Developing a Quantitative Framework to Track the Fate and Transport of Estrogens on a Watershed Scale

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Abstract

Estrogens are female sex hormones, and the major naturally occurring estrogens are estrone (E1), 17α-estradiol (E2α), 17β-estradiol (E2β), and estriol (E3). Concentrated animal feeding operations (CAFOs) and wastewater treatment facilities release a large amount of estrogens into surface water. Additionally, livestock manure and biosolids, which are widely used as fertilizers, have the potential to spread estrogens onto agricultural land. Estrogens either in surface water or on land surface go through prevalent and complex attenuations and transformations due to bio-transformation, sorption, photo-transformation, and plant uptake. Estrogens on the land surface can be transported into surface water through various pathways such as the surface runoff. Once estrogens get into surface water, they can impair the normal reproductive functions of aquatic animals at low concentrations. Thus, it is quite important to estimate estrogen levels in surface water in order to assess and mitigate the potential health risks caused by those estrogens. As a modeling framework can help to conduct this analysis, the goal of this study is to develop a quantitative modeling framework to simulate levels of the three most prevalent natural estrogens, E1, E2α, and E2β, in rivers. This study first adopted a wash-off model to quantify the transport of E1, E2α, and E2β from land to rivers by surface runoff. This study also developed a comprehensive transformation model to quantify the transformation and attenuation of E1, E2α, and E2β. This study then assembled these two mathematical models to develop a quantitative modeling framework, which can be implemented by the Hydrological Simulation Program - FORTRAN (HSPF), to simulate estrogen levels in rivers. Finally, this modeling framework was applied to the South River Watershed in Virginia and the Redwood River Watershed in Minnesota to track the fate and transport of estrogens from various sources such as wastewater

treatment plants (WWTPs), manure and biosolids used for land application, grazing farm animals, and septic systems. For both watersheds, a component analysis was conducted to quantify estrogens contributed by each source and a sensitivity analysis was conducted to investigate factors that can impact estrogen levels in rivers. The modeling results for both watersheds indicate that storm events just after manure land application can transport a large amount of estrogens to surface water and elevate estrogen levels. Buffer stripes are suggested in this case to reduce the mass of estrogens that are flushed into surface water. The modeling results for both watersheds also show that the simulated estrogen levels are sensitive to cattle grazing time in streams, and thus fencing off rivers to keep cattle out of the water is recommended to reduce the amount of estrogens that are directly released into streams by cattle. Additionally, the modeling results for both watersheds show that manure used for land application release a large amount of estrogens onto the land surface and the simulated estrogens levels are sensitive to the manure application rate, the manure storage before land application is thus encouraged in order to reduce the estrogen content in manure. This framework can be applied to watersheds to predict the temporal and spatial variation of estrogens in rivers, to quantify estrogens contributed by various sources, to investigate the factors that can lead to high estrogen levels, and to determine the best management practices (BMPs) of controlling estrogens in surface water.

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A	Land area of the agricultural land (m^2)
c	A dimensionless constant in the Empirical Model
C	Estrogen content in solid waste (ng/g)
C_A	Concentrations of E2α (ng/L)
C_B	Concentrations of E1 (ng/L)
C_{BR}	Estrogen content in dry biosolids (ng/g)
C_C	Concentrations of E2β (ng/L)
C_0	Initial concentration of estrogen (ng/L)
C_e	Aqueous concentration of estrogens at equilibrium (ng/L)
C_M	Estrogen content in manure (ng/g)
CN	Curve number
C_w	Estrogen concentration in WWTP effluents (ng/L)
D	Number of days in that month
d	A dimensionless constant in the Empirical Model
E_a	Daily estrogen excretion per animal (ng/day/animal)
E_F	Daily estrogen excretion by a female human (ng/day/female)
E_M	Daily estrogen excretion by a male human (ng/day/male)
f	Faction of estrogen that can be desorbed from manure and get into streams
F_C	Fraction of manure applied to cropland in a certain month
f_f	Failing rate of septic systems
F_P	Fraction of manure applied to pastureland in a certain month
fs	Fraction of housing units that use straight pipes

k	Lumped first-order sorption rate (day^{-1})
k_1	Transformation rate constant of E2α to E1 (day^{-1})
k_{-1}	Transformation rate constant of E1 to E2α (day^{-1})
k_2	Transformation rate constant of E2β to E1 (day^{-1})
k_{-2}	Transformation rate constant of E1 to E2β (day^{-1})
k_3	Transformation rate constant of E2a to E2β (day^{-1})
k_4	Transformation rate of E1 to other compounds (day^{-1})
k_{bio}	First-order decay rates via sorption, biotransformation (day^{-1})
K_d	Partition coefficient (Lg^{-1})
k_{direct}	First-order decay rates via direct photo-transformation (day^{-1})
K_f	Freundlich's constant ($mg^{1-N}L^{N}g^{-1}$)
$k_{indirect}$	First-order decay rates via indirect photo-transformation (day^{-1})
k_w	Wash-off coefficient ($min\ L^{-1}$)
k_{sorp}	First-order decay rates via sorption (day^{-1})
k_{upt}	First-order decay rates via plant uptake (day^{-1})
I	Measured rainfall intensity (cm/min)
L_{N1}	Daily estrogen load to cropland through manure application (ng/day/acre)
L_{N2}	Daily estrogen load to pastureland from domesticated animals (ng/day/acre)
L_{N3}	Daily estrogen load to land from failed septic systems (ng/day/acre)
L_{N4}	Daily estrogen load to agricultural land from biosolids (ng/day/acre)
L_{P1}	Direct daily estrogen load into streams by grazing farm animal (ng/day)
L_{P2}	Daily estrogen load to rivers from straight pipes (ng/day)
L_{P3}	Daily estrogen load into streams from WWTPs (ng/day)

M	Total mass of estrogens flushed from the land surface by surface runoff (ng)
N	A dimensionless constant in the Freundlich Equation
P	Population of livestock or poultry
P_F	Population of females within the study area
P_M	Population of males within the study area
Q	Surface runoff (L/min)
Q_w	Daily discharge of WWTPs (m^3/day)
R_B	Application rate of dry biosolids (g/day/acre)
R_{MC}	Annual manure/litter application rate to cropland (g/day/acre)
R_{MP}	Annual manure/litter application rate to pastureland (g/day/acre)
S	Total mass of estrogen on land (ng)
S_c	Solid content of fresh waste
S_e	Solid concentration of estrogens at equilibrium (mg/g)
t	Time (day)
T_p	Fraction of time spent by cows grazing on pastureland
T_s	Fraction of time spent by cows in streams
W	Daily fresh manure/litter production per animal (g/day/animal)

LIST OF ABBREVIATIONS

AFOs	Animal Feeding Operations
AMC	Antecedent Moisture Content
BASINS	Better Assessment Science Integrating Point and Non-point Sources
BMPs	Best Management Practices
CN	Curve Number
DOC	Dissolved Organic Carbon
DW	dry weight
E1	Estrogen
E1-3	Estrone-3-Glucuronide
E1-3S	Estrone-3-Sulphate
E2-3G	Estradiol-3-Glucuronide
E2-3S	Estradiol-3-Sulphate
E2-17S	Estradiol-17-Sulfate
E2α	17α-Estradiol
E2β	17β-Estradiol
E3	Estriol
EC50	Half Maximal Effective Concentration
EDs	Endocrine Disruptors
EDCs	Endocrine Disrupting Chemicals
EE2	17α-Ethynylestradiol
ERs	Estrogen Receptors
GIS	Geographic Information System

HA	Humic Acids
HSPF	Hydrological Simulation Program – FORTRAN
IPHT	Imminent Public Health Threats
LOEL	Lowest Observable Effect Level
LOOCV	Leave-One-Out Cross-Validation
MRLC	Multi-Resolution Land Characteristics Consortium
NLCD	National Land Cover Database
NMSE	Normalized Mean Square Error
NOAA	Oceanic and Atmospheric Administration
ODEs	Ordinary Differential Equations
PCA	Pollution Control Agency
ROS	Reactive Oxygen Species
SCS	Soil Conservation Service
SSTS	Subsurface Sewage Treatment Systems
STPs	Sewage Treatment Plants
TBA	Trenbolone Acetate
TMDL	Total Maximum Daily Load
TOC	Total Organic Content
USCB	United States Census Bureau
USEPA	United States Environmental Protection Agency
USGS	United States Geological Survey
VTG	Vitellogenin
WWTPs	Wastewater Treatment Plants

Chapter 1 Introduction

1.1 Background

Endocrine disruptors (EDs) or endocrine disrupting chemicals (EDCs) can bind to hormone receptors in aquatic organisms and disrupt normal hormone synthesis and metabolism (Birnbaum & Fenton, 2003; Bhandari et al., 2015). As a result, research interest in EDs, and estrogens in particular, has been increasing in recent years (Hutchinson & Pickford, 2002; Daston et al., 2003; Vandenberg et al., 2012). Estrone (E1), 17β-estradiol (E2β), 17α-estradiol (E2α), estriol (E3), and 17α-ethinyloestradiol (EE2) are the most prevalent estrogens found in the natural environment (De Rudder et al., 2004; Soto et al., 2004; Combalbert et al., 2012; Conley et al., 2017). The most apparent risk of estrogens is their effect on the reproductive functions of mammals, birds, fish, reptiles, amphibians and aquatic invertebrates (Tarrant, 2005; Waring & Harris, 2005; Lafont & Mathieu, 2007; Bhandari et al., 2015). Concentrations of E2β as low as 100 ng/L can cause a change of manifestation of the urogenital papillae in male zebrafish gonadal growth (Brion et al., 2004). EE2 concentrations as low as 4.5 ng/L can cause estrogenicity of male rainbow trout and promote the production of vitellogenin (VTG) mRNA and protein for both male and female fathead minnows, trout, and Japanese medaka (Sumpter & Jobling, 1995; Larsson et at., 1999; Kidd et al., 2007; Lei et al., 2013). In addition to fish, EE2 at a half-maximal effective concentration (EC50) dose as low as 1.8 ng/L can also cause feminization and sex reversal of the males of various amphibian species such as wood frogs (Pettersson et al., 2006; Hogan et al, 2008; Berg et al., 2009; Gyllenhammar et al., 2009; Tompsett et al., 2013; Bhandari et al., 2015). β-estradiol 17-valerate has been shown to disturb

the normal hatching processes of Japanese medaka embryos (Lei et al., 2013). There are inconsistencies in the literature as to which estrogens have the greatest ED potencies. For example, Nghiem et al., 2004 reports that E1 and E2β have the largest endocrine disrupting potency. However, Thorpe et al., 2003, Cao & Connell, 2010, and Bhandari et al., 2015 report that EE2 is 10 times more potent than E2β for estrogen receptors (ERs) ESR1 and ESR2 and imposes the highest level of health risks. Despite the inconsistencies as to which estrogens are most potent, all of the literature agrees on the fact that estrogens in the environment are a threat to aquatic health.

Estrogens exist and travel through the environment via a number of different pathways, as shown in Figure 1.1. Livestock such as cattle, pigs, and sheep are generally considered as the major sources of estrogens in the environment (Hanselman et al., 2003; Andaluri et al., 2012; Bartelt-Hunt et al., 2012; Bartelt-Hunt et al., 2013). Livestock excrete estrogens during natural metabolic processes and release them into the natural environment mainly through feces and urine (Palme et al., 1996; Lange et al., 2002; Ying et al., 2002; Raman et al., 2004; Schoenecker et al., 2004; Combalbert et al., 2012; Bai et al., 2015). In the U.K., the estimated average daily excretion of E1 and E2β by a dairy cow through feces and urine is 0.306 µg and 0.140 µg, respectively (Johnson et al., 2006). The dominant natural estrogens excreted by livestock are E2α, E2β, and E1, even though metabolites vary between species (Soto et al., 2004; Lorenzen et al., 2005; Kjær et al., 2007; Andaluri et al., 2012; Combalbert et al., 2012; Bartelt-Hunt et al., 2013). Poultry has also been shown to excrete E1, E2β, and E3 (Zhang, Shi, Liu, Zhan, Dang & Bo, 2014). The annual production of estrogens by cattle, poultry, pigs, and sheep in the U.S is about 45 - 64.9 tons, 3.44, 0.83 -1.2 tons, and 0.092 ton, respectively (Cromwell et al., 1993; Lange et al., 2002; Andaluri et al., 2012). Additionally, steroid hormones such as E2β, E2 benzoate-progesterone,

E2 benzoate-testosterone, E2 benzoate- trenbolone acetate (TBA) and E2- TBA are supplied to farm animals as growth promotants and have the potential to alter the estrogen excretion amount (Biswas et al., 2013). Livestock excretes natural estrogens in both free and conjugated formats (Combalbert et al., 2012; Zhang, Shi, Liu, Zhan, Dang & Bo, 2014; Zhang, Shi, Liu, Zhan & Chen, 2014; Bai et al., 2015). These conjugated estrogens include estradiol-17-sulfate (E2-17S), estradiol-3-sulphate (E2-3S), estrone-3-sulphate (E1-3S), estradiol-3-glucuronide (E2-3G), and estrone-3-glucuronide (E1-3G) and can be converted into other conjugated or free estrogens through hydrolysis and biotic transformation (Isobe et al., 2003; Combalbert et al., 2012; Bai et al., 2015).

Industrial wastewater and domestic sewage are considered another major source of estrogens into the environment (Standley et al., 2008; Johnson et al., 2000; Martinovic-Weigelt et al., 2013; Schultz et al., 2013). For example, manmade drugs such as oral contraceptives that contain synthetic estrogens such as EE2 are ingested by humans, excreted, and wind up in sewage treatment plants (STPs) and wastewater treatment plants (WWTPs) and eventually downstream (Crawford et al., 1990; Lai et al., 2000; Rosenfeldt & Linden, 2004). In addition to STPs and WWTPs, feedlot effluents have the potential to contribute and transport estrogens into water (Soto et al., 2004; Matthiessen et al., 2006; Kjær et al., 2007; Gall et al., 2011; Andaluri et al., 2012).

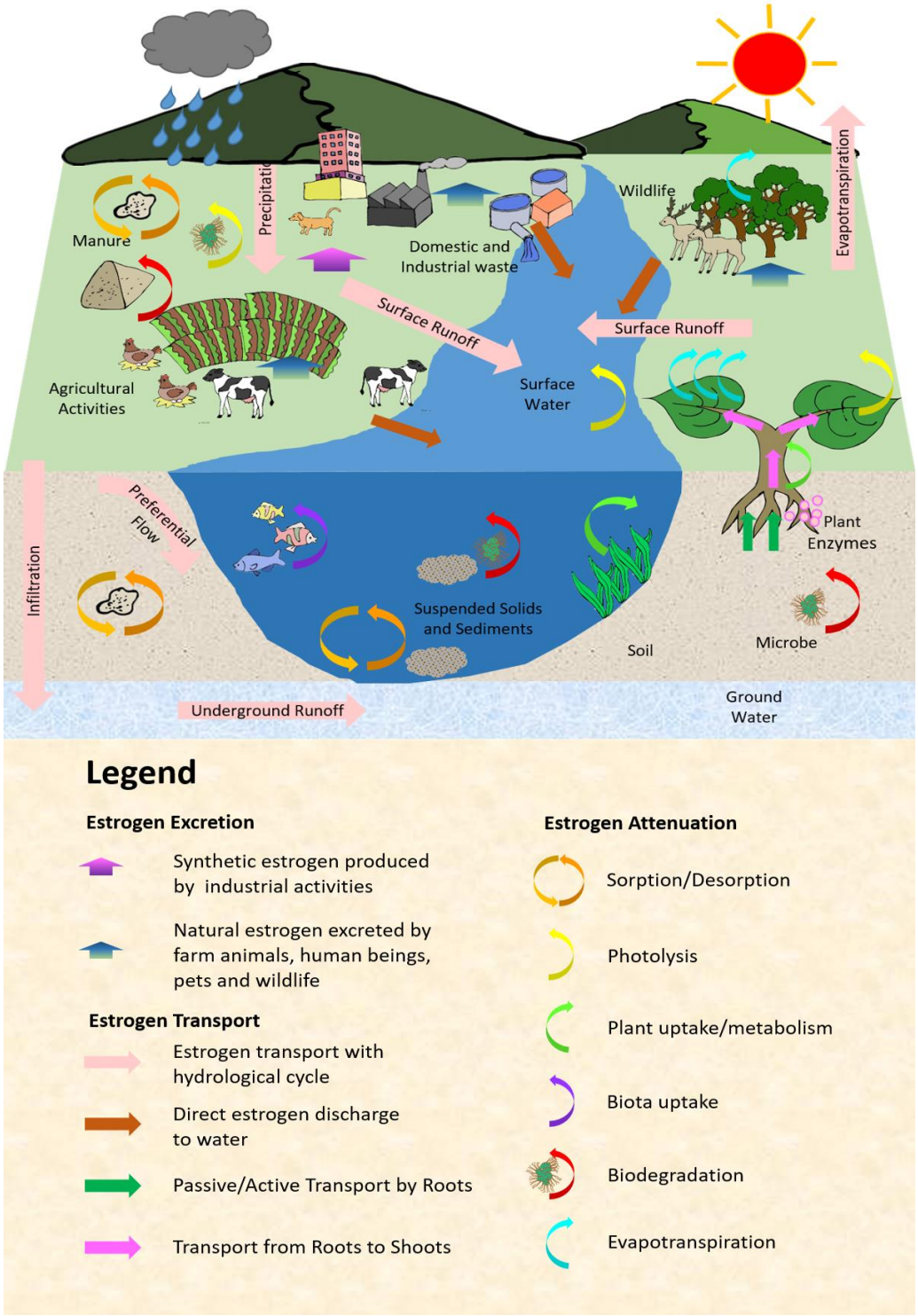

Figure 1.1 *The primary pathways for the occurrence, fate, and transport of estrogens in the natural environment.*

Estrogens are frequently detected in natural water bodies including rivers, estuaries, ponds, lakes, coastal oceans and groundwater across the world (Wicks et al., 2004; Mansell & Drewes, 2004; Zuo et al., 2006; Standley et al., 2008; Singh et al., 2010; Aris et al., 2014; Griffith et al., 2016). Estrogens can enter groundwater sources via improperly managed septic systems or manure storage systems (Fine et al., 2003; Swartz et al., 2006). Estrogens in surface water primarily originate in domestic wastewater and feedlot effluents (Baronti et al., 2000; Jafari et al., 2009; Song et al., 2009; Bartelt-Hunt et al., 2012). Surface waters in China have been observed to contain some estrogens of high concentrations (Peng et al., 2008; Lei et al., 2009; Wang et al., 2012; Yan et al., 2012; Zhang et al., 2012). For example, estrogen concentrations as high as 4100 ng/L have been measured in WWTP effluents in Beijing, China (Zhou, Zha, Xu, et al., 2012; Zhou, Zha & Wang, 2012). Comparatively, the maximum concentrations of E1, E2β, E2α, and E3 measured in cattle feedlot runoff for a study in Nebraska, USA, were 720 ng/L, 540 - 1250 ng/L, 1100-1360 ng/L, and 1050-2600 ng/L, respectively (Bartelt-Hunt et al., 2012). Conversely, estrogen concentrations in rivers are usually less than 10 ng/L due to dilution and attenuation processes (Colucci & Topp, 2002; Lee & Liu., 2002; Lucas & Jones, 2006; Cao & Connell, 2010; Dodgen et al., 2017).

Estrogens are also frequently detected in sludge from WWTPs (Mina et al., 2018). In the U.S., total detected estrogen concentrations have been reported up to 943 ng/g of dry solids from an activated sludge municipal WWTP (Andaluri et al., 2012). The estrogen concentrations measured in primary sludge are usually less than those in secondary and digested sludge (Muller et al., 2010; Martín et al., 2012). For example, in four WWTPs in Spain, the highest concentration of E2β in digested, secondary and primary sludge were 836 ng/g-dry weight (DW), 38 ng/g-DW and 25.4 ng/g-DW, respectively, and the highest concentration of E3 in digested,

secondary and primary sludge were 35.2 ng/g-DW, 23.4 ng/g-DW and 12.3 ng/g-DW, respectively (Martín et al., 2012). Concentrations of E1, E2β, E3 and EE2 in sludge from two STPs in Paris were detected at 5 ± 2 to 43 ± 10 ng/g-DW, 3 ± 1to 10 ± 8 ng/g-DW, 2 ± 2 ng/g-DW, and less than 3 to 5 ± 6 ng/g-DW, respectively (Muller et al., 2010). The highest concentration of E1, E2β, E3 and EE2 detected in sludge from six STPs in China were 13.4 ng/g-DW, 12.3 ng/g-DW, 1.5 ng/g-DW and 5.4 ng/g-DW, respectively (Huang et al., 2014).

Estrogens also exist widely in surface water sediments. A study measuring estrogen concentrations in sediments from 3 rivers in Tianjin, China found that the concentrations of E1, E2β, E3, and EE2 ranged from 0.98 to 21.6 ng/g-DW, below detection limit to 9.70 ng/g-DW, below detection limit to 7.29 ng/g-DW, and below detection limit to 9.26 ng/g-DW, respectively (Lei et al., 2009). The highest detected concentrations of E1, E2β, and EE2 in water sediments from Xiamen Bay, China were 7.38 ng/g, 2.35 ng/g, and 2.18 ng/g, respectively (Zhang et al., 2009). The highest detected concentrations of E1, E2β, E3, and EE2 in Dianchi Lake sediments were 13.2 ± 3.8 ng/g-W, 5.5 ± 2.3 ng/g-DW, 2.6 ± 2.5 ng/g-DW, and 2.5 ± 2.3 ng/g-DW, respectively (Huang et al., 2013).

Overall, the presence of estrogens in detectable concentrations in the environment and the variety of risks associated with them underscores the need for appropriate ways to best manage the release of estrogenic compounds.

1.2 Motivation and Objectives

In order to minimize the potential risks caused by estrogens to aquatic animals, it is quite important to assess variation patterns of estrogens in the surface water in order to mitigate the

potential health risks caused by those estrogens in aquatic environments and determine the best management practices (BMPs) for estrogen. Even though several studies tried to explore the distribution patterns of estrogens, they all have some limitations. On-site measurement studies usually cannot obtain enough data points to draw a whole picture for estrogen distribution characteristics (Conley et al., 2017). Most measurements of estrogens are conducted in the influents and effluents of WWTPs, as well as the surface runoff from the agricultural land, which are not the living environments of aquatic animals (Atkinson et al., 2012; Gall et al., 2014; Gall et al., 2015). Most measurements of estrogens in the living environments of aquatic animals such as rivers and streams are sporadic, making it difficult to quantify the spatial and temporal variations of estrogens (Soto et al., 2004; Bradley et al., 2009). In addition, the estrogen levels in surface waters are usually quite low, or even below the detection limits, and are thus hard to be accurately measured (Bradley et al., 2009).

Modeling work can effectively help to understand the variation of estrogens in the natural water bodies at low costs. However, most of such studies did not work on multiple estrogens due to the complex interconversion and transformation of estrogens (Johnson, 2010). E1 usually works as an intermediate of E2α and E2β attenuation (Colucci & Topp, 2002; Steiner et al., 2010). Zheng et al., 2012 also observed reversible conversions between E2α and E1, and between E2β and E1. Additionally, E2β is identified as a degradation product of E2α (Lee & Liu, 2002; Robinson et al., 2017). Those processes make the pattern of estrogen levels in streams really complicated. For example, one on-site study conducted in Minnesota tried to measure the attenuation rates of E1 and E2β in Redwood River. However, they observed an increase rather than a decrease of E1 levels, which is caused by the conversion of E2β into E1 (Writer et al., 2011). Thus more studies are still needed to address these issues.

Taking the issues listed above, a study to investigate both the spatial and temporal estrogen distributions integrating their excretion, transport, interconversion, and attenuation processes is thus necessary for a better understanding of estrogens. The goal of this study is to develop a quantitative framework to simulate estrogen levels in rivers on a watershed scale integrating these excretion, transport, interconversion, and attenuation processes. This framework is aimed at tracking the fate and transport of estrogens from various sources such as human actives, agricultural activities and other possible sources. This framework can be used to characterize the temporal and spatial variation of estrogens in streams, to explore the impacts of the interconversions on their levels in waters, and to investigate the factors that can lead to high estrogen levels and cause potential health risks to aquatic animals.

1.3 Outline of the Dissertation

The research presented here explains the rationale and methodology of developing a complete model framework to track the fate and transport of estrogens. This dissertation consists of eight chapters. Chapter 1 introduces the hazardous, generation and occurrence of estrogens in surface water and states the motivation and objectives. Chapter 2 reviews the literature related to attenuation and transport of estrogens. Chapter 3 presents the mathematical model to track the attenuation and transformation of estrogens in the natural environment. Chapter 4 presents the mathematical model to track the transport of estrogens from land to surface water by surface runoff. Chapter 5 presents the development of the model framework to track the fate and transport of estrogens on a water scale integrating their generation, attenuation/transformation, and transport using Hydrological Simulation Program – FORTRAN (HSPF) program. Chapter 6 provides an application of the model framework to a single estrogen, E2β, in the South River

Watershed, Virginia. Chapter 7 provides an application of an application of the model framework to multiple estrogens, E1, E2α, and E2β, in the Redwood River Watershed, Minnesota. Chapter 8 discusses the conclusions and recommendations for future research.

Chapter 2 Literature Review

2.1 Attenuation and Transformation Process

2.1.1 Adsorption

Estrogens can quickly sorb to soil and the apparent sorption equilibrium is observed to reach within a few hours (Lee et al., 2003). Estrogen sorption behaviors are determined by both their physicochemical properties and the soil or sorbent type (Ying et al., 2002; Casey et al., 2003; D'Alessio et al., 2014). In soils, estrogen adsorption rates increase with higher moisture content and temperature (Colucci et al., 2001). Additionally, estrogens typically have both low water solubilities and low K_{ow} values, meaning that they are hydrophobic and have a high potential to bind to sediments in water (Nghiem et al., 2004). Yu et al., 2004 and Brett et al., 2014 observed that 80 - 90% of E1 and EE2 can be adsorbed to suspended solids within one day and reach a steady-state within ten days. In addition to solid particles, estrogens can also be adsorbed to humic acids (HAs) in water (Chowdhury et al., 2011). Guo et al., 2018 also observed that the sorption of EE2 onto sediments is affected by rhamnolipidic biosurfactants. The presence of saline compounds such as NaCl induces flocculation and aggregation, which can promote sorption processes (Lai et al., 2000; De Mes et al., 2005). Estrogen sorption capacity is also positively related to total organic carbon (TOC) content as the sorption occurs through hydrogen bonding reactions between organic carbon and estrogen compounds (Lai, et al., 2000; Nghiem et al., 2004; D'Alessio et al., 2014).

The Freundlich sorption isotherm (Equation 2.1) is commonly used to describe the adsorption of estrogens to soils and sediments (Casey et al., 2005; Bai et al., 2015):

$$S_e = K_f C_e^{\,N} \tag{2.1}$$

where C_e and S_e are aqueous and solid concentrations at equilibrium, respectively, N is a dimensionless constant, and K_f is the Freundlich's constant. When $N = 1$, the sorption is a linear process, and K_f equals K_d, which is the partition coefficient; when $N > 1$, the sorption is a non-linear process; and when $N < 1$, the sorption process is limited (Lai et al., 2000; Bai et al., 2015). Many studies observed that the sorption of estrogens follows a non-linear pattern (Lai et al., 2000; Casey et a., 2003; Yu et al., 2004). However, some studies have found the linear model to accurately describe the estrogen sorption process (Casey et al., 2003; Casey et al., 2005). Typically, a larger octanol-water partition coefficient (K_{ow}) and a larger K_f indicate more significant sorption to particles. Casey et al., 2005 and Andaluri et al., 2012 observed log K_{ow} ranges for E2α, E2β, and E1 of 3.4-4.0, 2.59-3.29, 2.82-3.32, respectively and a log K_{ow} for 17α-dihydroeuilin of about 6.21. The similar log K_{ow}-values indicate that E1 and E2β have similar water solubility and therefore, similar parameter values for sorption (Jürgens et al., 2002).

2.1.2 Biotransformation

Estrogens can be chemically degraded or transformed via microbial interactions in a process known as biotransformation in both soils and water (Colucci et al., 2001; Mashtare et al., 2013). Historically, estrogens have often been observed to biotransform rapidly without an observed lag phase, even at very low concentrations (Colucci & Topp, 2002; Lee et al., 2003). Estrogens can be biotransformed by microbes under aerobic, anoxic, and anaerobic conditions in both soils and water (Colucci et al., 2001; De Mes et al., 2005; Mashtare et al., 2013). Usually, estrogen biotransformation rates and efficiencies under aerobic conditions are usually much higher than

those under anaerobic conditions (Lee & Liu., 2002; De Mes et al., 2005; Carr et al., 2011; Robinson et al., 2016). In the natural environment, aerobic biotransformation of estrogens is usually much greater than anaerobic biotransformation (Bradley et al., 2009). In addition to oxygen availability, biotransformation rates are also affected by estrogen properties, the initial estrogen concentration, temperature, moisture content, and biological activity in a particular environment (Zheng et al., 2012; Stadler & Love, 2016). The biological activity, which is assessed via biomass concentration and organic matter measurements, is the most important factor affecting the biotransformation rate (Lee & Liu., 2002; Xuan et al., 2008; Robinson et al., 2016). Zheng et al., 2012 observed the largest estrogen decay rate to occur at 35°C, at which temperature the biological activity was the greatest. The biotransformation rate also increases with higher moisture content (Xuan et al., 2008). At present, most studies on biotransformation of estrogens focus on WWTP processes. In a WWTP in Japan, Ermawati et al., 2007 reported that anaerobic biological treatment removed 80% of hormones while aerobic biological treatment removed up to 95% of hormones from the wastewater. The actual biotransformation rates and efficiencies in natural water are assumed to be lower than those measured in WWTPs due to lower microbial densities. It is also worth noting that biotransformation in sediments occurs mainly via combined biotransformation and sorption processes since the microbes that biotransform the estrogens also grow and attach to sediment surfaces (Bradley et al., 2009; Robinson et al., 2016).

Elucidation of the pathways for estrogen biotransformation reveals that many estrogen compounds are readily interconverted into other estrogenic compounds. Colucci & Topp, 2002 and Lee & Liu., 2002 investigated the biotransformation pathways of free estrogens and determined that E1 is an intermediate of E2β biotransformation. They observed that

microorganisms first convert E2β into E1 and then further degrade E1 into CO_2 or other polar compounds (Fan et al., 2007; D'Alessio et al., 2014; Huang et al., 2016). E1-3S, E3, 16α-hydroxyE1, 2-methoxyestradiol, 2-methoxyE1 and a lactone are also identified as biotransformation products of E2β (Goeppert et al, 2014). E3 is also identified as a biotransformation metabolite of E1 and E2α (Xuan et al., 2008; Li et al., 2013). However, these metabolites are unstable and can be further degraded by microorganisms (Lee & Liu., 2002). As a result of the frequent conversion of E2β to E1 during biotransformation, E2β typically exhibits higher biotransformation rates than E1 under the same conditions (Jürgens et al., 2002; Casey et al., 2005; Zheng et al., 2012). Under anaerobic conditions, E1, E2β, and E2α have also been observed to biotransform into one another (Mansell et al., 2011; Zheng et al., 2012). Under both aerobic and anaerobic conditions, Robinson et al., 2016 observed that E2α can be biotransformed into E1, E2β, and E3. Overall, pathways for estrogen biotransformation are complex due to the various intermediates and final products that depend on many factors.

Some studies have also elucidated the mechanisms for some estrogen biotransformations. Lee & Liu., 2002 reported that E2β biotransformation by sewage bacteria appeared to initiate at the hydroxy group at C-17 (ring D) of the molecule while Bradley et al., 2009 and Yu et al., 2013 observed that biotransformation of estrogens involves cleavage of the molecule's A-ring. Yu et al., 2013 proposed four microbial degradation pathways for E2β: 1) hydroxylation of the A-ring at C-4; 2) hydroxylation of the saturated ring; 3) dehydration of D-ring at C-17; and 4) dehydrogenation of D-ring at C-17. Likewise, Yu et al., 2013 also proposed five microbial degradation pathways for EE2: 1) A-ring C-2 hydroxylation; 2) A-ring 3-OH conversion to 3-keto; 3) B-ring C-6 hydroxylation; 4) D-ring C-17 conversion to keto; and 5) conjugation of EE2.

In addition to free estrogens, biotransformation also involves conjugated estrogens, which are estrogen that conjugated with glucuronide and/or sulfate groups, are converted into other conjugated or free estrogens through hydrolysis and biotic transformations (Combalbert et al., 2012; Bai et al., 2015). Bai et al., 2013 observed that E2-17S can be hydrolyzed into mono-E2-17S (OH-E2-17S) and di-hydroxy E2-17S (diOH-E2- 17S). In agricultural soils in New Zealand, Scherr et al., 2008 and Scherr et al., 2009 observed that E2-3S is first converted into E2β and E1-3S and then converted into E1 with the catalysis of arylsulphatase. Similarly, E2-3G is first converted into E1-3G and then converted into E1 and E2β. D'ascenzo et al., 2003 assessed biotransformation of conjugated estrogens in Italy and concluded that the deconjugation process is prevalent in sewage transport. Generally, the deconjugation rate is affected by the initial conjugated estrogen concentration and the conjugate moiety and sulfate conjugated estrogens are more resistant than glucuronide ones (Gomes et al., 2009; Bai et al., 2013). As a primarily biotic process, the deconjugation rate is also affected by temperature with the highest deconjugation rate observed at about 35°C (Scherr et al., 2008; Scherr et al., 2009; Zheng et al., 2013). In addition to the transformation of conjugated estrogens into free estrogens, free estrogens are also converted into conjugated formats (Shrestha et al., 2012). Goeppert et al., 2015 first proposed that microbes can convert E2β into E1 followed by E1 into E1-3S and later verified the assertion (Goeppert et al., 2017). In general, conjugated estrogens are more recalcitrant to biodegradation than free estrogens (Goeppert et al., 2015; Ben et al., 2017). Figure 2.1 summarizes some of the observed pathways for estrogen biotransformation reported in the literature.

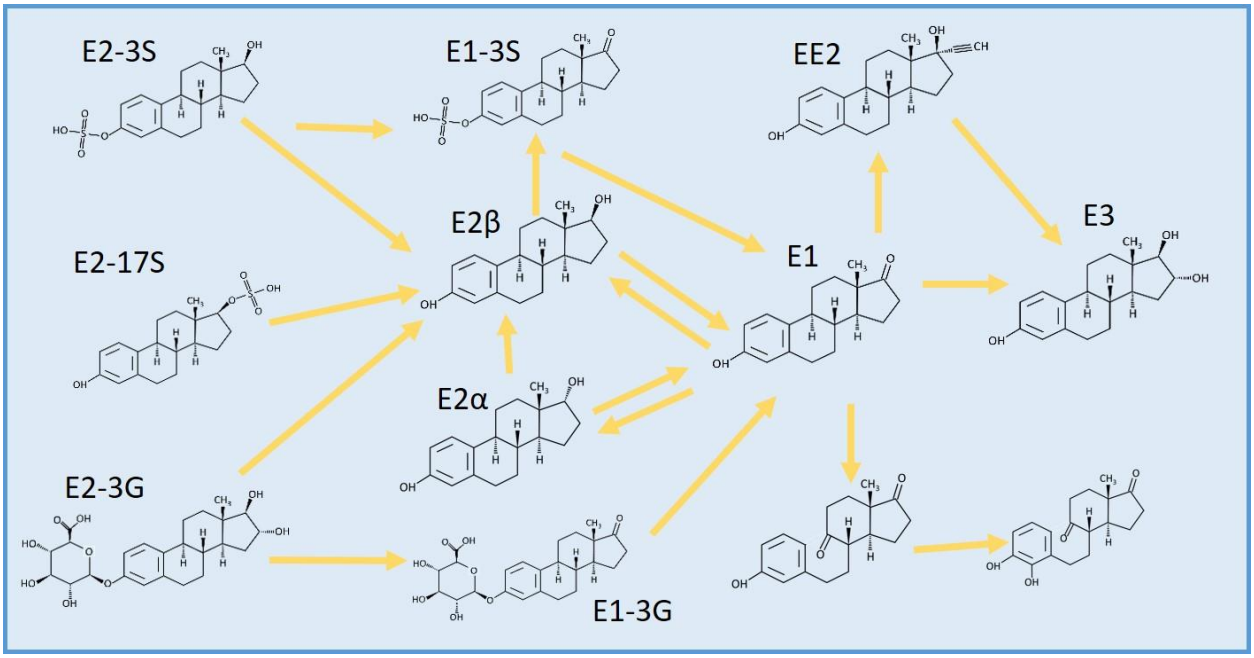

Figure 2.1 *Observed pathways for estrogen biotransformation as reported in previous studies.*

Fungi have also been shown to effectively biotransform estrogenic compounds. For example, Cajthaml et al., 2009 determined eight ligninolytic fungal strains including *Irpex lacteus 617/93*, *Bjerkandera adusta 606/93*, *Phanerochaete chrysosporium ME 446*, *Phanerochaete magnoliae CCBAS 134/I*, *Pleurotus ostreatus 3004 CCBAS 278*, *Trametes versicolor 167/93*, *Pycnoporus cinnabarinus CCBAS 595*, and *Dichomitus squalens CCBAS 750* can effectively degrade EE2. Blánquez & Guieysse, 2008 also observed *Trametes versicolor* to be effective in removing E2β and EE2. The enzymes contained in fungi may play an important role in the estrogen biotransformation process, as they can reduce their estrogenic potencies. For example, Suzuki et al., 2003 and Tamagawa et al., 2006 found ligninolytic enzymes from white rot fungi to be effective in removing the estrogenic potencies of E1, E2β, and EE2.

2.1.3 Photo-transformation

Photo-transformation processes, whereby a compound is transformed via a direct or indirect transfer of light energy, can also play an important role in estrogen removal in sunlit environments (Lin et al., 2005; Qu et al., 2012; Chen et al., 2013). Direct photo-transformation occurs when photons of a specific energy are absorbed by a compound and its impact depends on both the light absorption rate and the reaction quantum yield of the excited state of the compound (Whidbey et al, 2012). Indirect photo-transformation occurs when free radicals are produced from photosensitizers such as natural organic substances and mediate the photo-transformation reactions (Chowdhury et al., 2010). Indirect photo-transformation is generally thought to play a more important role than direct photo-transformation in estrogen degradation (Caupos et al., 2011; Writer et al., 2011). For example, Caupos et al., 2011 observed 60% removal of E1 via indirect photo-transformation. Conversely, Chowdhury et al., 2010 observed 67% removal of E1 via direct photo-transformation, which indicates that direct photo-transformation was more important than indirect photo-transformation for degradation of E1. Photo-transformation of estrogenic compounds is dependent on the available wavelengths of light energy and their specific light absorbances at those wavelengths.

The potential pathways of photo-transformation of estrogens have also been investigated. Whidbey et al., 2012 observed that E1, E2β, and EE2 generate inactive products of the phenol moiety through indirect photo-transformation and E2β and EE2 also generate inactive compounds through direct photo-transformation. Conversely, Whidbey et al, 2012 observed the generation of other estrogenically potent compounds during direct photo-transformation of E1, which were primarily identified as lumiE1. Leech et al., 2009 postulates that indirect photo-

transformation of E2β is caused by reactive oxygen species (ROS) formed by photo-transformation with dissolved organic carbon (DOC) (Leech et al., 2009; Caupos et al., 2011):

$$E2\beta + O_2 + h\nu \rightarrow \text{Products} + \text{ROS}$$

$$\text{ROS} + E2\beta \rightarrow \text{Products}$$

Photo-transformation of estrogens generates photo-products that are related to the hydroxylation of estrogens (Mazellier et al., 2008; Puma et al. 2010; Caupos et al., 2011; Chen et al., 2013). Mazellier et al., 2008 observed more than nine primary and secondary products of photo-transformation of E2β and EE2, which correspond with hydroxylated phenolic- or quinone-type compounds. Caupos et al., 2011 identified one direct photo-transformation product of E1 and four other DOC photo-induced (indirect) transformation products. They identified the major degradation product of E1 as an isomer of E1. During their observed photo-transformation of E1, the steroid moiety changed, but the aromatic moiety remained intact. In a study of photo-transformation of E2β, Chowdhury et al., 2011 inferred that the aliphatic rings of the compound were resistant to degradation while the aromatic ring was easily broken. Figure 2.2 presents some of the pertinent photo-transformation metabolites of estrogens.

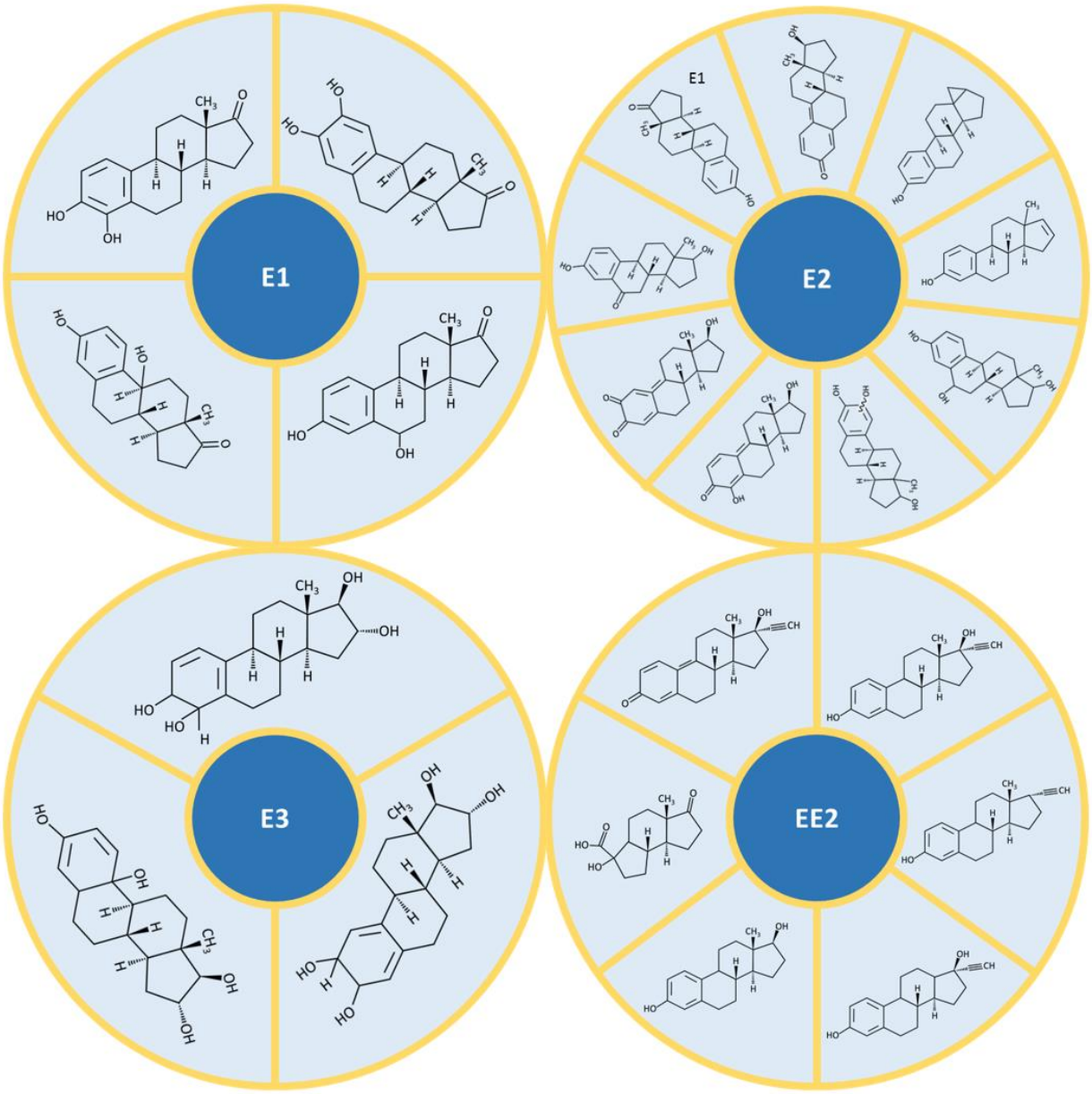

Figure 2.2 *The major identified metabolites of estrogen photo-transformation reported in the literature.*

Estrogen photo-transformation is also impacted by the presence of other chemical compounds in a system. For example, dissolved organic carbon, Fe^{3+}, TiO_2, H_2O_2, and HA can greatly enhance estrogen photo-transformation rates and efficiencies by generating OH·, a ROC that reacts with estrogens (Rosenfeldt & Linden, 2004; Feng et al., 2005; Leech et al., 2009; Chowdhury et al.,

2010; Puma et al., 2010; Chowdhury et al., 2011). However, Chowdhury et al., 2011 observed inhibition of E2β photo-transformation at HA concentrations higher than 8 mg/L. Feng et al., 2005 hypothesized a photo-transformation pathway for E1 due to OH radicals: first, reactions between OH radicals and E1 are initiated at the aromatic ring; second, the ring is cleaved off; and third, the organic molecule is mineralized.

Estrogen photo-transformation rates are also largely dependent on physical experimental conditions. The photo-transformation rate typically decreases when the initial concentration of an estrogen is high (Chowdhury et al., 2010; Chowdhury et al, 2011; Chen et al., 2013). Estrogen photo-transformation rates are also affected by pH and are typically greatest at a pH of 7 (Leech et al., 2009; Chowdhury et al., 2010; Chowdhury et al., 2011). The photo-transformation rate of E2β in alkaline conditions is slower than that in acidic conditions, while the rates of E1 and E3 in acidic conditions are slower than those in alkaline conditions (Chowdhury et al., 2010; Chowdhury et al., 2011; Chen et al., 2013). Light intensity is another factor impacting both photo-transformation rate and efficiency (Leech et al., 2009). Chowdhury et al., 2010 and Chowdhury et al., 2011 observed that the photo-transformation rate is proportional to light intensity for E1 and proportional to the square root of light intensity for E2β. The turbidity of water, which affects the light penetration, can also affect the photo-transformation rate of estrogens (Chowdhury et al., 2011). Maximum estrogen photo-transformation is greatest under full sunlight with UV-B (290-320 nm) typically occurs at the water surface and decreases with depth (Leech et al., 2009). Indirect photo-transformation via ROS generated by UV-A (320–400 nm) and visible light (400–720 nm) predominates in deep water (Leech et al., 2009). Puma et al., 2010 observed more rapid degradation of E1, E2β, EE2, and E3 via UVC (100–280 nm) wavelengths than UVA (315–400 nm) wavelengths.

2.2.4 Vegetation Uptake

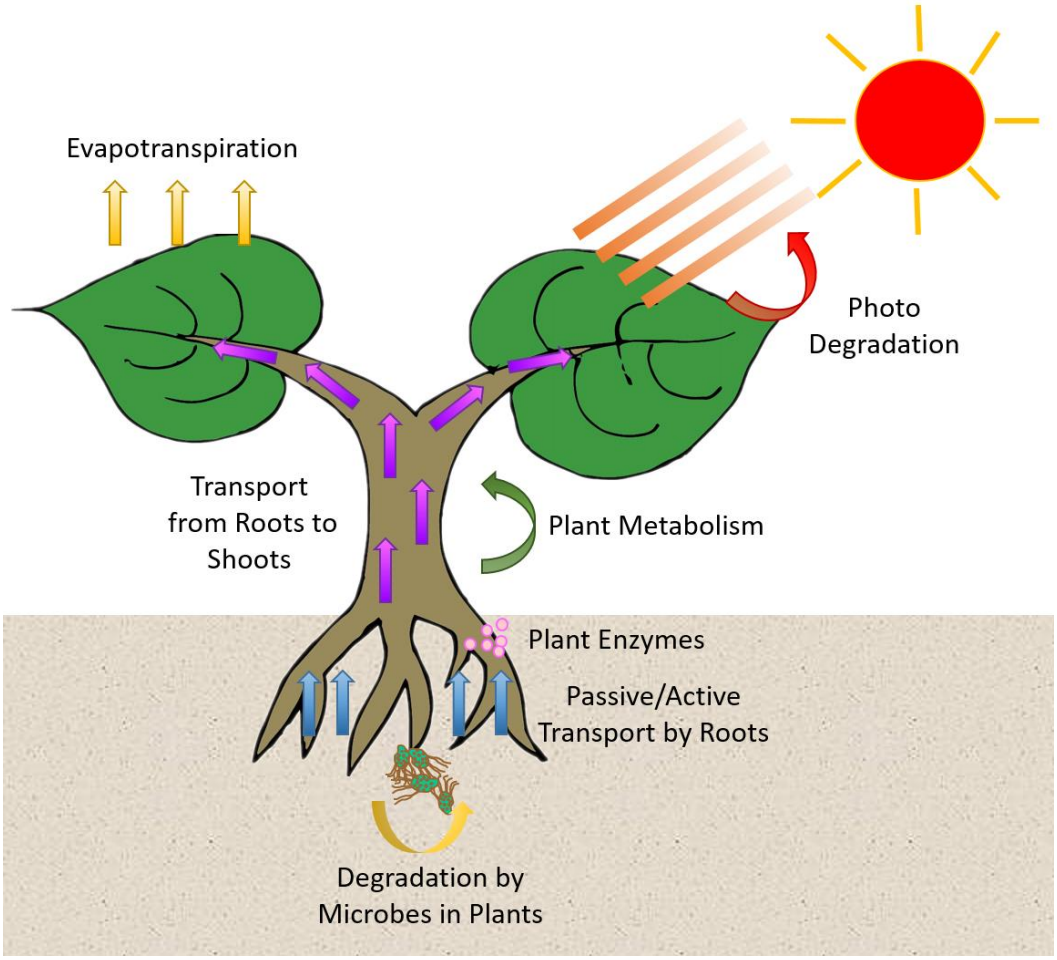

Figure 2.3 *Vegetation uptake of estrogens.*

Estrogens released into soils and streams can be assimilated by vegetation. Currently, there are few studies focusing on vegetation uptake of estrogens. Sabourin et al., 2012 performed an assessment in Canada on the vegetative uptake of estrogens and did not find detectable estrogen concentrations in sweet corn, carrots, tomatoes or potatoes. However, the accumulated estrogens may still be present at concentrations that are below the detection limit. Card et al., 2012 observed that E1 and E2β can be effectively transported to root tissues of maize seedlings from

autoclaved hydroponic solutions originally containing 2μM E1 or E2β. They detected both E1 and E2β in root tissues, but E2β was only detected in shoot tissues, indicating that the plant enzymes can promote estrogen transformation. They also reported half-lives for E1 and E2β of 1.44 days and 1.26 days via maize seedlings uptake, respectively. Bircher et al., 2015 found that poplars can rapidly absorb E2β and EE2 from aqueous solutions and transform them into E1 and E3. Imai et al., 2007 reported that in Japan, *portulaca oleracea* can effectively remove E2β and other phenolic estrogenic compounds from water. They indicated that the removal ability of *portulaca* was unaffected by E2β concentrations below 250 μM, sunlight, temperatures ranging from 15°C to 30°C, or pH ranging from 4 to 7 (Imai et al., 2007). In summary, estrogens are first absorbed by the roots through passive and active transport processes and travel upward to the shoots and other parts of the plant, as depicted in Figure 2.3 (Collins et al., 2006; Adeel et al., 2017). Some of the organic compounds assimilated by vegetation can be further degraded by plant metabolisms, but others are photo-transformed on the surface of leaves (Collins et al., 2006).

2.2 Transport of Estrogens from Land to Surface Water

Widespread manure and biosolids land application processes allow the estrogens present in manure to contaminant soils (Lorenzen et al., 2004; Khanal et al., 2006; Shargil et al., 2015). About 50% of estrogens are sorbed in the top 10 cm of soils and can persist for at least 4 months following land application (Sangsupan et al., 2006; Langdon et al., 2014). Following land application, estrogens can then be transported into nearby water bodies during hydrological events and through advection (Manshell et al., 2011; Chambers et al., 2014). Surface runoff during rainfalls is a major mechanism in the transport of estrogens from land to water. The

highest estrogen concentrations are usually detected during the first storm event following an animal manure land application (Shore, 2009; Gall et al., 2011). Subsurface tile drains installed in agricultural land where manure is applied receive particularly high loads of estrogens during storm events (Kjær et al., 2007; Gall et al., 2014). In addition to surface runoff, irrigation can promote the transport of estrogens from soils to water (Durán–Álvarez et al., 2014). Preferential flow and pronounced macropore flow are also important mechanisms in the transport of estrogens from soils to aquatic environments (Sangsupan et al., 2006; Kjær et al., 2007). Additionally, feedlot runoff can also directly deliver estrogens in farm animal wastes into streams and rivers (Soto et al., 2004; Mansell et al., 2011). Estrogens can also filter through soils and enter groundwater (Khanal et al., 2006). D'Alessio et al., 2014 observed rapid transport of estrogens in soils with large particles, limited fines contents, and low total organic carbon (TOC) contents. The transport rate of estrogens is also affected by vegetation cover and tillage (Jenkins et al., 2008; Dutta et al., 2010). However, the effects of these factors on the transport behaviors of estrogens are unclear.

2.3 Modeling Estrogen Attenuation

Models can be used to describe and predict the attenuation of estrogens in the environment. Casey et al., 2005, Goeppert et al., 2014, and Bai et al., 2015 have found that the attenuation of estrogens resulting from individual processes can be described as a pseudo-first-order kinetic model as below:

$$\frac{dC}{dt} = kC \tag{2.2}$$

and

$$k = k_{sorp} + k_{bio} + k_{upt} + k_{direct} + k_{indirect} \quad (2.3)$$

where C_0 is the initial concentration (ng/L), k is the lumped first-order sorption rate, k_{sorp}, k_{bio}, k_{upt}, k_{direct}, $k_{indirect}$ are the first-order decay rates via sorption, biotransformation, plant uptake, direct photo-transformation, and indirect photo-transformation, respectively (day^{-1}), C is estrogen concentrations (ng/L), and t is time (day).

Although the pseudo-first-order kinetics model can closely simulate the attenuation process of estrogens at low concentrations, it is not accurate to describe the complex attenuation of estrogens that includes the interconversion processes between estrogenic compounds. Colucci & Topp, 2002 and Steiner et al., 2010 further modified the pseudo-first-order kinetic model by assuming that E1 is a major intermediate of E2β degradation and that the conversion of E2β to E1 is irreversible. Therefore, the concentration of E1 at a certain time is determined by both the E2β βtransformation rate and the E1 attenuation rate, as shown in Equation 2.4:

$$\frac{dC_{E1}}{dt} = -k_{E1}C_{E1} + k_{E2}C_{E2} \quad (2.4)$$

where k_{E1} is the first-order attenuation rate of E1 (day^{-1}), k_{E2} is the first-order attenuation (conversion) rate of E2β (day^{-1}), and C_{E1} and C_{E2} are concentrations of E1 and E2β, respectively. Although this model includes the conversion of E2β to E1 during the attenuation process, it has several limitations. First, this model assumes that all of the E2β degrades into E1 before further degradation. Second, it assumes that the conversion of E2β to E1 is irreversible and does not consider the conversion process of E1 to E2β. Third, this model does not include E2α. To address these issues, Zheng et al. 2012 further developed the attenuation model of estrogens to describe the reversible conversion process among E2α, E2β, and E1 as follows:

$$\frac{dC_{E2\alpha}}{dt} = -k_{E2\alpha,E1}C_{E2\alpha} + k_{E1,E2\alpha}C_{E1} \tag{2.5}$$

$$\frac{dC_{E2\beta}}{dt} = -k_{E2\beta,E1}C_{E2\beta} + k_{E1,E2\beta}C_{E1} \tag{2.6}$$

$$\frac{dC_{E1}}{dt} = k_{E2\alpha,E1}C_{E2\alpha} + k_{E2\beta,E1}C_{E2\beta} - k_{E1,E2\alpha}C_{E1} - k_{E1,E2\beta}C_{E1} \tag{2.7}$$

where $C_{E2\alpha}$, $C_{E2\beta}$, and C_{E1} are the initial concentration or mass of E2α, E2β (μg), respectively, $k_{E2\alpha,E1}$ is the transformation rate of E2α to E1(day^{-1}), $k_{E2\beta,E1}$ is the transformation rate of E2β to E1 (day^{-1}), $k_{E1,E2\alpha}$ is the transformation rate of E1 to E2α (day^{-1}), $k_{E1,E2\beta}$ is the transformation rate of E1 to E2β (day^{-1}), and t is time (days). Compared to Colucci & Topp's model, Zheng's model includes more estrogen categories and describes more complex attenuation processes. However, it also has limitations. As shown in Figure 2.2, the estrogen transformation and interconversion processes are more complex than those described by Zheng's model. Zheng's model fails to include the degradation processes other than the interconversion between E1, E2α, and E2β. Therefore, the model results in a constant total mass of E1, E2α, and E2β, but the measured data shows a decrease in the total mass of the three estrogens due to attenuation and conversion into other compounds (Zheng et al. 2012). Despite these limitations, Zheng's model provides the methodology for further model development by including the interconversion processes for a greater number of estrogens than previously described. However, a further mathematical model is still needed to address the complex transformation of estrogens.

2.4 Modeling Estrogen Transport from Land to Surface Water

Estrogen transport in the environment can also be described via modeling. Jones et al., 2014a derived a one-dimensional diffusion model to estimate the mass of the metabolite trenbolone acetate (TBA) leaching from manure due to irrigation:

$$L(t) = (\frac{4D}{\pi f})^{1/2} \varphi C_w t^{1/2} \tag{2.8}$$

where L(t) is the area-normalized mass leached (ng/cm^2), C_w is the aqueous equilibrium concentration (ng/cm^3) in manure, D is the steroid diffusivity (cm^2/s), f is the dissolved fraction of TBA metabolites (unitless), φ is the porosity (unitless), and t is the exposure contact time (s). Jones et al., 2014b then further modified this model to estimate the TBA concentration in irrigation runoff:

$$C_r = AMSV^{-1} \left(\frac{4D}{\pi f}\right)^{1/2} \varphi C_m (\frac{1}{\frac{\varphi}{1-\varphi} + K_D}) t^{1/2} \tag{2.9}$$

where Cr is irrigation runoff concentration of TBA (ng/L), A is the interfacial manure/water surface area (cm^2/kg- DW), M is the manure mass excreted onto the land surface (kg-DW/AU), S is the stocking density (AU/ha), V is the applied irrigation volume (L/ha), C_m is the total mass of 17α-TBOH in manure (ng/g-DW), t is the total manure/water contact time (s), and K_D is the manure/water equilibrium partitioning coefficient (cm^3/g). Although these two models were developed for TBAs, they can be adapted for estrogens since TBAs and estrogens are chemically similar. However, those models are complicated and involve multiple parameters. Thus those modeling can be hardly applied to large-scale watersheds.

Lee et al., 2015 developed a model to describe a more complex transport mechanism. This model assumes that the estrogens are classified into adsorbed and dissolved estrogens. The adsorbed and dissolved estrogen masses can be calculated by the Freundlich sorption isotherm, as shown in Equation 2.1 (Bai et al., 2015). The dissolved estrogens are transported through surface runoff, percolate through, and become available in soil water. The dissolved estrogens mass can be calculated using the equation below (Lee et al., 2015):

$$M_w = (\frac{Q}{P})C_e \tag{2.10}$$

where M_w is the dissolved estrogen mass transported from land to water, P is the rainfall depth (mm), and Q is the discharge in units of mm. The adsorbed estrogens are transported via soil transport and can be calculated using the equation below (Lee et al., 2015):

$$M_s = \frac{11.8}{A}(V_t q_t)^{0.56} K L_s C S_p S_e \rho / 100 \tag{2.11}$$

Where M_s is the adsorbed estrogen mass transported from land to water, K, L_S, C, and S_p are the standard soil erodibility, topographic, cover, and supporting practice factors, respectively, A is the field area (ha), V_t is the runoff volume (m^2), q_t is the peak runoff (m^2/s), and ρ is the soil bulk density (g/cm). This considers the sorption of estrogens and assumes that the adsorption is occurring at a steady state. However, those models are also complicated and involve multiple parameters. Thus those modeling can be hardly applied to large-scale watersheds.

Compared to those complicated models, an empirical relationship developed by Gall et al., 2015 is simple. The model is expressed as:

$$M = aQ^b \tag{2.12}$$

where M is the estrogens mass transported by surface runoff (μg), Q is the discharge (L/min), and a and b are unitless constants. Gall et al., 2015 reported values of a ranging from 0.562 to 0.955, and values of b ranging from 0.92 to 0.98 for E1.

The empirical model simplifies the transport of estrogens from land to water by discharge and does not consider the realistic and complex transport mechanisms of estrogens. Compared to other models, this model involves fewer parameters and can be easily adapted to large-scale modeling for estrogens, and are thus more applicable to large-scale modeling. However, this model does not consider the impact of the total estrogen mass on land. The estrogen mass storage on land during storm events can greatly impact the mass transported by the surface runoff and the large mass transported by surface runoff is observed during the first storm event just after the manure land application (Gall et al., 2014). Additionally, a widely used wash-off model has not been adapted to estrogens. Thus, further studies are needed to explore simple transport models for estrogens.

Chapter 3 Attenuation and Transformation of Estrogen

3.1 Development of the Comprehensive Transformation Model

The attenuation and transformation process of estrogens is complex. E1 is a prevalent biodegradation intermediate of E2β and E2α during attenuation and degradation, and E1 is then further degraded into other polar compounds by microorganisms, such as E3 and EE2 (Fan et al., 2007; D'Alessio et al., 2014; Robinson et al., 2017). Reversible conversions between E2α and E1, and between E2β and E1 are also observed (Zheng et al. 2012). Additionally, E2β is identified as a degradation product of E2α, whereas E2α is not identified as a major degradation metabolite of E2β by bacteria (Lee & Liu, 2002; Robinson et al., 2017). Thus, the degradation and transformation of E1, E2α, and E2β can be described through four processes: first-order reversible conversion between E1 and E2α, as well as between E1 and E2β; the irreversible transformation of E2α into E2β; and irreversible degradation of E1 into other compounds. The complex interconversion and degradation processes depicted by this model is illustrated in Figure 3.1.

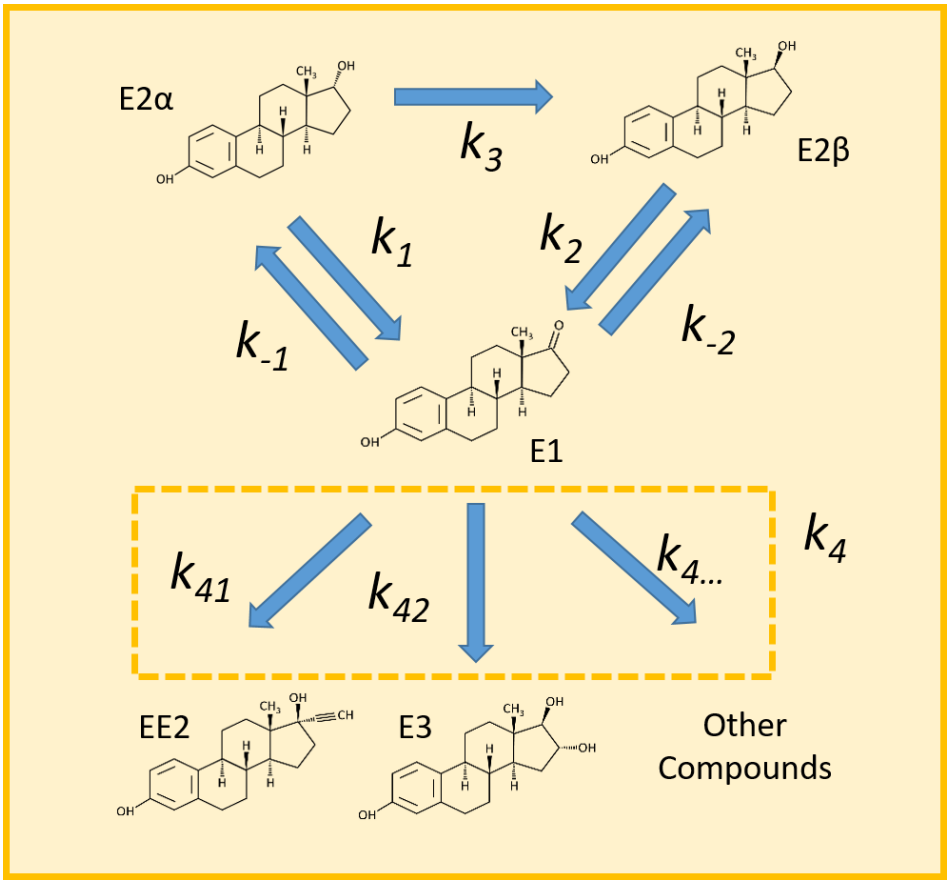

Figure 3.1 *The kinetics and mechanism of the attenuation and degradation of estrogens.*

The transformation and degradation of E1, E2α, and E2β can be described by the three first-order equations as below:

$$\frac{dC_A}{dt} = -(k_1 + k_3)C_A + k_{-1}C_B \tag{3.1}$$

$$\frac{dC_B}{dt} = k_1C_A - (k_{-1} + k_{-2} + k_4)C_B + k_2C_c \tag{3.2}$$

$$\frac{dC_c}{dt} = k_3C_A + k_{-2}C_B - k_2C_c \tag{3.3}$$

where C_A, C_B, and C_C are the concentrations of E2α, E1, and E2β, respectively, t is time, k_1 is the transformation rate constant of E2α to E1, k_{-1} is the transformation rate constant of E1 to E2α, k_2 is the transformation rate constant of E2β to E1, k_{-2} is the transformation rate constant of E1 to E2β, k_3 is the transformation rate constant of E2α to E2β, and k_4 is the transformation rate of E1 to other compounds.

This model assumes that the transformation and degradation of the estrogens occur in stable environments and conditions, such as oxygen supply, temperature, and biomass. Additionally, this model assumes that the transformation rate of estrogens is only affected by the estrogen concentrations and the rate constants. This model also assumes that the rate constants are independent of estrogen concentrations. Equations 3.1 to 3.3 can be solved by a mathematical approach such as the Laplace Transform. In this study, a matrix method was employed to solve this system of ordinary differential equations (ODEs) (Holt, 2012).

Eqs. 3.1 to 3.3 can be rewritten as a matrix format:

$$\begin{pmatrix} \frac{dC_A}{dt} \\ \frac{dC_B}{dt} \\ \frac{dC_c}{dt} \end{pmatrix} = \begin{pmatrix} -k_1 - k_3 & k_{-1} & 0 \\ k_1 & -(k_{-1} + k_{-2} + k_4) & k_2 \\ k_3 & k_{-2} & -k_2 \end{pmatrix} \times \begin{pmatrix} C_A \\ C_B \\ C_A \end{pmatrix} = M_1 \times \begin{pmatrix} C_A \\ C_B \\ C_A \end{pmatrix} \tag{3.4}$$

The eigenvalues of the matrix M_1 can be calculated using the equation below:

$$\begin{vmatrix} k_1 + k_3 - \lambda & -k_{-1} & 0 \\ -k_1 & (k_{-1} + k_{-2} + k_4) - \lambda & -k_2 \\ -k_3 & -k_{-2} & k_2 - \lambda \end{vmatrix} = 0 \tag{3.5}$$

The characteristics polynomial of the matrix M_1 was calculated as below:

$$\lambda^3 - (k_1 + k_{-1} + k_{-2} + k_2 + k_3 + k_4)\lambda^2 + (k_1k_2 + k_1k_{-2} + k_1k_4 + k_2k_3 + k_3k_{-1} + k_3k_{-2} + k_3k_4 + k_{-1}k_2 + k_4k_2)\lambda - (k_1k_4k_2 + k_4k_2k_3) = 0 \quad (3.6)$$

The roots of Equation 3.6 are the eigenvalues of the matrix M_1:

$$\lambda_1 = -\frac{a}{3} + 2\sqrt{\beta}\cos[\frac{arccos\frac{\alpha}{\beta^{1.5}}}{3}] \quad (3.7)$$

$$\lambda_2 = -\frac{a}{3} + 2\sqrt{\beta}\cos[\frac{arccos\frac{\alpha}{\beta^{1.5}}+2\pi}{3}] \quad (3.8)$$

$$\lambda_3 = -\frac{a}{3} + 2\sqrt{\beta}\cos[\frac{arccos\frac{\alpha}{\beta^{1.5}}-2\pi}{3}] \quad (3.9)$$

Where,

$$a = -(k_1 + k_{-1} + k_2 + k_{-2} + k_3 + k_4) \quad (3.10)$$

$$b = k_1k_2 + k_1k_{-2} + k_1k_4 + k_2k_3 + k_3k_{-1} + k_3k_{-2} + k_3k_4 + k_{-1}k_2 + k_4k_2 \quad (3.11)$$

$$c = -(k_1k_4k_2 + k_4k_2k_3) \quad (3.12)$$

$$\alpha = -\frac{(a^3)}{27} - \frac{c}{2} + \frac{ab}{6} \quad (3.13)$$

$$\beta = \frac{a^2}{9} - \frac{b}{3} \quad (3.14)$$

M_1 can be converted into the following equations:

$$(k_1 + k_3 - \lambda)x - k_{-1}y = 0 \tag{3.15}$$

$$-k_1x + (k_{-1} + k_{-2} + k_4 - \lambda)y - k_2z = 0 \tag{3.16}$$

$$k_3x - k_{-2}y + (k_2 - \lambda)z = 0 \tag{3.17}$$

According to Equations 3.15 to 3.17, the relations of x, y, and z can be described by the following equations:

$$y_i = \frac{k_1+k_3-\lambda_i}{k_{-1}} \times x_i \tag{3.18}$$

$$z_i = \frac{k_{-2}(k_1-\lambda_1)+k_{-1}k_3+k_{-2}k_3}{k_{-1}(k_2-\lambda_1)} \times x_i \tag{3.19}$$

where, i = 1,2, and 3.

The initial concentrations of E2α, E1 and E2β are imposed as C_{A0}, C_{B0}, and C_{C0} at t = 0, x, y and z can be solved by the matrix below:

$$\left(\begin{array}{ccc|c} 1 & 1 & 1 & C_{A0} \\ \frac{(k_1+k_3-\lambda_1)}{k_{-1}} & \frac{(k_1+k_3-\lambda_2)}{k_{-1}} & \frac{(k_1+k_3-\lambda_3)}{k_{-1}} & C_{B0} \\ \frac{k_{-2}(k_1-\lambda_1)}{k_{-1}(k_2-\lambda_1)} & \frac{k_{-2}(k_1-\lambda_2)}{k_{-1}(k_2-\lambda_2)} & \frac{k_{-2}(k_1-\lambda_3)}{k_{-1}(k_2-\lambda_3)} & C_{C0} \end{array}\right) \tag{3.20}$$

By row reduction, the solutions of x_1, x_2, and x_3 were calculated to be:

$$x_1 = \frac{C_{C0}k_{-1}(k_2-\lambda_1)(k_2-\lambda_2)(k_2-\lambda_3)}{(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)(\lambda_3-\lambda_1)(\lambda_1-\lambda_2)} + \frac{C_{B0}k_{-1}(\lambda_1-k_2)}{(\lambda_3-\lambda_1)(\lambda_1-\lambda_2)} + C_{A0}[1 - \frac{(k_1+k_3-\lambda_1)(\lambda_1-k_2)}{(\lambda_1-\lambda_2)(\lambda_3-\lambda_1)} - \frac{(k_2-\lambda_2)(k_2-\lambda_3)(k_1k_{-2}-k_{-2}\lambda_1+k_{-1}k_3+k_{-2}k_3)}{(\lambda_1-\lambda_2)(\lambda_3-\lambda_1)(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)}] \quad (3.21)$$

$$x_2 = \frac{C_{C0}k_{-1}(k_2-\lambda_1)(k_2-\lambda_2)(k_2-\lambda_3)}{(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)(\lambda_1-\lambda_2)(\lambda_2-\lambda_3)} + \frac{C_{B0}k_{-1}(\lambda_2-k_2)}{(\lambda_2-\lambda_3)(\lambda_1-\lambda_2)} + \frac{C_{A0}(k_2-\lambda_2)}{(\lambda_1-\lambda_2)(\lambda_2-\lambda_3)}[(k_1+k_3-\lambda_1) - \frac{(k_1k_{-2}-k_{-2}\lambda_1+k_{-1}k_3+k_{-2}k_3)(k_2-\lambda_3)}{(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)}] \quad (3.22)$$

$$x_3 = \frac{C_{C0}k_{-1}(k_2-\lambda_1)(k_2-\lambda_2)(k_2-\lambda_3)}{(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)(\lambda_3-\lambda_1)(\lambda_2-\lambda_3)} - \frac{C_{B0}k_{-1}(k_2-\lambda_3)}{(\lambda_3-\lambda_1)(\lambda_2-\lambda_3)} + \frac{C_{A0}(k_2-\lambda_3)}{(\lambda_3-\lambda_1)(\lambda_2-\lambda_3)}[(k_1+k_3-\lambda_1) - (k_2-\lambda_2)\frac{(k_1k_{-2}-k_{-2}\lambda_1+k_{-1}k_3+k_{-2}k_3)}{(k_2k_{-2}-k_1k_{-2}-k_{-1}k_3-k_{-2}k_3)}] \quad (3.23)$$

Finally, the mass of E2α, E1, and E2β can be expressed as follows:

$$C_A = x_1exp(-\lambda_1t) + x_2exp(-\lambda_2t) + x_3exp(-\lambda_3t) \quad (3.24)$$

$$C_B = \frac{k_1+k_3-\lambda_1}{k_{-1}}x_1exp(-\lambda_1t) + \frac{k_1+k_3-\lambda_2}{k_{-1}}x_2exp(-\lambda_2t) + \frac{k_1+k_3-\lambda_3}{k_{-1}}x_3exp(-\lambda_3t) \quad (3.25)$$

$$C_c = \frac{k_{-2}(k_1-\lambda_1)+k_{-1}k_3+k_{-2}k_3}{k_{-1}(k_2-\lambda_1)}x_1exp(-\lambda_1t) + \frac{k_{-2}(k_1-\lambda_2)+k_{-1}k_3+k_{-2}k_3}{k_{-1}(k_2-\lambda_2)}x_2exp(-\lambda_2t) + \frac{k_{-2}(k_1-\lambda_2)+k_{-1}k_3+k_{-2}k_3}{k_{-1}(k_2-\lambda_2)}x_3exp(-\lambda_3t) \quad (3.26)$$

3.2 Model Validation and Determination of Parameter Values

In order to validate the general application of this model, the model was applied to data measured under various experimental conditions, which are summarized in Table 3.1. Those additional datasets include estrogen levels measured both in anaerobic and aerobic conditions as

well as in aqueous solutions and solids with various initial estrogen concentrations. The simultaneous transformation and degradation processes among various estrogens make it difficult to directly measure the values of rate constants in the Equations 3.24 to 3.26. Instead, those rate values can be estimated by optimizing the fit of the predicted values to the measured data. The measured estrogen concentrations reported by previous studies were fitted to the model in order to derive the values of the attenuation and conversion rates used in the model.

Table 3.1 *The data used to verify the general application of the model.*

ID	Initial E2α conc.	Initial E2β conc.	Initial E1 conc.	Oxygen condition	Tem. (°C)	Medium	Reference
Z-1	5×10^6 ng/L	0	0	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
Z-2	0	5×10^6 ng/L	0	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
Z-3	0	0	5×10^6 ng/L	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
Z-4	5×10^3 ng/L	0	0	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
Z-5	0	5×10^3 ng/L	0	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
Z-6	0	0	5×10^3 ng/L	Anaerobic	35	Aqueous solutions mixed with dairy lagoon water	Zheng et al. 2012
M-1	5×10^4 ng/L	0	0	Aerobic	21 ± 2	Coloma soil	Mashtare et al., 2013
M-2	0	5×10^4 ng/L	0	Aerobic	21 ± 2	Coloma soil	Mashtare et al., 2013
M-3	5×10^4 ng/L	0	0	Aerobic	21 ± 2	Drummer soil	Mashtare et al., 2013
M-4	0	5×10^4 ng/L	0	Aerobic	21 ± 2	Drummer soil	Mashtare et al., 2013
R-1	10^6 ng/L	0	0	Aerobic	20 ± 2	Taunton River water - loam	Robinson et al., 2017
R-2	10^6 ng/L	0	0	Anaerobic	20 ± 2	Taunton River water - loam	Robinson et al., 2017
R-3	10^6 ng/L	0	0	Aerobic	20 ± 2	Weweantic River water - sand	Robinson et al., 2017
R-4	10^6 ng/L	0	0	Anaerobic	20 ± 2	Weweantic River water - sand	Robinson et al., 2017
B-1	0	1.1 ng/g	24 ng/g	Aerobic	6-60	Cattle manure	Bartelt-Hunt et al., 2012

Table 3.2 *The values of degradation/transformation rates derived from the measured data*

ID	Transformation/Degradation rate (day^{-1})						r^2
	k_1	k_{-1}	k_2	k_{-2}	k_3	k_4	
Z-1	0.41 ± 0.0030	0.44 ± 0.00050	0.075 ± 0.00050	0.045 ± 0.0014	0.011 ± 0.00010	0.0086 ± 0.00022	0.92
Z-2	0.041 ± 0.0014	0.012 ± 0.00025	1.3 ± 0.0050	0.37 ± 0.0010	0.026 ± 0.0015	0.013 ± 0.00010	0.94
Z-3	0.074 ± 0.0024	0.013 ± 0.00015	1.12 ± 0.0050	0.24 ± 0.0055	0.015 ± 0.0036	0.0060 ± 0.00015	0.99
Z-4	0.18 ± 0.00050	0.12 ± 0.00050	3.0 ± 0.0050	1.8 ± 0.025	0.018 ± 0.00085	0.018 ± 0.0018	0.95
Z-5	0.050 ± 0.0060	0.028 ± 0.00030	2.2 ± 0.0050	0.92 ± 0.015	0.050 ± 0.0013	0.019 ± 0.00030	0.93
Z-6	0.026 ± 0.0040	0.015 ± 0.0011	0.52 ± 0.013	0.23 ± 0.012	0.048 ± 0.0052	0.013 ± 0.00055	0.96
M-1	2.5 ± 0.050	2.1 ± 0.20	5.3 ± 0.31	6.7 ± 0.25	0.011 ± 0.00065	0.22± 0.0050	0.98
M-2	2.7 ± 0.24	1.09 ± 0.049	2.52 ± 0.057	1.30 ± 0.22	0.071 ± 0.0075	0.18 ± 0.00025	0.97
M-3	3.50 ± 0.090	2.9 ± 0.28	5.9 ± 0.55	7.9 ± 1.85	0.70 ± 0.10	1.1 ± 0.443	0.98
M-4	2.6 ± 0.050	3.1 ± 0.16	3.4 ± 0.015	1.6 ± 0.065	0.10 ± 0.0030	0.98 ± 0.00025	0.96
R-1	0.087 ± 0.005	0.055 ± 0.017	0.12 ± 0.0060	0.032 ± 0.012	0.0042 ± 0.00080	1.7 ± 0.245	0.94
R-2	0.059 ± 0.013	0.13 ± 0.010	0.11 ± 0.0098	0.024 ± 0.015	0.0046 ± 0.00060	0.24 ± 0.036	0.96
R-3	0.0089 ± 0.00035	0.00047 ± 0.00040	0.010 ± 0.00015	0.0037 ± 0.0014	0.0024 ± 0.000065	0.046 ± 0.013	0.99
R-4	0.0075 ± 0.00042	9.6E-07 ± 5.0E-08	0.0080 ± 1.0E-04	4.01E-08 ± 0.00	0.0026 ± 0.00012	0.020 ± 0.00050	0.96
B-1	0.078 ± 0.0010	0.0021 ± 0.0011	0.0036 ± 0.0040	0.0052 ± 5.0E-0.5	0.0014 ± 0.00035	0.049 ± 0.0045	0.84

The transformation and degradation rate constants estimated by each dataset are summarized in Table 3.2. The r^2-values for most of the datasets are high, while the r^2-value for data measured in cattle manure is lower than others. As only one dataset measured in cattle manure was used, and

the low r^2-value for data in solid formats may be random. Despite the relatively low r^2-value for the dataset B-1, the model fits the data well for estrogen transformation under both anaerobic and aerobic conditions as well as in aqueous solutions and solids. The rate constants vary greatly under these different experimental conditions, showing that the transformation and degradation rates change with the environment. This proves that the CTM developed in this study can be applied to estrogen transformation and interconversion in various environments and conditions. Based on the assumption of the model, the datasets Z-1 to Z-6 are expected to derive the same rate constant as they were measured at identical experimental conditions, and datasets Z-2, Z-3, Z-5, and Z-6 induced comparable rate constants.

However, datasets Z-1 and Z-4, which use E2α as the source of estrogen, induce rate constants which are distinct from those of other datasets. This result may be explained by the fact that E2α has additional degradation and transformation pathways that are not investigated in this model. These additional degradation pathways have little impact on the model results when E2α is at relatively low concentrations compared to E1 and E2β. Conversely, these additional pathways are significant when E2α has a relatively larger concentration. The values of k_1 and k_2 are larger than other rate constants, which is consistent with the observed rapid transformation of E2α and E2β into E1. The relatively smaller values of k_{-1} and k_{-2} than those of k_1 and k_2 show that the transformation of E1 into E2α and E2β is not as significant as their corresponding reverse processes. This agrees with the fact that the biodegradation process of E2α and E2β can be estimated using the simple pseudo-first-order kinetics when E1 is at low concentrations. Small values of k_3 imply that the transformation of E2α to E2β is not as significant as that of E2α to E1.

The modeling results with the derived rate constants for each set of data by Zheng et al., 2012 are shown in Figure 3.2. The solid line represents the simulation results by the comprehensive transformation model developed in this study, and the dashed line represents the simulation results by the reversible transformation model adapted by Zheng et al., 2012. The difference between the simulating results of these two models is not significant at the initial stage. However, the reversible transformation model tends to overestimate the estrogen concentrations at later stages. The difference between the two models is apparent for the total estrogens. The reversible transformation model results in a constant total mass of E1, E2α, and E2β over time, while the comprehensive transformation model produces more accurate simulation results for the decreasing total mass by inducing the further degradation of E1 into other compounds. Overall, the comprehensive transformation model generates more precise prediction results for biodegradation of estrogens under anaerobic conditions.

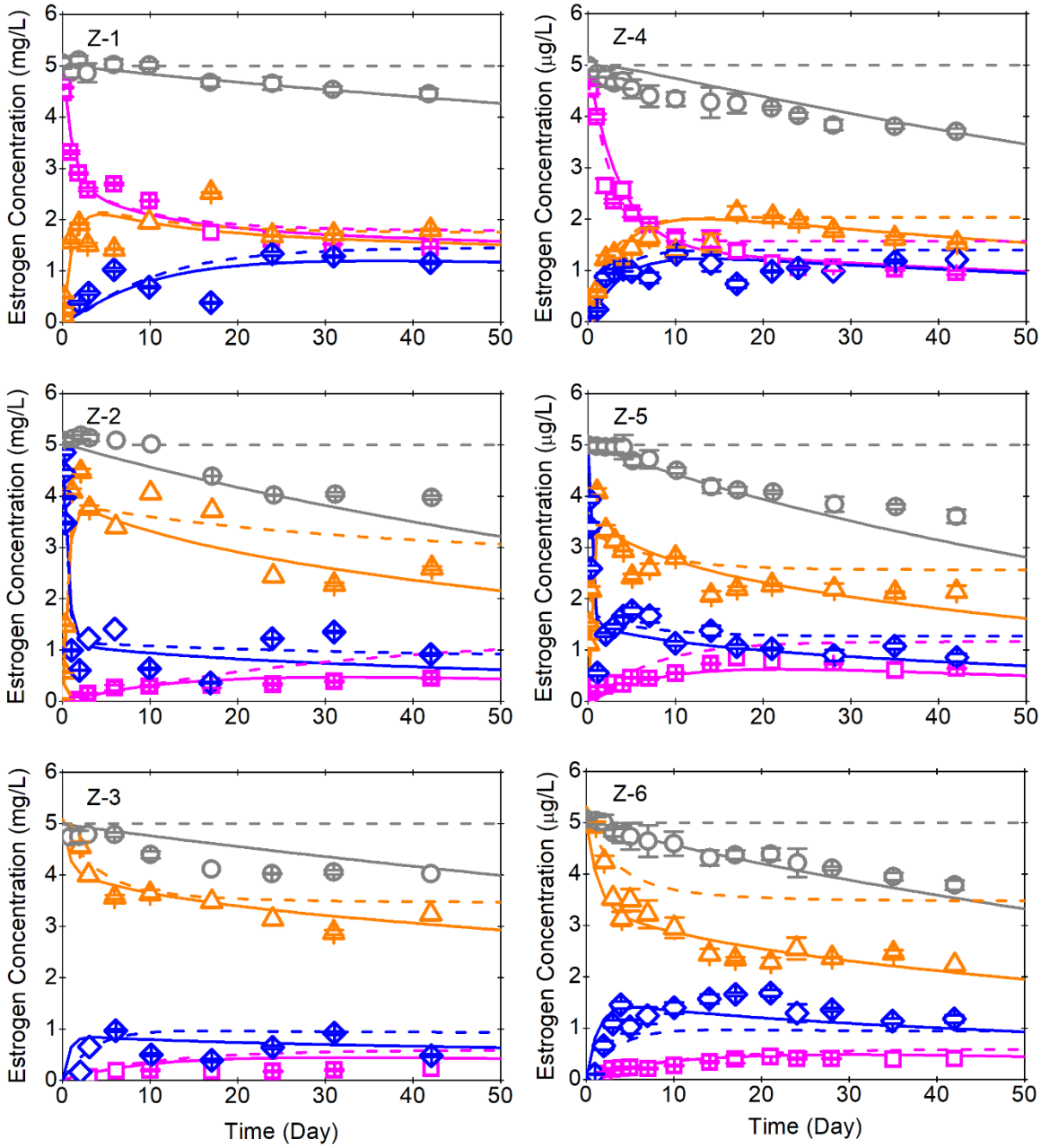

Figure 3.2 *The simulation results from the comprehensive transformation model (solid line) and the reversible transformation model by Zheng et al., 2012 (dashed line). Symbols represent measured data by Zheng et al., 2012 with IDs of Z-1 to Z-6: ○- E1+E2α+E2β, ◊---E2β, □---E2α, ⊿---E1.*

However, the optimization of the model-fitting method can induce the overfitting problem. In order to avoid the overfitting issue, a leave-one-out cross-validation (LOOCV) was employed to evaluate the prediction ability of the comprehensive transformation model (Friedman et al., 2001; James et al., 2013). In the LOOCV, one of the datasets reported by Zheng et al., 2012 was used as the validating data, while the others were considered as calibrating data. The calibrating data was used to fit the model in order to get the largest modeling fitting accuracy, and the validating data was used to evaluate the performance of the model with the derived model parameters. The average rate constants obtained for 5 calibrating datasets were then applied to the validating dataset to estimate the prediction accuracy. The prediction ability for each validating dataset was evaluated using the normalized mean square error (*NMSE*):

$$NMSE = \frac{n\sum_{i=1}^{n}(y_i - x_i)}{\sum_{i=1}^{n} x_i \times \sum_{i=1}^{n} y_i} \tag{3.27}$$

where x_i is the measured value, y_i is the simulated value, and n is the number of the data points in the validating data set.

The process was repeated 6 times until each dataset was used as the validating data. The configuration of the LOOCV is illustrated in Figure 3.3. The prediction ability of the comprehensive transformation model can be estimated by the average value of the *NMSE* for each validating dataset.

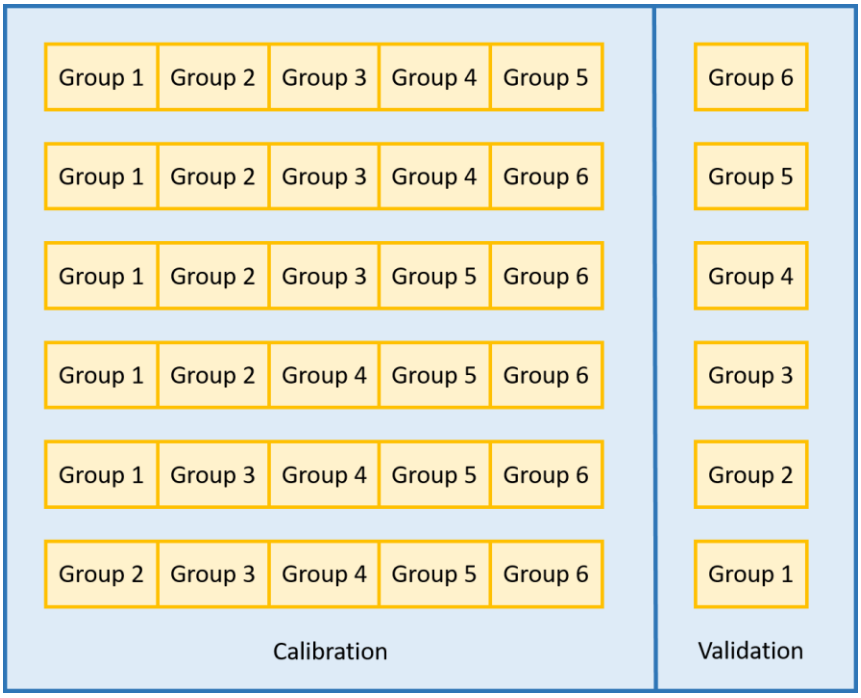

Figure 3.3 *The LOO cross-validation used in the study.*

The results for the LOOCV for data by Zheng et al., 2012 are summarized in Table 3.3. The overall averaged values of *NMSE* and r^2 of all the datasets are 0.201 and 0.776, respectively. These values show that the prediction performance of the comprehensive transformation model is acceptable. However, lower r^2-values of LOOCV than those in Table 3.2 imply that rate constants derived from each dataset varied, rather than staying constant as was expected by the model assumption. The variation of the rate constants at constant experiment conditions and environments may be caused by the uncertainty of the data measurement. Additionally, transformation and degradation rates may be impacted by the estrogen concentrations in the natural environment; these rates are not constants. However, the clear relationship between the transformation rates and the estrogen concentrations cannot be quantified by known mathematical models. Additionally, E2α and E2β have additional transformation and degradation processes which are not described in this comprehensive transformation model (Lee & Liu,

2002; Robinson et al., 2017). For example, E2α can be transformed into E3 in both aerobic and anaerobic conditions, and E2β can be transformed into E3, 16α-hydroxyestone, 2-methoxyestrone, and 2-methoxyestradiol under aerobic conditions (Lee & Liu, 2002; Robinson et al., 2017). Even though the transformation of E2α and E2β into E1 are dominant, these additional transformations can nonetheless induce uncertainties. These additional transformation and degradation processes can be estimated by adding more first-order processes into the model. The analytical solution of the model with more degradation and transformation processes can be derived using the method set up in Section 3.1. The complexity of the solution can be greatly increased by adding new parameters. However, the complex system of OED equations can be easily and quickly calculated by numerical computing programs such as Matlab® and Python.

Table 3.3 *The leave-one-out cross-validation prediction accuracy for each validating dataset.*

Calibrating data	**Validating data**	***NMSE***	r^2
1, 2, 3, 4, 5	6	0.234	0.712
1, 2, 3, 4, 6	5	0.123	0.729
1, 2, 3, 5, 6	4	0.077	0.903
1, 2, 4, 5, 6	3	0.410	0.691
1, 3, 4, 5, 6	2	0.235	0.786
2, 3, 4, 5, 6	1	0.129	0.837
Average		0.201	0.776

3.3 Importance of the Model Parameters

This comprehensive transformation model involves multiple attenuation and conversion rates, which cause difficulties in the application to real cases. Thus, analysis of the importance or significance of those attenuation and conversion rates can indicate the weight of those rate

constants. Correlations, which represent the statistical relationship and association between two random variables, were calculated. The importance or the significance of the parameters to the model results were analyzed using the random forests model. The random forests model is a substantial modification of bagging, which is a technique for reducing the variance of an estimated prediction function. This statistical analytical technique builds a large collection of de-correlated trees and then averages them. Random forests model also use the out-of-bag (OOB) samples to measure the prediction strength of each variable in a model. When a tree is grown, the OOB samples are passed down the tree, and the prediction accuracy is recorded. Then the values for each variable are randomly permuted in the OOB samples, and the accuracy is again computed. In this way, the random forests model can estimate and rank the percentage of the variable importance in model prediction (Friedman et al., 2001; James et al., 2013).

The correlations between the model parameters were analyzed, and the correlation between parameters are shown as a matrix in Figure 3.4. In Figure 3.4, large dark blue circles represent large positive correlations, large red circles represent large negative correlations, and small circles in light colors represent small correlations. For all three estrogens, large positive correlations are observed between k_1 and k_{-1}, and between k_2 and k_{-2}, which are paired reversible rate constants. Large positive correlations are also observed between the initial concentration of the target estrogen and the simulating results, which are denoted by C_t in Figure 3.4. In addition to the initial concentrations, E2α and E2β are also greatly impacted by the transformation rate from E2β to E1, and from E2α to E1. The matrix also shows that the correlation between rate constants and the time is not zero as the model assumes. Instead, they show a weak negative relation, implying that those rates may decrease with time and with lower estrogen concentrations in the solution.

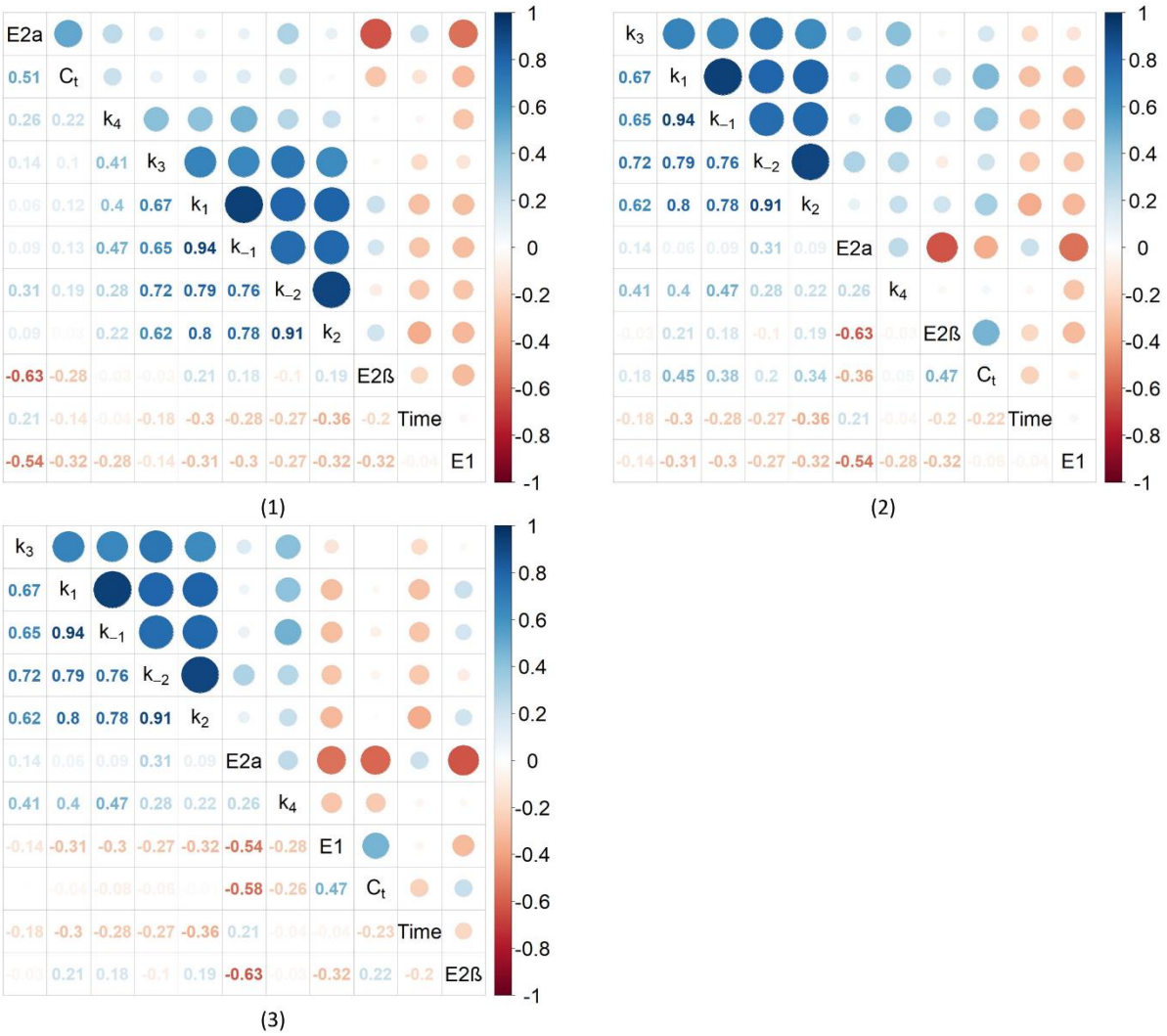

Figure 3.4 *The correlation matrix for estrogens. (1) to (3) are the matrices for E2α, E2β, and E1, respectively. Large circles represent large correlations, and small circles represent small correlations. The colors denote the positive or negative correlation, with red corresponding to negative correlations and blue corresponding to positive correlations. E2α, E2β, and E1 in (1) to (3) represent the initial normalized concentrations of E2α, E2β, and E1, respectively. Ct represents the simulated estrogen concentrations with time.*

The random forests model was performed using Python to analyze and compare the importance and significance of parameters, including time, initial concentrations, and transformation rates, which are listed in Tables 3.1 and 3.2. In the random forests algorithm, trees are developed using random sub-datasets, and the parameter importance analyzed by this method is random. In order to reduce the variation, a large tree number of 1000 was used. The analyzing results for each kind of estrogen are listed in Figure 3.5 in order of decreasing importance. In this analysis, a large portion of data was measured using E2α as the starting compounds, causing bias to the results. As a result, a large importance of $C_{E2\alpha,0}$, k_1, and k_{-1} of the simulation for E2β and E1 may be a result of overestimation. Figure 3.5 shows that time and initial concentrations are significant in the concentration estimation for all three estrogens. The results also show that the transformation rate of E1 to other compounds is not as significant as other transformation processes in estrogen estimation. This indicates that the further degradation processes of E1 to other compounds can be ignored and the model can be simplified when E2α or E2β works as the only target compound. In general, a large data size is essential to generate accurate analytical results in statistics. However, the data size used in this study is not large enough and may cause uncertainties in this parameter-importance analysis.

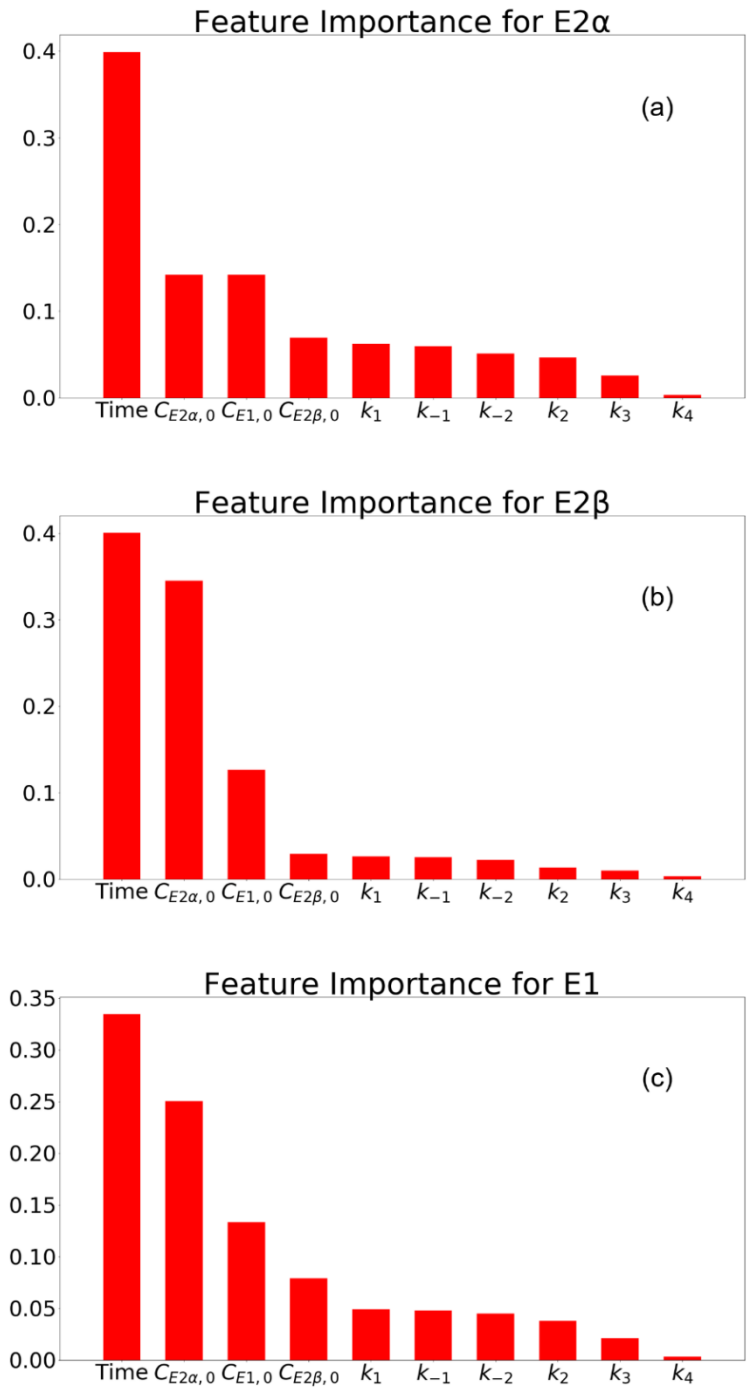

Figure 3.5 *The importance of the parameters in the comprehensive transformation model for E2α, E2β, and E1 calculated using the random forests model.* $C_{E2\alpha,0}$, $C_{E2\beta,0}$ *and* $C_{E1,0}$ *in (1) to (3) represent the initial normalized concentrations of E2α, E2β, and E1, respectively. Ct represents the simulated estrogen concentrations with time.*

Chapter 4 Transport of Estrogens by Surface Runoff

4.1 Development of Transport Models by Surface Runoff

Estrogens contained in manure can get access to surface water following land application. One important transport pathway of estrogens from agricultural land into the water environment is through surface runoff during storm events (Kjær et al., 2007; Jenkins et al., 2008; Dutta et al., 2010; Gall et al., 2011). Studies have proven that the estrogen mass transported by storm events is highly related to the rainfall amount (Yang et al., 2012; Jones et al., 2014a). Rainfall events of high intensities usually induce large estrogen mass loads into water (Kjær et al., 2007; Dutta et al., 2010; Gall et al., 2011).

The wash-off model, which has been widely used for transport of chemicals by surface runoff, is adapted to track the transport of estrogens following rainfall events (Shaw et al., 2009; Hossain et al., 2011):

$$\frac{dS}{dQ} = -k_w S \tag{4.1}$$

Where S is the total mass of estrogen on land during the storm events (ng), k_w is the wash-off coefficient (min L^{-1}), and Q is the surface runoff (L min^{-1}).

Integrating Equation 4.1 yields:

$$M = S[1 - \exp(-k_w \times Q)] \tag{4.2}$$

where M is the total mass of estrogens flushed from the land surface by surface runoff during storm events.

In addition to the wash-off model, an empirical model has been used to estimate the mass of hormones exported during rainfall events (Jones et al., 2014a; Gall et al., 2015):

$$M = c(SQ)^d \tag{4.3}$$

where c and d are dimensionless constants.

4.2 Determination of Parameter Values

The data reported by Yang et al., 2012 was used to evaluate the performance and determine the parameter values of the wash-off and empirical models in this study. Yang et al., 2012 assessed the potential for runoff of hormones and sterols, including androgens, estrogens, and progestogens from three adjacent agricultural test plots (Plots 1, 2 and 3). The area of each plot was 6 m^2 and the soil types of the study area were mainly classified as Vona loamy sand and Vona sandy loam. Yang's study modeled four identical precipitation events with the intensity of 65 mm of one-hour duration 5 days before the biosolids application, as well as 1 day, 8 days and 35 days after the application. From each plot, the runoff rates and the hormone mass loads in surface water flow during artificial events on Day 1, Day 8 and Day 35 were measured and recorded. Even though these artificial precipitations were of the same intensity, the measured surface flow rate greatly varied due to the variation of antecedent moisture content (AMC). The variations of AMC on these three days were caused by the interference of the natural storm events and the effects of the previous artificial events. In this study, the mass loads of E1, E2β,

and E2α in surface water flow reported on Days 1 and 8 were used to quantify key model parameters, and the mass loads Day 35 was used to validate the models.

The Soil Conservation Service (SCS) rainfall-runoff model was used to estimate surface runoff during the simulated storm events (NRCS, 2010):

$$Q = \frac{[I - 0.2(\frac{2540}{CN} - 25.4)]^2}{I + 0.8(\frac{2540}{CN} - 25.4)} \times A \times 10 \quad (4.4)$$

where I is the measured rainfall intensity (cm/min), Q is the surface runoff (L/min), A is the land area of the agricultural land (m^2), and CN is the curve number, a parameter used in hydrology to predict direct runoff, and CN is a function of the soil type, hydrologic conditions, land use and the soil treatment. The CN values were determined by fitting the SCS model to the measured flow rate curve.

The measured and simulated flow rates using the SCS model are illustrated in Figure 4.1, and the derived CN values are summarized in Table 4.1. Low r^2-values for Day 1 indicates that the SCS model fails to catch the low flows and has a better performance for high flows. Besides, as shown in Figure 4.1, the measured flow rates on Day 8 from Plot 1 showed a sudden decrease after 25 minutes, and this observation cannot be explained by the SCS model. However, the SCS model overall effectively simulates the measured flow rate during the artificial rainfall events with r^2-values higher than 0.90 in most cases.

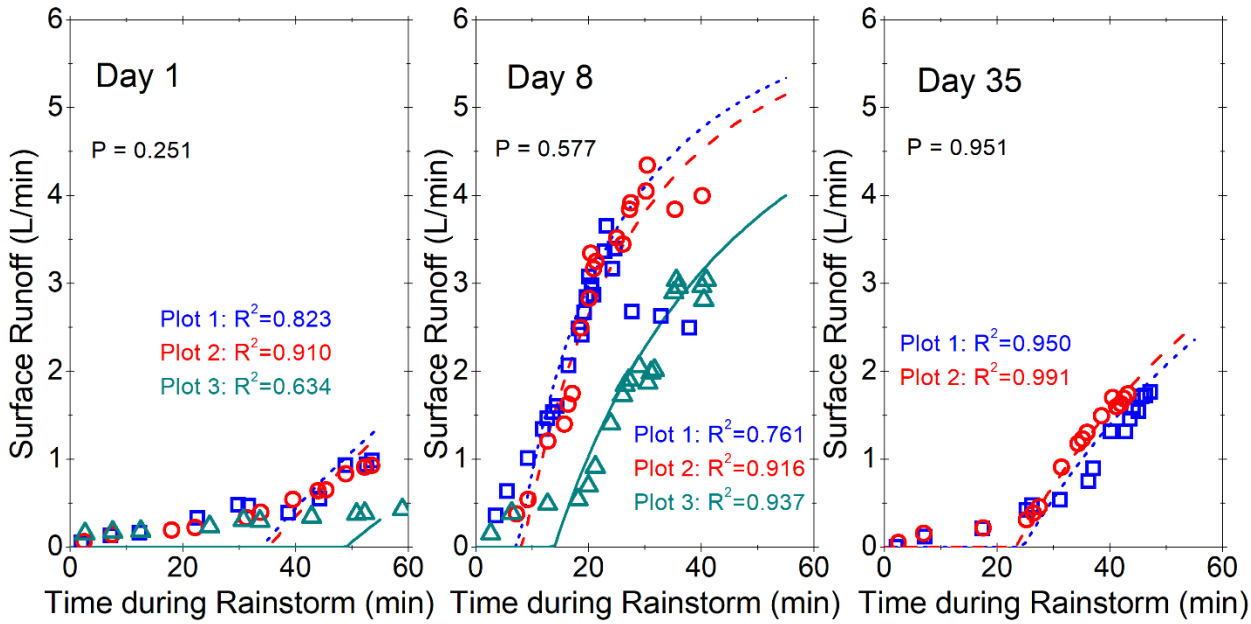

Figure 4.1 *Simulated and measured surface runoff at three plots on Days 1, 8 and 35: the squares, circles, and triangles denote the measured surface runoff at Plot 1, Plot 2 and Plot 3, respectively; the short-dashed, dashed, and solid lines denote the surface runoff simulated using Equation 4.4 at Plot 1, Plot 2 and Plot 3, respectively. [Data from Yang et al., 2012].*

Table 4.1 *The CN Values Estimated by Model Fit. n = not reported.*

Day	Plot 1	Plot 2	Plot 3
Day 1	49	58	58
Day 8	78	67	66
Day 35	86	87	--

The calculated total masses of three estrogens on the agricultural land over time using the model developed by Zheng et al., 2012. The calculated masse are shown in Figure 4.2. The mass of E1 decreases after the biosolids land application, while the masses of E2α and E2β increase as a

result of the E1 transformation. Finally, equilibrium is reached among the three estrogens as they approach a steady state.

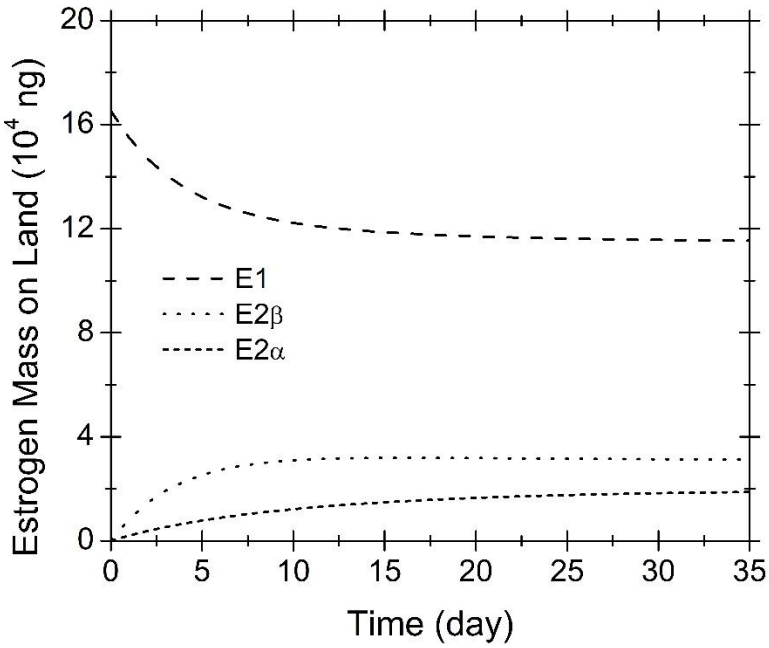

Figure 4.2 *The calculated mass of estrogens at each plot after land application of biosolids using the model described by Eqs. S4 to S10 with interconversion of E1, E2α, and E2β.*

Model coefficients k_w, c, and d were estimated by linear regression using the measured data. For the wash-off model, Equation 4.2 can be rewritten as follows:

$$Ln\left(1-\frac{M}{S}\right) = -k_w Q \tag{4.5}$$

For the empirical model, Equation 4.3 can be rewritten as follows:

$$Ln(M) = dLn(QS) + Ln(c) \tag{4.6}$$

A linear regression analysis was performed using the measured mass loads, measured surface runoff and the calculated mass storage on Days 1 and 8. The results and regression equations are summarized in Figure 4.3. Although the linear regression of the wash-off model has poor performance for E2α with a low r^2-value of 0.136, the linear regression shows good overall performance with r^2-values higher than 0.80 for E1 and E2β. The parameters derived and collected as described above are summarized in Table 4.2. Based calculation results, k_w-values are constant for these three estrogens, varying from 0.00015 to 0.00021 min/L, while the magnitudes of *c* and *d* values change drastically for these three estrogens.

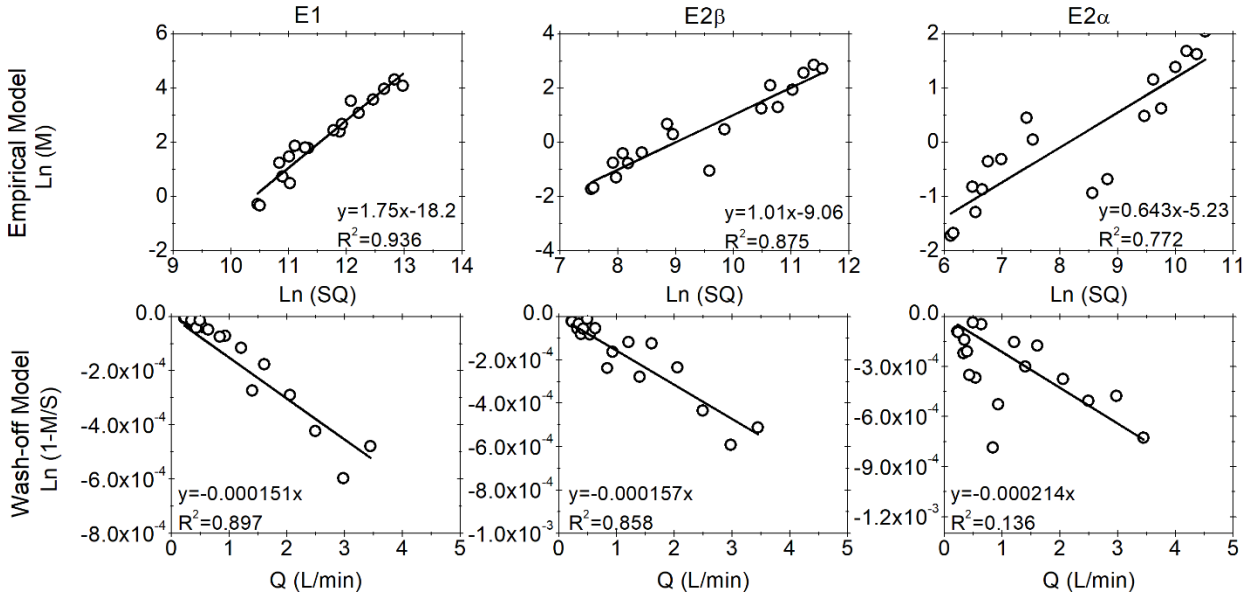

Figure 4.3 *Linear regression to determine values of parameters k_w, c, and d of three estrogenic compounds using Equations 4.5 and 4.6.*

Table 4.2 *Parameters Values for the Wash-off and Exponential Models*

Parameter	Unit	Value	Source
$k_{w,E1}$	min/L	0.00015	by model calibration
$k_{w,E2\alpha}$	min/L	0.00021	by model calibration
$k_{w,E2\beta}$	min/L	0.00016	by model calibration
c_{E1}	-	1.2E-8	by model calibration
$c_{E2\alpha}$	-	0.0053	by model calibration
$c_{E2\beta}$	-	0.00012	by model calibration
d_{E1}	-	1.8	by model calibration
$d_{E2\alpha}$	-	0.64	by model calibration
$d_{E2\beta}$	-	1.0	by model calibration

The derived coefficients were applied to the wash-off and empirical models to estimate the mass loads of three estrogens from three plots on Day 35 to validate and evaluate the predicted performance of the two models. A comparison of the estimated and measured mass loads of E1, E2α, and E2β by the two models is summarized in Table 4.3. The empirical model shows higher r^2-values for E1 and E2β than the wash-off model, and the wash-off model shows a higher r^2-value for E2α than the empirical model. The r^2-values for E1 and E2β of both the models are higher than 0.90, which indicates that both models are better at predicting the mass loads of E1 and E2β than E2α. The comparable r^2-values show that the empirical and wash-off models have similar abilities for estimating the mass loads for the three estrogens.

Table 4.3 *The coefficient of determination (r^2) for the measured and estimated mass load of three estrogens by two models from three plots on Day 35.*

Estrogen	Empirical model and observed data	Wash-off model and observed data
E1	0.95	0.91
E2β	0.94	0.93
E2α	0.64	0.74

4.3 Evaluation and Comparison of the Two Models

To evaluate the overall performance of the two models during the whole study period, the simulated mass loads and the measured values on Day 1, Day 8, and Day 35 are illustrated in Figure 4.4. Simulated results of both models match the measured values during rainfall events. On Days 1 and 35 with low flows, the difference between the simulated results of the two models is not significant. Conversely, the difference between the simulation results of the two models is larger on Day 8 with high flows, especially for E1 and E2α. The comparison of the two models is illustrated in Figure 4.5. In general, a greater overlap between the fitted line and the 45° line indicates a better performance. All of the fitted lines for the wash-off model for all three estrogens are closer to the 45° line than those of the empirical model. Thus, for this specific case, the wash-off model shows a better performance in predicting the mass loads of E1, E2α, and E2β than the empirical model.

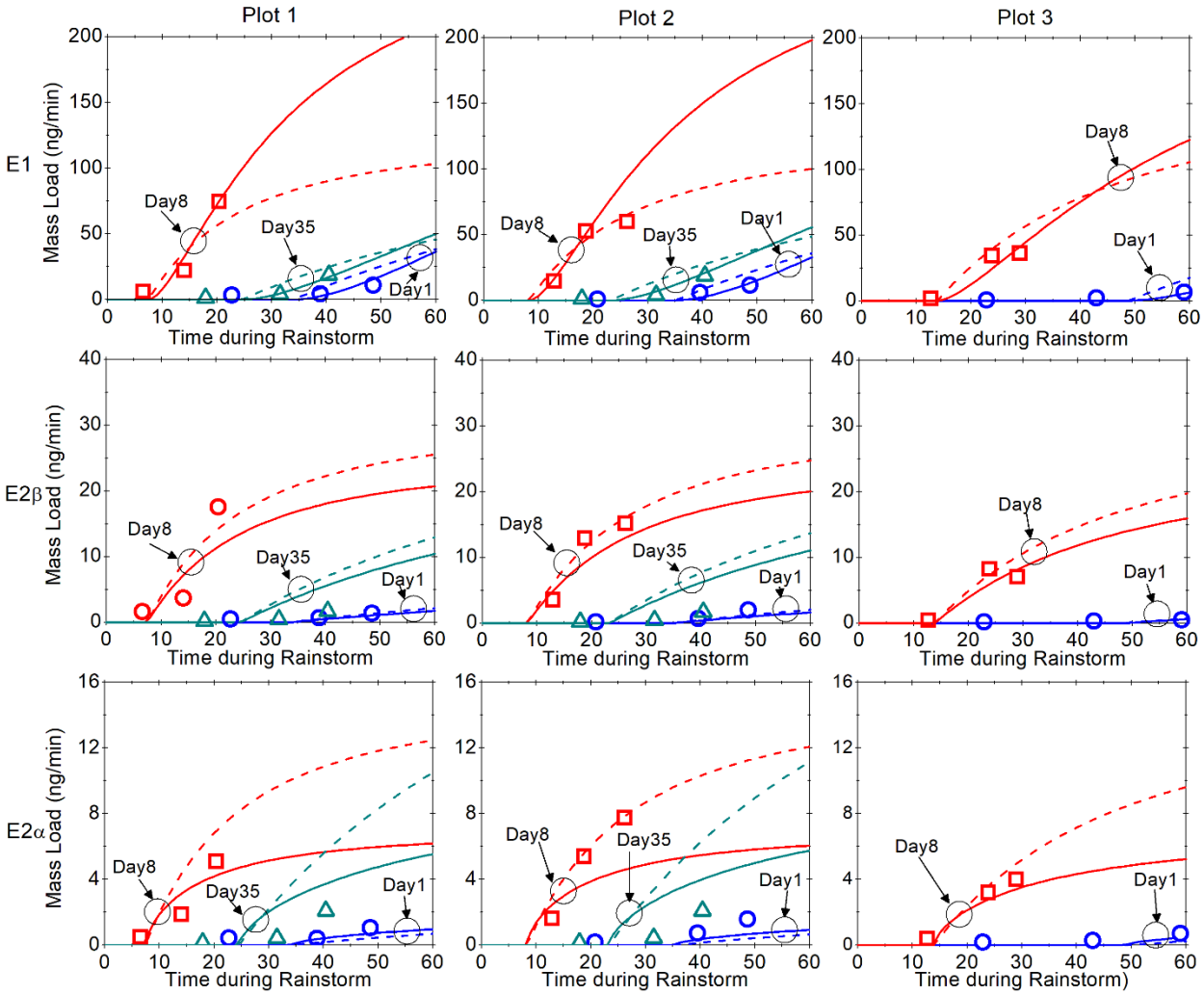

Figure 4.4 *Simulated and measured mass loads of estrogens during rainfall events from three plots: the squares, circles, and triangles denote the measured mass loads on Days 1, 8 and 35, respectively; the solid and dashed lines denote the mass loads simulated by the empirical and wash-off model, respectively. [Data from Yang et al., 2012].*

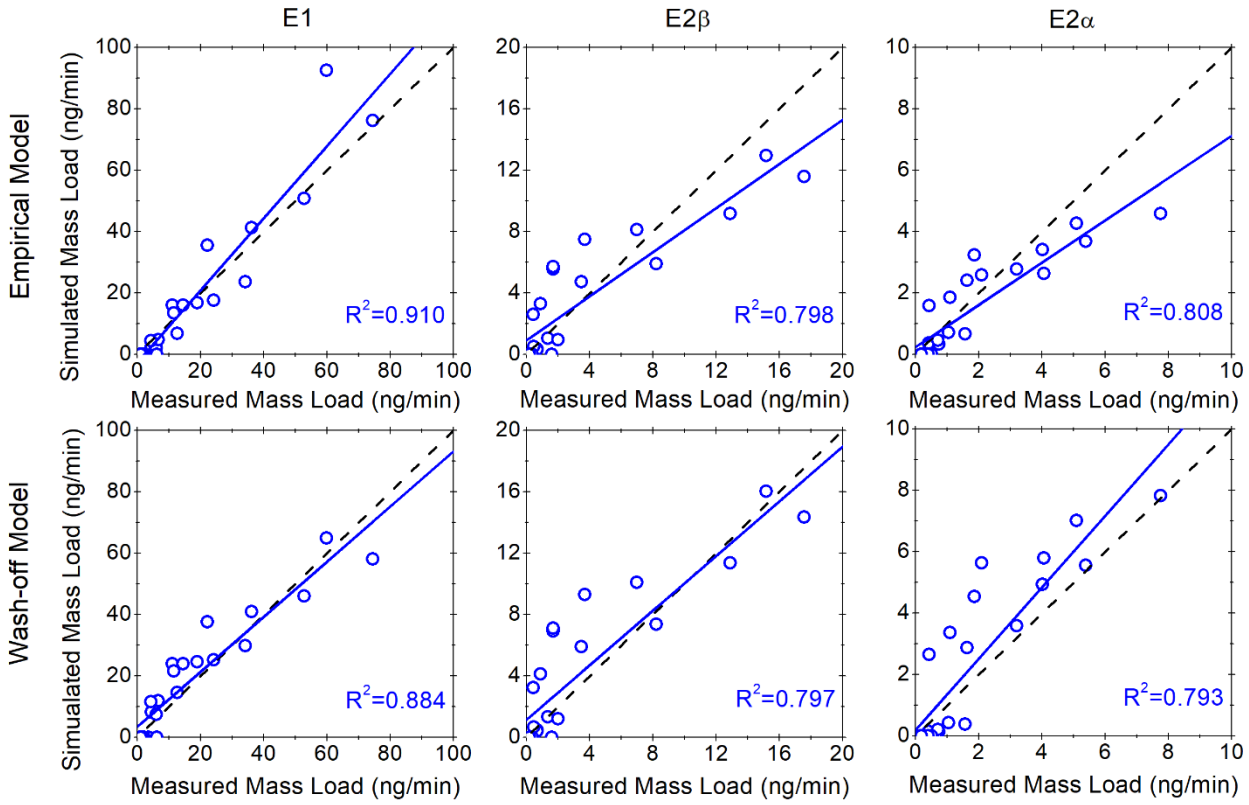

Figure 4.5 *Comparison of the performance of the empirical and the wash-off models for the three estrogenic compounds.*

A statistical analysis was conducted to evaluate the performance of the empirical model and the wash-off model on the transport of estrogens during rainfall events. The numerical difference between the modeled and measured estrogen mass loads was calculated first, then the difference between these two models was analyzed. *P*-values for the difference between the simulated and the measured masses are summarized in the first two columns in Table 4.4. The empirical model has the highest *P*-value for E2β and the lowest *P*-value for E2α. The wash-off model has the highest *P*-value for E1 and the lowest *P*-value for E2α. The *P*-values for the difference between the simulation results of these two models are summarized in the third column in Table 4.4. As shown in Table 4.4, the *P*-value is low for E2β and E2α, indicating that there is a large statistical difference in the performance between the two models for E2β and E2α. Both the wash-off and

empirical models had large variations from the true values, resulting in large differences between these two models. The numerical accuracy of these two models depends on the parameters used in this study. For example, the actual attenuation rate of estrogens varies with the environment as it is affected by several factors, such as the temperature, estrogen concentrations and solar radiation (Bradley et al., 2009; Chowdhury et al., 2011). However, the attenuation rates of three estrogens were assumed to be constant in this study. In addition, model coefficients are sensitive to the environment such as soil properties, and the accuracy of the model coefficient estimation may be impaired by the sparse data used in this study. Furthermore, the actual interconversion of estrogens involves other estrogens rather than just the three investigated in this study, such as the conjugated estrogens, and thus may be more complicated than hypothesized in this study (Bai et al., 2015).

Table 4.4 *P-values for two-tailed paired t-test for the empirical model results and the measured values, the wash-off model results and the measured values, and the empirical model results and the wash-off model results (α = 0.05).*

Estrogen	Empirical model and observed data	Wash-off model and observed data	Two models
E1	0.798	0.246	0.478
E2β	0.937	0.116	0.000439
E2α	0.103	0.0699	0.00309

In general, both models do well in estimating the estrogen loads exported by surface runoff from agricultural land. Compared to the empirical model, the wash-off model performs better when applied to this specific case. Additionally, the wash-off model is more compatible with hydrological modeling software such as HSPF for large-scale application of estrogen modeling

(Zhao & Lung, 2017). In general, the performances of the wash-off and empirical models can be affected by several factors, such as the estrogen type, data quality, and surface flow rates. Thus this comparison of results only applies to this specific study and a more general conclusion comparing these two models requires additional studies.

Chapter 5 Development a Model Framework to Track the Fate and Transport of Estrogens on a Watershed Scale

5.1 The HSPF Model

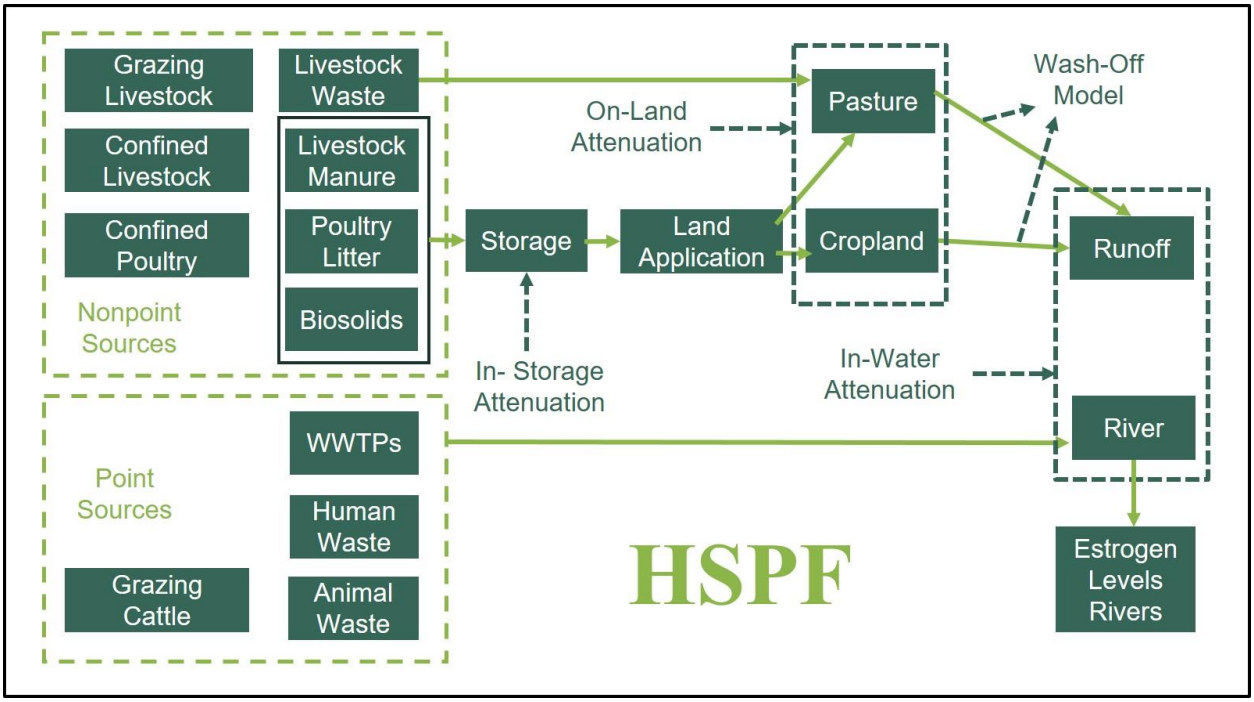

Figure 5.1 *The HSPF modeling frame to track the fate and transport of estrogens.*

The modeling of estrogens on a watershed scale can be accomplished by the HSPF (Hydrological Simulation Program—Fortran). The execution of HSPF can be accomplished through Better Assessment Science Integrating Point and Non-point Sources (BASINS). BASINS integrates the Geographical Information System (GIS), data analysis and modeling system to create watershed-based analysis and is mainly composed of the GIS interface, WinHSPF, and WDMUtil (US EPA, 2015). To accomplish the modeling work for estrogens, the GIS interface can be used to select the watershed, download the hydrology data, delineate the watershed and prepare input

files for HSPF. WDMUtil is then used to input meteorological data, flow rate data and estrogen loads. HSPF is used for the hydrological calibration and estrogen analysis. The HSPF modeling frame to track the fate and transport of estrogens is depicted in Figure 5.1. HSPF utilizes a buildup-wash off model to estimate substance levels in rivers (US EPA, 2015). This model assumes that substance accumulates on the land surface during dry periods and can be flushed into rivers by surface runoff during storm events. To accomplish the modeling, HSPF requires users to provide substance monthly accumulation rates, the monthly storage limit, the rate of surface runoff which will remove 90% of stored estrogens on land per hour, and the decay rate in water. The monthly accumulation rates are the daily loads of estrogens to land surface from non-point sources in a unit area. The monthly storage limit is the amount of estrogens that can be accumulated within one month without the interference of surface runoff. The amount of estrogens that are directly released into rivers are loaded into the HSPF using the "Point Sources" module.

5.2 Estrogen Load Estimations

Livestock, poultry, biosolids, septic systems, and WWTPs are considered as primary sources of estrogens within a watershed. Solid manure produced by livestock and poultry during confinement is collected for land application (USDA NRCS, 1995). Livestock and poultry manure produced during grazing can be either deposited onto pastureland or directly released into streams (Lucas & Jones, 2006; Shappell et al., 2016). In addition to livestock manure, biosolids are also used for land application within the study area and thus work as an additional source of estrogens (Lorenzen et al., 2004). Additionally, septic systems can release human feces, which contains estrogens, to the natural environment (Swartz et al., 2006). Failed septic

systems can release human wastes to land and straight pipes can directly release estrogens into streams (Virginia Department of Environmental Quality, 2009). WWTPs are also considered as sources of estrogens. Among the estrogen sources described above, sources releasing estrogens onto land are considered as nonpoint sources and the mass loads to land surface are simulated by the HSPF using the daily accumulation rates and monthly storage limit, whereas those directly releasing estrogens into streams are considered as point sources and the mass loads directly into rivers are simulated by the HSPF using the "Point Sources" module.

5.2.1 Estrogen Loads from Domesticated Animals

Estrogen loads from the manure land application are estimated by multiplying the manure application rate by the estrogen content in manure. Estrogen loads to cropland through land application were calculated using the equation below:

$$L_{N1} = \frac{R_{MC} C_M F_C}{D} \tag{5.1}$$

where L_{N1} is the daily estrogen load to cropland through manure application, R_{MC} is the annual manure/litter application rate to cropland, C_M is the estrogen content in manure, F_C is the fraction of manure applied to cropland in a certain month, and D is the number of days in that month.

Estrogen loads to pasture land include those through land application and those directly released by grazing animals. Direct estrogen deposition onto pastureland by grazing animals is quantified by multiplying the daily estrogen production of livestock or poultry by the time fraction that livestock or poultry spent on pastureland. Estrogen loads to pastureland from cattle were calculated using the equation below:

$$L_{N2} = \frac{R_{MP}C_M F_P + PE_a T_P D}{D} \quad (5.2)$$

where L_{N2} is the daily estrogen load to pastureland from domesticated animals, R_{MP} is the annual manure/litter application rate to pastureland; F_P is the fraction of manure applied to pastureland in a certain month, P is the population of livestock or poultry, T_p is the fraction of time spent by cows grazing on pastureland, and E_a is the daily estrogen excretion per animal. The daily estrogen excretion by livestock is calculated using the equation below:

$$E_a = WS_c C \quad (5.3)$$

where W is the daily fresh manure/litter production per animal, S_c is the solid content of fresh waste, and C is the estrogen content in solid waste.

Cattle grazing on the pastureland have the chance to get access to streams and excrete wastes directly into streams. Direct estrogen deposition into streams is estimated by multiplying the daily estrogen production of livestock or poultry by the time fraction that livestock or poultry spent in streams and the faction of estrogen that can be desorbed from manure and released into streams. Thus the estrogen loads from the cattle wastes directly excreted into streams were calculated using the equation below:

$$L_{P1} = PE_a T_s f \quad (5.4)$$

where L_{P1} is the daily estrogen load into streams from cattle manure which is directly excreted into streams, T_s is the fraction of time spent by cows in streams, and f is the faction of estrogen that can be desorbed from manure and released into streams.

5.2.2 Estrogen Loads from Septic Systems

Loads of estrogen from the failed septic system and straight pipes are calculated by multiplying the number of households with failed septic systems/straights pipes by the number of people per household and the estrogen excretion amount per human. The household and population data can be obtained from the U.S. Census Bureau (USCB). The housing units within a watershed can be estimated using the Arc geographic information system (ArcGIS) tools.

The daily loads of estrogen from failed septic systems were calculated using the equation below:

$$L_{N3} = f_f \times (P_F \times E_F + P_M \times E_M) \tag{5.5}$$

where L_{N3} is the daily estrogen load to land from failed septic systems, f_f is failing rate of septic systems, P_F is the population of females within the study area, P_M is the population of males within the study area, E_F is the daily estrogen excretion by a female human, and E_M is the daily estrogen excretion by a male human.

The daily loads of estrogen from straight pipes were calculated using the equation below:

$$L_{P2} = f_s \times (P_F \times E_F + P_M \times E_M) \tag{5.6}$$

where L_{P2} is the daily estrogen load to rivers from straight pipes, fs is the fraction of housing units that use straight pipes.

5.2.3 Estrogen Loads from WWTPs

The daily loads of estrogens from WWTPs were calculated using the equation below (Gall et al., 2014).

$$L_{P3} = Q_w C_w \tag{5.7}$$

where L_{P3} is the daily estrogen load into streams from WWTPs, Q_w is the daily discharge of WWTPs, and C_w is the estrogen concentration in WWTP effluents.

5.2.4 Estrogen Loads from Biosolids

The daily estrogen loads from biosolids were calculated using the equation below:

$$L_{\mathrm{N4}} = R_B C_{BR} \tag{5.8}$$

where L_{N4} is the daily estrogen load to agricultural land from biosolids, R_B is the application rate of dry biosolids, and C_{BR} is the estrogen content in dry biosolids.

The accumulation rate of estrogens on land in the HSPF equals the sum of the daily loads of estrogens to land from all of the nonpoint sources. The load of estrogens directly into surface water in the HSPF equals the sum of the daily loads of estrogens to surface water from all of the point sources

5.3 Attenuation and Transformation of Estrogens in the Environment

The attenuation and transformation of estrogens occur during storage, on the land surface, and in water. The attenuation and transformation of estrogens occur during storage and on land surface is reflected by the monthly storage limit in HSPF. In this modeling framework, the monthly storage limit equals the accumulated mass of estrogens within one month with the attenuation process during storage and on the land surface. Manure can be stored as piles or in aerobic lagoons (Moyer & Hyer, 2003). Following land application, estrogens on agricultural land undergo an attenuation process that includes sorption to solids, biodegradation, photodegradation, and plant uptake during the dry periods after land application (Das et al., 2004; Bradley et al., 2009; Caupos et al., 2011; Card et al., 2012).

When only one kind of estrogen is considered as the target compounds, the attenuation of this estrogen in the natural environment can be described by the pseudo-first-decay model:

$$C_t = C_0 e^{-kt} \tag{5.9}$$

where k is the lumped first-order attenuation rate of estrogen, C_t is the estrogen concentration or mass at time t, and C_0 is the original concentration or mass of estrogen.

However, when more than one kinds of estrogens are considered as target compounds, the pseudo-first-decay model cannot accurately estimate the attenuation of estrogens. In this case, the attenuation and interconversion of estrogens during storage, on land and in water can be estimated by the comprehensive transformation model developed in Chapter 3 (Equations 3.24 to 3.26).

5.4 Transport of Estrogens from Land into Streams

Estrogen contained in manure can be transported into streams by the surface runoff during storm events (Lucas & Jones, 2009). Previous studies found that the wash-off behavior of estrogens can be described by the first order wash-off model (Gall et al., 2014; Luo, 2014; Gall et al., 2015). Thus the Equation 4.2 was used to describe the transport of estrogens from land into streams. HSPE requires the value of WSQOP, the rate of surface runoff which will remove 90 percent of estrogens per hour. The WSQOP can be estimated using the equation below:

$$WSQOP = \frac{2.3}{k_w} \tag{5.10}$$

As the HSPF program utilizes a WSQOP unit of in/hr, the unit of k_w should be converted from min/L to hr/in by dividing to the volumetric flow rate by the land area.

Chapter 6 Case Study 1: Tracking the Fare and Transport of E2β in the South River Watershed

6.1 Study Site and Extent of Available Data

This study area is located at the south end of Augusta County, Virginia (Figure 6.1). The selected area was divided into nine sub-watersheds based on flow line features and elevations which were obtained from the United States Geological Survey (USGS). The main river system in this area is the South River. There are two major tributaries located upstream of the South River in this study area; one originates from the sub-watersheds 3 and 4 and flows across the sub-watersheds 6, 2 and 7, and the other one originates from the sub-watershed 5. These two branches then merge into the sub-watershed 8 and finally flow out of the study area through the sub-watershed 9. The north end of the South River in this area, which is located in the sub-watershed 9, was selected as the outlet point as there is a USGS Site (01627500) (38°13’07” N, 78°50’13” W), making it convenient for the hydrological calibration. The outlet point is marked as a green triangle in Figure 6.1. Four municipal WWTPs: Stuarts Draft WWTP, Waynesboro WWTP, Vesper View WWTP and Harrisonburg WWTP are located within the selected study area and discharge into the South River. E2β concentrations in WWTP effluents were estimated from the literature review based on the wastewater treatment process. The design information of four major WWTPs within the study area was obtained from the Augusta County Service Authority (ACSA), and City of Waynesboro government website. The information of these four WWTPs is summarized in Table 6.1.

Table 6.1 *Design flows and treatment processes of four major WWTPs located within the South River Watershed*

Name	Sub-watershed	Design flow (m^3/day)	Treatment type	Treatment process	Estimated E2β concentration in effluent (ng/L)
Stuarts Draft WWTP	6	15100	Tertiary	An Enhanced Nutrient Reduction (BNR) plant; 5 stage Bardenpho that removes BOD, solids, nitrogen and phosphorous (alum used); Deep bed denitrification filter; UV disinfection	4.6
Waynesboro WWTP	8	22700	Tertiary	Two BNR tanks; two secondary clarifiers; denitrification filters; UV disinfection; solids dewatering facilities	1.42
Vesper View WWTP	9	379	Secondary	An extended aeration package plant designed to remove BOD and solids	10.6
Harriston WWTP	9	379	Secondary	A four cell lagoon with mechanical aerators designed to remove BOD and solids	15.2
Reference				Servos et al. (2005)	

National Land Cover Database 2011 (NLCD 2011) data was obtained from the Multi-Resolution Land Characteristics Consortium (MRLC) to investigate the land uses of the study area. The dominant land uses in this area are forest and agricultural land, which account for 60.6% and 24.4% of the total area, respectively. Land uses of each sub-watershed are summarized in Table 6.2. Hourly precipitation, wind speed, temperature, dew point temperature and cloud cover data at Shenandoah Region Airport (38°15'50.4" N, 78°53'45.6" W, 111.59 m), Charlottesville-Albemarle Airport (38°08'16.8" N, 78°27'10.8" W, 195.38 m) and Roanoke Regional Airport (37°18'57.6" N, 79°58'26.4" W, 1175 m) from 2010 to 2015 were obtained from the National Oceanic and Atmospheric Administration (NOAA). Hourly solar radiation, evaporation, and evapotranspiration data were calculated and disaggregated by WDMUtil. Cloud cover data was originally reported as clear, scattered, broken or overcast, and was converted to a scale ranging

from 1 to 10 using the strategy listed in Appendix B (Perez et al., 2002). Daily average flow data from 2010 to 2015 at the USGS gage 01627500 was obtained from the USGS website. The cattle and poultry populations in the study area in 2012 were obtained from Virginia's Animal Feeding Operations (AFO) database. The livestock populations were assumed to keep constant throughout the three-year simulation period. The cattle and poultry populations in each sub-watershed are summarized in Table 6.3.

Table 6.2 *Land uses and area in 9 sub-watersheds within the study area*

Sub-watershed	Area (km^2)							
	Built-up	Forest	Barren	Range	Water/Wetland	Pastureland	Cropland	Total
1	2.21	23.9	0	0	0	1.05	0.0306	27.2
2	2.16	22.8	0.009	0	0.0801	2.60	0.711	28.4
3	4.85	10.9	0.0135	0	0.172	23.1	1.33	40.4
4	2.75	26.0	0	0.0045	0.0945	3.55	0.579	33.0
5	6.47	87.8	0.0288	0	0.439	5.34	1.49	102
6	7.69	29.3	0.0711	0.0135	0.0882	10.7	3.35	51.3
7	7.55	16.5	0.0153	0	0.434	15.1	7.41	47.0
8	29.2	27.7	0.0261	0	0.169	13.8	1.04	71.9
9	12.8	67.6	0.0117	0	0.435	31.3	3.18	115
Total	75.6	313	0.176	0.018	1.91	107	19.1	516

Table 6.3 *Livestock populations in the South River Watershed*

Sub-watershed	Beef cow	Dairy cow	Broilers	Turkeys	Layers
1	292	83	0	0	0
2	321	80	9225	0	1610
3	570	134	0	19175	1806
4	465	110	0	15663	1475
5	394	164	0	0	0
6	687	164	31	4142	2442
7	531	133	15266	0	2664
8	771	221	0	0	0
9	1439	753	92780	323115	0
Total	6469	1842	117302	362095	9996

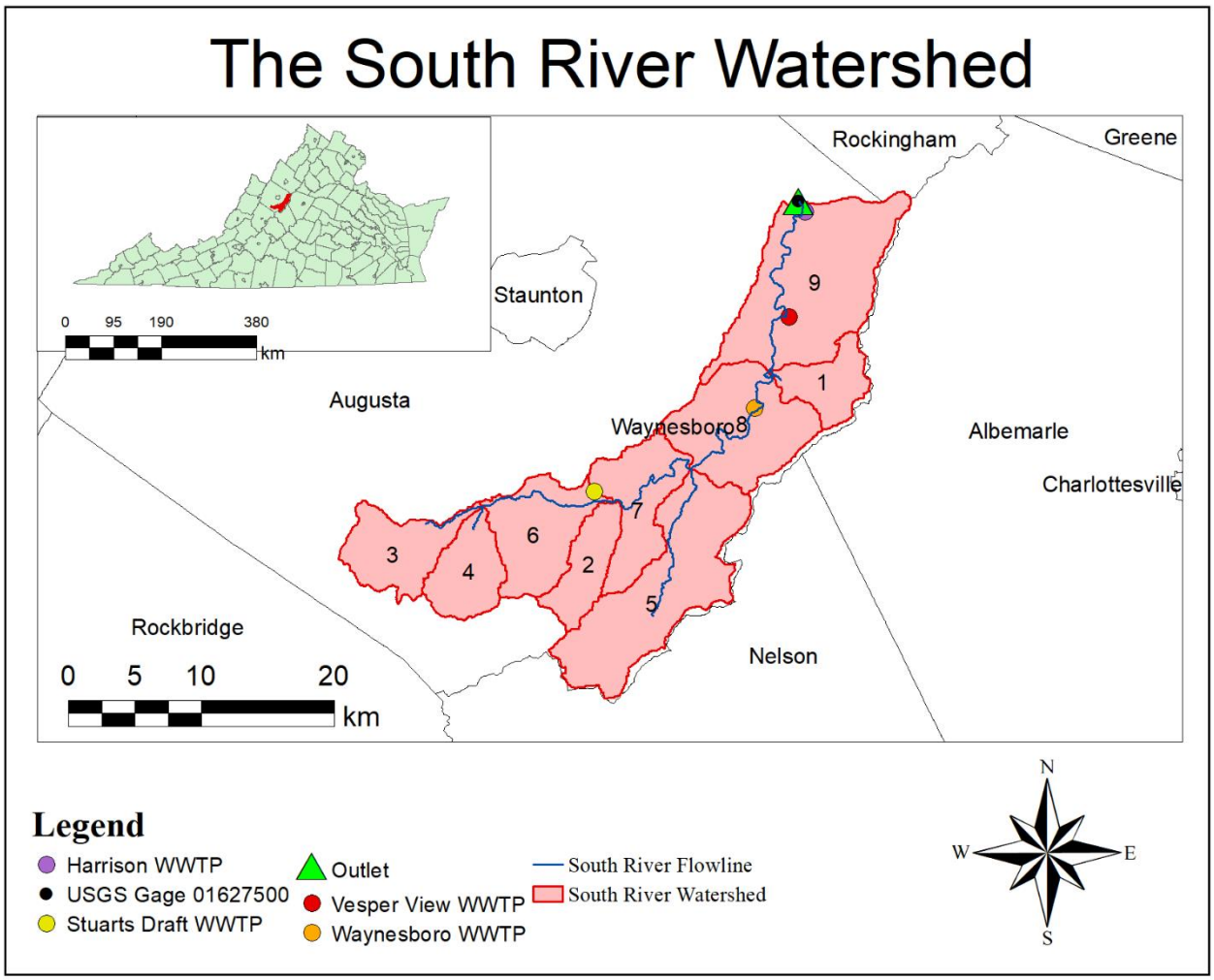

Figure 6.1 *The South River Watershed located in Augusta County, Virginia.*

6.2 E2β Loads to the South River

In this study, cattle and poultry were considered as primary animal sources of E2. Solid manure produced by cattle and poultry during confinement is collected for land application (Virginia Department of Environmental Quality, 2009). Cattle manure produced during grazing can be either deposited onto pastureland or directly released into streams (Lucas & Jones, 2006; Shappell et al., 2016). The desorption rate of estrogens from cattle manure that are directly released into streams was observed to be 18% (Andaluri et al., 2012). E2β loads from manure

land application were estimated by multiplying the manure application rate by the E2β content in manure. The annual application rates of dairy cattle manure, beef cattle manure, and poultry litter are 2040, 2700 and 673 g/m^2-year to cropland, and are 1200, 2700 and 673 g/m^2-year to pastureland, with priority given to cropland (Virginia Department of Environmental Quality, 2009). Liquid dairy manure receives priority over poultry litter and poultry receives priority over solid cattle manure (Virginia Department of Environmental Quality, 2009). It was estimated that liquid dairy manure was applied to 7.01 km^2 of cropland, poultry litter was applied to 12.1 km^2 of cropland and 20.7 km^2 of pastureland, and solid beef manure was applied to 1.57 km^2 of pastureland. The daily schedule of cattle is summarized in Table 6.4. The E2β productions by cattle and poultry are summarized in Table 6.5. The schedule of manure application to agricultural land in the South River Watershed is listed in Table 6.6.

Table 6.4 *Daily schedule for beef cattle and dairy cattle in confinement, on pastureland and in streams in the South River Watershed*

Month	**Beef cattle**			**Dairy cattle**		
	Confined (h)	**Pasture (h)**	**Streams (h)**	**Confined (h)**	**Pasture (h)**	**Streams (h)**
January	9.60	13.9	0.50	18.0	5.50	0.50
February	9.6	13.9	0.50	18.0	5.50	0.50
March	0	23.25	0.75	9.60	13.65	0.75
April	0	23.0	1.00	7.20	15.80	1.00
May	0	22.5	1.50	7.20	15.30	1.50
June	0	20.75	3.25	7.20	13.55	3.25
July	0	20.75	3.25	7.20	13.55	3.25
August	0	20.75	3.25	7.20	13.55	3.25
September	0	22.5	1.50	7.20	15.30	1.50
October	0	23.0	1.00	7.20	15.80	1.00
November	0	23.25	0.75	9.60	13.65	0.75
December	9.60	13.9	0.50	18.0	5.50	0.50
Reference	Virginia Department of Environmental Quality, 2009					

Table 6.5 *Cattle manure and poultry litter production rate and the E2β content in manure/litter*

Cow	Wet manure production (kg/day)	Solid content in manure (%)	E2β content in solid waste (ng/g)	Reference
Beef cow	50.8	15	7.3	USDA NRCS (1992); Bartelt-Hunt et al., (2013)
Dairy cow	21.3	16.2	16.6	USDA NRCS (1992); Andaluri et al. (2012)
Broiler litter (females)	0.224	0.26	65	USDA NRCS (1992); Shore, 2009a
Broiler litter (males)	0.224	0.26	14	USDA NRCS (1992); Shore, 2009a
Layers	0.140	0.25	533	USDA NRCS (1992); Shore, 2009a
Turkey	0.474	0.26	13.3	USDA NRCS (1992); Shore, 2009a

Table 6.6 *Schedule of manure application to agricultural land in the South River Watershed*

Month	Liquid manure applied (%)	Solid manure and poultry litter applied (%)
January	0	0
February	5	5
March	25	25
April	20	20
May	5	5
June	10	5
July	0	5
August	5	5
September	15	10
October	5	10
November	10	10
December	0	0
Reference	Virginia Department of Environmental Quality, 2009	

The daily loads of E2β from WWTPs were calculated using the daily E2β concentrations in WWTP effluents and the daily discharge of WWTPs. In this estimation, the design flow rate is used as the daily flow rate for each WWTP.

The household and population data were obtained from the USCB. The 5-year averaged (2011-2015) total population in Augusta County is 74314, and the total housing units in Augusta County in 2016 is 74314. There are 2.30 people per household on average. And the female-male ratio is 0.49:0.51. The housing units in the South River Watershed were estimated using the ArcGIS tool. The failure rate of septic systems within the South River Watershed was assumed to be three percent (Virginia Department of Environmental Quality, 2008). Three percent of the houses located within 200 ft of streams were assumed to dispose sewage directly into rivers using straight pipes (Virginia Department of Environmental Quality, 2008; Virginia Department of Environmental Quality, 2009). The estimated numbers of failed septic systems and straight pipes in the South River Watershed are summarized in Table 6.7.

Table 6.7 *Estimated numbers of housing units, failed septic systems and straight pipes in each sub-watershed*

Sub-watershed	Total household	Failed septic system	Straight pipes
1	521	16	3
2	701	21	5
3	428	13	2
4	475	14	3
5	1079	32	6
6	1686	51	13
7	1641	49	7
8	10661	320	34
9	2512	75	14
Total	19704	591	86

Approximately 65,000 acres of permitted land application sites in Virginia. The application rate of dry biosolids in Virginia was estimated to be 0.759 dry kg/m^2-year. The identified permitted land area within the study area is 0.675 km^2 in sub-watershed 8 and 0.372 km^2 in sub-watershed 9 based on VDEQ records. The content of E2β in biosolids is quite low and the highest content is smaller than 0.48 ng/g-DW (Yang et al., 2012). In this study, an E2β content of 0.48 ng/g-DW was used.

The calculated contributions of E2β from each source are summarized in Table 6.8.

Table 6.8 *Loads of E2β into the study area from various sources*

Source	E2β loads (g/year)	Percentage
Direct loading to streams		
Cattle	8.56	2.65%
Straight pipes	0.162	0.0501%
WWTPs	40.7	12.6%
Loading to land surfaces		
Cattle	124	38.3%
Poultry	149	46.0%
Biosolids	0.381	0.118%
Failed septic systems	1.11	0.343%
Total	135	100%

6.3 Transformation and Transport of Estrogens in the Watershed

In this case study, E2β attenuation process was characterized by the first-order kinetics. The attenuation rate of E2β in Virginia rivers is 3 day^{-1} (Pagsuyoin et al., 2012). The average on-surface attenuation rate of E2β is 0.37 day^{-1} on warm days and 0.21 day^{-1} on cold days, respectively (Colucci et al., 2001). The attenuation rate of E2β in lagoon water at 15 °C under

anaerobic conditions is about 0.34 day^{-1} (Zheng et al., 2012; Hakk et al., 2014), and the attenuation rate of E2β in cattle manure piles under natural conditions is about 0.1 day^{-1} (Zheng et al., 2007). In this study, the dairy cattle manure and poultry litter were considered as liquid manure and were stored in anaerobic lagoons for 100 days on average (Moyer & Hyer, 2003). The beef cattle manure was considered as solid manure and was stored as piles for 30 days on average (Moyer & Hyer, 2003).

The estrogens on cropland and pastureland are transported into streams by the surface runoff during storm events (Jenkins et al., 2006; Yang et al., 2012; Schoenborn et al., 2015). The wash-off coefficient, k_w, was estimated by fitting the data obtained from Yang et al., 2012 by assuming a constant total storage of E2β. The k_w value was determined to be 1.73 hr/in for E2β after a unit conversion from min/L to hr/in. The wash-off coefficient on pastureland was assumed to be the same.

6.4 Hydrological Calibration and Validation

The hydrological calibration was conducted first. The simulated flow rates at the outlet point for 2013 to 2014 were compared with the observed data at the USGS Gage 01627500 for hydrological calibration. The flow rates were simulated by adjusting the hydrological parameters within the possible range allowed by BASINS (Technical Note 6). The key parameters included the upper zone nominal soil moisture storage (UZSN), the lower zone nominal soil moisture storage (LZSN), the index to infiltration capacity (INFILT), the interflow inflow parameter (INTFW), and the lower zone evapotranspiration parameter (LZETP). The calibrated model was then used to simulate the flow rates in 2015 for the hydrological validation. Then the model was run with 110% and 90% of calibration values of the hydrological parameters for the hydrological

sensitivity analysis (Virginia Department of Environmental Quality, 2008). The correlation coefficients from 2013 to 2015 were calculated to evaluate the impact of each model parameter. The observed and the simulated average daily flow rates at the USGS Gage 01627500 from 2013 to 2015 are illustrated in Figure 6.2. The major hydrological parameter values used in this model are provided in Appendix C. Hydrological calibration results are summarized in Table 6.9. The correlation coefficient values for the calibration and the validation phases were 0.70 and 0.74, respectively. The Nash–Sutcliffe model efficiency coefficient values for the calibration and the validation phases were 0.46 and 0.48, respectively. The simulated flow was acceptable and the calibration was considered to be accomplished. The results of the hydrological sensitivity analysis are summarized in Table 6.10. The hydrological model was most sensitive to AGWRC, and INFILT and LZSN. The effects of UZSN, INTFW IRC and LZETP were not apparent in this study.

Table 6.9 *Comparison of the measured and the simulated flow rates at the USGS gage 01627500 in Augusta County from 2013 to 2015.*

Flow	**Calibration**		**Error (%)**	**Validation**		**Error (%)**	**Criteria (%)**
	Observed ($\times 10^6$ m^3)	**Simulated ($\times 10^6$ m^3)**		**Observed ($\times 10^6$ m^3)**	**Simulated ($\times 10^6$ m^3)**		
Total volume	500	450	-10	210	230	9.5	10
Low flows	107	111	3.7	25	28	12	10
High flows	350	290	-17	110	107	2.7	15
Volume-spring	220	160	-27	76	88	16	30
Volume-summer	91	75	-18	22	29	32	30
Volume-fall	46	42	-8.7	78	78	0.0	30
Volume-winter	150	170	13	49	49	0.0	30
Storm Volume	250	220	-12	97	110	13	20

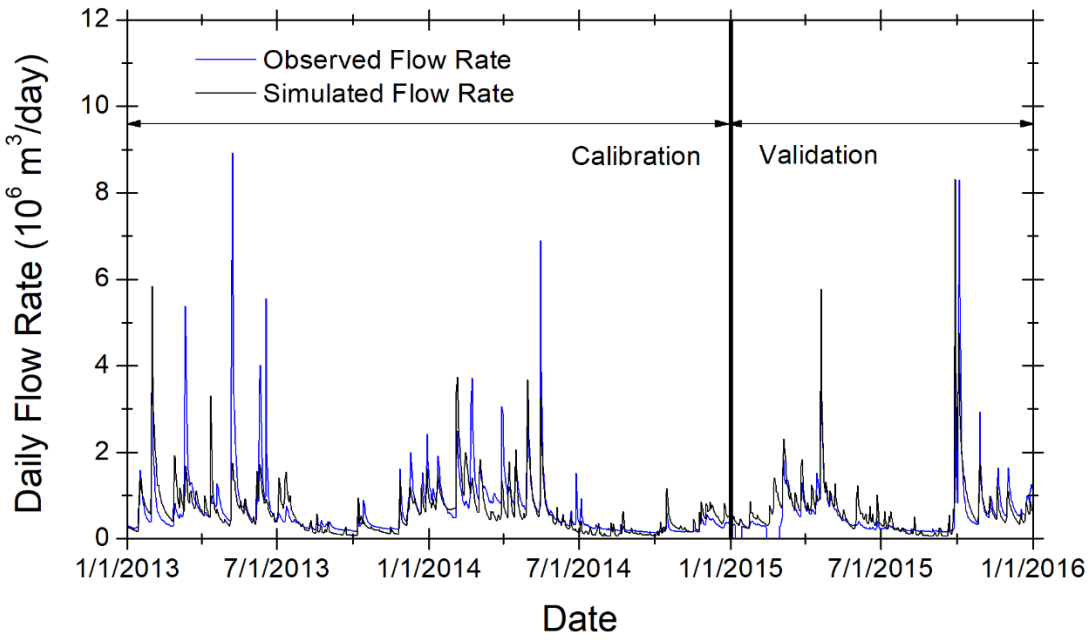

Figure 6.2 *The observed and the simulated daily flow rates at the USGS Gage 01627500 from 2013 to 2015. The gray curve denotes the observed flow rates and the black curve denotes the simulated flow rates. The flow rates in 2013 – 2014 were used for hydrological calibration, and the flow rates in 2015 were used for hydrological validation.*

Table 6.10 *Values of the coefficient of determination with respect to the variations of parameters increased or decreased by 10%.*

Parameter	***r***	
	+10%	**-10%**
LZSN	0.701	0.691
INFILT	0.706	0.686
AGWRC	0.569	0.718
UZSN	0.697	0.697
INTFW	0.697	0.697
IRC	0.697	0.697
LZETP	0.697	0.697
Original	0.700	

6.5 E2β Simulation

The following hypothetical scenarios were conducted to quantify the E2β contribution of each source into the South River after the hydrological calibration:

1) Only E2β loads from each single source added into the model;
2) E2β loads from all nonpoint sources added into the model;
3) E2β loads from all point sources added into the model;
4) E2β loads from both nonpoint and point sources added into the model.

The results of each model were then compared to the lowest observable effect level (LOEL) of estrogens for fish and plants, which was reported to be ranging from 2.9 ng/L to 50 ng/L (Thorpe et al., 2003; Barel-Cohen et al., 2006; Seki et al., 2006).

Figure 6.3 presents the simulated E2β concentrations in the South River from 2013 to 2015. The simulated E2β in the South River from all nonpoint sources, all point sources and both combined are illustrated in Figures 6.3a, 6.3b, and 3c, separately. The simulated E2β concentrations were below the smallest reported LOEL (2.8 ng/L) throughout the study period. The simulated E2β concentrations ranging from 0.0602 ng/L to 0.509 ng/L in the South River are comparable to the measured values of field studies, which reported aqueous E2β concentrations ranging from below the detection limit to 8.4 ng/L in rivers in the U.S. (Soto et al., 2004; Bradley et al., 2009). All of the simulated concentration peaks appeared with small tails as a result of the large in-stream attenuation rate of E2β used in this study (Pagsuyoin et al., 2012). The simulated concentrations from each point source and non-point sources are summarized in Figures 6.4 and 6.5, respectively. The simulated concentrations from each point source showed similar profiles with different magnitudes. A similar tendency also appeared for the simulated concentrations

from each nonpoint source. However, the simulated concentrations from nonpoint sources (Figure 6.3a) and point sources (Figure 6.3b) showed completely different trends. Figure 6.3a shows drastic variations of the concentrations over time while Figure 6.3b shows more subtle variations in concentrations over time. The concentration profile in Figure 6.3a shows an inverse trend to the profile shown in Figure 6.3b. Specifically, the peak concentrations appear in Figure 6.3a when the low concentrations appear in Figure 6.3b, while the low concentrations appear in Figure 6.3a when the peak concentrations appear in Figure 6.3b. As shown in Figure 6.3, nonpoint sources mainly affected the peak concentrations, while point sources mainly affected the base-flow concentrations. In total, the contributions of point sources and nonpoint sources to the simulated E2β in the South River were 83.7% and 16.3%, respectively.

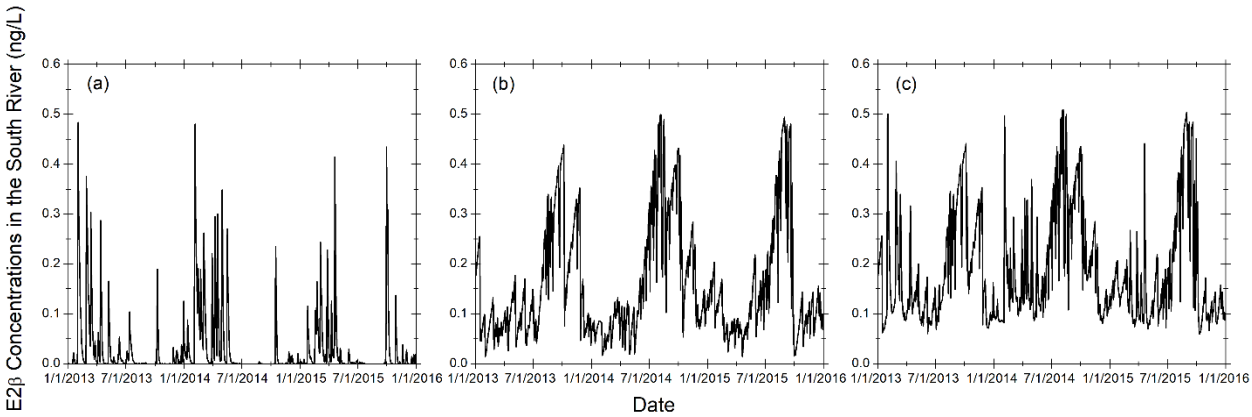

Figure 6.3 *Simulated E2β concentrations at the outlet point in the South River Watershed from 2013 to 2015. (a) Simulated E2β levels from nonpoint sources; (b) Simulated E2β from point sources; (c) Simulated E2β levels from both point and non-point sources.*

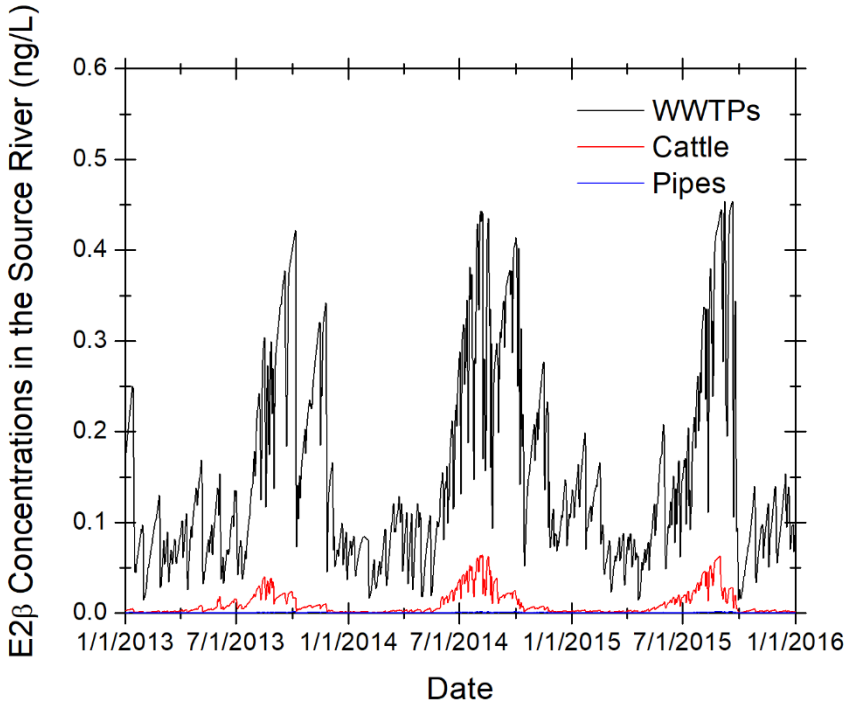

Figure 6.4 *The simulated E2β levels at the outlet point in the South River from 2013 to 2015 from three point sources: the black curve represents the contribution of WWTPs, the red curve represents the contribution of cattle in streams, and the blue curve represents the contribution of straight pipes.*

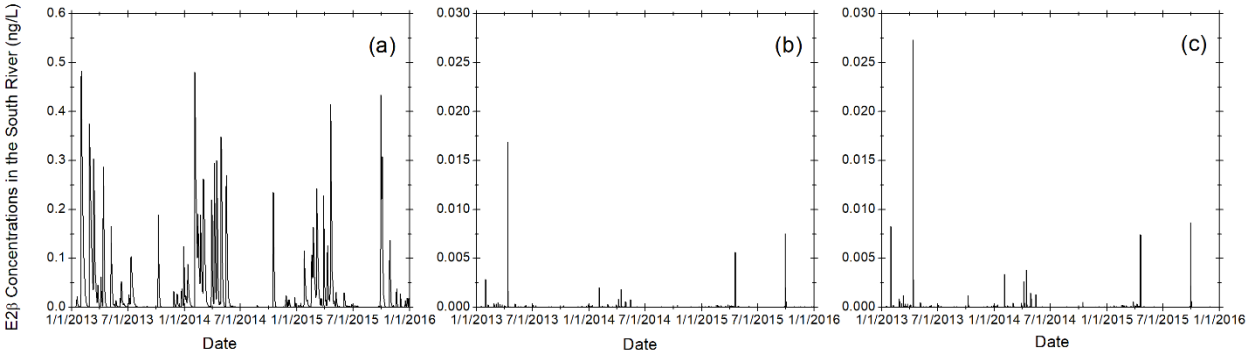

Figure 6.5 *The simulated E2β levels at the outlet point in the South River from 2013 to 2015 from three nonpoint sources: (a) the contribution of cattle manure; (b) the contribution of biosolids; and (c) the contribution of failed septic systems. The contribution of poultry litter is invisible due to rapid E2β attenuation in poultry litter during storage before land application.*

Figure 6.6a and 6.6b present the correlation coefficients (*r*) between the precipitation and E2β loads from nonpoint and point sources, respectively. Figure 6.6c and 6.6d present the correlation coefficients between the flow rates and E2β loads from nonpoint and point sources, respectively. The small absolute values of *r* in Figures 6.6a and 6.6b indicate that the impact of the precipitation on the simulation results was not significant. The transport of E2β driven by the rainfall was described by an exponential equation in this study, thus the relationship between the precipitation and E2β loads from nonpoint sources cannot be described by a simple linear model. Conversely, the large absolute values of *r* in Figures 6.6c and 6.6d show that the flow rate affected the simulation results significantly. The positive *r*-value in Figure 6.6c implies a positive relationship between the flow rates and the simulated in-stream E2β concentrations from nonpoint sources, as overflow delivers more E2β into streams from the land surface during the storm events according to the wash-off model. This result was consistent with previous field studies, which observed that the transport of E2β from nonpoint sources is strongly driven by rainfalls (Gall et al., 2011; Gall et al., 2014; Gall et al., 2015; Lee et al., 2015). The negative *r*-value in Figure 6.6d implies a negative relationship between the flow rates and the simulated in-stream E2β concentrations from point sources. We suppose that this modeling result was mainly caused by the dilution of E2β in receiving streams. A similar trend was observed in field studies due to more complicated transport processes of E2β. For example, the suspended solids during the storm events can increase the sorption of E2β (Casey et al., 2003; Das et al., 2004). Additionally, the dissolved oxygen in the water during the drought period is lower than that during the high flow period, leading to a slower biodegradation rate of E2β (Fan et al., 2007). Also, the sediments may work as a sink and release the sorbed E2β back into the water column during the drought period (Gall et al., 2014). The correlation coefficients between the simulated in-stream E2β concentrations and the E2β loads

from nonpoint and point sources were 0.177 and 0.405, respectively (not shown in figures), indicating that the impact of the loads was smaller than that of the flow rate. In short, the rainfall events can promote the transport of E2β from nonpoint sources, but dilute E2β from point sources.

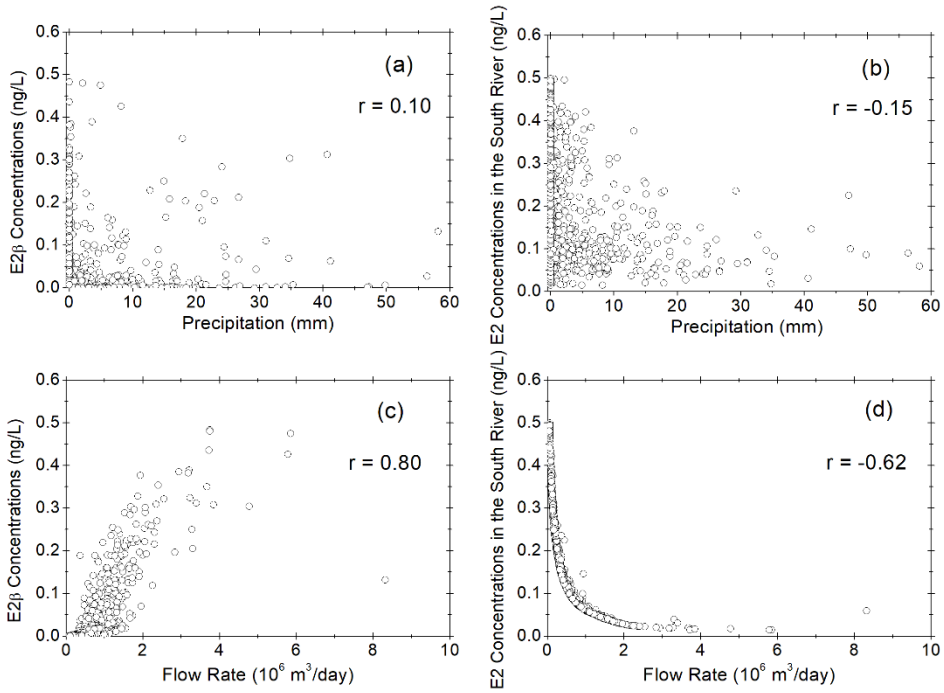

Figure 6.6 *The Correlation coefficient between the simulated E2β levels and precipitation and flow rates. (a) The correlation coefficient between the simulated E2β levels from nonpoint sources and the precipitation; (b) the correlation coefficient between the simulated E2β levels from point sources and the precipitation; (c) the correlation coefficient between the simulated E2β levels from nonpoint sources and the daily flow rates; (d) the correlation coefficient between the simulated E2β levels from point sources and the daily flow rates.*

6.6 Sensitivity Analysis

As there were no measured data in the South River to fit the model, sensitivity and uncertainty analyses were used to substantiate the model results. In the sensitivity analysis, the model was run with 80% and 120% of the stocking density, the manure concentrations, the grazing time on the pastureland, the grazing time in streams, the area of the agricultural land, the on-surface decay rate, the in-stream decay rate, the WWTP effluent concentrations, and the wash-off coefficient (Virginia Department of Environmental Quality, 2008). Then, the highest and average simulated E2β levels at the outlet point from 2013 to 2015 were used to evaluate the impact of each model parameter. Studies reported a wide range of on-surface decay rates and WWTP effluent concentrations: the detected concentrations of E2β in WWTP effluents range from below the detection limit to 26.7 ng/L in North America (Reddy et al., 2005; Esperanza et al., 2007; Atkinson et al., 2012; Vajda et al., 2011; Griffith et al., 2014), and the decay rates of E2β on agricultural land range from 0.07 to 3.33 day^{-1} (Colucci et al., 2001; Fan et al., 2007; Jacobsen et al., 2005). Thus, the uncertainty analysis was used to determine the extents of the modeling results. The Monte Carlo method was used for the uncertainty analysis (Mishra, 2011; Xie & Lian, 2013). The lower and upper limits of the modeling variables are listed in Appendix D. 1000 parameter sets were randomly selected by Matlab®, then these random value sets were used to run HSPF for 1000 times. These modeling results were then used to determine the possible range of E2β levels in the South River.

The results of the sensitivity analysis of the E2β simulation are listed in Table 6.11 and illustrated in Figure 6.7. The simulation results were most sensitive to the in-stream decay rate, WWTP effluent concentrations, agricultural land area, the stocking density, the manure concentrations,

and the time spent in streams by cattle. In comparison, the grazing time on the pastureland, the on-surface decay rate, and the wash-off coefficient weakly affected the model results. These results indicated that point sources played a much more important role in the simulated in-stream E2β concentrations than nonpoint sources for two reasons. First, the chosen outlet point was located just at the downstream of a WWTP. Second, the E2β applied to the land had decreased drastically as a result of the natural attenuation processes before entering streams. The total estimated E2β loads to the land comprised more than 80% of the total E2β loads into the study area (Table 6.8). However, only 13.6% of the E2β in the South River at the outlet point came from nonpoint sources. Modeling results also showed that E2β concentrations in the South River were more sensitive to WWTP effluents than to cattle manure directly excreted into streams.

Table 6.11 *Variation in the simulated average and highest E2β levels in the South River with respect to variation in parameters increased or decreased by 20% in 2013-2015*

Parameter	**Change in average concentrations**		**Change in highest concentrations**	
	+20%	**-20%**	**+20%**	**-20%**
Stocking Density	+1.39%	-0.870%	+2.36	-2.36
Manure Concentration	+1.39%	-0.870%	+2.36	-2.36
Grazing Time on Pastureland	0	0	0	0
Grazing Time in Streams	+1.22%	-0.870%	+2.36	-1.77%
Agricultural Land Area	+1.74%	0	0	0
On-Surface Decay Rate	0	0	0	0
In-Stream Decay Rate	-16.7%	+23.0%	-12.6%	+28.5%
Loads from WWTPs	+15.8%	-15.7%	+17.3%	-2.55%
Wash-off coefficient	0	+0.174	0	0

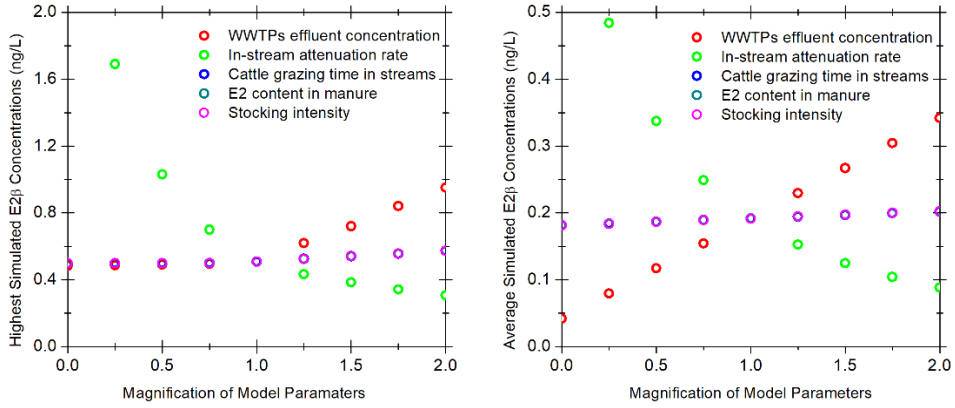

Figure 6.7 *Variations of the average and the highest simulated E2β levels in the South River with respect to the variations of parameters.*

6.7 Implications of the Modeling Results: BMPs for E2β Control

E2β is unstable in the natural environment, which means that E2β has the largest impact on the adjacent environment (Bartelt-Hunt et al., 2012; Pagsuyoin et al., 2012). E2β concentrations may be above the LOEL at the mixing zone receiving WWTP effluents and in streams receiving the feedlot runoff. Thus, the BMPs are necessary for E2β control in the study area. The estimated costs for various BMPs within the study area are summarized in Table 6.12. In this study, the practice cost for the study area was simply calculated by multiplying the numbers of the units by the unit cost obtained from the literature review. The actual costs of the BMPs can be complex and different from the calculated values as they include costs for capital construction, fixed assets, operations, and energy consumption, and are affected by various factors such as service area and service years (Edmonds et al., 2003; Zhu et al., 2004). The simulation results indicated that WWTPs contributed most of the E2β to the South River. E2β concentrations smaller than 20.6 ng/L in WWTP effluents are essential to keep the simulated in-stream E2β concentrations below the LOEL. Membrane methods and advanced oxidation processes can effectively remove

estrogens from wastewater (Feng et al., 2005; Kim et al., 2007; Snyder et al., 2007). However, there is no need for WWTP upgradations based on the simulation results for two reasons. First, the two largest WWTPs located within the study area both use tertiary treatment methods that can effectively remove E2β from wastewater. Second, additional treatment processes come with high costs for WWTPs. Additionally, the BMPs are necessary to keep E2β concentrations in streams receiving feedlot effluent below the LOEL. Based on the calculation results, more than 95.0% and 99.9% of the E2β contained in the manure was degraded during storage as piles for 30 days and in lagoons for 100 days, indicating that the manure storage before the land application is effective in E2β removal and thus should be encouraged. E2β concentration can also be effectively reduced by manure composting (Hakk et al., 2005; Andaluri et al., 2012; Bartelt-Hunt et al., 2013). In addition to land application of manure, cattle grazing on the pastureland contribute a large amount of E2β onto pastureland and into streams. Thus, methods to reduce E2β loads from grazing cattle are necessary. Buffer stripes have been observed to effectively reduce E2β loads by surface runoff from land (Shore, 2009b). Fencing off rivers to keep cattle out of the water is the most economical practice with consideration for cost and practicality.

Table 6.12 *The estimated annual cost in the study area using various E2β management practice*

Treatment method	Unit cost	Annual cost in the study area ($)	Reference
O_3	$0.33/1000 gallons	1230000	Sarkar et al., 2014
O_3/UV	$3.21/1000 gallons	12000000	Sarkar et al., 2014
O_3/H_2O_2	$0.34/1000 gallons	1270000	Sarkar et al., 2014
$O_3/UV/H_2O_2$	$2.72/1000 gallons	10100000	Sarkar et al., 2014
Buffer strips	$0.0154/m^2	1940000	Helmers, 2008
Composting	$6.98/m	111000	Bass et al., 2012

Chapter 7 Case Study 2: Tracking the Fare and Transport of E1, E2α, and E2β in the Redwood River Watershed

7.1 Study Site and Extent of Available Data

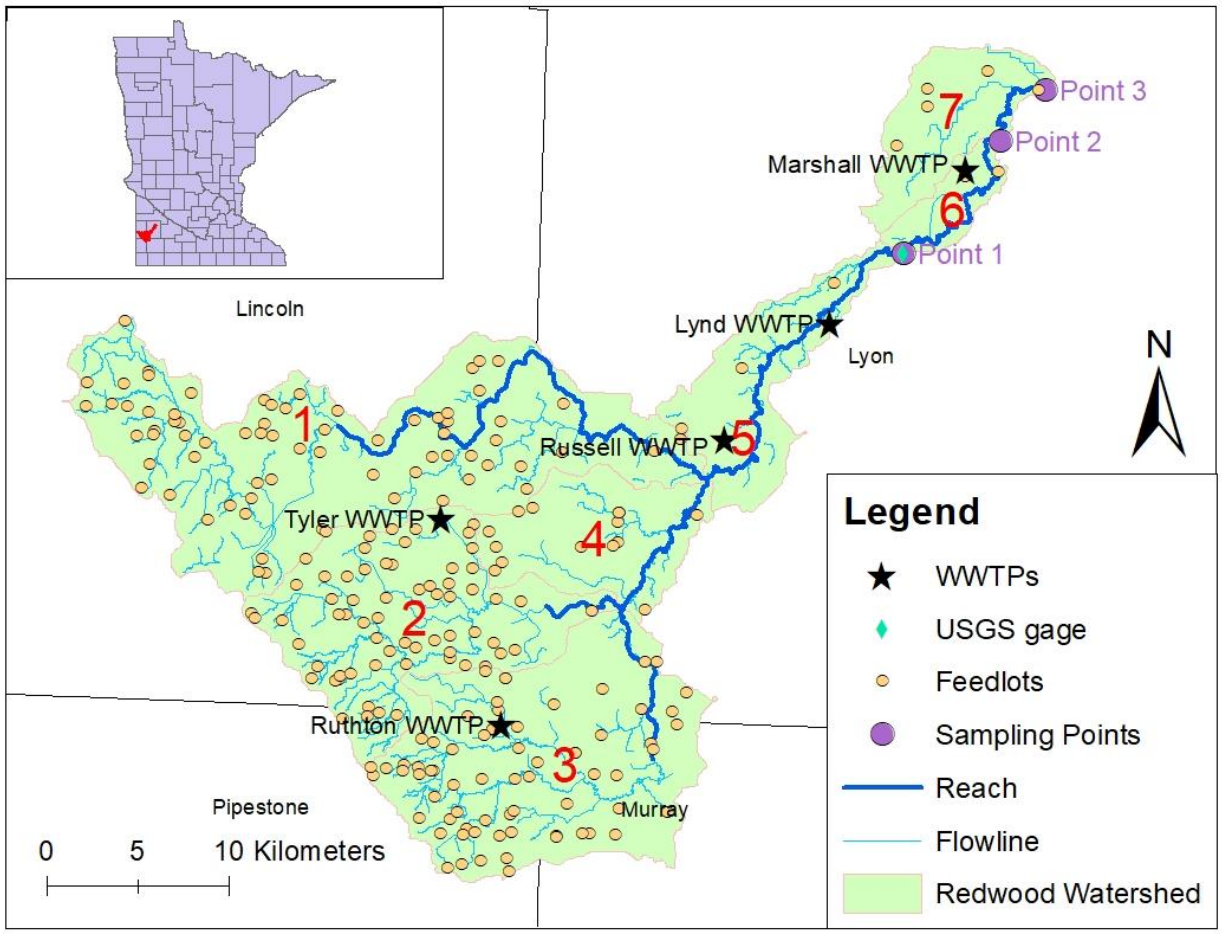

Figure 7.1 *The Redwood Watershed located in southwestern Minnesota.*

This study area is located in southwestern Minnesota and is part of the hydrological unit of 07020006. This study area is illustrated in Figure 7.1. The total area of this watershed is 768 km^2. The selected area is divided into seven watersheds based on flow line features and

elevations. The main river system in this area is the Redwood River which originates from the mountain area in Lincoln County, Pipestone County, Murray County, and Lyon County. The streams flow northeastwards and merge in Lyon County. There are two hundred and twenty-nine feedlots within the study area. Additionally, five WWTPs discharge into the Redwood River. These are Tyler WWTP, Lynd WWTP, Marshall WWTP, Russell WWTP, and Ruthton WWTP.

NLCD 2011 data was obtained from the MRLC to investigate the land uses of the study area. The dominant land uses in this area is agricultural land, which accounts for 72.3% of the total area. Land uses of each sub-watershed are summarized in Table 7.1. Meteorological data at Pipestone Municipal Airport and Southwest Minnesota Regional Airport were obtained from the NOAA. The data measured at Pipestone Municipal Airport was applied to sub-watersheds 1, 2, and 3 and the data measured at Southwest Minnesota Regional Airport was applied to sub-watersheds 4, 5, 6, and 7 according to the Thiessen Polygon analysis. Daily average flow data from May 2006 to April 2009 at the United States Geological Survey (USGS) gage 05315000 (green triangle), which is located in the sub-watershed 5, was obtained from the USGS website. The measured estrogen levels in 2007 at three locations along the Redwood River in the sub-watersheds 5, 6 and 7, respectively, were obtained from Lee et al., 2014. The feedlot data in Minnesota was obtained from the Minnesota Geospatial Commons, and livestock populations within the study area were estimated using the ArcGIS tool. The beef cattle, dairy cattle, and swine were identified as the dominant livestock within the study area. The livestock populations were assumed to keep constant throughout the simulation period. The livestock populations in each sub-watershed are summarized in Table 7.2.

Table 7.1 *The land use and area of the Redwood River Watershed in the unit of km^2.*

Sub-watershed	Water and wetland	Urban and build-up	Rangeland	Pasture	Cropland	Forest	Barren	Sum
1	23	12	39	9	162	2	0	248
2	4	10	20	2	100	0	0	135
3	12	10	29	3	127	0	0	181
4	1	4	8	4	50	1	0	68
5	3	6	7	10	34	6	0	66
6	1	10	0	0	9	0	0	20
7	1	4	0	0	45	0	0	50
Sum	44	57	102	29	526	10	1	768

Table 7.2 *The livestock population within the Redwood River Watershed.*

Livestock	Sub-watershed						
	1	2	3	4	5	6	7
Dairy cattle less than 1000 pounds	80	30	125	20	0	0	50
Dairy cattle - heifers	380	189	872	20	0	30	50
Dairy cattle - calves	350	145	147	21	0	30	50
Dairy cattle greater than 1000 pounds	1007	454	435	73	0	65	100
Beef cattle - slaughter/stock	3175	3688	1480	844	650	0	975
Beef cattle - feeder/heifer	1585	1955	2305	725	85	0	0
Beef cattle - cow/calf pairs	1708	831	829	212	0	0	60
Beef cattle - calves	395	980	200	375	0	0	0
Swine greater than 300 pounds	15	205	310	0	0	45	0
Swine 55-300 pounds	10710	3735	7000	54	0	2900	4900
Swine less than 55 pounds	695	1790	3425	0	0	0	500

7.2 Estrogen Loads to the Redwood River

In this study, cattle, swine, septic systems, and WWTPs were considered as primary sources of estrogens. Based on the United States Environmental Protection Agency (USEPA), biosolids are applied to less than 1% of the nation's agricultural land and are thus not considered as a major source of estrogens.

In this study, beef and dairy cattle were assumed to be either kept in feedlots or allowed to graze, and swine was assumed to be only kept in feedlots. Cattle manure produced during grazing can be either deposited onto pastureland or directly released into streams (Lucas & Jones, 2006; Shappell et al., 2016). Manure produced by cattle and swine during confinement is collected for land application to agricultural land. This study assumed that the cattle manure collected during confinement was applied to both the cropland and the pasture land, while the swine manure collected during confinement was only applied to cropland. 74%, 35%, and 5% of the total dairy, beef, and swine manure were estimated to be applied to agricultural land, and 25% and 60% of the dairy and beef manure were estimated to be released to the pasture land in the Redwood River Watershed (Doering et al., 2013). Beef and dairy cattle are assumed to graze from May to October (University of Maine Cooperative Extension, 2014). Cattle were also assumed to get access to surface water during grazing. The grazing schedule for dairy cattle was summarized in Table 7.3.

Table 7.3 *Daily schedule for beef cattle and dairy cattle on pastureland and in streams in the Redwood River Watershed*

Month	Dairy cattle		Beef cattle	
	Pasture (h)	Streams (h)	Pasture (h)	Streams (h)
January	0	0	0	0
February	0	0	0	0
March	0	0	5.8	0.2
April	0	0	11.5	0.5
May	11.5	0.5	22	2
June	10.75	1.25	20.75	3.25
July	10.75	1.25	20.75	3.25
August	10.75	1.25	20.75	3.25
September	11	1	22.5	1.50
October	11.5	0.5	23	1.00
November	0	0	5.8	0.2
December	0	0	0	0

Direct estrogen deposition onto pastureland during cattle grazing was quantified by multiplying the daily estrogen production of cows by the time fraction that cows spent on pastureland. Direct estrogen deposition into streams was estimated by multiplying the daily waste production of cows by the time fraction that cows spent in streams and the estrogen amount that can be desorbed from cattle manure in water. The amount of E1, E2α, and E2β desorbed from solid waste matrices are 7.7, 2.5 and 3.0 ng/g, respectively (Andaluri et al., 2012). Estrogen loads from manure land application were estimated by multiplying the manure application rate by the estrogen content in manure. The manure application rate in Minnesota was estimated by the nitrogen needs of crops. Corn-soybean crop rotation is a typical tillage method used in Minnesota and swine manure is used for land application from early October to mid-November (Vetsch & Lamb, 2011). In this study, the cattle manure is assumed to be used for land application both in spring and autumn. The estimated manure application rate is summarized in Table 7.4.

Table 7.4 *Schedule of manure application to agricultural land in the U.S.*

Month	Cattle manure applied (%)	Swine manure applied (%)
January	0	0
February	0	0
March	0	0
April	20	0
May	20	0
June	0	0
July	0	0
August	0	0
September	15	0
October	30	67
November	15	33
December	0	0

Manure is considered to be liquid when the manure has a solid content less than 15% based on Minnesota PCA regulations. The characteristics of cattle and swine manure are summarized in Table 7.5, and they all have a solid content less than 15%, thus cattle and swine were considered liquid manure and stored in an anaerobic lagoon for 180 days before land application (Edmonds et al., 2003). Injection is a suggested method for liquid manure application (Hernandez, 2012).

Table 7.5 *Cattle and swine manure solid content and the estrogen content in manure.*

Livestock	Dry solids percentage (%)	Estrogen content (ng/g-DW)			Reference
		E2α	E2β	E1	
Dairy cattle	14.0	1416	153	535	Zheng et al., 2007; ASAE, 2003
Beef cattle	14.7	4.7	7.3	150	Bartelt-Hunt et al., 2013; ASAE, 2003
Swine	13.1	290.9	619.7302	1774.874	Ramen et al., 2014; ASAE, 2003

Loads of estrogen from the failed septic system and straight pipes were calculated by multiplying the population by failing rate of septic systems and the estrogen excretion amount per human. The household and population data were obtained from the USCB. The population in the Redwood River Watershed were estimated using the ArcGIS tool. The estimated human population within the study area is listed in Table 7.6. Based on the information provided by the Minnesota Pollution Control Agency (PCA), 16% of the subsurface sewage treatment systems (SSTS) are failing and 4% are imminent public health threats (IPHT). Thus, a total of 20% of septic systems were assumed to discharge estrogens into the surface water within the study area. The main estrogen excreted by human wastes are E1 and E2β, and the excretion of E2α is quite limited (Moos et al., 2009). The daily excretions of E1 and E2β by per male are about 2500 and 2865 ng, respectively, and the daily excretions of E1 and E2β by per female are about 6607 and 5513 ng, respectively (Adeel et al., 2017).

Table 7.6 *The estimated population within the Redwood River Watershed.*

Watershed	Male	Female
1	484	473
2	525	535
3	253	243
4	118	114
5	200	196
6	2851	2745
7	345	304

Tyler WWTP, Lynd WWTP, Marshall WWTP, Russell WWTP, and Ruthton WWTP contribute estrogens into rivers through effluents. The daily loads of estrogen from WWTPs were calculated by multiplying the discharge by the estrogen content in the effluent discharge. The estrogen

levels in the effluents of Lynd WWTP and Marshall WWTP were obtained from Ferrey, 2011. The estrogen levels in the effluents of Russell WWTP, Tyler WWTP, and Ruthton WWTP were estimated based on the measured data for WWTPs with similar or same treatment units by Ferrey, 2011. The detailed information of these WWTPs is provided in Table 7.7. For estrogen levels which were below the detection limit and were thus not accurately measured, the minimum detection limit of 0.21 ng/L divided by the square root of 2 was used (Lee et al., 2011).

Table 7.7 *The discharge, treatment unit, and estimated effluent estrogen levels in the five WWTPs located in the Redwood River Watershed. nd- not detected.*

Name	**Sub-watershed**	**Annual average flow (m^3/day)**	**Treatment unit**	**E1 (ng/L)**	**E2α (ng/L)**	**E2β (ng/L)**
Tyler WWTP	2	683	Stabilization Pond	18	1.03	6.8
Ruthton WWTP	3	160	Stabilization Pond	18	1.03	6.8
Lynd WWTP	5	79	Stabilization Pond	18	1.03	6.8
Russell WWTP	5	218	Cutting Screen, Activated Sludge Extended Aeration, Final Settling Tank, Chlorination, Sludge Storage Tank for Hauling by Tank Truck	nd	nd	1.24
Marshall WWTP	6	13242	Grit Chamber Aerated, Mechanical Bar Screens, Primary Settling Tank, Aerated Pond, Digester, Activated Sludge Extended Aeration, Final Settling Tank, Solids Contact Clarifier, Mixed Media Gravity Filters, Chlorination, Sludge lagoon	3.04	nd	0.91

7.3 Transformation and Transport of Estrogens in the Watershed

The attenuation and interconversion of estrogens during storage in anaerobic lagoons and on land during dry periods were estimated using the comprehensive transformation model depicted in Chapter 3. The dominant soil within the Redwood River Watershed is silt-clay loam based on the information by the Web Soil Survey (WSS). The transformation and attenuation rates of estrogens were estimated by fitting the model to the measured data in water sampled from an anaerobic lagoon by Zheng et al., 2012 and in the silt-clay loam soil by Mashtare et al., 2013. The estimated transformation and degradation rates are summarized in Table 7.8.

Table 7.8 *The estimated transformation and interconversion rates of estrogens in an anaerobic lagoon and on land during dry periods.*

Transformation /Degradation rate (day^{-1})	**Anaerobic lagoon**	**Silt-clay loam**
k_1	0.18	0.050
k_{-1}	0.12	0.028
k_2	3.0	2.2
k_{-2}	1.8	0.92
k_3	0.018	0.050
k_4	0.018	0.019
Reference	Zheng et al., 2012	Mashtare et al., 2013

The degradation of estrogens in rivers is rapid, and interconversion rates are hard to be directly measured. Thus, estrogen attenuations in surface water are estimated by the step degradation model developed by Colucci & Topp, 2002 and Steiner et al., 2010. In this simplified model, E2α and E2β are first degraded into E1, and then into other compounds. The degradation rates of E2β and E1 in the Redwood River were estimated to be 3.2 and 0.85 day^{-1}, respectively, using

the data reported by Writer et al., 2011. The degradation rate of E2α was estimated to be 0.62 day^{-1} using the data measured in water sampled from Taunton River, Massachusetts (Robinson et al., 2017).

Surface runoff during storm events was considered as the major mechanism to transport estrogens from land to surface water. The transport of estrogens from land to surface water was estimated by the wash-off model depicted in Chapter 3. The wash-off coefficient, k_w, was estimated by fitting the data obtained from Yang et al., 2012 and the estrogen mass on land was estimated using the comprehensive transformation model. As the HSPF program utilizes a k_w-value measured in hr/in, the unit of k_w was converted from min/L to hr/in in this case study. The k_w-values for E1, E2α, and E2β were estimated to be 0.686, 0.117, and 0.406 hr/in, respectively. Those values were used for estrogen modeling work in the Redwood River Watershed.

7.4 Hydrological Calibration and Validation

The hydrological calibration and validation were conducted before estrogen stimulation. The simulated flow rates for May 2006 to April 2008 were compared to the observed data at the USGS Gage 05315000 for hydrological calibration. The flow rates were simulated by adjusting the hydrological parameters within the possible range allowed by BASINS (Technical Note 6). The key parameters included the upper zone nominal soil moisture storage (UZSN), the lower zone nominal soil moisture storage (LZSN), the index to infiltration capacity (INFILT), the interflow inflow parameter (INTFW), variable groundwater recession (KVARY), interflow inflow parameter (INTFW), and the lower zone evapotranspiration parameter (LZETP). Then the calibrated model was then used to simulate the flow rates in May 2008 to April 2009 for the hydrological validation.

The observed and the simulated daily flow rates at the USGS Gage 05315000 from May 2006 to April 2009 are illustrated in Figure 7.2. The statistical hydrological calibration results are summarized in Table 7.9. The major hydrological parameter values used in this model are provided in Appendix C. The HSPF model simulated two high flows in August 2007 and October 2008 which were not measured in the Redwood River. It leads to a significant difference between the simulated and measured flow rates during the validation period. The difference is caused by the large precipitation depth measured at the Southwest Minnesota Regional Airport during this period. It indicates that the meteorological data measured at the two stations cannot represent the precipitation variations across the study area.

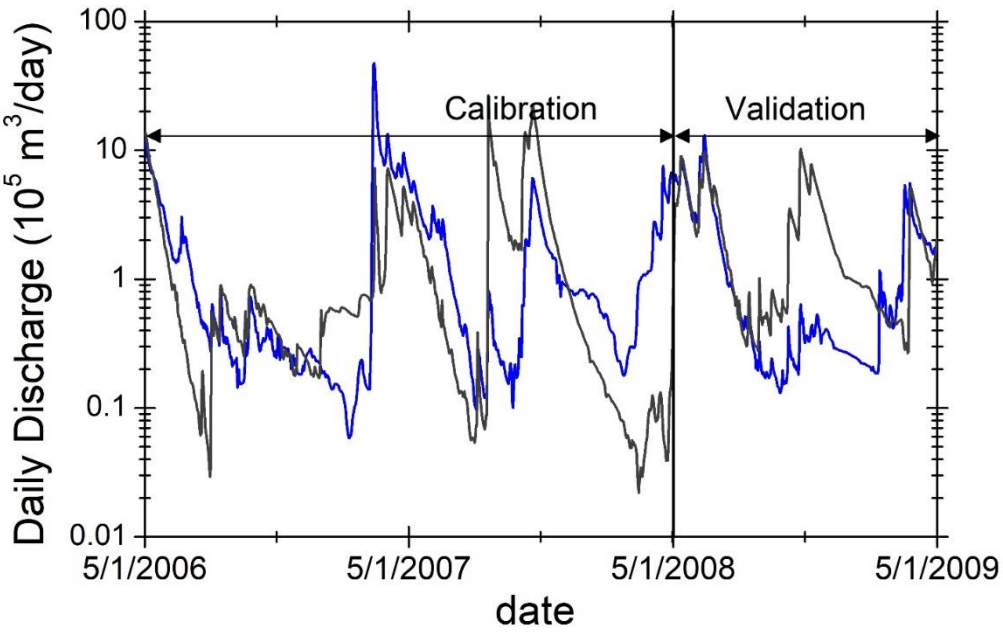

Figure 7.2 *The observed and the simulated daily flow rates at the USGS Gage 05315000 from May 2006 to April 2009. The blue dashed curve denotes the observed flow rates and the gray solid curve denotes the simulated flow rates. The flow rates in May 2006 to April 2008 were used for hydrological calibration, and the flow rates in May 2008 to April 2009 were used for hydrological validation.*

Table 7.9 *Comparison of the measured and the simulated flow rates in the unit of 10^5 m^3/day at the USGS gage 05315000 in the Redwood River Watershed from May 2006 to April 2009*

Flow	Calibration			Validation			Criteria (%)
	Observed (10^5 m^3/day)	Simulated (10^5 m^3/day)	Percentage difference	Observed (10^5 m^3/day)	Simulated (10^5 m^3/day)	Percentage difference	
Total Flow	1450	1303	-10	587	855	46	10
High Flow	722	685	-5	191	239	25	15
Low Flow	94	87	-7	58	34	-42	10
Summer Flow	177	216	22	231	224	-3	30
Winter Flow	70	69	0	31	89	191	30
Storm Flow	156	160	3	79	67	-15	15

As the difference between the measured and simulated winter flow during the validation period is significant, a hydrological sensitivity analysis was conducted to explore the uncertainties caused by the hydrological parameters. In this study, each hydrological parameter was increased or decreased by 20%. Then the change the total flow, high flow, low flow, summer flow, winter flow, and storm flow from May 2006 to April 2009 were assessed. The analytical results are summarized in Table 7.10. The analyzing results show that AGWRC, INFILT, LZSN, and UZSN can effectively affect the total flow, high flow, low flow, and seasonal flow. In this case study, AGWRC has the largest impact on the simulated flow rates. Increasing the value of AGWRC decreases high flow, increases low flow, and decreases storm flow. Additionally, the hydrological sensitivity analysis shows that LZSN has a large impact on both summer and winter flow, while UZSN has a large impact only on summer flow. Compared to other hydrological parameters, INTFW and LZETP have little impact on simulating results.

Table 7.10 *Percentage Difference between the Simulated and Measured Flow from May 2006 to April 2009*

Hydrological parameter		Total flow	High flow	Low flow	Summer flow	Winter flow	Storm flow
Original		6	1	-20	8	58	-3
LZSN	+20%	-1	-14	-5	-2	50	-5
	-20%	14	16	-35	21	71	-7
INFILT	+20%	7	4	-23	5	58	-3
	-20%	5	1	-17	13	60	-4
KVARY	+20%	6	9	-20	6	43	-2
	-20%	6	-7	-22	10	78	-2
AGWRC	+20%	-34	-87	-14	-31	179	-5
	-20%	8	77	-45	25	-4	-36
UZSN	+20%	3	-5	-19	0	58	-3
	-20%	9	7	-20	18	59	-4
INTFW	+20%	6	1	-20	8	58	-3
	-20%	6	1	-20	9	59	-3
LZETP	+20%	6	1	-20	8	58	-3
	-20%	6	1	-20	8	58	-3

7.5 Estrogen Simulation

After the hydrological calibration, the estrogen simulation was conducted. The estrogen levels in 2007 at three points along the Redwood River in the sub-watersheds 5, 6 and 7 were simulated using the HSPF program and compared to the measured levels by Lee et al., 2014. The simulated estrogen equivalency was also estimated and compared to the average levels estimated by Lee et al., 2014. One unit of E1, E2α, and E2β equals 0.2, 0.125 and 1 unit of estrogen equivalency, respectively (Lee et al., 2014). Then the following hypothetical scenarios were conducted to quantify the estrogens contributed from each source into the Redwood River:

1) Estrogen loads from only beef manure used for land application were added into the model;

2) Estrogen loads from only grazing beef cattle were added into the model;

3) Estrogen loads from only dairy manure used for land application were added into the model;

4) Estrogen loads from only grazing dairy cattle were added into the model;

5) Estrogen loads from only swine manure used for land application were added into the model;

6) Estrogen loads from failed septic systems were added into the model;

7) Estrogen loads from WWTPs were added into the model.

Figure 7.3 presents the simulated and measured E1, E2α, and E2β concentrations in the Redwood River in 2007 including all of the sources that are described in Section 7.2. The simulated E1, E2α, and E2β levels were highest at Point 1 located in the sub-watershed 5 and lowest at Point 3 located in the sub-watershed 7. The simulated concentrations of E1 are higher than those of E2α and E2β throughout the study period in all three sub-watersheds. It is due to the assumed rapid irreversible transformation of E2α and E2β into E1 in surface water. Additionally, the simulated concentrations of E2α are higher than those of E2β. It is caused by the lower degradation rates in water used for E2α than E2β in this study. As the degradation rate of E2α used in this study was not measured in the Redwood River, it induces uncertainties in the modeling results.

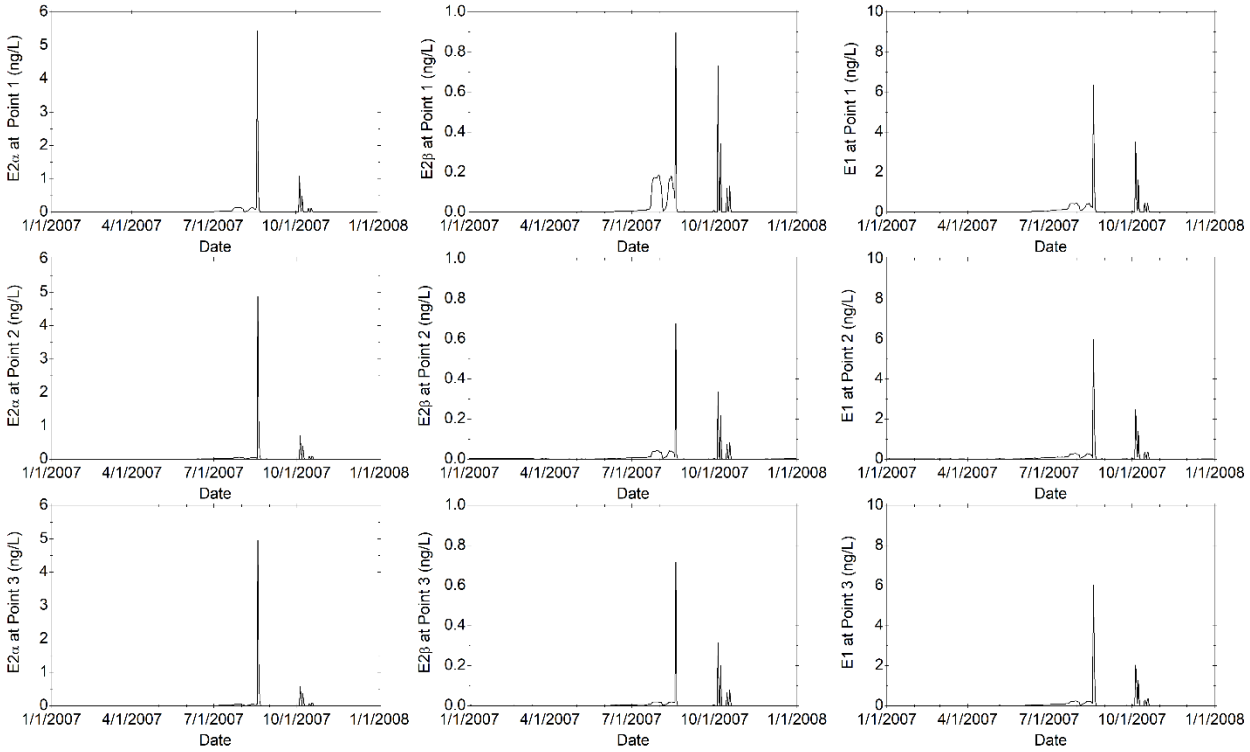

Figure 7.3 *Simulated estrogen levels at three points along the Redwood River.*

Figures 7.4 to 7.6 show the measured and simulated estrogen levels with the simulated flow rates at the three points. The simulated estrogens at the three points have a similar pattern. The highest concentrations occur in August and October, which coincident with high simulated flow rates. The simulated estrogen levels in sub-watershed 5 in storm events are higher than those in sub-watershed 6 and 7. It is caused by the larger agricultural land area in sub-watershed 5 than those in sub-watersheds 6 and 7. Large effluents from Marshall WWTP located in the sub-watershed 6 with low estrogen concentrations further decrease simulated estrogen levels in the sub-watershed 6 due to dilution. Additionally, the simulated estrogen levels in sub-watershed 7 in storm events are slightly higher than those in sub-watershed 6. This is due to the larger agricultural land area in the sub-watershed 7 than that in the sub-watershed 6, as shown in Table 7.1. In general, the simulated estrogen profiles in the Redwood River show a positive relationship with the flow rate.

Specifically, the peak concentrations of estrogens appear with the peak flow, while the concentrations of estrogens are quite low when the low flow appears. The high simulated estrogen levels are also impacted by the manure land application time. The modeling results show that the high surface runoff just after land application can lead to high estrogen levels in rivers. The estrogen levels were measured in sub-watersheds 5, 6 and 7 on February 27th, March 20th, May 10th, June 21st, August 28th, and September 25th in 2007. The measured total estrogen levels in these three sub-watersheds range from below the detection limit to 1.16 ng/L, from below the detection limit to 1.75 ng/L, and from below the detection limit to 1.6 ng/L, respectively. These measured values are comparable to the total simulated estrogen levels, which range from 0.001 ng/L to 12.7 ng/L, from 0.002 ng/L to 11.5 ng/L, and from 0.002 ng/L to 11.7 ng/L in sub-watersheds 5, 6, and 7, respectively. However, the model underestimates the estrogen levels in February and May at Point 1 and in March at Points 2 and 3, it may be caused by the underestimated surface runoff at this time. The high measured estrogen levels in March may also be caused by the high manure application rate occurred at this time in the real practice. The estimated manure application rate may fail to represent variations of real manure application rate across the study area and cause uncertainties to modeling results. Additionally, the peak simulated estrogen levels in August and October may overestimate the actual estrogen levels due to the overestimated surface runoff. However, such an overestimation cannot be verified by the measured data due to the lack of the measured data at this time. Figures 7.4 to 7.6 do not show an evident relationship between the WWTP discharge and simulated estrogen levels or between the WWTP discharge and observed estrogen levels. This indicates that the impact of WWTPs on estrogen levels in the Redwood River is not significant.

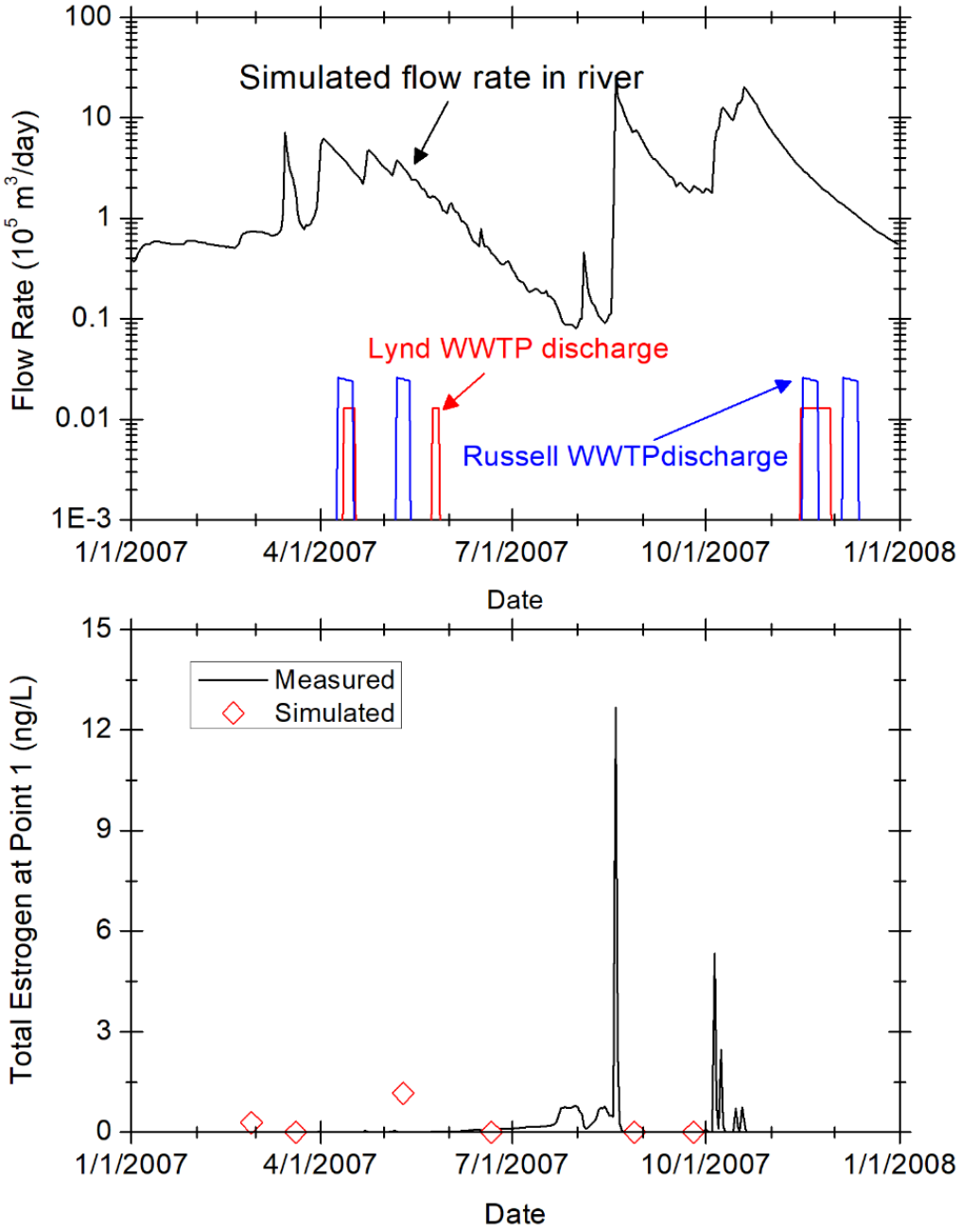

Figure 7.4 *Simulated and measured estrogen concentrations at Point 1 in 2007. The solid curve represents the simulated total estrogen levels in the Redwood River, and the red diamonds denote the measured estrogen levels by Lee et al., 2014.*

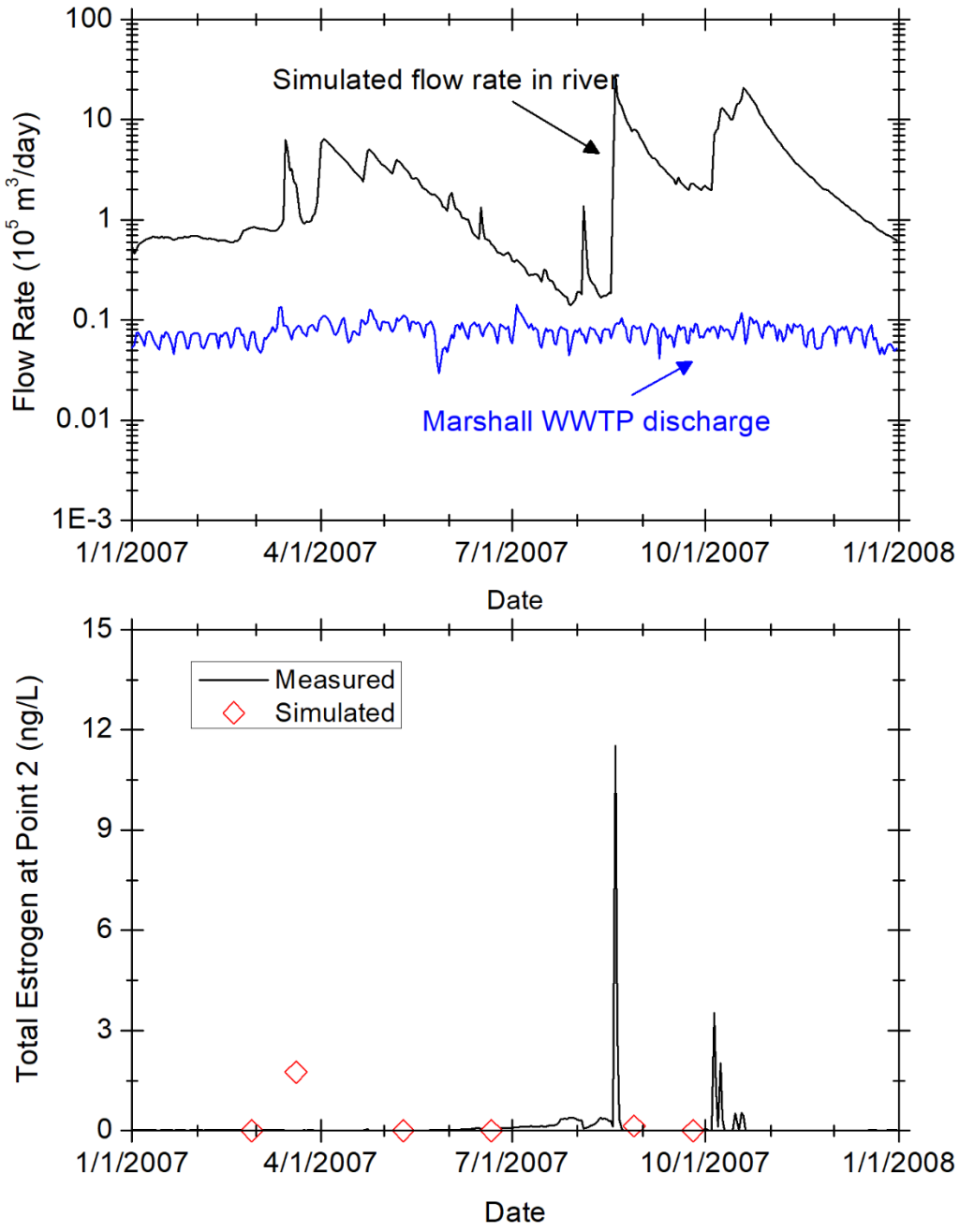

Figure 7.5 *Simulated and measured estrogen concentrations at Point 2 in 2007. The solid curve represents the simulated total estrogen levels in the Redwood River, and the red diamonds denote the measured estrogen levels by Lee et al., 2014.*

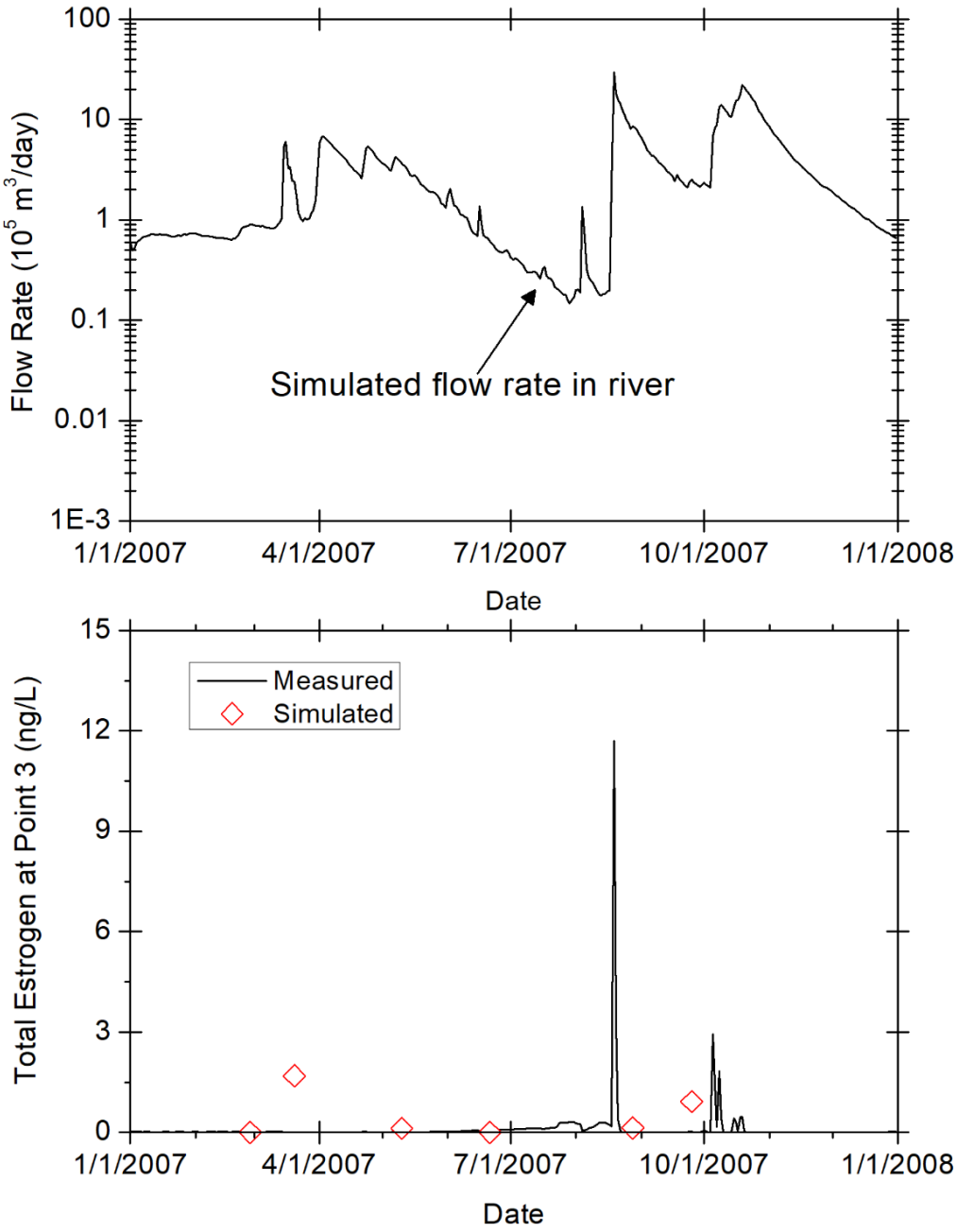

Figure 7.6 *Simulated and measured estrogen concentrations at Point 3 in 2007. The solid curve represents the simulated total estrogen levels in the Redwood River, and the red diamonds denote the measured estrogen levels by Lee et al., 2014.*

The estradiol equivalency was calculated from the simulated estrogen levels and compared to the predicted-no-effect-concentration (PNEC). The simulated estrogen equivalence in this three sub-watersheds ranges from 0.0002 to 2.85 ng/L, from 0.0008 to 2.45 ng/L and from 0.0007 to 2.54 ng/L, respectively. The average measured estrogen equivalence in Sub-watersheds 5, 6 and 7 in 2017 are 0.032 ng/L, 0.082 ng/L and 0.056 ng/L respectively. The PNEC of E2β is estimated to be 2 ng/L (Caldwell et al., 2012). As the simulated estradiol equivalency is below the PNEC during most of the time, the adverse effects of estrogens to the aquatic animals is negligible.

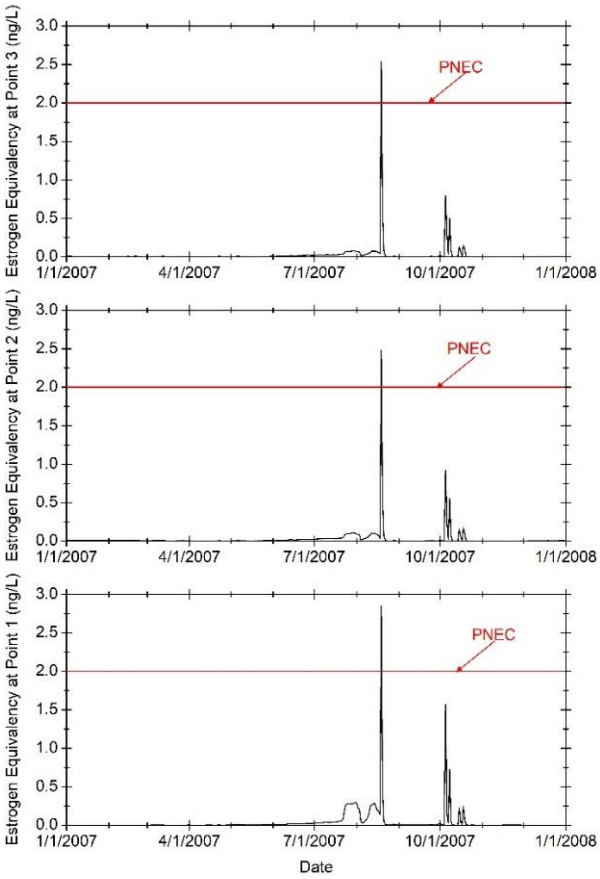

Figure 7.7 *Simulated and measured estrogen equivalence at the three sampling points along the Redwood River Watershed in 2007. The solid curve represents the simulated estrogen equivalence in the Redwood River, and the red line denotes the average measured estrogen equivalence by Lee et al., 2014.*

The contribution of estrogens from each source is summarized in Table 7.11. The simulated estrogen levels from each source are shown in Appendix D. This analysis shows that the nonpoint sources contribute the majority of estrogens to the surface water and the contribution of WWTPs is smaller than 1%. However, WWTPs contribute a large amount of estrogens to surface water in the South River Watershed study. It was due to the high WWTPs loads used in the South River Watershed case study. In this case study, the measured estrogen levels in the discharge of the largest WWTP, Marshall WWTP, are low. Even though the measured estrogen levels in other small WWTPs are high, they only have periodic discharge to rivers in April, May, October, and November. Thus the contribution of WWTPs in this case study is not significant.

Table 7.11 *The contribution of estrogens from each source.*

Estrogen source	**Initial loads of estrogens from each source**			**Total simulated estrogen mass in the river from each source**			**Percentage of the simulated each kind of estrogens from each source**		
	E1	**E2α**	**E2β**	**E1**	**E2α**	**E2β**	**E1**	**E2α**	**E2β**
	g/year	**g/year**	**g/year**	**g/year**	**g/year**	**g/year**	**%**	**%**	**%**
Release to land									
Beef Cattle	4040	127	197	4.61	0.195	0.501	8.71	0.920	4.30
Dairy Cattle	4710	12500	1350	14.9	18.0	3.32	28.2	85.0	28.5
Swine	30200	4950	10500	32.6	2.94	7.70	61.6	14.0	66.1
Release to river									
Beef Cattle	229	7.00	11.0	0.177	0.021	0.021	0.335	0.099	0.182
Dairy Cattle	98.0	259	28.0	0.167	0.013	0.031	0.316	0.061	0.264
WWTPs	52.0	3.00	17.0	0.253	0.008	0.040	0.478	0.039	0.339
Septic Systems	3.00	--	3.00	0.167	--	0.034	0.316	--	0.296

7.6 Sensitivity Analysis

The accuracy of this complex model is impacted by several factors including the flow rate, the degradation rates of E2α and E2β into E1, the degradation rate of E1 into other compounds, livestock density, the WWTP effluent concentration and wash-off coefficient. In order to quantify the uncertainty caused by these factors, a sensitivity analysis was conducted for hydrological and estrogen parameters. The hydrological parameters include precipitation, LZSN, INFILT, KVARY, AGWRC, UZSN, INTFW, and LZETP. The estrogen parameters include the degradation rate of E2α and E2β into E1, the degradation rate of E1 into other compounds, the livestock density, the WWTP effluent concentration, manure application rates, and the grazing time. In the sensitivity analysis, each of the parameters listed above decreases or increases by 20%, and the changes of the annual mean of the simulated total estrogen levels were compared.

Figures 7.8 and 7.9 show the results of the sensitivity analysis as the percent change in mean for the hydrological parameters and the estrogen parameters at Point 1. Figure 7.8 indicates that the simulated estrogens are sensitive to AGWRC and precipitation. This is due to the fact that the modeling results are strongly affected by the flow rates, which is sensitive to AGWRC and precipitation. Figure 7.9 shows that the simulation results are most sensitive to the decay rate of estrogens in rivers, manure application rates, and the cattle grazing time. This result shows that the assumption of estrogen decay rates in rivers, manure application time and rates, and the cattle grazing schedule can largely impact the simulation results. The sensitivity analysis for Points 2 and 3 show the similar results and is shown in Appendix E.

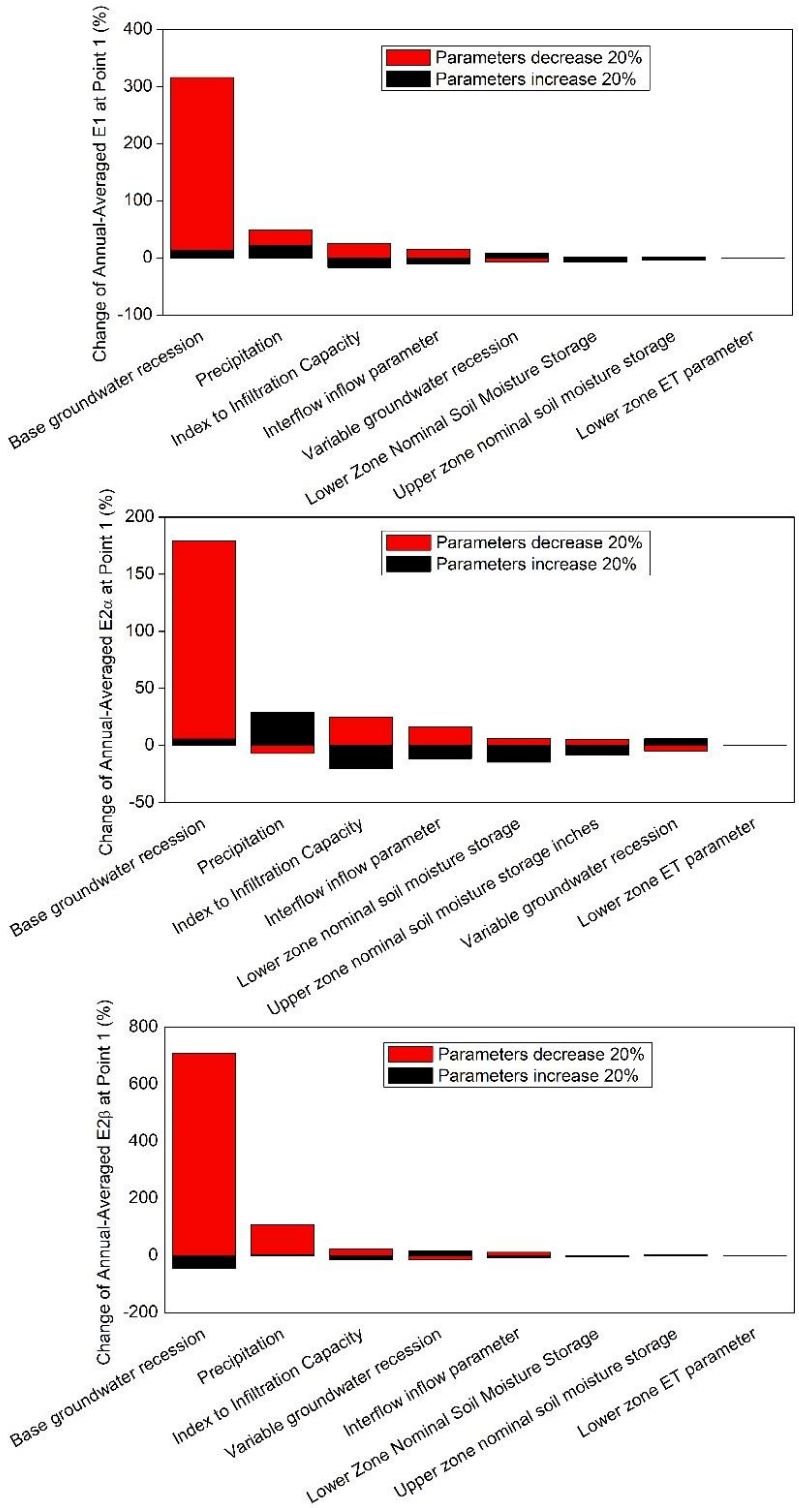

Figure 7.8 *The percentage change of the simulated annual-averaged estrogens at Point 1 with a change of the hydrological parameters.*

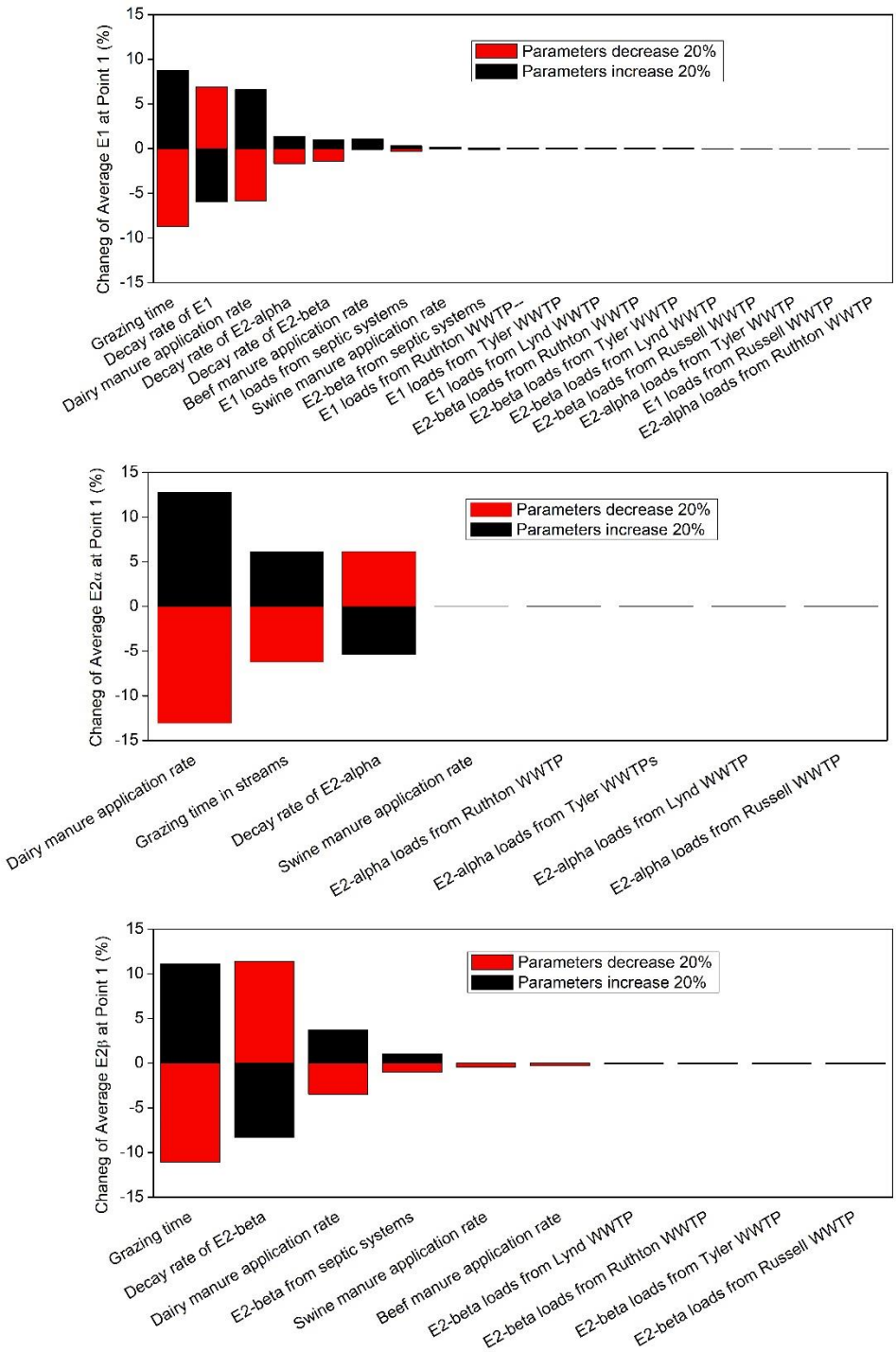

Figure 7.9 *The percentage change of the simulated annual-averaged estrogens at Point 1 with a change of the estrogen parameters.*

7.7 Implications of the Modeling Results

The simulated estrogen levels in the Redwood River are highly affected by the hydrological conditions. The surface runoff during the rainfall events has the potential to transport estrogens from land to water. At the same time, the increased river flow has the potential to dilute estrogen concentrations in the Redwood River (Zhao & Lung, 2017). The large WWTPs usually use tertiary treatments and the contributions of WWTPs is small. However, WWTPs with only primary or secondary treatments have the potential to elevate estrogen levels in rivers during dry periods. Additionally, the storm events just after manure land application can transport a large amount of estrogens to surface water. Buffer stripes, which have been observed to effectively reduce estrogen loads by surface runoff from land, are recommended (Shore, 2009b).

Chapter 8 Summary, Conclusion, and Recommendations

8.1 Summary and Conclusions

This study first developed a comprehensive transformation model to describe the complex transformation of E1, E2α, and E2β during attenuation. This provides the possibility to simultaneously simulate various kinds of estrogens which may impact the levels of each other. This comprehensive transformation model fits the data well for estrogen transformation under both anaerobic and aerobic conditions as well as in aqueous solutions and solids. Compared to previous models, this model can accurately estimate the complex variations of estrogen levels with the interference of the complex conversion among estrogens. This study then compared two simple models, the wash-off, and empirical models, to investigate the transport of estrogens from agricultural land by surface runoff during rainfall events. The data reported by Yang et al., 2012 was used to evaluate the performance of the wash-off and empirical models. While both models can closely simulate the mass loads of estrogens during rainfall events, the wash-off model is more compatible with hydrological modeling software such as HSPF for large-scale modeling. Study results prove that the wash-off model is suitable for modeling the transport of estrogens following rainfall events. Finally, the validated wash-off model and the comprehensive transformation model were employed to develop a framework to assess estrogen levels in the natural environment using the HSPF program. This modeling framework assumes that estrogens released to agricultural accumulate during dry periods, and are then transported into rivers by surface runoff during storm events. Meanwhile, some estrogens are directly released into surface water. These estrogens go through complex transformation and attenuation processes both on land and in water. This framework was applied to the South River Watershed in Virginia and the

Redwood River Watershed in Minnesota to simulate estrogen levels in the surface water. For the estrogen simulation, cattle and poultry manure, biosolids, human feces and WWTP effluents were considered as the primary sources of estrogens.

This modeling framework was used to identify the factors that lead to high estrogen levels in rivers and assess potential risks of estrogens on a watershed and determine BMPs. The analyzing results of both case studies show that flow rate and point sources affected the simulated in-stream estrogen concentrations significantly. The simulation results are most sensitive to the in-stream decay rate, manure application rate, and livestock grazing time. They also show that estrogen on the land surface is transported to the receiving water by the surface runoff and the estrogen released into streams can be diluted by the high water flow. The reduction of loads from the point sources on dry days and the reduction of loads from the nonpoint sources on rainy days are two practical mechanisms to control estrogens. The BMPs for the cattle manure management is desired in this area to prevent the potential for high estrogen levels in streams from grazing livestock. These results indicate that fencing off rivers to keep cattle out of the water is the most economical practice with consideration for cost and practicality. Manure storage before the land application is also encouraged to reduce estrogen content. Modeling results also indicate that the surface runoff just after manure application can cause elevated estrogen levels in rivers. Buffer stripes have been observed to effectively reduce estrogen loads by surface runoff from manure land application and is thus recommended.

The two case studies show that the modeling framework developed in this study can be applied to various watersheds to assess the temporal and spatial variations of estrogens levels along rivers. This modeling framework can also be used to quantify estrogen contributions from

various estrogen sources. Such quantification can be further used to develop the total maximum daily loads (TMDL) of estrogens for a watershed. In addition to estrogens, the modeling framework can be applied to compounds that have the similar fate and transport characteristics and processes, such as androgens.

8.2 Recommendations and Future Directions

Although recent studies reveal rich information about estrogens, many gaps still remain in our understanding of estrogen fate and transport on a watershed scale. First, most studies focusing on the attenuation of estrogens are conducted under ideal experimental conditions and very few of them investigate the attenuation rates in the natural environment. Second, at present, most of the studies investigating the transport of estrogens from agricultural land quantitatively conclude that the transport process is affected by precipitation, but they fail to quantify the transport of estrogens by additional processes, such as irrigation leaching, preferential flow, pronounced macropore flow, and discharge from the soil (Shore et al., 2004; Sangsupan et al., 2006; Kjær et al., 2007; Durán–Álvarez et al., 2014; Jones et al., 2014a). Third, animals can excrete different amounts of estrogens at different estrous states or with different diets, which is often not accounted for when estimating the yearly production of estrogens from livestock (Tucker, 2009; Zhang, Shi, Liu, Zhan & Chen, 2014). Additionally, the major focus has been on measuring the fecal excretion of estrogens, but there are other less studied pathways for estrogen excretion such as through emesis (Cantarow et al., 1943). Furthermore, in addition to farm animals, wild animals can also contribute estrogens to surface water (Rolland et al., 2005; Pereira et al., 2006). Thus, the estrogen load estimation in this study may be inaccurate and incomplete due to the lack of knowledge. Fourth, current studies assume constant attenuation and transformation rates of estrogens. However, they are not constants

and affected by the estrogen concentrations. Fifth, this framework only works for E1, E2α, and E2β. Compared to these estrogens, EE2 has a larger estrogenic potency and can impair the reproductive function of *Gobiocypris rarus* at a concentration of 0.2 ng/L. Thus, the modification is needed to quantify the potential hazardous of EE2. In order to address the issues listed above and further improve the modeling framework developed in this study, further studies are needed to more accurately quantify the estrogen amount excreted by both farm animals and wildlife. Studies to investigate additional pathway to transport estrogens from land to rivers in addition to surface runoff are also needed. Additionally, the attenuation and transformation rates measured in the natural environment are essential to accurately estimate estrogen levels in the natural water bodies. More importantly, studies to track the fate and transport of EE2 are encouraged to more effectively assess the potential risks of estrogen to aquatic animals.

References

Adeel, M., Song, X., Wang, Y., Francis, D., & Yang, Y. (2017). Environmental impact of estrogens on human, animal and plant life: A critical review. *Environment International*. DOI: 10.1016/j.envint.2016.12.010

Andaluri, G., Suri, R. P., & Kumar, K. (2012). Occurrence of estrogen hormones in biosolids, animal manure and mushroom compost. *Environmental Monitoring and Assessment*, *184*(2), 1197-1205. DOI: 10.1007/s10661-011-2032-8

Aris, A. Z., Shamsuddin, A. S., & Praveena, S. M. (2014). Occurrence of 17α-ethynylestradiol (EE2) in the environment and effect on exposed biota: a review. *Environment International*, *69*, 104-119. DOI: 10.1016/j.envint.2014.04.011

ASAE Standard, 2003. Manure production and characteristics. ASAE D384.1 FEB03, USA, pp. 683–685.

Atkinson, S. K., Marlatt, V. L., Kimpe, L. E., Lean, D. R., Trudeau, V. L., & Blais, J. M. (2012). The occurrence of steroidal estrogens in south-eastern Ontario wastewater treatment plants. *Science of the Total Environment*, *430*, 119-125. DOI: 10.1016/j.scitotenv.2012.04.069

Bai, X., Casey, F. X., Hakk, H., DeSutter, T. M., Oduor, P. G., & Khan, E. (2013). Dissipation and transformation of 17β-estradiol-17-sulfate in soil–water systems. *Journal of Hazardous Materials*, *260*, 733-739. DOI: 10.1016/j.jhazmat.2013.06.036

Bai, X., Casey, F. X., Hakk, H., DeSutter, T. M., Oduor, P. G., & Khan, E. (2015). Sorption and degradation of 17β-estradiol-17-sulfate in sterilized soil–water systems. *Chemosphere*, *119*, 1322-1328. DOI: 10.1016/j.chemosphere.2014.02.016

Barel-Cohen, K., Shore, L. S., Shemesh, M., Wenzel, A., Mueller, J., & Kronfeld-Schor, N. (2006). Monitoring of natural and synthetic hormones in a polluted river. *Journal of Environmental Management*, *78*(1), 16-23. DOI:10.1016/j.jenvman.2005.04.006

Baronti, C., Curini, R., D'Ascenzo, G., Di Corcia, A., Gentili, A., & Samperi, R. (2000). Monitoring natural and synthetic estrogens at activated sludge sewage treatment plants and in a receiving river water. *Environmental Science & Technology*, *34*(24), 5059-5066. DOI: 10.1021/es001359q

Bartelt-Hunt, S. L., DeVivo, S., Johnson, L., Snow, D. D., Kranz, W. L., Mader, T. L., ... & Zhang, T. C. (2013). Effect of composting on the fate of steroids in beef cattle manure. *Journal of Environmental Quality*, *42*(4), 1159-1166. DOI: 10.2134/jeq2013.01.0024

Bartelt-Hunt, S. L., Snow, D. D., Kranz, W. L., Mader, T. L., Shapiro, C. A., Donk, S. J. V., ... & Zhang, T. C. (2012). Effect of growth promotants on the occurrence of endogenous and synthetic steroid hormones on feedlot soils and in runoff from beef cattle feeding operations. *Environmental Science & Technology*, *46*(3), 1352-1360. DOI: 10.1021/es202680q

Bass, T., Dafoe, J., & Schumacher. J. (2012). *Manure Composting for Livestock & Poultry Production.*

Ben, W., Zhu, B., Yuan, X., Zhang, Y., Yang, M., & Qiang, Z. (2017). Transformation and fate of natural estrogens and their conjugates in wastewater treatment plants: Influence of operational parameters and removal pathways. *Water Research*, *124*, 244-250. DOI: 10.1016/j.watres.2017.07.065

Berg, C., Gyllenhammar, I., & Kvarnryd, M. (2009). Xenopus tropicalis as a test system for developmental and reproductive toxicity. *Journal of Toxicology and Environmental Health, Part A*, *72*(3-4), 219-225. DOI: 10.1080/15287390802539079

Bhandari, R. K., Deem, S. L., Holliday, D. K., Jandegian, C. M., Kassotis, C. D., Nagel, S. C., ... & Rosenfeld, C. S. (2015). Effects of the environmental estrogenic contaminants bisphenol A

and 17α-ethinyl estradiol on sexual development and adult behaviors in aquatic wildlife species. *General and Comparative Endocrinology*, *214*, 195-219. DOI: 10.1016/j.ygcen.2014.09.014

Bircher, S., Card, M. L., Zhai, G., Chin, Y. P., & Schnoor, J. L. (2015). Sorption, uptake, and biotransformation of 17β-estradiol, 17α-ethinylestradiol, zeranol, and trenbolone acetate by hybrid poplar. *Environmental Toxicology and Chemistry*, *34*(12), 2906-2913. DOI: 10.1002/etc.3166

Birnbaum, L. S., & Fenton, S. E. (2003). Cancer and developmental exposure to endocrine disruptors. *Environmental Health Perspectives*, *111*(4), 389.

Biswas, S., Shapiro, C. A., Kranz, W. L., Mader, T. L., Shelton, D. P., Snow, D. D., ... & Ensley, S. (2013). Current knowledge on the environmental fate, potential impact, and management of growth-promoting steroids used in the US beef cattle industry. *Journal of Soil and Water Conservation*, *68*(4), 325-336. DOI: 10.2489/jswc.68.4.325

Blánquez, P., & Guieysse, B. (2008). Continuous biotransformation of 17β-estradiol and 17α-ethynylestradiol by Trametes versicolor. *Journal of Hazardous Materials*, *150*(2), 459-462. 10.1016/j.jhazmat.2007.09.085

Bradley, P. M., Barber, L. B., Chapelle, F. H., Gray, J. L., Kolpin, D. W., & McMahon, P. B. (2009). Biotransformation of 17β-estradiol, estrone and testosterone in stream sediments. *Environmental Science & Technology*, *43*(6), 1902-1910. DOI: 10.1021/es802797j

Brett, T. K. (2014). The fate of estrone (E1), 17beta-estradiol (E2), E3 (E3) and 17alpha-ethinylestradiol (EE2) in surface waters. *Electronic Theses and Dissertations (ETDs) 2008+*.

Brion, F., Tyler, C. R., Palazzi, X., Laillet, B., Porcher, J. M., Garric, J., & Flammarion, P. (2004). Impacts of E2, including environmentally relevant concentrations, on reproduction after exposure during embryo-larval-, juvenile-and adult-life stages in zebrafish (Danio rerio). *Aquatic Toxicology*, *68*(3), 193-217. DOI: 10.1016/j.aquatox.2004.01.022

Cajthaml, T., Křesinová, Z., Svobodová, K., & Möder, M. (2009). Biotransformation of endocrine-disrupting compounds and suppression of estrogenic activity by ligninolytic fungi. *Chemosphere*, *75*(6), 745-750. DOI: 10.1016/j.chemosphere.2009.01.034

Caldwell, D. J., Mastrocco, F., Anderson, P. D., Länge, R., & Sumpter, J. P. (2012). Predicted-no-effect concentrations for the steroid estrogens estrone, 17β-estradiol, estriol, and 17α-ethinylestradiol. *Environmental toxicology and chemistry*, *31*(6), 1396-1406. DOI: 10.1002/etc.1825

Cantarow, A., Rakoff, A. E., Paschkis, K. E., Hansen, L. P., & Walkling, A. A. (1943). Excretion of Exogenous and Endogenous Estrogen in Bile of Dogs and Humans. *Experimental Biology and Medicine*, *52*(3), 256-257. DOI: 10.3181/00379727-52-14109P

Cao, Q., Yu, Q., & Connell, D. W. (2010). Fate simulation and risk assessment of endocrine disrupting chemicals in a reservoir receiving recycled wastewater. *Science of the Total Environment*, *408*(24), 6243-6250. DOI: 10.1016/j.scitotenv.2010.08.059

Card, M. L., Schnoor, J. L., & Chin, Y. P. (2012). Uptake of natural and synthetic estrogens by maize seedlings. *Journal of Agricultural and Food Chemistry*, *60*(34), 8264-8271. DOI: 10.1021/jf3014074

Carr, D. L., Morse, A. N., Zak, J. C., & Anderson, T. A. (2011). Microbially mediated degradation of common pharmaceuticals and personal care products in soil under aerobic and reduced oxygen conditions. *Water, Air, & Soil Pollution*, *216*(1-4), 633-642. DOI: 10.1007/s11270-010-0558-y

Casey, F. X., Larsen, G. L., Hakk, H., & Šimunek, J. (2003). Fate and transport of 17β-estradiol in soil− water systems. *Environmental Science & Technology*, *37*(11), 2400-2409. DOI: 10.1021/es026153z

Casey, F. X., Šimůnek, J., Lee, J., Larsen, G. L., & Hakk, H. (2005). Sorption, mobility, and transformation of estrogenic hormones in natural soil. *Journal of Environmental Quality*, *34*(4), 1372-1379. DOI:10.2134/jeq2004.0290

Caupos, E., Mazellier, P., & Croue, J. P. (2011). Photodegradation of estrone enhanced by dissolved organic matter under simulated sunlight. *Water Research*, *45*(11), 3341-3350. DOI: 10.1016/j.watres.2011.03.047

Chambers, K. B., Casey, F. X., Hakk, H., DeSutter, T. M., & Shappell, N. W. (2014). Potential bioactivity and association of 17β-estradiol with the dissolved and colloidal fractions of manure and soil. *Science of the Total Environment*, *494*, 58-64. DOI: 10.1016/j.scitotenv.2014.06.121

Chen, Y., Zhang, K., & Zuo, Y. (2013). Direct and indirect photodegradation of estriol in the presence of humic acid, nitrate and iron complexes in water solutions. *Science of the Total Environment*, *463*, 802-809. DOI: 10.1016/j.scitotenv.2013.06.026

Chowdhury, R. R., Charpentier, P. A., & Ray, M. B. (2010). Photodegradation of estrone in solar irradiation. *Industrial & Engineering Chemistry Research*, *49*(15), 6923-6930. DOI: 10.1021/ie901796x

Chowdhury, R. R., Charpentier, P. A., & Ray, M. B. (2011). Photodegradation of 17β-estradiol in aquatic solution under solar irradiation: Kinetics and influencing water parameters. *Journal of Photochemistry and Photobiology A: Chemistry*, *219*(1), 67-75. DOI: 10.1016/j.jphotochem.2011.01.019

Collins, C., Fryer, M., & Grosso, A. (2006). Plant uptake of non-ionic organic chemicals. *Environmental Science & Technology*, *40*(1), 45-52. DOI: 10.1021/es0508166

Colucci, M. S., Bork, H., & Topp, E. (2001). Persistence of estrogenic hormones in agricultural soils. *Journal of Environmental Quality*, *30*(6), 2070-2076. DOI:10.2134/jeq2001.2070

Colucci, M. S., & Topp, E. (2002). Dissipation of part per trillion concentrations of estrogenic hormones from agricultural soils. *Canadian Journal of Soil Science*, *82*(3), 335-340. DOI: 10.4141/S01-079

Combalbert, S., Bellet, V., Dabert, P., Bernet, N., Balaguer, P., & Hernandez-Raquet, G. (2012). Fate of steroid hormones and endocrine activities in swine manure disposal and treatment facilities. *Water Research*, *46*(3), 895-906. DOI: 10.1016/j.watres.2011.11.074

Conley, J. M., Evans, N., Cardon, M. C., Rosenblum, L., Iwanowicz, L. R., Hartig, P. C., ... & Wilson, V. S. (2017). Occurrence and in vitro bioactivity of estrogen, androgen, and glucocorticoid compounds in a nationwide screen of united states stream waters. *Environmental Science & Technology*, *51*(9), 4781-4791. DOI: 10.1021/acs.est.6b06515

Crawford, P., Chadwick, D. J., Martin, C., Tjia, J., Back, D. J., & Orme, M. (1990). The interaction of phenytoin and carbamazepine with combined oral contraceptive steroids. *British Journal of Clinical Pharmacology*, *30*(6), 892-896. DOI: 10.1111/j.1365-2125.1990.tb05457.x

Cromwell, G. L., Stahly, T. S., Coffey, R. D., Monegue, H. J., & Randolph, J. H. (1993). Efficacy of phytase in improving the bioavailability of phosphorus in soybean meal and corn-soybean meal diets for pigs. *Journal of Animal Science*, *71*(7), 1831-1840. DOI:10.2527/1993.7171831x

D'Alessio, M., Vasudevan, D., Lichwa, J., Mohanty, S. K., & Ray, C. (2014). Fate and transport of selected estrogen compounds in Hawaii soils: Effect of soil type and macropores. *Journal of Contaminant Hydrology*, *166*, 1-10. DOI: 10.1016/j.jconhyd.2014.07.006

Das, B. S., Lee, L. S., Rao, P. S. C., & Hultgren, R. P. (2004). Sorption and degradation of steroid hormones in soils during transport: Column studies and model evaluation. *Environmental Science & Technology*, *38*(5), 1460-1470. DOI: 10.1021/es034898e

D'ascenzo, G., Di Corcia, A., Gentili, A., Mancini, R., Mastropasqua, R., Nazzari, M., & Samperi, R. (2003). Fate of natural estrogen conjugates in municipal sewage transport and

treatment facilities. *Science of the Total Environment*, *302*(1), 199-209. DOI: 10.1016/S0048-9697(02)00342-X

Daston, G. P., Cook, J. C., & Kavlock, R. J. (2003). Uncertainties for endocrine disruptors: our view on progress. *Toxicological Sciences*, *74*(2), 245-252. DOI: 10.1093/toxsci/kfg105

De Mes, T., Zeeman, G., & Lettinga, G. (2005). Occurrence and fate of estrone, 17β-estradiol and 17α-ethynylestradiol in STPs for domestic wastewater. *Reviews in Environmental Science and Bio/Technology*, *4*(4), 275-311. DOI: 10.1007/s11157-005-3216-x

De Rudder, J., Van de Wiele, T., Dhooge, W., Comhaire, F., & Verstraete, W. (2004). Advanced water treatment with manganese oxide for the removal of 17α-ethynylestradiol (EE2). *Water Research*, *38*(1), 184-192. DOI: 10.1016/j.watres.2003.09.018

Dodgen, L. K., Kelly, W. R., Panno, S. V., Taylor, S. J., Armstrong, D. L., Wiles, K. N., ... & Zheng, W. (2017). Characterizing pharmaceutical, personal care product, and hormone contamination in a karst aquifer of southwestern Illinois, USA, using water quality and stream flow parameters. *Science of the Total Environment*, *578*, 281-289. 10.1016/j.scitotenv.2016.10.103

Doering, J., Wohnoutka, S., Goodrich, D., & Oliver, K. (2013). Redwood River Fecal Coliform Total Maximum Daily Load Report.

Durán–Álvarez, J. C., Prado, B., Ferroud, A., Juayerk, N., & Jiménez-Cisneros, B. (2014). Sorption, desorption and displacement of ibuprofen, estrone, and 17β estradiol in wastewater irrigated and rainfed agricultural soils. *Science of the Total Environment*, *473*, 189-198. DOI: 10.1016/j.scitotenv.2013.12.018

Dutta, S., Inamdar, S., Tso, J., Aga, D. S., & Sims, J. T. (2010). Free and conjugated estrogen exports in surface-runoff from poultry litter–amended soil. *Journal of Environmental Quality*, *39*(5), 1688-1698. DOI:10.2134/jeq2009.0339

Edmonds, L., Gollehon, N., Kellogg, R. L., Kintzer, B., Knight, L., Lander, C., ... & Schaeffer, J. (2003). Costs associated with development and implementation of comprehensive nutrient management plans. *USDA Natural Resources Conservation Service*.

Ermawati, R., Morimura, S., Tang, Y., Liu, K., & Kida, K. (2007). Degradation and behavior of natural steroid hormones in cow manure waste during biological treatments and ozone oxidation. *Journal of Bioscience and Bioengineering*, *103*(1), 27-31. DOI: 10.1263/jbb.103.27

Esperanza, M., Suidan, M. T., Marfil-Vega, R., Gonzalez, C., Sorial, G. A., McCauley, P., & Brenner, R. (2007). Fate of sex hormones in two pilot-scale municipal wastewater treatment plants: Conventional treatment. *Chemosphere*, *66*(8), 1535-1544. DOI:10.1016/j.chemosphere.2006.08.020

Fan, Z., Casey, F. X., Hakk, H., & Larsen, G. L. (2007). Persistence and fate of 17β-estradiol and testosterone in agricultural soils. *Chemosphere*, *67*(5), 886-895. DOI: 10.1016/j.chemosphere.2006.11.040

Feng, X., Ding, S., Tu, J., Wu, F., & Deng, N. (2005). Degradation of estrone in aqueous solution by photo-Fenton system. *Science of the Total Environment*, *345*(1), 229-237. DOI: 10.1016/j.scitotenv.2004.11.008

Ferrey, M. (2011). *Wastewater treatment plant endocrine disrupting chemical monitoring study*. Minnesota Pollution Control Agency. DOI: 10.1021/es034410+

Fine, D. D., Breidenbach, G. P., Price, T. L., & Hutchins, S. R. (2003). Quantitation of estrogens in ground water and swine lagoon samples using solid-phase extraction, pentafluorobenzyl/trimethylsilyl derivatizations and gas chromatography–negative ion chemical ionization tandem mass spectrometry. *Journal of Chromatography A*, *1017*(1), 167-185. DOI: 10.1016/j.chroma.2003.08.021

Friedman, J., Hastie, T., & Tibshirani, R. (2001). *The elements of statistical learning* (Vol. 1, pp. 337-387). New York: Springer series in statistics.

Gall, H. E., Sassman, S. A., Jenkinson, B., Lee, L. S., & Jafvert, C. T. (2014). Hormone loads exported by a tile-drained agroecosystem receiving animal wastes. *Hydrological Processes*, *28*(3), 1318-1328. DOI: 10.1002/hyp.9664

Gall, H. E., Sassman, S. A., Jenkinson, B., Lee, L. S., & Jafvert, C. T. (2015). Comparison of export dynamics of nutrients and animal-borne estrogens from a tile-drained Midwestern agroecosystem. *Water Research*, *72*, 162-173. DOI: 10.1016/j.watres.2014.08.041

Gall, H. E., Sassman, S. A., Lee, L. S., & Jafvert, C. T. (2011). Hormone discharges from a midwest tile-drained agroecosystem receiving animal wastes. *Environmental Science & Technology*, *45*(20), 8755-8764. DOI: 10.1021/es2011435

Goeppert, N., Dror, I., & Berkowitz, B. (2014). Detection, fate and transport of estrogen family hormones in soil. *Chemosphere*, *95*, 336-345. DOI: 10.1016/j.chemosphere.2013.09.039

Goeppert, N., Dror, I., & Berkowitz, B. (2015). Fate and transport of free and conjugated estrogens during soil passage. *Environmental Pollution*, *206*, 80-87. DOI: 10.1016/j.envpol.2015.06.024

Goeppert, N., Dror, I., & Berkowitz, B. (2017). Spatial and Temporal Distribution of Free and Conjugated Estrogens During Soil Column Transport. *CLEAN–Soil, Air, Water*, *45*(2). DOI: 10.1002/clen.201600048

Gomes, R. L., Scrimshaw, M. D., & Lester, J. N. (2009). Fate of conjugated natural and synthetic steroid estrogens in crude sewage and activated sludge batch studies. *Environmental Science & Technology*, *43*(10), 3612-3618. DOI: 10.1021/es801952h

Griffith, D. R., Soule, M. C. K., Eglinton, T. I., Kujawinski, E. B., & Gschwend, P. M. (2016). Steroidal estrogen sources in a sewage-impacted coastal ocean. *Environmental Science: Processes & Impacts*, *18*(8), 981-991. DOI: 10.1039/C6EM00127K

Guo, Y. P., Hu, Y. Y., Lin, H., & Ou, X. L. (2018). Sorption and desorption of 17α-ethinylestradiol onto sediments affected by rhamnolipidic biosurfactants. *Journal of Hazardous Materials*, *344*, 707-715. DOI: 10.1016/j.jhazmat.2017.11.005

Gyllenhammar, I., Holm, L., Eklund, R., & Berg, C. (2009). Reproductive toxicity in Xenopus tropicalis after developmental exposure to environmental concentrations of ethynylestradiol. *Aquatic Toxicology*, *91*(2), 171-178. DOI: 10.1016/j.aquatox.2008.06.019

Hakk, H., Millner, P., & Larsen, G. (2005). Decrease in water-soluble 17β-estradiol and testosterone in composted poultry manure with time. *Journal of Environmental Quality*, *34*(3), 943-950. DOI: 10.2134/jeq2004.0164

Hakk, H., Sikora, L., Casey, F. X., & Larsen, G. L. (2014). Fate of 17β-estradiol in anaerobic lagoon digesters. *Journal of Environmental Quality*, *43*(2), 701-708. DOI:10.2134/jeq2013.06.0245

Hanselman, T. A., Graetz, D. A., & Wilkie, A. C. (2003). Manure-borne estrogens as potential environmental contaminants: a review. *Environmental Science & Technology*, *37*(24), 5471-5478. DOI: 10.1021/es034410+

Helmers, M. J. (2008). 4. *Buffers and Vegetative Filter Strips*.

Hernandez, J. A., & Schmitt, M. A. (2012). Manure management in Minnesota. *Univ. of Minnesota Ext. Publ. WW-03553. Univ. of Minnesota Ext., St. Paul*.

Hogan, N. S., Duarte, P., Wade, M. G., Lean, D. R., & Trudeau, V. L. (2008). Estrogenic exposure affects metamorphosis and alters sex ratios in the northern leopard frog (Rana pipiens): identifying critically vulnerable periods of development. *General and Comparative Endocrinology*, *156*(3), 515-523. DOI: 10.1016/j.ygcen.2008.03.011

Holt, J. (2012). *Linear Algebra with Applications*. York: WH Freeman.

Hossain, I., Imteaz, M. A., & Hossain, M. I. (2011). Application of build-up and wash-off models for an East-Australian catchment. *Analysis*, *266*, 3320. DOI: 10.1504/IJW.2014.057784

Huang, B., Li, X., Sun, W., Ren, D., Li, X., Li, X., ... & Pan, X. (2014). Occurrence, removal, and fate of progestogens, androgens, estrogens, and phenols in six sewage treatment plants around Dianchi Lake in China. *Environmental Science and Pollution Research*, *21*(22), 12898-12908. DOI: 10.1007/s11356-014-3236-6

Huang, B., Wang, B., Ren, D., Jin, W., Liu, J., Peng, J., & Pan, X. (2013). Occurrence, removal and bioaccumulation of steroid estrogens in Dianchi Lake catchment, China. *Environment International*, *59*, 262-273. DOI: 10.1016/j.envint.2013.06.018

Huang, P., Mukherji, S. T., Wu, S., Muller, J., & Goel, R. (2016). Fate of 17β-Estradiol as a model estrogen in source separated urine during integrated chemical P recovery and treatment using partial nitritation-anammox process. *Water Research*, *103*, 500-509. DOI: 10.1016/j.watres.2016.07.044

Hutchinson, T. H., & Pickford, D. B. (2002). Ecological risk assessment and testing for endocrine disruption in the aquatic environment. *Toxicology*, *181*, 383-387. DOI: 10.1016/S0300-483X(02)00471-7

Imai, S., Shiraishi, A., Gamo, K., Watanabe, I., Okuhata, H., Miyasaka, H., ... & Hirata, K. (2007). Removal of phenolic endocrine disruptors by Portulaca oleracea. *Journal of Bioscience and Bioengineering*, *103*(5), 420-426. DOI: 10.1263/jbb.103.420

Isobe, T., Shiraishi, H., Yasuda, M., Shinoda, A., Suzuki, H., & Morita, M. (2003). Determination of estrogens and their conjugates in water using solid-phase extraction followed by liquid chromatography–tandem mass spectrometry. *Journal of Chromatography A*, *984*(2), 195-202. DOI: 10.1016/S0021-9673(02)01851-4

Jacobsen, A. M., Lorenzen, A., Chapman, R., & Topp, E. (2005). Persistence of testosterone and 17β-estradiol in soils receiving swine manure or municipal biosolids. *Journal of Environmental Quality*, *34*(3), 861-871. DOI:10.2134/jeq2004.0331

Jafari, A., Abasabad, R., & Salehzadeh, A. (2009). Endocrine disrupting contaminants in water resources and sewage in Hamadan City of Iran. *Journal of Environmental Health Science & Engineering*, *6*(2), 89-96.

James, G., Witten, D., Hastie, T., & Tibshirani, R. (2013). *An introduction to statistical learning* (Vol. 112). New York: Springer.

Jenkins, M. B., Endale, D. M., Schomberg, H. H., & Sharpe, R. R. (2006). Fecal bacteria and sex hormones in soil and runoff from cropped watersheds amended with poultry litter. *Science of the Total Environment*, *358*(1), 164-177. DOI: 10.1016/j.scitotenv.2005.04.015

Jenkins, M. B., Truman, C. C., Siragusa, G., Line, E., Bailey, J. S., Frye, J., ... & Sharpe, R. R. (2008). Rainfall and tillage effects on transport of fecal bacteria and sex hormones 17β-estradiol and testosterone from broiler litter applications to a Georgia Piedmont Ultisol. *Science of the Total Environment*, *403*(1), 154-163. DOI: 10.1016/j.scitotenv.2008.05.014

Johnson, A. C. (2010). Natural variations in flow are critical in determining concentrations of point source contaminants in rivers: an estrogen example. *Environmental Science & Technology*, *44*(20), 7865-7870. DOI: 10.1021/es101799j

Johnson, A. C., Belfroid, A., & Di Corcia, A. (2000). Estimating steroid oestrogen inputs into activated sludge treatment works and observations on their removal from the effluent. *Science of the Total Environment*, *256*(2), 163-173. DOI: 10.1016/S0048-9697(00)00481-2

Johnson, A. C., Williams, R. J., & Matthiessen, P. (2006). The potential steroid hormone contribution of farm animals to freshwaters, the United Kingdom as a case study. *Science of the Total Environment*, *362*(1), 166-178. DOI: 10.1016/j.scitotenv.2005.06.014

Jones, G. D., Benchetler, P. V., Tate, K. W., & Kolodziej, E. P. (2014a). Mass balance approaches to characterizing the leaching potential of trenbolone acetate metabolites in agro-ecosystems. *Environmental Science & Technology*, *48*(7), 3715-3723. DOI: 10.1021/es405701f

Jones, G. D., Benchetler, P. V., Tate, K. W., & Kolodziej, E. P. (2014b). Trenbolone acetate metabolite transport in rangelands and irrigated pasture: observations and conceptual approaches for agro-ecosystems. *Environmental Science & Technology*, *48*(21), 12569-12576. DOI: 10.1021/es503406h

Jürgens, M. D., Holthaus, K. I., Johnson, A. C., Smith, J. J., Hetheridge, M., & Williams, R. J. (2002). The potential for estradiol and ethinylestradiol degradation in English rivers. *Environmental Toxicology and Chemistry*,*21*(3), 480-488. DOI: 10.1002/etc.5620210302

Khanal, S. K., Xie, B., Thompson, M. L., Sung, S., Ong, S. K., & Van Leeuwen, J. (2006). Fate, transport, and biotransformation of natural estrogens in the environment and engineered systems. *Environmental Science & Technology*, *40*(21), 6537-6546. DOI: 10.1021/es0607739

Kidd, K. A., Blanchfield, P. J., Mills, K. H., Palace, V. P., Evans, R. E., Lazorchak, J. M., & Flick, R. W. (2007). Collapse of a fish population after exposure to a synthetic estrogen. *Proceedings of the National Academy of Sciences*, *104*(21), 8897-8901. DOI: 10.1073/pnas.0609568104

Kim, S. D., Cho, J., Kim, I. S., Vanderford, B. J., & Snyder, S. A. (2007). Occurrence and removal of pharmaceuticals and endocrine disruptors in South Korean surface, drinking, and waste waters. *Water Research*, *41*(5), 1013-1021. DOI: 10.1016/j.watres.2006.06.034

Kjær, J., Olsen, P., Bach, K., Barlebo, H. C., Ingerslev, F., Hansen, M., & Sørensen, B. H. (2007). Leaching of estrogenic hormones from manure-treated structured soils. *Environmental Science & Technology*, *41*(11), 3911-3917. DOI: 10.1021/es0627747

Lafont, R., & Mathieu, M. (2007). Steroids in aquatic invertebrates. *Ecotoxicology*, *16*(1), 109-130. DOI: 10.1007/s10646-006-0113-1

Lai, K. M., Johnson, K. L., Scrimshaw, M. D., & Lester, J. N. (2000). Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. *Environmental Science & Technology*, *34*(18), 3890-3894. DOI: 10.1021/es9912729

Langdon, K. A., Warne, M. S., Smernik, R. J., Shareef, A., & Kookana, R. S. (2014). Persistence of estrogenic activity in soils following land application of biosolids. *Environmental Toxicology and Chemistry*, *33*(1), 26-28. DOI: 10.1002/etc.2395

Lange, I. G., Daxenberger, A., Schiffer, B., Witters, H., Ibarreta, D., & Meyer, H. H. (2002). Sex hormones originating from different livestock production systems: fate and potential disrupting activity in the environment. *Analytica Chimica Acta*, *473*(1), 27-37. DOI: 10.1016/S0003-2670(02)00748-1

Larsson, D. G. J., Adolfsson-Erici, M., Parkkonen, J., Pettersson, M., Berg, A. H., Olsson, P. E., & Förlin, L. (1999). Ethinyloestradiol—an undesired fish contraceptive?. *Aquatic Toxicology*, *45*(2), 91-97. DOI: 10.1016/S0166-445X(98)00112-X

Lee, B., Kullman, S. W., Yost, E., Meyer, M. T., Worley-Davis, L., Williams, C. M., & Reckhow, K. H. (2014). A Bayesian network model for assessing natural estrogen fate and transport in a swine waste lagoon. *Integrated Environmental Assessment and Management*, *10*(4), 511-521. DOI: 10.1002/ieam.1538

Lee, B., Kullman, S. W., Yost, E. E., Meyer, M. T., Worley-Davis, L., Williams, C. M., & Reckhow, K. H. (2015). Predicting characteristics of rainfall driven estrogen runoff and transport from swine AFO spray fields. *Science of the Total Environment*, *532*, 571-580. DOI: 10.1016/j.scitotenv.2015.06.051

Lee, H. B., & Liu, D. (2002). Degradation of 17β-estradiol and its metabolites by sewage bacteria. *Water, Air, and Soil Pollution*, *134*(1-4), 351-366. DOI: 10.1023/A:1014117329403

Lee, K. E., Langer, S. K., Barber, L. B., Writer, J. H., Ferrey, M. L., Schoenfuss, H. L., ... & Martinovic, D. (2011). *Endocrine active chemicals, pharmaceuticals, and other chemicals of*

concern in surface water, wastewater-treatment plant effluent, and bed sediment, and biological characteristics in selected streams, Minnesota-design, methods, and data, 2009 (No. 575). US Geological Survey.

Lee, L. S., Strock, T. J., Sarmah, A. K., & Rao, P. S. C. (2003). Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. *Environmental Science & Technology*, *37*(18), 4098-4105. DOI: 10.1021/es020998t

Leech, D. M., Snyder, M. T., & Wetzel, R. G. (2009). Natural organic matter and sunlight accelerate the degradation of 17ß-estradiol in water. *Science of the Total Environment*, *407*(6), 2087-2092. DOI: 10.1016/j.scitotenv.2008.11.018

Lei, B., Huang, S., Zhou, Y., Wang, D., & Wang, Z. (2009). Levels of six estrogens in water and sediment from three rivers in Tianjin area, China. *Chemosphere*, *76*(1), 36-42. DOI: 10.1016/j.chemosphere.2009.02.035

Lei, B., Kang, J., Yu, Y., Zha, J., Li, W., & Wang, Z. (2013). β-estradiol 17-valerate affects embryonic development and sexual differentiation in Japanese medaka (Oryzias latipes). *Aquatic Toxicology*, *134*, 128-134. DOI: 10.1016/j.aquatox.2013.03.011

Li, J., Jiang, L., Liu, X., & Lv, J. (2013). Adsorption and aerobic biotransformation of four selected endocrine disrupting chemicals in soil–water system. *International Biodeterioration & Biotransformation*, *76*, 3-7. DOI: 10.1016/j.ibiod.2012.06.004

Lin, A. Y. C., & Reinhard, M. (2005). Photodegradation of common environmental pharmaceuticals and estrogens in river water. *Environmental Toxicology and Chemistry*, *24*(6), 1303-1309. DOI: 10.1897/04-236R.1

Lorenzen, A., Chapman, R., Hendel, J. G., & Topp, E. (2005). Persistence and pathways of testosterone dissipation in agricultural soil. *Journal of Environmental Quality*, *34*(3), 854-860. DOI: 10.2134/jeq2004.0330

Lorenzen, A., Hendel, J. G., Conn, K. L., Bittman, S., Kwabiah, A. B., Lazarovitz, G., ... & Topp, E. (2004). Survey of hormone activities in municipal biosolids and animal manures. *Environmental Toxicology*, *19*(3), 216-225. DOI: 10.1002/tox.20014

Lucas, S. D., & Jones, D. L. (2006). Biotransformation of estrone and 17 β-estradiol in grassland soils amended with animal wastes. *Soil Biology and Biochemistry*, *38*(9), 2803-2815. DOI: 10.1016/j.soilbio.2006.04.033

Lucas, S. D., & Jones, D. L. (2009). Urine enhances the leaching and persistence of estrogens in soils. *Soil Biology and Biochemistry*, *41*(2), 236-242. DOI: 10.1016/j.soilbio.2008.10.022

Luo, Y. (2014). Review of modeling approaches for pesticide washoff from impervious surfaces. In *Describing the Behavior and Effects of Pesticides in Urban and Agricultural Settings* (pp. 65-82). American Chemical Society. DOI: 10.1021/bk-2014-1168.ch004

Mansell, D. S., Bryson, R. J., Harter, T., Webster, J. P., Kolodziej, E. P., & Sedlak, D. L. (2011). Fate of endogenous steroid hormones in steer feedlots under simulated rainfall-induced runoff. *Environmental Science & Technology*, *45*(20), 8811-8818. DOI: 10.1021/es202072f

Mansell, J., & Drewes, J. E. (2004). Fate of Steroidal Hormones During Soil-Aquifer Treatment. *Groundwater Monitoring & Remediation*, *24*(2), 94-101. DOI: 10.1111/j.1745-6592.2004.tb00717.x

Martín, J., Camacho-Muñoz, D., Santos, J. L., Aparicio, I., & Alonso, E. (2012). Occurrence of pharmaceutical compounds in wastewater and sludge from wastewater treatment plants: removal and ecotoxicological impact of wastewater discharges and sludge disposal. *Journal of Hazardous Materials*, *239*, 40-47. DOI: 10.1016/j.jhazmat.2012.04.068

Martinovic-Weigelt, D., Minarik, T. A., Curran, E. M., Marchuk, J. S., Pazderka, M. J., Smith, E. A., ... & Schoenfuss, H. L. (2013). Environmental estrogens in an urban aquatic ecosystem: I. Spatial and temporal occurrence of estrogenic activity in effluent-dominated systems. *Environment International*, *61*, 127-137. DOI: 10.1016/j.envint.2013.07.018

Mashtare, M. L., Green, D. A., & Lee, L. S. (2013). Biotransformation of 17α-and 17β-estradiol in aerobic soils. *Chemosphere*, *90*(2), 647-652. DOI: 10.1016/j.chemosphere.2012.09.032

Matthiessen, P., Arnold, D., Johnson, A. C., Pepper, T. J., Pottinger, T. G., & Pulman, K. G. T. (2006). Contamination of headwater streams in the United Kingdom by oestrogenic hormones from livestock farms. *Science of the Total Environment*, *367*(2), 616-630. DOI: 10.1016/j.scitotenv.2006.02.007

Mazellier, P., Méité, L., & De Laat, J. (2008). Photodegradation of the steroid hormones 17β-estradiol (E2) and 17α-ethinylestradiol (EE2) in dilute aqueous solution. *Chemosphere*, *73*(8), 1216-1223. DOI: 10.1016/j.chemosphere.2008.07.046

Miller, K. J., & Meek, J. (2006). *Helena Valley ground water: pharmaceuticals, personal care products, endocrine disruptors (PPCPs), and microbial indicators of fecal contamination*. Helena, MT: Montana Department of Environmental Quality.

Mina, O., Gall, H. E., Elliott, H. A., Watson, J. E., Mashtare, M. L., Langkilde, T., ... & Boyer, E. W. (2018). Estrogen occurrence and persistence in vernal pools impacted by wastewater irrigation practices. *Agriculture, Ecosystems & Environment*, *257*, 103-112. 10.1016/j.agee.2018.01.022

Mishra, A. (2011). *Estimating uncertainty in HSPF based water quality model: Application of Monte-Carlo based techniques* (Doctoral dissertation).

Moos, W. H., Dykens, J. A., Nohynek, D., Rubinchik, E., & Howell, N. (2009). Review of the effects of 17α-estradiol in humans: a less feminizing estrogen with neuroprotective potential. *Drug Development Research*, *70*(1), 1-21. DOI: 10.1002/ddr.20284

Moyer, D. L., & Hyer, K. E. (2003). *Use of the Hydrological Simulation Program-FORTRAN and bacterial source tracking for development of the fecal coliform total maximum daily load (TMDL) for Christians Creek, Augusta County, Virginia*. US Department of the Interior, US Geological Survey.

Muller, M., Combalbert, S., Delgenès, N., Bergheaud, V., Rocher, V., Benoît, P., ... & Hernandez-Raquet, G. (2010). Occurrence of estrogens in sewage sludge and their fate during plant-scale anaerobic digestion. *Chemosphere*, *81*(1), 65-71. DOI: 10.1016/j.chemosphere.2010.06.062

Nghiem, L. D., Manis, A., Soldenhoff, K., & Schäfer, A. I. (2004). Estrogenic hormone removal from wastewater using NF/RO membranes. *Journal of Membrane Science*, *242*(1), 37-45. DOI: 10.1016/j.memsci.2003.12.034

NRCS (2010). *Estimation of direct runoff from storm rainfall*. National Engineering Handbook, Section 4– Hydrology.

Pagsuyoin, S. A., Lung, W. S., & Colosi, L. M. (2012). Predicting EDC concentrations in a river mixing zone. *Chemosphere*, *87*(10), 1111-1118. DOI:10.1016/j.chemosphere.2012.02.004

Palme, R., Fischer, P., Schildorfer, H., & Ismail, M. N. (1996). Excretion of infused 14 C-steroid hormones via faeces and urine in domestic livestock.*Animal Reproduction Science*, *43*(1), 43-63. DOI: 10.1016/0378-4320(95)01458-6

Peng, X., Yu, Y., Tang, C., Tan, J., Huang, Q., & Wang, Z. (2008). Occurrence of steroid estrogens, endocrine-disrupting phenols, and acid pharmaceutical residues in urban riverine water of the Pearl River Delta, South China. *Science of the Total Environment*, *397*(1), 158-166. DOI: 10.1016/j.scitotenv.2008.02.059

Pettersson, I., Arukwe, A., Lundstedt-Enkel, K., Mortensen, A. S., & Berg, C. (2006). Persistent sex-reversal and oviducal agenesis in adult Xenopus (Silurana) tropicalis frogs following larval exposure to the environmental pollutant ethynylestradiol. *Aquatic Toxicology*, *79*(4), 356-365. DOI: 10.1016/j.aquatox.2006.07.004

Pereira, R. J. G., Polegato, B. F., de Souza, S., Negrão, J. A., & Duarte, J. M. B. (2006). Monitoring ovarian cycles and pregnancy in brown brocket deer (Mazama gouazoubira) by

measurement of fecal progesterone metabolites. *Theriogenology*, *65*(2), 387-399. DOI: 10.1016/j.theriogenology.2005.02.019

Perez, R., Bonaventura-Sparagna, J. A., Kmiecik, M., George, R., & Renne, D. (2002). Indications of biases in reporting cloud cover at major airports. Preprints. In *Sixth Symp. on Integrated Observing Systems*.

Puma, G. L., Puddu, V., Tsang, H. K., Gora, A., & Toepfer, B. (2010). Photocatalytic oxidation of multicomponent mixtures of estrogens (estrone (E1), 17β-estradiol (E2), 17α-ethynylestradiol (EE2) and estriol (E3)) under UVA and UVC radiation: photon absorption, quantum yields and rate constants independent of photon absorption. *Applied Catalysis B: Environmental*, *99*(3), 388-397. DOI: 10.1016/j.apcatb.2010.05.015

Qu, S., Kolodziej, E. P., & Cwiertny, D. M. (2012). Phototransformation rates and mechanisms for synthetic hormone growth promoters used in animal agriculture. *Environmental Science & Technology*, *46*(24), 13202-13211. DOI: 10.1021/es303091c

Raman, D. R., Williams, E. L., Layton, A. C., Burns, R. T., Easter, J. P., Daugherty, A. S., ... & Sayler, G. S. (2004). Estrogen content of dairy and swine wastes. *Environmental Science & Technology*, *38*(13), 3567-3573. DOI: 10.1021/es0353208

Reddy, S., Iden, C. R., & Brownawell, B. J. (2005). Analysis of steroid conjugates in sewage influent and effluent by liquid chromatography-tandem mass spectrometry. *Analytical Chemistry*, *77*(21), 7032-7038. DOI: 10.1021/ac050699x

Robinson, J. A., Ma, Q., Staveley, J. P., Smolenski, W. J., & Ericson, J. (2016). Degradation and transformation of 17α-estradiol in water-sediment systems under controlled aerobic and anaerobic conditions. *Environmental Toxicology and Chemistry*. DOI: 10.1002/etc.3383

Robinson, J. A., Ma, Q., Staveley, J. P., Smolenski, W. J., & Ericson, J. (2017). Degradation and transformation of 17α-estradiol in water-sediment systems under controlled aerobic and

anaerobic conditions. *Environmental Toxicology and Chemistry*, 36(3), 621-629. DOI: 10.1002/etc.3383

Rolland, R. M., Hunt, K. E., Kraus, S. D., & Wasser, S. K. (2005). Assessing reproductive status of right whales (Eubalaena glacialis) using fecal hormone metabolites. *General and Comparative Endocrinology*, *142*(3), 308-317. DOI: 10.1016/j.ygcen.2005.02.002

Rosenfeldt, E. J., & Linden, K. G. (2004). Degradation of endocrine disrupting chemicals bisphenol A, ethinyl estradiol, and estradiol during UV photo-transformation and advanced oxidation processes. *Environmental Science & Technology*, *38*(20), 5476-5483. DOI: 10.1021/es035413p

Sabourin, L., Duenk, P., Bonte-Gelok, S., Payne, M., Lapen, D. R., & Topp, E. (2012). Uptake of pharmaceuticals, hormones and parabens into vegetables grown in soil fertilized with municipal biosolids. *Science of the Total Environment*, *431*, 233-236. DOI: 10.1016/j.scitotenv.2012.05.017

Sangsupan, H. A., Radcliffe, D. E., Hartel, P. G., Jenkins, M. B., Vencill, W. K., & Cabrera, M. L. (2006). Sorption and transport of 17β-estradiol and testosterone in undisturbed soil columns. *Journal of Environmental Quality*, *35*(6), 2261-2272. DOI:10.2134/jeq2005.0401

Sarkar, S., Ali, S., Rehmann, L., Nakhla, G., & Ray, M. B. (2014). Degradation of estrone in water and wastewater by various advanced oxidation processes. *Journal of Hazardous Materials*, *278*, 16-24. DOI: 10.1016/j.jhazmat.2014.05.078

Scherr, F. F., Sarmah, A. K., Di, H. J., & Cameron, K. C. (2008). Modeling degradation and metabolite formation kinetics of estrone-3-sulfate in agricultural soils. *Environmental Science & Technology*, *42*(22), 8388-8394. DOI: 10.1021/es801850a

Scherr, F. F., Sarmah, A. K., Di, H. J., & Cameron, K. C. (2009). Degradation and metabolite formation of 17β-estradiol-3-sulphate in New Zealand pasture soils. *Environment International*, *35*(2), 291-297. DOI: 10.1016/j.envint.2008.07.002

Schoenborn, A., Kunz, P., & Koster, M. (2015). Estrogenic activity in drainage water: a field study on a Swiss cattle pasture. *Environmental Sciences Europe*, *27*(1), 17. DOI 10.1186/s12302-015-0047-4

Schoenecker, K. A., Lyda, R. O., & Kirkpatrick, J. (2004). Comparison of three fecal steroid metabolites for pregnancy detection used with single sampling in bighorn sheep (Ovis canadensis). *Journal of Wildlife Diseases*, *40*(2), 273-281. DOI: 10.7589/0090-3558-40.2.273

Schultz, M. M., Minarik, T. A., Martinovic-Weigelt, D., Curran, E. M., Bartell, S. E., & Schoenfuss, H. L. (2013). Environmental estrogens in an urban aquatic ecosystem: II. Biological effects. *Environment International*, *61*, 138-149. DOI: 10.1016/j.envint.2013.08.006

Seki, M., Yokota, H., Maeda, M., & Kobayashi, K. (2005). Fish full life-cycle testing for 17β-estradiol on medaka (Oryzias latipes). *Environmental Toxicology and Chemistry*, *24*(5), 1259-1266. DOI: 10.1897/04-379R.1

Servos, M. R., Bennie, D. T., Burnison, B. K., Jurkovic, A., McInnis, R., Neheli, T., ... & Ternes, T. A. (2005). Distribution of estrogens, 17β-estradiol and estrone, in Canadian municipal wastewater treatment plants. *Science of the Total Environment*, *336*(1), 155-170. DOI:10.1016/j.scitotenv.2004.05.025

Shargil, D., Gerstl, Z., Fine, P., Nitsan, I., & Kurtzman, D. (2015). Impact of biosolids and wastewater effluent application to agricultural land on steroidal hormone content in lettuce plants. *Science of the Total Environment*, *505*, 357-366. DOI: 10.1016/j.scitotenv.2014.09.100

Shappell, N. W., Billey, L. O., & Shipitalo, M. J. (2016). Estrogenic activity and nutrient losses in surface runoff after winter manure application to small watersheds. *Science of the Total Environment*, *543*, 570-580. DOI: 10.1016/j.scitotenv.2015.10.130

Shaw, S. B., Stedinger, J. R., & Walter, M. T. (2009). Evaluating urban pollutant buildup/wash-off models using a Madison, Wisconsin catchment. *Journal of Environmental*

Engineering, *136*(2), 194-203. DOI: 10.1061/(ASCE)EE.1943-7870.0000142#sthash.CYiRg2DZ.dpuf

Shore, L. (2009a). Steroid hormones generated by CAFOs. In *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations* (pp. 13-21). Springer US.

Shore, L. (2009b). Transport of steroids in surface waters. In *Hormones and Pharmaceuticals Generated by Concentrated Animal Feeding Operations* (pp. 23-27). Springer US.

Shore, L. S., Reichmann, O., Shemesh, M., Wenzel, A., & Litaor, M. I. (2004). Washout of accumulated testosterone in a watershed. *Science of the Total Environment*, *332*(1), 193-202. DOI:10.1016/j.scitotenv.2004.04.009

Shrestha, S. L., Casey, F. X., Hakk, H., Smith, D. J., & Padmanabhan, G. (2012). Fate and transformation of an estrogen conjugate and its metabolites in agricultural soils. *Environmental Science & Technology*, *46*(20), 11047-11053. DOI: 10.1021/es3021765

Singh, S. P., Azua, A., Chaudhary, A., Khan, S., Willett, K. L., & Gardinali, P. R. (2010). Occurrence and distribution of steroids, hormones and selected pharmaceuticals in South Florida coastal environments. *Ecotoxicology*, *19*(2), 338-350. DOI: 10.1007/s10646-009-0416-0

Snyder, S. A., Adham, S., Redding, A. M., Cannon, F. S., DeCarolis, J., Oppenheimer, J., ... & Yoon, Y. (2007). Role of membranes and activated carbon in the removal of endocrine disruptors and pharmaceuticals. *Desalination*, *202*(1-3), 156-181. DOI: 10.1016/j.desal.2005.12.052

Song, H. L., Nakano, K., Taniguchi, T., Nomura, M., & Nishimura, O. (2009). Estrogen removal from treated municipal effluent in small-scale constructed wetland with different depth. *Bioresource Technology*, *100*(12), 2945-2951. DOI: 10.1016/j.biortech.2009.01.045

Soto, A. M., Calabro, J. M., Prechtl, N. V., Yau, A. Y., Orlando, E. F., Daxenberger, A., ... & Sonnenschein, C. (2004). Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in Eastern Nebraska, USA. *Environmental Health Perspectives*, *112*(3), 346.

Stadler, L. B., & Love, N. G. (2016). Impact of microbial physiology and microbial community structure on pharmaceutical fate driven by dissolved oxygen concentration in nitrifying bioreactors. *Water Research*, *104*, 189-199. DOI: 10.1016/j.watres.2016.08.001

Standley, L. J., Rudel, R. A., Swartz, C. H., Attfield, K. R., Christian, J., Erickson, M., & Brody, J. G. (2008). Wastewater-contaminated groundwater as a source of endogenous hormones and pharmaceuticals to surface water ecosystems. *Environmental Toxicology and Chemistry*, *27*(12), 2457-2468. DOI: 10.1897/07-604.1

Steiner, L. D., Bidwell, V. J., Di, H. J., Cameron, K. C., & Northcott, G. L. (2010). Transport and modeling of estrogenic hormones in a dairy farm effluent through undisturbed soil lysimeters. *Environmental Science & Technology*, *44*(7), 2341-2347. DOI: 10.1021/es9031216

Sumpter, J. P., & Jobling, S. (1995). Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. *Environmental Health Perspectives*, *103*(Suppl 7), 173.

Suzuki, K., Hirai, H., Murata, H., & Nishida, T. (2003). Removal of estrogenic activities of 17β-estradiol and ethinylestradiol by ligninolytic enzymes from white rot fungi. *Water Research*, *37*(8), 1972-1975. DOI: 10.1016/S0043-1354(02)00533-X

Swartz, C. H., Reddy, S., Benotti, M. J., Yin, H., Barber, L. B., Brownawell, B. J., & Rudel, R. A. (2006). Steroid estrogens, nonylphenol ethoxylate metabolites, and other wastewater contaminants in groundwater affected by a residential septic system on Cape Cod, MA. *Environmental Science & Technology*, *40*(16), 4894-4902. DOI: 10.1021/es052595+

Tamagawa, Y., Yamaki, R., Hirai, H., Kawai, S., & Nishida, T. (2006). Removal of estrogenic activity of natural steroidal hormone estrone by ligninolytic enzymes from white rot fungi. *Chemosphere*, *65*(1), 97-101. DOI: 10.1016/j.chemosphere.2006.02.031

Tarrant, A. M. (2005). Endocrine-like signaling in cnidarians: current understanding and implications for ecophysiology. *Integrative and Comparative Biology*, *45*(1), 201-214. DOI: 10.1093/icb/45.1.201

Thorpe, K. L., Cummings, R. I., Hutchinson, T. H., Scholze, M., Brighty, G., Sumpter, J. P., & Tyler, C. R. (2003). Relative potencies and combination effects of steroidal estrogens in fish. *Environmental Science & Technology*,*37*(6), 1142-1149. DOI: 10.1021/es0201348

Tompsett, A. R., Wiseman, S., Higley, E., Giesy, J. P., & Hecker, M. (2013). Effects of exposure to 17α-ethynylestradiol during larval development on growth, sexual differentiation, and abundances of transcripts in the liver of the wood frog (Lithobates sylvaticus). *Aquatic Toxicology*, *126*, 42-51. DOI: 10.1016/j.aquatox.2012.10.003

Tucker, H. A. (2009). *Factors affecting estrogen excretion in dairy heifers* (Doctoral dissertation, Virginia Polytechnic Institute and State University).

University of Maine Cooperative Extension, (2014). *Four Seasons Grazing in Minnesota.*

US EPA, (2015). *BASINS 4.1 (Better Assessment Science Integrating point & Non-point Sources) Modeling Framework*. National Exposure Research Laboratory, RTP, North Carolina.

USDA NRCS, (1992). *Agricultural Waste Management Handbook.*

USDA NRCS, (1995). Animal Manure Management

Vandenberg, L. N., Colborn, T., Hayes, T. B., Heindel, J. J., Jacobs Jr, D. R., Lee, D. H., ... & Zoeller, R. T. (2012). Hormones and endocrine-disrupting chemicals: low-dose effects and nonmonotonic dose responses. *Endocrine Reviews*, *33*(3), 378-455. DOI: 10.1210/er.2011-1050

Vajda, A. M., Barber, L. B., Gray, J. L., Lopez, E. M., Bolden, A. M., Schoenfuss, H. L., & Norris, D. O. (2011). Demasculinization of male fish by wastewater treatment plant effluent. *Aquatic Toxicology*, *103*(3), 213-221. DOI:10.1016/j.aquatox.2011.02.007

Vetsch, J. & Lamb, J. (2011). *Applying Instinct™ as a nitrogen stabilizer for fall applied manure*.

Virginia Department of Environmental Quality, (2008). *Bacteria TMDL Development for the Dan River, Blackberry Creek, Byrds Branch, Double Creek, Fall Creek, Leatherwood Creek, Marrowbone Creek, North Fork Mayo River, South Fork Mayo River, Smith River, Sandy Creek, and Sandy River Watersheds*.

Virginia Department of Environmental Quality, (2009). *Bacteria and Benthic Total Maximum Daily Load Development for South River*.

Wang, L., Ying, G. G., Chen, F., Zhang, L. J., Zhao, J. L., Lai, H. J., ... & Tao, R. (2012). Monitoring of selected estrogenic compounds and estrogenic activity in surface water and sediment of the Yellow River in China using combined chemical and biological tools. *Environmental Pollution*, *165*, 241-249. DOI: 10.1016/j.envpol.2011.10.005

Waring, R. H., & Harris, R. M. (2005). Endocrine disruptors: a human risk?.*Molecular and Cellular Endocrinology*, *244*(1), 2-9. DOI: 10.1016/j.mce.2005.02.007

Webster, J. P., Kover, S. C., Bryson, R. J., Harter, T., Mansell, D. S., Sedlak, D. L., & Kolodziej, E. P. (2012). Occurrence of trenbolone acetate metabolites in simulated confined animal feeding operation (CAFO) runoff. *Environmental Science & Technology*, *46*(7), 3803-3810. DOI: 10.1021/es204529v

Whidbey, C. M., Daumit, K. E., Nguyen, T. H., Ashworth, D. D., Davis, J. C., & Latch, D. E. (2012). Photochemical induced changes of in vitro estrogenic activity of steroid hormones. *Water Research*, *46*(16), 5287-5296. DOI: 10.1016/j.watres.2012.07.016

Wicks, C., Kelley, C., & Peterson, E. (2004). Estrogen in a karstic aquifer. *Ground Water*, *42*(3), 384-389. DOI: 10.1111/j.1745-6584.2004.tb02686.x

Williams, R. J., Johnson, A. C., Smith, J. J., & Kanda, R. (2003). Steroid estrogens profiles along river stretches arising from sewage treatment works discharges. *Environmental Science & Technology*, *37*(9), 1744-1750. DOI: 10.1021/es0202107

Writer, J. H., Ryan, J. N., Keefe, S. H., & Barber, L. B. (2011). Fate of 4-nonylphenol and 17β-estradiol in the Redwood River of Minnesota. *Environmental Science & Technology*, *46*(2), 860-868. DOI: 10.1021/es2031664

Xie, H., & Lian, Y. (2013). Uncertainty-based evaluation and comparison of SWAT and HSPF applications to the Illinois River Basin. *Journal of Hydrology*, *481*, 119-131. DOI:10.1016/j.jhydrol.2012.12.027

Xuan, R., Blassengale, A. A., & Wang, Q. (2008). Degradation of estrogenic hormones in a silt loam soil. *Journal of Agricultural and Food Chemistry*, *56*(19), 9152-9158. DOI: 10.1021/jf8016942

Yan, Z., Lu, G., Liu, J., & Jin, S. (2012). An integrated assessment of estrogenic contamination and feminization risk in fish in Taihu Lake, China. *Ecotoxicology and Environmental Safety*, *84*, 334-340. DOI: 10.1016/j.ecoenv.2012.08.010

Yang, Y. Y., Gray, J. L., Furlong, E. T., Davis, J. G., ReVello, R. C., & Borch, T. (2012). Steroid hormone runoff from agricultural test plots applied with municipal biosolids. *Environmental Science & Technology*, *46*(5), 2746-2754. DOI: 10.1021/es203896t

Ying, G. G., Kookana, R. S., & Ru, Y. J. (2002). Occurrence and fate of hormone steroids in the environment. *Environment International*, *28*(6), 545-551. DOI: 10.1016/S0160-4120(02)00075-2

Yu, C. P., Deeb, R. A., & Chu, K. H. (2013). Microbial degradation of steroidal estrogens. *Chemosphere*, *91*(9), 1225-1235. DOI: 10.1016/j.chemosphere.2013.01.112

Yu, Z., Xiao, B., Huang, W., & Peng, P. A. (2004). Sorption of steroid estrogens to soils and sediments. *Environmental Toxicology and Chemistry*,*23*(3), 531-539. DOI: 10.1897/03-192

Zhang, H., Shi, J., Liu, X., Zhan, X., & Chen, Q. (2014). Occurrence and removal of free estrogens, conjugated estrogens, and bisphenol A in manure treatment facilities in East China. *Water Research*, *58*, 248-257. DOI: 10.1016/j.watres.2014.03.074

Zhang, H., Shi, J., Liu, X., Zhan, X., Dang, J., & Bo, T. (2014). Occurrence of free estrogens, conjugated estrogens, and bisphenol A in fresh livestock excreta and their removal by composting in North China. *Environmental Science and Pollution Research*, *21*(16), 9939-9947. DOI: 10.1007/s11356-014-3002-9

Zhang, X., Zhang, D., Zhang, H., Luo, Z., & Yan, C. (2012). Occurrence, distribution, and seasonal variation of estrogenic compounds and antibiotic residues in Jiulongjiang River, South China. *Environmental Science and Pollution Research*, *19*(5), 1392-1404. DOI: 10.1007/s11356-012-0818-z

Zhao, X. & Lung W.S. (2017). Modeling the fate and transport of 17β-estradiol in the South River watershed in Virginia. *Chemosphere*, *186*, 780-789. 10.1016/j.chemosphere.2017.08.058

Zhao, X. & Lung W.S. (2018). Tracking the Fate and Transport of Estrogens following Rainfall Events. *Water Science & Technology*. DOI: 10.2166/wst.2018.204

Zheng, W., Li, X., Yates, S. R., & Bradford, S. A. (2012). Anaerobic transformation kinetics and mechanism of steroid estrogenic hormones in dairy lagoon water. *Environmental Science & Technology*, *46*(10), 5471-5478. DOI: 10.1021/es301551h

Zheng, W., Yates, S. R., & Bradford, S. A. (2007). Analysis of steroid hormones in a typical dairy waste disposal system. *Environmental Science & Technology*, *42*(2), 530-535. DOI: 10.1021/es0718

Zheng, W., Zou, Y., Li, X., & Machesky, M. L. (2013). Fate of estrogen conjugate 17α-estradiol-3-sulfate in dairy wastewater: Comparison of aerobic and anaerobic degradation and metabolite formation. *Journal of Hhazardous Materials*, *258*, 109-115. DOI: 10.1016/j.jhazmat.2013.04.038

Zhou, Y., Zha, J., Xu, Y., Lei, B., & Wang, Z. (2012). Occurrences of six steroid estrogens from different effluents in Beijing, China. *Environmental Monitoring and Assessment*, *184*(3), 1719-1729. DOI: 10.1007/s10661-011-2073-z

Zhou, Y., Zha, J., & Wang, Z. (2012). Occurrence and fate of steroid estrogens in the largest wastewater treatment plant in Beijing, China. *Environmental Monitoring and Assessment*, *184*(11), 6799-6813. DOI: 10.1007/s10661-011-2459-y

Zhu, N., Deng, C., Xiong, Y., & Qian, H. (2004). Performance characteristics of three aeration systems in the swine manure composting. *Bioresource Technology*, *95*(3), 319-326. DOI: 10.1016/j.biortech.2004.02.021

Zuo, Y., Zhang, K., & Deng, Y. (2006). Occurrence and photochemical degradation of 17α-ethinylestradiol in Acushnet River Estuary. *Chemosphere*, *63*(9), 1583-1590. DOI: 10.1016/j.chemosphere.2005.08.063

Appendix A

Table A1. *Observed Estrogen Concentrations in Surface Water*

Name	Country	Surface Water Type	Sampling Times	Detected times	Detected concentration (ng/L)	Reference
		Pond	9	5	1.2 - 3.0	Standley et al., 2008
		Pond	9	0	<1.0	Standley et al., 2008
		Creek	na[1]	0	<0.5	Bradley et al., 2009
		WWTP effluent	na[1]	0	<0.5	Bradley et al., 2009
		WWTP effluent	na[1]	na[1]	110	Bradley et al., 2009
		River	na[1]	2	2.1 ± 0.1	Bradley et al., 2009
		River	na[1]	3	22.1 ± 0.7	Bradley et al., 2009
		River	na[1]	na[1]	0.90 - 2.9	Singh et a., 2010
		River	na[1]	na[1]	2.536	Soto et al., 2004
		River	na[1]	na[1]	8.3	Soto et al., 2004
		Ditch	683	608	LOD[2] - 40	Gall et al., 2011
		Feedlot runoff	50	12-13	<5 - 720	Bartelt-Hunt et al., 2012
		Estuarine	na[1]	na[1]	0.78 - 1.2	Zuo et al., 2006
		Coastal seawater	na[1]	na[1]	0.66 - 5.2	Singh et a., 2010
		Offshore seawater	na[1]	na[1]	nd[3] - 0.88	Singh et a., 2010
		Groundwater	na[1]	na[1]	LOD[2] - 120	Swartz et al., 2006
		Groundwater	49	1	nd[3] -1	Miller & Meek, 2006
		Groundwater	na[1]	1	nd[3] - 4.5	Fine et al., 2003
	Denmark	Tile-drained loamy field	7	4	LOD[2] - 68.1	Kjær et al., 2007
		Tile-drained loamy field	20	11	LOD[2] - 10.9	Kjær et al., 2007
	Iraq	Sewage	150	na[1]	6 - 20	Jafari et al., 2009
		River	100	na[1]	2 - 9	Jafari et al., 2009
		Groundwater	100	na[1]	0.1 0.2	Jafari et al., 2009
	Italy	River	na[1]	na[1]	1.5	Baronti et al., 2000
	Japan	STW effluent	27	27	0.39 - 10.49	Song et al., 2009
	England	STW effluent	28	28	0.8 - 11.2	Williams et al., 2003
		STW effluent	14	9	<0.4 - 2.2	Williams et al., 2003
		STW effluent	14	14	3.5 - 12.2	Williams et al., 2003
		Streams	na[1]	na[1]	0.10 - 9.31	Matthiessen et al., 2006
	South Korea	WWTPs effluent	7	5	2.2 - 36	Kim et al., 2007
		River	8	3	1.7 - 5.0	Kim et al., 2007
	China	WWTP effluent	na[1]	na[1]	nd[3] - 140	Zhou, Zha & Wang, 2012

Name	Country	Surface Water Type	Sampling Times	Detected times	Detected concentration (ng/L)	Reference
		WWTP effluent	17	17	0.7 - 1200	Zhou, Zha, Xu et al., 2012
		STP effluent	30	30	10.1 - 29.4	Huang et al., 2014
		Lake	16	14	nd[3] - 1.45	Yan et al., 2012
		River	na[1]	na[1]	0.64 - 55.3	Lei et al., 2009
		River	8	8	8 - 65	Peng et al., 2008
		River	15	12	nd[3] - 15.6	Wang et al., 2012
		River	44	35	<2.4 - 321.02	Zhang et al., 2012
E2β	USA	Pond	9	0	<2.0	Standley et al., 2008
		Pond	7	1	2.2	Standley et al., 2008
		Creek	na[1]	0	<0.5	Bradley et al., 2009
		Creek	na[1]	0	<0.5	Bradley et al., 2009
		River	na[1]	0	< 0.5	Bradley et al., 2009
		River	na[1]	3	8.4 ± 1.1	Bradley et al., 2009
		River	na[1]	na[1]	nd[3]	Singh et a., 2010
		River	na[1]	na[1]	3.2	Soto et al., 2004
		Spring water	39	39	13.4 - 79.7	Wicks et al., 2004
		Ditch	683	294	LOD[2] - 20.9	Gall et al., 2011
		Feedlot runoff	50	7-12	<5 - 1250	Bartelt-Hunt et al., 2012
		Estuarine	na[1]	na[1]	0.56 - 0.83	Zuo et al., 2006
		Coastal seawater	na[1]	na[1]	LOD[2] - 5.5	Singh et a., 2010
		Offshore seawater	na[1]	na[1]	nd[3]	Singh et a., 2010
		Groundwater	na[1]	na[1]	LOD[2] - 45	Swartz et al., 2006
		Groundwater	49	2	nd[3] - 29	Miller & Meek, 2006
	Denmark	Tile-drained loamy field	7	1	1.8	Kjær et al., 2007
		Tile-drained loamy field	20	5	LOD[2] - 2.5	Kjær et al., 2007
	Iraq	Sewage	150	na[1]	12 - 35	Jafari et al., 2009
		River	100	na[1]	3 - 10	Jafari et al., 2009
		Groundwater	100	na[1]	0.2 - 0.3	Jafari et al., 2009
	Italy	River	na[1]	na[1]	0.11	Baronti et al., 2000
	Japan	STW effluent	27	27	1.35 - 9.05	Song et al., 2009
	England	STW effluent	28	14	<0.4 - 2	Williams et al., 2003
		STW effluent	9	4	<0.4 - 1.7	Williams et al., 2003
		STW effluent	14	12	<0.4 - 4.3	Williams et al., 2003
		Streams	na[1]	na[1]	0 - 0.89	Matthiessen et al., 2006
	South Korea	WWTPs effluent	7	0	<1.0	Kim et al., 2007
		River	8	0	nd[3]	Kim et al., 2007
	China	WWTP effluent	na[1]	na[1]	nd[3] - 8.4	Zhou, Zha & Wang, 2012
		WWTP effluent	17	15	nd[3] - 67.4	Zhou, Zha, Xu et al., 2012

Name	Country	Surface Water Type	Sampling Times	Detected times	Detected concentration (ng/L)	Reference
		STP effluent	30	30	1.5 - 10.8	Huang et al., 2014
		Lake	16	16	4.41 - 9.96	Yan et al., 2012
		River	na[1]	na[1]	nd[3] - 32.4	Lei et al., 2009
		River	15	1	nd[3] - 2.3	Wang et al., 2012
		River	8	2	nd[3] - 2	Peng et al., 2008
		River	44	9	<2.5 - 74.4	Zhang et al., 2012
E2α	USA	Ditch	589	100	LOD[2] - 51.8	Gall et al., 2011
		River	na[1]	na[1]	3.8	Soto et al., 2004
		Feedlot Runoff	50	39-40	<103 - 1360	Bartelt-Hunt et al., 2012
		Groundwater	49	0	nd[3]	Miller & Meek, 2006
	China	River	8	3	nd[3] - 2	Peng et al., 2008
E3	USA	Ditch	537	14	LOD[2] - 19.6	Gall et al., 2011
		Feedlot Runoff	50	15-23	<243 - 2600	Bartelt-Hunt et al., 2012
		Groundwater	49	3	nd[3] - 6.4	Miller & Meek, 2006
	Italy	River	na[1]	na[1]	0.33	Baronti et al., 2000
	South Korea	WWTPs effluent	7	3	8.9 - 25	Kim et al., 2007
		River	8	0	nd[3]	Kim et al., 2007
	China	WWTP effluent	na[1]	na[1]	nd[3] - 11	Zhou, Zha & Wang, 2012
		WWTP effluent	17	11	nd[3] - 1200	Zhou, Zha, Xu et al., 2012
		STP effluent	30	na[1]	nd[3] - 7.6	Huang et al., 2014
		Lake	16	16	1.02 - 1.65	Yan et al., 2012
		River	na[1]	na[1]	nd[3] - 46.4	Lei et al., 2009
		River	8	1	nd[3] - 1	Peng et al., 2008
		River	44	7	<3.1 - 39.8	Zhang et al., 2012
EE2	USA	Estuarine	na[1]	na[1]	3.01 - 4.67	Zuo et al., 2006
		Groundwater	49	0	nd[3]	Miller & Meek, 2006
	Iraq	Sewage	150	na[1]	2 - 12	Jafari et al., 2009
		River	100	na[1]	0.01- 2	Jafari et al., 2009
		Groundwater	100	na[1]	0.5 - 1	Jafari et al., 2009
	Italy	River	na[1]	na[1]	0.04	Baronti et al., 2000
	Japan	STW effluent	27	27	0.59 - 6.56	Song et al., 2009
	England	STW effluent	9	28	<0.5 - 1.9	Williams et al., 2003
		STW effluent	4	9	<0.5 - 1.1	Williams et al., 2003
		STW effluent	7	14	<0.5 - 3.4	Williams et al., 2003
	South Korea	WWTPs effluent	7	1	1.3	Kim et al., 2007
		River	8	0	nd[3]	Kim et al., 2007
	China	WWTP effluent	na[1]	na[1]	nd[3] - 5.8	Zhou, Zha & Wang, 2012

Name	Country	Surface Water Type	Sampling Times	Detected times	Detected concentration (ng/L)	Reference
		WWTP effluent	17	16	nd[3] - 4100	Zhou, Zha, Xu et al., 2012
		STP effluent	30	na[1]	nd[3] - 9.7	Huang et al., 2014
		Lake	16	10	nd[3] - 10.20	Yan et al., 2012
		River	na[1]	na[1]	nd[3] - 35.6	Lei et al., 2009
		River	8	1	nd[3] - 1	Peng et al., 2008

Note: [1]data not available; [2]limit of detection; [3]not detected.

Appendix B

Table B1. *Descriptive and corresponding numeral cloud cover*

Cloud cover in description	Cloud cover in eighth	Cloud cover in tenth
Clear	0/8	0
Scattered	1/8-4/8	3
Broken	5/8-7/8	7.5
Overcast	8/8	10
Reference	Perez et al. (2002)	

Appendix C

Table C1. *The hydrological parameters used in the model for the South River Watershed*

Parameter	Definition	Units	Min	Max	Model value
LZSN	Lower Zone Nominal Soil Storage	inches	2	15	2.0
INFILT	Index to Infiltration Capacity	in/h	0.001	0.5	0.1-0.15
AGWRC	Base groundwater recession	none	0.85	0.999	0.96
CEPSE	Interception storage capacity	inches	0.01	0.4	0.01
UZSN	Upper Zone Nominal Soil Storage	inches	0.05	2	0.5-1
INTFW	Interflow inflow parameter	none	1	10	3
IRC	Interflow recession parameter	none	0.3	0.85	0.3
LZETP	Lower zone ET parameter	none	0.1	0.9	0.1

Table C2. *The hydrological parameters used in the model for the Redwood River Watershed*

Parameter	Definition	Units	Min	Max	Model value
LZSN	Lower Zone Nominal Soil Storage	inches	2	15	2.0
INFILT	Index to Infiltration Capacity	in/h	0.001	0.5	0.15-0.25
AGWRC	Base groundwater recession	none	0.85	0.999	0.98
CEPSE	Interception storage capacity	inches	0.01	0.4	0.03-1
UZSN	Upper Zone Nominal Soil Storage	inches	0.05	2	0.4
INTFW	Interflow inflow parameter	none	1	10	3
IRC	Interflow recession parameter	none	0.3	0.85	0.85
LZETP	Lower zone ET parameter	none	0.1	0.9	0.2

Appendix D

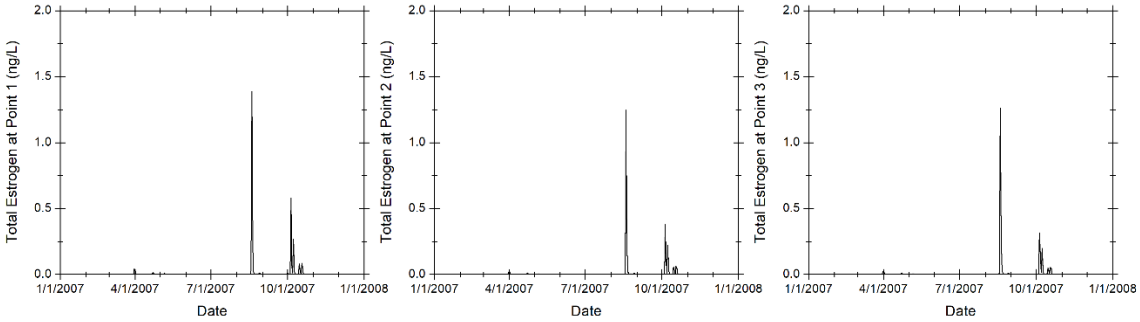

Figure D1. *The simulated total estrogens from land-applied beef cattle manure.*

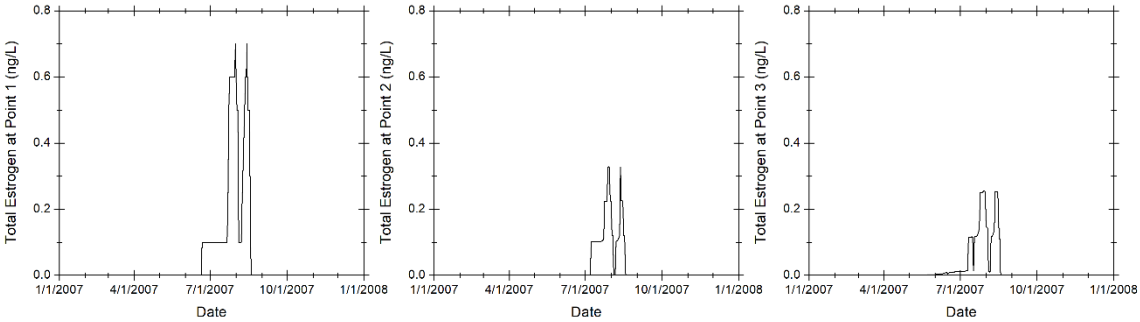

Figure D2. *The simulated total estrogens from beef cattle waste directly released into streams.*

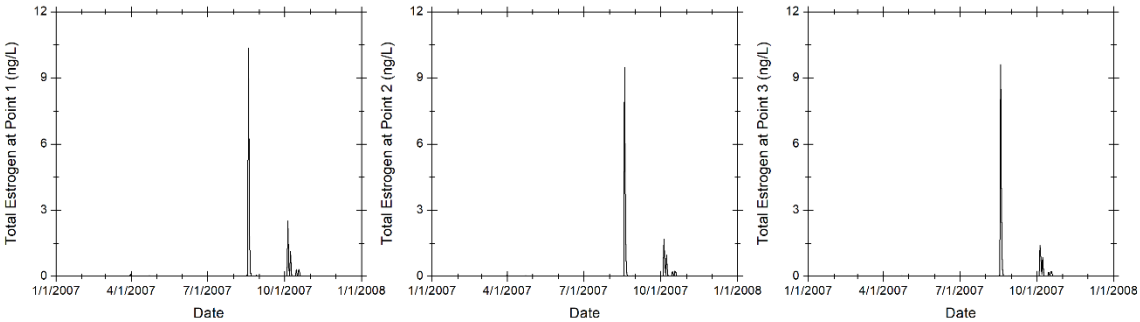

Figure D3. *The simulated total estrogens from land-applied dairy cattle manure.*

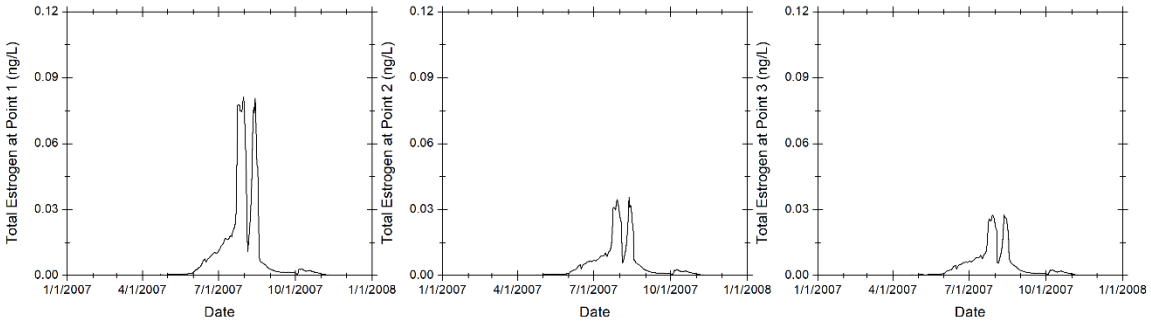

Figure D4. *The simulated total estrogens from dairy cattle waste directly released into streams.*

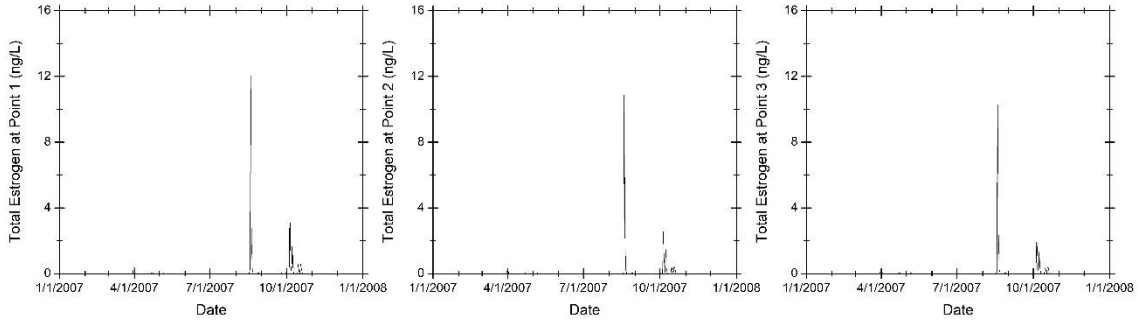

Figure D5. *The simulated total estrogens from land-applied swine manure.*

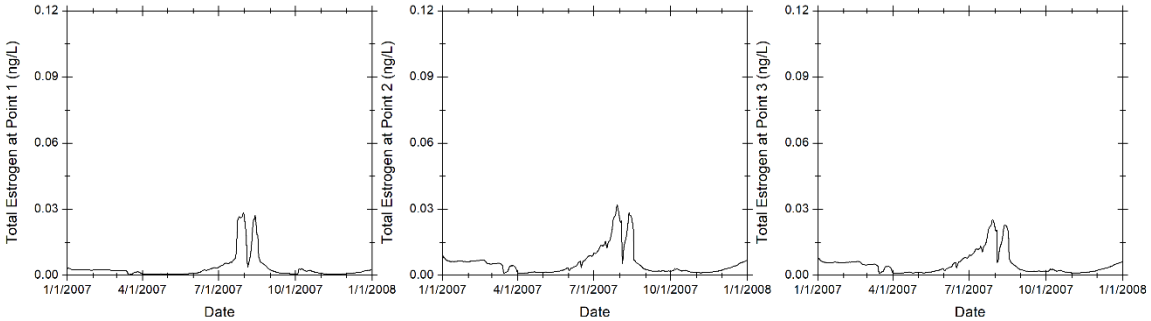

Figure D6. *The simulated total estrogens from septic systems.*

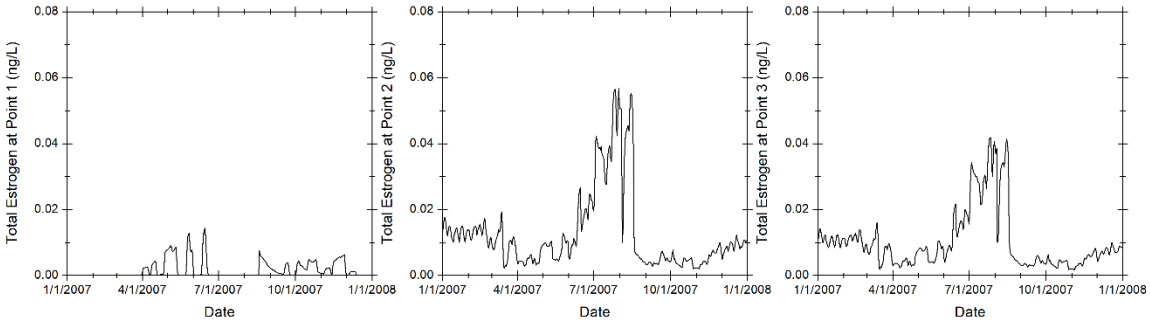

Figure D7. *The simulated total estrogens from WWTPs.*

Appendix E

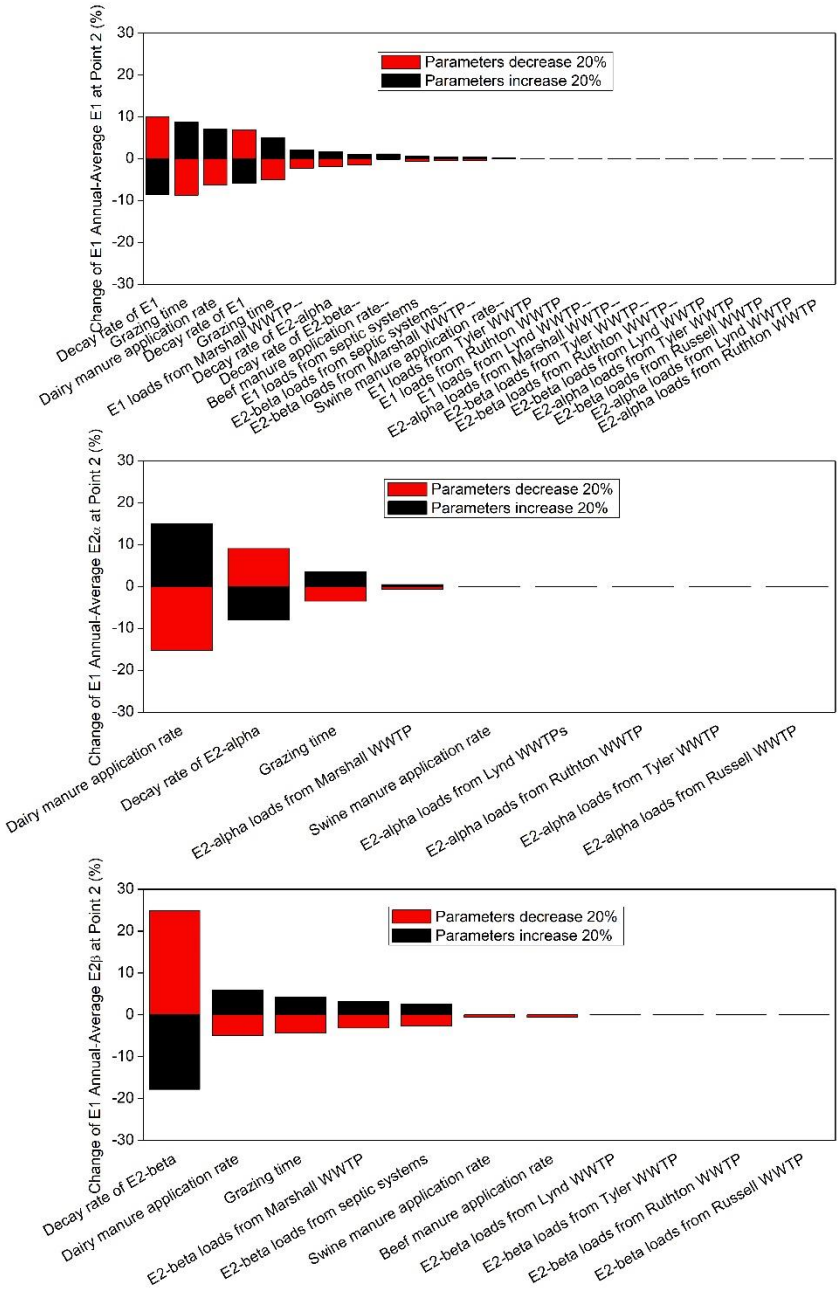

Figure E1. *The sensitivity analysis at Point 2 using estrogen parameters.*

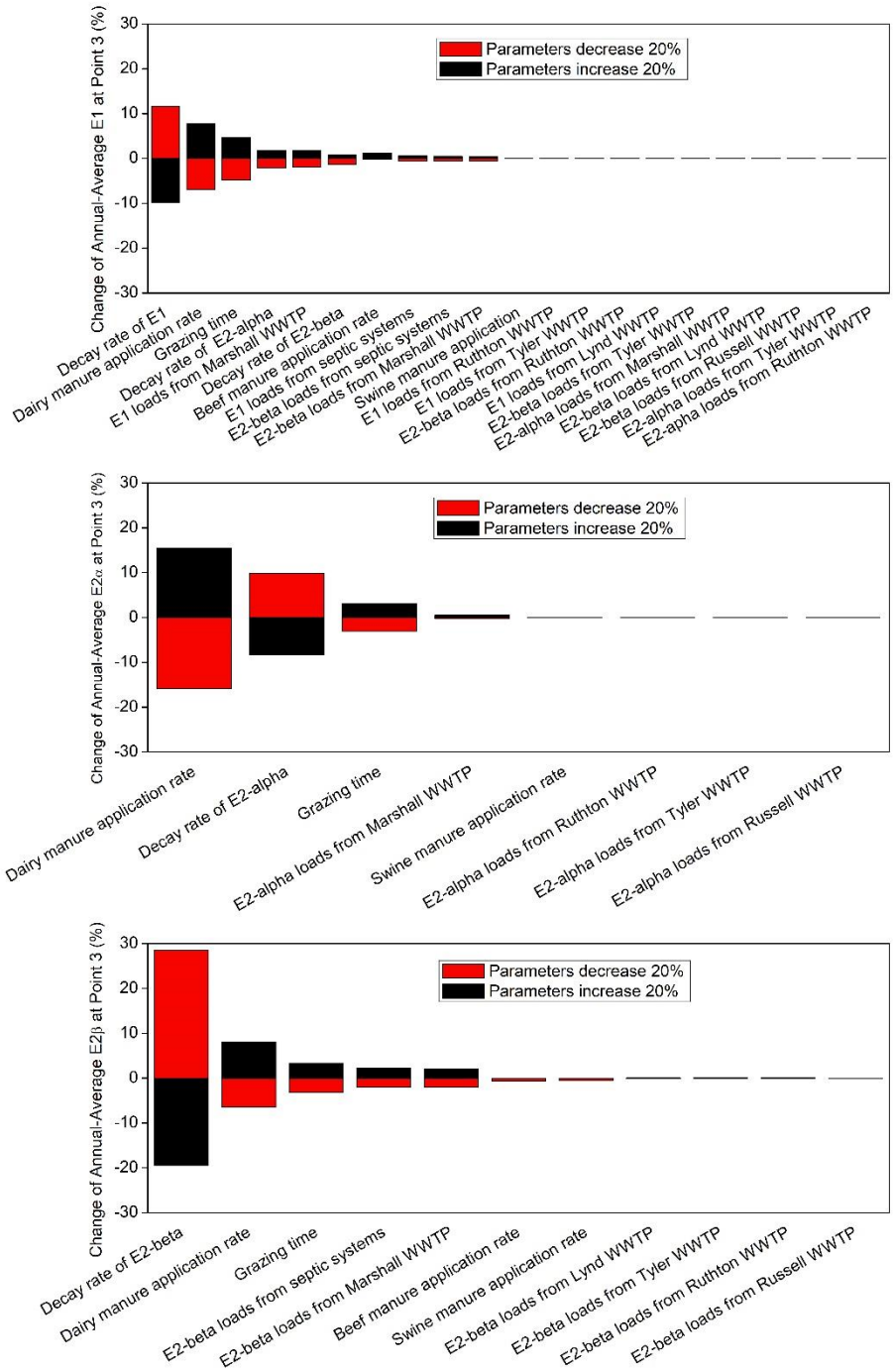

Figure E2. *The sensitivity analysis at Point 3 using estrogen parameters.*

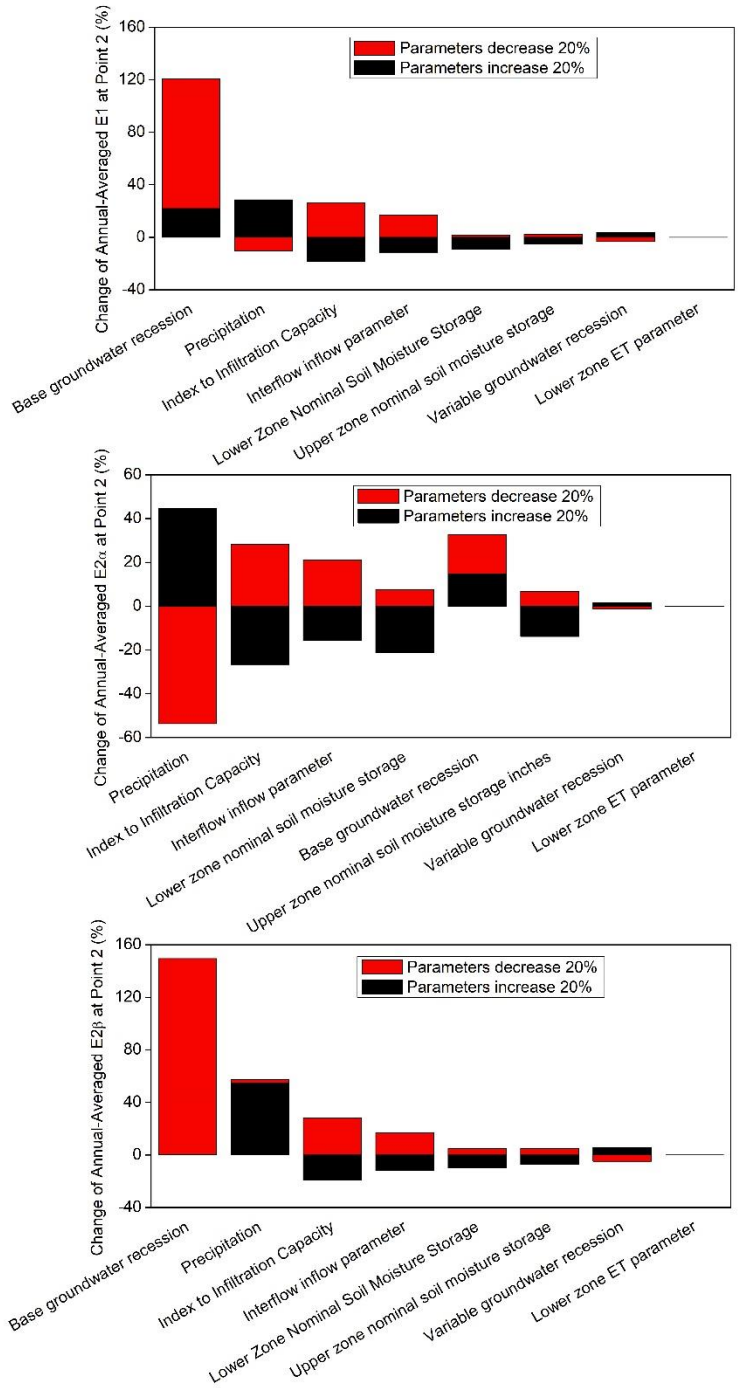

Figure E3. *The sensitivity analysis at Point 2 using hydrological parameters.*

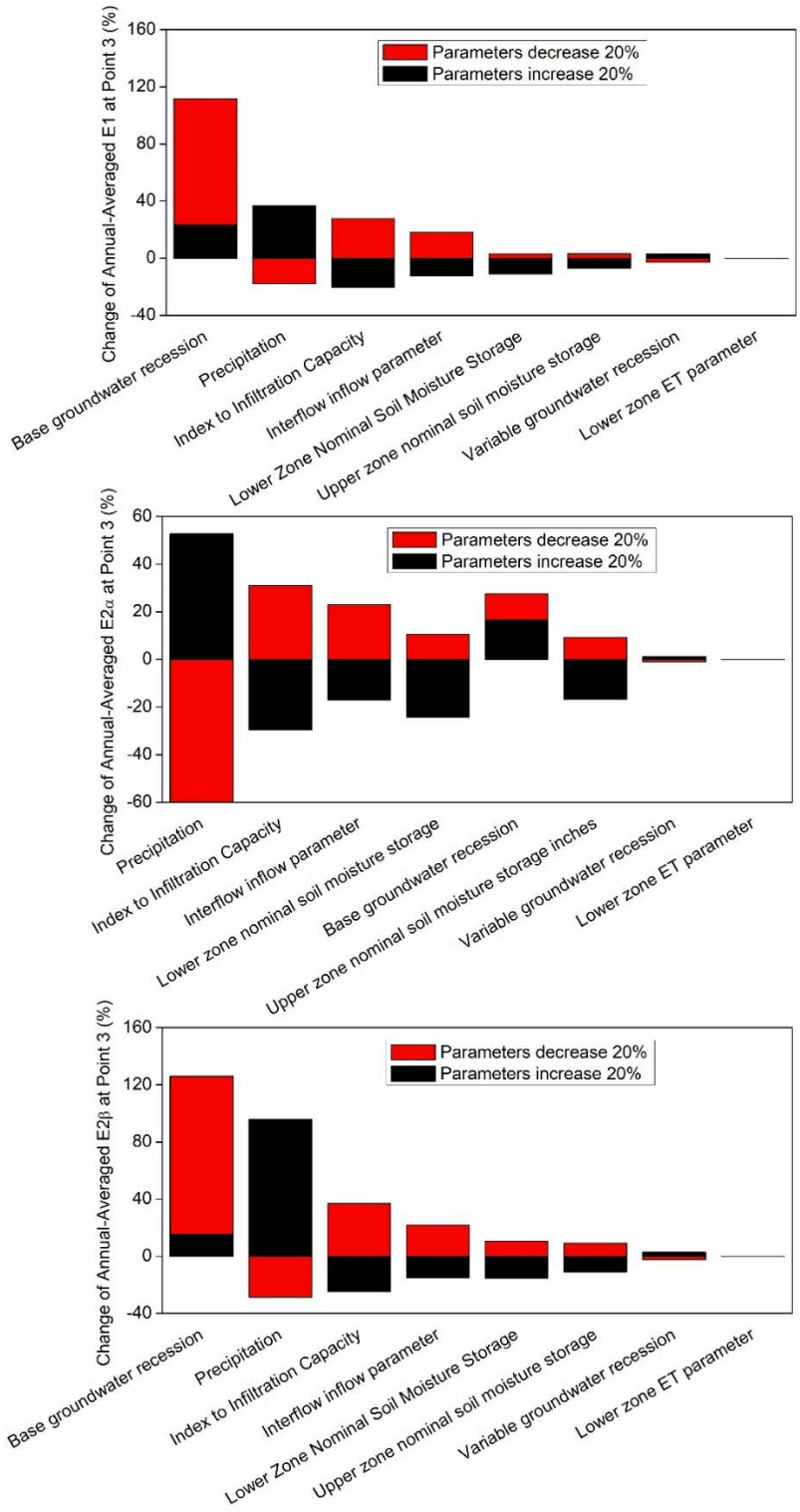

Figure E4. *The sensitivity analysis at Point 3 using hydrological parameters.*