THE CHEMICAL BEHAVIOR OF ESTRONE AND

17β-ESTRADIOL IN THE ENVIRONMENT

A Dissertation

by

JEFFREY LAYTON ULLMAN

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

DOCTOR OF PHILOSOPHY

May 2006

Major Subject: Biological & Agricultural Engineering

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Approved by:

Co-Chairs of Committee,	Saqib Mukhtar
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ABSTRACT

The Chemical Behavior of Estrone and 17β-estradiol in the Environment. (May 2006)

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The endogenous hormones estrone and 17β-estradiol support vertebrate growth and development, but slight increases above ambient concentrations may paradoxically induce endocrine disruption, leading to increased frequencies of reproductive disorders and cancer in humans and wildlife. Livestock excrete estrogenic compounds which can lead to surface and groundwater contamination. Limited information exists concerning estrogen fate and transport, as exposure concerns have recently arisen. This study examined the chemical behavior of estrone and 17β-estradiol with the goal of elucidating manure management principles applicable to animal feeding operations (AFOs).

Both compounds indicated that they are susceptible to physicochemical and biological decomposition. Photolysis yielded 27- and 35-day half-lives for estrone and 17β-estradiol at pH 7, respectively, based on test conditions. 17β-estradiol photolysis exhibited a slight pH-dependent behavior. Mineralization produced half-lives ranging from 7 to 15 days for estrone and 3 to 7 days for 17β-estradiol. Indigenous microbial populations did not demonstrate a lag phase and therefore appeared to have been well acclimated to degrading these compounds. Anaerobic lagoon supplements did not affect mineralization rates. The compounds had partition coefficients ranging from 2 to 4.4

mL g^{-1}, depending on soil characteristics. Estrone and 17β-estradiol had a higher absorptivity to soils with greater clay content and organic matter. Once sorbed, binding appeared largely irreversible with minimal desorption.

Column experiments detected no estrone in the leachate for the finer textured soils, while estrone had completely migrated through the loamy fine sand after 7 pore volumes. Hypothetical scenarios, simulated using HYDRUS-1D, evaluated the combined effects of soil texture and the values obtained for sorption and mineralization on leaching. Model results indicated rapid leaching in loamy sand, while sandy clay loam and clay yielded significantly slower estrone and 17β-estradiol movement with concentrations 1 to 3 orders of magnitude lower.

Data suggest that estrogens applied to sandy soil may leach and contaminate groundwater, especially in the presence of shallow water tables. Sandy clay loam and clay likely present minimal risk for subsurface mobility, but simulated accumulation near the soil surface may promote transport via overland flow. Sound manure management practices will likely reduce off-site transport of estrogens originating from AFOs.

DEDICATION

To the prospect of sustainable agriculture, integrating the stewardship of both natural and human resources.

ACKNOWLEDGMENTS

I would like to express my gratitude to Dr. Mukhtar and Dr. Senseman for their counsel and assistance during my tenure at Texas A&M University. Their guidance was integral to my development in both the engineering and environmental chemistry fields. Dr. Kenimer has served as a trusted mentor and her patience and encouragement were instrumental to the completion of my degrees. I also extend my thanks to Dr. Zhan for his support, both in and out of the classroom.

I have been blessed to have had more than my fair share of advocates up to this point in my life. Although too many to mention here, I would like to thank all those at the Cleveland Museum of Natural History, University School, Cornell University, Duke University and Texas A&M University who made a lasting impression on me. The nurturing optimism of my parents deserves special mention, as without their sacrifice I would never have gotten this far.

Appreciation is extended to the United States Environmental Protections Agency and the Texas Water Resources Institute for their financial assistance in this project. Finally I would like to thank David Liere and all the other livestock producers that have helped me in my pursuits.

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1. INTRODUCTION TO ESTROGENS OF AGRICULTURAL ORIGIN: A LITERATURE REVIEW OF EFFECTS, SOURCES AND CHEMICAL BEHAVIOR IN THE ENVIRONMENT

1.1. INTRODUCTION

Endocrine disrupting chemicals (EDCs) represent a class of substances that interact with the endocrine system, potentially impairing cell proliferation and differentiation, which can consequently contribute to infertility (Nash et al., 2004), developmental abnormalities (Migliaccio et al., 1995) or hormone-related cancer (Jones and Hajek, 1995; Lai et al., 2002) in humans and wildlife. Endocrine disruptors include both agonist (a chemical that can combine with a receptor to produce a physiologic reaction typical of a naturally occurring substance) and antagonist (a substance that interferes with the physiological action of another) compounds. The EPA Office of Research and Development has addressed the risks of EDCs and the Office of Science Coordination and Policy currently lists endocrine disruptor screening as a priority setting. A myriad of hormone-mimicking compounds exist in the environment, including pesticides, herbicides, PCBs, dioxins, alkyl-phenols, pharmaceuticals and other synthetic compounds. Although much attention has focused on anthropogenic EDCs (xenoestrogens), natural hormones present a potentially significant and potent environmental load.

This dissertation follows the style of Journal of Environmental Quality.

Although endogenous, natural estrogens such as estrone and 17β-estradiol can similarly exert detrimental impacts on growth and development when present in excessive concentrations. It is becoming increasingly apparent that these hormonal compounds reach elevated levels in both municipal and agricultural wastestreams, as humans and animals excrete estrone and 17β-estradiol via urine and feces. Large estrogen loads associated with animal feeding operations (AFOs) arise not only from steroidal treatments given to livestock to enhance growth and fecundity, but also from the current trend towards concentrating animals into small geographic areas, magnifying waste related issues. Manure disposal presents a potential source for surface and groundwater contamination, consequently threatening human health and imperiling wildlife communities. This warrants further investigation into environmental exposure to estrogenic agents. The following literature review will briefly describe chemical properties, physiological impacts, agricultural sources and the environmental transport of these compounds.

1.2. CHEMICAL PROPERTIES

Steroidal compounds, including estrogens, represent a hormonal class generally synthesized from cholesterol. Therefore, 17β-estradiol and estrone display molecular structures similar to cholesterol, with a five-carbon ring attached to three six-carbon rings (Figure 1.1). Henceforth, the terms estrogen, estrogenic compound and estrogenic agent refer to the general group of steroid compounds that function as the primary

female sex hormones while 17β-estradiol and estrone refer to the specific compounds shown here.

Estrogens display characteristics typical of organic compounds, including low solubility and high affinity to organic matter (Table 1.1). These lipophilic traits lead to ready diffusion through the cell membrane and into the cytoplasm of target cells. Although typically excreted in a conjugated form, quick conversion to unconjugated molecules occurs in the environment and discussion will be limited to these relevant structures.
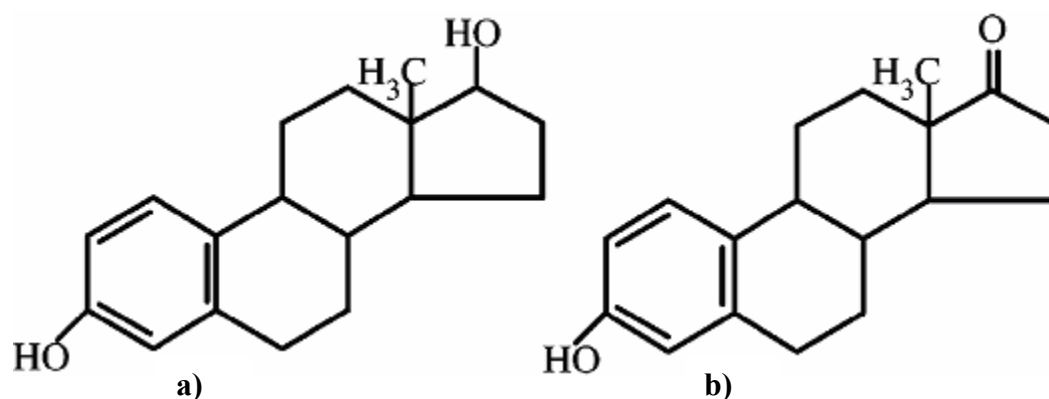
Fig. 1.1. Molecular structure of a) 17β-estradiol and b) estrone.

Table 1.1. Selected physicochemical properties of estrone and 17β-estradiol (Hanselman et al., 2003; Lai et al., 2000).[†]

Compound	MW	S_W	Vapor pressure	log K_{OW}	pK_a
	(g mol^{-1})	(mg L^{-1})	(Pa)		
Estrone	270.4	13	3×10^{-8}	3.1-3.4	10.3-10.8
17β-estradiol	272.4	13	3×10^{-8}	3.1-4.0	10.5-10.7

[†] **MW – molecular weight, S_W – solubility in water, K_{OW} – octanol-water partition coefficient, K_a – acid ionization constant.**

1.3. PHYSIOLOGICAL EFFECTS

Hormones, including the natural estrogenic compounds estrone and 17β-estradiol, can result in endocrine disrupting effects in human and wildlife populations, impacting reproduction and development (2000; Crews et al., 1995; Routledge et al., 1998; Sone et al., 2004; Sumpter and Jobling, 1995). Although endogenous, the potency of these compounds can lead to detrimental health consequences at concentrations as low as 1 ng L^{-1} (Irwin et al., 2001; Routledge et al., 1998). Despite corresponding concerns over the presence of hormones in aquatic systems, a thorough understanding of both acute and chronic exposure remains deficient. Nonetheless, a number of studies have begun to address this paucity.

1.3.1. Wildlife Impacts

It remains unclear if estrone or 17β-estradiol presents a risk to invertebrates (Hutchinson et al., 1999; Segner et al., 2003); however, the detrimental effects induced by natural hormones on vertebrates have been well documented. Much of the research conducted on higher-level organisms has focused on fish, partially due to the palpable impacts imparted by estrogens. The protein vitellogenin, synthesized in liver cells, acts as a precursor to egg yolk proteins in non-mammalian vertebrates. Since estrogens regulate vitellogenisis it serves as an excellent marker to indicate exposure to environmental estrogens.

Sumpter and Jobling (1995) authored a landmark paper describing vitellogenin synthesis in male fish maintained in municipal wastewater treatment effluent.

Furthermore, this study documented that a mixture of various estrogenic contaminants enhanced this development compared to each of the compounds when tested individually. Subsequently, Routledge et al. (1998) demonstrated that estrone and 17β-estradiol led to a demasculinization of male rainbow trout (*Oncorhynchus mykiss*) at 25 ng L^{-1} concentrations, suggesting the potency of these compounds. Estrogenic effects on fish have since been shown in zebrafish (*Danio rerio*; (Van den Belt et al., 2003), carp (Cyprinus carpio; (Higashitani et al., 2003) and sheepshead minnows (*Cyprinodon variegates*; (Folmar et al., 2000), among others. Wild fathead minnows (*Pimephales promelas*) exposed to cattle feedlot effluent similarly displayed feminization, exhibiting diminished testosterone synthesis, altered head morphometrics and decreased testis size, indicating the potential risk associated with AFO runoff (Orlando et al., 2004). Cases of altered sex ratios and sex reversals in fish have also been observed (Lai et al., 2002; Nakamura, 1984).

Estrogenic activity in amphibians and reptiles similarly occurs. Laboratory studies have induced vitellogenesis in male red-eared turtles (*Trachemys scripta*) and African clawed frogs (*Xenopus laevis*) through 17β-estradiol injections (Palmer and Palmer 1995). Other researchers investigated the influence of low endocrine disruptor concentration in amphibians both *in vivo* and *in vitro*. Kloas et al. (1999) demonstrated 17β-estradiol exposure at 2.7 μg L^{-1} generated physiological changes at three organizational levels in *Xenopus laevis*, including binding to liver estrogen receptors, induction of vitellogin-mRNA in cultured liver cells and altered larvae sexual development. Similarly, *Xenopus laevis* embryos reared in 2.2 mg L^{-1} 17β-estradiol

concentrations exhibited short body length, microcephaly (abnormal smallness of the head), edema (serous fluid accumulation in tissue or body cavity) and abnormal gut coiling (Sone et al., 2004). Although field caught male and juvenile painted turtles (*Chrysemys picta*) residing in waters that displayed 17β-estradiol concentrations around 1.0 ng L^{-1} failed to exhibit vitellogenesis, elevated vitellogin levels in females resulted at these low levels, suggesting alterations in reproductive fitness by shifting energy allocations, physiology and egg production (Irwin et al. 2001).

Most studies on the impacts of natural estrogens have revolved around the impacts at a species level, while higher-level investigations remain limited. A lentic microcosm study suggests that shifts in ecosystem structure and function may occur following 17α-ethynylestradiol dosing, an estrogen that closely resembles estrone and 17β-estradiol (Hense et al., 2004). Although zooplankton populations deviated ambiguously from controls, displaying only a decrease in copepod density, phytoplankton characteristics displayed diminished species diversity, biomass and cell density. Effective risk assessments necessitate further ecological studies, but these preliminary results indicate a potential threat of natural hormones that transcends single species.

1.3.2. Human Impacts

Conclusive cause-effect relationships between environmental chemical exposure and adverse physiological impacts are difficult to establish due to the preponderance of unknown and conflicting variables. Evidence indicates detrimental trends in health and

reproductive vigor exist in association with EDCs; however, the plethora of compounds humans are exposed to makes it often unfeasible to isolate the effects of a single compound. The following discussion on estrogenic impacts on humans will refer to estrogenic agents in general, unless otherwise specified.

Suggestions for linkage between endocrine disruptor exposure and human health implications include (Harrison et al., 1997):

- increased incidents of breast, testicular and prostate cancer

- reduced sperm counts and quality

- increased cases of cryptorchidism (undescended testes) and hypospadias (malformation of the penis)

- increased occurrence of polycystic ovaries in women.

Estrogens and estrogen-mimickers may cause or contribute to a number of these physiological alterations. For instance, the incidence of breast cancer in industrialized nations has inarguably risen over the last several decades. Although research continues to reveal the scope and magnitude of issues related to hormone exposure, evidence suggests 17β-estradiol induces breast cancer (Dickson 1986).

Reports of early female maturity in humans is supported by the parallel occurrence of estrogenically induced premature udder development and lactation in heifers (Ball et al., 2000). Premature human sexual development in Puerto Rico has raised concern that environmental estrogens now impact much of the population. Reports include precocious pseudo-puberty at ages as low as 24 months, while

premature breast development has occurred before age 8 in young girls (Rodriguez et al. 1985).

Some researchers theorize that diminished male fertility may be magnified by exposure to elevated estrogenic levels, such as estrone and 17β-estradiol, during embryonic, fetal and early postnatal periods (Colburn 1995). Parallel to this hypothesis, reduced sperm counts and increased incidents of testicular cancer in humans may be induced by environmental estrogen exposure (Carlsen et al., 1995). As demonstrated earlier with wildlife, some argue a degree of feminization is occurring in human society due to a conglomerate of chemicals, of which estrogens likely play a role.

Estrogenic impacts may not be overt and remain unidentified until effects materialize across the population (e.g., reduced sperm counts), but implications to human health are evident. The potency of 17β-estradiol, and to a lesser extent estrone, at such low concentrations make these compounds chemicals of concern.

1.4. ESTROGEN SOURCES

The preponderance of research in the field of environmental estrogens exists in the municipal wastewater treatment literature. When relating estrone and 17β-estradiol concentrations in municipal sewage effluent to potential wildlife impacts, Johnson and Sumpter (2001) listed these hormones as compounds of concern. These elevated levels originate from estrogen excretion rates of 25-100 μg per day by women depending on the phase of the menstrual cycle, a level that rises to a daily secretion of 30 mg by the end of pregnancy (Turan, 1995). Anxiety over high estrogen levels and other EDCs has

generated studies in many European nations, Brazil, Canada and the United States (Belfroid et al., 1999; Cargouet et al., 2004; Johnson et al., 2005; Ternes et al., 1999). Despite corresponding concerns over the presence of hormones originating from agricultural production in drinking water and aquatic systems, data collection on estrogen concentrations in livestock waste streams has only recently begun. Likewise, little information on the potential off-site transport of estrogens exists. Nevertheless, initial results find not only elevated estrogenic concentrations, but also evidence supporting migration to both surface and groundwater systems.

1.4.1. Manure Management Systems

Elevated concentrations of excreted steroidal compounds have been documented in livestock (Mathur and Common 1969) and poultry (Erb et al. 1977) wastes for some time, but recent concerns over hormonal exposure warrant further investigation of concentrations in animal manure and process generated wastewater. Birds excrete significantly higher hormone quantities compared to mammals, with poultry litter from laying hens exhibiting estrogen concentrations as high as 533 ng g^{-1} (Shore et al., 1995); nevertheless livestock manure management systems contain estrogen levels above ambient levels (Table 1.2).

A potentially significant environmental load of estrogens originating from milk cows exists, particularly in regions populated with large numbers of dairy operations. Estimates project that cattle in the United States excrete 45 tons of estrogen annually (Shore and Shemesh, 2003). Although this figure does not distinguish between beef and

Table 1.2. Concentrations of estrone and 17β-estradiol reported for different livestock manure management systems.

Estrogenic source	Estrogen	Concentration[†]	Reference
Swine lagoon	17β-estradiol	Up to 3 µg L^{-1}	(Fine et al., 2003)
Swine farrowing lagoon	Estrone	5.9 µg L^{-1}	(Raman et al., 2004)
	17β-estradiol	3.9 µg L^{-1}	
Milk cow slurried manure	Estrone	255-640 µg L^{-1}	(Shore and Shemesh, 2003)
	17β-estradiol	170-1230 µg L^{-1}	
Dairy lagoons & holding ponds	17β-estradiol	< 2 µg L^{-1}	(Williams et al., 2002)
Flushed dairy manure wastewater	Estrone	0.78 µg L^{-1}	(Hanselman et al., 2004) [‡]
	17β-estradiol	≤ 1.31 µg L^{-1}	
Dairy waste lagoon	Estrone & 17β-estradiol	≤ 0.65 µg L^{-1}	(Kolodziej et al., 2004)
Dairy holding pond	Estrone	2.5-5.6 µg L^{-1}	(Raman et al., 2004)
	17β-estradiol	0.8-1.9 µg L^{-1}	

[†] Values given as means unless otherwise stated.
[‡] Compared analysis methods which showed significant variability. Maximum values shown.

dairy animals, milk cows clearly excrete a substantial proportion as pregnant dairy cows excrete 163 mg daily through urine alone (Hanselman et al., 2003). Moreover, 17β-estradiol supplements may be given to cattle to increase meat quality, improve feed

conversion and increase weight gain (Beconi et al., 1995; Enright et al., 1990; Henricks et al., 1997; Herschler et al., 1995; Popp et al., 1997), raising urinary 17β-estradiol concentrations up to six-fold (Callantine et al., 1961). Raman et al. (2004) calculated that a typical dairy facility exhibits estrogen emission factors of 3.3 and 1.1 mg d^{-1} (1000 kg live animal weight)$^{-1}$ for estrone and 17β-estradiol, respectively, from dairy liquid manure holding ponds.

1.4.2. Environmental Exposure

Land application of animal manures represents an integral part of the livestock industry's waste management paradigm. Estrogen persistence and mobility following land disposal of manure and process generated wastewater correspondingly influences potential off-site transport to waters of concern.

Recent evidence suggests potential off-site migration of estrone and 17β-estradiol following manure application to agricultural soils. Initial studies examined land application of poultry litter. High litter application rates led to first-storm runoff 17β-estradiol concentrations of 1,280 ng L^{-1} (Nichols et al., 1997); however, a follow up study found grass filter strips reduced 17β-estradiol runoff concentrations averaging 3,500 ng L^{-1} by 98% (Nichols et al., 1998). Sizable edge-of-field 17β-estradiol losses occurred following broiler litter amendments to grasslands, but appreciable increases in soil concentration resulted reaching 675 ng kg^{-1} (Finlay-Moore et al., 2000).

Liquid dairy manure applied at 17β-estradiol concentrations of 3,300 ng L^{-1} displayed runoff values above ambient levels (Dyer et al., 2001). Manure amendments

corresponding to nitrogen and phosphorus based recommendation rates yielded runoff as high as 41 and 29 ng L^{-1}, respectively. Although runoff concentrations decreased with time, these values are above those known to imperil wildlife populations. Cattle grazing pastures contributed no detectable increase in 17β-estradiol runoff concentrations (Finlay-Moore et al., 2000), suggesting the main estrogen exposure risk likely comes from AFOs.

A number of studies demonstrate the potential for estrogen migration following manure application to agricultural soils. Evidence suggests cattle AFOs can release significant estrogenic activity into neighboring waterways (Soto et al., 2004). Female painted turtles (*Chrysemys picta*) residing in ponds receiving runoff from cattle operations exhibited signs of altered reproductive fitness at 17β-estradiol concentrations around 1.0 ng L^{-1} (Irwin et al., 2001), while wild male fathead minnows (*Pimephales promelas*) displayed characteristics of demasculinization following exposure to effluent (Orlando et al., 2004). Affirmation that local cattle operations generated elevated 17β-estradiol concentrations in karst aquifers denotes potential estrogenic contamination of groundwater systems (Peterson et al., 2000).

Treatment of agriculturally generated wastewater remains unexamined as a method for limiting human and wildlife exposure to estrogenic agents. Potential technological transfer from municipal systems may present an option, but practicality often dictates the necessity for more specific livestock waste management methodologies. One promising technology transfer includes the use of constructed wetlands. Utilizing the high biological activity inherent to wetlands, engineered

treatment systems mitigated 17β-estradiol levels by 41% in river water principally composed of tertiary wastewater effluent (Gray and Sedlak, 2005). Similarly, estrogenic activity underwent modest reduction in wastewater effluent subsequent to passage through treatment wetlands (Bjolseth et al., 2003). Variable treatment efficacy and alterations required for managing agricultural wastewater necessitates further research prior to implementing wetlands for AFO waste treatment. This first entails a better understanding of the chemical behavior of these estrogenic compounds.

1.5. ENVIRONMENTAL TRANSPORT

A number of mechanisms influence the sequestration and dissipation of estrogenic compounds, which consecutively impacts their environmental fate and transport. Although not an inclusive list, photolysis, sorption and microbial degradation processes regulate persistence and mobility, two factors inherent to deterministic and stochastic predictive models. The following section explores research conducted on estrone and 17β-estradiol in these fields and briefly describes several rudimentary models used to explain leaching potential.

1.5.1. Photolysis

Most papers dealing with light-induced estrogen degradation consider photolysis in association with a photocatalyst, which presents difficulty when considering this dissipation phenomenon in natural waters. Moreover, a wide range of light sources used in experiments further complicates attempts to compare studies. However, research has

begun to address this mechanism impacting the fate of estrogenic agents in the environment.

Photodegradation of 17β-estradiol in conjunction with a titanium dioxide (TiO$_2$) catalyst displayed 99% degradation after a 30-min UV exposure (mercury-xenon lamp, 200 W, 365-nm band-passed filter) designed to test photolysis as a water treatment technique (Ohko et al., 2002). Similarly, Nakashima et al. (2002) found 98% 17β-estradiol decomposition after 1 h in a reactor fitted with TiO$_2$-modified mesh sheets and black fluorescent lamps; however, no concentration attenuation occurred over the same time period when illuminated without a catalyst. Immobilized TiO$_2$ mitigated 17β-estradiol by 50% in 40 min and 98% in 3.5 h (xenon lamp, 150 W, filter to remove λ<300 nm), compared to non-catalytic degradation of 11% and 44% over the same time periods (Coleman 2000). Adjusting pH led to increased photolysis rates up to pH 7, followed by a decrease to pH 10 and a rapid intensification to pH 12. Corresponding hydroxyl radical generation associated with increasing pH was attributed to fostering 17β-estradiol oxidation in this case.

A paper considering direct photolysis in the absence of a catalyst examined estrogen irradiation in conjunction with two disparate light sources (Liu and Liu, 2004). Using a UV-light (disinfection lamp, 30 W, λ_{max} = 254 nm) and UV-Vis-light (high-pressure mercury lamp, 125 W, $\lambda_{max} \geq$ 365 nm), concentration profiles were determined. Half-lives generated for estrone and 17β-estradiol approached 10 and 40 min, respectively, under the UV-lamp. The UV-Vis-light resulted in an estrone half-life of about 50 min, but only produced minimal 17β-estradiol degradation. An absorbance

peak of 288 nm by 17β-estradiol may help explain this finding, as it exhibits generally weak absorption in the UV range; similarly, Rosenfeldt and Linden (2004) determined a maxima at about 280 nm. Altering initial concentration for both estrone and 17β-estradiol from 20 to 3 mg L^{-1} increased photolysis rates by about two-fold during UV-light illumination (Liu and Liu, 2004). Furthermore, Liu and Liu also demonstrated a pH dependence on estrogen photodegradation rates, with a minimum at pH 5 and a generally faster trend at higher pH values for both compounds.

Although focusing on photocatalytic efficacy, Coleman et al. (2004) found that minimal 17β-estradiol photolysis occurred in the presence of UVA light (high-pressure mercury lamp, 125 W; the UVA spectrum represents long-wave UV radiation spanning 315-380 nm) over the first 160 min, after which the concentration decreased abruptly. A half-life of 195 min was ultimately calculated and complete elimination occurred after 485 min. Estrone exhibited a more linear degradation that resulted in 50% and 100% dissipation in 68 and 360 min, respectively. Moreover, although not stated overtly, experimental controls indicated no hydrolysis occurred during the study. Temperature imparts no impact on 17β-estradiol photolysis from 0°C to 75°C (Kimura et al., 2004).

1.5.2. Sorption

Sorption represents a principle mechanism controlling contaminant mobility in natural systems. Research has begun examining this phenomenon in terms of estrogen removal from solution, but most studies only consider wastewater removal techniques, including partitioning by synthetic membranes (Chang et al., 2003; Nghiem et al., 2004;

Yamamoto and Liljestrand, 2004; Yoon et al., 2004), reverse osmosis (Schafer et al., 2003) and activated carbon (Chang et al., 2004; Yoon et al., 2003). A number of studies have also focused on the sorption of steroids to river sediments and aquifer materials (Holthaus et al., 2002; Lai et al., 2000; Ying et al., 2003; Yu et al., 2004).

Despite growing interest in the chemical behavior of manure-borne estrogens, research considering interactions of natural hormones with agricultural soils remains limited. Casey et al. (2003; 2005) investigated agricultural soils of mixed taxonomy and found sorption affinity correlated with organic matter, along with surface area and cation-exchange capacity, and demonstrated estrogens do not readily leach. Lee et al. (2003) also determined 17β-estradiol would undergo limited leaching and concluded runoff presented the most likely input to surface waters.

Specific sorption processes have yet to be identified, but organic carbon appears to provide a primary binding mechanism (Lee et al., 2003). The hydrophobic nature of estrone and 17β-estradiol likely promotes an affinity toward organic matter, yielding high K_{OC} values. Although estrogen sorption correlates strongly with soil organic matter content, this fails to preclude the existence of alternative sorption processes. Binding sites associated with clay particles likely contribute to the sorption of these hormones, as estrogen adsorption can correlate with clay content. In one instance the interaction between 17β-estradiol and fine sediments exceeded the correlation with organic carbon (Holthaus et al., 2002). Lai et al. (2000) suggest the importance of iron oxides as a binding mechanism, where ion exchange between the oxide hydroxyl group and the polar estrogen phenolic group regulates adsorption. Similarly, Casey et al. (2003) found

a correlation between 17β-estradiol sorption and soil cation exchange capacity (CEC) that was assumed to be associated with clay; however, this may be an artifact of organic material also exhibiting high CEC as estrogenic phenolic groups will form organic anions under basic conditions, but will never form cations (Lee et al., 2003).

A number of other soil properties and environmental factors likely contribute to the partitioning of estrogenic agents into a solid phase. One such instance includes the influence of salinity on binding capability. Looking at river waters, higher ionic strength solutions led to greater estrogen sorption; concurrently, elevated salt concentrations also enhance particle aggregation and flocculation (Lai et al., 2000). The coupling of these two phenomena likely promotes sedimentation of hormones out of the water column.

1.5.3. Microbial Degradation

An early investigation into the degradation of steroidal compounds by Turfitt (1947) examined the potential breakdown of estrone and 17α-estradiol by microorganisms indigenous to five disparate soil types. Isolated mold and bacterial strains cultured with the hormonal compounds provided as an energy source revealed no microflora associated with loam, marl or alkaline peat soils could metabolize estrone or estradiol. However, a strain of the bacterial genus *Proactinomyces* isolated from an arable soil degraded estrone, while strains of *Proactinomyces spp.* originating from the arable and acid soils metabolized estradiol. A strain of the gram-negative bacterium genus *Novosphingobium* isolated from activated sludge similarly decomposes 17β-estradiol (Fujii et al., 2002).

The prevalent view in the literature advocates a relatively rapid transformation of both estrone and 17β-estradiol in aquatic, terrestrial and sewage treatment systems (Casey et al., 2003; Jurgens et al., 2002; Lee et al., 2003; Ying et al., 2002; Ying et al., 2004). For instance, investigations of degradation rates in aquifer material found 17β-estradiol degraded rapidly, exhibiting a half-life of 2 d (Ying et al., 2003). Similarly, sewage bacteria rapidly transformed 17β-estradiol in municipal sewage effluent, with experiments showing complete removal of 17β-estradiol within 18 h (Lee and Liu, 2002).

Analysis of complete degradation (i.e., mineralization) reveals estrogens undergo a less aggressive response by microbial populations than simple transformation in soil systems, a salient point since certain steroidal metabolites continue to exhibit estrogenic activity. Application of estrone and 17β-estradiol to agricultural soils demonstrated both compounds formed non-extractable residues that only mineralized slowly (Colucci et al., 2001). A follow-up study conducted at more environmentally relevant concentrations yielded consistent results (Colucci and Topp, 2002). Jacobsen et al. (2005) found 17β-estradiol degraded rapidly, but mineralization similarly progressed slowly in soils receiving swine manure. Understanding estrogen behavior requires distinguishing between dissipation and complete elimination from a system.

Little information exists on environmental factors promoting or inhibiting estrogen degradation. Joss (2004) found estrone degraded slower under anaerobic conditions while 17β-estradiol exhibited high rates regardless, but the influence of oxidation-reduction conditions remains ambiguous. Factors such as soil type, nutrient

levels, pH and other recognized variables impacting microbial activity have gone largely neglected.

1.5.4. Modeling

Increased emphasis on determining estrogen fate and transport parameters provides more information on the potential persistence and mobility of these hormones in the environment. Data incorporation into predictive models presents the next step in developing a robust risk assessment investigating potential adverse health effects to human and wildlife populations. The recent recognition of problems associated with steroid contamination, however, largely hinders extrapolation of laboratory results to field settings. Even soil column studies designed to produce breakthrough curves for accompanying model simulation remain virtually nonexistent.

First modeling attempts looked at radiolabeled 17β-estradiol movement through columns using a loam or sand packing medium (Larsen et al., 2001). The loam soil prevented any estrogenic elution subsequent to displacement by 10 pore volumes, with most radioactivity accounted for in the upper levels of the column. In contrast, the sand matrix rapidly flushed 17β-estradiol, yielding 85% recovery in the effluent after 1.06 pore volumes. Although strongly bound by the loam soil, column K_d values were less than those found in a concurrent batch equilibrium study.

A follow-up project examining 17β-estradiol mobility in a column setting coupled results with the HYDRUS-1D transport model (Casey et al., 2003). Inclusion of sorption and transformation processes into the model allowed for good description of

breakthrough curves in five disparate soil types using the inverse modeling technique, but a number of limitations remained. Once more, sorption parameters failed to agree with those found in batch equilibrium experiments. In addition, analysis involved large confidence intervals for estimated parameters, with hydrodynamic dispersivities ranging from 3.6 to over 60 cm for a 15.2 cm long column. These disadvantages were largely corrected in a subsequent study (Casey et al., 2005). Using rate-limited sorption parameters, column and batch equilibrium results agreed, but needs for further degradation elucidation remain.

The other primary estrogen modeling publication to date also uses HYDRUS-1D (Das et al., 2004). Both pulse and flow interruption conditions imposed on columns strove to describe 17β-estradiol transport by forward modeling and to identify independent degradation estimates, respectively. Although this approach provided sufficient simulation efficacy, the authors noted a need for models that better explain compound transformation. It became evident the complex microbial degradation mechanism requires representation beyond a simple first-order kinetic process.

Clearly the rudimentary modeling conducted to date exhibits limitations in explaining laboratory studies. Although future endeavors should strive to accurately simulate estrogen fate and transport in the field, much research remains prior to transferring data into large scale models.

1.6. CONCLUSIONS

The estrogenic compounds estrone and 17β-estradiol can exert harmful health effects in humans and wildlife when in excess, despite their necessity in vertebrate development. In addition to municipal wastewater treatment plants, livestock production presents a significant environmental load of these hormones. Consequently, elevated off-site estrogen concentrations associated with AFOs have occurred in both surface and groundwater systems (Dyer et al., 2001; Peterson et al., 2000; Soto et al., 2004). Exposure pathways arising from the agricultural land application of estrogen-laden manures depend on the ultimate fate and transport of these compounds. Initial investigation indicates strong association of estrone and 17β-estradiol with soils and rapid degradation by photolytic and microbial processes; however, much ambiguity exists and conflicting studies abound. Elucidation of estrogen chemical behavior will not only allow for more rigorous environmental risk assessments through more accurate modeling, but will also aid in development of agricultural best management practices (BMPs).

2. ESTRONE AND 17β-ESTRADIOL PHOTOLYSIS IN AQUEOUS SOLUTION

2.1. INTRODUCTION

Increased awareness of environmental and human health impacts due to steroidal estrogens such as estrone and 17β-estradiol has led to concerns over surface and groundwater contamination. Research on the possible effects from estrogenic exposure continues; however, these bioactive agents appear to accentuate many developmental and reproductive disorders. Sumpter and Jobling (1995) documented feminization of male fish maintained in municipal wastewater treatment effluent. Many ensuing studies found similar characteristics in numerous fish species (Folmar et al., 2000; Higashitani et al., 2003; Orlando et al., 2004; Van den Belt et al., 2003), amphibians (Kloas et al., 1999; Sone et al., 2004) and reptiles (Irwin et al., 2001). Conclusive cause-effect relationships between environmental exposure and adverse physiological impacts are difficult to establish in humans due to a plethora of confounding variables, however potential links to breast cancer and male infertility exist (Carlsen et al., 1995; Dickson et al., 1986; Harrison et al., 1997).

A transition towards larger, more concentrated animal feeding operations (AFOs) has magnified problems related with managing animal manure. Although excessive nutrient and pathogen levels in process generated wastewater receive attention, potentially elevated estrogen concentrations in these waste-streams has recently spawned increased concern over hormonal contamination. For instance, pregnant milk cows

excrete approximately 170 mg of estrogen daily (Hanselman et al., 2003) which can lead to manure slurries exhibiting concentrations up to 640 and 1230 $\mu g \ L^{-1}$ for estrone and 17β-estradiol, respectively (Shore and Shemesh, 2003). Furthermore, estrogen supplements may be given to cattle to improve animal performance (Beconi et al., 1995; Enright et al., 1990; Henricks et al., 1997; Herschler et al., 1995; Popp et al., 1997), potentially raising urinary 17β-estradiol levels six-fold (Callantine et al., 1961). Although subsequent dilution occurs due to flushing, dairy lagoons and holding ponds still can maintain 17β-estradiol concentrations in the neighborhood of 2 $\mu g \ L^{-1}$ (Raman et al., 2004; Williams et al., 2002). Considering reports that waters receiving cattle feedlot effluent imparted detrimental health effects to aquatic life at 17β-estradiol concentrations as low as 1 $ng \ L^{-1}$, risk assessments require a better understanding of estrogenic behavior in AFO wastestreams prior to release into the environment.

Photolysis of organic compounds often remains neglected in studies investigating contaminant persistence. However, this mechanism can contribute significantly to pollutant degradation under certain conditions. Direct photolysis involves photon energy cleaving covalent bonds when compounds absorb light, a process that transforms the molecular structure (Schwarzenbach et al., 1993). Photolysis can occur on soil surfaces, but decomposition rates can be substantially less than in aqueous systems and diminish with decreasing soil moisture (Curran et al., 1992).

Most researchers have considered photochemical transformation of estrogenic compounds in association with a catalyst and have focused on removal strategies from municipal wastewater streams. For instance, photodegradation of 17β-estradiol in

conjunction with a titanium dioxide (TiO_2) catalyst displayed 99% degradation after a 30-min UV exposure (Ohko et al., 2002), while Nakashima et al. (2002) found 98% 17β-estradiol decomposition after 1 hr in a reactor fitted with TiO_2-modified mesh sheets and black fluorescent lamp. In the absence of a catalyst, half-lives calculated for estrone and 17β-estradiol approached 10 and 40 min, respectively, under a UV-lamp, but estrone displayed a half-life of about 50 min with only minimal 17β-estradiol degradation under a UV-Vis-light (Liu and Liu, 2004). This is likely due to differences in light wavelengths, as Rosenfeldt and Linden (2004) determined a 17β-estradiol absorbance maxima at about 280 nm while the UV-Vis-light reached a mechanism at 365 nm. Temperature imparted no impact on 17β-estradiol photolysis from 0°C to 75°C (Kimura et al., 2004).

Although most research to date has focused on municipal wastewater treatment as an estrogen source, AFOs clearly pose another potential contributor of hormonal contamination to natural waters. The lack of data under natural conditions and in livestock waste streams necessitates deeper examination of this mechanism. Therefore, the objectives of this study were to quantify photolysis rates across a number of pH conditions. This will act as a first step toward analyzing estrogen photodegradation in a livestock waste-stream context. The resulting information will not only aid in determining the persistence of these bioactive compounds, but also may aid in optimization of manure handling techniques to diminish associated risks.

2.2. MATERIALS AND METHODS

Estrone (99% pure) and 17β-estradiol (98% pure) were purchased from Sigma-Aldrich (St. Louis, MO) and solutions of the respective estrogens dissolved in high performance-grade methanol (EMD Chemicals, Gibbstown, NJ) were added to deionized water to obtain 10 µg mL^{-1} concentrations with 1% methanol content. These solutions were adjusted to pH 4, 7 and 9 using HCl and NaOH. Samples were placed in 1-mL borosilicate glass vials, capped and subjected to irradiation a distance of 12-cm below the light source. Two 100-W UV lamps (F72T12/VHO 5.0 Midday sun) simulated the UV portion of sunlight emission within a growth chamber devoid of external light. Estrone or 17β-estradiol concentrations were quantified after irradiation times of 0, 0.5, 1, 1.5, 2, 3, 6 and 12 h with a high performance liquid chromatography HPLC equipped with a photodiode array detector (Waters, Inc., Milford, MA). A dark control for 17β-estradiol was included to check for chemical hydrolysis. A Waters$^{®}$ Symmetryshield™ RP8 3.5-µm particle size 2.1 x 150 mm column (Waters, Inc., Milford, MA) with a mobile phase of 60% deionized water, 39% acetonitrile and 1% formic acid was used for chemical analysis.

The natural log of each estrogen concentration with respect to the initial concentration [ln (C/Co)] was calculated and plotted for each time period. The photolysis half-life (t$_{1/2}$) describes the time for which a concentration diminishes by half due to light induced reactions, such that:

$$t_{1/2} = \frac{\ln(2)}{k} \qquad (2.1)$$

where k represents the first-order rate constant and is determined by the absolute value of slope, assuming the reaction follows first-order degradation.

2.3. RESULTS AND DISCUSSION

Estrone exhibited a faster photolysis rate than 17β-estradiol across all three pH conditions tested (Figure 2.1). The greater susceptibility to photodegradation by estrone corresponds to other studies (Coleman et al., 2004; Liu and Liu, 2004). Although the actual rates found here differed from Liu and Liu, estrone degradation in both studies was about 33% faster than 17β-estradiol at pH 7.

Additional peaks failed to develop on the chromatograph following irradiation at the absorbance analyzed. This finding concurs with Liu and Liu (2004) who demonstrated benzene ring breakage led to the formation of carbonyl-containing compounds following estrogen irradiation. Since several estrone and 17β-estradiol breakdown products maintain estrogenic activity, albeit to a lesser degree, the oxidation of the basic chemical structure likely indicates elimination of the endocrine disrupting potential for these hormones subsequent to photolysis. The estrone concentration profile determined at pH 4 has been included for reference (Figure 2.2).
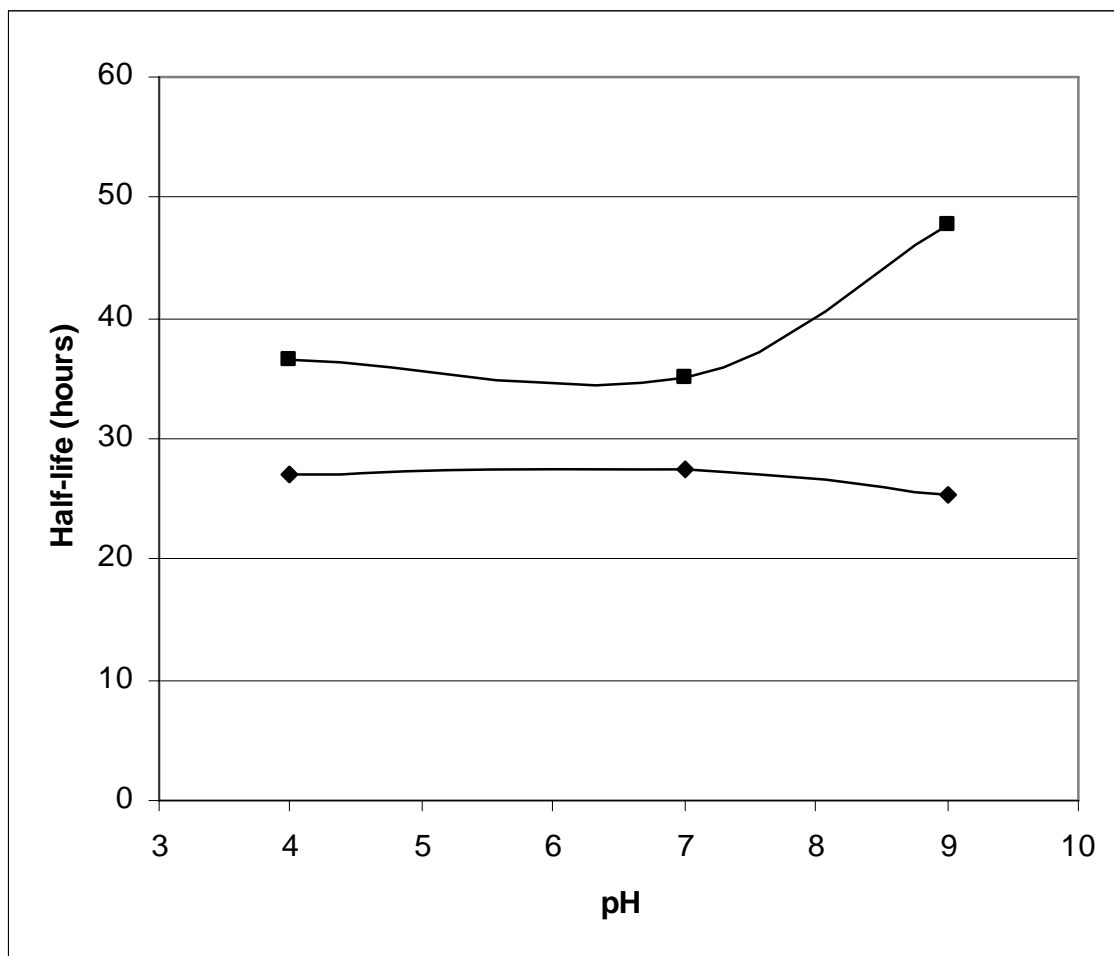
Fig. 2.1. Photolysis half-lives for estrone (♦) and 17β-estradiol (■) in aqueous solutions of varying pH.
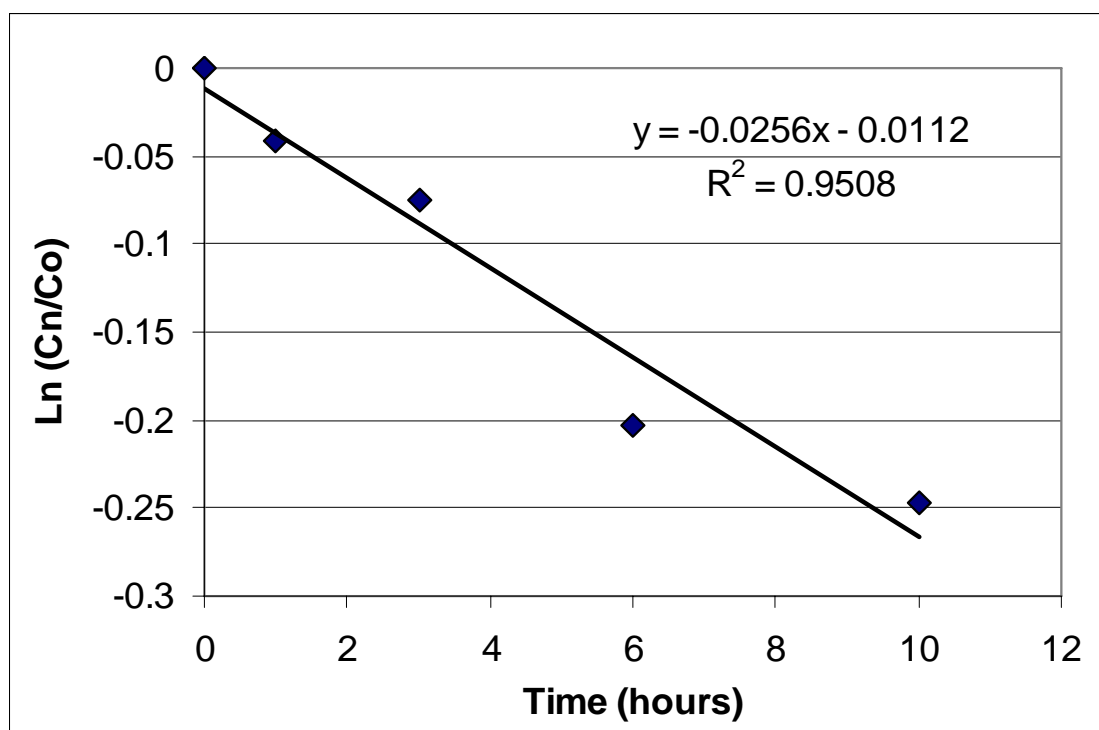
Fig. 2.2. Estrone concentration profile at pH 4 during irradiation with UV-light.

The use of photocatalysts and a wide variety of light sources in estrogen photolysis experiments complicates comparisons to other studies. As mentioned earlier, half-lives on the order of minutes dominate the literature rather than hours as found here. Coleman et al. (2004) found that minimal 17β-estradiol photolysis occurred in the presence of UVA light (UVA spectrum represents long-wave UV radiation spanning 315-380 nm) over the first 160 min, after which the concentration decreased to yield a 195 min half-life and a complete elimination by 485 min. Estrone exhibited a more linear degradation that resulted in 50% and 100% dissipation in 68 and 360 min, respectively. Although photolysis was still faster than found here, this study demonstrates the range of half-lives and chemical behavior determined to date. The slow degradation rates found here may be an artifact of the light source, as the wavelengths generated by the UV-lamps exceeded 310 nm and displayed a maximum near 340 nm. Since these wavelengths lie considerably above the 288-nm peak absorbance for 17β-estradiol, the slow photodegradation rates are resonable and are supported by the negligible 17β-estradiol degradation found by Liu and Liu (2004).

Estrone displayed no pH dependent degradation rate, whereas 17β-estradiol half-lives decreased slightly from pH 4 to 7 and then increased at pH 9. This trend differed from that shown by Liu and Liu (2004) where both estrone and 17β-estradiol had a similar pH dependence. In that case, photolysis decreased from pH 2 to 5 and subsequently increased to pH 8. Although Coleman (2000) only examined 17β-estradiol, the pH dependence corresponded well with the pattern here since pH adjustments led to increased photolysis from pH 3 to 7, followed by a decrease to pH 10.

A rapid intensification then occurred moving toward pH 12 where photolysis was 3-fold greater than at pH 7, a phenomenon attributed to hydroxyl radical generation associated with high pH levels.

The hydrolysis of 17β-estradiol was negligible in this study as determined by the dark control. The 17β-estradiol half-life at pH 7 exceeded 1385 h (~ 58 d), and showed no degradation in the pH 4 solution. Coleman et al. (2004) indicated no hydrolysis in dark controls.

These findings suggest moderate photodegradation rates under the conditions tested. For comparison, the herbicide imazethapyr exhibited a 4-h half-life in aqueous solution (Curran et al., 1992). Even if more rapid photolysis occurs under a light emitting a broader wavelength, the high organic matter inherent to anaerobic lagoon water presents a parameter that may influence estrogen degradation during treatment. The associated turbidity will inhibit light penetration and restrict the photo-reactive zone to the lagoon surface. On the other hand, indirect photolysis may occur where energy transfers from one excited species to another, a process that may abound in AFOs waste streams.

Photodegradation following land application may present an additional mechanism that warrants consideration when determining the fate of estrone and 17β-estradiol. Anaerobic lagoon effluent applied via center-pivot irrigation will expose estrogens previously stored below the photo-reactive zone that withstood photolysis, chemical degradation and microbial transformation. Direct sunlight will react with some of the molecules on the soil surface, but exposure duration may vary greatly. Following

land application of animal manures, estrogenic compounds will migrate into the soil profile. Diffusion coefficients for estrone and 17β-estradiol are not available, but pesticide values range from 0.05 to 50 mm^2 d^{-1} (Katagi, 2004). The small photic depth in soil will limit light penetration to approximately 1.5 mm, with 95% attenuation rate at 0.5 mm (Frank et al., 2002). Therefore, photodegradation at the soil surface may play a key role in estrogen degradation prior to migration into the soil profile depending on site-specific conditions.

2.4. CONCLUSIONS

Half-lives determined for estrone and 17β-estradiol photolysis indicated moderate photodegradation under UV-light, with estrone exhibiting slightly faster decomposition. The limited wavelength spectrum emitted by the light source may have resulted in underestimates for the degradation rates, as the maximum light absorption for estrogens exists below the range available for analysis. Estrone displayed no significant pH dependent reaction rate, but 17β-estradiol photolysis decreased from pH 7 to 9. Appreciable hydrolysis was not detected for 17β-estradiol.

3. SORPTION-DESORPTION BEHAVIOR OF ESTRONE AND 17β-ESTRADIOL IN AGRICULTURAL SOILS

3.1. INTRODUCTION

Hormonally active agents represent a class of chemicals that interact with the endocrine system, potentially impairing cell proliferation and differentiation. Endocrine disruption may contribute to infertility (Nash et al., 2004), developmental abnormalities (Migliaccio et al., 1995) or hormone-related cancer (Jones and Hajek, 1995; Lai et al., 2002) in humans and wildlife. Although endogenous, natural estrogens such as estrone and 17β-estradiol can similarly impact vertebrate growth and development at concentrations as low as 1.0 ng L^{-1} (Irwin et al., 2001). Detrimental effects induced by excessive natural hormones include altered sex ratios and sex reversals in fish (Lai et al., 2002; Nakamura, 1984), premature udder development and lactation in heifers (Ball et al., 2000) and reproductive disorders in wildlife (Crews et al., 1995; Routledge et al., 1998; Sone et al., 2004; Sumpter and Jobling, 1995). Evidence links these compounds to breast cancer (Dickson et al., 1986), while reduced sperm counts and increased incidents of testicular cancer in humans may be induced by environmental estrogen exposure (Carlsen et al., 1995).

Land application of manure originating from animal feeding operations (AFOs) presents an environmental estrogen load that may result in regional surface or ground water concentrations significantly above ambient levels. Excreted steroidal compounds have been quantified in livestock (Mathur and Common, 1969) and poultry (Erb et al.,

1977) wastes since the late 1960's, but environmental impacts continued unrecognized for years. Recent evidence suggests estrone and 17β-estradiol may migrate off-site following manure application to agricultural soils. Raman et al. (2004) found estrone concentrations ranged up to 10^5 ng L^{-1} in various dairy and swine waste management systems, while Nichols et al. (1998) measured 17β-estradiol runoff concentrations of 3,500 ng L^{-1} from fields receiving poultry litter. Similarly, confirmation that local cattle and poultry operations generated elevated 17β-estradiol concentrations in karst aquifers indicates AFOs can contaminate groundwater supplies (Peterson et al., 2000).

Initial studies indicate hormonal agents associated with livestock waste can promote detrimental health effects in wildlife. Female painted turtles (*Chrysemys picta*) residing in ponds that received runoff from beef cattle pastures exhibited greater vitellogenin synthesis, a precursor to egg yolk proteins in non-mammalian vertebrates (Irwin et al., 2001). Higher vitellogenisis, a process regulated by estrogens, may subsequently modify reproductive fitness by shifting energy allocations within individuals and altering egg production. Wild male fathead minnows (*Pimephales promelas*) exposed to cattle feedlot effluent displayed diminished testosterone synthesis, altered head morphology and decreased testis size, denoting demasculinization (Orlando et al., 2004). Concern over hormonal exposure to human and wildlife populations warrants further research on the fate and transport of estrogenic chemicals following land application of animal wastes.

Sorption represents a principle mechanism controlling contaminant mobility in natural systems. Research has begun examining this phenomenon, but a preponderance

of studies consider wastewater removal techniques, including partitioning by synthetic membranes (Chang et al., 2003; Nghiem et al., 2004; Yamamoto and Liljestrand, 2004; Yoon et al., 2004), reverse osmosis (Schafer et al., 2003) and activated carbon (Chang et al., 2004; Yoon et al., 2003). A number of studies have focused on the sorption of selected steroids to river sediments and aquifer materials (Holthaus et al., 2002; Lai et al., 2000; Ying et al., 2003; Yu et al., 2004). Organic carbon appears to provide a strong binding mechanism for estrogenic compounds in sediments, while associations with iron oxides and small-sized particles also occur. Despite growing interest in the chemical behavior of manure-borne estrogens, research considering interactions of natural hormones with agricultural soils remains limited. Casey et al. (2003; 2005) investigated agricultural soils of mixed taxonomy and found sorption affinity correlated with organic matter, surface area and cation-exchange capacity; column studies subsequently demonstrated limited estrogen leaching. Lee et al. (2003) also suggested restricted 17β-estradiol leaching and concluded runoff presented the most likely input to surface waters.

An understanding of the sorption behavior of estrone and 17β-estradiol across a spectrum of agricultural soils remains vital in determining the potential mobility of estrogenic compounds following land disposal of livestock manure and wastewater. Furthermore, desorption parameters present an important consideration when modeling potential chemical migration, as future release of these compounds from a soil matrix would lead to unforeseen contamination of waterbodies. Consequentially, knowledge of sorption-desorption dynamics remains critical to fully assess estrogen fate and transport

in agricultural soils. Therefore, the objectives of this study were to (1) quantify estrone and 17β-estradiol sorption parameters for soils representative of those that may receive manure applications, and (2) measure the corresponding desorption parameters, an aspect not explicitly considered to date.

3.2. MATERIALS AND METHODS

3.2.1. Soils

Three agricultural soils exhibiting disparate texture and physicochemical properties were selected to determine sorption characteristics for estrone and 17β-estradiol (Table 3.1). Samples of Tremona loamy fine sand (thermic Aquic Arenic Palenstalfs), Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) and Houston Black clay (fine, smectitic thermic Udic Haplusterts) were collected from the top 5 cm of the soil profile. These soils had no known history of receiving estrogenic amendments. The soil samples were air dried at room temperature and subsequently passed through a 2-mm sieve.

Chemical properties for estrone and 17β-estradiol can be found in Table 3.2. To achieve the analytical detection limits required when using low estrogenic concentrations typical in the environment, ^{14}C-labeled estrone and 17β-estradiol were used in conjunction with non-radioactive technical-grade chemicals. Stock solutions consisted of [4-^{14}C]-estrone (2035 MBq mmol^{-1}) and [4-^{14}C]17β-estradiol (2035 MBq mmol^{-1}) purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO).

Table 3.1. Properties of soils used for estrone and 17β-estradiol adsorption-desorption experiments.

Soil	Textural class	Sand	Silt	Clay	Organic matter	Organic carbon[†]	pH
				%			
Tremona	Loamy fine sand	81	10	9	0.73	0.42	7.0
Weswood	Silty clay loam	11	68	31	1.82	1.05	8.1
Houston black clay	Clay	19	38	43	2.60	1.51	8.1

†Percentage of organic carbon (OC) based on percentage organic matter (OM): %OC = 0.58 × OM (Weber et al., 2004).

3.2.2. Chemicals

Parent solutions were prepared in high performance-grade methanol to achieve 3.33×10^5 disintegrations min^{-1} mL^{-1}. The chemical purity of the non-labeled technical-grade estrone and 17β-estradiol was 99% and 98%, respectively. Apposite amounts of the radioactive and technical-grade parent solutions were diluted in 0.01 *M* $CaCl_2$/methanol (99:1 v/v) to produce 0.005, 0.01, 0.03, 0.05 and 0.1 µg mL^{-1} batch solutions. These concentrations were selected because they represent environmentally relevant concentrations associated with land application of animal manures (Colucci and Topp, 2002; Finlay-Moore et al., 2000; Nichols et al., 1998).

Table 3.2. Selected physicochemical properties of estrone and 17β-estradiol (Hanselman et al., 2003; Lai et al., 2000).[†]

Compound	MW	S_W	Vapor pressure	log K_{OW}	pK_a
	(g mol^{-1})	(mg L^{-1})	(Pa)		
Estrone	270.4	13	3×10^{-8}	3.1-3.4	10.3-10.8
17β-estradiol	272.4	13	3×10^{-8}	3.1-4.0	10.5-10.7

[†] MW – molecular weight, S_W – solubility in water, K_{OW} – octanol-water partition coefficient, K_a – acid ionization constant.

3.2.3. Adsorption

The batch equilibrium method was used to characterize the adsorption isotherms for estrone and 17β-estradiol at 22 ± 2°C. A 5-mL aliquot of each batch solution was added to 1 g of soil to obtain a 5:1 water to soil ratio in a 50-mL glass centrifuge tube. Slurries were placed on a reciprocal shaker for 72 h in order to reach equilibrium, which was determined through preliminary experimentation (data not shown). The slurries were subsequently centrifuged for 20 min at 2000 x g. Three milliliters of supernatant were drawn from the vials and mixed with 3 mL of Ecolte (+) liquid scintillation cocktail. A Beckman 6500 liquid scintillation spectrometer operated in the auto-disintegrations per minute mode was used to analyze ^{14}C content in each sample, correcting for counting efficiency. The calculated difference between the supernatant concentration and the initial estrogenic compound content indicated the amount of estrone and 17β-estradiol adsorbed. Four replicates were used for each treatment.

3.2.4. Desorption

Desorption was examined in conjunction with adsorption at the 0.1 and 0.03 μg mL^{-1} concentrations. Three milliliters of 0.01 M CaCl$_2$ solution was added back into the vials which were placed back on the reciprocal shaker for 24 h to disperse the soil pellets. The vials were then centrifuged for 20 min at 2000 x g and the same analytical procedure described for the adsorption study was followed. The desorption process was repeated three times to yield four desorption observations. The amount of estrone and 17β-estradiol remaining sorbed to the soil was calculated as the difference between the supernatant concentration at each stage and the remaining total compound content determined after subtracting the amount of chemical removed.

3.2.5. Isotherm Analysis

Adsorption and desorption isotherm parameters were determined using the linear form of the Freundlich equation:

$$\log C_S = \log K_F \times 1/n \log C_W \qquad (3.1)$$

where C_S is the amount of estrogenic compound adsorbed per mass soil (μg g^{-1}), C_W is the concentration in solution (μg mL^{-1}) and K_F and $1/n$ are empirical constants. K_F represents the Freundlich constant, whereas $1/n$ indicates adsorption linearity (i.e., sorption intensity) as a function of solution concentration (Socias-Viciana et al., 1999). A value of $1/n = 1$ designates a linear isotherm, while $1/n > 1$ signifies compound adsorption increases without limit and $1/n < 1$ signifies adsorption approaches a limit.

Hereafter, K_{Fads} and n_{ads} refer to adsorption coefficients and K_{Fdes} and n_{des} refer to desorption coefficients.

Adsorption distribution coefficients (K_d) indicating the partition between the solid and liquid phases were determined by dividing the adsorbed concentration by the solution concentration, such that:

$$K_d = C_S / C_W \tag{3.2}$$

Distribution coefficients were calculated for each solution equilibrium concentration and then averaged across all concentrations to yield a single estimate for K_d. Adsorption was normalized to the organic carbon (OC) content of the soil (K_{OC}) using the equation:

$$K_{OC} = (K_d/\%OC) \times 100 \tag{3.3}$$

Hysteresis was quantified using the expression described by (Ma et al., 1993):

$$\omega = \{[(1/n_{ads})/(1/n_{des})] - 1\} \times 100 \tag{3.4}$$

which is based on the difference between the adsorption and desorption isotherms. Regression analysis was also performed on the adsorption and desorption isotherms using StatView 5.0.1 (SAS Institute, 1998) to assess how well the linear model explained data variability.

3.3. RESULTS AND DISCUSSION

3.3.1. Equilibrium Determination

The estrogenic compounds in this study are relatively nonvolatile and moderately hydrophobic (Table 3.2) and therefore were expected to associate predominantly with the soil fraction of the soil-water slurry. However, the rates at which estrone and 17β-

estradiol reach equilibrium in batch equilibrium studies vary and require examination for different media prior to analysis. A 72 h period was required to reach equilibrium in this study. A multi-day attainment of a sorption equilibrium was supported by Holthaus et al. (2002) and Casey et al. (2003) who found that equilibrium was not reached within 48 h for river sediments and soil, respectively. Similarly, Yu et al. (2004) observed equilibrium required anywhere between 2 and 14 d depending on estrogen concentration relative to the solubility limit. In contrast, maximum estrogen sorption to river and estuary sediments occurred after 1 h of shaking (Lai et al., 2000). Equilibrium periods of 24 h or less have also been reported for these compounds (Yamamoto et al., 2003; Ying et al., 2003).

Soil material has a spectrum of binding site types, each exhibiting discrete equilibrium rates that contribute to chemical equilibration for a soil. This heterogeneity may therefore create the disparity in equilibrium times observed between this and prior studies. Furthermore, Holthaus et al. (2002) assert a true equilibrium can not be achieved by labile compounds such as estrone and 17β-estradiol in a biologically active system. Therefore, practical considerations dictate the equilibrium period used during sorption experiments, but necessitate notation when comparing studies.

3.3.2. Adsorption

The adsorption isotherms for estrone and 17β-estradiol generally followed linear behavior over the concentration ranges considered (Figures 3.1).
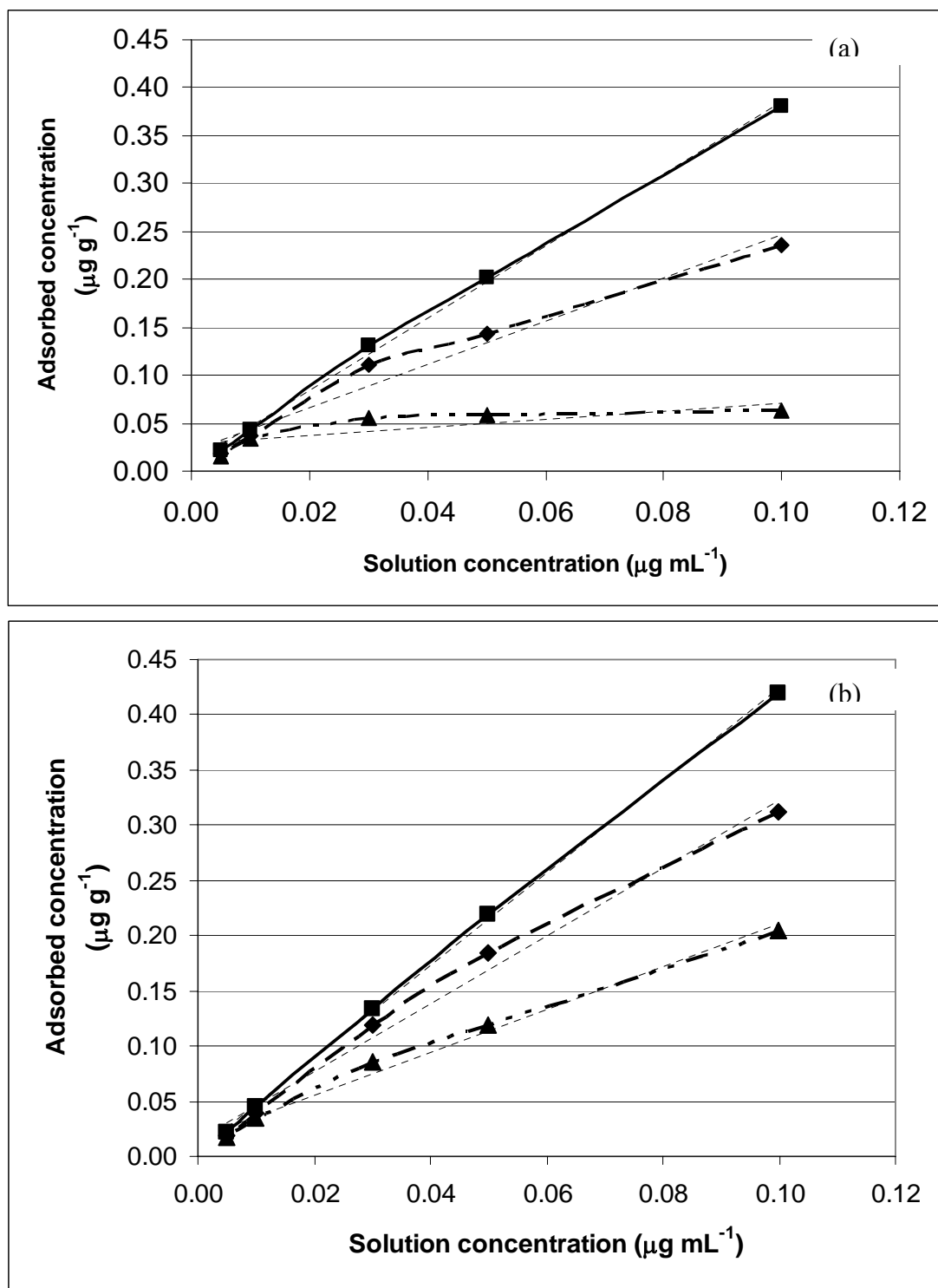
Fig. 3.1. Sorption isotherms for (a) estrone and (b) 17β-estradiol in Tremona loamy fine sand (▲), Weswood silty clay loam (♦) and Houston Black clay (■).

Linear distribution coefficients reveal moderate sorption to the selected soils, with the degree of sorption increasing from loamy fine sand to silty clay loam to clay (Table 3.3). The relationship determined through regression analysis was strong, as most r^2 values equaled or approximated 1.0 with the only exception being the estrone-loamy fine sand treatment. Standard deviations were greater for the Tremona loamy fine sand compared to the Houston Black clay.   The K_d values for 17β-estradiol corresponded with those determined for soils containing 0.22% organic carbon content (K_d = 3.6 ml g^{-1}), but were less than the K_d = 83.2 ml g^{-1} value associated with earlier work by Lee et al. (2003) on soils exhibiting 2.91% organic carbon.

Table 3.3. Linear distribution coefficients (K_d), soil adsorption coefficients normalized for organic carbon (K_{OC}) and the coefficient of determination (r^2) for the linear isotherm fit for estrone and 17β-estradiol in selected agricultural soils. [†]

Soil series	K_d	K_{OC}	r^2
	--------------- mL g^{-1} ---------------		
	Estrone		
Tremona loamy fine sand	2.02 (1.10)	481.46 (261.73)	0.51
Weswood silty clay loam	3.24 (0.56)	308.69 (53.11)	0.96
Houston Black clay	4.18 (0.24)	277.12 (15.71)	1.00
	17β-Estradiol		
Tremona loamy fine sand	2.90 (0.63)	690.01 (150.99)	0.98
Weswood silty clay loam	3.70 (0.33)	352.30 (31.73)	0.99
Houston Black clay	4.39 (0.12)	290.74 (8.04)	1.00

[†]**Values in parentheses represent standard deviations.**

The K_{OC} values indicated a strong association of estrone and 17β-estradiol with organic carbon (Table 3.3). The most pronounced affinity for organic matter occurred in the Tremona loamy fine sand, which has only 10% silt and 9% clay composition. The preponderance of coarse textured particles suggests that estrogen sorption depends on binding to organic matter in this soil, whereas alternative binding sites may exist in the other soils exhibiting greater clay content in addition to organic matter. The K_{OC} values obtained here fell within the lower range of values found in other studies. Holthaus et al. (2002) determined K_{OC} values for 17β-estradiol ranged from 212 to 1,614 mL g^{-1} in sediments, but Yu et al. (2004) obtained values up to three orders of magnitude greater for both estrone and 17β-estradiol in an uncharacterized topsoil. Strong correlations between estrogen sorption and organic carbon content have been found for both sediments (Lai et al., 2000) and soils (Lee et al., 2003).

Where the Freundlich model provides a 1/n value approaching 1, data were analyzed for Freundlich isotherm parameters to provide a more accurate description. Values for K_{Fads} and 1/n are summarized for estrone and 17β-estradiol in Table 3.4. Freundlich isotherms achieved essentially perfect fits for all soil-compound treatments except for estrone in the Tremona loamy fine sand.

Although the linear model accurately describes most combinations of estrogen and soil, the loamy fine sand and silty clay loam soils exhibited Freundlich 1/n values less than 1.0 which signifies a finite number of specific binding sites or a potentially high concentration. On the contrary, the Houston Black clay displayed 1/n values that approximated 1.0 for both estrone and 17β-estradiol; this may indicate cooperative

interactions between the clay surface and other sorbed organic species which promote a stabilized binding surface (O'Loughlin et al., 2000).

The K_{Fads} and 1/n parameters obtained for estrone and 17β-estradiol agreed reasonably with published values (Table 3.5). A salient consideration when comparing partition coefficients between studies is that these are operational parameters restricted to describing a specific system. Many factors influence these parameters. For instance, if the soils used generate significant colloidal material compared to those in other studies, the distribution coefficients could be artificially lowered because adsorbed molecules associated with colloids might be removed from the system and considered part of the aqueous phase when actually in a sorbed phase. Yamamoto and Liljestrand (2003) found 17β-estradiol K_{OC} values on the order of 10^5 for fulvic, humic and tannic acids, respectively, indicating a high affinity to colloidal organic matter. More likely, differences in soil characteristics may result in adsorption differences due to variations in organic carbon content, clay composition and particle size.

Table 3.4. Freundlich adsorption isotherm parameters for estrone and 17β-estradiol in selected agricultural soils.

Soil series	K_{Fads}	1/n	r^2
	$(\mu g^{1-1/n}\, mL^{1/n}\, g^{-1})$		
	Estrone		
Tremona loamy fine sand	0.50	0.44	0.75
Weswood silty clay loam	1.33	0.8	0.99
Houston Black clay	1.74	0.96	1.00
	17β-estradiol		
Tremona loamy fine sand	1.16	0.82	0.99
Weswood silty clay loam	1.60	0.94	1.00
Houston Black clay	1.85	0.98	1.00

The effects of different soil parameters on adsorption could not be distinguished by the analysis conducted; however, correlation with organic matter content suggests binding with organic carbon acts as a key estrogen adsorption mechanism (Lee et al., 2003). The hydrophobic nature of estrone and 17β-estradiol promotes association with organic matter, yielding the high K_{OC} values shown in Table 3.3. Although estrone and 17β-estradiol sorption correlates with soil organic matter content, this does not preclude the existence of alternative sorption processes. Binding sites associated with clay particles likely contribute to sorption of these hormonal compounds, as estrone and 17β-estradiol adsorption correspondingly correlated with clay content. Furthermore, iron oxides may play a factor in estrogen binding through ion exchange between the oxide hydroxyl group and the polar estrogenic phenolic group (Lai et al., 2000). Similarly, Casey et al. (2003) found a correlation between 17β-estradiol sorption and soil cation exchange capacity (CEC) that was assumed to be associated with clay. However, this may be an artifact of organic material exhibiting high CEC as the estrogenic phenolic groups form organic anions under basic conditions, but will never form cations (Lee et al., 2003).

The composition of the clay component also requires consideration to more accurately estimate sorption parameters. The large surface-to-volume ratios exhibited by fine clay particles may enhance estrogen affinity to clay materials. In one instance the interaction between 17β-estradiol and fine sediments exceeded the correlation with organic carbon content (Holthaus et al., 2002). Grant et al. (1998) found a kaolinite clay

Table 3.5. Freundlich isotherm parameters from literature.

Matrix	Compound	K_F	$1/n$	Source
River sediment	Estrone	53.7	0.73	Lai et al. (2000)
	17β-estradiol	36.3	0.67	
River sediment	17β-estradiol	4 o 74	0.97[†]	Holthaus et al. (2002)
Aquifer material	17β-estradiol	21.8	0.40	Ying et al. (2003)
Sediment	Estrone	0.1 to 0.3	0.66 to 0.88	Yu et al. (2004)
	17β-estradiol	0.6 to 1.0	0.48 to 0.73	
Topsoil	Estrone	1.4	0.63	
	17β-estradiol	1.5	0.59	
Sand		4	2.00	
Sandy clay loam	17β-estradiol	86	1.15	Casey et al. (2003)
Clay loam		332	1.30	
Kaolinite		10	1.05	

† Value represents average value

had a total surface area of 23 m^2 g^{-1} compared to a montmorillonite clay which had a significantly greater surface area at 800 m^2 g^{-1}; when collapsed the montmorillonite exhibited a surface area of 84 m^2 g^{-1}. This disparity can be attributed to negligible spacing between platelets in kaolinite, whereas smectite clays such as montmorillonite can display considerable spacing between layers. The principal clay type may therefore impact the degree of estrogen adsorption. Furthermore, since smectite clays exhibit such a wide range of surface areas based on the state of the soil, other soil solution parameters may have an influence on sorption of estrone and 17β-estradiol. Interlayer spacing in smectites change based on the predominant cation in solution and the degree of hydration. Environmental factors such as these may not only impact the extent of adsorption, but may also change soil parameters and consequently influence estrogen desorption mechanisms.

Increased media salinity may similarly enhance estrogen sorption. Although not fully investigated in soils, higher ionic strength solutions led to greater estrogen sorption in river waters; concurrently elevated salt concentrations also enhance aggregation and flocculation of solid particles which likely promotes sedimentation of hormones out of the water column (Lai et al., 2000). This finding also has ramifications on estrogen fate prior to land application of animal manures using liquid waste systems. Certain management practices lead to high salinity in some livestock lagoons (Ullman and Mukhtar, 2006), which correspondingly implies deposition and burial in lagoon sludge. Other environmental factors influencing sorption mechanisms of estrone and 17β-estradiol to soils require further examination.

The addition of estrone to the loamy fine sand represented the only treatment in this study where neither the linear distribution ($r^2 = 0.51$) nor the Freundlich ($r^2 = 0.75$) isotherm models adequately explained sorption behavior. The estrone adsorption isotherm in the loamy fine sand was more effectively described by the Langmuir equation, which can be expressed as:

$$C_S = \frac{\alpha K_L C_W}{(1 + K_L C_W)} \tag{3.5}$$

where C_S is the amount adsorbed per unit of soil ($\mu g\ g^{-1}$), C_W is the concentration in solution ($\mu g\ mL^{-1}$), K_L represents an empirical Langmuir partition coefficient and α is the maximum amount of compound that can be adsorbed. Figure 3.2 contrasts the three adsorption isotherm models.

One assumption of the Langmuir model states adsorption occurs on planar surfaces (i.e., monolayer coverage) with a fixed number of binding sites (Sparks, 1995). The low organic matter (0.73%) and high sand content (81%) of the Tremona loamy fine sand correspond well with this assumption. Sand largely consists of relatively non-reactive quartz which exhibits a low surface area, both characteristics indicative of a planar matrix with finite sorption potential. This finding may denote sandy soils as having a higher potential for estrone transport following land disposal of estrogen-laden manures. Even at low concentrations, repeated applications may facilitate saturation of binding sites which would consequently maintain estrone in solution and enable migration.
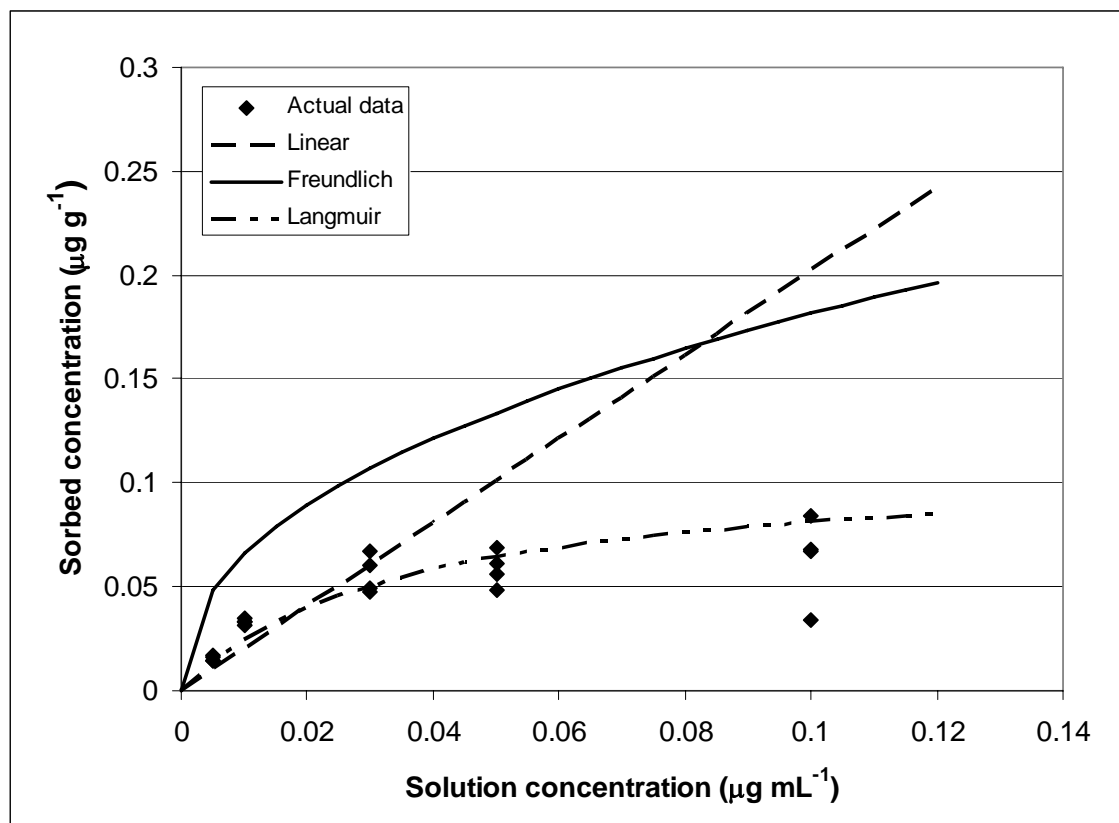
Fig. 3.2. Adsorption isotherms for estrone in Tremona loamy fine sand.

3.3.3. Desorption

The estrone and 17β-estradiol adsorption-desorption isotherms for loamy fine sand, silty clay loam and clay soils are presented in Figure 3.3, while desorption isotherm parameters can be found in Table 3.6. The Freundlich desorption isotherm parameters were lower than the adsorption parameters for all treatments, indicating that the adsorption-desorption process was hysteretic. This trend was concentration independent, illustrated by the roughly parallel curves obtained at 0.1 and 0.3 μg mL^{-1}. The large difference between the adsorption and desorption curves implies the estrogens

become increasingly difficult to desorb, resulting in a recalcitrant fraction. This has a

number of implications on the fate and transport of these compounds. The hormonal

compounds may remain unavailable to microbial degradation, thus extending their
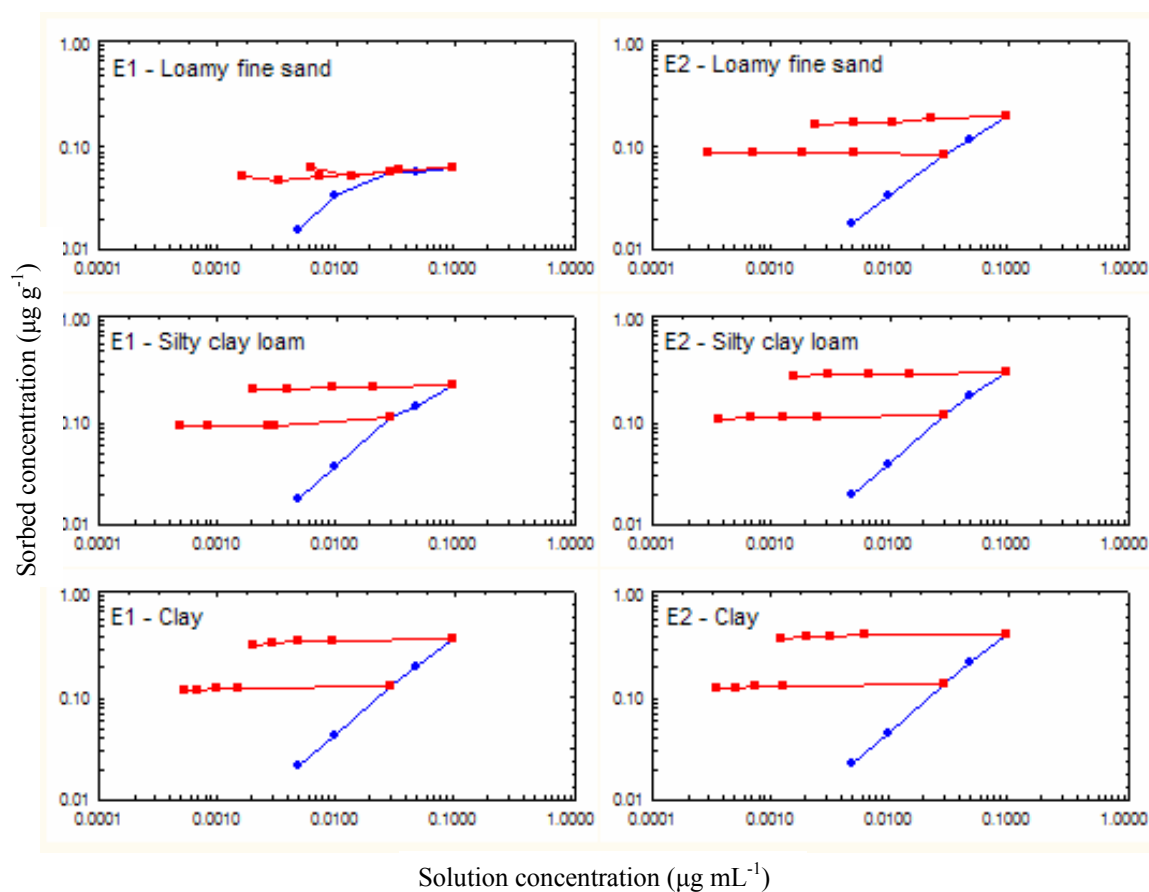
Fig. 3.3. Sorption isotherms (●) and four sequential desorption steps (■) for estrone and 17β-estradiol in selected agricultural soils.

Table 3.6. Freundlich desorption isotherm parameters for estrone and 17β-estradiol in selected agricultural soils.

Soil series	K_{Fdes}	$1/n$	r^2	ω
	($\mu g^{1-1/n} mL^{1/n} g^{-1}$)			
	Estrone			
Tremona loamy fine sand	0.30	0.02	na†	2133
Weswood silty clay loam	0.55	0.03	0.98	2679
Houston Black clay	0.68	0.03	0.86	2894
	17β-estradiol			
Tremona loamy fine sand	0.53	0.05	0.99	1395
Weswood silty clay loam	0.62	0.02	0.99	4316
Houston Black clay	0.70	0.02	0.84	6010

† Not applicable (NA) since re-adsorbed estrone during last stage; when data from the last stage was removed obtained $r^2 = 0.90$.

persistence. On the other hand, strong affinity for the solid phase likely precludes biological uptake by humans and wildlife, thus decreasing the risk to these populations.

Hysteresis was greatest for the Houston Black clay, exhibiting a ω value for 17β-estradiol over four-fold larger than that observed for the Tremona loamy fine sand. Therefore, the Houston Black clay not only exhibits greater sorption capacity for estrogens, but also demonstrates more effective retention of sorbed hormones. This may be due to entrapment by organic matter or association with fine particles discussed earlier. The K_{Fdes} values also signify the amount of compound retained by the soil matrix, with larger measures denoting a larger proportion of estrogen retained (Mersie and Seybold, 1996). As shown in Table 3.6, K_{Fdes} values increased in the order Tremona > Weswood > Houston Black clay.

The estrone and 17β-estradiol desorption isotherm parameters for all soils showed a low desorption rate, as 1/n values ranged from 0.02 to 0.05 for all soil-compound combinations. This parameter estimates desorption intensity with low values indicate a lesser degree of desorption. Thus, these 1/n values support the presupposition that binding of estrogens to these soils is strong (Mersie and Seybold, 1996).

The r^2 values indicated the Freundlich model adequately described all of the sequential desorption events except for estrone in the Tremona loamy fine sand. The representative desorption isotherm for this compound-soil interaction described an initial desorption, but subsequently indicated re-adsorption of estrone. The reason for this phenomenon is unclear; however, the shaking intrinsic to this method may have broken up soil particles over the course of this experiment, exposing more binding sites and thereby removing additional estrone from solution. When the last desorption stage was removed for analysis, the isotherm followed Freundlich behavior.

The octanol-water partition coefficients (K_{OW}) for estrone and 17β-estradiol provided reasonable estimates for the respective K_{OC} values based on the regression equation developed by Chiou et al. (1983):

$$\log K_{OM} = 0.904 \log K_{OW} - 0.779 \qquad (3.6)$$

Agreement between experimentally derived K_{OC} values and those estimated using this equation have been achieved over a range of organic compounds (Sharer et al., 2003). This finding suggests initial binding may be due to hydrophobic interactions taking place on the interfacial region. Correspondingly, Yamamoto and Liljestrand (2003) advocated the importance of interactions between estrogenic and organic matter phenolic groups.

However, Yamamoto and Liljestrand (2003) found a poor correlation between K_{OW} and K_{OC} for estrogenic compounds and indicated the contribution of other sorption mechanisms. This discord in findings is not necessarily exclusive, as hydrophobic interactions may initially predominate, but limited desorption suggests a configuration that may include other mechanisms. Over time partitioning into organic matter may occur (i.e., *ab*sorption), a mechanism that transcends hydrophobic interactions.

3.4. CONCLUSIONS

Estrogen sorption-desorption dynamics play a significant role in determining chemical mobility. This study demonstrated that estrone and 17β-estradiol sorption to selected agricultural soils is moderate, but fairly irreversible. Both compounds exhibited greater binding affinity with soils containing higher organic carbon and clay content; however, the specific mechanisms remain unknown. These findings allude to a strong association between estrogens and agricultural soils following land application of animal manure, which may retain these biologically active hormones on-site and inhibit migration to waters of concern. Despite the affinity of estrone and 17β-estradiol for soil materials, the potential for off-site hormone transport should not be discounted. Colloidal particles may enhance movement through the soil profile and erosion may transport the compounds overland. Further research should consider the influence of natural (e.g., temperature, anaerobic conditions) and anthropogenic (e.g., manure application methods, erosion control) parameters on the fate and transport of hormonal compounds.

4. ESTRONE AND 17β-ESTRADIOL MINERALIZATION IN SOILS RECEIVING DAIRY LAGOON EFFLUENT

4.1. INTRODUCTION

The occurrence of steroidal agents in human and animal waste streams presents a potential estrogenic contaminant source to environmental systems. These hormones, including the natural hormonal compounds estrone and 17β-estradiol, can result in endocrine disrupting effects in human and wildlife populations, impacting reproduction and development (Ball et al., 2000; Crews et al., 1995; Routledge et al., 1998; Sone et al., 2004; Sumpter and Jobling, 1995). Although endogenous, the potency of these compounds can lead to detrimental health consequences at concentrations as low as 1 ng L^{-1} (Irwin et al., 2001; Routledge et al., 1998). Despite corresponding concerns over the presence of hormones in aquatic systems, a thorough understanding of the fate and transport mechanisms influencing environmental exposure pathways remains deficient.

Land application of animal manures represents an integral part of the livestock industry's waste management paradigm. Elevated estrogen concentrations associated with animal feeding operations (AFOs) arise not only from steroidal treatments given to livestock to enhance growth and fecundity, but also from the trend towards concentrating animals into small geographic areas, potentially magnifying contamination problems. Anaerobic lagoons treating dairy waste-streams also present a significant environmental load of estrone and 17β-estradiol (Hanselman et al., 2004; Kolodziej et al., 2004; Raman et al., 2004). Consequently, elevated off-site estrogen concentrations associated with

cattle operations have occurred in both surface and groundwater systems (Dyer et al., 2001; Peterson et al., 2000; Soto et al., 2004). Detrimental effects on human and wildlife health arising from agricultural disposal of estrogen-laden manures depends on the ultimate fate of these hormones prior to exposure.

Estrogen persistence following land application of animal manure and process generated wastewater correspondingly influences potential off-site transport to sensitive water resources. Microbial activity in soils presents a primary decomposition mechanism for many organic contaminants. Thus, assessments of estrone and 17β-estradiol susceptibility to microbial degradation will provide insight into the long-term persistence of these compounds following land application, but a dearth of data exists on hormone dissipation.

The prevalent view in the literature advocates a relatively rapid transformation of both estrone and 17β-estradiol in aquatic, terrestrial and sewage treatment systems (Casey et al., 2003; Jurgens et al., 2002; Lee et al., 2003; Ying et al., 2002; Ying et al., 2004). For instance, investigations of degradation rates in aquifer material showed 17β-estradiol exhibited a half-life of 2 days (Ying et al., 2003). Similarly, sewage bacteria rapidly transformed 17β-estradiol in municipal sewage effluent, with experiments showing complete removal of 17β-estradiol within 18 h (Lee and Liu, 2002). Estrogen mineralization in soil reveals a less aggressive response by microbial populations, a salient point since certain steroidal metabolites continue to exhibit estrogenic activity. Application of estrone and 17β-estradiol to agricultural soils demonstrated both compounds formed non-extractable residues that mineralized slowly (Colucci et al.,

2001). A follow-up study conducted at more environmentally relevant concentrations (2.7 µg kg^{-1}) yielded consistent results (Colucci and Topp, 2002). Jacobsen et al. (2005) found 17β-estradiol degraded rapidly, but mineralization progressed slowly in soils receiving swine manure. Understanding estrogen behavior requires distinguishing between dissipation and mineralization from a system.

Elucidation of the ultimate fate of estrogenic agents remains imperative, as ongoing research reveals potential detrimental impacts to human health and aquatic ecosystems. A thorough assessment of hormonal chemical behavior following land application entails determining degradation rates of these estrogenic compounds across a spectrum of soils. Therefore, the objectives of this study were to (1) evaluate the mineralization rates of estrone and 17β-estradiol in three distinct agricultural soils, and (2) analyze the impact of dairy lagoon effluent on degradation of these steroids, as anaerobic lagoon effluent comprises a complex mixture of organic and inorganic compounds that may promote or impede microbial degradation of hormones. Quantitative information pertaining to estrogen mineralization would not only provide data on the persistence of estrone and 17β-estradiol following land application, but also insight into the amount of hormone available for transport to surface and groundwater resources.

4.2. MATERIALS AND METHODS

Three agricultural soils exhibiting disparate texture and physicochemical properties were selected to determine mineralization characteristics for estrone and 17β-estradiol (Table 4.1). Samples of Tremona loamy fine sand (thermic Aquic Arenic Palenstalfs), Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) and Houston Black clay (fine, smectitic thermic Udic Haplusterts) were collected from the top 5 cm of the soil profile. The soil samples were air dried at room temperature and subsequently passed through a 2-mm sieve.

Table 4.1. Properties of soils used for sorption and microbial degradation analysis for estrone and 17β-estradiol.

Soil	Textural class	Sand	Silt	Clay	Organic matter	pH
			%			
Tremona	Loamy fine sand	81	10	9	0.73	7.0
Weswood	Silty clay loam	11	68	31	1.82	8.1
Houston black clay	Clay	19	38	43	2.60	8.1

The microcosms created for this study consisted of 5 g soil subsamples contained in 80-mL glass test tubes sealed with a rubber stopper. The soils were re-wetted gravimetrically to 20% moisture using either deionized water or dairy lagoon effluent taken from a center-pivot irrigation system used for land application; this treatment is referred to as "water source" hereafter. Table 4.2 presents effluent physicochemical

properties as analyzed by the Texas Cooperative Extension Soil, Water and Forage Testing Laboratory.

Table 4.2. Physicochemical properties of dairy lagoon effluent collected from center-pivot irrigation system during land application.

pH	7.7	Na	135 mg L^{-1}
N	89 mg L^{-1}	Zn	0.13 mg L^{-1}
P	23 mg L^{-1}	Fe	0.97 mg L^{-1}
K	142 mg L^{-1}	Cu	0.06 mg L^{-1}
Ca	97 mg L^{-1}	Mn	0.47 mg L^{-1}
Mg	36 mg L^{-1}	Conductivity	1506 μmhos cm^{-1}

Radiolabeled hormones in distilled water solutions were added to the soil microcosms following incubation in the dark at 25°C for 10 days to re-establish steady-state microbial activity, bringing the final moisture content to 30% and the estrogenic dry weight concentration to 1 μg kg^{-1}. This estrogen quantity corresponded with a 10 μg L^{-1} effluent concentration and a total pore water concentration of 3.33 μg L^{-1}, amounts representative of a typical dairy operation (Raman et al., 2004). Stock solutions consisted of [4-^{14}C]-estrone (2035 MBq mmol^{-1}) and [4-^{14}C]17β-estradiol (2035 MBq mmol^{-1}) purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). Background estrogen concentrations in the lagoon effluent were not accounted for and only radiolabeled estrogens were considered. The experimental design thus comprised four treatments for each soil, consisting of hormones added to soil wetted with either distilled water or dairy lagoon effluent for both estrone and 17β-estradiol.

Two scintillation vials were suspended over the soil profile; one contained water to maintain a humid atmosphere to prevent soil desiccation while the other contained 3 mL 1 M KOH to trap evolved $^{14}CO_2$. Incubation continued as described above and the CO_2 traps were periodically removed and replaced with fresh vials containing KOH after the experiment began. A Beckman 6500 liquid scintillation spectrometer operated in the auto-disintegrations per minute mode analyzed ^{14}C content in each sample, correcting for counting efficiency. All treatment combinations were replicated four times and appropriate quality assurance/quality control protocol was followed.

Approximate mineralization rates for unbound estrogen were calculated to delineate the mobility potential in instances where microbial processes predominate (i.e., sorption sites become saturated). Using the first-order model:

$$\ln C_n/C_0 = kt \tag{4.1}$$

where C_n is the concentration at time t, C_0 is the initial concentration and k is the rate constant, a description of estrogen dissipation can be estimated. Regression analysis then yields a half-life by:

$$t_{1/2} = \frac{\ln(2)}{k} \tag{4.2}$$

in which k is obtained from the absolute value of the slope.

Analysis of variance (ANOVA) was conducted using StatView 5.0.1 (SAS Institute, 1998). The ANOVA determined the main effects and interactions of the treatments on estrogen degradation which were in a completely randomized design in a 2 × 2 × 3 factorial arrangement. The Tukey multiple comparison test was performed as a

post-hoc analysis to determine statistical differences between treatment combinations. A probability level of $P < 0.05$ was selected to delineate treatment differences.

4.3. RESULTS AND DISCUSSION

Mineralization of estrone and 17β-estradiol was investigated under aerobic conditions by trapping $^{14}CO_2$ emitted to the microcosm atmosphere subsequent to cleavage of the phenolic ring, a process typically microbially regulated. Although the specific definition of degradation denotes only the transformation of a compound into simpler compounds, this method of quantifying the complete breakdown of estrone and 17β-estradiol to inorganic components was selected because certain metabolites of these hormones maintain estrogenic activity (Lee and Liu, 2002). The broad spectrum of chemical structures that continue to pose a potential threat to human and wildlife health following microbial transformation, in addition to their transient nature, makes it impracticable to isolate all breakdown products. The location of the radiolabeled carbon atom in position 4 on the aromatic structure likely denotes mineralization when trapped, as opposed to the loss of a carbon situated on a side chain that undergoes cleavage during initial degradative processes. Recognizing the specific difference between the terms degradation and mineralization, hereafter degradation will refer to the complete decomposition and thus equivalent to mineralization unless otherwise specified.

After a 43-d incubation, 10% to 40% of the estrogen compounds were mineralized, regardless of the soil-water source treatment combination (Figures 4.1-4.3). Degradation progressed at a relatively rapid rate over the first 3 days in all cases,

subsequently slowing until essentially reaching a plateau after 9 days. In each instance,

17β-estradiol mineralization occurred more rapidly than estrone, ultimately yielding a

total 17β-estradiol removal approximately twice that of estrone.
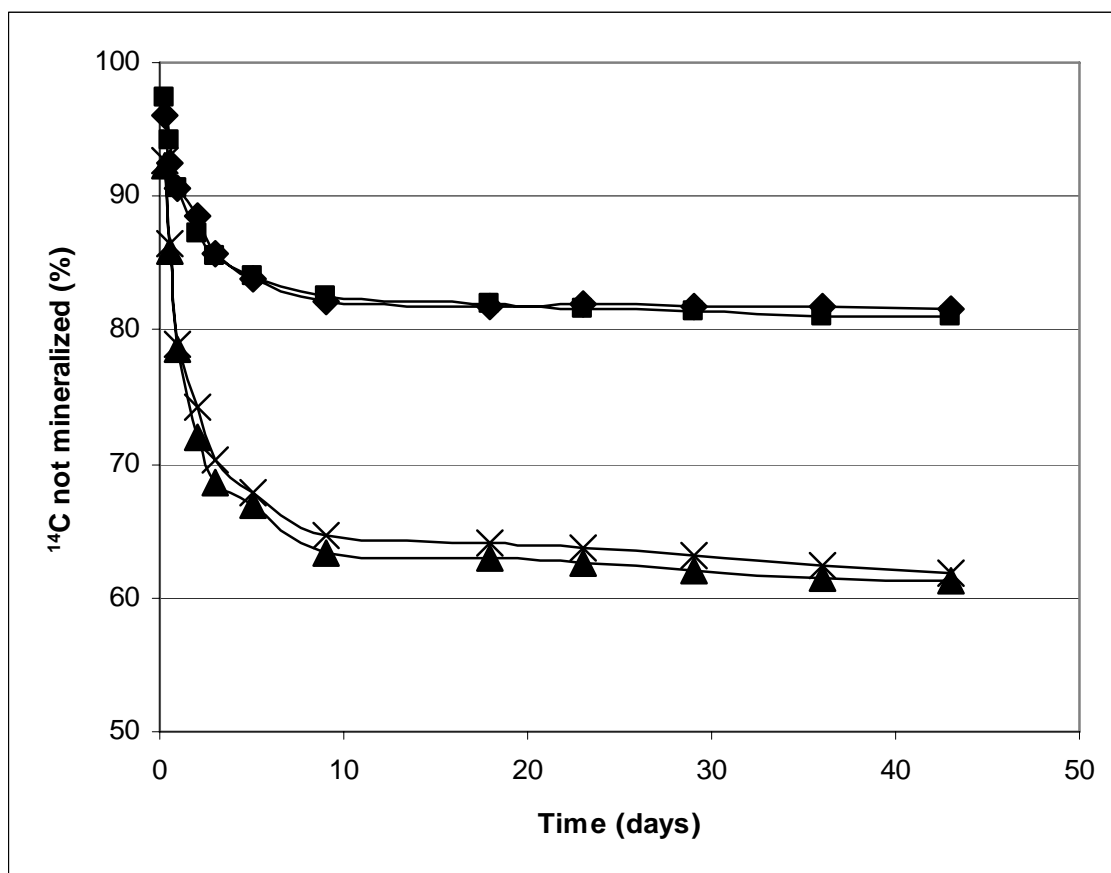
Fig. 4.1. Estrogen degradation expressed as the percent ^{14}C added to Tremona loamy fine sand not mineralized (i.e., not collected as $^{14}CO_2$). Treatments include estrone-lagoon effluent (♦), estrone-water (■), 17β-estradiol-lagoon effluent (▲) and 17β-estradiol-water (×).
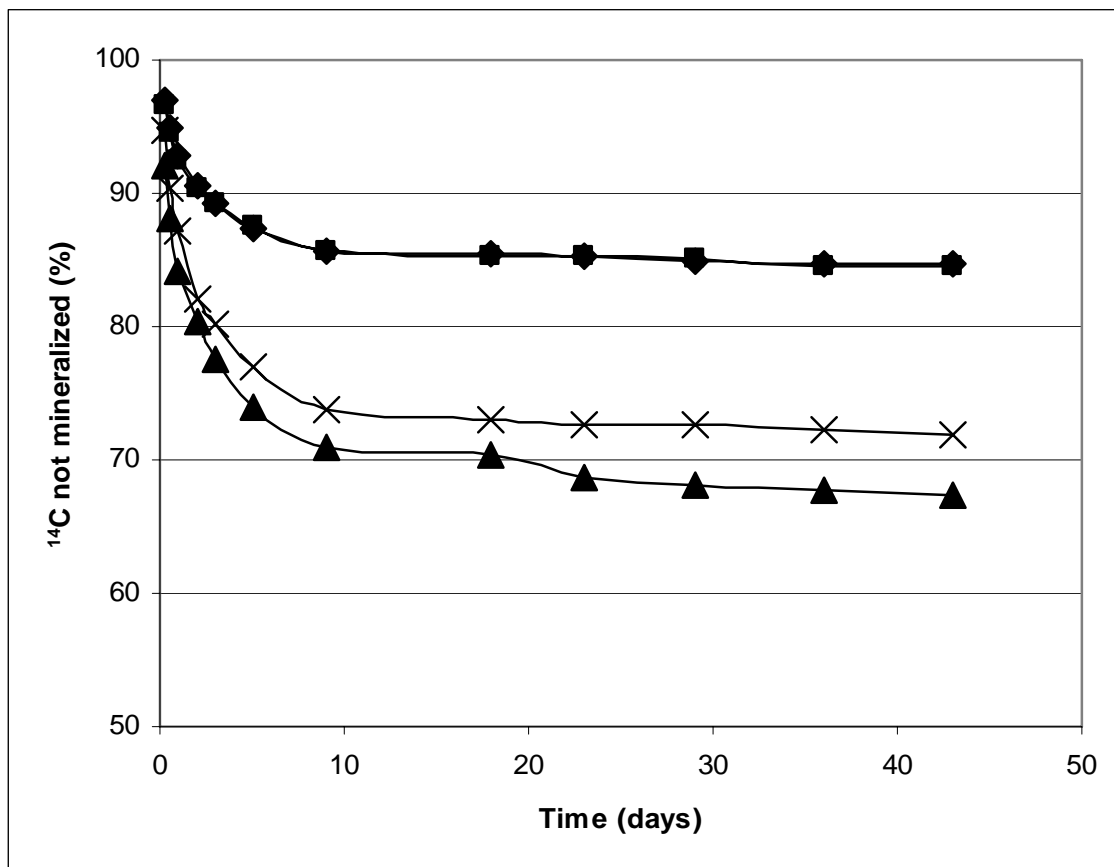
Fig. 4.2. Estrogen degradation expressed as the percent ^{14}C added to Weswood silty clay loam not mineralized (i.e., not collected as $^{14}CO_2$). Treatments include estrone-lagoon effluent (♦), estrone-water (■), 17β-estradiol-lagoon effluent (▲) and 17β-estradiol-water (×).
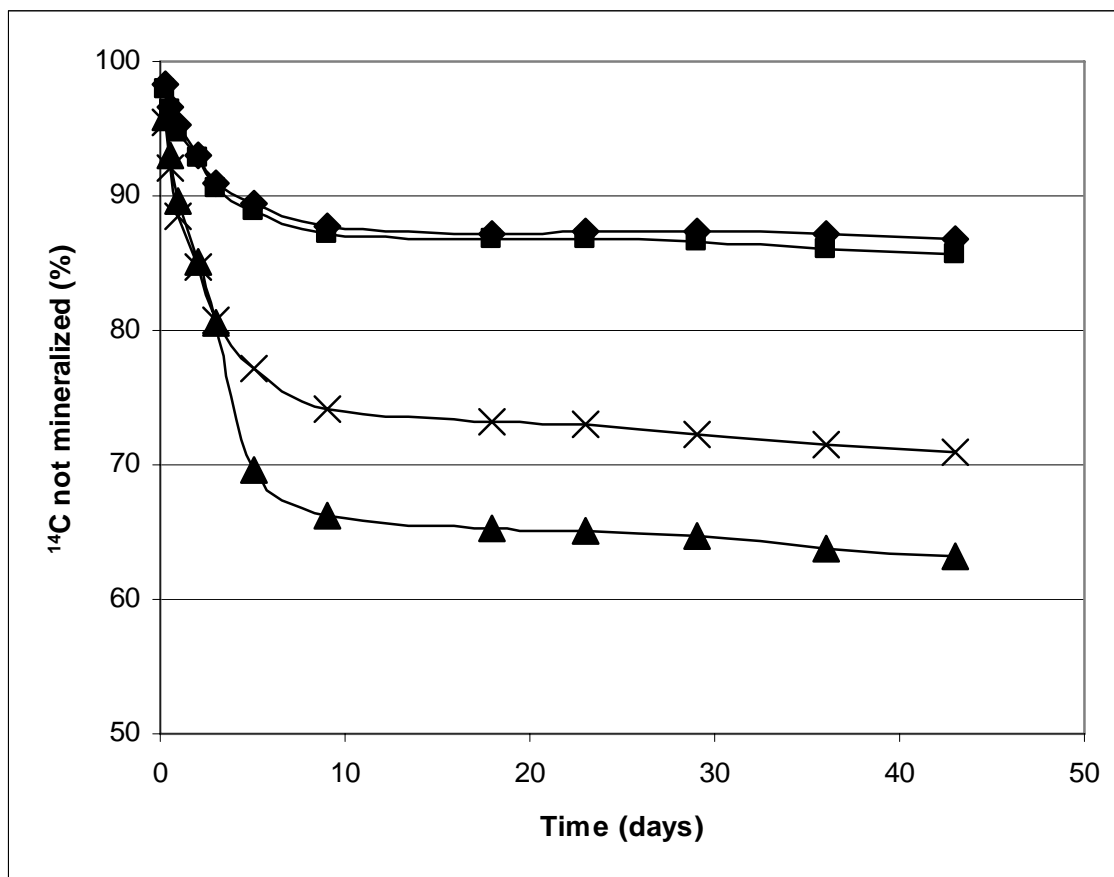
Fig. 4.3. Estrogen degradation expressed as the percent ^{14}C added to Houston Black clay not mineralized (i.e., not collected as $^{14}CO_2$). Treatments include estrone-lagoon effluent (♦), estrone-water (■), 17β-estradiol-lagoon effluent (▲) and 17β-estradiol-water (×).

Significant differences in estrogen degradation were found within each treatment, as well as a compound by soil and compound by water source interaction (Table 4.3). Post-hoc analysis delineated specific effects of treatment combinations (Table 4.4). As Figures 4.1 - 4.3 indicate, a strong disparity existed between estrone and 17β-estradiol degradation rates. When considering the influence of soil characteristics on estrone degradation, significant differences existed among all soils; however, although there were interactions between the Tremona loamy fine sand and the other two soils, no such interaction occurred between the Weswood silty clay loam and Houston Black clay for 17β-estradiol mineralization. Water source exhibited no influence on estrone degradation rates, whereas a significant difference was found between the deionized water and lagoon effluent application for 17β-estradiol in the Houston Black clay soil. Although not significant at the 95% confidence level, the Weswood sandy clay loam showed disparity between the water sources at $P = 0.0599$.

Table 4.3. P-values obtained by analysis of variance comparing the effects of the different treatments for the compound, soil and water source used.

Comparison	P-value†
Compound	0.0001
Soil	0.0001
Water source	0.0365
Compound × Soil	0.0463
Compound × Water source	0.0172
Soil × Water source	0.5880
Compound × Soil × Water source	0.4108

†Italics represent significant difference at $P < 0.05$.

Table 4.4. P-values for simple effects of compound, soil and water source on degradation rates for estrone and 17β-estradiol.[†]

Compound	Treatment combination	P-value[‡]
Estrone & 17β-estradiol	Between compounds at:	*0.0001*
Estrone	Between soils	
	LFS-SCL	*0.0001*
	LFS-Clay	*0.0001*
	SCL-Clay	*0.0032*
	Water source	
	LFS	0.9805
	SCL	0.7928
	Clay	0.5889
17β-estradiol	Between soils	
	LFS-SCL	*0.0001*
	LFS-Clay	*0.0001*
	SCL-Clay	0.9029
	Water source	
	LFS	0.6102
	SCL	0.0599
	Clay	*0.0428*[§]

[†] LFS = Tremona loamy fine sand, SCL = Weswood silty clay loam.
[‡] Italics represent significant difference at $P < 0.05$.
[§] Calculated from end of first day until end of study; $P = 0.08$ for complete data set due to initially equivalent values.

Identifying all factors influencing the degradation trends exhibited by estrone and 17β-estradiol proved elusive due to the inherent complexity of soil, which includes many biological, chemical and physical parameters that promote or inhibit mineralization of organic compounds. However, basic inferences can be made for estrogen degradation based on the physicochemical characteristics associated with the different soil-water source combinations tested. The following section attempts to synthesize existing knowledge into a coherent description of the processes taking place on estrone and 17β-estradiol at environmentally relevant concentrations.

The predominant current position states that estrone and 17β-estradiol dissipate rapidly with half-lives typically ranging on the order of hours to a few days (Casey et al., 2003; Casey et al., 2005; Colucci and Topp, 2002; Colucci et al., 2001; Lee et al., 2003). This statement may be misconstrued to mean complete elimination of estrogenic activity, an assertion the findings in this study refute. The term dissipation constitutes the formation of soil-bound, non-extractable residues, as well as transformation products. The incomplete mineralization found in this study agrees with similar studies of estrone and 17β-estradiol decomposition (Colucci and Topp, 2002; Colucci et al., 2001; Jacobsen et al., 2005). Although a number of abiotic factors likely occur, Jacobsen et al. (2005) report hormonal mineralization to CO_2 requires microbial activity, an aspect that will be the focus of the following discussion.

The immediate evolution of $^{14}CO_2$ indicates mineralization requires no acclimation period by the indigenous microbial population. Although successive enrichments of estrogen-degrading bacteria identified from activated sludge diminished

the time required to completely degrade estrone and 17β-estradiol (Yu et al., 2005), application of estrogenic agents to agricultural soils generated the same response demonstrating no lag phase (Colucci and Topp, 2002; Colucci et al., 2001). The microorganisms responsible for estrogen decomposition apparently are ubiquitous in soils, since the absence of a lag phase transpired in distilled water treatment, as well as the lagoon effluent. An early investigation into the degradation of steroidal compounds by Turfitt (1947) examined the potential breakdown of estrone and 17α-estradiol by microorganisms indigenous to five disparate soils and established a strain of the bacterial genus *Proactinomyces* isolated from an arable soil degraded estrone, while strains of *Proactinomyces* spp. originating from the arable and acid soils metabolized estradiol. A strain of the gram-negative bacterium genus *Novosphingobium* isolated from activated sludge similarly decomposed 17β-estradiol (Fujii et al., 2002).

The greater mineralization of 17β-estradiol compared to estrone corresponds with a number of other studies looking at the degradative behavior of estrogenic substances. Direct comparisons between land applied estrogens and those in municipal sewage have limitations; however, many studies to date have considered estrogenic agents in a wastewater treatment setting and these preliminary results provide insight into fundamental behavior of these steroids. For instance, a pilot scale study found a 94.7% average removal rate for 17β-estradiol from wastewater, but only a 69.2% attenuation rate for estrone (Onda et al., 2003). Similarly, sewage treatment plants in Germany and Brazil exhibited higher removal efficiencies for 17β-estradiol compared to estrone (Ternes et al., 1999).

Despite these earlier findings, the strong disparity between estrone and 17β-estradiol degradation rates was notable when considering complete mineralization to CO_2, as estrone represents the primary metabolite of 17β-estradiol in environmental systems (Colucci et al., 2001; Jacobsen et al., 2005). Transformation of 17β-estradiol involves the substitution of the phenol group (-OH) with a carbonyl group (C=O) to form estrone. This well documented pathway leads to the logical expectation that estrone would mineralize at least at the same rate as 17β-estradiol if not faster, since 17β-estradiol has to be transformed to estrone before further decomposition. Therefore, investigation into an alternative degradative pathway should receive consideration. Although an abiotic mechanism may play a role in degradation of these compounds, complete mineralization typically requires microbial activity (Jacobsen et al., 2005).

The decelerated mineralization rate observed after approximately three days corresponds with the 3-d equilibrium period determined for sorption mechanisms in the selected soils. Competitive sequestration of estrone and 17β-estradiol between microbial metabolism and soil sorption appears to occur, corresponding with the observation that the microbial degradation rate of organic compounds frequently displays a negative correlation with soil adsorption (Blumhorst, 1996). Although typically considered largely as a retardation factor in contaminant transport, binding processes also have implications on bioavailability. The data suggest that estrone and 17β-estradiol readily degrade during initial introduction of the estrogenic agents to the soil, a time when the hormones primarily remain in solution. As the hormonal compounds begin to associate

with soil particles, formation of a resilient soil-bound fraction likely occurs that diminishes the availability of estrone and 17β-estradiol for microbial degradation.

The high estrogenic affinity for soils may play a dual role in the fate, and ultimately on the potential environmental exposure, of the hormones. Although strong sorption characteristics impede migration of organic chemicals, they concurrently limit bioavailability to microorganisms and promote compound persistence in soil. On the contrary, a low adsorptive soil may increase hormone mobility potential, but concurrently enhance estrogen decomposition. Therefore, the greater mineralization rates found in the loamy fine sand may indicate more rapid estrogen dissipation overall, whereas stronger association with the Houston Black clay may allow for long-term resilience. Therefore, potential future release of bound estrogens, particularly in instances following transport of clay particles to aquatic ecosystems via erosion processes, may present greater exposure to humans and wildlife.

Two methods known to augment biodegradation include inoculation with an enriched microbial population capable of metabolizing a compound and supplementation of limiting nutrients. Lagoon effluent fulfills both of these factors, but this amendment failed to demonstrate a significant acceleration of mineralization for estrone and only minor enhancement for 17β-estradiol. The hypothesis that indigenous microorganisms readily degrade the estrogenic compounds, substantiated by the lack of a lag phase prior to mineralization, suggests microbes added to the soil via lagoon effluent provide minimal, if any, stimulus. Likewise, greater nutrient concentrations associated with the lagoon effluent did not contribute to estrogen breakdown.

It is difficult to make a general statement regarding the increased mineralization of 17β-estradiol in the presence of lagoon effluent for the Houston Black clay. Clay minerals may stimulate, decrease or impart no effect on degradation of organic compounds. Moreover, this soil also exhibits the highest amount of organic matter. The formation of clay aggregates stabilized by organic matter may present an explanation for heightened mineralization in the clay soil, assuming different breakdown pathways for estrone and 17β-estradiol. These amalgamations, which provide microhabitats for microbial growth, may depend on diffusive forces to supply both 17β-estradiol and nutrients for biodegradation, potentially leading to nutrient limitation during 17β-estradiol decomposition. Presuming 17β-estradiol maintains equivalent diffusion rates irrespective of the fluid, elevated nutrient levels associated with the lagoon effluent may promote enhanced nutrient transport into the aggregate domain, alleviating nutrient deprivation. The higher clay and organic matter content of the sandy clay loam, compared to the loamy fine sand, may similarly form aggregates that lead to the substantial difference in mineralization rates in this soil depending on the water source.

Although it is unreasonable to assume a simple rate law will adequately describe a complex soil system, fitting a first-order model to the data permits calculation of half-lives for the respective compounds in the various soils. Since it appears that a competitive phenomenon takes place between mineralization and sorption mechanisms, $^{14}CO_2$ evolution over the first day (measurements at 0, 6, 12 and 24 hours) was chosen for analysis since this data followed a linear model and sorption equilibrium was found to take place after three days. Half-lives for the respective treatments were found as

displayed in Table 4.5. Refining these values should be a priority in future research to allow better assessment of the fate of estrogens.

Table 4.5. Half-lives (in days) approximated for estrone and 17β-estradiol applied to microcosms in deionized water and anaerobic lagoon effluent assuming limited sorption to soil particles using first day data.[†]

	LFS		SCL		Clay	
Compound	Water	Lagoon	Water	Lagoon	Water	Lagoon
Estrone	7.0	7.2	9.3	9.8	13.3	14.5
17β-estradiol	3.0	2.9	5.2	4.2	5.9	6.5

[†] LFS = Tremona loamy fine sand, SCL = Weswood silty clay loam.

The estrogen concentration in solution prior to application was 10 ppb, achieving a 1 ppb soil concentration. These levels correspond with those found for dairy liquid manure management systems (Table 4.6). Estimates project that cattle in the United States excrete 45 tons of estrogens annually (Shore and Shemesh, 2003). These compounds bind strongly to the soils tested here as shown previously, thus inhibiting bioavailability. Contrarily, the limited estrone and 17β-estradiol mineralization found may allow for the accumulation of estrogenic agents in the soil after repetitive irrigation with lagoon effluent. This may be particularly true in regions populated with large numbers of AFOs with inadequate land available for land application.

Table 4.6. Concentrations of estrone and 17β-estradiol reported for dairy liquid manure management systems.

Estrogenic source	Estrogen	Concentration	Reference
		$(\mu g\ L^{-1})^{\dagger}$	
Milk cow slurried manure	Estrone	255-640	(Shore and Shemesh, 2003)
	17β-estradiol	170-1230	
Lagoons & holding ponds	17β-estradiol	Below 2	(Williams et al., 2002)
Flushed manure wastewater	Estrone	0.781	(Hanselman et al., 2004) [‡]
	17β-estradiol	Up to 1.31	
Dairy waste lagoon	Estrone & 17β-estradiol	Up to 0.650	(Kolodziej et al., 2004)
Dairy holding pond	Estrone	2.5-5.6	(Raman et al., 2004)
	17β-estradiol	0.8-1.9	

[†] Values given as means unless otherwise stated.
[‡] Compared analysis methods which showed significant variability. Maximum values shown.

A number of studies demonstrate the potential for estrogen migration following manure application to agricultural soils. Evidence suggests cattle AFOs can release significant estrogenic loads into neighboring waterways (Soto et al., 2004). Female painted turtles (*Chrysemys picta*) residing in ponds receiving runoff from cattle operations exhibited signs of altered reproductive fitness at 17β-estradiol concentrations around 1.0 ng L^{-1} (Irwin et al., 2001), while wild male fathead minnows (*Pimephales promelas*) displayed characteristics of demasculinization following exposure to effluent

(Orlando et al., 2004). Affirmation that local cattle operations generated elevated 17β-estradiol concentrations in karst aquifers denotes potential estrogenic contamination of groundwater systems (Peterson et al., 2000).

As emphasized previously, lack of mineralization does not eliminate the possibility of estrogen transformation. Although the potential cumulative and synergistic activity between estrone-17β-estradiol and the associated estrogenic metabolites remains in question, the breakdown products of 17β-estradiol exhibit progressively less estrogenic activity, with estrone being half as potent as 17β-estradiol (Colucci et al., 2001). Therefore, surface applied manures and process generated wastewater present a potential mode by which estrogens may be introduced into the environment, but this may or may not significantly increase exposure to estrogenic activity by humans or aquatic organisms.

4.4. CONCLUSIONS

Although it appears that sorption mechanisms play a predominant role in the fate of estrone and 17β-estradiol, quantification of mineralization rates provides critical information on the amount of hormone in the pore water accessible for migration through the soil profile. This may be particularly salient in soils that exhibit lower partition coefficients, where evidence suggests a saturation of sorption sites may occur at environmentally relevant concentrations. The loamy fine sand soil demonstrated a higher degree of decomposition than the sandy fine loam and clay soils, ascribed to greater bioavailability associate with less estrogenic binding potential. Microbial

inoculation and nutrient supplementation via dairy lagoon effluent additions showed neither significant exacerbation nor mitigation for mineralization except for 17β-estradiol in the clay soil. This finding, combined with the lack of a lag phase for estrogen breakdown, implies no need for acclimation of indigenous microbial populations. Irrespective of the treatment, estrone consistently underwent lower mineralization rates than 17β-estradiol.

The high decomposition rates found in the early time periods subsequent to soil application, presumably representing degradation of estrogens in solution, indicate a rapid elimination of non-bound hormones. Thus, a minimal risk likely exists for surface and groundwater contamination from adjoining pastures receiving land applied lagoon effluent, presuming no particulate transport by surface or preferential flow pathways. However, more rigorous assessment of potential environmental exposure to hormones requires greater consideration of the natural and anthropogenic parameters impacting soil microbial populations and the corresponding effect on fate and transportation processes.

5. ESTROGEN TRANSPORT IN AGRICULTURAL SOILS: SOIL COLUMNS AND MODELING

5.1. INTRODUCTION

The endogenous estrogens estrone and 17β-estradiol play a key role in vertebrate sexual development. Environmental exposure to this hormonally active agent may engender abnormal vertebrate development when present in excessive levels, inducing reproductive disorders in wildlife (Crews et al., 1995; Lai et al., 2002; Routledge et al., 1998; Sone et al., 2004; Sumpter and Jobling, 1995). In addition, these compounds ostensibly contribute to hormone-related cancers and infertility in humans (Dickson et al., 1986; Harrison et al., 1997; Jones and Hajek, 1995). Evidence suggests adverse physiological alterations may occur at concentrations as low as 1.0 ng L^{-1} (Irwin et al. 2001), leading to concerns over water resource contamination.

Concerns over organic agrochemical contamination historically focus on herbicide and pesticide use and distribution. This paradigm is evolving to encompass not only pharmaceuticals, but also natural steroidal compounds originating from animal feeding operations (AFOs). Initial reconnaissance considering agriculturally generated estrogens examined poultry operations, revealing estrogen runoff above ambient levels (Finlay-Moore et al., 2000; Nichols et al., 1997; Nichols et al., 1998). Although birds excrete significantly higher hormone quantities compared to mammals, milk cows generate a significant environmental load. Pregnant cows excrete approximately 170 mg daily (Hanselman et al., 2003), while a typical dairy facility exhibits 17β-estradiol

holding pond emission factors of 1.1 mg d^{-1} (1000 kg live animal weight)$^{-1}$ (Raman et al., 2004). Runoff concentrations following land amendments consisting of liquid dairy manure yielded values above levels known to imperil wildlife populations. Evidence suggests cattle AFOs can release significant estrogenic activity into neighboring waterways (Soto et al., 2004), with both painted turtle (*Chrysemys picta*) and fathead minnow (*Pimephales promelas*) populations displaying characteristics indicative of diminished reproductive fitness subsequent to effluent exposure (Irwin et al., 2001; Orlando et al., 2004). Furthermore, affirmation local cattle operations generated elevated estrogen concentrations in karst aquifers denotes potential estrogenic contamination of groundwater systems (Peterson et al., 2000).

The extent of estrogen exposure following manure land application depends largely on the chemical behavior of these compounds. Sorption and degradation mechanisms represent the primary governing mechanisms controlling contaminant mobility in natural systems. Estrogens display a distinct affinity for soil and sediment particles (Holthaus et al., 2002; Lai et al., 2000; Ying et al., 2003; Yu et al., 2004). Organic carbon appears to provide a strong binding mechanism for estrogens (Lee et al., 2003), while associations with iron oxides and small-sized particles also occur (Lai et al., 2000). Despite rapid transformation of both estrone and 17β-estradiol under a variety of conditions (Casey et al., 2003; Jurgens et al., 2002; Lee et al., 2003; Ying et al., 2002; Ying et al., 2004), application of these compounds to agricultural soils demonstrated they formed non-extractable residues that mineralized slowly (Colucci and Topp, 2002; Colucci et al., 2001; Jacobsen et al., 2005). Although further research into

estrogenic fate and transport parameters remains, modeling represents the next step to fully assess the potential off-site migration through leaching and surface-flow.

Despite a recent proliferation of research examining the chemical behavior of estrogens in the environment and their occurrence and distribution in natural and man-made systems, soil column studies designed to produce breakthrough curves for accompanying model simulation remain virtually nonexistent. An initial column investigation found 17β-estradiol failed to elute from a loam soil following displacement by 10 pore volumes, while a sand medium yielded 85% recovery in the effluent after only 1.06 pore volumes (Larsen et al., 2001). A follow-up paper coupled the HYDRUS-1D transport model with column data for five disparate soil types, obtaining adequate agreement using inverse modeling techniques (Casey et al., 2003). A number of limitations were evident, however, as analysis involved large confidence intervals for estimated fate and transport parameters. Using rate-limited sorption parameters helped eliminate some of the problems encountered during inverse modeling (Casey et al., 2005). Das et al. (2004) expanded simulation efforts by incorporating both pulse and flow interrupted conditions into forward modeling techniques. Simple first-order kinetic processes describing degradation provided sufficient efficacy, but more accurate predictions require better understanding of the complex mechanisms influencing estrogen persistence and mobility.

The potential migration of estrogen to surface and groundwater resources following land application of AFOs generated wastewater warrants further investigation across a spectrum of agricultural soils. Sorption properties evaluated earlier indicate

estrogenic compounds readily partition into the particulate fraction of the soil matrix, while molecules remaining in the pore water rapidly mineralize. These findings correspond well with the literature, yet estrogen concentrations elevated above ambient levels continue in natural systems. Consequentially, comprehension of estrogen leaching dynamics remains critical to fully assess the fate and transport of this compound. Therefore, this study comprised two main objectives: (1) to evaluate sorption and degradation characteristics and their impact on estrone transport in selected soils, and (2) test the predictive efficacy of calculated soil parameters in describing small-scale mobility using a one-dimensional transport model. This approach integrates previously obtained fate and transport parameters to address the fundamental issue of estrogen mobility.

5.2. MATERIALS AND METHODS

5.2.1 Soils

Three distinct soils that represent those typical of agricultural operations conducting land application of AFOs generated wastewater were used as packing material in the column experiment. Samples of Tremona loamy fine sand (thermic Aquic Arenic Palenstalfs), Weswood silty clay loam (fine-silty, mixed, superactive, thermic Udifluventic Haplustepts) and Houston Black clay (fine, smectitic thermic Udic Haplusterts) were collected from the top 5 cm of the soil profile. These soils had no known history of receiving estrogenic amendments. The soil samples were air dried at

room temperature and subsequently passed through a 2-mm sieve. Each soil exhibits disparate textural and physicochemical properties (Table 5.1).

Table 5.1. Properties of soils used for estrone leaching analysis in column experiment.

Soil	Textural class	Sand	Silt	Clay	Organic matter	Organic carbon[†]	pH
				%			
Tremona	Loamy fine sand	81	10	9	0.73	0.42	7.0
Weswood	Silty clay loam	11	68	31	1.82	1.05	8.1
Houston black clay	Clay	19	38	43	2.60	1.51	8.1

†Percentage of organic carbon (OC) based on percentage organic matter (OM):
  %OC = 0.58 × OM (Weber et al., 2004).

5.2.2. Column Experiments

Soils were lightly packed into 7.62 cm diameter stainless steel columns fitted with a screen over the top to allow even water distribution during additions and a screen and layer of cheese-cloth at the bottom to retain soil. A funnel lay attached to the bottom of each column provided leachate collection. Soil depths were 1 cm for the Weswood and Houston Black clay and 3 cm for the Tremona.

Prior to estrone application, each soil column underwent a saturation and flushing procedure with 0.01 M $CaCl_2$ solution. Estrone additions consisted of 10 mL of a 0.03 µg mL^{-1} solution comprised of radiolabeled [4-^{14}C]-estrone (2035 MBq mmol^{-1}) purchased from American Radiolabeled Chemicals, Inc. (St. Louis, MO). This 0.3 µg total estrone quantity represented a typical irrigation event using anaerobic lagoon

effluent from a dairy operation (Raman et al., 2004). After this single pulse addition applied evenly over the top screen, the column was constantly flushed with 0.01 M CaCl$_2$ in an attempt to maintain a hydraulic head of 5 mm. For every 10 mL of leachate, 3 mL of effluent was drawn and mixed with 3 mL of Ecolte (+) liquid scintillation cocktail. A Beckman 6500 liquid scintillation spectrometer operated in the auto-disintegrations per minute mode analyzed ^{14}C content in each sample, correcting for counting efficiency.

5.2.3. Modeling

Perhaps Darcy's Law represents the best known description of water movement in saturated systems; however, this model principally considers saturated systems. Buckingham modified Darcy's Equation to account for hydraulic conductivity of unsaturated soil as a function of media water content, as opposed to a constant value as under saturated conditions, to yield:

$$q_W = -K(\psi)\frac{\partial H}{\partial z} \tag{5.1}$$

where q_W is the water flow rate (L T^{-1}), $K(\psi)$ is the unsaturated hydraulic conductivity and H equals the total hydraulic head comprised of the pressure head (ψ) and elevation head (z).

Since the vadose zone is a dynamic system, the transient water flow necessitates a more robust expression. Combining water conservation with the Buckingham-Darcy flux equation, the Richards Equation describes water flow by (in one of several forms):

$$\nabla \cdot K(\psi)\nabla H = C(\psi)\frac{\partial \psi}{\partial t} \qquad (5.2)$$

where the symbol ∇ represents a gradient, $C(\psi)$ is the specific moisture capacity and t is time.

A number of hydraulic mechanisms dictate subsurface contaminant transport in soils. Advection represents the primary factor, describing the mass transport of a pollutant due to water flow. Mechanical dispersion, which results from mixing due to localized variations in the flow velocity, further impacts contaminant transport, while molecular movement results in a degree of diffusion. Hydrodynamic dispersion describes both mechanical dispersion and diffusion, as these two processes are often difficult to describe separately.

In the case of non-conservative pollutants, incorporation of a retardation factor can account for sorption to the soil matrix. Using the partitioning coefficient (K_d), the retardation factor (R_f) can be determined by:

$$R_f = V_c/V = 1 + (\rho_b/\theta)\, K_d \qquad (5.3)$$

where Vc/V is the ratio of the chemical velocity relative to the mean pore-water velocity, ρ_b is the bulk density of the media and θ is the volumetric water content. Including a decay constant (λ) the advection-dispersion equation becomes:

$$\nabla \cdot D'\nabla C - \nabla \cdot CV = R_f \frac{\partial C}{\partial t} + \lambda C \tag{5.4}$$

where D′ is the hydrodynamic dispersion coefficient and C is the aqueous contaminant concentration.

The software package HYDRUS-1D incorporates each of these aspects of water flow and contaminant movement to simulate transport in the vadose zone. Due to this applicability, HYDRUS-1D (version 2.02) was selected to simulate estrone migration through the soil columns. This finite element model calculates solute movement based on advective-dispersion principals in conjunction with the Richard's equation for variably saturated water flow. Provisions for nonequilibrium sorption and first-order degradation allow for incorporation of the distribution coefficients and mineralization rates calculated earlier for each soil (Šimůnek et al., 2005).

Due to unforeseen difficulties encountered during the soil column experiment, hypothetical simulations designed to mimic estrone migration in a field setting replaced modeling the soil column results as originally intended. Theoretical soil profiles modeled in HYDRUS-1D allowed comparison of estrone leaching through different soils and analysis of the impact of soil layers on solute transport.

The selected soil hydraulic model consisted of the van Genuchten-Mualem single porosity archetype with no hysteresis (Šimůnek et al., 2005). Default values for loamy

sand, silty clay loam and clay from the soil catalog represented the water flow parameters (e.g., porosity, hydraulic conductivity, etc.) for the Tremona, Weswood and Houston Black clay soils, respectively. A constant 1-cm pressure head characterized the upper water flow boundary condition while a seepage face denoted the lower boundary condition. Initial moisture conditions for the soil profile were -100 cm. A 1-min pulse addition of a 0.00037 mmol mL^{-1} (100 μg mL^{-1}) estrone solution was applied at the beginning of the simulation. A 0.1-m dispersivity was selected and diffusion was assumed negligible. Table 5.2 indicates the soil specific parameters used in the model as determined in the previous sections. A 1-m profile scenario represented each soil was simulated in addition to a run considering 0.9 m loamy sand overlying 0.1 m a clay pan.

Table 5.2. Adsorption and microbial decay coefficients used in HYDRUS-1D simulations.

Soil equivalent	Textural class	K_d[†]	λ[‡]
		mL mmol^{-1}	d^{-1}
Tremona	Loamy sand	0.55	0.10
Weswood	Silty clay loam	0.87	0.07
Houston Black clay	Clay	1.13	0.05

[†]Linear partition coefficient.
[‡]First-order transformation rate constant.

5.3. RESULTS AND DISCUSSION

5.3.1. Column Experiments

Attainment of breakthrough curves describing leachate (i.e., outflow) concentration over time proved difficult for this experimental setup due to the high estrone partition coefficients for Weswood silty clay loam and Houston Black clay. Furthermore, calculated hydraulic conductivities were slow (0.023 and 0.011 cm min^{-1} for Weswood and Houston Black clay, respectively). Surprisingly, the silty clay loam displayed slower water flow than the clay. This phenomenon was attributed to an observed sheen that looked as though the soil surface had formed a slick seal. Analysis failed to detect any ^{14}C in the collected leachate for these two soils.

Tremona loamy fine sand exhibited extremely fast hydraulic conductivity (too rapid to calculate) and advective flow governed estrone transport to yield the breakthrough curve shown in Figure 5.1. A mass balance revealed 44% estrone recovery after 7.5 pore volumes, but a greater amount would likely have eluted had more water been flushed through the soil column. The maximum concentration recorded in the leachate was 0.007 μg mL^{-1}, occurring after 2.4 pore volumes.

An additional factor that likely contributed to the rapid breakthrough curve displayed by the loamy fine sand entails the intrinsically lower sorption reaction rates which led to curtailed retardation factors. The smaller estrone affinity for the coarse textured soil allowed for less retention of estrone in the soil profile and higher breakthrough concentrations. In contrast, clay soils impart greater retardation due to

enhanced estrone binding, represented by the greater K_d values, which in turn impedes mobility.

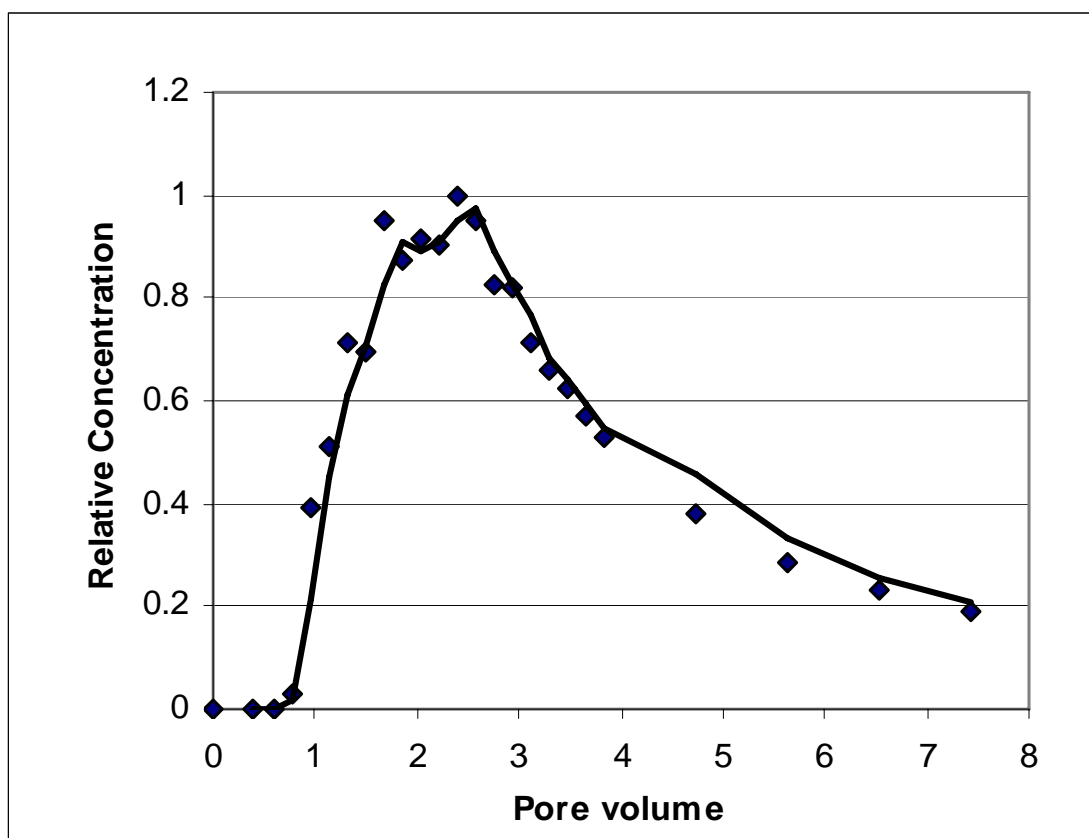**Fig. 5.1. Estrone breakthrough curve for Tremona loamy fine sand.**

Detection of radiolabeled ^{14}C prior to the passage of a pore volume indicates estrone actually moved faster through the column than the average water velocity. This more rapid estrone transmittance may result from a degree of preferential flow. On the fine scale inherent to column studies, particularly analyses considering homogeneous

soils, preferential flow paths primarily represent pore spaces that manifest high connectivity formed by irregular packing of the media. This modification of vertically-percolating water flow would accordingly lead to altered results, perhaps inducing the irregular shaped breakthrough curves observed here.

Preferential flow similarly transpires under unstable flow conditions that promote wetting front fragmentation into distinct narrow-flow channels. These areas of localized flux can exhibit significantly higher flow rates than the average of a uniform front, since these discrete fingers act as conduits (Jury et al., 1991). This phenomenon appears most prevalently in sandy soils, as course-textured matrices demonstrate significantly greater hydraulic conductivity differences between the wet and dry state compared to fine-textured soils (Kung, 1990).

Interpretation of the early and asymmetric breakthrough curve exhibited by the loamy fine sand may provide insight into the transport mechanisms acting on estrone, particularly if the actual solute breakthrough would precede that projected by models. Breakthrough curves that exhibit behavior deviant from idyllic expectations denote nonequilibrium processes that comprise time-dependent reactions in addition to the prevailing advection-dispersion processes (Bajracharya and Barry, 1997). One corresponding mechanism includes a time-dependent sorption reaction that may signify intra-particle diffusion, such as inclusion into organic matter. The K_{OC} values determined indicate a pronounced affinity of estrone with organic carbon for the Tremona soil, which exhibits only 10% silt and 9% clay composition. The preponderance of coarse textured particles suggests sorption depends on binding to

organic matter, whereas alternative binding sites associated with greater clay content may exist in the other two soils (Lai et al., 2000). Previous analysis revealed sorption equilibrium times approached 3 days for these soils. Therefore, the sorption occurring in this experiment likely represents more rapid estrone binding through "adsorption" surface mechanisms, whereas slower "absorption" processes that incorporate estrone into organic matter probably remain incomplete during the relatively short transport time which took several hours (Stevenson, 1994). Such a scenario would thus yield lower effective partition coefficients for the column systems than those found by the batch equilibrium technique. Further investigation into specific binding mechanisms may elucidate estrone soil sequestration that influences long-term persistence.

5.3.2. HYDRUS-1D Modeling

Breakthrough curves generated by HYDRUS-1D varied substantially between soil type in terms of both time and maximum estrone effluent concentration (Figure 5.2; Table 5.3). The results imply an inverse relation between theoretical water flow and sorption for these soils, likely attributed in part to innate clay characteristics. The minuscule spacing between platelets of fine textured soils yield low hydraulic conductivities under normal conditions, while large surface-to-volume ratios concurrently provide numerous binding sites as previously seen.
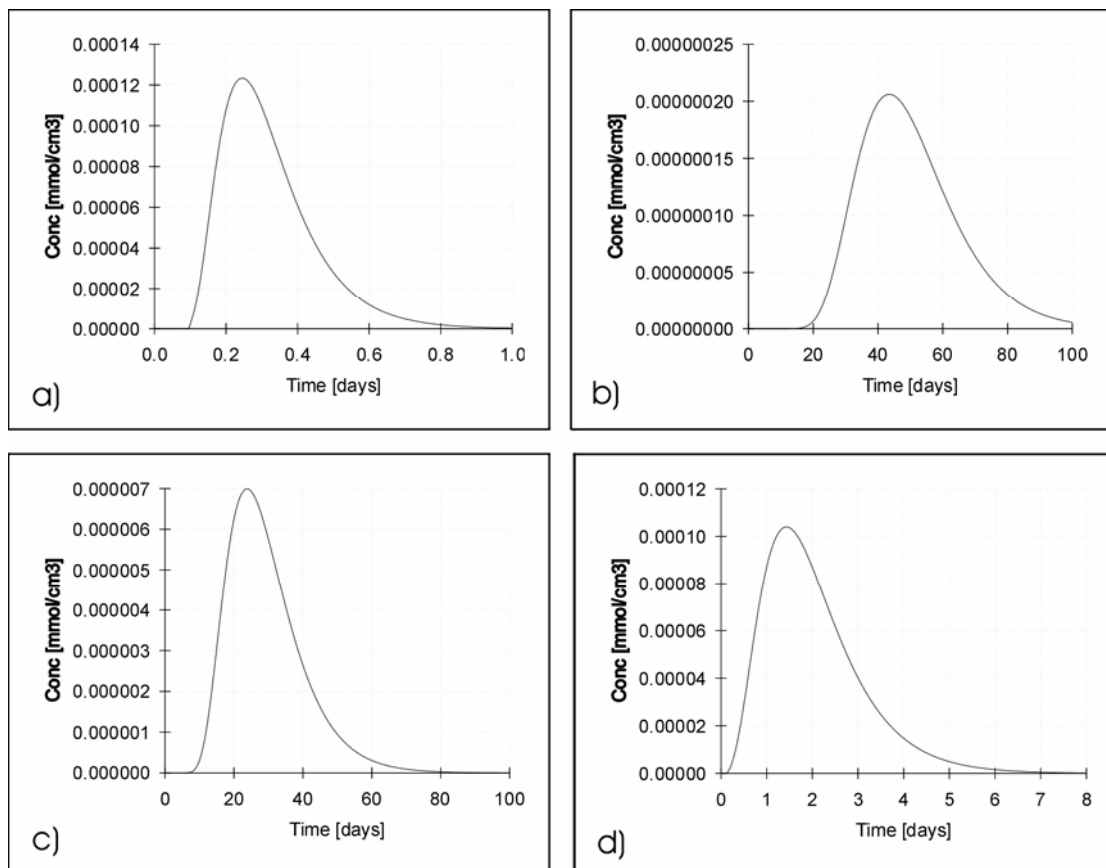
Fig. 5.2. Theoretical breakthrough curves for a 1-min pulse of 100 μg L^{-1} estrone solution applied to a 1-m thick soil profile of a) loamy sand, b) silty clay loam, c) clay and d) 0.9 m loamy fine sand overlying 0.1 m clay pan.

Table 5.3. Maximum concentration and time until maximum for theoretical estrone breakthrough curves projected by HYDRUS-1D.

Soil	Maximum concentration[†]	Time until maximum
	$\mu g\ mL^{-1}$	d
Loamy fine sand	37.8	0.25
Silty clay loam	0.056	43.5
Clay	1.89	23.5
0.9 m loamy sand over 0.1 m clay	28.1	1.4

[†] Concentrations converted from mmol mL^{-1} units used in HYDRUS-1D.

The slower breakthrough curve progression and lower concentration for the silty clay loam compared to the clay soil was counterintuitive, as the higher K_d value for the clay would be expected to yield greater estrone retardation. Furthermore, the comparably larger estrone quantity not sorbed by the silty clay loam would unlikely degrade sufficiently to inhibit estrone migration more than in the clay since the silty clay loam exhibits only a marginally greater decay rate. However, the HYDRUS-1D soil catalog revealed the saturated hydraulic conductivities as 1.68 and 4.8 cm d^{-1} for the silty clay loam and clay, respectively. Although clays typically display lower hydraulic conductivities than other soil types (Hillel, 1998), in this instance the clay soil has a hydraulic conductivity almost 3-fold greater than the silty clay loam. Anecdotal evidence noted during the failed column experiments with the Weswood and Houston Black clay soils support such a situation; the observation may be due to ped formation in the clay that led to soil aggregates and associated preferential flow paths. Therefore, the estrone leaching potential appears to be greater in clay soils than silty clay loams. Clay

desiccation occurring in dry environments may result in crack-induced preferential flow pathways, exacerbating this discrepancy.

Although the loamy fine sand reached a maximum outflow leachate concentration 175-fold faster than the silty clay loam, the shapes of the breakthrough curves were basically the same sigmoidal shape. Finer-textured soils tend to deviate from symmetry more than coarse-textured material (Hillel, 1998), but HYDRUS-1D eliminates the inherent structural features during simulation and the resulting homogeneity will idealize the curves. This similarity in shape signifies that the K_d values used in the model do not introduce variations in the retreating slope of the breakthrough curve, only differences in the retardation factor. However, the idyllic nature of the model likely imparted at least partially to these symmetrically-shaped breakthrough curves, as Figure 5.1 indicated an asymmetric shape under actual conditions.

Layered soil profiles, such as sand overlying a clay pan, represent typical circumstances found in lands receiving AFO lagoon effluent. Estrone migration modeled through a 0.9-m thick loamy sand layer overlying 0.1 m of clay exhibited a 5-fold slower breakthrough time compared to a 1-m loamy sand profile; however, maximum concentrations were on the same order. Infiltration rates typically diminish in systems comprised of multiple layers due to variations in texture and permeability (Jury et al., 1991). When a coarse soil (e.g., sand) overlies a fine soil (e.g., clay) infiltration declines due directly to restricted permeability. On the contrary, the greater permeability inherent to a coarse soil situated beneath a fine soil propagates a low matric potential

that restrains water from filling the more conductive pores, resulting in a smaller unsaturated conductivity than the saturated conductivity of the overlying fine textured medium. Although the matric inhibition eventually wanes and allows for increased infiltration, the new rate remains lower than the isolated fine soil. Furthermore, prior to the system reaching matric equilibrium, the accumulated water at the interface may induce unstable flow due to the wetting front progressing irregularly into the coarse soil layer, resulting in discrete fingers that act as conduits (Hillel, 1998).

Estrone concentrations found in the modeled soils after completion of the respective breakthrough curves indicate sorbed estrone predominates lower in the profile for the loamy sand compared to the silty clay loam and clay where the bound estrone remains in the top 0.2 m (Figure 5.3). The competing mechanisms of advective movement and adsorption explain this phenomenon, as rapidly infiltrating water will transport contaminants further into the soil profile before sorption becomes more dominant. On the other hand, the low hydraulic conductivity of the silty clay loam and clay allow for adsorption to predominate in the upper layers. This has implications in the field, as even though estrone strongly adsorbs to soils containing greater clay content the compound remains near the soil surface and more available for over-land flow in association with erosion.
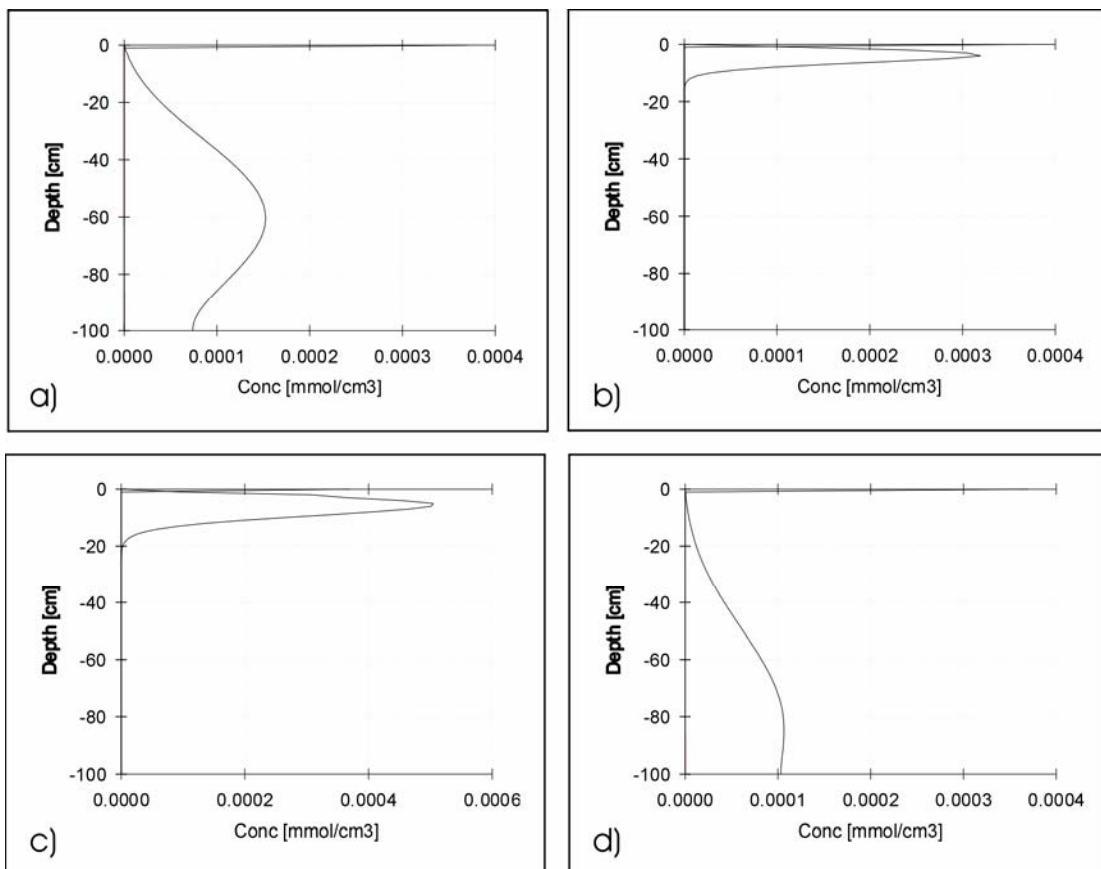
Fig. 5.3. Theoretical soil profile concentrations following completion of breakthrough curves for a 1-min pulse of 100 µg L^{-1} estrone solution applied to a 1-m thick soil profile of a) loamy sand, b) silty clay loam, c) clay and d) 0.9 m loamy fine sand overlying 0.1 m clay pan.

5.4. CONCLUSIONS

Soil profiles modeled as a single-continuum inadequately capture the heterogeneity and nonequilibrium processes inherent to natural systems, necessitating further examination of the retardation-preferential flow relationship under disparate field conditions. The constant head applied over saturated conditions represents special cases, such as low lying areas exhibiting high water tables or after a prolonged precipitation event. Although controlled modeling conditions prevent direct transfer of results to field scenarios, these findings contribute to the limited research that incorporates estrone transport parameters into models describing mobility in soil systems.

It became clear in both the column experiment and modeling exercise that sandy soil presents significant leaching potential which may lead to breakthrough of estrogenic compounds from the root zone and introduction into groundwater. On larger field scales, preferential pathways emanate from animal and worm burrows, root channels and desiccation cracks in the upper soil horizons, along with fractures, faults and karst features in deeper soil and rock formations. These preferential flow conduits can promote rapid downward seepage and connect the surface more directly to underlying water tables, especially in the presence of shallow groundwater. Therefore, the flow demonstrated here likely underestimates transport under field conditions with prominent preferential flow attributes. Furthermore, estrogen associated with highly adsorptive soils appears to accumulate near the soil surface and may experience overland flow if erosion occurs. Thus, distinct potential for estrogen contamination of surface and groundwater systems exists on a site-specific basis.

6. SUMMARY: IMPLICATIONS AND FUTURE RESEARCH NEEDS

6.1. APPLICABILITY TO AGRICULTURAL OPERATIONS

Concern over health risks arising from environmental estrogen contamination has promoted increased attention to these compounds by academia, government and private citizens. Vertebrate development requires the endogenous hormones estrone and 17β-estradiol, but increases above ambient concentrations can induce endocrine disruption that may lead to reproductive disorders and cancer proliferation. This paradox precludes poultry and livestock producers from avoiding controversy surrounding these steroids, as concentrated animal populations excrete significant estrogen loads. Despite growing interest in manure-borne hormones, research remains limited and conflicting findings pervade the literature. This paucity of data creates impetus to more rigorously examine estrogen fate and transport processes to alleviate latent calls for regulatory action prior to adequate risk characterization. Therefore, this dissertation strove to examine several different mechanisms impacting estrone and 17β-estradiol behavior in an agricultural context.

Although much research remains, this study produced salient insight into estrone and 17β-estradiol photolysis, sorption-desorption, mineralization and leaching potential. This discernment primarily involves the following findings:

1) Photolysis at pH 7, representative of an anaerobic lagoon, resulted in half-lives on the order of 27 to 35 days.

2) Estrogen sorption exhibited greater partition coefficients in soils containing higher organic carbon and clay content.

3) Once sorbed, binding appeared largely irreversible with minimal desorption.

4) The coarse textured soil analyzed demonstrated greater mineralization than the finer textured soils.

5) Dairy lagoon effluent additions to soil neither exacerbated nor mitigated estrogen mineralization in most cases. No lag phase occurred before degradation began.

6) Sandy soil displayed significant leaching potential in both soil columns and theoretical models.

7) No leaching occurred in the soil columns containing the loamy and clay soils and the theoretical models indicated accumulation near the soil surface following land application.

Preliminary guidelines can be derived from this information to assist animal feeding operations (AFOs) in limiting estrogen contamination of adjoining waterbodies due to land application of manure and process generated wastewater.

These results indicate that neither estrone nor 17β-estradiol likely present a propensity for subsurface migration in finer textured loamy and clay soils, as both compounds displayed a high affinity for the soil material and exhibited limited leaching potential in the soil columns and modeling exercise. However, this does not preclude off-site transport to waters of concern. On larger field scales preferential pathways emanate from animal and worm borrows, root channels and desiccation cracks in the

upper soil horizons, along with fractures, faults and karst features in deeper soil and rock formations. The resultant preferential flow conduits can promote rapid downward seepage and a direct hydraulic connection with the underlying water table, especially in the presence of shallow groundwater. Therefore, the flow demonstrated here likely underestimates transport under field conditions exhibiting prominent preferential pathways. Moreover, lower mineralization rates may indicate a greater persistence in finer textured soils. Since estrone and 17β-estradiol sorb strongly to soils they may not be bioavailable, but erosion induced particle transport may present a surface migration mechanism for bound estrogens. Estrone accumulation in the upper soil horizon during leaching simulations may accentuate this overland transport process.

On the other hand, sandy soil demonstrated a higher mineralization rate than the loamy and clay soils, probably due to the greater bioavailability associate with smaller partition coefficients. However, this enhanced degradation does not reduce rapid estrone mobility in the soil column and modeling scenario. Advective transport dominates estrone migration in soils with high hydraulic conductivity. Sandy soils thus present a significant estrogen leaching potential which may lead to breakthrough from the root zone into groundwater systems.

Implications for AFOs primarily involve best judgment during manure application. Producers should avoid pasture or crop irrigation with effluent containing nutrients and estrogens in locations characterized by shallow water tables or near surface waters. Management should evaluate site specific criteria, such as soil characteristics, to assess areas at risk. For instance, sandy soils will likely promote migration to

waterbodies in close proximity. Altered application rates and durations may also limit estrogen mobility. Similarly, timing presents an important consideration. Application prior to anticipated precipitation events may prove unsound, as estrogen will not have had time to sorb to the soil, which will promote overland flow via surface runoff. Likewise, producers in colder regions should avoid applying manures on frozen ground or snow-pack, since this also encourages overland transport. Essentially, basic best management practices (BMPs) intended for manure application or designed to limit organic agrochemical migration will likely work well for limiting contamination by estrogenic agents. Since microbial inoculation via dairy lagoon effluent additions essentially imparted no effect on mineralization and no lag phase occurred, this finding likely indicates operators need not be concerned with indigenous microbial populations requiring special treatment. In addition, AFOs may seek to reduce the estrogen load by limiting estrogenic supplements when appropriate.

At this juncture, the information collected prevents much speculation on treatment processes or estrogen behavior in anaerobic lagoons and other manure storage structures. Although estrone appears more susceptible to photodegradation than 17β-estradiol, the increased resistance to microbial mineralization may increase its persistence in anaerobic lagoons. The high organic matter inherent to anaerobic lagoon constituents likely presents an inhibiting parameter to photolysis that would decrease the influence of this degradation mechanism during wastewater processing. Furthermore, high microbial activity dominates in these treatment systems. Once in natural waters, photochemical decomposition may play a greater role in estrogenic fate. Diluted

wastewaters and aquatic ecosystems containing more diffuse microbial populations may alter the degradation dynamic.

6.2. POTENTIAL FUTURE RESEARCH

Increasing concerns for potential adverse human and ecological health effects resulting from hormone exposure warrants further research on the environmental fate and transport of estrone and 17β-estradiol. Key factors that demand consideration include a more thorough examination of breakdown pathways and characterization of metabolite behavior. Although such general analysis helps elucidate potential risk arising from the release of these bioactive compounds, AFOs-specific aspects require particular attention due to limited research on associated steroidal loads. Better determination of average estrogen concentrations in livestock waste-streams and further investigations into the influence of natural (e.g., temperature, anaerobic conditions) and anthropogenic (e.g., manure application methods, erosion control) parameters from an agricultural perspective will help delineate the true contribution of environmental estrogens by poultry and livestock operations. The following section outlines a fraction of the myriad research opportunities that exist in this context.

Although photolysis has been established as a degradative mechanism that acts on estrone and 17β-estradiol, the extent that this process influences the fate of these estrogenic agents in natural and engineered settings remains ambiguous. Clearly, photodegradation rates under sunlit conditions require greater attention, since illumination by artificial light sources fails to embody the large wavelength spectrum

that would interact with estrogens in the environment. Quantum yield, a parameter that relates the number of molecules reacting to the number of photons absorbed, permits an assessment of direct photolysis under disparate light intensities and conditions. Accurate determination of estrone and 17β-estradiol quantum yields will allow scientists to project varying photolysis rates in a water column due to light attenuation, as well as at different regional locations. Light-induced decomposition in natural waters and various waste-streams (e.g., primary anaerobic lagoons) may help identify potential factors that enhance or interfere with estrogen photolysis. Assessment of indirect photolysis, degradation due to energy transfer from one excited species to another, will provide insight into whether organic matter imparts a synergistic or antagonistic influence on photolysis. This finding pertains especially to AFOs generated waste-steams due to the inherently high organic matter content which may convey energy to other chemical species. Determining estrogen photolysis on soil surfaces will discern decomposition subsequent to land application of treatment lagoon effluent, a mechanism that may play a significant role in the fate of estrone and 17β-estradiol.

The estrogen sorption-desorption dynamic presents a principal mechanism controlling mobility and bioavailability. The fundamental role sorption confers on estrone and 17β-estradiol transport dictates determination of partition coefficients across a wide assortment of agricultural soils. The current catalog documenting linear and Freundlich parameters depicts a small soil subset which requires a more expansive representation. Moreover, sorption characteristics inherent to differing mineralogical types implore inspection, as disparity may exist between constituents within a soil class

(e.g., kaolinite versus montmorillonite clays). A larger data set also allows one to identify correlations with different physicochemical soil properties that may offer an indication of specific binding mechanisms. More rigorous analytical chemistry will similarly help discriminate the particular sorption mechanisms acting on estrone and 17β-estradiol and their temporal changes.

Although this study determined minimal estrogen desorption, examination of this phenomenon over a range of environmental conditions may reveal potential release of bound compounds in certain circumstances. Such a situation would not only alter chemical mobility, but could also make previously immobilized estrogenic agents bioavailable. Quantification of colloidal sorption coefficients presents an additional research opportunity. These minute particles (diameters <10 μm) essentially act as a third stage that bridges the distinction between dissolved and solid particle forms. Colloids typically remain in solution and can enhance contaminant transport in subsurface systems. Therefore, estrogens association with colloids may demonstrate increased mobility even in a sorbed state. All of these aspects of sorption chemistry contribute to the body of knowledge that will be drawn on by future models designed to assess off-site transport and exposure pathways following land application of estrogen-laden manures.

Microbial transformation plays a governing role in the persistence of AFOs generated estrogens, but inadequate characterization of this degradative mechanism across varied conditions currently exists. Environmental determinants, including but not limited to temperature, moisture, salinity, nutrient availability and redox potential, solicit

further examination. Metabolite determination also requires attention, as some estrone and 17β-estradiol breakdown products continue to exhibit estrogenic behavior. The use of mineralization in this study alleviated this uncertainty; however, degradation to a stable but non-estrogenic intermediate may prove a more accurate standard for assessing exposure to endocrine disrupting chemicals. Degradation to a benign metabolite likely occurs more rapidly and completely than mineralization to CO_2. Investigation of microbial transformation in process generated wastewater will further explain processes influencing estrone and 17β-estradiol fate. Analysis of microbial degradation in anaerobic lagoons, coupled with facility-wide estrogen mass balance calculations, may reveal significant elimination o f excreted estrogens during effluent storage and treatment.

Rigorous environmental risk assessments considering livestock facilities necessitate application of accurate models across a range of conditions; however, current simulation of estrogen transport remains limited to only a few researchers. Although incomplete data on the environmental behavior of these compounds inhibits extensive extrapolation to real-world settings, as scientists obtain more laboratory and field data, ongoing model development can compliment these efforts. Predictive models entail formulating an operative conceptual framework comprised of parameters that adequately describe spatial and temporal trends and variability. The developmental process for creating effective mathematical models takes time and rudimentary attempts alleviate this lengthy progression.

The small number of modeling attempts leaves this area wide open to future labor. A basic tenet that deserves consideration includes incorporation of heterogeneity into solute transport simulations. Models need to expand on the one-dimensional, homogenous approach since transfer of results to a field setting will be unrealistic. Preferential and anisotropic flow represents characteristics innate to contaminant migration in heterogeneous soils. Considering the large travel times present under field conditions compared to sorption kinetic time scales, preferential flow paths likely dominate the estrogen transport dynamic. Investigation of different temporal scales presents another modeling aspect needing refinement. Intermittent flow analysis will provide a closer simulation of estrone and 17β-estradiol transport under field conditions where periodic irrigation and evapotranspiration processes lead to redistribution of the moisture regime for surface soils between precipitation events. Although it is presently impractical to include most of these suggestions, this partial list outlines some future needs for development of accurate predictive tools.

Inclusion of field studies will provide not only a more realistic depiction of estrogen behavior in the environment, but will also serve to validate model simulations using parameters ascertained in the laboratory. For instance, field studies considering phyto-degradative mechanisms will likely observe enhanced rhizosphere microbial activity due to increased water flow and gas diffusion through macropores. Estrogenic compounds incorporated into the soil during manure application may thus exhibit a corresponding increase in microbial degradation over laboratory rates, thus allowing for corrections in model parameters. Furthermore, field studies provide a venue for testing

conceptualized BMPs and engineering treatment designs derived from assimilated laboratory data and model projections. Examples may include altering anaerobic lagoon management criteria to maximize microbial degradation or the use of highly adsorptive industrial clays as an innovative treatment technology for estrogen immobilization.

A more robust investigation of estrogen behavior that integrates laboratory and field experiments will elucidate the ultimate fate of these compounds and allow for BMP development intended to minimize human and wildlife exposure. In addition to estrone and 17β-estradiol, other natural hormones (e.g., testosterone, the principle make sex hormone), synthetic steroids (e.g., trenbolone, a bovine growth promoter) and pharmaceuticals (e.g., tetracyclines, a class of antibiotics) are now beginning to receive attention as environmental pollutants. Thus, the knowledge gained through estrogen research will provide a basis to analyze future emerging contaminants generated by livestock operations. Viewed through this prism, AFOs will likely be able to decrease the hormonal load entering the environment while avoiding unnecessary alterations to the current manure management archetype. Such an approach corresponds with the evolving paradigm of sustainable management that aspires to maintain and restore ecological integrity while allowing for compatible agricultural profitability.

REFERENCES

Bajracharya, K., and D.A. Barry. 1997. Nonequilibrium solute transport parameters and their physical significance: Numerical and experimental results. J. Contam. Hydrol. 24:185-204.

Ball, S., K. Polson, J. Emeny, W. Eyestone, and R.M. Akers. 2000. Induced lactation in prepubertal Holstein heifers. J. Dairy Sci. 83:2459-2463.

Beconi, M.G., M.D. Howard, T.D.A. Forbes, R.B. Muntifering, N.W. Bradley, and M.J. Ford. 1995. Growth and subsequent feedlot performance of estradiol-implanted vs nonimplanted steers grazing fall-accumulated endophyte-infested or low-endophyte tall fescue. J. Anim. Sci. 73:1576-1584.

Belfroid, A.C., A. Van der Horst, A.D. Vethaak, A.J. Schafer, G.B.J. Rijs, J. Wegener, and W.P. Cofino. 1999. Analysis and occurrence of estrogenic hormones and their glucuronides in surface water and waste water in The Netherlands. Sci. Total Environ. 225:101-108.

Bjolseth, I.M., D.M. Quanrud, M. Karpiscak, W.P. Ela, K.E. Lansey, R.G. Arnold. 2003. Fate of estrogenic activity during wetland treatment of wastewater effluent and stormwater runoff. *In* 11th Biannual Symposium on the Groundwater Recharge, Phoenix, AZ. June 5-7.

Blumhorst, M.R. 1996. Experimental parameters used to study pesticide degradation in soil. Weed Tech. 10:169-173.

Callantine, M.R., M. Stob, and F.N. Andrews. 1961. Fecal elimination of estrogens by cattle treated with diethylstilbestrol and hexestrol. Am. J. Vet. Res. 22:462-465.

Cargouet, M., D. Perdiz, A. Mouatassim-Souali, S. Tamisier-Karolak, and Y. Levi. 2004. Assessment of river contamination by estrogenic compounds in Paris area (France). Sci. Total Environ. 324:55-66.

Carlsen, E., A. Giwercman, N. Keiding, and N.E. Skakkebaek. 1995. Declining semen quality and increasing incidence of testicular cancer - Is there a common cause? Environ. Health Persp. 103:137-139.

Casey, F.X.M., G.L. Larsen, H. Hakk, and J. Simunek. 2003. Fate and transport of 17 beta-estradiol in soil-water systems. Environ. Sci. Technol. 37:2400-2409.

Casey, F.X.M., J. Simunek, J. Lee, G.L. Larsen, and H. Hakk. 2005. Sorption, mobility, and transformation of estrogenic hormones in natural soil. J. Environ. Qual. 34:1372-1379.

Chang, S., T.D. Waite, A.I. Schafer, and A.G. Fane. 2003. Adsorption of the endocrine-active compound estrone on microfiltration hollow fiber membranes. Environ. Sci. Technol. 37:3158-3163.

Chang, S., T.D. Waite, P.E.A. Ong, A.I. Schafer, and A.G. Fane. 2004. Assessment of trace estrogenic contaminants removal by coagulant addition, powdered activated carbon adsorption and powdered activated carbon/microfiltration processes. J. Environ. Eng. 130:736-742.

Chiou, C.T., P.E. Porter, and D.W. Schmedding. 1983. Partition Equilibria of Nonionic Organic Compounds between Soil Organic Matter and Water. Environ. Sci. Technol. 17:227-231.

Coleman, H.M., B.R. Eggins, J.A. Byrne, F.L. Palmer, and E. King. 2000. Photocatalytic degradation of 17-beta-oestradiol on immobilised TiO_2. Appl. Cat B-Environ. 24:L1-L5.

Coleman, H.M., E.J. Routledge, J.P. Sumpter, B.R. Eggins, and J.A. Byrne. 2004. Rapid loss of estrogenicity of steroid estrogens by UVA photolysis and photocatalysis over an immobilised titanium dioxide catalyst. Water Res. 38:3233-3240.

Colucci, M.S., and E. Topp. 2002. Dissipation of part-per-trillion concentrations of estrogenic hormones from agricultural soils. Can. J. Soil Sci. 82:335-340.

Colucci, M.S., H. Bork, and E. Topp. 2001. Persistence of estrogenic hormones in agricultural soils: I. 17 beta-estradiol and estrone. J. Environ. Qual. 30:2070-2076.

Crews, D., J.M. Bergeron, and J.A. McLachlan. 1995. The role of estrogen in turtle sex determination and the effect of PCBs. Environ. Health Persp. 103:73-77.

Curran, W.S., M.M. Loux, R.A. Liebl, and F.W. Simmons. 1992. Photolysis of imidazolinone herbicides in aqueous solution and on soil. Weed Sci. 40:143-148.

Das, B.S., L.S. Lee, P.S.C. Rao, and R.P. Hultgren. 2004. Sorption and degradation of steroid hormones in soils during transport: Column studies and model evaluation. Environ. Sci. Technol. 38:1460-1470.

Dickson, R.B., M.E. McManaway, and M.E. Lippman. 1986. Estrogen-induced factors of breast cancer cells partially replace estrogen to promote tumor growth. Science. 232:1540-1543.

Dyer, A. R., D.R. Raman, M.D. Mullen, R.T. Burns, L.B. Moody, A.C. Layton, G.S. Sayler. 2001. Determination of 17β-estradiol concentrations in runoff plots receiving dairy manure. ASAE Paper 01-2107. Am. Soc. Agric. Eng., St. Joseph, MI.

Enright, W.J., J.F. Quirke, P.D. Gluckman, B.H. Breier, L.G. Kennedy, I.C. Hart, J.F. Roche, A. Coert, and P. Allen. 1990. Effects of long-term administration of pituitary-derived bovine growth hormone and estradiol on growth in steers. J. Anim. Sci. 68:2345-2356.

Erb, R.E., B.P. Chew, and H.F. Keller. 1977. Relative concentrations of extrogen and progesterone in milk and blood, and excretion of estrogen in urine. J.Anim. Sci. 46:617-626.

Fine, D.D., G.P. Breidenbach, T.L. Price, and S.R. Hutchins. 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. J. Chrom. A. 1017:167-185.

Finlay-Moore, O., P.G. Hartel, and M.L. Cabrera. 2000. 17 beta-estradiol and testosterone in soil and runoff from grasslands amended with broiler litter. J. Environ. Qual. 29:1604-1611.

Folmar, L.C., M. Hemmer, R. Hemmer, C. Bowman, K. Kroll, and N.D. Denslow. 2000. Comparative estrogenicity of estradiol, ethynyl estradiol and diethylstilbestrol in an *in vivo*, male sheepshead minnow (Cyprinodon variegatus), vitellogenin bioassay. Aqu. Tox. 49:77-88.

Frank, M.P., P. Graebing, and J.S. Chib. 2002. Effect of soil moisture and sample depth on pesticide photolysis. J. Agric. Food Chem. 50:2607-2614.

Fujii, K., S. Kikuchi, M. Satomi, N. Ushio-Sata, and N. Morita. 2002. Degradation of 17 beta-estradiol by a gram-negative bacterium isolated from activated sludge in a sewage treatment plant in Tokyo, Japan. Appl. Environ. Microbiol. 68:2057-2060.

Grant, P.G., S.L. Lemke, M.R. Dwyer, and T.D. Phillips. 1998. Modified Langmuir equation for S-shaped and multisite isotherm. Langmuir. 14:4292-4299.

Gray, J.L., and D.L. Sedlak. 2005. The fate of estrogenic hormones in an engineered treatment wetland with dense macrophytes. Water Environ. Res. 77:24-31.

Hanselman, T.A., D.A. Graetz, and A.C. Wilkie. 2003. Manure-borne estrogens as potential environmental contaminants: A review. Environ. Sci. Technol. 37:5471-5478.

Hanselman, T.A., D.A. Graetz, and A.C. Wilkie. 2004. Comparison of three enzyme immunoassays for measuring 17 beta-estradiol in flushed dairy manure wastewater. J. Environ. Qual. 33:1919-1923.

Harrison, P.T.C., P. Holmes, and C.D.N. Humfrey. 1997. Reproductive health in humans and wildlife: Are adverse trends associated with environmental chemical exposure? Sci. Total Environ. 205:97-106.

Henricks, D.M., R.T. Brandt, E.C. Titgemeyer, and C.T. Milton. 1997. Serum concentrations of trenbolone-17 beta and estradiol-17 beta and performance of heifers treated with trenbolone acetate, melengestrol acetate, or estradiol-17 beta. J. Anim. Sci. 75:2627-2633.

Hense, B.A., G.F. Severin, G. Welzl, and K.W. Schramm. 2004. Effects of 17 alpha-ethinylestradiol on zoo- and phytoplankton in lentic microcosms. Anal. Bioanal.l Chem. 378:716-724.

Herschler, R.C., A.W. Olmsted, A.J. Edwards, R.L. Hale, T. Montgomery, R.L. Preston, S.J. Bartle, and J.J. Sheldon. 1995. Production responses to various doses and ratios of estradiol benzoate and trenbolone acetate implants in steers and heifers. J. Anim. Sci. 73:2873-2881.

Higashitani, T., H. Tamamoto, A. Takahashi, and H. Tanaka. 2003. Study of estrogenic effects on carp (Cyprinus carpio) exposed to sewage treatment plant effluents. Water Sci. Technol. 47:93-100.

Hillel, D. 1998. Environmental soil physics. Academic Press, San Diego.

Holthaus, K.I.E., A.C. Johnson, M.D. Jurgens, R.J. Williams, J.J.L. Smith, and J.E. Carter. 2002. The potential for estradiol and ethinylestradiol to sorb to suspended and bed sediments in some English rivers. Environ. Toxicol. Chem. 21:2526-2535.

Hutchinson, T.H., N.A. Pounds, M. Hampel, and T.D. Williams. 1999. Impact of natural and synthetic steroids on the survival, development and reproduction of marine copepods (Tisbe battagliai). Sci. Total Environ. 233:167-179.

Irwin, L.K., S. Gray, and E. Oberdorster. 2001. Vitellogenin induction in painted turtle, *Chrysemys picta*, as a biomarker of exposure to environmental levels of estradiol. Aqu. Tox. 55:49-60.

Jacobsen, A.M., A. Lorenzen, R. Chapman, and E. Topp. 2005. Persistence of testosterone and 17 beta-estradiol in soils receiving swine manure or municipal biosolids. J. Environ. Qual. 34:861-871.

Johnson, A.C., and J.P. Sumpter. 2001. Removal of endocrine-disrupting chemicals in activated sludge treatment works. Environ. Sci. Technol. 35:4697-4703.

Johnson, A.C., H.R. Aerni, A. Gerritsen, M. Gibert, W. Giger, K. Hylland, M. Jurgens, T. Nakari, A. Pickering, M.J.F. Suter, A. Svenson, and F.E. Wettstein. 2005. Comparing steroid estrogen, and nonylphenol content across a range of European sewage plants with different treatment and management practices. Water Res. 39:47-58.

Jones, L.A., and R.A. Hajek. 1995. Effects of estrogenic chemicals on development. Environ. Health Persp. 103:63-67.

Jurgens, M.D., K.I.E. Holthaus, A.C. Johnson, J.J.L. Smith, M. Hetheridge, and R.J. Williams. 2002. The potential for estradiol and ethinylestradiol degradation in English rivers. Environ. Toxicol. Chem. 21:480-488.

Jury, W.A., W.R. Gardner, and W.H. Gardner. 1991. Soil physics. 5th ed. John Wiley & Sons, New York.

Katagi, T. 2004. Photodegradation of pesticides on plant and soil surfaces. p. 1-189 *In* Reviews of environmental contamination and toxicology. Vol. 182. Springer-Verlag, New York.

Kimura, A., M. Taguchi, H. Arai, H. Hiratsuka, H. Namba, and T. Kojima. 2004. Radiation-induced decomposition of trace amounts of 17 beta-estradiol in water. Rad. Phys. Chem. 69:295-301.

Kloas, W., I. Lutz, and R. Einspanier. 1999. Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals *in vitro* and *in vivo*. Sci. Total Environ. 225:59-68.

Kolodziej, E.P., T. Harter, and D.L. Sedlak. 2004. Dairy wastewater, aquaculture, and spawning fish as sources of steroid hormones in the aquatic environment. Environ. Sci. Technol. 38:6377-6384.

Kung, K.J.S. 1990. Preferential flow in a sandy vadose zone. 2. Mechanism and implications. Geoderma. 46:59-71.

Lai, K.M., M.D. Scrimshaw, and J.N. Lester. 2002. The effects of natural and synthetic steroid estrogens in relation to their environmental occurrence. Crit. Rev. Toxicol. 32:113-132.

Lai, K.M., K.L. Johnson, M.D. Scrimshaw, and J.N. Lester. 2000. Binding of waterborne steroid estrogens to solid phases in river and estuarine systems. Environ. Sci. Technol. 34:3890-3894.

Larsen, G., F. Casey, B. Magelky, C. Pfaff, H. Hakk. 2001. Sorption, mobility and fate of 17 β-estradiol and testosterone in loam soil and sand. *In* Second International Conference on Pharmaceuticals and Endocrine Disrupting Chemicals in Water, Minneapolis, MN. October 9-11, 2001.

Lee, H.B., and D. Liu. 2002. Degradation of 17β-estradiol and its metabolites by sewage bacteria. Water Air Soil Pollut. 134:353-368.

Lee, L.S., T.J. Strock, A.K. Sarmah, and P.S.C. Rao. 2003. Sorption and dissipation of testosterone, estrogens, and their primary transformation products in soils and sediment. Environ. Sci. Technol. 37:4098-4105.

Liu, B., and X.L. Liu. 2004. Direct photolysis of estrogens in aqueous solutions. Sci. Total Environ. 320:269-274.

Ma, L.W., L.M. Southwick, G.H. Willis, and H.M. Selim. 1993. Hysteretic characteristics of atrazine adsorption-desorption by a Sharkey soil. Weed Sci. 41:627-633.

Mathur, R.S., and R.H. Common. 1969. A note on the daily excretion of estradiol-17 and estrone by the hen. Poultry Sci. 48:100-104.

Mersie, W., and C. Seybold. 1996. Adsorption and desorption of atrazine, deethylatrazine, deisopropylatrazine, and hydroxyatrazine on Levy wetland soil. J. Agric. Food Chem. 44:1925-1929.

Migliaccio, S., R.R. Newbold, J.A. McLachlan, and K.S. Korach. 1995. Alterations in estrogen levels during development affects the skeleton: Use of an animal model. Environ. Health Persp. 103:95-97.

Nakamura, M. 1984. Effects of estradiol-17β on gonadal sex differentiation in two species of salmonids, the masu salmon, *Oncorhynchus masou*, and the chum salmon, *O. Keta*. Aquaculture. 43:83-90.

Nakashima, T., Y. Ohko, D.A. Tryk, and A. Fujishima. 2002. Decomposition of endocrine-disrupting chemicals in water by use of TiO_2 photocatalysts immobilized on polytetrafluoroethylene mesh sheets. J. Photochem. Photobiol. A-Chem. 151:207-212.

Nash, J.P., D.E. Kime, L.T.M. Van der Ven, P.W. Wester, F. Brion, G. Maack, P. Stahlschmidt-Allner, and C.R. Tyler. 2004. Long-term exposure to environmental concentrations of the pharmaceutical ethynylestradiol causes reproductive failure in fish. Environ. Health Persp. 112:1725-1733.

Nghiem, L.D., A.I. Schafer, and M. Elimelech. 2004. Removal of natural hormones by nanofiltration membranes: Measurement, modeling, and mechanisms. Environ. Sci. Technol. 38:1888-1896.

Nichols, D.J., T.C. Daniel, P.A. Moore, D.R. Edwards, and D.H. Pote. 1997. Runoff of estrogen hormone 17β-estradiol from poultry litter applied to pasture. J. Environ. Qual. 26:1002-1006.

Nichols, D.J., T.C. Daniel, D.R. Edwards, P.A. Mooe, and D.H. Pote. 1998. Use of grass filter strips to reduce 17β-estradiol in runoff from fescue-applied poultry litter. J. Soil Water Conserv. 53:74-77.

Ohko, Y., K.I. Iuchi, C. Niwa, T. Tatsuma, T. Nakashima, T. Iguchi, Y. Kubota, and A. Fujishima. 2002. 17β-estrodial degradation by TiO_2 photocatalysis as means of reducing estrogenic activity. Environ. Sci. Technol. 36:4175-4181.

O'Loughlin, E.J., S.J. Traina, and G.K. Sims. 2000. Effects of sorption on the biodegradation of 2-methylpyridine in aqueous suspensions of reference clay minerals. Environ. Toxicol. Chem. 19:2168-2174.

Onda, K., Y. Nakamura, C. Takatoh, A. Miya, and Y. Katsu. 2003. The behavior of estrogenic substances in the biological treatment process of sewage. Water Sci. Technol. 47:109-116.

Orlando, E.F., A.S. Kolok, G.A. Binzcik, J.L. Gates, M.K. Horton, C.S. Lambright, L.E. Gray, A.M. Soto, and L.J. Guillette. 2004. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. Environ. Health Persp. 112:353-358.

Palmer, B.D., and S.K. Palmer. Vitellogenin induction by xenobiotic estrogens in the red-eared turtle and African clawed frog. Environ. Health Persp. 103:19-25.

Peterson, E.W., R.K. Davis, and H.A. Orndorff. 2000. 17 beta-estradiol as an indicator of animal waste contamination in mantled karst aquifers. J. Environ. Qual. 29:826-834.

Popp, J.D., T.A. McAllister, W.J. Burgevitz, R.A. Kemp, J.P. Kastelic, and K.J. Cheng. 1997. Effect of trenbolene acetate estradiol implants and estrus suppression on

growth performance and carcass characteristics of beef heifers. Canadian J. Anim. Sci. 77:325-328.

Raman, D.R., E.L. Williams, A.C. Layton, R.T. Burns, J.P. Easter, A.S. Daugherty, M.D. Mullen, and G.S. Sayler. 2004. Estrogen content of dairy and swine wastes. Environ. Sci. Technol. 38:3567-3573.

Rodriguez, C.S., A.M. Bongiovanni and L.C. Borrego. 1985. Effects of environment hormone contamination in Puerto Rico. *In*. J.A. McLachlan (ed). Estrogens in the environment II: Influences in development. Elsevier, New York.

Rosenfeldt, E.J., and K.G. Linden. 2004. Degradation of endocrine disrupting chemicals bisphenol A, ethinyl estradiol, and estradiol during UV photolysis and advanced oxidation processes. Environ. Sci. Technol. 38:5476-5483.

Routledge, E.J., D. Sheahan, C. Desbrow, G.C. Brighty, M. Waldock, and J.P. Sumpter. 1998. Identification of estrogenic chemicals in STW effluent. 2. *In vivo* responses in trout and roach. Environ. Sci. Technol.32:1559-1565.

Schafer, A.I., L.D. Nghiem, and T.D. Waite. 2003. Removal of the natural hormone estrone from aqueous solutions using nanofiltration and reverse osmosis. Environ. Sci. Technol. 37:182-188.

Schwarzenbach, R.P., P.M. Gschwend, and D.M. Imboden. 1993. Environmental organic chemistry. John Wiley & Sons, Inc., New York.

Segner, H., K. Caroll, M. Fenske, C.R. Janssen, G. Maack, D. Pascoe, C. Schafers, G.F. Vandenbergh, M. Watts, and A. Wenzel. 2003. Identification of endocrine-disrupting effects in aquatic vertebrates and invertebrates: report from the European IDEA project. Ecotoxicol. Environ. Safety. 54:302-314.

Sharer, M., J.H. Park, T.C. Voice, and S.A. Boyd. 2003. Aging effects on the sorption-desorption characteristics of anthropogenic organic compounds in soil. J. Environ. Qual. 32:1385-1392.

Shore, L.S., and M. Shemesh. 2003. Naturally produced steroid hormones and their release into the environment. Pure Appl. Chem. 75:1859-1871.

Shore, L.S., D.L. Correll, and P.K. Chakraborty. 1995. Relationship of fertilization with chicken manure and concentrations of estrogens in small streams, *In* K. Steele (ed.) Animal waste and the land-water interface. Lewis Publishers, Boca Raton.

Socias-Viciana, M.H., M. Fernandez-Perez, R. Villafranca-Sanchez, E. Gonzalez-Pradas, and F. Flores-Cespedes. 1999. Sorption and leaching of atrazine and MCPA

in natural and peat-amended calcareous soils from Spain. J. Agric. Food Chem.47:1236-1241.

Sone, K., M. Hinago, A. Kitayama, J. Morokuma, N. Ueno, H. Watanabe, and T. Iguchi. 2004. Effects of 17 beta-estradiol, nonylphenol, and bisphenol-A on developing Xenopus laevis embryos. Gen. Comp. Endocrinol. 138:228-236.

Soto, A.M., J.M. Calabro, N.V. Prechtl, A.Y. Yau, E.F. Orlando, A. Daxenberger, A.S. Kolok, L.J. Guillette, B. le Bizec, I.G. Lange, and C. Sonnenschein. 2004. Androgenic and estrogenic activity in water bodies receiving cattle feedlot effluent in eastern Nebraska, USA. Environ. Health Persp.112:346-352.

Sparks, D.L. 1995. Environmental soil chemistry. Academic Press, San Diego.

Stevenson, F.J. 1994. Humus chemistry: Genesis, composition, reactions. John Wiley & Sons, New York.

Sumpter, J.P., and S. Jobling. 1995. Vitellogenesis as a biomarker for estrogenic contamination of the aquatic environment. Environ. Health Persp.103:173-178.

Ternes, T.A., M. Stumpf, J. Mueller, K. Haberer, R.D. Wilken, and M. Servos. 1999. Behavior and occurrence of estrogens in municipal sewage treatment plants - I. Investigations in Germany, Canada and Brazil. Sci. Total Environ. 225:81-90.

Turan, A. 1995. Excretion of natural and synthetic estrogens and their metabolites: Occurrence and behavior in water. *In* Endocrinically active chemicals in the environment, Vol. UBA Texte 3/96. Umweltbundesamt, Berlin.

Turfitt, G.E. 1947. Microbiological agencies in the degradation of steroids. 2. Steroid utilization by the microflora of soils. J. Bacteriol. 54:557-562.

Van den Belt, K., R. Verheyen, and H. Witters. 2003. Comparison of vitellogenin responses in zebrafish and rainbow trout following exposure to environmental estrogens. Ecotoxicol. Environ. Safety 56:271-281.

Weber, J.B., G.G. Wilkerson, and C.F. Reinhardt. 2004. Calculating pesticide sorption coefficients (K-d) using selected soil properties. Chemosphere 55:157-166.

Williams, E. L., D.R., Raman, R.T. Burns, A.C. Layton, A.S. Daugherty, M.D. Mullen, 2002. Estrogen concentrations in dairy and swine waste storage and treatment structures in and around Tennessee. ASAE Paper 024150. Am. Soc. Agric. Eng., St. Joseph, MI.

Yamamoto, H., and H.M. Liljestrand. 2003. The fate of estrogenic compounds in the aquatic environment: sorption onto organic colloids. Water Sci. Technol. 47:77-84.

Yamamoto, H., and H.M. Liljestrand. 2004. Partitioning of selected estrogenic compounds between synthetic membrane vesicles and water: Effects of lipid components. Environ. Sci. Technol. 38:1139-1147.

Yamamoto, H., H.M. Liljestrand, Y. Shimizu, and M. Morita. 2003. Effects of physical-chemical characteristics on the sorption of selected endocrine disrnptors by dissolved organic matter surrogates. Environ. Sci. Technol. 37:2646-2657.

Ying, G.G., R.S. Kookana, and Y.J. Ru. 2002. Occurrence and fate of hormone steroids in the environment. Environ. Internat. 28:545-551.

Ying, G.G., R.S. Kookana, and P. Dillon. 2003. Sorption and degradation of selected five endocrine disrupting chemicals in aquifer material. Water Res. 37:3785-3791.

Ying, G.G., R.S. Kookana, and P. Dillon. 2004. Attenuation of two estrogen compounds in aquifer materials supplemented with sewage effluent. Ground Water Monit. Remed. 24:102-107.

Yoon, Y., P. Westerhoff, J. Yoon, and S.A. Snyder. 2004. Removal of 17β-estradiol and fluoranthene by nanofiltration and ultrafiltration. J. Environ. Eng. 130:1460-1467.

Yoon, Y.M., P. Westerhoff, S.A. Snyder, and M. Esparza. 2003. HPLC-fluorescence detection and adsorption of bisphenol A, 17β-estradiol, and 17α-ethynyl estradiol on powdered activated carbon. Water Res.37:3530-3537.

Yu, C.P., R. Ahuja, G. Sayler, and K.H. Chu. 2005. Quantitative molecular assay for fingerprinting microbial communities of wastewater and estrogen-degrading consortia. Appl. Environ. Microbiol.71:1433-1444.

Yu, Z.Q., B.H. Xiao, W.L. Huang, and P. Peng. 2004. Sorption of steroid estrogens to soils and sediments. Environ. Toxicol. Chem. 23:531-539.

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