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Fate and Transport of Steroid Hormones in the Environment

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Abstract

As they interfere with the normal functioning of endocrine system, a continuous occurrence of steroid hormones in our water resources have a growing environmental and public health concerns. Few studies depict that these compounds have a strong tendency to adsorb to the soil organic matter or degrade rapidly both in water and soil. It was also assumed that they have little mobility and no persistence in the environment, and therefore the possibility of contaminating our water resources would have been due to accidental spill or leakage from poorly constructed animal waste lagoons. Nonetheless, these compounds are consistently detected in surface and ground waters, and the recorded concentrations in the environment significantly exceeded the reported low observable effect concentration 1 ng L^{-1} for 17 β -estradiol (E2). This study reviews the current knowledge of the occurrence, fate and transport of 17 β -estradiol and testosterone in the environment. Factors and processes that affect the mobility of these compounds in the environment are also highlighted. It appears that these hormones together with their metabolites may persist in soil or sediments for several months, and their mobility and behavior in the environment need to be well understood. Furthermore, despite the fact that the largest potential source of these compounds is Concentrated Animal Feeding Operations (CAFO), the current generally accepted livestock waste management practices are not adequately or effectively protect our water resources. Thus, further researches about how to enhance their removal efficiency in animal manures as well as wastewater effluent is of paramount importance.

Introduction

The widespread occurrence of steroid hormones and their metabolites in the natural water resources as well as drinking water is gaining a growing concern. 17 β -estradiol (E2) and testosterone are some of the naturally produced steroid estrogens and androgens, respectively. These compounds including their metabolites are relatively the most potent endocrine disruptors that may interfere with the normal functioning of the endocrine system at a very low concentration. For example, E2 was reported to reduce sperm fertility very drastically and induce vitellogenin in male trout at about 1 ng L^{-1} (Lahnsteiner *et al.*, 2006). In addition, exposure to E2 and its metabolite estrone (E1) in the environment was linked to feminization of male fish, decreased sperm counts, increased testicular, prostate and breast cancer and male reproductive disorder (Dickson *et al.*, 1986; Harrison *et al.*, 1997), whereas the presence of testosterone and its metabolite androstenedione in the aquatic ecosystem was linked to the masculinization of female mosquito fish (Thomas *et al.*, 2002).

Despite those environmental and public health concerns, the processes and factors that affect the fate and transport of these compounds is not well understood. In laboratory studies, E2 and testosterone were shown to have a strong tendency to be sorbed into the soil or sediment organic matter, and degrade rapidly in soil and water. Subsequently the possibility of leaching into the ground water was indicated to be limited (Lee *et al.*, 2003). Nevertheless, the persistence of these compounds in soils, ground water and surface water for several months is not consistent with the high sorption and degradation rate constants reported by the laboratory studies. Thus, for better

characterization of their potential risk to the environment as well as human health, a better understanding of the factors and processes that affect the fate and transport of these compounds in the environment is imperative.

In this study, the available primary and secondary publications were reviewed and the current knowledge about the fate and transport of E1, E2, testosterone and androstenedione in the environment is summarized. Also, an overview of the potential sources and the recorded environmental concentrations in the environment is given.

Physicochemical properties

The selected physicochemical properties of E2 and Testosterone together with their selected metabolites are indicated in Table 1, which shows that E1 and E2 are less water-soluble than testosterone and androstenedione. That is consistent with their hydrophobic property except for the androstenedione whose $\log K_{ow}$ is higher than the $\log K_{ow}$ of E1. Testosterone is relatively the least in hydrophobic property. Vapor pressure of androgens is not available, but E2 and E1 have low volatility with very low vapor pressure (2.3×10^{-13} mm Hg) indicating that loss of these compounds through volatilization process is not likely.

Table 1. Selected properties of estrogens and androgens

Hormone	Molecular Weight	Water Solubility at 20°C (mg L ⁻¹)	Vapor pressure (mm Hg)	$\log K_{ow}$
17β-estradiol	272.4	13 ^a	2.3×10^{-13}	3.94 ^d
Estrone	270.4	13 ^a	2.3×10^{-13}	3.43 ^d
Testosterone	288.4	18-25 ^b	NA	3.32 ^e
Androstenedione	286.4	37-41 ^c	NA	3.69 ^e

^aLai *et al.* (2000); ^bSugaya *et al.* (1997); ^cSuzuki *et al.* (2001); ^dYing *et al.* (2005); ^eTabak *et al.* (1981); NA: Not Available

Source of contamination

Ground water as well as surface water contamination by steroid hormones can occur several ways, but the main potential sources and pathways are illustrated in Figure 1. The two main sources of contamination include wastewater effluents (Ternes *et al.*, 1999), and runoff from soil amended with sewage sludge or biosolids, and animal manure from concentrated animal feeding operations (Finlay-Moore *et al.*, 2000). Both animals and humans excrete significant quantities of E2 and testosterone (Shemesh and Shore, 2004), which may find its way to surface waters or ground water in considerable quantity. This is due to the fact that conventional biological wastewater treatment plants may not completely remove all these hormones present in the influent, and consequently wastewater effluent that may contain significant concentrations of these compounds may directly be discharged to receiving water.

Considerable amount of these hormones may contaminate agricultural soil and then water through application of wastewater effluent for irrigation and soil amendment with poultry manure and animal wastes (Figure 1). Overland flow is not the only source of surface water contamination, as steroid hormones that infiltrated into the subsurface, and were transported via soil and groundwater to the stream channel may contaminate surface waters (Herman and Mills, 2003).

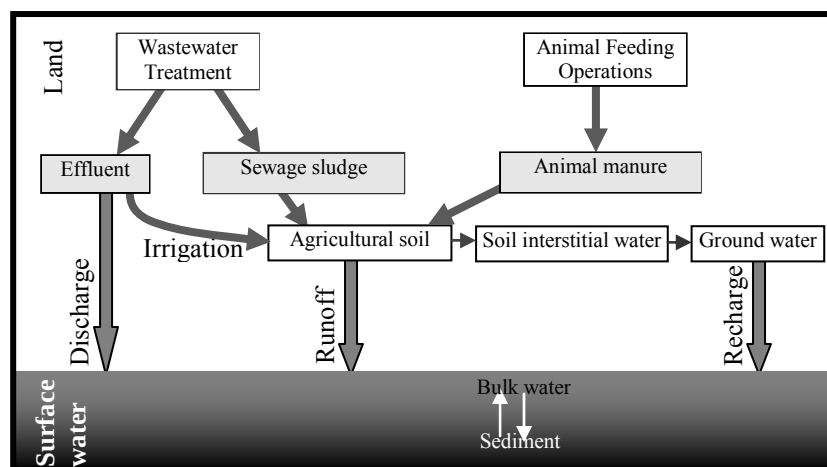


Figure 1. Schematic presentation of main sources of contamination

As abiotic processes such as granulated activated carbon and reverse osmosis can treat wastewater effluent further, manure-born steroid hormones is becoming of concern for five main reasons. *First*, they are the most potent endocrine disrupting compounds other than industrial products such as alkylphenol ethoxylates (Jobling and Sumpter, 1993). *Second*, animal farms produce a significant quantity of manure together with these highly potent endocrine disrupters each year. For example, E2 ranges between 14 and 533 ng g⁻¹ with average 44 ng g⁻¹ on the dry weight basis (Shemesh and Shore 1994). Persistence of E2 and testosterone in the animal manure for prolonged durations (more than 2 years) was also reported; reaches up to 904 ng g⁻¹ and 670 ng g⁻¹ on the dry weight basis, respectively (Fisher *et al.* 2005). *Third*, assuming the bioavailable environmental concentration would be very low due to possible biotic or abiotic transformation, these compounds are not yet regulated. *Fourth*, they are continuously present in soil and aquatic environments, including soil that is not amended with animal manures. This might be due to their possible persistence in the environment or an external input by wild animals or birds (Finlay-Moore *et al.*, 2000). *Fifth*, the current generally accepted livestock waste management practices are not adequately or effectively protecting our water resources (Burkholder *et al.*, 2006). Consequently, both poultry compost and cattle manure remain the potential sources of steroid hormones, including synthetic estrogens, unless proper alternative actions are taken. This suggests that it requires further studies that focus on the methods used to reduce the concentrations of these hormones in the poultry compost or cattle manures most efficiently.

Recorded concentrations in the environment

Few monitoring data are available for steroid hormones in the environment, as it is not yet required by EPA to monitor them in our water supply. Nevertheless, significant concentration of these compounds has been reported in the environment including in drinking water. These compounds have been found in streams, ground water, soil water, effluent, runoff water from agricultural site, drinking water, soil and sediments (Table 2). Typical concentration of E2 ranges from 0.2 ng L⁻¹ in drinking water (Kuch and Ballschmitter, 2001) to 120 ng L⁻¹ in streams (Kolpin *et al.*, 2002). The concentration in the stream is about two orders of magnitude as high as effect concentration, 1 ng L⁻¹. Limited data are available for testosterone, E1 and androstenedione. Typical wastewater effluent concentration in the United States ranges from 6.2 to 54.8 ng L⁻¹ for E2. Testosterone concentration ranges from 0.2 ng L⁻¹ in drinking water to 1,

Table 2. Environmental concentration of natural steroid hormones

System	E2 (ng/l)	Testosterone (ng/l)	E1 (ng/l)	Androstenedione (ng/l)
Stream	19-120 ^a	200 ^a	17 ^a	20-60 ^b
Soil water	86-160 ^c			
Ground water	66 ^d			
Effluent	6-53 ^e	5-10 ^e	4 ^e	4.5 ^e
Runoff*	20-2,330 ^f	10-1,830 ^f		
Drinking water	0.2 – 2.1 ^g	0.2-0.6 ^g		
Soil	675 ^f ng kg ⁻¹	260 ^f ng kg ⁻¹		100 ^j ng kg ⁻¹
River sediment	50 – 2520 ng/kg ⁱ		22800 ng/kg ^h	500-900 ^b

*Runoff from soil amended with poultry manure; ^aKolpin *et al.*, 2002; ^bJenkins *et al.*, 2003; ^cHerman and Mills, 2003; ^dDorabawila and Gupta, 2005; ^ePeterson *et al.*, 2000; ^fKuch & Ballschmitter, 2001; ^gTanaka *et al.*, 2000; ^hSchlenk *et al.*, 2005; ⁱLee *et al.*, 2003; ^jKuster *et al.*, 2004; ^kFinlay-Moore *et al.*, 2000; ^lShore *et al.*, 2007

830 ng L⁻¹ in runoff water from soil amended with poultry manure. In sediment considerable amount of these hormones was also reported, which shows the potential persistence of E1, E2 and androstenedione in the river sediment (Jenkins *et al.*, 2003; Tanaka *et al.*, 2000; Schlenk *et al.*, 2005).

Persistence of steroid hormone in the soil amended with animal manure and poultry compost may determine the contamination of ground waters. If these chemicals are persistent in soil or water in some conditions, any surface land activities may influence the leaching potential of steroids into the ground water. For example, tillage and grass strips may increase percolation (Finlay-Moore *et al.*, 2000); Herman and Mills (2003) reported 86-160 ng L⁻¹ E2 in the interstitial water. When a chemical is able to percolate deep into the subsoil, it may reach the ground water.

Fate

Sorption

Sorption of E2 and testosterone to the sediment or soil may determine the fate and transport of these compounds in the environment. Sorption to the soil organic matter reduces the soluble form of these compounds and thereby probably limits their leaching to the ground water. Several sorption studies have been done for E1 and E2, but little was done for testosterone and androstenedione (Table 3). On the basis of the $\log K_{oc}$ values depicted in Table 3, testosterone is more mobile than E2, which is consistent with most batch equilibrium and soil column studies (Stumpe and Marschner, 2006; Lee *et al.*, 2003). Androstenedione is the least mobile among four steroid hormones presented herein. This is consistent with the $\log K_{ow}$, but inconsistent with its water solubility (Table 1).

While studying the sorption of steroid hormones, six important factors need to be taken into consideration: content of soil organic carbon, type and content of clay mineral, salinity, method of sorption test, initial concentration of the test compounds, and understanding of mechanism of interaction. Several studies indicated that sorption of the four hormones under consideration is correlated with the presence of organic carbon and clay content (Casey *et al.*, 2005; Lee *et al.*, 2003; Mansell *et al.*, 2004). The more organic carbon and clay that is present in the soil or sediment, the higher the quantity of these steroid hormones would be sorbed to the soil or

Table 3. Typical $\log K_{oc}$

References	$\log K_{oc}$			
	E1	E2	Testosterone	Androstenedione
Hildebrand <i>et al.</i> (2006)	4.64	3.69	NA	NA
Ying and Kookana (2005)	3.34-3.57	3.49-3.71	NA	NA
Yu <i>et al.</i> (2004)	3.3-5.25	3.14-5.38	NA	NA
Lee <i>et al.</i> (2003)	3.18-3.22	3.16-3.52	3.24-3.44	3.67-3.77
Stumpe and Marschner (2006)	NA	3.22-3.75	2.59-2.88	NA
Lai <i>et al.</i> (2000)	3.69	3.5	NA	NA

sediment. It should also be noted that the presence of organic carbon is not a prerequisite for sorption of the steroid hormones. For example, iron oxide alone is shown to account for 40% of sorption to organic carbon (Lai *et al.*, 2000), which indicates that the presence of iron oxide and salinity may increase sorption of these compounds.

Application of different methods may result in significantly different results in $\log K_{oc}$. In an undisturbed soil column study, sorption estimates were indicated to be lower than the batch equilibrium as the result of rate-limiting sorption due to the advective transport (Casey *et al.*, 2004). In the batch equilibrium test, which involves an aqueous phase, the sorbed phase is usually estimated on the basis of mass balance instead of measuring directly (Case *et al.* 2005). Such easy approach may not be reliable in some conditions when one deals with a chemical that can be biotransformed easily. In addition, the method of sterilization of the soil sample for sorption study can also affect the results of the sorption test, e.g., using sodium azide (Mansell *et al.* 2004) instead of autoclaving. According to Hildebrand *et al.* (2006), autoclaving was indicated to be the most effective sterilizer. Consequently, while most studies indicated that testosterone was more mobile than E2, Mansell *et al.* (2004) suggested that testosterone was less mobile than E2. As testosterone is relatively easily degraded, the estimated sorbed phase in Mansell *et al.* might be overestimated, and it might have included biotransformation. Therefore, for an appropriate sorption test, autoclaving and determination of the sorbed phase is essential in order to ensure the mass balance.

In the most of the sorption studies conducted for steroid hormones, higher initial concentrations were applied in order to ensure a concentration range of above the detection limit of the analytical technique under consideration. In application of such a high concentration rather than an environment related concentration, sorption equilibrium was reached within a day. However, when 20 times lower than their solubility limit (0.1 mg/l) is applied, equilibrium is reached within two weeks (Yu *et al.*, 2004). When 0.4% of their water solubility limit was applied for testosterone and androstenedione, sorption equilibrium was reached within three weeks (Kim *et al.*, 2007). Moreover, Kim *et al.* indicated that under such a low concentration, $\log K_{oc}$ could vary from 6.30 to 6.80 and 6.16 to 6.92 for testosterone and androstenedione, respectively. This is significantly different from the data presented in Table 2, and thus further investigation on the basis of environment relevant concentrations is essential.

In-addition of hydrophobic interaction, it is important to consider other possible ways of interaction of steroids and soil particles includes hydrogen bonds, covalent bonds and intercalation (sorption between the kaolinite structure). The polar groups of E2 and testosterone may react with humic acids and mineral surfaces by hydrogen or covalent bonds (Yamamoto *et*

al., 2003). The author indicated that the measured $\log K_{oc}$ was independent of $\log K_{ow}$ for sorption of E2 to humic acids. In the case of intercalation, soils or sediments rich in swelling clay or montmorillonite are effective in sorbing E2 (van Emmerik *et al.*, 2003), minimizing the likelihood of ground water contamination. It indicates that sorption can lead to significant retardation during leaching in soils; however, strong sorption into soil can increase the potential for surface runoff losses and surface water contamination during storm events.

Desorption

Limited studies are available for the desorption study of estrogens, but no data is available for the androgens. On the basis of limited data available in literature, two important factors can be considered as the determining factors for desorption of these hormones. Firstly is the initial concentration of the compound of concern applied for desorption study. In sandy soil, rapid desorption of E1 and E2 was reported, and the greatest degree of desorption occurred within the lowest initial concentration of $10 \mu\text{g L}^{-1}$ (Hildebrand *et al.*, 2006). At initial concentration of 10 ng L^{-1} , there was $> 85 \%$ desorption reported for both E1 and E2. The author also showed that desorption of estrogens was being increased with decreasing initial concentration. This suggests that at environmentally relevant concentration, desorption may be the determining factor for the fate and transport of estrogen in soil or sediments.

Secondly the type of dominating clay minerals in soil or sediment may play a great role in determining desorption of steroid hormones in soil. According to van Emmerik *et al.* (2003), E2 was indicated to desorb rapidly (within a few seconds) in the kaolinite and illite. On the other hand, in the swelling clay dominating soil or sediment, lack of desorption from montmorillonite due to intercalation into interlayer regions was reported (van Emmerik *et al.*, 2003). It appears that in soil containing significant quantity of montmorillonite, sorption may limit leaching of E2 into the ground water. Note that at an environmentally relevant concentration and in soil that has no swelling clay, estrogens exhibit rapid sorption and desorption (Hildebrand *et al.*, 2006), and therefore leaching to the ground water is most likely. For a better understanding of the kinetics and mechanisms of desorption for all of four steroid hormones under consideration, further investigation is required.

Biodegradation

Although natural steroid hormones can be degraded by abiotic processes such as photolysis, the biodegradation process is mainly responsible for the elimination of E2 and testosterone including their metabolites in soils as well as in the receiving water. Laboratory studies have shown that these compounds would degrade rapidly at various conditions of moisture content and temperature (Culucci *et al.*, 2001). Nevertheless, their continuous presence in agricultural soil for several months indicates the potential persistence of these compounds in the environment. There are several factors that may affect biodegradation of steroid hormones in the environment: temperature, aeration, soil moisture and organic matter content. In well-aerated soil, rapid degradation of E2 and testosterone was reported at temperatures higher than 12°C (Culucci *et al.*, 2001). Under aerobic conditions, typical available biodegradation rate constants in soil are indicated in Table 4, which shows that testosterone degrades relatively faster than E1 and E2. Soil microcosm study showed that, at soil temperature between 20 and 30°C , aerobic biodegradation of E2, E1 and testosterone increases with soil moisture; higher at 15% than air-dried or moistened to the field capacity (Culucci *et al.*, 2001; Lorenzen *et al.*, 2005). The authors

showed that temperature below 12°C, dried soil, and moisten soil to the field capacity could reduce the biodegradation of steroid hormones. Note that soil moistened to field capacity limits oxygen supply to aerobic biodegradation. This suggests that the persistence of steroid hormones is more likely in dried sandy soil, moistened soil at field capacity or saturated soils.

Under anaerobic conditions, biotransformation of both E2 and testosterone is indicated to be limited. In anoxic or anaerobic conditions, E2 was shown to degrade slowly and E1 accumulates (Fan *et al.*, 2007; Jürgens *et al.*, 1999); complete degradation is not likely. This is consistent with the tests conducted under strict anaerobic conditions including sulphate, nitrate and iron reducing, and methanogenic conditions in which E2 degradation was limited (Czajka and Londry, 2006). The author found even slower degradation of E2 under nitrate reducing conditions. Occurrence of a high concentration of androstenedione in anoxic sediment of the Fenholloway River (Jenkins *et al.*, 2003) could also suggest that this compound can persist in anaerobic or anoxic conditions. If steroid hormones are persistent in anoxic sediment, which is mostly the case for E1 and androstenedione, the sorbed phase may desorb and get released into the overlying water where it can pose a risk to the pelagic fish. This shows that anoxic or anaerobic sediment can be a potential source for steroid hormones.

Moreover, the presence of viable microbial biomass and associated inhibiting factors, including antibiotics in the manure or biosolids, and agricultural practices may affect the biotransformation rate of steroid hormones in the amended soil. According to Shi *et al.* (2004), ammonia-oxidizing bacteria degrade E2 without E1 accumulation, whereas other heterotrophic bacteria transform E2 to E1. It indicates that any inhibiting factors that affect the activities of ammonia oxidizers may determine the accumulation of E1. The presence of antibiotics (chlortetracycline) and biosolids application can affect the degradation of testosterone (Chun *et al.*, 2005).

Agricultural practices including tillage, grass strips and soil aeration may affect the degradation of steroids in soils. Tillage, grass strips and soil aeration increase soil permeability, enhance incorporation of manures into the soil, reduce surface runoff, and increase the biotransformation of these compounds (Fisher *et al.*, 2005; Herman and Mills, 2003). In the non-till and grazing lands where there is no incorporation, groundwater contamination was shown to be limited, which may play a great role in surface water contamination through runoff (Fisher *et al.*, 2005). Soil aeration would be required when there is a significant surface compaction, slow water infiltration, low earthworm activity, and low microbial activities. It is also important to investigate the effect of other agricultural practices including the application of crop-residue-derived ash on the soil bed, which may reduce biodegradation of these hormones by reducing bioavailability (Zhang *et al.*, 2004).

Table 4. Aerobic biodegradation of steroid hormones in soil

Soil type	Organic Mater (%)	pH	Moisture (%)	Temperature (°C)	Degradation rate constant (d ⁻¹)		
					E1 ¹	E2 ¹	T ²
Loam	3.2	7.4	15	30	0.75	0.52	6.4
Silty loam	2.9	6.7	35	30	0.41	0.45	1.17
Sandy loam	0.8	5.8	15	30	0.47	0.47	NA

¹Culucci *et al.* (2001); ²Lorenzen *et al.* (2005)

It must also be noted that most biodegradation tests were conducted under higher initial concentrations, rather than the concentration that can be detected in the real environment. Culucci *et al.* (2001), for example, applied 10 mg steroid hormone per kg soil. Similarly, Czajka and Londry (2006) applied 5 mg L⁻¹. It is not known if environment related concentration would show similar results. At environmentally relevant concentration, they might be too low to be utilized by heterotrophic microbial biomass as a carbon source. In that case, the availability of dissolved organic carbon may affect the degradation of organic contaminants positively as well as negatively (Deksissa and Vanrolleghem, 2005). With a limited carbon source, which is the case with low loaded water, small quantity of additional organic matter may enhance biodegradation of trace organic contaminants. According to Herman and Mills (2003), addition of 1 mg L⁻¹ dissolved organic carbon increased the biotransformation of E2. The author also showed that higher concentrations of readily available carbon sources inhibited E2 degradation. This suggests that the availability of higher concentration of readily biodegradable organic carbon in water, as well as in soil or sediment may negatively affect the degradation of E2. A similar investigation need to be done for testosterone too. Thus, further assessment on biodegradation of these hormones need to be with under environment relevant concentrations, and would have to involve a different approach such as the use of ¹⁴C-labelled estrogens and androgens.

Summary

This review study indicates that there is a widespread occurrence of E2 and testosterone in the environment, but little is known about the behavior of these endocrine disrupting hormones as well as their metabolites in the environment. Significant concentrations of these compounds were reported to reach ground water as well as surface water. Sorption, desorption and biodegradation processes are the governing processes of fate and transport of these compounds in the environment. Factors that affect sorption and desorption include type and initial concentration of the compound, soil type, and content of organic carbon and swelling clay in soil. In the absence of swelling clay, at environmentally relevant concentrations, estrogens may have a rapid desorption rate constant. Factors that affect sorption may also affect biodegradation. In addition, other factors such as moisture content, aeration, inhibiting factors including antibiotics, temperature, and presence of viable microbial biomass may determine the degradation of these hormones. Under aerobic conditions, all three steroid hormones may be degraded rapidly, provided that the carbon source, temperature and soil moisture are not the limiting factors. Under anaerobic conditions, biodegradation of all four steroid hormones under consideration is limited, E2 and testosterone tends to degrade very slowly, and their metabolites E1 and androstenedione may accumulate. Thus, persistence of E1 and androstenedione in anoxic conditions, together with their possible rapid desorption, may govern the fate and transport of these compounds in soil and surface water.

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