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Topic 2.2

Naturally produced steroid hormones and their release into the environment*

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Abstract: Steroidal hormones produced by humans and animals are constantly excreted into the environment in their active forms. The primary steroid hormones are progesterone, estrone, estradiol, testosterone, and cortisol, all of which are lipophilic and poorly soluble in water. The steroids of major concern are estrone and estradiol-17β, since they exert their physiological effects at a lower concentration than other steroids and can be found in the environment in concentrations above their lowest observable effect level for fish and plants (10 ng/l). The steroid hormones can be readily measured in run-off, soil, and groundwater, but each steroid has its distinct pathway of transport. Since the major source of steroids in the environment appears to be cattle and chickens, the hormonal steroid input into the environment could be drastically reduced by well-established techniques such as buffer strips and composting.

INTRODUCTION

Hormones produced by humans and animals are constantly excreted into the environment. Many of these hormones are peptides and are rapidly destroyed. However, the steroid hormones are chemically very stable and are excreted in the free form or as conjugates, which very readily biotransform to the free form [1,2]. The primary steroid hormones are estrone, estradiol, progesterone, testosterone, and cortisol, all of which are lipophilic and poorly soluble in water (log P_{ow} between 3 to 4 [3]). The steroids of major concern are estrone and estradiol-17β, since they exert their physiological effects at lower concentrations than other steroids and can be found in the environment in concentrations above their lowest observable effect level (LOEL) for fish and plants (10 ng/l) [4–8]. Estradiol-17β is considered 10 times more potent than estrone as measured by uterotropic activity in the standard mouse assay [9]. However, estrone has about 1/5 the activity of estradiol-17β in inducing trout vitellogenin synthesis [10], and both compounds had the same activity in a mutagenesis test [11] and in increasing body weight in cattle [12]. Estrone is more potent than estradiol-17β in increasing alfalfa growth [8] and uterine imbibition test [13]. The other free estrogens excreted by animals and humans, estriol (significant quantities only in pregnant women), estradiol-17α (produced by cattle) and equilin (produced by pregnant horses) are considered weak estrogens, but estradiol-17α can be a potent estrogen in the neonatal mouse [14]. Therefore, unless otherwise stated, estrogens in this presentation is used to mean free estrone and estradiol-17β. Progesterone and testosterone are also excreted in the free active form but the concentrations measured in the environment (ppt) are at least a magnitude below their LOEL. Although comparable amounts of corticosterone and cortisol as other steroids are also produced by the various species, nearly all corticoids are excreted as inactive metabolites [15].

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PRODUCTION FROM HUMANS

Human females excrete about 5 μg/day each of estrone and estradiol and males about 10 mg/day of androgens (primarily testosterone and androstenedione) (Table 1) [16]. (A recent review has indicated the amounts shown for daily production in pre-pubertal children are substantial overestimations [17].) The amount of excreted estrogens of pregnant women can be 1000 times higher (late gestation production: 26 mg estradiol/24 h; 37 mg estrone/24 h [16]), depending on the stage of pregnancy. In addition, substantial amounts of natural estrogens are consumed as pharmaceuticals. This consumption has been calculated to be about 100 kg/yr/5 million inhabitants [18]. Using a formula to include the amount of estrogen produced by each segment of population (e.g., males, pregnant women), it has been calculated that estrone and estradiol excreted in human urine is in the order of 4.4 kg/yr/one million inhabitants. This estimation, which ignores the contribution of fecal estrogens or pharmaceutical consumption, could account for 50 % of the observed estrogen in the influents to sewage water plants [19].

Table 1 Human production and excretion of estrogens (from ref. [16]).

Sex steroid	Amount excreted in urine (μg/day)	Amount produced (μg/day)	Sex
17β-Estradiol	0.3–5	82–695	Female (cycling)
17β-Estradiol	–	13	Female (pre-pubertal)
17β-Estradiol	1.5	48	Male
17β-Estradiol	–	6.5	Male (pre-pubertal)
Estriol	3–65	–	Female (pregnant)
Estrone	2–20	110–497	Female (cycling)
Estrone	–	41	Female (pre-pubertal)
Estrone	3	88	Male
Estrone	–	35	Male (pre-pubertal)
Androgens	2100–23 100	6500 (testosterone)	Male
Androgens	800–10 500	240 (testosterone)	Female

PRODUCTION FROM LIVESTOCK

Animal manure is a major source of the natural steroids, estrogen and testosterone, reaching the environment. Manure is a mixture of feces, urine, and bedding. If the percentage of water is above 20 %, this mixture is referred to as slurry. When chicken manure has a high percentage of bedding, it is referred to as poultry litter. The use of animal manures for fertilization of fields and the production by concentrated animal feeding operations (CAFOs) has increased the impact of the manures on watersheds [8,20]. The animal manure most used for fertilization of fields in the United States is chicken manure. About 12 000 000 Mg/year of poultry litter was produced in the United States, most of which is applied to grasslands as fertilizer [21]. With the increased emphasis on manure management programs by various governments, it is expected that the use of poultry litter and cattle manure for fertilizing fields will increase substantially in the next decade. Some manure is used for feeding cattle in the form of silage (mixing manure with wheat or corn stalks under anaerobic conditions). Silaging increases the available protein as opposed to composting, which is a destructive process.

Production of estrogen by livestock

Most of the estrogen excreted in cow feces is in the last trimester of pregnancy, and nearly all of the estradiol-17β and estrone are in the free form [22] (Table 2). In addition to estradiol-17β and estrone, comparable amounts of estradiol-17α are produced. The contribution of urine where estrogen is present mostly in the form of conjugates to the total estrogen excreted is usually less than 20 %. However,

these conjugates rapidly convert to the free active form after excretion [23]. Studies with injection of labeled steroid indicate that domestic animals differ widely in their routes of excretion [24] (Table 2).

Table 2 Percentage of infused labeled steroid excreted in feces (from ref. [24]).

Steroid	Sheep	Ponies	Pigs
Progesterone	77	75	34
Testosterone	44	28	14
Cortisol	28	59	7
Estrone	89	2	4

Similar to sheep in Table 2, in the cow actual measurement of daily sampling of urine and feces (Table 3) indicated the amount of steroidal estrogen excreted in the urine was less than 10 %. It has been calculated that a pregnant cow excretes 0.76 g of estrogen per pregnancy (mostly in the form of estradiol-17α) and that for Austria, which has about 700 000 milk cows, the yearly excretion of estrogen is in the order of 540 kg [25].

Table 3 Hormone content in cattle and swine manure.

Source	Estrone (μg/kg)	Estradiol 17β (μg/kg)	Comments	Ref.
Milk cows (slurry)	255–640	170–1230	Total solids	[2]
Bulls (slurry)	<2	<2	Total solids	[2]
Milk cows (feces, late gestation)	840 (estrone + estradiol)		Dry wt	[25]
Milk cows (manure pile)	700–1000 (estrone + estradiol)		Dry wt	[25]
Milk cows (feces)				
–100 days before parturition	0.9	9.0	Fresh wt	[22]
–60 days before parturition	0.1	13.9	Fresh wt	[22]
–30 days before parturition	4.1	19.1	Fresh wt	[22]
–10 days before parturition	9.4	42.2	Fresh wt	[22]
–5 days before parturition	11.4	60.0	Fresh wt	[22]
Swine (slurry)	<2–84	<2–64	Dry wt	[2]
Sow (feces, late gestation)	15–28		Dry wt	[32]
Mare (feces, late gestation)	50–200 mg		Dry wt	[33]
Milk cows (urine)	44 mg/24 h or 1.4 mg/kg	41 mg/24 h or 1.3 mg/kg	About 30 l urine/day	[31]
Pony mares (pregnant, urine)	200–800 mg/24 h (estrone+equilin) 400 mg/kg		About 2 l urine/day	[34]

The principle estrogens excreted by chickens are estrone and estradiol-17β. The excretion in urine of estrogen in laying and nonlaying hens was about 3 and 0.5 μg/d for estrone and 3 and 2 μg/d for estradiol-17β, respectively [27]. The clearance rate for estradiol in the laying hen has been calculated to be 6.3 ng/min [27] or 9 μg/d. This can be compared with a calculated excreted value of estrogen measured in manure (from Table 4) of 30 μg/d.

Hormones in manure

In slurries from dairy farms values of about 600–1600 μg estrogen/kg total solids have been reported [2]. This would agree with the value of 800–1300 μg of estrogen/kg dry matter (dm) manure found for pregnant cows [25,28]. Chicken manure contains up to 533 μg estrogen/kg dm and 670 μg testos-

terone/kg (Table 4) [29]. The amount of testosterone in chicken manure that was silaged or left in open pits was found to be constant over several months, and thermal processing had little effect on the hormone concentrations [29]. In contrast, in a study of a manure pile containing estrogen from cows in the peri-parturient period, it was found that after two months, the interior of the pile had little estrogen and estrogen was present in significant quantities only in the outer crust [25]. No published information for manure content is available for other livestock included in manure management programs (turkeys, ducks, sheep, horse), but turkey and ducks apparently produce much less estrogen/g manure than chickens (Shore, unpublished observations).

Table 4 Hormones content in chicken manure.

Source	T (μg/kg d.w.)	E (μg/kg d.w.)	Ref.
Immature broilers			
Females	133	65	[30]
Males	133	14	[30]
Laying Hens	254	533	[30]
Roosters	670	93	[30]
Chicken litter		133	[35]

Based on similar observations, Lange et al. [36] have calculated the total output of domestic animals in tons/yr (Table 5).

Table 5 Estimated yearly steroid hormone excretion by farm animals in the European Union and the United States—Year 2000 (from ref. [36]).

Species	European Union				USA			
	Million heads	Estrogens (tons)	Androgens (tons)	Gestagens (tons)	Million heads	Estrogens (tons)	Androgens (tons)	Gestagens (tons)
Cattle	82	26	4.6	185	98	45	1.9	253
Pigs	122	3.0	1.0	79	59	0.83	0.35	22
Sheep	112	1.3		58	7.7	0.092		3.9
Chickens	1002	2.8	1.6		1816	2.7	2.1	
Total	1318	33	7.1	322	1981	49	4.4	279

PRODUCTION FROM WILDLIFE

There is no information on the contribution of nondomestic animals to the environmental estrogen load. However, fields in which wild turkeys were present for several months showed high levels of estradiol and testosterone in the soil [21]. Water in fish aquariums reached equilibrium values ranging from 3.5 to 15 ng estradiol-17β/l [4], and fish ponds studied over a four-year period had equilibrium concentrations of 5 to 7 ng/l estrogens and comparable amounts of testosterone (Shore, unpublished observations). In a pond with 150 wild birds, it was found that the concentration in the pond of estrogen and testosterone remained at between 2–5 ng/l over a six-month period in spite of an input of 1250 μg estrogen/day (as measured in the feces and calculated from ref. [37]). The half-life of estrogen and testosterone in the water was in the order of an hour (Shore, unpublished observations).

Due to its importance as a noninvasive technique to monitoring wildlife, there is considerable information on the fecal concentrations of progesterone and estrogen in a variety of wildlife [38]. Concentrations of about 5 μg progestagens (20α progesterone, progesterone)/g dry feces is a common finding for the largest mammals during the luteal phase or late gestation, while estrogen concentrations vary widely between species from 0.1 μg/g in the elephant [39] to 17 μg/g in the musk ox [28].

HORMONES IN FOOD AND FEED

Hormones in food

Average adult consumption of hormones in food has been calculated to be 10 µg progesterone/day, 0.1 µg estrogen/day, and 0.05 µg testosterone/day [40], which is quite small compared to human endogenous production. The principle sources are meat and milk products. Meat can contains small amounts of steroids, e.g., 0.5 µg testosterone/kg for bulls [41], 7 µg progesterone/kg for heifers [42], but the levels of estrogen are barely detectable even in pregnant heifers (3–5 ng/kg, [16]). The highest levels of testosterone in edible tissues observed in the bulls were 3 µg/kg in kidney and 11 µg/kg in fat. Bovine milk is rich in a variety of hormones reflective of the plasma values, but steroid hormones, being lipophilic, can concentrate in the milk and milk products, depending on fat content [43,44] (Table 6). Although estrogenic hormones in milk from nonpregnant cows are in the pg/ml range, milk from pregnant cows can contain 500 ng estradiol/l, 1 µg estrone/l (mostly as conjugated sulfate), and 10 µg progesterone/l [43] (and approximately half of dairy herd is in late pregnancy during milking). Human breast milk for infants contains little estrogen or progesterone since nursing humans are generally not pregnant. Since young children consume about 300–700 ml/day of bovine milk, they may ingest 40 to 100 ng/day of estrogen (estradiol, estrone, and estrone sulfate), and whether this can be considered a safe level is a matter of debate [45]. However, there is little data on the incidence of the cardinal sign of hyperestrogenism, premature thelarche, except for Puerto Rico [46] where there is a high incidence of premature thelarche due to estrogenic substances in the environment.

Table 6 Estrogens (pg/ml or pg/g) in milk and milk products (from ref. [44]).

Source	Estrone	Estradiol	Estrone + estrone sulfate
Milk from cows			
Estrus	58	84	
Luteal	45	29	
Late pregnant	45	49	200–1000
Store milk	55	10	500
Butter	539	82	1470
Cheese	35	10	170
Cream		<30	260

Hormones in feed

Although plants contain a variety of compounds that are defined as steroids [47], finding of significant amounts of vetebrate steroids, such as estrone in apple seeds (130 µg estrone/kg [48]), is rare. However, plants produce phytoestrogens, which are well documented to cause reproductive problems in domestic animals [49]. The endogenous level of the phytoestrogens is raised when legumes are irrigated with sewage effluent as well as other forms of stress such as a fungal infection. An increase in endogenous phytoestrogen content can be induced with the levels of estrone and estradiol found in sewage effluent [50]. Chicken manure does have substantial estrogen and testosterone and when fed as silage can cause hyperestrogenism and delayed puberty in cattle [49,51].

INITIAL CONCENTRATIONS IN THE ENVIRONMENT

Initial concentrations in soil

Estradiol and testosterone both bind to the soil (at a depth of 5 cm) with a reported concentration of about 650 ng/kg on manured plots as opposed to 150 ng/kg on control fields [21]. However, only testosterone reaches the groundwater, while estrogen remains bound to the upper crust of the soil, probably because of its phenolic group binding to soil particles [8,52].

Run-off from manured fields and chicken manure stacks

Following rain events, both estrogen and testosterone are found in the run-off [20,21,35,53]. Run-off following a rain event from the fields contained substantial amounts of estrogen and testosterone (1–3 μg/l) [53]. Exposure of chum salmon to such levels (2 μg estradiol-17β/l for a month) has been reported to be lethal [54]. The amount of estradiol in the run-off increased linearly with the increasing application rate of the litter (1.76 to 7.05 Mg/hectacre), increase in pH, and TOC (total organic carbon) [35]. The concentration and mass losses of estradiol-17β in the run-off can be remarkably reduced using buffer strips or addition of alum to the litter. In a pond, which received run-off from a manured field, estrogen levels were above 5 ng/l (maximum 25 ng/l) for several months with a half-life of about 2.5 months [20]. This can be compared with the very short half-life of an hour in a wild bird pond (Shore, unpublished observations) and 6 days in English rivers [55]. Apparently, as described below for sludge, microbial adaptation plays a major role in destruction of the hormones.

Substantial quantities of estrogen can also elute from stack manure piles. It was found that eluants from chicken manure piles were found to contain 630 ng/l of testosterone and 730 ng/l of estrogen (L. Shore and C. Oshins, unpublished observations). Composting was found to virtually destroy the steroid hormone content in the eluant and in the pile itself.

Sewage

The levels of hormones in raw sewage are by nature highly variable depending on source and amount of rainfall.

Values of 40–130 ng estrogen/l over a six-month period were observed in sewage water consisting primary of septic tank effluents from a population of about 7000 inhabitants [20]. In an extensive survey of influent produced by populations of 3500 to 1.2 million, it was found the range of estradiol-17β was <0.5 to 48 ng/l, estrone 17–102 ng/l and estriol from <0.5 to 10 ng/l [19]. Sewage influent at five sewage treatment plants (STPs) was 1.5 ng/l for estradiol and 5.5 ng estrone/l. After hydrolysis, the levels were 3 ng/l estradiol and 13 ng/l estrone [56]. Conjugates can therefore contribute up to 50 % of the total concentration in the influent.

Treated sewage waste

Treatment of sewage water can be classified as primary, secondary, and tertiary. Primarily and secondary treatment (sedimentation and oxygenation ponds) are used for agricultural irrigation. Tertiary treatment is usually in an activated sludge (biosolids) plant that utilizes both anaerobic and aerobic digestion at high temperatures. Alternatively, constructed wetlands (which are considered to be more environmentally friendly) consisting primarily of bulrushes, are used. If the water is used to recharge the aquifer, ground filtration through sand, chalk, or other soils (geofiltration) results in further purification. The concentration of hormones found in the effluent depends on the concentration of hormones in the initial sewage, the type of treatment and physicochemical parameters such as flow rate and time of incubation. The solid materials left after the processing of the sewage water is termed sludge.

Irrigation water from sedimentation ponds

Water after treatment in sedimentation ponds was analyzed for the presence of testosterone and estrogen (Table 7). The source and season were factors in the hormone content. The irrigation water contained from 40 to 340 ng/l of estradiol-17β and estrone as measured by radioimmunoassay. This was well within the range shown to increase the phytoestrogen content and growth of alfalfa plants.

Table 7 Estrogen concentrations in primary treated sewage water used for irrigation (from ref. [7]).

Source	Season	Estradiol and estrone (ng/l)
Agricultural	Dry season	341
Agricultural	Wet season	152
Municipal	Dry season	116
Municipal	Wet season	39

Effluent from sewage treatment plants—artificial wetlands and activated sludge

Activated sludge

The concentrations of estrogen (estradiol+estrone) were studied in an activated sewage treatment plant over a one-year period [7]. During a high flow time the initial amount of estrogen and testosterone in the sewage water after primary and secondary treatment were 54 and 19 ng/l, respectively. In the supernatant of the digestion tank the values for both compounds were between 10 and 20 ng/l and the concentration in the interstitial water of the sludge was similar. In the effluent the values for estrogen and testosterone were 6.4 and 7.2 ng/l respectively. This corresponded to 90 % digestion for estrogen and 60 % digestion for testosterone. However, during low flow periods, where the initial concentration was between 50 and 140 ng estrogen/l and 200–300 ng testosterone/l, the resultant effluent contained 38–50 ng/l of estrogen and 46–121 ng testosterone/l testosterone indicating a lower (60 %) reduction in estrogen, but a comparable amount of digestion as observed at high flow for testosterone. Laboratory studies using active biosolids have shown that although 80–90 % of estrogens are destroyed in the first week, it requires three to four weeks to reduce the estrone, estradiol, and estriol levels to nondetectable levels [57]. The nature of the adapted microbiological populations is an important factor in the removal of testosterone and estrogen. Biosolids from municipal plants (84 %/24 h) are much more effective in reducing estrogen levels than those from industrial plants (4 %) [23]. The nature of the influent and degree of processing also have a effect. Comparison of Brazilian influent comparable influents (15–20 ng estradiol and 30–40 ng estrone/l) showed that elimination in Brazil was higher (99 and 83 % for estradiol and estrone, respectively) than in Germany (64 and 68 %) [58]. In 17 out of 38 sewage sludges natural estrogens could be detected, 17β-estradiol was found in 10 sludges in a concentration range between 4.2 and 111 μg/kg dm (median 12.7 μg/kg dm), estrone concentrations were detected between 3.3 and 328 μg/kg dm in 7 samples, and estriol could be analyzed in 3 samples at 18.1–31.4 μg/kg dm (mean 26 μg/kg dm) [2].

Artificial wetlands

An artificial wetland site was sampled five times over a 10-month period (Shore, unpublished observations). The water was first filtrated through gravel and sand (trickling filtration), which substantially reduced testosterone from 166 to 7 ng/l and estrogen (estradiol+estrone) from 73 to 2 ng/l. Passing through the artificial wetlands had little effect, and the effluent contained 5 ng testosterone/l and 2 ng estrogen/l. Passing through peat did reduce the testosterone further to 2 ng/l, but had no effect on the estrogen level.

Geofiltration

Percolation of tertiary treated sewage water through sandy soil to recharge an aquifer reduced the hormone concentration to undetectable levels (<0.1 ng/l) [7]. Water in springs from a mantled karst aquifer recharged after rain events contained levels of 6 to 66 ng/l, and the estradiol concentration correlated with the *E. coli* and fecal coliform counts [59].

Springs and wells

Although extensive surveys indicate that testosterone and estrogen can be detected in springs and wells used for drinking water, the levels are usually well under 1 ng/l. In an old survey of 64 wells in southern Germany in 1977, levels from <0.1 to 0.9 ng estradiol/l with an average of 0.2 were found [60]. Five wells from under a farm with extensive animal husbandry and manured fields had no detectable levels of estrogen (<0.1), but did have some testosterone (about 1 ng/l) [52]. In a survey of wells receiving contaminated water, it was found that six wells receiving water from rocky strata had detectable estrogen in the range of 1.7–5 ng/l. However, estrogen was undetectable (<0.5 ng/l) in six wells that received water filtered through sandy strata (Shore, unpublished observations). Municipal water supplies in Arkansas, a state with extensive use of poultry manure for land reclamation, had a concentration of 30 ng estradiol-17β/l [36].

Estrogen concentrations in surface waters

In an attempt to identify possible sources of steroid hormones, 17 streams in the Conestoga River Valley of the mid-Atlantic region of the United States were surveyed [20]. Results can be summarized as follows: For stream sampling, four of ten sites had testosterone concentrations of above 1 ng/l. Three of these sites were in areas with heavy use of chicken manure as fertilizer and one site received effluent from an STP. Comparison of a stream dominated by forest with a stream dominated by cropland indicated that there was a gradient of estrogen discharge downstream along the stream dominated by cropland (0.54–1.83 ng/l). Therefore, two sources of pollution were identified—run-off from fields fertilized with manure and discharge into streams from STPs. The levels in freely flowing streams apparently do not exceed 5 ng/l estradiol+estrone, but this level in the same magnitude of the LOEL and harbors a potential for environmental effects.

Several studies of estrogen content in surface waters have recently been reported [2,54]. In general, concentrations of estrogens in surface water were generally low (below 1 ng/l). In one study [2], estradiol-17β was detected in 6 out of 117 samples in a concentration range between 0.8 and 29 ng/l with a median concentration of the positive samples of 1.7 ng/l. In 14 samples, at least one of the metabolites estrone and estriol were determined. Estrone was found in 8 surface waters with a median concentration of the positive samples of 2.3 ng/l, and estriol was detectable in 7 samples with a median concentration of the positive samples of 3.0 ng/l. In marked contrast, an extensive reconnaissance study by the USGS (U.S. Geological Survey) [61] reported very high levels of hormones (<5 ng/l) in 10 to 20 % of the 139 stream studies. Specifically, in the positive samples, the following maximum and median values in ng/l were obtained: estrone 117, 27; 17 β estradiol 93, 9; estriol 51, 19; 17 α estradiol 74, 30; testosterone 214, 116 and progesterone 119, 111. Equilenin, a natural estrogen produced by horses and widely used as a replacement for estrogen, had a maximum of 278 and a median of 140 ng/l.

BIOABSORTION AND MINERALIZATION

Steroidal hormones leave the aqueous phase though absorption to particulate matter (sediment or sludge) or by mineralization (conversion of organic compounds to inorganic compounds). Mineralization (reduction of organic compounds to inorganic compounds) is usually the result of mi-

crobiologically activity, but photodegradation or other physicochemical reactions may take place. These compounds are heat stable (mp 175° for estradiol-17β, 252° for estrone).

Bioabsorption

Sediments

In experiments with river sediments, it was found that the initial rapid absorption of estrogen (4 μg/g/h) reached maximal absorption within an hour after which the rate of sorption remained the same or decreased. This absorption increased with the TOC of the sediments and the salinity of the water [62]. In a survey of 12 lake sediments, estradiol-17β was found in 3 sediments with a mean concentration of 8.5 μg/kg dm. Estrone was only found in one sediment at 13.7 μg/kg dm, and estriol was not detectable [2].

Soil

Estradiol rapidly dissipates into all the soils that have been tested (silt loam, sandy loam, and loam) [63]. In sterile soil, estradiol is converted abiotically to estrone with a half-life of about 50 h. The transformation of estradiol to estrone is apparently strongly favored as similar conversion takes place in sewage [6,58] and rivers [62].

Mineralization

Biosolids from sewage treatment plants

The importance of adapted microbiological populations was shown using biosolids from sewage treatment plants [23]. The mineralization of added estradiol-17β was 84 %/24 h by biosolids from a municipal plant, but only 4 % by biosolids from an industrial plant. Biosolids from municipal plants mineralized 70–80 % C^{14}-labeled estradiol to carbon dioxide within 24 h. Removal of estadiol from the aqueous phase by biogradation and/or biosorption to cell matter was greater than 90 %. Testosterone was mineralized in amounts ranging from 55–65 %/24 h, and its removal from the aqueous phase was also greater than 90 %. The rates of mineralization were first-order k's of 0.0042 min for estradiol and 0.0152 min for testosterone which could be calculated as an half-lives of 2.75 h for estradiol-17β and 46 min for testosterone. A 17β-estradiol degrading bacterium, which may be a new Novosphingobium species, has been isolated from activated sludge [64].

Soil

Although estradiol is converted to estrone in autoclaved soil, estrone remains stable. In nonsterile soils (loam, silt loam, and sand loam), by 72 h, both estrone and estradiol form nonextractable residues (57 to 90 %) which are only slowing mineralized. Using labeled steroids, it was found that after 61 d, only 10 to 15 % of the estrogen was mineralized as indicated by labeled CO_2 [63].

Rivers

Water samples from English rivers were studied to measure the biodegradation potential of the key steroid estrogen, estradiol-17β [55,62]. Microorganisms in the river water samples were capable of transforming estradiol-17β to estrone with half-lives of 0.2–9 days when incubated at 20 °C. Estrone was then further degraded at similar rates. Estradiol-17β degradation rates were similar for spiking concentrations throughout the range of 20 ng/l to 500 μg/l. Microbial cleavage of the steroid ring system was demonstrated by release of radiolabeled CO_2 from the aromatic ring of estradiol-17β (position 4). When estradiol-17β was degraded the loss of estrogenicity, measured by the yeast estrogen screen assay (YES), closely followed the loss of the parent molecule. Thus, apart from the transient formation of estrone, the degradation of estradiol-17β does not form other significantly estrogenic intermediates. Estradiol-17β could also be degraded when incubated with anaerobic bed-sediments. Estradiol-17β is susceptible to photodegradation, with half-lives in the order of 10 days under ideal conditions [55]. The

half-life of estradiol in river sediments under aerobic conditions was 0.11 days (2.7 h), which is very close to that observed for digestion with biosolids, and under anaerobic conditions the half-life was 0.37 days.

IMPACT

Substantial quantities of estrogen are constantly excreted in the environment. The major source of measured environmental estrogen appears to be domestic animal manures as human sewage is generally degraded in sewage treatment plants. The contribution of wildlife and domestic pets is not known. However, equilibrium concentrations of 5 ng estrogen/l are found in duck and fish ponds and levels above this are not usually found in freely flowing streams. This level is not much below the NOEL 10–50 ng estrogens/l on plants and fish. Little is known of the environmental fate of these hormones, but they apparently do not accumulate in the environment. Any estimation of xenoestrogen impact on the environment must take into account the background level of natural estrogen, which can be significant in areas of concentrated animal husbandry and areas receiving sewage plant effluent from densely populated areas. The effects of the hormones are not necessarily direct. Estrogen in the irrigation water can cause legumes to produce high amounts of phytoestrogen, which in turn cause reproductive problems when ingested by cattle.

STATE OF THE ART

As opposed to many of the compounds discussed in this book, a great deal is known about the possible effects of estradiol and estrone on many animal species including multigenerational studies and NOEL levels. The metabolism of these compounds has also been extensively described in several species, particularly in humans. The amount released into the environment is also quantifiable as manure management programs extensively monitor the amount of manure produced by a variety of farm animals in many countries, especially the United States (e.g., ref. [37]) and Germany [65], and the amounts of sewage passing through sewage treatment plant are documented (e.g., for the United States, ref. [66]).

RECOMMENDATIONS FOR MANAGEMENT OF THE PROBLEM

The relevant environmental agencies should formulate predictable no-effect levels for steroid compounds similar to the proposed predicted-no-effect-concentrations (PNECs) for natural and synthetic steroid estrogens in surface waters by the UK Environmental Agency [67].

Simple low-technology processes such as buffer strips and composting can drastically reduce the amount of steroid hormones. Although not necessary economically justified for the sole purpose of removal of estrogens, buffer strips and composting have many other beneficial effects in protecting the environment. Therefore, composting and buffer strips should be an integral part of manure management programs.

RECOMMENDATIONS FOR FUTURE RESEARCH

The environmental fate and rate of mineralization of the steroid hormones is poorly characterized. Virtually no information is available on what bacteria digest the compound or how their physicochemical degradation are accomplished. Such information should be the subject of immediate research as (1) the technology to do it is readily available; (2) the effects of all estrogenmimetic compounds found in the environment need to be measured against the steroid estrogen background; and (3) since estrogen and testosterone are ubiquitous in human and animal excreta, it could be a standard monitor for sewage pollution and help identify the sources of various pollutants.

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