Manure-Borne Estrogens as Potential Environmental Contaminants: A Review

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Livestock wastes are potential sources of endocrine disrupting compounds to the environment. Steroidal estrogen hormones such as estradiol, estrone, and estriol are a particular concern because there is evidence that low nanogram per liter concentrations of estrogens in water can adversely affect the reproductive biology of fish and other aquatic vertebrate species. We performed a literature review to assess the current state of science regarding estrogen physicochemical properties, livestock excretion, and the fate of manure-borne estrogens in the environment. Unconjugated steroidal estrogens have low solubility in water (0.8−13.3 mg L^{-1}) and are moderately hydrophobic (log K_{ow} 2.6−4.0). Cattle excrete mostly 17α-estradiol, 17β-estradiol, estrone, and respective sulfated and glucuronidated counterparts, whereas swine and poultry excrete mostly 17β-estradiol, estrone, estriol, and respective sulfated and glucuronidated counterparts. The environmental fate of estrogens is not clearly known. Laboratory-based studies have found that the biological activity of these compounds is greatly reduced or eliminated within several hours to days due to degradation and sorption. On the other hand, field studies have demonstrated that estrogens are sufficiently mobile and persistent to impact surface and groundwater quality. Future research should use standardized methods for the analysis of manure, soil, and water. More information is needed about the types and amounts of estrogens that exist in livestock wastes and the fate of manure-borne estrogens applied to agricultural lands. Field and laboratory studies should work toward revealing the mechanisms of estrogen degradation, sorption, and transport so that the risk of estrogen contamination of waterways can be minimized.

Introduction

Livestock manure contains appreciable amounts of natural steroidal estrogen hormones, particularly estradiol, estrone, and estriol, that can potentially contaminate surface and groundwater resources (*1*−*8*). Estrogen contamination of waterways is a concern because low part per trillion (10−100 ng L^{-1}) concentrations of these chemicals can adversely affect the reproductive biology of aquatic wildlife (fish, turtles, frogs, etc.) by disrupting the normal function of their endocrine systems (*9*, *10*). For example, concentrations of 17β-estradiol or estrone in water as low as 30 ng L^{-1} for 21 days induced vitellogenin (an egg yolk precursor protein that is normally produced only by adult females) synthesis and abnormal testicular growth in male fathead minnows (*Pimephales promelas*) (*11*, *12*).

Few studies have specifically examined the relationship between manure-borne estrogens from livestock and adverse effects on aquatic wildlife. Irwin et al. (*13*) measured the concentrations of 17β-estradiol in farm ponds impacted by beef cattle runoff and studied the effects of estradiol on vitellogenin production in painted turtles (*Chrysemys picta*). Estradiol concentrations in the ponds ranged from <1 to 7 ng L^{-1}. Juvenile and male turtles did not synthesize vitellogenin during 28 d of exposure, but female turtles collected from the runoff-impacted ponds had significantly greater concentrations of vitellogenin than female turtles from nonimpacted (control) ponds.

The human health risk associated with environmental estrogen exposure is not clearly known, but the potential risk of endocrine disruption to fish and wildlife requires a better understanding of the sources and fate of estrogens in the environment. The objective of this review is to assess the current state of science regarding estrogen physicochemical properties, livestock excretion, and the biogeochemical fate of manure-borne estrogens in the environment for the purpose of identifying priority research needs.

The scope of this review is limited to the natural estrogen steroids estradiol, estrone, estriol, and their conjugated metabolites. The trivial names and systematic nomenclature for the main chemical compounds that are described in this text are as follows: 17α-estradiol (1,3,5(10)-estratrien-3,17α-diol), 17β-estradiol (1,3,5(10)-estratrien-3,17β-diol), estrone (1,3,5(10)-estratrien-3-ol-17-one), estriol (1,3,5(10)-estratrien-3,16α,17β-triol).

Structure and Physicochemical Properties

Estradiol, estrone, estriol, and other natural steroidal estrogens contain an aromatic A-ring as a distinctive part of their tetracyclic molecular framework (Figure 1) (*14*, *15*). Key structural differences arise in the D-ring structure owing to the type and stereochemical arrangement of functional groups at the C-16 and C-17 positions. Estradiol can have either a hydroxyl group at C-17 that points downward from the molecule (α configuration) or a hydroxyl group that projects upward from the molecule (β configuration). Estrone differs from estradiol because there is a carbonyl group at C-17 rather than a hydroxyl. Estriol features hydroxyl groups at both the C-16 and C-17 position and, thus, has four epimers. Conjugated estrogens are analogous in structure to estradiol, estrone, or estriol, except that a sulfate and/or

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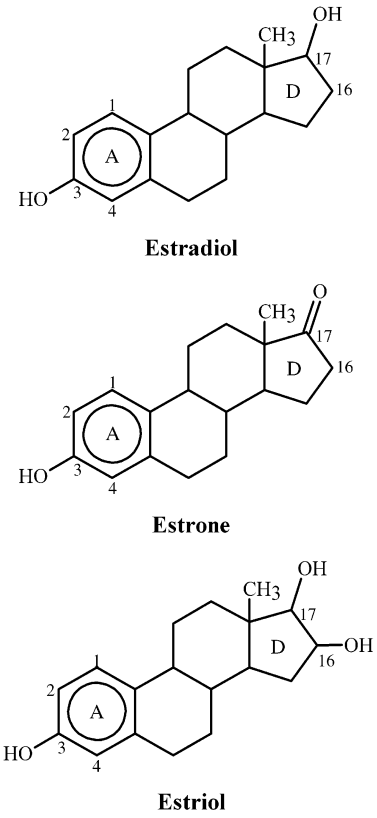

FIGURE 1. Molecular structures of estradiol, estrone, and estriol. The letters and numbers indicate the ring assignments and carbon numbers, respectively.

TABLE 1. Selected Physicochemical Properties of Steroidal Estrogens[a]

property	estradiol	estrone	estriol	reference
formula	$C_{18}H_{24}O_2$	$C_{18}H_{22}O_2$	$C_{18}H_{24}O_3$	
MW (g mol^{-1})	272.4	270.4	288.4	(*22*)
S_w (mg L^{-1})	3.9−13.3	0.8−12.4	3.2−13.3	(*18*, *20*, *21*)
VP (Pa)	3×10^{-8}	3×10^{-8}	9×10^{-13}	(*22*, *24*)
log K_{ow}	3.1−4.0	3.1−3.4	2.6−2.8	(*19*, *22*, *24*)
pK_a	10.5−10.7	10.3−10.8	10.4	(*20*, *23*)

[a] MW − molecular weight, S_w − solubility in water, VP − vapor pressure, K_{ow} − octanol−water partition coefficient, K_a − acid ionization constant.

glucuronide group is substituted at the C-3 and/or C-17 positions of the parent compound (e.g. 17β-estradiol-3-sulfate, 17β-estradiol-17-sulfate, 17β-estradiol-3,17 disulfate). An in-depth description of the electronic structure, crystal geometry, and spectral characteristics of the different estrogens is beyond the scope of this review but is available in Salole (*16*) and Kubli-Garfias (*17*).

The physicochemical properties of estradiol, estrone, and estriol are given in Table 1. Unconjugated estrogens have low aqueous solubility (0.8−13.3 mg L^{-1}) and are moderately hydrophobic (log K_{ow} 2.6−4.0) (*18*−*22*). They are nonvolatile (vapor pressure 9×10^{-13}−3×10^{-8} Pa), weak acids (pK_a, 10.3−10.8) (*20*, *23*, *24*). Physicochemical data for the conjugated estrogens were not found in the literature. However, estrogen conjugates likely have much greater aqueous solubility than unconjugated estrogens due to their polar glucuronide or sulfate functional groups.

Livestock Excretion

Steroidal estrogen hormones are excreted to the environment in the urine and feces of all species, sexes, and classes of farm animals (*25*). However, different estrogens are associated with different livestock species. Cattle (*Bos taurus*) excrete ≥90% of estrogens as 17α-estradiol, 17β-estradiol, and estrone as free and conjugated metabolites (*26*−*31*). The 17α-estradiol epimer is much more prevalent than 17β-estradiol. Conversely, 17α-estradiol rarely occurs in the excreta of swine (*Sus scrofa*) or poultry (*Gallus domesticus*) (*32*−*34*). They excrete 17β-estradiol, estrone, and estriol plus conjugates (*34*). The α and β stereochemical distinction of estradiol might be useful for identifying the livestock species contributing to waterway contamination (cattle vs poultry or swine), but this possibility has not been studied.

Different species also excrete estrogens by different routes. Radiotracer studies showed that cattle excrete estrogens mostly in feces (58%), whereas swine and poultry excrete estrogens mostly in urine (96% and 69%, respectively) (*28*, *32*, *35*). However, these ratios change during pregnancy (*27*). Since urine and feces are not usually handled separately in commercial animal production systems, the route of excretion would not appear to be an important environmental consideration (*25*). However, urinary estrogens are mostly conjugates, whereas fecal estrogens are excreted as unconjugated "free" steroids (*35*). At present, the environmental significance of conjugated vs unconjugated estrogens is debatable due to a lack of information regarding conjugate fate (discussed later).

Estimates, calculated from literature values, of the estrogen excretion rates of cattle, swine, and poultry are given in Tables 2−4, respectively. The various studies of urinary and fecal estrogen excretion were originally intended for describing the patterns of hormonal changes that occur during estrus and pregnancy with the practical purpose of establishing calibrated tests that could be used for fertility control or diagnosing pregnancy (*27*, *29*, *31*, *36*−*45*). The usefulness of the data for environmental purposes is limited because the data represent only sexually mature, female animals from a few breeds. Several factors (e.g. age, mass, diet, season, health status, circadian variation) may contribute to excretion rates that are not easily accounted for (*42*). Furthermore, few data were found which address estrogen excretion by sexually immature, sexually modified (ovariectomized, castrated), or male animals (*46*, *47*). The contribution of estrogens from these animals needs to be better resolved.

Another criticism of the excretion data is that ambiguous quantification methods were used. The studies done in the 1950s to 1970s era relied on colorimetric techniques which inherently lacked sensitivity and selectivity for estrogens (*48*). Colorimetric procedures were phased out in the 1970s and 1980s in favor of highly sensitive radioimmunoassay and enzyme immunoassay methods, but their accuracy is affected by cross reactivity. Furthermore, enzyme immunoassays can suffer from false-positive interferences due to endogenous enzymes, matrix effects, and chromagens (*49*, *50*).

We conclude that the data are insufficient for accurately calculating the total mass flux of estrogens to the environment from whole populations of cattle, swine, or poultry. Other researchers have not been so apprehensive. Lange et al. (*51*) calculated estrogen excretion for various livestock species. They reported that cattle, pigs, and chickens contribute 45, 0.8, and 2.7 Mg estrogens yr^{-1}, respectively, in the United States.

Another way of estimating the risk posed by manure-borne estrogens is to measure the concentrations of estrogens in livestock wastes that are land-applied as soil amendments. This approach takes into consideration the degradation of estrogens during storage and accounts for losses associated with manure handling and treatment practices. However, extensive surveys of different animal production systems are required to establish approximate ranges of estrogens in

TABLE 2. Estimated Rates of Fecal and Urinary Estrogen Excretion from Dairy Cattle[d]

reproductive stage	*N*	excretion rate/ 1000 kg LAM[a] (μg d^{-1})	estrogens measured	method	reference
		Fecal Excretion			
nonpregnant	21	600 ± 200	E2α	RIA	(*31*)
nonpregnant	7	400 ± 10	E1, E2α,[b] E2β	RIA	(*37*)
0−80 d pregnant	10	300 ± nd	E1, E2α, E2β	RIA	(*27*)
0−84 d pregnant	7	400 ± 20	E1, E2α, E2β[b]	RIA	(*37*)
80−210 d pregnant	10	1500 ± nd	E1, E2α, E2β	RIA	(*27*)
140−200 d pregnant	7	11400 ± 1200	E1, E2α,[b] E2β	RIA	(*37*)
210−240 d pregnant	10	5400 ± nd	E1, E2α, E2β	RIA	(*27*)
		Urinary Excretion			
nonpregnant	7	500 ± 40	E1, E2α,[c] E2β	RIA	(*30*)
55−81 d pregnant	5	700 ± 60	E1, E2α,[c] E2β	RIA	(*30*)
101−123 d pregnant	13	14400 ± nd	E1, E2α, E2β, E3	FL	(*109*)
111 d pregnant	3	34300 ± nd	E1, E2α, E2β, E3	FL	(*110*)
107−145 d pregnant	4	3400 ± 1200	E1, E2α,[c] E2β	RIA	(*30*)
165−175 d pregnant	5	28800 ± nd	E1, E2α, E2β, E3	FL	(*109*)
205−209 d pregnant	4	22300 ± 2500	E1, E2α,[c] E2β	RIA	(*30*)
250−254 d pregnant	5	86800 ± 28000	E1, E2α, E2β, E3	FL	(*109*)
271−285 d pregnant	13	163000 ± 20000	E1, E2α, E2β, E3	FL	(*109*)

[a] LAM − live animal mass; calculations based on typical animal weight of 640 kg for dairy (*111*). [b] 11% 17α-estradiol cross reactivity. [c] 32% 17α-estradiol cross reactivity. [d] *N* − number of animals, nd − no data, E1 − estrone, E2 − estradiol, E3 − estriol, RIA − radioimmunoassay, FL − fluorimetry.

TABLE 3. Estimated Rates of Fecal and Urinary Estrogen Excretion from Sows[c]

reproductive stage	*N*	excretion rate/ 1000 kg LAM[a] (μg d^{-1})	estrogens measured	method	reference
		Fecal Excretion			
nonpregnant	4	800 ± nd	E1, E2β, E3	RIA	(*57*)
nonpregnant	69	100 ± 70	E1	EIA	(*45*)
nonpregnant	6	600 ± 250	E1,[b] E2α, E2β, E3	RIA	(*36*)
nonpregnant	27	900 ± nd	not specified	RIA	(*44*)
14−34 d pregnant	6	1500 ± nd	E1, E2β, E3	RIA	(*57*)
25−33 d pregnant	466	1000 ± 680	E1	EIA	(*45*)
0−35 d pregnant	30	1600 ± nd	E1,[b] E2α, E2β, E3	RIA	(*36*)
		Urinary Excretion			
nonpregnant	4	600 ± 350	E1	FL	(*40*)
nonpregnant	2	500 ± 600	E1	FL	(*41*)
nonpregnant	2	400 ± 300	E1	FL	(*39*)
0−42 d pregnant	2	4400 ± 6200	E1	CL	(*112*)
42−77 d pregnant	2	5000 ± 6200	E1	CL	(*112*)
77−105 d pregnant	2	108000 ± 106000	E1	CL	(*112*)

[a] LAM − live animal mass; calculations based on typical animal weight of 61 kg for swine (*111*). [b] 122% estrone, 30% 17α-estradiol, 100% 17β-estradiol, 64% estriol cross reactivity. [c] *N* − number of animals, nd − no data, E1 − estrone, E2 − estradiol, E3 − estriol, RIA − radioimmunoassay, EIA − enzyme immunoassay, FL − fluorimetry, CL − colorimetry.

livestock wastes. Few studies have characterized the estrogen (estradiol, estrone, and estriol) profile of cattle, swine, or poultry wastes (Table 5). Concentrations of 17β-estradiol in various dairy, swine, and poultry wastes range from below detectable limits (BDL) to 239 ± 30 μg kg^{-1}, BDL to 1215 ± 275 μg kg^{-1}, and 33 ± 12 to 904 μg kg^{-1}, respectively (*3*, *5*, *52*). More characterization data are needed to determine which type of livestock wastes are most estrogenic and if manure treatment strategies are needed to reduce estrogen concentrations to environmentally acceptable levels.

Environmental Fate

Conjugate Hydrolysis. The fate of estrogen conjugates is not clearly known. It is often assumed that common fecal microorganisms such as *Eschericia coli* are capable of hydrolyzing estrogen conjugates via glucuronidase and sulfatase enzymes to unconjugated forms (*53*). The assumption appears valid for estrogen glucuronides but is questionable for estrogen sulfates since measurable concentrations (ng L^{-1}) of these conjugates have been reported in sewers, sewage treatment works, and river water (*45*, *54*−*56*). D'Ascenzo et al. (*55*) demonstrated that estrogen sulfates are slowly hydrolyzed in septic tank wastewater. After a 10 h lag-phase, half-lives of estradiol-3-sulfate and estrone-3-sulfate were approximately 2.5 d at 20 °C. Estriol-3-sulfate was more stable, with a lag-phase of 70 h and half-life of 5 d.

Limited research has evaluated the stability of conjugated estrogens in manure. Vos (*57*) incubated ^{3}H-estrone-sulfate and ^{3}H-estrone-glucuronide with sow feces ≤30 min at 20 °C. Estrone glucuronide was rapidly deconjugated (90% in 30 min) in the fecal suspension, but estrone sulfate was not hydrolyzed. Raman et al. (*6*) incubated dairy waste with *Helix pomatia* glucuronidase-sulfatase to hydrolyze conjugated estrogens. No differences in free estrogen concentrations were found between hydrolyzed and nonhydrolyzed samples. These results suggest that estrogen sulfates were not present in the dairy manure. However, enzyme hydrolysis is not an efficient method for deconjugating estrogen sulfates and often requires the application of more rigorous techniques such as acid hydrolysis, ammonolysis, or methanolysis (*58*−*61*). Finlay-Moore et al. (*3*) tried to measure estradiol conjugates in poultry waste-impacted runoff water using the meth-

TABLE 4. Estimated Rates of Urinary Estrogen Excretion from Nonlaying and Laying Hen Chickens[b]

reproductive stage	N	excretion rate/ 1000 kg LAM[a] (μg d^{-1})	estrogens measured	method	reference
nonlaying	3	600 ± 30	E1	CL	(*113*)
nonlaying	1	500 ± nd	E1, E3	CL	(*114*)
nonlaying	1	400 ± 20	E1	CL	(*113*)
nonlaying	2	1400 ± 550	E1, E2β	CL	(*115*)
nonlaying	2	900 ± nd	E1, E3	CL	(*114*)
laying	1	1600 ± nd	E1, E2β	FL	(*116*)
laying	1	2100 ± 80	E1	CL	(*113*)
laying	1	2700 ± 130	E1, E3	CL	(*114*)
laying	1	1400 ± 50	E1	CL	(*113*)
laying	2	3500 ± 430	E1, E2β	CL	(*115*)
laying	3	1600 ± nd	E1, E3	CL	(*114*)

[a] LAM − live animal mass; calculations based on typical animal weight of 1.8 kg for layers (*111*). [b] *N* − number of animals, nd − no data, E1 − estrone, E2 − estradiol, E3 − estriol, CL − colorimetry, FL − fluorimetry.

anolysis procedure of Tang and Crone (*61*). They reported that the method was unsuitable, however, because measured values of unconjugated 17β-estradiol increased ≤150% in some cases and decreased ≤63% in other samples.

Degradation of Unconjugated Estrogens. The biodegradation and transformation of unconjugated estrogens has been studied in soil, water, and manure for several years. In 1947, Turfitt (*62*) examined the biodegradation of 17α-estradiol and estrone using 355 different cultures of bacteria isolated from five different soil types. No culturable bacteria were found in loam, marl, or alkaline peat soils that could metabolize estradiol. However, one *Proactinomyces spp*. was isolated from an acid sand, and two strains were found in arable soil that could use estradiol as a carbon source. Estrone was degradable by one species of *Proactinomyces spp*. in the arable soil, but no degradation was observed with organisms from the other four soils.

More recently, Colucci et al. (*63*) studied the dissipation (decrease in extractable/bioavailable concentrations and mineralization) of ^{14}C-17β-estradiol in loam, sandy loam, and silt loam soils from Canada. The biological activity (determined by a yeast assay) of estradiol was rapidly dissipated in all soils, and 17β-estradiol was rapidly converted to estrone. The accumulation of estrone in the loam soil was maximal at 6 h but was undetectable thereafter. In the silt loam and sandy loam soils, however, estrone was detectable for 3 months. Autoclaving the soils did not prevent the oxidation of estradiol to estrone. This result suggests that either there was an incomplete sterilization of the soil, the enzyme responsible for estradiol transformation survived autoclaving, or that estradiol oxidation can proceed abiotically. The mineralization (cleavage of the phenolic ring) of estradiol in the soils tested was relatively slow compared with the rates of dissipation; only 12−17% of added ^{14}C-17β-estradiol was evolved as $^{14}CO_2$ after 3 months of incubation at 30 °C. The highest rates of mineralization were observed in the sandy loam soil, and the lowest rates were observed in the silt loam soil. A comparison of soil pH, organic matter content, and texture did not reveal any consistent effect of these soil properties. When the soil temperature was increased from 4 °C to 37 °C, mineralization in the loam soil increased from 4 to 15% after 61 days of incubation. Mineralization also increased from <1% to 20% after 73 d of incubation when the moisture content of the sandy loam soil was increased from air-dry to 15%. However, when moisture content of the same soil was increased to field capacity (24%), the amount of estradiol mineralized decreased sharply to 8%. The authors concluded that estrogens are biodegradable in soils by ubiquitous microorganisms that require no prior adaptation (*63*).

Rapid biodegradation of estrogens in river water was reported by Jurgens et al. (*64*). The half-lives of estradiol and estrone at 20 °C ranged from 0.2 to 9 d and from 0.1 to 11 d, respectively. No significant losses of estradiol were found in sterile controls. Lai et al. (*65*) reported that common freshwater algae (*Chlorella vulgaris*) are capable of oxidizing 17β-estradiol to estrone.

Jarvenpaa et al. (*66*) found that aerobic and anaerobic microflora isolated from the human intestinal tract and human feces were capable of transforming estrogens during 24−72 h incubation. *Alcaligenes faecalis, Pseudomonas aeruginosa*, *Staphylococcus aureus*, and *Mycobacterium smegmatis* converted estradiol to estrone and vice versa. *Streptococcus faecalis* (four strains) oxidized estradiol to estrone, and one strain transformed estrone to 16α-hydroxyestrone. *Bacteroides fragilis* reduced estrone to estradiol, but

TABLE 5. Concentrations of Estradiol and Estrone Reported in Various Types of Dairy, Swine, and Poultry Wastes (Dry Weight Basis)[b]

waste type	N	17α-estradiol (μg kg^{-1})	17β-estradiol (μg kg^{-1})	estrone (μg kg^{-1})	method	reference
Dairy						
press cake solids	1	139 ± 7	BDL	426 ± 78	GC-MS	(*6*)
dry-stack (semisolid)	36	603 ± 358	236 ± 216	349 ± 339	GC-MS	(*52*)
dry-stack (solid)	24	289 ± 207	113 ± 67	203 ± 176	GC-MS	(*52*)
holding ponds	48	370 ± 59	239 ± 30	543 ± 269	GC-MS	(*52*)
Swine						
finishing lagoon	48	BDL	BDL	1507 ± 382	GC-MS	(*52*)
finishing hoops	18	BDL	160 ± 26	217 ± 52	GC-MS	(*52*)
farrowing lagoon	16	BDL	BDL	1295 ± 168	GC-MS	(*52*)
farrowing pit	32	890 ± 120	1215 ± 275	4728 ± 427	GC-MS	(*52*)
Poultry						
broiler litter	3	ND	33 ± 12	ND	EIA	(*3*)
broiler litter	3	ND	133 ± 6	ND	EIA	(*4*)
broiler litter (Al treat.)	3	ND	101 ± 2	ND	EIA	(*4*)
broiler litter	1	ND	904	ND	EIA	(*5*)
broiler litter (females)	10	ND	65 ± 7[a]		RIA	(*67*)
broiler litter (males)	10	ND	14 ± 4[a]		RIA	(*67*)
layer litter	17	ND	533 ± 40[a]		RIA	(*67*)
rooster litter	10	ND	93 ± 13[a]		RIA	(*67*)

[a] Reported as 17β-estradiol plus estrone. [b] *N* − number of samples, BDL − below detectable limits, ND − not determined, GC-MS − gas chromatography−mass spectrometry, EIA − enzyme immunoassay, RIA − radioimmunoassay.

also converted estrone to 16α-hydroxyestrone. *Staphylococcus aureus* and *M. smegmatis* reduced 16α-hydroxyestrone to estriol. *Candida albicans*, *Enterobacter cloacae*, *E. coli* (two strains), *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Proteus vulgaris* were unable to metabolize estrone, estradiol, or 16α-hydroxyestrone to any other products (*66*).

Shore et al. (*67*) incubated broiler litter for 1 week at different pH values, with and without the addition of antibiotics (penicillin/streptomycin), and found significant reductions in estrogen concentrations at pH 5 and 7 but no change at pH 1 or 12. When antibiotics were added to the litter, estrogens persisted. Schlenker et al. (*68*) studied the degradation of estrogens in cattle feces by incubating manure samples for 12 weeks at 20−23 °C. The median concentrations of total estrogens extracted from the manure were unchanged for 9 weeks but were reduced by 80% after 12 weeks. Schlenker et al. (*69*) tested *E. coli* and *Clostridium perfringens* for their ability to degrade fecal estrone in cattle manure. The *E. coli* had no effect on estrone concentrations, but the *C. perfringens* reduced the average concentration of estrone from ∼16 μg L^{-1} to ∼11 μg L^{-1} during the 48 h incubation. Schlenker et al. (*70*) evaluated the influence of temperature on the stability of estrogens in the feces of cattle. At 5 °C, the median concentrations of total estrogens extracted from the manure fell below initial concentrations after 12 weeks of incubation. At 30 °C, however, estrogen was almost completely eliminated from the samples within 3 weeks. Similar studies of estrogen degradation in dairy cattle manure were done by Raman et al. (*6*). Press cake samples were spiked with 17β-estradiol and incubated at temperatures ranging from 5° to 50 °C. The effects of acidification on estrogen transformation and degradation during sample storage were also evaluated. At all temperatures, estradiol concentrations rapidly declined during the first 24 h of incubation, and estrone accumulated. Total estrogen removal rates followed the pattern of estrone degradation, and these data were fitted to a first-order decay model. Rate constants increased from ∼0.03 d^{-1} at 5 °C to ∼0.12 d^{-1} at 50 °C. Acidification to pH 2 reduced rates of estrogen transformations at both 5 °C and 30 °C, but a 15% and 31% loss, respectively, of total estrogen was still observed when samples were stored for 7 days. The authors speculated that *Cornybacterium spp.* were partially responsible for the estrogen transformations in their study (*6*).

Based on the data available, it appears that estrogens are biodegraded in the environment by many different types of microorganisms. Few degradation mechanisms have been proposed, but the oxidation of estradiol (C-17 alcohol) to estrone (C-17 ketone) is frequently reported (*6*, *22*, *24*, *54*, *63*). It can be hypothesized that the reaction is catalyzed by bacterial or fungal dehydrogenases (*71*−*74*). Further degradation of estrone may involve C-2 or C-4 hydroxylation of the phenolic A-ring and subsequent ring cleavage and/or C-16 hydroxylation of the D-ring (*66*, *75*, *76*). The phenol-oxidase group of enzymes (e.g. laccase, tyrosinase, and peroxidase) that are produced by bacteria, white-rot fungi, and plants might be critical for the degradation process (*77*−*79*). If so, the phenolic estrogens may be oxidized to quinones, which may polymerize into humus-like macromolecules (*80*−*92*).

If estrogens behave like other phenolic compounds in the environment, they may also oxidize abiotically. For example, Lehmann et al. (*93*) demonstrated that the oxidation of phenolic acids in soils can be coupled with the reduction of Fe and Mn oxides. The catalytic effects of Mn (IV), Fe(III), aluminum, and silicon oxides on the formation of phenolic polymers in soils was studied by Shindo and Huang (*94*). Mn oxides caused phenolic compounds to be converted to humic acid with a high degree of humication via oxidative polymerization. Mn oxide reduction is an important mechanism in the oxidation of phenols in aquatic systems (*95*). We are not aware of studies that have examined the role of Mn in the environmental fate of estrogens.

Sorption and Mobility. Estrogens are nonvolatile, slightly hydrophobic compounds that do not ionize at normal environmental pH and should be extensively sorbed by aquatic sediments and soils. Holthaus et al. (*19*) studied the sorption of 17β-estradiol to river sediments. They reported sorption coefficients (K_d) that ranged from 4 to 74 L kg^{-1} for bed sediments and from 21 to 122 L kg^{-1} for suspended sediments. Casey et al. (*96*) reported that ^{14}C-17β-estradiol is strongly sorbed by soils. Sorption coefficients (K_d) ranged from 86 to 6670 L kg^{-1} as determined by batch equilibrium studies with four Mollisols. Positive correlations were found between estradiol sorption and silt content ($r^2 = 0.92$) and organic carbon ($r^2 = 0.62$). Column experiments demonstrated that estradiol is not easily leached through the soil. Colucci et al. (*63*) also reported a strong retention of estrogens to soil particles. Within 3 days of contact between ^{14}C-estradiol and loam, sandy loam, and silt loam soils, 91, 70, and 56% of the radioactivity, respectively, was nonextractable from the soils using ethyl acetate or acetone. Variations in soil properties (soil pH 5.8−7.4, organic matter 0.8−3.2%) were not consistently related to sorption capacity. However, when soils were autoclaved, the amount of extractable radioactivity remained constant for several days. This result suggested that the formation of nonextractable (bound) residues in the soils was microbially mediated. Colucci and Topp (*97*) concluded that estrogen dissipation via the formation of soil-bound residues greatly reduces the risk of contamination of water adjacent to agricultural soils treated with municipal biosolids or livestock wastes.

Though laboratory-based experiments have suggested that ^{14}C estrogens are rapidly sorbed by soil particles, it should be recognized that sorption was evaluated without additions of manure. The information thus gained does not allow assessment of the effects of the chemical, physical, and microbiological changes that can occur in a soil following a manure application. It can be speculated that natural surfactants and colloids might increase the mobility of estrogens in soils and together with erosion and preferential flow mechanisms could lead to the transport of manure-borne estrogens to waterways.

Occurrence in Manure-Impacted Water. Field studies with manure have demonstrated that estrogens are sufficiently mobile to impact surface and groundwater quality. For example, Shore et al. (*8*) surveyed estrogen (17β-estradiol plus estrone) concentrations in a few small streams, an irrigation pond, and a farm well impacted by the land application of poultry litter (no estrogen concentrations reported or application rates specified). Estrogen concentrations in the streams increased from <0.5 ng L^{-1} to 5 ng L^{-1} following poultry litter application, whereas concentrations in the pond decreased from 23 to 5 ng L^{-1} during the study period (9 months). Low concentrations (<0.10 ng L^{-1}) of estrogens were found in the well water samples.

Nichols et al. (*4*) tested the hypothesis that land-applied poultry litter contributes 17β-estradiol to runoff water. They reported that the water-soluble 17β-estradiol contents of normal and alum treated litter were 133 and 102 μg kg^{-1} (dry weight basis), respectively. Estradiol concentrations in the runoff water increased with litter application rate (1.76−7.05 Mg ha^{-1}) for both untreated and aluminum sulfate treated amendments. A maximum concentration of 1280 ng estradiol L^{-1} was detected in first-storm runoff water from plots amended with normal poultry litter. Aluminum sulfate treatment of the litter significantly reduced 17β-estradiol concentrations in first-storm runoff by 42%, presumably due to the flocculation of soluble organic compounds with aluminum. An additional study by these authors compared the effectiveness of varying lengths of grass filter strips to

help reduce concentrations of 17β-estradiol in runoff water from fescue-applied poultry litter (*5*). The water-soluble 17β-estradiol concentration of the litter sample was 904 μg kg^{-1}. The litter application rate of 5 Mg ha^{-1} was consistent with the recommendation for tall fescue in Arkansas. Concentrations of 17β-estradiol in runoff from plots without a grass filter (controls) averaged 3500 ng L^{-1}. Compared with the control plots, estradiol concentrations were reduced by 58, 81, and 94% after transport through 6.1, 12.2, and 18.3 m long grass filters, respectively. Bushee et al. (*1*) investigated runoff concentrations of 17β-estradiol from plots amended with horse bedding or municipal sludge. The horse bedding and municipal sludge contained 35 μg kg^{-1} and 5 μg kg^{-1} (author did not indicate wet or dry weight basis) of 17β-estradiol, respectively. The horse bedding was applied to fescue grass plots at a rate of 9.1 Mg ha^{-1} and the sludge at a rate of 7.7 Mg ha^{-1}. The cumulative transport of estradiol from the plots after 30 min of simulated rainfall was 70 and 12 mg ha^{-1} for horse bedding and municipal sludge, respectively. In contrast to the findings of Nichols et al. (*4*), alum treatment of either material did not significantly reduce estradiol losses.

Finlay-Moore et al. (*3*) measured 17β-estradiol concentrations in runoff and soil from grazed and ungrazed pastures fertilized with broiler litter. The ethyl acetate extractable concentrations of 17β-estradiol in three poultry litter samples ranged from 20 to 35 μg kg (dry weight basis). After litter was applied, concentrations of 17β-estradiol in runoff were 20−2530 ng L^{-1}, depending on litter application rate and time between application and runoff. High background estradiol concentrations were found in runoff that ranged from 50 to 150 ng L^{-1}. Prior to the addition of litter, the concentration of 17β-estradiol in the soil was ∼55 ng kg^{-1}. Immediately following the application of litter, elevated levels of 17β-estradiol were detected (≤675 ng kg^{-1}). The high concentrations did not persist in surface (upper 2.5 cm) soil for more than a few weeks. No samples were collected from lower soil depths, so leaching of estradiol into the soil profile or degradation in the soil could not be determined. There were no significant effects of grazing cattle on the concentrations of 17β-estradiol in the runoff (*3*).

Dyer et al. (*2*) measured 17β-estradiol concentrations in runoff from bermudagrass plots fertilized with liquid dairy manure. They applied manure containing 3300 ng L^{-1} (wet weight basis) of 17β-estradiol to plots at rates equivalent to 0, 65, and 142 kg N ha^{-1}. Runoff samples were collected from the plots following natural rainfall events (rainfall dates or amounts not reported). Estradiol concentrations from control plots ranged from below detectable limits (1.6 ng L^{-1}) to 2.1 ng L^{-1}. At the highest rate of manure application, estradiol concentrations reached as 41 ng L^{-1} but decreased steadily to background (control) concentrations by the end of the study (3 months). These results suggested that N-based application rates of dairy manure could potentially increase 17β-estradiol concentrations in runoff.

Nationwide reconnaissance data by the U.S. Geological Survey showed estradiol and estrone concentrations were ≤200 and ≤112 ng L^{-1}, respectively, in a network of 139 streams in 30 states impacted by animal wastes (*98*). Peterson et al. (*99*) sampled five springs from the mantled karst aquifer system of northwest Arkansas (a major poultry and cattle production region) for fecal coliforms and 17β-estradiol. Concentrations of 17β-estradiol ranged from 6 to 66 ng L^{-1}. At all locations, there was a positive correlation between estradiol concentrations and the concentrations of both fecal coliform (r^2 ranging from 0.49 to 0.86) and *E. coli* (r^2 ranging from 0.40 to 0.88), suggesting that estradiol and bacteria were moving through the aquifer system in a similar fashion. The authors concluded that estradiol of livestock origin was directly affecting the groundwater quality of the springs.

The concentrations of 17β-estradiol reported in the abovementioned studies of surface and groundwater warrant careful attention due to the previously stated 10−100 ng L^{-1} range of biological significance for aquatic organisms. We noticed that all of the field studies, except for Kolpin et al. (*98*), determined 17β-estradiol using immunoassay. The authors provided few quality control details (besides manufacturer's statements) regarding the sensitivity, accuracy, precision, and reliability of the analytical methods used. As previously stated, immunoassays can be affected by a number of interferences, especially when chromatographic purification is not done. Surface water is known to contain natural organic matter that can interfere with immunoassays in a manner that causes false positive signals (*58*). We suspect that the runoff concentrations are overestimated. If not, the contamination of surface and groundwater by manure was probably worse than predicted by the evaluation of 17β-estradiol alone due to the unmeasured contribution of estrone. In either case, the validation of immunoassay results by the use of nonambiguous quantification methods such as LC-MS or GC-MS would add credibility to the measured estrogen concentrations. A number of mass spectrometry methods have recently been proposed for the analysis of estrogens in sewage, sewage effluent, and other water samples (*100*−*108*). These techniques may be useful, if not directly applicable, for the quantification of estrogens in manure and manure-impacted soil and waterways.

Future Research Needs

In light of the information presented in this review, a number of research priorities can be suggested: (i) There is a critical need to use standardized methods for the analysis of estrogens in manure, soil, and water. Juridical proof of estrogen contamination will require LC-MS or GC-MS quantification methods. (ii) More national, state, and local surveys of manure-impacted surface and groundwater resources need to be conducted to determine if estrogen contamination is a widespread phenomenon or is localized to intensive livestock production areas. Other water quality indicators (e.g. fecal coliforms, nitrates, phosphorus) should also be measured during these surveys so that maximum information can be gained about any estrogen pollution attributable to manure. Wildlife exposed to estrogen-contaminated waterways and/or test organisms should be studied for evidence of reproductive abnormalities. (iii) More information is needed about the types and amounts of estrogens that exist in fresh livestock excreta (urine and feces) and manure. Characterization experiments should be broad in scope to reflect a wide range of livestock production techniques and manure handling and storage practices. Better estimates of the total mass flux of estrogens to the environment could therefore be made. (iv) More work needs to be done regarding the fate of conjugated (especially estrogen sulfates) and unconjugated estrogens in manure, soil, and water. The rates of deconjugation reactions, the oxidation/reduction relationship between estradiol and estrone, and the kinetics of biodegradation should be measured in the various matrices. Experiments that reveal the influence of temperature, moisture, pH, and microbial activity would also improve knowledge of estrogen persistence under various environmental conditions. Ideally, the specific enzyme(s) and/or soil mineral(s) participating in estrogen transformation and mineralization reactions should be identified so that degradation and sorption mechanisms can be proposed. Partitioning experiments need to identify the surfaces responsible for estrogen sorption (organic matter, Fe and Al oxides, etc.) and the chemical conditions (pH, salinity, etc.) that enhance binding of estrogens to solid phases in manure, soils, and aquatic systems. Desorption kinetics and aging phenomena should also be evaluated because

estrogens may form nonextractable (bound) residues in soils. More field and laboratory studies are needed to determine the mechanisms of estrogen transport (surface runoff vs leaching) to waterways. (v) Besides estrogens, other hormonally active agents in manure (e.g. androgens, gestagens, growth promoters, and antibiotics) need to be characterized and studied. Ultimately, it may be necessary to develop cost-effective manure treatment strategies to reduce or eliminate manure-borne endocrine disruption hazards.

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