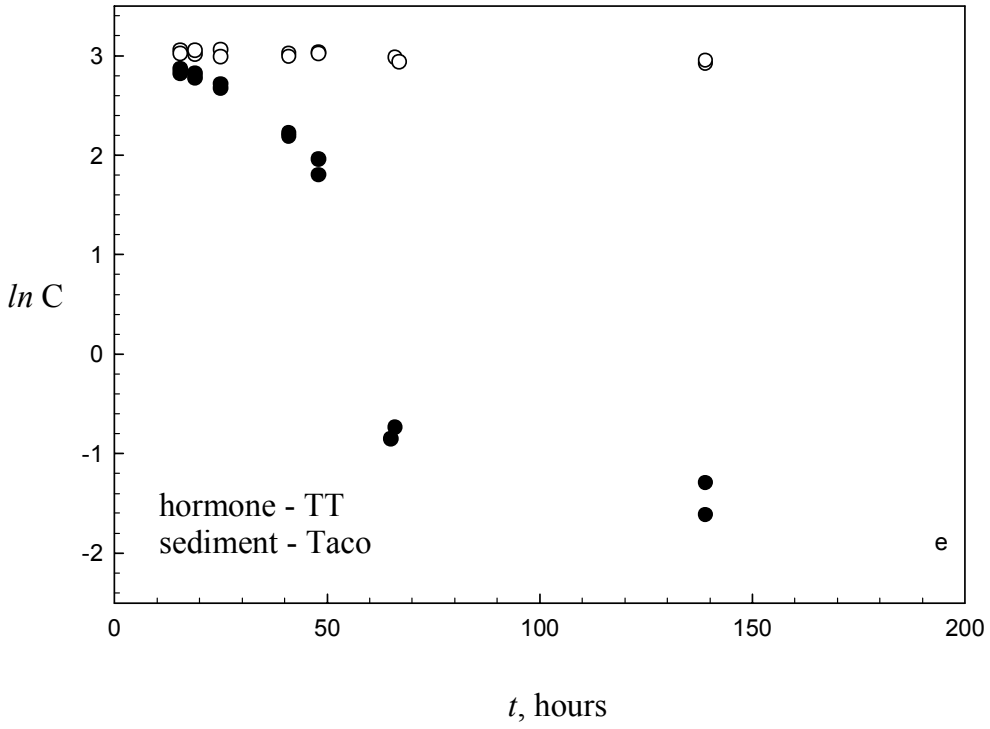


Figure 4.2 (cont.) Biotransformation of TT by e) Taco. Open circles are killed controls.

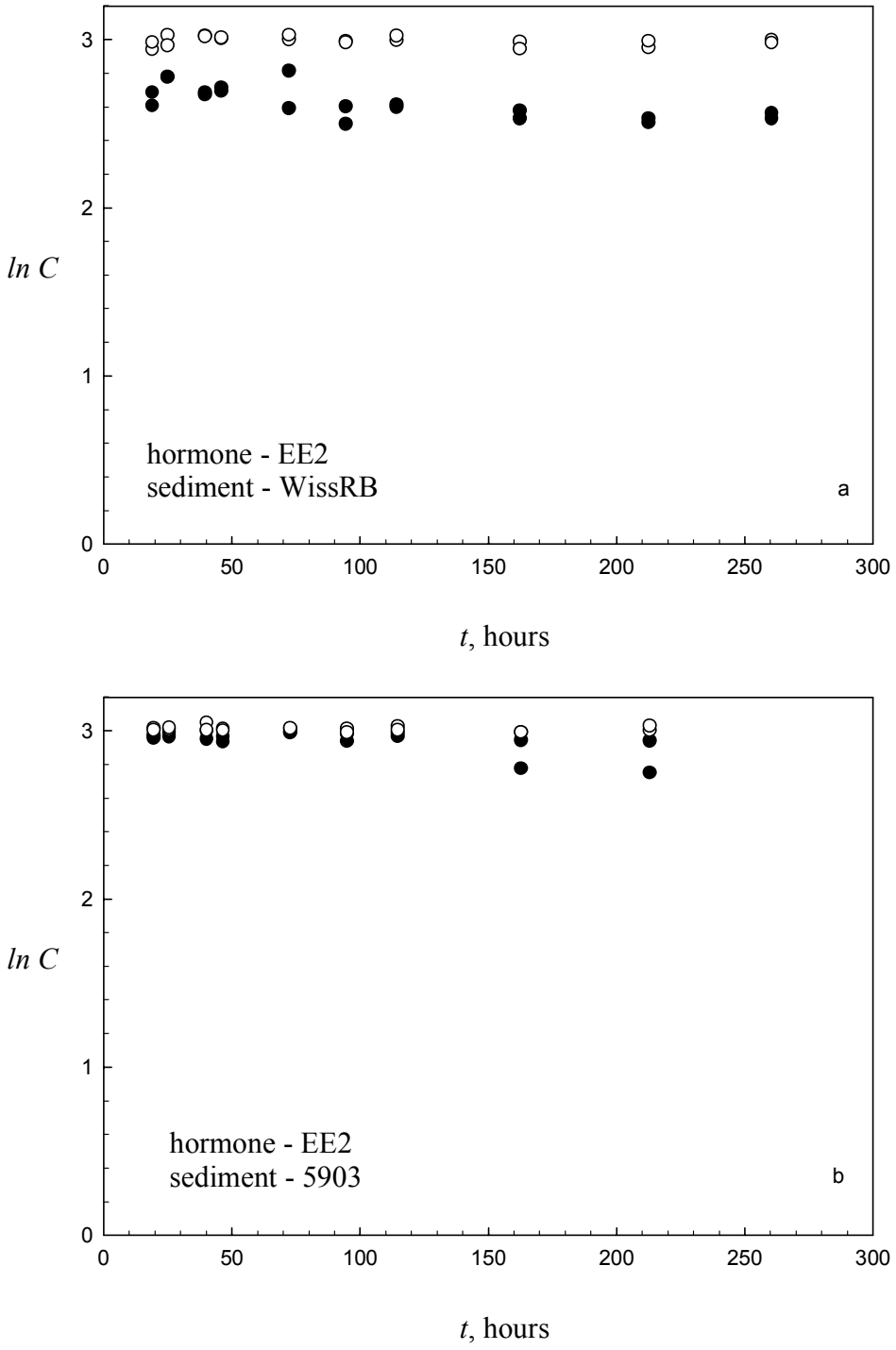
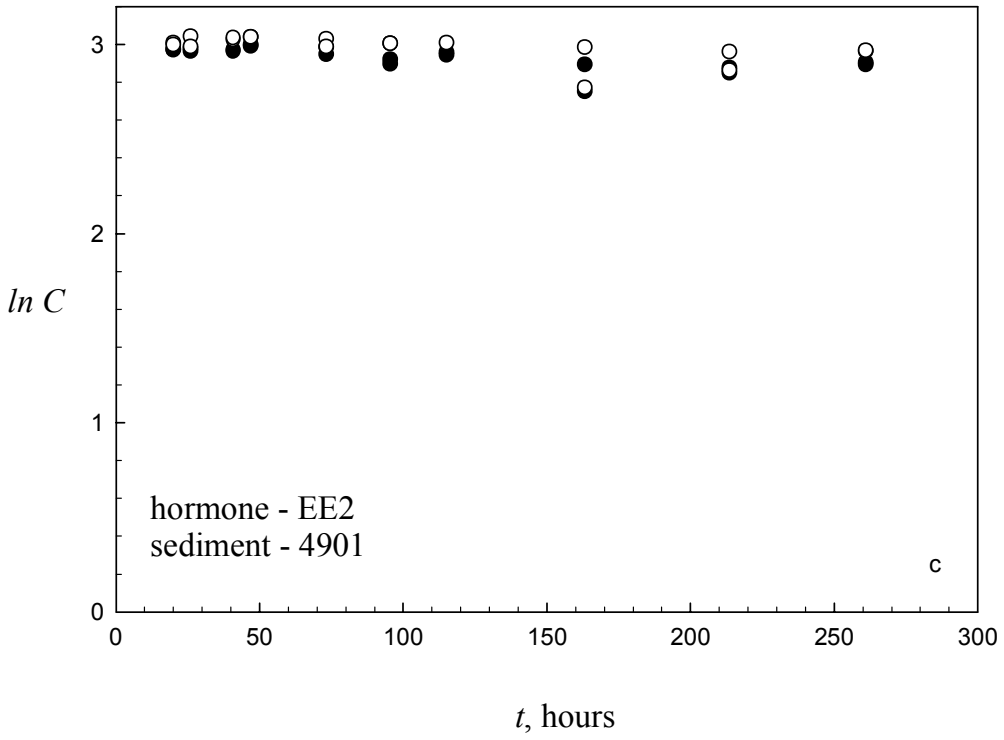


Figure 4.3 Biotransformation of EE2 by a) WissRB and b) 5903. Open circles are killed controls. (continued on next page)



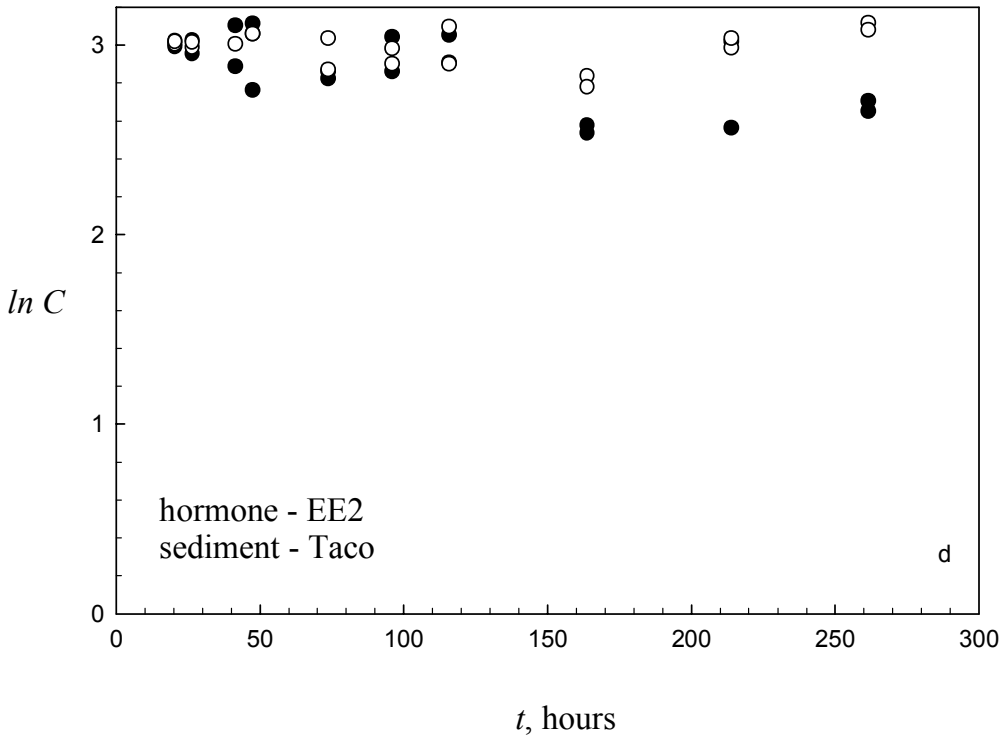


Figure 4.3 (cont.) Biotransformation of EE2 by c) 4901 and d) Taco. Open circles are killed controls. (continued on next page)

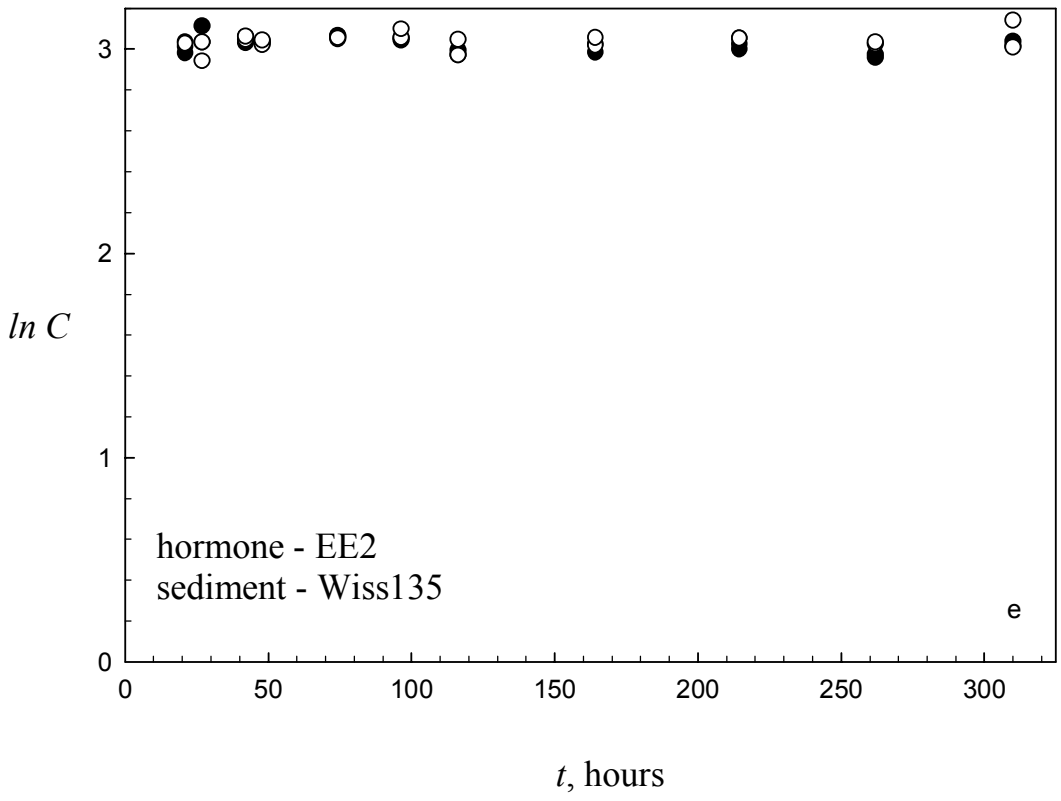


Figure 4.3 (cont.) Biotransformation of EE2 by e) Wiss135. Open circles are killed controls.

CHAPTER 5 – DETECTION OF HORMONES IN THE ENVIRONMENT

Overview

The purpose of this chapter was to collect actual hormone field data to see if there were any correlations between the field data and the laboratory study results (sorption and biotransformation) discussed in Chapters 3 and 5. Sampling plan and method development for both SPE and chemical analysis are provided and the environmental detections are summarized. A discussion of the findings follows as well as a comparison of our results with those of others.

5.1 Rationale and Objectives

The purpose of this field study was to determine the concentration of hormones (E1, E2, EE2, AD, TT) in the effluents of select STPs, two major rivers in central and northern New Jersey, and a CSO. The results obtained from sample analysis will hopefully shed light on the presence of these common endocrine disrupting chemicals in a population dense state of the U.S.

5.2 Sampling Locations

Eight sampling sites (Figure 5.1) were selected from along the Raritan and Passaic Rivers in central and northern New Jersey. Four STP effluents and four river water samples were collected and sampled three times each. One duplicate CSO sample was collected during a single rain event from Perth Amboy, NJ, on May 16, 2008. The two STPs chosen along the Raritan river were the Clinton (40.625842,-74.911416) and Flemington (Three Bridges 40.517535,-74.807003) STPs. The two STPs chosen along the Passaic River were the Two Bridges (40.903896,-74.273865) and Livingston

(40.808157,-74.342594) STPs. The Two Bridges plant is actually near the confluence of the Pompton and Passaic Rivers. River water samples along the Raritan were collected at Hamden Rd. (Hamden, NJ; 40.612079,-74.908798) and Studdiford Dr. (South Branch, NJ; 40.54676,-74.696286) while the river samples collected along the Passaic River were taken from Eagle Rock Ave. (East Hanover, NJ 40.82764,-74.335041) and Rt. 23 (Paterson, NJ; 40.88779,-74.246421) . River sample collection sites were chosen first for geographically representative reasons and secondly, convenience purposes (i.e., the collection point needed to have an easily accessible bridge).

STP choice was also related to geography, but was also based on whether the STP received mostly municipal as opposed to industrial wastewater (the latter would most likely not contain hormones). In addition, the STP needed to be receptive to the idea of the sampling plan. The three rounds of STP effluent and river samples from each site were collected between 5/19/08 and 7/1/08. The flow into each of the STPs ranged from 2-7 MGD. See Figure 5.1 for the locations of each sampling point.

5.3 Sample Collection

Duplicate samples were collected in 4L amber glass bottles containing approximately 600 mg of NaN_3 to prevent biotransformation. Samples from STP effluents were taken either straight from the effluent chamber using a plastic bucket or from a sampling hose that was connected directly to the effluent pipe. In the latter case, the hose was allowed to run for a few minutes in order to flush out any stagnant water prior to sample collection. River water samples were collected from a bridge using a plastic bucket tied to nylon rope. Samples were immediately placed on ice and refrigerated upon return to the laboratory.

5.4 Sample Processing

5.4.1 Filtration and Extraction

Within 24 hours of collection the samples were vacuum pre-filtered using Whatman 934AH glass fiber filter disks (1.5 μm, 12.5 cm). The filtrate was then immediately spiked with 100 or 200 ng of the surrogate compound, estrone-2,4,16,16-d4 (E1-D4) (200 μg/L methanol stock solution). The filtrate was then shaken for one hour on an orbital shaker to ensure proper distribution of the surrogate throughout the solution prior to extraction.

A Kontes SPE manifold was employed for extraction of hormones from the filtered CSO sample, STP effluent samples, and river samples. Extraction disks, 47 mm 3M Empore SDB-XC, Polystyrenedivinylbenzene, were preconditioned with 10 mL acetone, 10 mL isopropanol, 10 mL methanol, and 10 mL Milli-Q water prior to the extraction of 4 L of sample. The samples (loaded gradually into a 1 L glass reservoir attached to the manifold) were allowed to flow through the extraction disk at approximately 5 drops per second. After extraction was completed, the extraction disk was allowed to dry for up to 30 minutes prior to sample elution. A glass vial was placed inside of the manifold and the sample was eluted with 10 mL of methanol and 10 mL of DCM. The DCM/methanol mixture was then immediately covered with a Teflon-lined lid and refrigerated until blow-down.

5.4.2 Cleanup

All samples were blown down to approximately 1 mL of solvent prior to clean up. Silica gel was used to remove interfering substances from the concentrated sample. The

silica gel column was pre-conditioned with 1 bed volume of DCM prior to sample loading. One bed volume of DCM followed by 1 bed volume of methanol was used to elute the sample into a glass conical flask and subsequently rotovapped gently down to approximately 1 mL. Finally this aliquot of sample-containing solvent was transferred to glass GC vials and gently blown down to dryness with nitrogen. Then 300 μL of methanol was used to resuspend the sample followed by addition of 24.84 ng of internal standard, deuterated E2 (E2-D3) (60 μL of 414 μg/L methanol stock solution), for a final total volume of 360 μL.

5.5 Chemical Analysis

The LC-MS/MS instrument utilized in this study was an Agilent 1200 LC system coupled to an Agilent triple quadrupole mass spectrometer using Atmospheric Pressure Chemical Ionization (APCI) (Agilent Technologies, USA). Instrument control and data processing were conducted with MassHunter software (Agilent). All of the hormones were separated by an Agilent XDB C_{18} column (3.0 × 15 mm, 3.5 mm). APCI was employed in the positive mode. The flow rate was set to 0.6 mL/min with water (0.1% formic acid) and methanol as the two solvents. Instrument parameters were as follows: 350°C gas and 400°C vaporizer temperatures; 5L/min gas flow, 60 psi nebulizer pressure, 4500V capillary. The gradient settings were as follows: 0~20 min/ 0%~80% mobile phase B; 20~23 min/ 80%~0% mobile phase B; post-run time of 7 minutes. Equilibrium was reestablished prior to the next injection. The limit of detection (LOD) for TT, AD, EE2, E1, and E2 were 0.05, 0.04, 0.91, 0.09, and 0.09 ng/L, respectively. There was difficulty encountered while developing the method for EE2, hence the fact that it was the hormone with the highest LOD. See Table 5.1 for the LC/MS/MS parameters, Figure

5.2 for the total ion chromatogram (TIC), and Figure 5.3 for the individual chromatographs of the precursor and product ions. While the male hormones could be easily separated from each other, it was a challenge to separate the 3 female hormones and the surrogate standard.

5.6 QA/QC

In addition to the use of a surrogate standard for the sample itself, matrix spike and blank samples were processed as well. Three matrix spike samples were prepared with 4 L of Milli-Q water and 40 ng each of the target analytes. Four blanks consisting of solely 4L of Milli-Q water were analyzed as well. The results of the blank sample analysis can be found in Table 5.2 and the matrix spike results can be found in Table 5.3. There were two AD and TT detections in two of the blank samples, which may have been injection needle cross-contamination from samples containing said hormones. The average surrogate recovery of E1-D4 was 7.51% and ranged from non-detect to 11.31%. Final results were not corrected for surrogate standard (SS) due to low recovery and fluctuations from sample to sample. A signal to noise ratio of at least three was the minimum to confirm the presence of the peaks. In addition, the ratio of the precursor to product ion needed to be within 25% of the minimum and maximum precursor to product ion ratios determined from amongst the series of external standards prepared.

5.7 Results and discussion

5.7.1 Detections

All detections are summarized in Table 5.4 with maximum detections depicted on the map in Figure 5.1. At least one hormone was detected at all nine sampling locations during at least one of the three sampling events. All concentrations were found to be in the low ng/L range or less. Average concentrations, when detected, of TT, AD, EE2, E1, and E2 were 0.62, 2.51, 4.61, 4.86, and 2.19 ng/L, respectively. The frequency of detection of each of the hormones in all samples increased in the order of EE2 = E2 < TT < E1 < AD. Between the two male hormones investigated, AD was detected more frequently than TT and it was also found at higher concentrations (max = 15.2 ng/L). Amongst the female hormones, E1 was found most often and also had the highest concentrations (max = 12.8 ng/L) compared to E2 and EE2. Overall, AD was the most frequently detected hormone (found in 76% of samples).

There was a high degree of variability amongst the samples collected in this study, both among the duplicates and amongst sampling events. Variability in the quality of STP effluents is not unusual. In Williams et al.'s (2003) study of sewage treatment works (STW) effluent, they found that E1 and E2 concentrations could vary by 2- or 3-fold for samples collected on consecutive days.

Our female hormone detections appear to follow the same trend as those of other research groups. Williams et al. (2003) also found that E1 was the greatest compared to E2 and EE2 in their investigation of both river water and STP effluent in the U.K. It appears that EE2 and E2 are detected sporadically based on our results and others. Snyder et al. (1999) collected samples from a river channel and found EE2 at only one

location. When Noppe et al. (2007) searched for the female hormones in an estuary, they did not find E2 at all. In their particular study, both EE2 and E2 were all below their limit of quantification (LOQ). Desbrow et al. (1998), in their British STW study also noticed that EE2 was detected the least frequently.

There have been very few studies that attempted to detect both male and female hormones simultaneously. In a Japanese study, Yamamoto et al. (2006) noted that E1 was more prevalent than E2 and that AD was more prevalent than TT in their river and estuarine water samples, which is similar to the frequency of these hormones in this study. Kolodziej et al. (2003) determined the levels of male and female steroid hormones in the effluent of STPs and also found that E1 far exceeded E2 but that TT was more common than AD. It may be that the microbial communities responsible for the biotransformation of TT are more common in natural waters than in STPs.

The lack of E2 detections in the STP effluent may be due to its ability to biotransform rapidly. Results presented earlier in this document have shown that E2 can quickly biotransform in a simple soil – Milli-Q water slurry. It can only be expected that the microorganism-rich STP sludge (and even natural water) would enhance the biotransformation reaction of E2. When Ternes et al. (1999) evaluated the biotransformation of E2 in activated sludge, they found that E2 was rapidly oxidized to E1. On a similar note, AD concentrations were much higher than TT and detected much more frequently, probably for the same reason that E2 was found infrequently. As mentioned earlier in this document (Chapter 4), TT, like E2, biotransformed rapidly in a simple soil-Milli-Q water slurry, so its transformation would most likely be further enhanced in an STP and in natural waters.

It was interesting to observe that the single E2 and EE2 detections happened to be at a river sampling site (Rt. 23), and not an STP effluent site. According to Barel-Cohen et al. (2006), the presence of EE2 distinguishes hormones from human and non-human sources. While the STP sampled upstream of this site (Two Bridges) did not contain EE2 or E2 in its effluent, it is possible that another discharging STP upstream of Rt. 23 is contributing to the presence of EE2 and E2 along this stretch of the Passaic River. Runoffs from biosolids application or leaking septic systems are other potential, but unlikely, sources of this synthetic hormone in the river.

5.7.2 Raritan Versus Passaic

In Table 5.5, the sum of all hormones and number of detections is summarized for both the Raritan and Passaic. When comparing the overall presence of hormones associated with the Raritan versus Passaic River, it appears that total hormone concentration and the frequency of detections were comparable between both polluted rivers.

5.7.3 STP Effluent Versus River Water

When comparing the overall presence of hormones in STP effluent versus river water, it was interesting to see that there were nearly twice as many detections in river water compared to the STP effluent. Further, the sum of all hormones was found to be equivalent in the rivers compared to the STP effluents as well, meaning that dilution may not be playing much of a role in the hormones' fate. There could be two reasons for this: a) delayed deconjugation of conjugated steroids (discharged from STPs) while in river

transit and b) the contribution of other STPs or non-point sources (i.e., agriculture) discharging upstream from the river sampling point.

Estrogens are mostly excreted from the body as inactive polar conjugates (forming glucuronic and sulfate moieties) that can be cleaved by the appropriate microorganisms. STPs obviously have a high concentration of microorganisms such as *Escherichia coli*, which display glucuronidase and sulphatase activity (Ternes et al., 1999). While this cleavage of the conjugated hormones is most notable within an STP, it should not be impossible for this transformation to take place in surface waters as well. Yamamoto et al. (2006) detected conjugated E1 (E1 3-sulfate) in many of their water samples. Thus, the presence of the conjugated hormone in rivers has a potential to be a delayed active hormone "sink," as it continues in transit. Therefore, in addition to cumulative effects of potential upstream sources and possible backflow conditions in rivers, a delayed "release" of the active form of hormone could lead to surface water concentrations that are similar to STP effluent concentrations (which otherwise are expected to be more diluted once it enters the surface water body).

5.7.4 CSO

The CSO sample contained only TT. Because the conjugated hormones in the combined sewer overflow were freshly discharged and only in transit briefly before sample collection, there may not have been time for a substantial portion of the conjugated female hormones to become cleaved by microorganisms. This study only analyzed for the free unconjugated hormones and would therefore not be able to detect any inactive conjugated hormones in the CSO sample. Ternes et al. (1999) investigated

the loads of estrogens during their passage through a municipal STP and noticed that the estrogens were higher during preliminary clarification compared to the raw influent and final effluent. Thus it does not come as a surprise that the raw CSO sample collected in this study would not have high quantities of free hormones at that point in the sewage collection system. Furthermore, excess rain water (which triggered the event in the first place) may have also diluted the hormones, making it even more difficult to detect them.

5.8 Summary

It appears from the results of this field study that hormones definitely have a presence in the rivers and STP effluents of central and northern New Jersey. As mentioned earlier, hormones have the potential to have pheromonal affects on fish (Kolodziej et al., 2003) and since it was found in many of the samples collected, this may pose an issue for wildlife in the Raritan and Passaic Rivers. As mentioned earlier, TT, AD, and E1 are known to have pheromonal effects at concentrations of 3, 300, and 30 ppt (Adams et al., 1987; Poling et al., 2001; Murphy et al., 2001) With TT and E1 detections of 1.10 and 12.84 ppt, respectively, it appears that the concentrations of these two hormones fall close to the concentrations needed to have a pheromonal effect. More importantly, EE2 was found at concentrations up to 4.61 ppt, which is greater than the 1 ppt needed to induce vitellogenin production in fish (Purdom et al., 1994). E1 was frequently detected and although it has the least potency of the female hormones (Sumpter et al., 2005) its commonness and higher concentrations compared to the more potent E2 and EE2 may cause it to have as a strong an impact on the health of ecosystems. Given the biotransformation results in Chapter 4, it made sense that the biotransformation products of TT and E2, AD and E1, were more prevalent.

Table 5.1 LC/MS/MS parameters

Hormone	Retention Time (min)	Precursor Ion	Product Ion	Fragment (V)	Collision Energy (eV)
E1	19.4	271.5	253.1	100	10
E2	19.4	255.1	158.8	100	15
EE2	19.4	279.2	132.9	100	15
E1-D4	19.4	275.3	257.3	100	10
TT	19.9	289.2	97.0	160	20
AD	19.2	287.0	96.8	140	25

Table 5.2 Blank sample analysis

	Blank Sample Analysis (ng/L)					
	TT	AD	EE2	E1	E2	E1-D4
Blank 1	3.84	7.08	nd	nd	nd	nd
Blank 2	nd	nd	nd	nd	nd	nd
Blank 3	0.41	4.88	nd	nd	nd	nd
Blank 4	nd	nd	nd	nd	nd	nd

nd = non detect

Table 5.3 Matrix spike recovery

	Matrix Spike Analysis (% Recovery)[a]					
	TT	AD	EE2	E1	E2	E1-D4
MS 1	26.02	32.57	25.52	0.0	19.11	11.31
MS 2	17.10	17.46	14.77	14.14	12.32	11.23
MS 3	73.09	89.30	66.29	88.15	72.61	0.0

[a] Recoveries are based on the addition of 40 ng of each hormone to each sample

Table 5.4 Summary of hormone detections in STP effluents and rivers

				Mean Concentration (ng/L)[bc]				
Sampling Site	River	N[a]	Date	TT	AD	EE2	E1	E2
Clinton STP Effluent	Raritan	2	5/19/8	nd	nd	nd	11.41	nd
		1	5/28/8	nd	4.73	nd	nd	nd
		1	6/17/8	0.35	nd	nd	12.84	nd
Flemington STP Effluent	Raritan	2	5/19/8	0.29	1.48	nd	nd	nd
		1	6/10/8	nd	3.57	nd	nd	nd
		1	6/17/8	nd	4.07	nd	nd	nd
Livingston STP Effluent	Passaic	1	5/28/8	nd	0.09	nd	0.11	nd
		2	6/13/8	1.10	6.52	nd	nd	nd
		2	6/23/8	nd	nd	nd	nd	nd
Two Bridges STP Effluent	Passaic	1	5/22/8	nd	nd	nd	nd	nd
		2	6/13/8	nd	1.50	nd	nd	nd
		2	6/23/8	nd	nd	nd	nd	nd
Studdiford Dr Bridge	Raritan	1	5/28/8	nd	0.84	nd	nd	nd
		2	6/10/8	nd	0.82	nd	nd	nd
		1	6/27/8	nd	1.55	nd	3.91	nd
Hamden Rd Bridge	Raritan	2	6/10/8	nd	1.06	nd	3.24	nd
		2	6/23/8	nd	0.50	nd	1.94	nd
		2	6/27/8	nd	0.63	nd	0.74	nd
Eagle Rock Ave Bridge	Passaic	1	5/22/8	nd	1.23	nd	nd	nd
		2	6/13/8	0.02	0.88	nd	nd	nd
		2	7/1/8	1.00	1.03	nd	nd	nd
Rt. 23 Bridge	Passaic	2	5/22/8	nd	0.66	nd	nd	nd
		2	6/17/8	nd	15.19	4.61	4.72	2.19
		2	6/30/8	nd	1.39	nd	nd	nd
Perth Amboy CSO		2	5/16/8	0.92	nd	nd	nd	nd
Limit of Detection (ng/L)				0.05	0.04	0.91	0.09	0.09
Mean Concentration[e] (ng/L)				0.62	2.51	4.61	4.86	2.19
Max Concentration (ng/L)				1.10	15.19	4.61	12.84	2.19
Frequency (%) [d]				24	76	4	32	4

[a] N = number of samples

[b] nd = non-detect

[c] sample concentrations not corrected for recovery

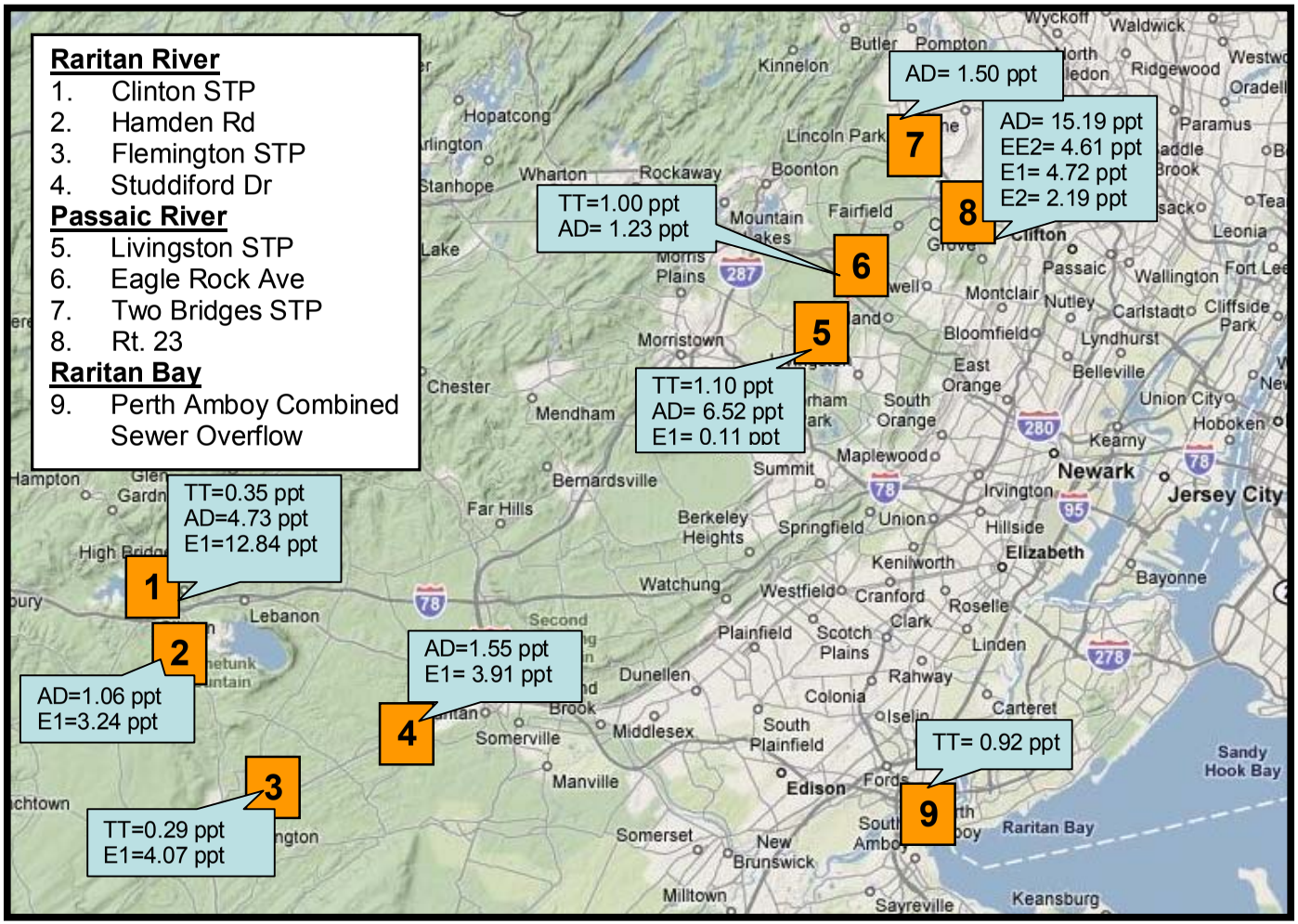
[d] based on a total of 25 non-duplicate samples collected

[e] when detected

Table 5.5 Overall Concentrations and Total Hits

Sum all rivers (ng/L)	48.15
Sum all STPs (ng/L)	48.06
Total hits in all rivers	21
Total hits in all STPs	13

Figure 5.1 Sampling Locations and Maximum Concentration Detected at each site (not corrected for SS recovery)



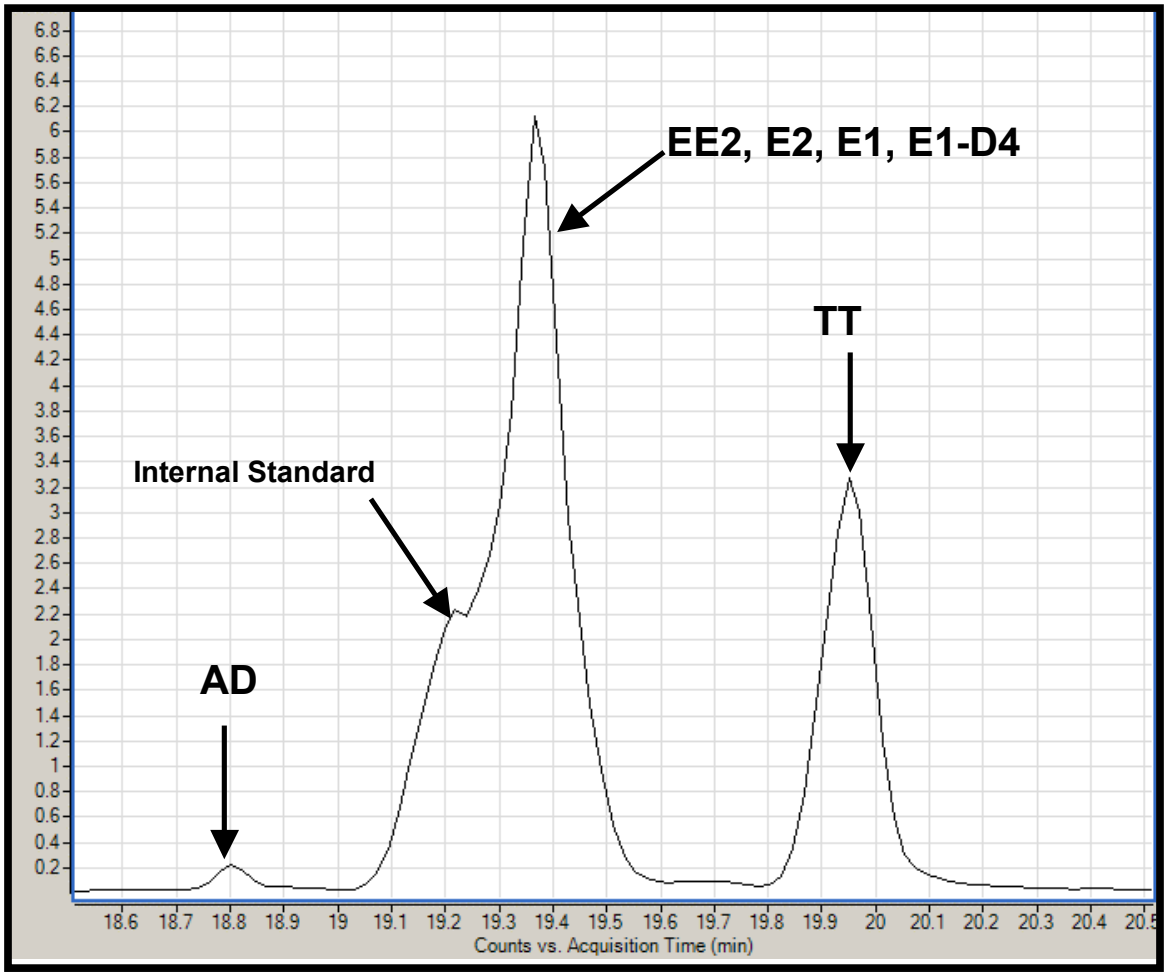


Figure 5.2 LC/MS/MS Total Ion Chromatogram. Separation of male and female hormones as well as surrogate.

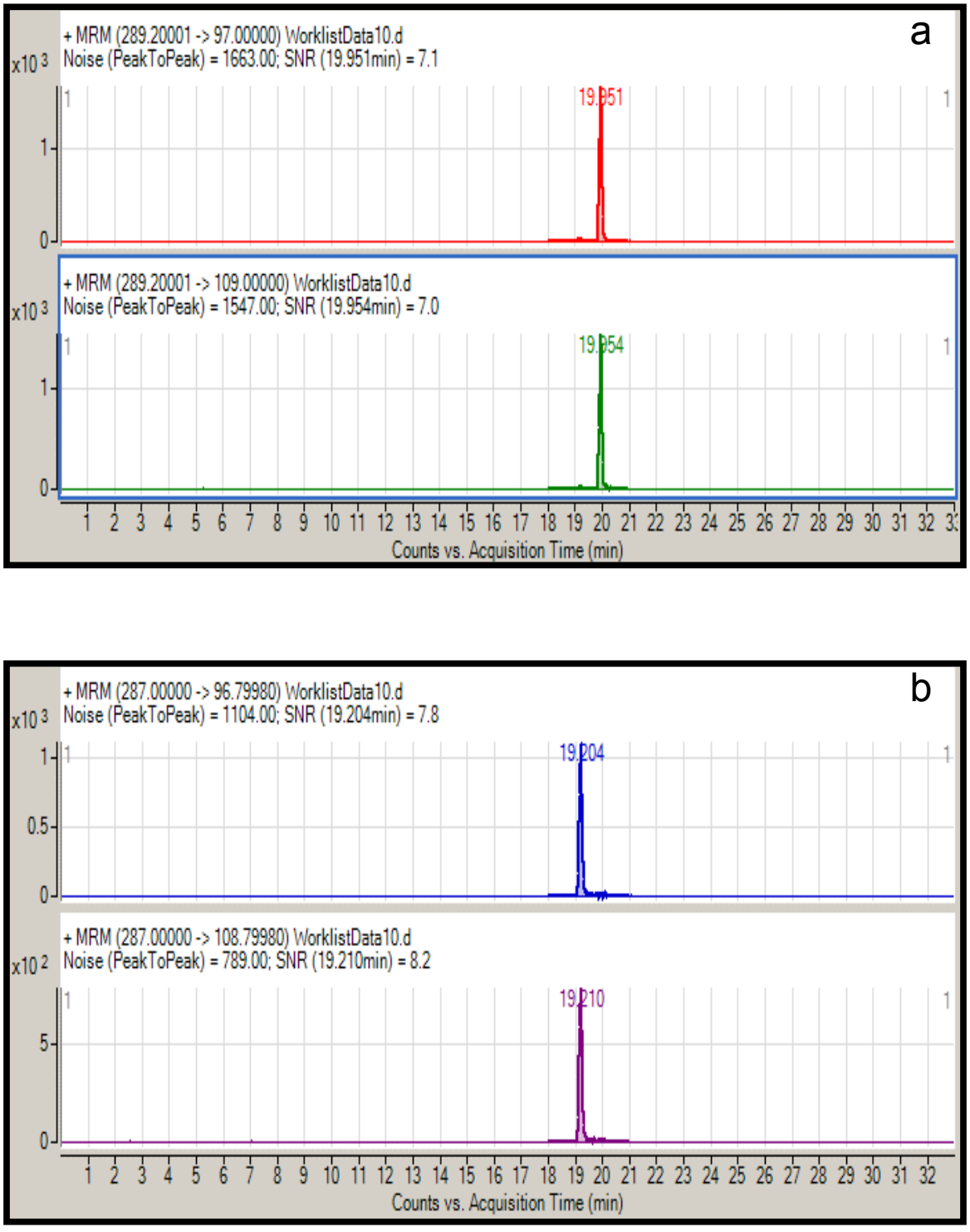
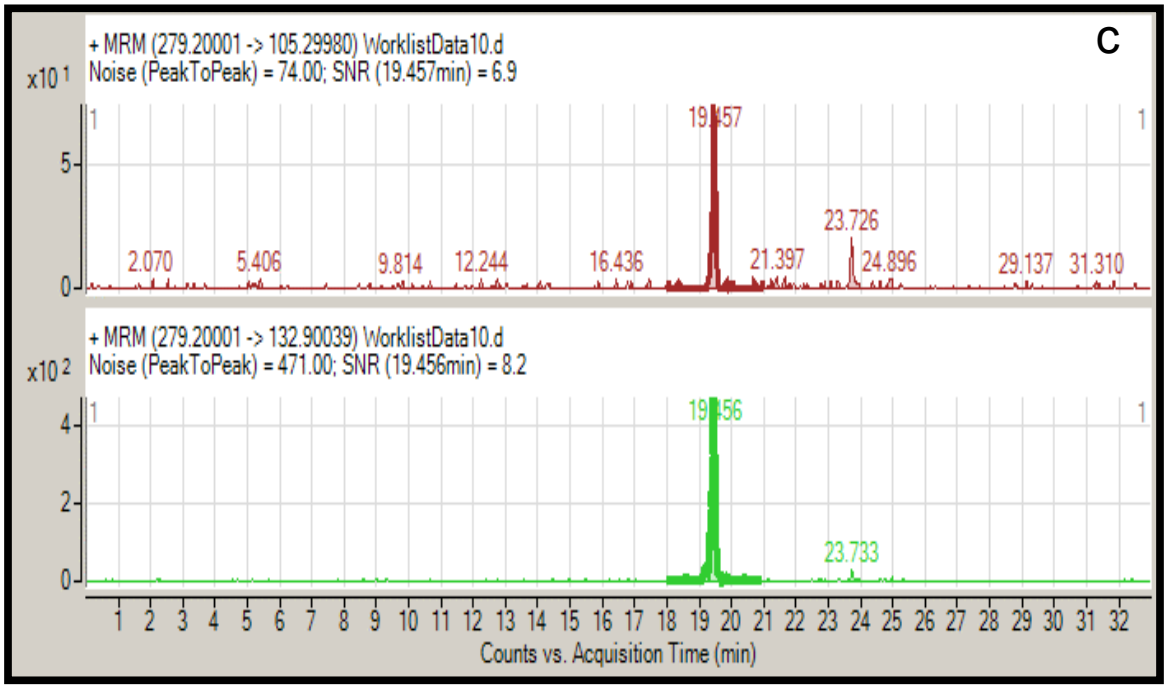


Figure 5.3 LC/MS/MS chromatograms for a) TT and b) AD (continued on next page)



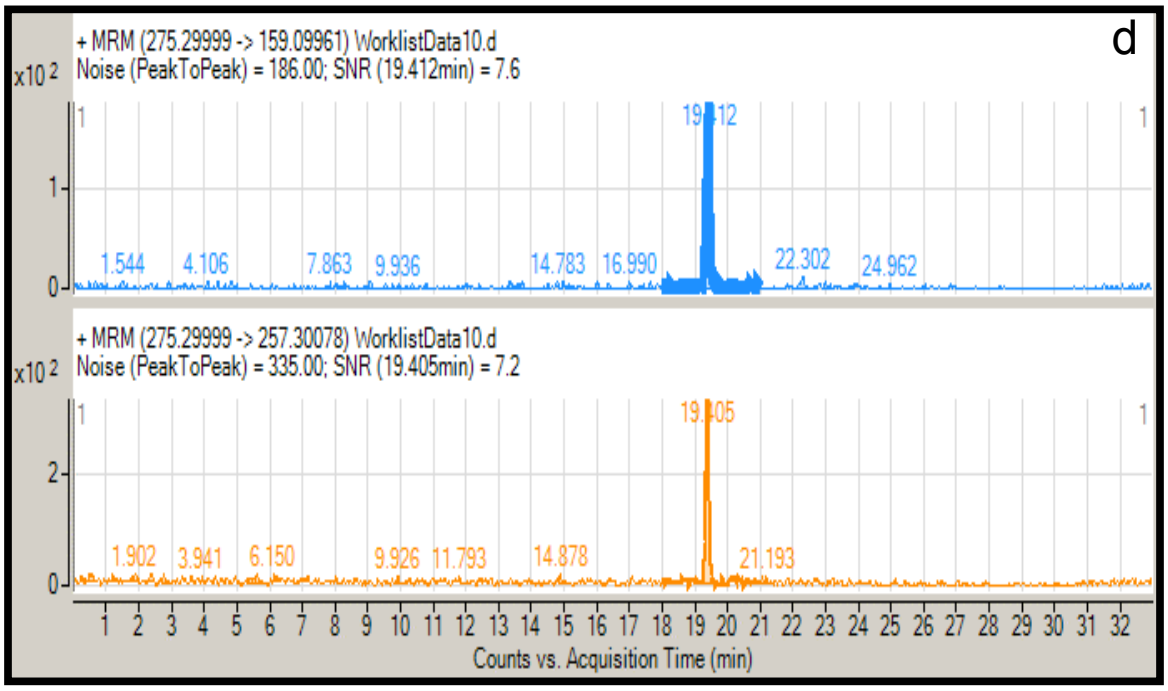


Figure 5.3 (cont.) LC/MS/MS chromatograms for c) EE2 and d) Surrogate Standard
(continued on next page)

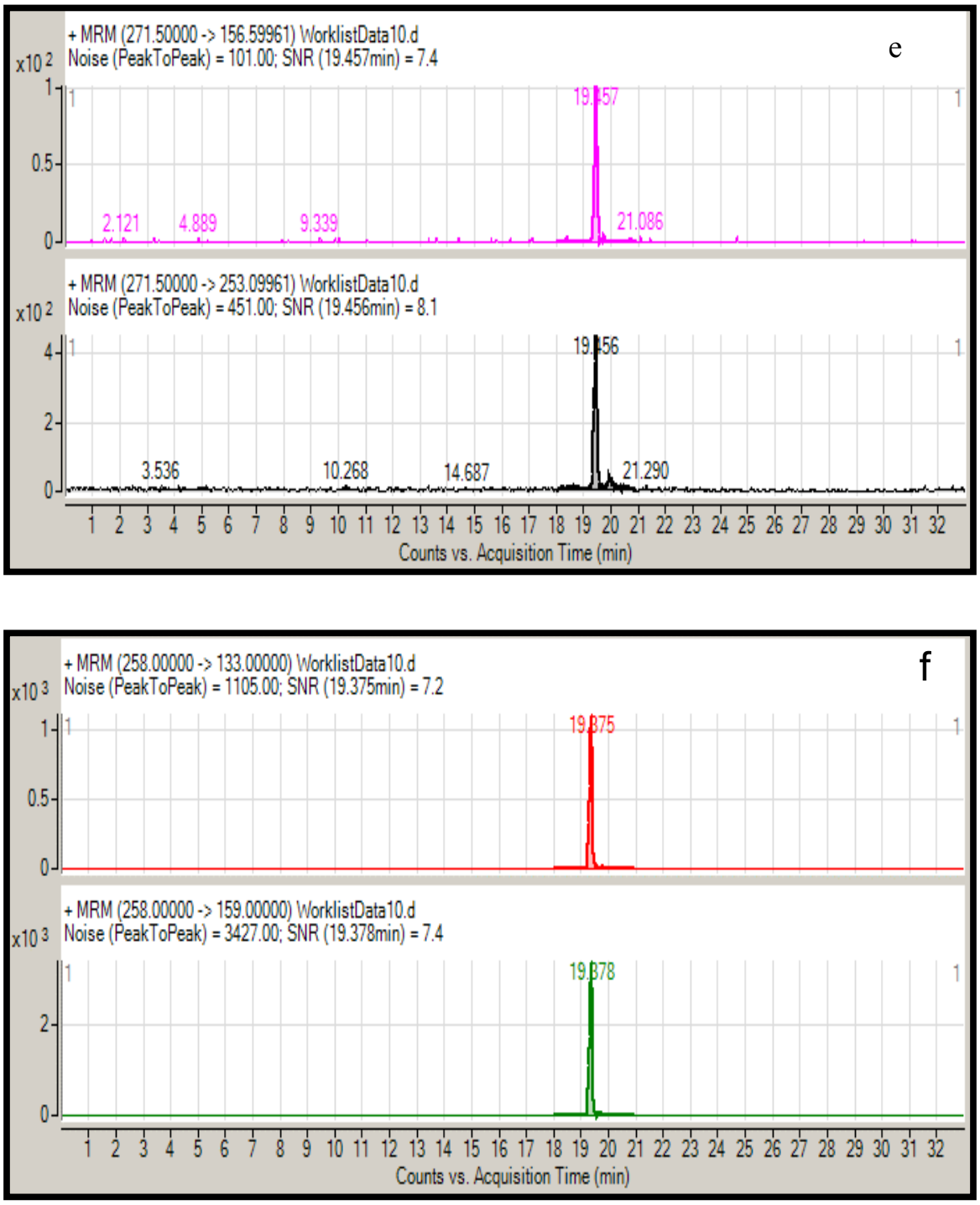


Figure 5.3 (cont.) LC/MS/MS chromatograms for e) E1 and f) Internal Standard
(continued on next page)

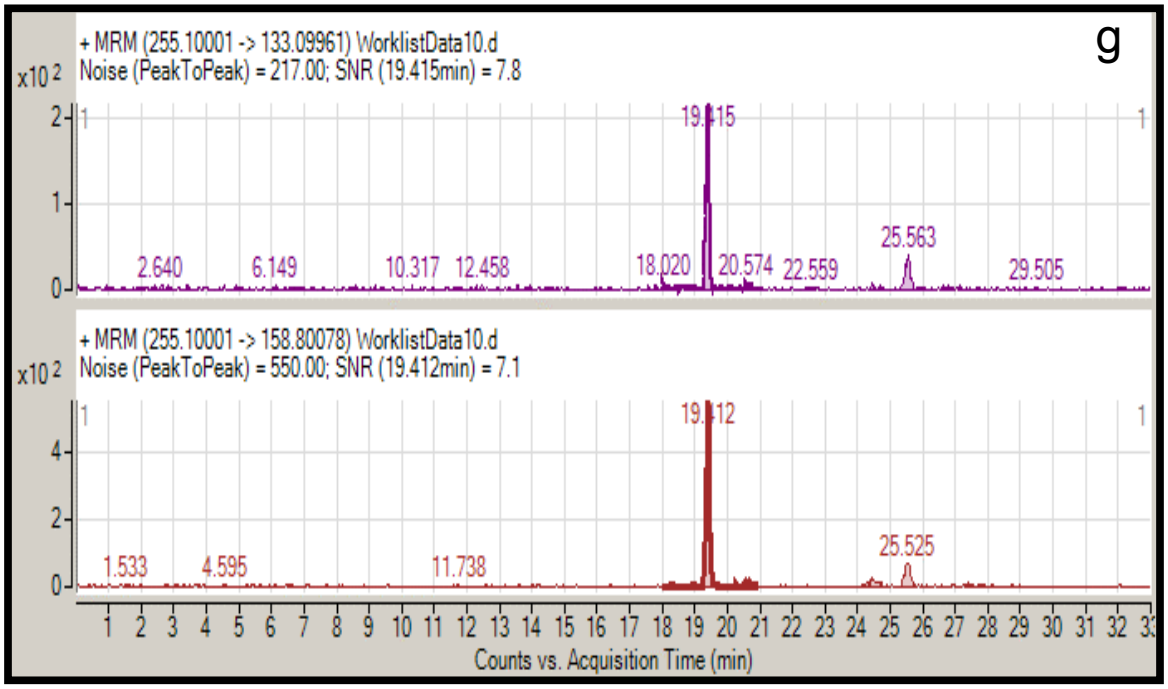


Figure 5.3 (cont.) LC/MS/MS chromatograms for g) E2

CHAPTER 6 – CONCLUSIONS, IMPLICATIONS, AND RECOMMENDATIONS

6.1 Conclusions

The studies carried out in this dissertation have shed light on the issues of male and female hormone sorption, biotransformation, and presence in the environment.

1) Due to the slightly more soluble nature of androstenedione (AD), this daughter product of testosterone (TT) has a lower propensity for sorption compared to the parent.

2) The order in which sorption increases for the hormones is 17α-ethinylestradiol (EE2) < TT < 17β-estradiol (E2). The order in which the Philadelphia area river sediments are able to take up hormones is Tacony (Taco) < Queen's Lane DWTP Intake (5903) < Belmont DWTP Intake (4901) < Wissahickon (Wiss135) < Wissahickon River Bank (WissRB).

3) It is apparent that the short-term experiments conducted by other researchers underestimate the sorption capacities for steroid hormones. Depending on the initial solute concentration, our data on male hormones suggests at least one to three weeks may be required for sorption to reach equilibrium.

4) The natural tendency for log K_{OC} values to increase as aqueous concentrations of the hormones decrease (i.e., non-linear character) may be beneficial, especially since they are found at such low concentrations in the environment. They will be more likely immobilized if they are present at lower concentrations.

5) The data from the sorption of male and female hormones to the Philadelphia area

sediments suggests that there is little correlation between sorption capacity and total organic carbon (TOC); thus site specific interactions between the functional groups of the hormones and sediment surface may be predominating.

6) The sorption of hormones to various soil fractions varied greatly from one fraction to another. Particulate organic matter (POM) exhibited the highest sorption capacity and non-linearity for the hormones despite the fact that they are only mildly hydrophobic. Humic acid (HA) is obviously the main contributor to hormone sorption linearity and low sorption capacity.

7) TT biotransformed at a faster rate than E2. EE2 is comparatively recalcitrant.

8) The biotransformation of hormones appeared to adhere to pseudo-first order kinetics ($R^2 > 0.84$)

9) Sediments containing lower organic carbon levels yield longer biotransformation lag times.

10) Two major rivers of New Jersey, the Passaic and the Raritan, contain hormones in the low ng/L range as well as the effluents of various New Jersey municipal sewage treatment plants (STPs), and a combined sewer overflow (CSO). At least one hormone was detected at all 9 sampling locations in central and northern New Jersey. AD and E1 were the most frequently detected and found at the highest concentrations. The low levels of unconjugated hormone at the CSO may have been due to the lack of deconjugation in the freshly discharged sewage/rain water mixture.

11) With TT and E1 detections of 1.10 and 12.84 ng/L, respectively, it appears that the concentrations of these two hormones fall close to the concentrations needed to have a

pheromonal effect. More importantly, EE2 was found at concentrations up to 4.61 ng/L, which is greater than the 1 ng/L needed to induce vitellogenin production in fish.

12) The presence of AD and E1 in the STP effluents and rivers of northern and central New Jersey are in alignment with the biotransformation data observed in the biotransformation study (Chapter 4). Since E2 and TT were very rapidly biotransformed, it makes sense that their biotransformation products AD and E1 were found in greater abundance in the environment. The low frequency of EE2 in our field study was in agreement with several other groups. It is mostly likely due to the fact that EE2 is not discharged into municipal STPs as frequently as the other steroids since it is a synthetic hormone not naturally excreted by the body. This does not mean that EE2 is not a concern in New Jersey – as mentioned earlier, it was still detected at deleterious levels. Depending on the time of day that STPs are sampled, this birth control compound could fluctuate greatly (especially the "morning flush"); it is also more potent.

6.2 Implications

The presence of the synthetic female hormone could lead to vitellogenin production in wildlife whereas the presence of TT and AD have pheromonal consequences. Being that the male hormones are sorbed less strongly to sediment and soil compared to the natural female hormone means that they will be more mobile in the environment. On the other hand, male hormones also exhibit faster biotransformation rates compared to the natural female hormones, so this factor may help offset the negatives associated with their ease of movement.

As mentioned earlier, the sediments with lower organic carbon content experienced longer lag times for biotransformation. This could imply that in swiftly moving waterways where less organic matter is found near the centroid of flow, hormones may be more likely to persist. Microbial communities with a preference for the solid phase may not be able to thrive in such nutrient-depleted soils.

In the grand scheme of things, elucidation of hormone sorption and biotransformation characteristics would be useful when considering land application of manure and municipal biosolids. Their impact could be assessed so farmers may alter their manure application methods to minimize estrogen runoff into watersheds (Hildebrand et al., 2003). As far as comparisons to other EDCs are concerned, Ying et al. (2003) determined that E2 and EE2 exhibited sorption characteristics somewhere in between Bisphenol-A (lowest of their three categories) and surfactant transformation products (4-t-OP and 4-n-NP) (highest of their three categories).

The fact that hormones were found in rivers at concentrations close to those of the STP effluent is a cause for concern. Improper land management practices may be responsible for what appears to be non-point source contributions of hormones to the rivers. Stricter controls on agricultural activities, animal husbandry practices, and biosolids applications may need to be employed. Another reason for similar river and STP effluent concentrations could be delayed deconjugation of the hormones in the rivers. In that case, longer retention times in the STP would assist in the matter and future field studies must include the analysis of both conjugated and unconjugated hormones.

6.3 Recommendations

Considering that hormones are initially released mainly as inactive conjugates, sorption studies need to be carried out on this form of the hormones, especially since there is a chance that conjugated steroids could survive STP treatment. Future research on the fate and transport of hormones needs to be geared toward daughter product biotransformation and detection of AD and E1 considering that they were nearly ubiquitous at the sampling sites evaluated in this study. It should be noted that in this study the filtrate from effluent and river samples was collected and processed for analysis as well. However, it was realized afterwards that even the gentle heat of the soxhlet extraction was too intense for the hormones, and resulted in their transformation during the extraction step, rendering the samples unusable. Thus, this study should be expanded to other STPs along the Passaic and Raritan in the future, but the collected filtrate must be extracted via a room temperature method instead of the conventional soxhlet method.

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