**Estrogens: Properties, Behaviors, and Fate in Dairy
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Estrogens: Properties, Behaviors, and Fate in Dairy Manure-Amended Soils

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Abstract: In 2012 there were 63% fewer dairies in the United States than there were in 1997 as a result of conglomeration of the dairy industry into concentrated animal feeding operations (CAFOs) at the expense of smaller farms (USDA 2015). Today, 60% of all milk produced in the U.S. comes from 5% of the nation's dairies (operations with ≥ 500 cows) (USDA 2014). Concentrated animal feeding operations are touted as economically efficient agricultural business models, hailed for their increased milk yields. Yet, with an average daily manure production of over 27,000 kg for a 500-head dairy, manure storage and disposal are serious management and environmental concerns. A common economical mode of manure disposal is application to nearby agricultural fields. However, a major concern with land-application of dairy manure is the fate of manure-borne hormones, compounds considered chemicals of emerging concern, and the potential threat these hormones pose to humans and the environment. The fate of these chemicals in the soil environment is complicated by multiple edaphic variables including pH, mineralogy, organic matter, microbial activity, and redox status. Estrogens are sorbed by soil organic matter and transformed to non-bioactive, highly soluble conjugated forms or to metabolites that exhibit yet additional properties distinct from their parent compounds. However, deconjugation frequently occurs, regenerating endocrine-disrupting free estrogen compounds. It is challenging to fully understand the behavior and predict the fate of estrogenic compounds from dairy manure in soils because of variable and complex interactions with soil factors, as well as possible interactions among the different chemicals of emerging concern. This review focuses on the behavior of naturally occurring estrogen hormones present in dairy manure in the soil environment. Heightened understanding of the fate of these compounds in soil will enhance our ability to reduce their potential risks.

Key words: dairy manure, hormones, estrogen, soil

1. Introduction

A consequence of the increasing replacement of small farms with concentrated animal feeding operations is the generation of large quantities of manure in a confined location. While application of manure to nearby agricultural fields has been utilized for centuries as a means of animal waste disposal, the application of such vast amounts of manure to a localized area has led to growing concerns for the unknown environmental risks posed by hormones present in the manure. Estrogenic hormones have been detected in surface and groundwaters in the immediate vicinity of manure-applied fields in concentrations ranging from micrograms to nanograms per liter, illustrating the mobility of these chemicals in soil, and thus their possible threat to the greater environment (Li et al. 2012). However, few studies have investigated the fate of hormones in soil following land-application of dairy waste specifically (Lucas and Jones 2006; Kjær et al. 2007; Steiner et al. 2010). This question is complicated by the variety of distinct chemicals present in dairy waste (Zheng et al. 2008; Noguera-Oviedo and Aga 2016), the numerous different soil types to which the waste is applied (Colucci et al. 2001; Caron et al. 2010; Mashtare et al. 2011), and the different waste application methods (Huijsmans et al. 2003; Gall et al. 2011; Mina et al. 2016). Due to their prevalence in dairy waste (Table 2) (Raman et al. 2004; Gadd et al. 2010) and their strong links to endocrine disruption (Hanselman et al. 2003; Vethaak et al. 2005), the fate of naturally occurring estrogenic hormones in soil is addressed.

2. Estrogens

Hormones are synthesized in the endocrine systems of mammals and regulate metabolism and development. To control these physiological processes, hormones naturally are biologically active at very low concentrations (ng L^{-1}). The primary hormones produced by dairy cows are progestogens, androgens, and estrogens. In addition to these naturally produced hormones, dairy cows may be given additional natural and synthetic hormones to increase milk production and control reproduction (Pursley 1997;

Bartelt-Hunt et al. 2012). Of these three hormone classes, estrogens have received the most attention in the literature because of their links to endocrine disruption. Endocrine disrupting compounds have a strong affinity for endocrine receptors and accordingly have very high estrogenic activity at low concentrations, contributing to their biological risk. At concentrations below 1 ng L^{-1} in aquatic environments, estrogens have been implicated in reduced reproductive fitness, the presence of both male and female sex characteristics, skewed population sex ratios, and abnormal mating behavior in fish and other aquatic organisms (Metcalf et al. 2001; Lange et al. 2002; Leet et al. 2011; Van Donk et al. 2013). Moreover, extensive research has focused on the associations between endocrine disrupting compounds and human male and female reproductive disorders (Diamanti-Kandarakis et al. 2009) such as decreased sperm quantity and quality in males (Vested et al. 2013), and to breast cancer (Diamanti-Kandarakis et al. 2009; Teitelbaum et al. 2015; Giulivo et al. 2016). Despite a wealth of research suggesting the influence of endocrine disrupting compounds on human development and disease incidence, concrete evidence of the link remains sparse and numerous studies over the past decades have produced conflicting results (Sharpe and Irvine 2004; Delbès et al. 2006; Fenton 2006; Bonde et al. 2016). Further research is needed to address the gap in scientific understanding of this broad and significant human health topic (Teitelbaum et al. 2015; Bijlsma and Cohen 2016).

The primary estrogens excreted by dairy cows are estrone (E1), 17α -estradiol ($E2\alpha$), 17β -estradiol ($E2\beta$), and to a lesser extent, estriol (E3) (Sarmah et al. 2008). They are synthesized mainly in the ovaries, but also in the brain, hypothalamus, adipose tissue, and placenta. Estrogens derive initially from cholesterol and pregnenolone, which are themselves then transformed into androstenedione and testosterone, the immediate precursors to the three primary estrogenic compounds: E1, E2, and E3. The steroidal estrogens are each composed of 18 carbon atoms, composing one aromatic ring, two cyclohexane rings, and one cyclopentane ring (Fig. 1). The individual hormones are differentiated by the position of hydroxyl groups on the cyclopentane. The $E2\alpha$ and $E2\beta$ epimers are distinguished, respectively, by having the C17 hydroxyl group either outside of or aligned with the molecular plane, attributing different chemical properties to the epimers (Hanselman et al. 2003). The pK_a values of E1,

E2, and E3 range from 10.3 to 10.8 (Hurwitz and Liu 1977; Lewis and Archer 1979) (Table 1), making them non-ionic in most natural soils. They have a low vapor pressure, and thus low volatility (Combalbert and Hernandez-Raquet 2010). They are poorly water soluble, with solubility of about 13 mg L^{-1} , and the K_{OW} (octanol-water partition coefficient) for all natural estrogens ranges from 2.8 to 4.6, demonstrating their hydrophobic character (Table 1; Lai et al. 2000).

Pregnant and cycling dairy cows contribute 90% of all endogenous hormones present in the environment in the U.S., bringing annual dairy estrogen excretion by dairy cows to over 88 t (Lange et al. 2002; Khanal et al. 2006). However, the amount of hormones in manure varies drastically according to the dairy operation and depends on factors such as type of facility and the manner in which manure is collected and removed (Combalbert and Hernandez-Raquet 2010). The amount and distribution profile of the excreted hormones in manure also vary according to sex, species, age, weight, and reproductive status of the animal, with highest excretion levels from pregnant cows (Combalbert and Hernandez-Raquet 2010; Mashtare et al. 2011). 17α -Estradiol, the dominant steroid hormone in dairy waste (Hoffmann et al. 1997), is not excreted by humans or other animals (Hanselman et al. 2003). However, $E2\beta$ exhibits the greatest estrogenic activity of natural free estrogens, followed by E3, E1, and $E2\alpha$ (Gadd et al. 2010).

Because of its high estrogenic activity, much research has focused on the environmental fate of $E2\beta$, with little attention given to $E2\alpha$. The estrogenic activity of $E2\alpha$ in comparison to $E2\beta$ varies according to the target species. In mammals, $E2\alpha$ is reported as being 500 times less estrogenic than $E2\beta$ (Huang et al. 2010), but aquatic species appear to be much more sensitive. 17α -Estradiol was found to be 11-30 times less estrogenic than $E2\beta$ in Medaka fish (Huang et al. 2010) and was only 8-9 times less potent than $E2\beta$ in flathead minnows (Shappel et al. 2010). These studies support the suggestion that although the estrogenic activity of $E2\alpha$ is far less than $E2\beta$, its high concentration in dairy waste (Table 2) increases its potential as a biological risk (Hajek et al. 1997; Mashtare et al. 2011). Although imparting weak estrogenic activity, $E2\alpha$ was found to be potentially teratogenic and to cause morphological alterations in mammals according to developmental stage at time of exposure (Hajek et al. 1997). 17α -Estradiol can also undergo partial metabolism to the more estrogenically active form of $E2\beta$ (Hajek et al. 1997).

Evidence suggests that these stereoisomers may behave differently in the environment and thus potentially have significantly different biological and environmental effects (Stanley and Brooks 2009). For these reasons, the environmental behavior of E2 α must also be considered when evaluating the fate of estrogens in soil.

As the excreted hormone quantity and composition differ between animal species, it is challenging to directly compare study results from different animals. For example, cattle excrete primarily E2 α , E2 β , and E1, while pigs and poultry excrete mainly E1, E2 β , and E3 (Fine et al. 2003; Hanselman et al. 2003). Furthermore, the microorganisms present in different livestock may also influence estrogen degradation and metabolite formation in manure. While E1 is commonly transformed into E3 in pig and poultry manure, E3 was never detected in dairy manure samples in various studies (Hanselman et al. 2003; Hutchins et al. 2007; Zheng et al. 2008; Li et al. 2012). Therefore, the limited research on estrogen fates in dairy manure-amended soils necessitates making inferences from studies involving waste from other animal types.

2.1 Estrogen Conjugates

In addition to the free estrogens E1, E2 α , E2 β , and E3 produced and excreted by livestock, conjugated forms are also present in animal manure. Estrogen conjugates are naturally formed in the liver, whereby sulfate, glucuronide, and/or sulfoglucuronide groups are attached to the hydroxyl or phenoxy groups at the C3 and/or C17 position on the free estrogens (Hutchins et al. 2007; Combalbert and Hernandez-Raquet 2010). The conjugated forms are more polar, giving them increased solubility and thus are primarily excreted in urine (D'Ascenzo et al. 2003). Though free forms dominate in excreted feces, some conjugated forms are also present in feces (Combalbert and Hernandez-Raquet 2010). The hydrophilic nature of estrogen conjugates also facilitates their mobility in soil (Hutchins et al. 2007). In a comparison of E1 and E1-3-sulfate (E1-3S) sorption to three New Zealand soils, Scherr et al. (2009a) found the sorption of E1-3S to be one order of magnitude less than the free form. Conjugated estrogens

have very low estrogenic activity compared to free forms, but they are readily transformed back to the unconjugated, bioactive forms by enzymatic processes of intestinal, fecal, and soil microbes present in manure and the environment (Dray et al. 1972; Hutchins et al. 2007; Yu et al. 2013). The frequent reversible transformations between free and conjugated estrogen species (Fig. 2) are influenced by the properties of the environmental matrix including pH and redox status, which complicates the prediction of hormone load and estrogenic activity in manure-applied soils (Wang et al. 2014). Moreover, very little research has investigated sorption dynamics of conjugated estrogens in soil (Scherr et al. 2009b; Gadd et al. 2010).

The most prevalent estrogen conjugates are the sulfate forms of E1, E2 α , and E2 β (Hutchins et al. 2007; Gadd et al. 2010), with glucuronide forms rarely detected in animal manure as they are readily hydrolyzed (D'Ascenzo et al. 2003; Hutchins et al. 2007; Kumar et al. 2012). 17 β -Estradiol degrades to the primary metabolite E1, which is then transformed into the conjugate E1-3-sulfate (E1-3S) (Goeppert et al. 2015) (Fig. 2). The E1-3S secondary metabolite is one of the most commonly detected conjugates and has been identified in swine, poultry, and dairy manure lagoon samples (Hutchins et al. 2007; Gadd et al. 2010; Noguera-Oviedo and Aga 2016). E1-3-sulfate is also the dominant estrogen present in urine of pregnant cattle, comprising 48-92% of total estrogens (Hoffmann et al. 1997). It has been found in surface waters and effluents of wastewater treatment plants, representing one of the most persistent estrogen conjugates (Scherr et al. 2009b). E1-3-sulfate has even been detected in swine manure samples that had been stored at 4 °C for up to 5 years (Hutchins et al. 2007). In a study comparing estrogens in swine, poultry, and dairy manure lagoons, dairy manure had the highest concentration of conjugated estrogens at 57% of total estrogens, and all of them the recalcitrant sulfate form (Hutchins et al. 2007). In another study looking at conjugated estrogens, conjugates comprised no more than 22% of total estrogen concentration in dairy manure (Gadd et al. 2010). The difference between Hutchins et al. (2007) and Gadd et al.'s (2010) studies could be attributed to different degrees of conjugation or deconjugation.

17 β -Estradiol-17-sulfate (E2 β -17S) is another common estrogen conjugate detected in animal waste and in runoff from poultry litter-applied fields (Dutta et al. 2010; Bai et al. 2013). Soil batch studies

171 indicate that E2 β -17S is most commonly hydroxylated to other metabolites in soil, notably E1-3S, yet
172 approximately 1% is deconjugated back to the free estrogen forms of E1 and E2 β (Fig. 2). Although the
173 percentage is small, this quantity is sufficient to raise concerns considering the vast quantities of manure
174 typically applied and the high bioactivity of E2 β and E1 at low concentrations. The minimal amount of E1
175 and E2 β formation from deconjugation of E2 β -17S may be due to the dependence of this process on
176 microbial arylsulphatase activity, which is likely distributed non-uniformly in soils and decreasingly with
177 soil depth (Scherr et al. 2009b; Bai et al. 2015). In topsoil with 1.29% organic carbon, the dissipation
178 half-life for E2 β -17S was 2.5-9.3 hours. In contrast, 14 days after application, approximately 80% of
179 initial E2 β -17S remained in subsoil containing 0.26% organic carbon (Bai et al. 2015). Though high
180 organic matter content in manure-amended soils will likely increase conjugate degradation rates,
181 enzymatic degradation activities may also be hindered by strong interactions between organic matter and
182 enzymes.

183 17 α -Estradiol-3-sulfate (E2 α -3S) is also present in high quantities in cattle urine and it degrades to
184 the primary metabolites E1-3S and E2 α , and the secondary metabolite E1 (Fig. 2). Degradation occurs
185 under both aerobic and anaerobic conditions, though the transformation rates vary drastically depending
186 on oxygen status and temperature. Breakdown rates were significantly faster under aerobic compared to
187 anaerobic conditions (Zheng et al. 2013). This could result in much greater persistence of E2 α -3S in
188 saturated soils following manure application.

189 Although the glucuronide conjugates receive less attention in the literature, these conjugated forms
190 need to be considered. If readily hydrolyzed to free forms as multiple studies report, they could be an
191 additional source of toxic exposure. However, Shrestha et al. (2012) found the conjugate 17 β -estradiol-3-
192 glucuronide (E2 β -3G) persisted in soil solution following application of swine manure for 21-28 days,
193 after which time it transformed into the free estrogens E2 β and E1 (Fig. 2). The increased persistence in
194 soil solution could increase the soluble transport of this chemical through the soil profile and into
195 groundwater.

Conversion of the conjugate 17 β -estradiol-3-glucuronide (E2 β -3G) to the free form E2 β (Fig. 2) occurs via activity of microbial β -glucuronidase, similar to microbial hydrolysis of E2 β -17S. Because of the dependence on microorganisms for glucuronide conjugate hydrolysis, the reaction rate may be faster in high organic matter soils (Shrestha et al. 2012), though organic matter content may also impede enzyme activity due to strong interaction forces.

Conjugate formation can occur via biotic and abiotic processes. Goeppert et al. (2014) demonstrated in column studies that either biotic or abiotic processes cause degradation of E2 β to E1. Conversely, formation of the metabolite E1-3S from E2 β occurs only in the presence of soil microbes. Arylsulfotransferase was recently implicated as the microbial enzyme responsible for transforming the free estrogen E1 to its conjugate E1-3S in soils (Goeppert et al. 2015). Via abiotic interactions with manganese oxides and clay surfaces, E2 β -17S is degraded to various hydroxyl metabolites and the free estrogens E2 β and E1 (Bai et al. 2015).

Research on the behavior of estrogen conjugates in the environment has increased in recent years, though much more is needed to inform best management practices of field-applied manure. Quantifying estrogen conjugates in soils and their metabolite production through use of radioisotope tracer studies is a potential method for improved analysis of estrogen conjugate fate in soils (Wang et al. 2014). Mathematical modeling offers an additional tool for evaluating the various processes influencing estrogen conjugate behavior and transport in soils (Bai et al. 2014).

2.2. Mechanisms of Estrogen Removal

The fate of estrogens in soil is determined largely by mineralization and sorption mechanisms (Stumpe and Marschner 2010a; Zheng et al. 2012; Mashtare et al. 2013), though abiotic transformations also occur and influence the fate of estrogens (Stumpe and Marschner 2010a) (Fig. 3). Estrogens are biomolecules that are readily degraded and transformed by microbes under both aerobic and anaerobic

conditions (Zheng et al. 2012; Yu et al. 2013). Additionally, they are strongly adsorbed to soil organic matter (SOM) due to their hydrophobic nature (Stumpe and Marschner 2010a and 2010b).

Mineralization

Numerous long-term field studies have shown an increase in biological activity and soil respiration from inputs of organic amendments (Fauci and Dick 1994; Fernandes et al. 2005; Stumpe and Marschner 2010a). Moreover, many studies report increased diversity in soil microbial communities as a result of organic waste inputs (de Graaff et al. 2010; Sradnicka et al. 2013). Therefore, addition of dairy manure may impact estrogen mineralization in soils by: 1) increased nutrients affecting microbial activity, 2) alteration of the microbial community structure affecting microbial activity, and 3) increased sorption sites affecting bioavailability of estrogens (Stumpe and Marschner 2010a). Physicochemical properties of manure during storage and after land-application may influence the rate and extent of estrogen mineralization. These factors include redox, moisture, temperature, age, type, and composition of manure (Lucas and Jones 2006; Mansell et al. 2011).

Type and properties of soils also impact the rate of estrogen mineralization (Lucas and Jones 2006). Correlation between degradation rate and estrogen concentration varies among studies. In general, it appears that estrogen concentration does not impact or slow degradation under aerobic conditions; whereas under anaerobic conditions, high concentrations of estrogens may slow degradation (Combalbert and Hernandez-Raquet 2010). The degradation rate of E1 and E2 β in soils was significantly greater when applied in a manure matrix, as compared to the degradation rate when applied only with water or livestock urine (Lucas and Jones 2006). This is likely a result of the native manure microbial community that has adapted to those conditions and has evolved to degrade hormonal compounds (Lucas and Jones 2006).

The main steroidal estrogens E1, E2 α , E2 β , and E3 undergo a multitude of transformations under both aerobic and anaerobic conditions. Aerobic and anaerobic soil and fecal bacteria are responsible for the dehydrogenation on C17 of E2 β to produce E1 (Hutchins et al. 2007; Zheng et al. 2008; Yu et al. 2013), which explains the increase in E1 concentration that accompanies E2 β degradation (Colucci et al. 2001; Lee et al. 2007). Greater percent recovery was measured for E1 than for E2 β from soil lysimeters treated with estrogen-spiked dairy manure, likely due to the transformation of E1 from E2 β in soils (Steiner et al. 2010). In an analysis of estrogen concentrations along a typical dairy waste disposal system, E2 α and E1 comprised >80% and 10%, respectively, of total hormones in fresh manure wastewater directly exiting the milking parlor. However, in wastewater sampled approximately 1 km further from the parlor, those concentrations had changed to <30% for E2 α and >70% for E1, illustrating the transformation of E2 α to E1 by manure microbes (Zheng et al. 2008). Not only does E2 β degrade to E1 under anaerobic conditions, albeit at a slower rate (Lee and Liu 2002; Combalbert and Hernandez-Raquet 2010), but E1 and E2 β also transform to E2 α under anaerobic conditions (Yu et al. 2013). The rate of E2 β to E1 transformation increases under dark conditions compared to in the light (Lai et al. 2002). Estrone can undergo further transformation to produce E2 α (Czajka and Londry 2006; Combalbert and Hernandez-Raquet, 2010; Yu et al. 2013). At a waste water treatment plant, for example, removal of E1 ranged from 19% to 94%, and from 76% to 92% for E2 (Baronti et al. 2000).

Polymerase chain reaction-based molecular research over the past decade has steadily increased our knowledge of the organisms and pathways responsible for biodegradation of estrogens, yet relatively few specifics are currently understood (Yu et al. 2013; Blunt et al. 2017). Moreover, no published studies have researched the specific microbial organisms, mechanisms, or pathways involved in estrogen mineralization in dairy manure-amended soils. Therefore, our understanding of this topic draws heavily from studies on activated sludge from sewage treatment plants (STP) (Lee and Liu 2002; Shi et al. 2004; Liu et al. 2016; Yu et al. 2016; Li et al. 2017) and from environmental assessments (Blunt et al. 2017). Environmental conditions can impact the abilities of microorganisms to mineralize estrogens. For example, two *Rhodococcus* sp. bacterial strains, JX-2 (Liu et al. 2016) and DS-201 (Yu et al. 2016), and a

Novosphingobium sp. bacterial isolate (Li et al. 2017), all isolated from activated sludge of STPs, significantly degraded E2 β , but with peak performance at 30 °C and pH 7.0. They each demonstrated distinct tolerances to temperature and pH ranges, with JX-2 demonstrating much greater temperature and pH tolerance than DS-201. While these environmental parameters do not match typical agricultural field conditions, data from these studies enhance our understanding of microbial estrogen degradation and can be applied to dairy manure applications. For example, when *Rhodococcus* sp. JX-2 was immobilized in a protective sodium alginate matrix, it was capable of degrading over 80% of E2 β in fresh dairy manure within 7 days (Liu et al. 2016).

Estrogen-degrading bacteria have also been isolated from natural environmental samples. Kurisu et al. (2010) isolated five strains of *Rhodococcus* sp. and *Sphingomonas* sp. from soils that were capable of mineralizing E2 β and E1. A degradation pathway was proposed, but the influence of typical environmental field conditions on degradation is unknown. Estrone and E2 β were also degraded by bacterial isolates from an aquatic source (Blunt et al. 2017). Whereas *Pseudomonas* sp. have been previously identified as estrogen-degraders (Isabelle et al. 2011), this was the first reporting of isolates from the *Rhizobium* and *Sphingopyxis* genera that were capable of mineralizing estrogens (Blunt et al. 2017). Although these isolates were discovered in water, the three genera include common soil-inhabiting bacterial species, which expands the phylogenetic pool of potential soil-borne microbes capable of degrading estrogen.

Degradation Pathways

Microbial degradation has been identified as central to estrogen elimination in both aerobic and anaerobic systems, though knowledge of the specific degradation pathways is sparse (Lorenzen et al. 2006; Lucas and Jones 2006; Yu et al. 2013). As a result of numerous heterotrophic and autotrophic microorganisms representing diverse phylogenies (Yu et al. 2013), estrogens undergo complex

biodegradation pathways in the environment, a result of which can be non-steroidal transformation products (Yoshimoto et al. 2004; Yu et al. 2007; Yu et al. 2016). Soil microbes rely on a variety of enzymes to degrade estrogenic compounds intracellularly and extracellularly, such as cytochrome P450s, catechol-*o*-methyltransferases, polyphenol oxidases, and ammonia monooxygenase (Shi et al. 2004; Lucas and Jones 2006; Caron et al. 2010).

Though estrogen biodegradation research has focused on bacterial species, various fungi and algae are known to degrade estrogens (Yu et al. 2013). Microbial degradation of estrogens can be either a metabolic or cometabolic mechanism (Lucas and Jones 2006; Yu et al. 2007). In metabolic degradation, the estrogens are used as an energy and/or carbon source for microbes, whereas no carbon or energy gains are attained in cometabolic pathways, thus requiring an additional nutrient source to carry-out the reaction. While metabolic degradation mechanisms are assumed to dominate, studies show that E2 degradation occurs at a slower rate when alternative carbon sources are present (Muller et al. 2010), as would be the case in land-application of manure. Therefore, the pathways and organisms involved in cometabolic processes cannot be discounted.

Estrogen Sorption

Numerous studies have investigated sorption dynamics of estrogens in soil, producing diverse results (Scherr et al. 2009a; Combalbert and Hernandez-Raquet 2010; Amin et al. 2012), but with a general consensus that soil organic carbon (SOC) content is the primary influence on estrogen sorption dynamics in soil (Sarmah et al. 2008; Caron et al. 2010; Stumpe and Marschner, 2010a and 2010b). In Caron et al.'s (2010) extensive survey of estrogen sorption in soils across Alberta, Canada they concluded that K_d (soil sorption coefficient) values of E1, E2 β , and E3 were positively correlated (r^2 values of 0.50, 0.75, and 0.69) to SOC, which is in agreement with numerous studies that E1 and E2 β sorption is positively correlated to SOC content (Sarmah et al. 2008; Stumpe and Marschner 2010a). In soils with

<1% SOC, K_d values were significantly smaller than in soils with greater SOC concentration (Caron et al. 2010). Multiple studies have determined the K_d values of E2 β to be nearly two-fold greater than those of E2 α (Caron et al. 2010; Mashtare et al. 2011).

The strong correlation between estrogen K_d values and SOC implies that hydrophobic partitioning is the mechanism controlling sorption, but data attained in numerous studies suggest that sorption mechanisms other than hydrophobic partitioning are also involved (Yamamoto and Liljestrand 2003; Sarmah et al. 2008; Caron et al. 2010). The octanol-water partition coefficient (K_{ow}) is used to estimate the amount of a substance that is partitioned into SOM and is usually directly related to K_{oc} (organic carbon-normalized sorption coefficient). However, the K_{oc} and K_{ow} of E1, E2 β , and E3 are poorly associated (Yamamoto and Liljestrand 2003; Sarmah et al. 2008; Caron et al. 2010). Poor association of K_{oc} and K_{ow} for E2 β has likewise been reported in other studies (Lai et al. 2000; Holthaus et al. 2002). In a comparative study of the stereoisomers E2 β and E2 α , Qiao et al. (2012) found their K_{ow} values to be very similar (3.76 and 3.73). If hydrophobic partitioning was the sole mechanism, the K_{ow} for E2 β would be lower than that of E2 α in accordance with E2 β 's significantly lower measured K_{hw} values (hexane-water partition coefficient). Mashtare et al. (2011) suggest this contradiction could result from increased H-bonding of E2 β . Electron donor-acceptor interactions have also been suggested as a potential sorption mechanism (Yamamoto and Liljestrans 2003). The differing position of the OH group on the E2 isomers may also be responsible for their differing sorption strengths. The OH group on E2 β lies within the plane of the molecule and therefore may be able to form additional sorption interactions than E2 α whose OH group is oriented away from the molecular plane (Mashtare et al. 2011). Furthermore, reported K_{ow} values for estrogens from numerous studies were highly diverse, making it difficult to know for certain the accurate values.

While SOC exerts the dominant influence on estrogen sorption, mineralogical and chemical properties of soil may also influence estrogen sorption processes. Estrone, E2 α , and E2 β partition readily and strongly to soil and sediments, while E3 does not (Casey et al. 2003 and 2005; Li et al. 2012).

Conflicting data exist regarding the correlation between estrogen sorption and cation exchange capacity (CEC). Caron et al. (2010) attributed the correlation between CEC and K_d values of E2 β and E3 ($r = 0.73$ and 0.63 , respectively) to the high correlation between SOC and CEC. Similarly, Mashtare et al. (2011) concluded that the moderate associations between CEC and K_d of E2 α and E2 β ($r = 0.67$) were artifacts from the strong association between percent OC and CEC ($R^2 = 0.74$) and between percent OC and K_d ($R^2 = 0.99$). Considering estrogens are weak acids with pK_a values greater than 10 and therefore non-ionic at typical soil pH, a strong correlation between CEC and estrogen sorption is highly unlikely (Sarmah et al. 2008; Mashtare et al. 2011). Moreover, Sarmah et al. (2008) found that in soils with 2.4 to 9.7% organic carbon, soil clays had little influence on estrogen sorption. However, in soils with high oxide concentrations, there may be increased interaction between soil oxides and estrogens (Sarmah et al. 2008). For example, high oxide concentrations in soils may catalyze abiotic chemical reactions of estrogens, such as hydrolysis, redox, substitution, or polymerization (Karthikeyan et al. 1999; Bai et al. 2015). No significant correlation has been found between estrogen sorption in soil and pH (Hanselman et al. 2003; Caron et al. 2010; Mashtare et al. 2011). Neither has any correlation been found between K_d values of estrogens and percent clay, percent silt, $CaCO_3$, or EC (Caron et al. 2010; Mashtare et al. 2011). A significant negative correlation between K_d values for E2 β and E1 to percent sand was found by Caron et al. (2010), while no correlation was found by Mashtare et al. (2011).

Kaolinite does not bind E1 and only weakly binds E2 (Casey et al. 2003; Bonin and Simpson 2007), while both sorb more strongly to montmorillonite, with greater E2 binding (Bonin and Simpson 2007). The difference in sorption to kaolinite and montmorillonite may be caused by increased surface area of the latter and intercalation of estrogens into interlayer space of montmorillonite (Bonin and Simpson 2007). X-Ray diffraction studies confirm that increasing d-spacing of montmorillonite allows estradiol to enter the interlayer (Van Emmerik 2003). Moreover, slow uptake of estradiol by montmorillonite and minimal desorption support intercalation as a primary mechanism of estrogen sorption on expanding clays (Shareef et al. 2006). The sorption behavior of estrogen conjugates has only minimally been investigated. With a pK_a of -3.0, the weak acid conjugate E1-3-sulfate is always negatively charged and binds to

positively charged soil particles via electrostatic interactions. The presence of the sulfate group on E1-3-sulfate reduces hydrophobic interactions with SOM, though it may interact weakly with SOM and clays via ligand exchange, intercalation, and H-bonding (Scherr et al. 2009a).

The variability in experimental protocols makes it challenging to compare sorption results across studies. A common procedure to measure estrogen sorption is to take the difference between the initial aqueous estrogen input and the final aqueous concentration. However, this simplistic approach does not consider microbial or abiotic degradation or other transformation products (Sarmah et al. 2008), as it assumes that sorption is the only fate of the initial hormone input. In addition, most studies on estrogen sorption in soils do not include manure applications in the experimental design (Casey et al. 2005; Hildebrand et al. 2006; Mashtare et al. 2011), thereby rendering the study conclusions possibly incongruent with field conditions. Considering the widely supported conclusion that soil organic matter significantly influences estrogen sorption and degradation in soils (Bonin and Simpson 2007; Caron et al. 2010; Stumpe and Marschner 2010b; Prater et al. 2016), it follows that inclusion of organic matter-rich animal waste in experiments could contribute markedly to study results. Furthermore, numerous studies (Stumpe and Marschner 2010a; Chambers et al. 2014; Prater et al. 2016) show that dissolved organic carbon and the soil colloidal fraction decrease sorption of estrogenic compounds to soil, thereby enhancing estrogen transport. Therefore, our understanding of the sorption mechanisms of manure-borne estrogens in soils following land application of dairy waste would benefit from more research in which manure was incorporated into the experimental design.

2.3. Estrogen Transport Processes

Though biodegradation and sorption are the primary mechanisms influencing estrogen fate in soils, detection of estrogens in groundwater and surface water indicates that transport processes are also determining factors. Free estrogens have been found in runoff following land application of dairy manure

(Dyer et al. 2001; Li et al. 2012; Mina et al. 2016) or beef heifer manure (Biswas et al. 2017), in soil drainage water following application of dairy manure (Li et al. 2012) or swine manure (Kjær et al. 2007), in streams draining cattle-grazed fields (Kolodziej and Sedlak 2007), and in runoff from poultry manure-amended fields following storm events (Dutta et al. 2010). The detection of estrogens 32 m below clay-lined dairy farm lagoons suggests that preferential flow is responsible for estrogen transport through the vadose zone and into groundwater (Arnon et al. 2008). In a tile-drained Indiana field site, preferential flow was determined to be the dominant transport process for estrogens in soil, evidenced by the coordination of peak hormone load detection with hydrograph data (Gall et al. 2014). Many of the laboratory studies from which sorption behavior is inferred use packed soil columns, which may not accurately reflect realistic soil profiles. Kjær et al. (2007) detected concentrations of E1 and E2 β of 68.1 and 2.5 ng L⁻¹ one m below soil following land-application of swine manure. Concentrations of E2 β between 16 to 100 ng L⁻¹ have additionally been detected at depths of 2-3 m following manure application to fields (Thompson et al. 2009). Preferential flow through soil macropores and/or via colloidal enhanced transport are mechanisms responsible for significant leaching of estrogens (Steiner et al. 2010). Based on early estrogen breakthrough prior to the inert bromide tracer in undisturbed soil lysimeters treated with hormone-spiked dairy manure, Steiner et al. (2010) concluded that rapid estrogen movement through soil was caused by colloidal-enhanced transport. Colloids are between one nm and one μ m in size and can be of organic, inorganic, or biological origin. The availability of colloids for estrogen sorption would likely be high in dairy manure application scenarios. Hydrophobic estrogens can rapidly sorb to colloids via both hydrophobic and non-hydrophobic associations (Steiner et al. 2010). Increased soil pH from manure application or a decrease in soil solution ionic strength may stimulate increased colloidal release from soil and into solution, thereby furthering estrogen movement through the profile (McGechan et al. 2002; Steiner et al. 2010). Up to 60% of E2 β can be associated with soil colloids in wastewater (Holbrook et al. 2004). The aqueous matrix by which estrogens enter the soil, whether water or animal urine, also impacts their transport through soil, with urine enhancing movement of estrogens through the soil profile (Lucas and Jones 2009).

The application method and seasonal timing also impact estrogen transport following land application of dairy manure. Only one published study addresses the effect of dairy manure application method on the transport of manure-borne estrogens (Mina et al. 2016), requiring inferences to be drawn from the wealth of research on nutrient transport processes following different dairy manure application methods (Kleinman et al. 2009; Johnson et al. 2011; Williams et al. 2011). Traditional application practice includes broadcasting manure onto fields and then deeply tilling it into the soil (Johnson et al. 2011; Duncan et al. 2017). Soil incorporation of dairy manure has been shown to decrease surface runoff of nutrients (Kleinman et al. 2009; Johnson et al. 2011; Duncan 2017), but due to increased potential of soil erosion (Pimentel et al. 1995), incompatibility of stony soils to deep tilling (Webb et al. 2010; Maguire et al. 2011), and increased adoption of reduced tillage systems (Knowler and Bradshaw 2007), alternative manure application methods are being actively researched (Chen et al 2001; Huijsmans et al. 2003; Johnson et al. 2011; Duncan et al. 2017). In their comparison of estrogen transport in surface runoff following either surface broadcasting or shallow disk injection of dairy manure, Mina et al. (2016) found that total estrogen load in surface runoff from broadcast application was about two orders of magnitude greater than the estrogen load from shallow disk injection application. Moreover, precipitation events differentially influenced estrogen transport under the two application methods, with broadcast application being most susceptible to rainfall occurring soon after application and estrogen in the shallow disk injection treatments being most susceptible to runoff loss following intense rainfall events. In an assessment of estrogen concentrations under tile-drained agricultural fields that received dairy and beef waste using various application methods, Gall et al. (2011) concluded that greater hormone runoff occurred from broadcasted solid wastes compared to pivot irrigation of lagoon effluent. The typically low yet variable concentrations of estrogens in groundwater indicate that estrogen transport through soils depends on site-specific soil characteristics, local hydrology, manure composition, and manure application timing and method (Lucas and Jones 2009; Gall et al. 2011; Williams et al. 2011; van Donk et al. 2013; Mina et al. 2016).

443

444 **2.4. Influence of Manure Storage on Estrogen Fate**

445 The environmental fate of manure-borne estrogens is highly influenced by the storage conditions
446 of the manure prior to land application. The microbial community and activity in manure (Stumpe and
447 Marschner 2007 and 2010a; Zhang et al. 2014; Blunt et al. 2017), the physicochemical properties of
448 estrogens (Stumpe and Marschner 2007), the physical and chemical properties of the manure treatment
449 process (Zheng et al. 2008; Prater et al. 2015) and the environmental conditions of the treatment or
450 storage system (Blunt et al. 2017) all impact the fate of estrogens in manure storage. Estrogen
451 concentration in manure varies greatly with the type of storage facility (Table 2) due to biodegradation,
452 photodegradation, settling of particles with attached hormones, dilution, residence time, and
453 environmental parameters such as oxygen content and pH (Zheng et al. 2008; Combalbert and
454 Hernandez-Raquet 2010). For example, the hormone levels in a tertiary dairy lagoon were two orders of
455 magnitude less than levels detected in a primary lagoon, suggesting that the longer residence time of
456 manure in the third lagoon allowed for increased estrogen degradation and greater settling of estrogen-
457 associated manure solids (Zheng et al. 2008). Attenuation of estrogens in dry-pile manure also occurs. In
458 comparison to fresh manure (<2 hr), E2 β and E2 α concentrations in dairy manure dry-piled for 2 weeks
459 had decreased by 80% and 88%, respectively. In accordance with previous studies attributing microbial
460 metabolism to degradation of E2 to E1, the concentration of the latter concomitantly increased by 30%
461 (Zheng et al. 2008). Zheng et al. (2008) also found that the levels of estrogen in 2-week-old dry-stacked
462 manure were up to 15 times higher than hormone levels in dairy lagoon waste. This finding suggests that
463 field application of solid manure, compared to dairy wastewater application, may be associated with
464 increased environmental risks from higher hormone loading. However, as the stability of E2 α in
465 anaerobic lagoons is unknown, it can transform back to E1 and then to the highly estrogenically active
466 E2 β (Czajka and Londry 2006; Hutchins et al. 2007). Reversible transformations readily occur between
467 E1, E2 α , and E2 β , making it difficult to assess the environmental effects of application of lagoon manure

because transformations to more or less biologically active forms may occur (Hutchins et al. 2007; Zheng et al. 2008; Prater et al. 2015). The extent of estrogen elimination from biodegradation varies drastically as a result of estrogen concentration, and different manure holding and treatment system conditions and processes (Table 2) (Yu et al. 2013). The time required for adequate hormone attenuation in dry-piled or composted manure is not clear. Whereas Zheng et al. (2008) found a nearly 90% reduction in E2 β and E2 α concentrations following dry-piling dairy manure, no significant difference in estrogen levels was seen between stockpiled and composted beef heifer manure after 12 weeks (Biswas et al. 2017). However, the latter study did not measure hormone levels at additional time points during the 12-week period.

3. Research Gaps

The presence of endocrine disrupting estrogenic compounds in the environment has garnered scientific attention for decades (Hanselman et al. 2003). While research has made significant strides in our understanding of estrogen behavior in soils, research gaps remain. Moreover, even greater is the knowledge gap regarding the properties and behaviors of dairy manure-borne estrogens following land application of manure. Considering the complex transformations that estrogenic compounds undergo in the environment, much more research is needed to clarify how environmentally relevant conditions impact estrogen degradation pathways, including how diverse microbial communities differentially affect estrogen transformations. Furthermore, the estrogenic bioactivity of the numerous metabolites and degradation products need to be understood. In general, data on estrogen fate in soil following field application of dairy waste would benefit substantially from experimental designs that more closely replicate natural field conditions, such as using in-tact soil cores and including manure. Numerous waste management practices exist at dairy operations and the influence that these variable manure storage and handling methods have on estrogen behavior following field application has been minimally researched. Increased research on this topic could lead to broad adoption of improved best management practices that

could ultimately minimize the amount of estrogens entering soils. Given the increasing prevalence of large-scale US dairies that generate exorbitant quantities of manure, it is imperative that research voids are critically addressed.

4. Conclusion

Estrogenic hormones enter agricultural fields from the application of dairy manure. Anticipating the fate of these chemicals in the soil environment is complicated by the multiple variables influencing their behavior including soil pH, soil mineralogy, organic matter, microbial community, and redox status (Fig. 3). Moreover, estrogens degrade to form numerous metabolites that exhibit yet additional properties distinct from their parent compounds. Consideration of the fate of estrogens in soil must include assessment of these degradation products. Estrogens are readily sorbed by soil organic matter and degrade rapidly to non-bioactive, highly soluble conjugated forms (Fig. 3). However, as part of the complex estrogen transformation pathways, deconjugation frequently occurs, regenerating the endocrine-disrupting free estrogen compounds. Considering the complex environmental interactions influencing the behavior of estrogens in soils, further research is needed to develop best management practices for the prudent application of dairy manure to agricultural soils.

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Estrogens: Properties, Behaviors, and Fate in Dairy Manure-Amended Soils

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22 Table 1. Physicochemical properties of naturally-occurring estrogens in dairy waste

Compound	Molecular Weight	pK _a ^{a, b}	Log K _{ow} ^c	Water Solubility ^{d, e} (mg L ⁻¹)	K _d (L kg ⁻¹) ^f	K _{oc} (L kg ⁻¹) ^f
Estrone (E1)	270.37	10.3-10.77 ^b	3.13 - 3.43	1.3 - 12.4	33	1557
Estradiol (E2)	272.39	10.5-10.71 ^b	3.10 - 4.01	1.48 - 12.96	23	1082
Estriol (E3)	288.39	10.4	2.60 - 2.81	3.2 - 13.3	23	1059

23 *a*, Lewis and Archer 1979; *b*, Hurwitz and Liu 1977; *c*, Lai et al. 2000; *d*, Shareef et al. 2006; *e*,
 24 Hanselman et al. 2003; *f*, Caron et al. 2010.

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40 Table 2. Estrogen concentrations in dairy waste*

Storage Type	E1	E2 α	E2 β	E3	Method	Reference
Liquid manure (ng L⁻¹)						
Dairy raw wastewater	879	2,282	643	nd	GC-MS	Hanselman et al. 2006
Dairy primary lagoon	260	80	85	nm	GC-MS	Zheng et al. 2008
Dairy primary lagoon ^a (Nov collection)	1,816	100	27	<LOD	LC/ESI/MS/MS	Gall et al. 2014
Dairy primary lagoon ^a (May collection)	2,700	833	nm	nm	LC-MS-MS	Shappell et al. 2010
Dairy secondary lagoon	146	452	300	<8	GC-MS-MS	Hutchins et al. 2007
Dairy secondary lagoon ^a (Nov collection)	25	<LOD	<LOD	<LOD	LC/ESI/MS/MS	Gall et al. 2014
Dairy secondary lagoon ^a (Feb-April collection)	75	212	167	<LOD	HPLC-ESI-MS/MS	Gall et al. 2011
Dairy holding pond	4,500	3,000	1,460	nm	GC-MS	Raman et al. 2004
Dairy ponds ^b	1,163	356	132	nd	GC-MS	Sarmah et al. 2006
Solid manure (µg kg⁻¹ dw)						
Cow press cake manure	98	32	nd	nd	GC-MS	Raman et al. 2001
Dairy fresh manure	535	1,416	153	nd	GC-MS	Zheng et al. 2008
Dairy manure piled (2 weeks age)	697	172	37	nm	GC-MS	Zheng et al. 2008
Dairy solids ^d (April collection)	11595	2617	815	<LOD	LC/ESI/MS/MS	Gall et al. 2014
Dairy solids ^a (May collection)	99667	33333	<LOD	<LOD	LC-MS-MS	Shappell et al. 2010
Dairy solids ^a (Dec collection)	31260	88197	7895	<LOD	LC/ESI/MS/MS	Gall et al. 2014
Dairy dry-stack semisolid ^c	300	500	160	nm	GC-MS	Raman et al. 2004
Dairy dry-stack solid ^c	180	180	<100	nm	GC-MS	Raman et al. 2004

41 * Table adapted from Combalbert and Hernandez-Raquet 2010.

42 LOD: limit of detection; nm: not measured; nd: not detected; HPLC/ESI/MS/MS: high performance

43 liquid chromatography electrospray ionization tandem mass spectrometry; *a*, average of 3 samples; *b*,

44 average of six ponds; *c*, estimated from the graph provided in the reference; *d*, average of 6 samples.

Figure 1. Molecular structures of estradiol, estrone, and estriol.

Draft

Estradiol

Estrone

Estriol

Figure 2. Transformations which commonly occur among free and conjugated estrogens under variable soil environmental conditions.

Figure 3. Fate of estrogens in soil. Estrogens enter agricultural fields from the application of dairy manure. Degradation, especially microbial mineralization, and sorption are the primary mechanisms influencing estrogen fate in soils. SOC is the dominant influence on sorption dynamics. High concentrations of soil oxides may catalyze abiotic chemical reactions of estrogens, including hydrolysis, redox, substitution, or polymerization. Estrogens also intercalate into interlayer spaces of 2:1 expanding clays such as montmorillonite. Estrogens undergo complex transformation pathways, forming numerous metabolites and conjugation products in the process. Transport processes, dominated by preferential flow through soil macropores, likewise affect estrogen fate in soils. Highly soluble estrogen conjugates readily move through the soil profile.