Chapter 10

Livestock Hormones in Aquatic Ecosystems

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We begin this article with a general description of sources of hormones followed by their transport and fate in the environment. We then focus on occurrences and effects of livestock estrogens in aquatic ecosystems. Two case studies from our research are presented in details to contrast levels of estrogens in a concentrated animal farming area in northern Missouri with those in a relatively pristine area in southern Missouri. Estimation of human exposure to estrogenic compounds from various sources would be informative to the general public. Thus, we estimate exposures from environmental estrogens, medicinal estrogens, and dietary phytoestrogens. Estimation of exposure via these three sources was made using 17β-estradiol equivalent concentrations. Finally, we discuss the limited available data on other livestock hormones and then conclude with future research that should be conducted to further understand the impact of livestock hormones on aquatic ecosystems.
Introduction

Humans and wildlife are exposed to numerous chemicals that have the potential to modulate their endocrine systems (1-6). Some of the most potent of the identified endocrine modulators are natural and synthetic estrogens. These modulators are especially potent because of their strong binding affinity to estrogen receptors, which leads to biological effects even at very low concentrations (7). Additionally, they may exert their estrogenic effects via estrogen receptor-independent pathways, most of which are not well understood. Natural and synthetic hormones may reach the environment through confined animal feedlot effluent, sewage treatment plant effluent, and run-off from land-applied manure. Aquatic wildlife species face the greatest threat of exposure, although terrestrial wildlife may be exposed through soil and humans may be exposed through drinking water contamination and food sources.

One of the best examples of endocrine disruption in wildlife from natural and synthetic hormones is in the United Kingdom, where sewage effluent entering rivers was causing feminization of fish. The chemicals responsible for this endocrine disruption were found to be 17α-ethynyl estradiol (a synthetic estrogen in contraceptives), 17β-estradiol, and estrone (8-11). Due to these and other studies, concern has been raised about possible contamination of surface waters by hormones from livestock. The situation with livestock differs from that of human sewage in that while large scale livestock operations are required to treat animal excretion before release into the environment, land applied manure may enter surface waters via run-off. Furthermore, the cumulative effect of animal excretion from traditional family-run livestock farms can not be ignored because they are not required to have an animal waste treatment system. Additionally, livestock manure differs from human sewage because most livestock animals raised in the United States are treated with growth hormones (12). Therefore, it is necessary to assess the possibility of contamination and effects on humans and wildlife with these issues in mind.

Hormones in Livestock

The three major types of livestock raised in major operations are cattle, swine, and poultry. These animals all naturally produce the estrogens 17α- and 17β-estradiol, estrone, estriol, and progesterone, which are responsible for the development of female secondary sex characteristics and regulation of the menstrual cycle, pregnancy, and embryogenesis. To date, six hormones are approved for use in the United States as growth hormones in livestock: 17β-estradiol and the synthetic estrogen zeranol, progesterone and the synthetic progesterone melengestrol acetate (used to synchronize or induce estrus), and testosterone and the synthetic androgen trenbolone acetate. These hormones are administered as subcutaneous implants or feed additives. Typical treatments with growth hormones increase growth rates by 5-20% and feeding efficiency by 3-10% (13). Hormones are not approved for use in the European Union.

Excretion of natural sex steroid hormones occurs via the feces or urine. Lange et al. (14) estimate that yearly excretion of estrogens, progesterones, and
androgens by livestock in the U.S. to be 49, 4.4, and 279 tons, respectively. Before or after excretion, hormones may be metabolized. The major metabolite of zearanol is zearalanone and the minor metabolite is taleronol. Progesterone is typically excreted as metabolites including pregnane diones, prenanolones, and prenanediols (15). Melengestrol acetate is excreted largely unaltered. Trenbolone acetate is mainly excreted as the metabolites 17α- and 17β-trenbolone. It has been estimated that approximately 8-12% of added growth hormones is excreted (14). Little is known about the total amount of growth hormones excreted on a yearly basis compared to natural hormones.

A portion of the cattle, swine, and poultry in the U.S. are raised in concentrated animal feeding operations (CAFOs). These facilities contain large numbers of livestock in small areas. Concerns about such operations include problems with manure storage and land application, Escherichia coli outbreaks, groundwater and surface water contamination, excess nutrients, fish kills, air quality, and the use of antibiotics and growth hormones. Such facilities are considered point sources for hormones, nutrients, and other pollutants.

**Transport and Fate in the Environment**

Natural and synthetic steroid hormones from livestock excretion reach the environment through feedlot effluent and runoff from land applied manure. Factors affecting the concentrations of hormones in the environment include inputs of hormones to the system, sorption to soils and sediments, degradation, and dilution (14-17). Inputs are controlled by the number, age, sex, reproductive status, and type of the livestock, as well as the runoff potential of the soil and the amount of precipitation in the region. Sorption is controlled by soil characteristics such as particle size and organic matter content, as well as the solubility of the hormone. Degradation is affected by temperature and light, as well as nutrient availability and biological activity of the soil (18). Dilution is affected by precipitation, stream discharge, and other stream characteristics. Based on these factors, hormones may accumulate either in soil or surface water.

Natural estrogens are rapidly degraded under aerobic conditions, often within several hours to several days (18-22). Estradiol will be degraded to estrone under aerobic conditions (23), but estrone will not be broken down further. Estrogens also sorb strongly to soils and sediments, which may serve as a sink in the environment (24-28). Testosterone easily leaches from the soil because it does not sorb strongly compared to estrogens (12, 14). Although testosterone is fairly mobile, it degrades even faster than natural estrogens. Metabolites of testosterone include 4-androstene-3,17-dione, 5α-androstane 3,17-dione, and 1,4-androstadiene-3,17-dione (12, 18, 20, 21,23). Generally, progesterone is excreted as a biologically inactive metabolite. Little is known about its fate in the environment (15).

Synthetic hormones are considered to be more persistent than natural steroid hormones. For example, soils that received manure application had detectable concentrations of melengestrol acetate for the entire growing season and trenbolone for 8 weeks. The half-life of trenbolone was approximately 260 days (29). Both compounds adsorb strongly to organic matter and are unlikely
to leach in large quantities (30). There is no information regarding the fate of zeranol in the environment.

Based on sorption and degradation potentials, the occurrence of hormones in the environment might be expected to be low. However, the abundance of liquid and solid manure from concentrated animal feedlot operations and the widespread use of growth hormones increase the likelihood of short-term and even chronic contamination of surface waters by hormones. Additionally, hormones are the most potent of endocrine disrupting chemicals, and even low concentrations in the environment might have detrimental effects on humans and wildlife, particularly if contamination occurs during crucial developmental periods affected by such hormones.

**Occurrence and Effects of Livestock Estrogens in the Environment**

Several studies have measured the concentrations of natural and synthetic estrogens from livestock in the water. The results of these studies are summarized in **Table 1**. Concentrations of 17α- and 17β-estradiol in water ranged from not detected to 2,530 ng/L. Relatively little is known about the occurrence of hormones in drinking water and meat. Concentrations in meat would be expected to vary based on time of treatment with hormones, when or if the subcutaneous hormone implant was removed, and the sex and reproductive status of the animal.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Livestock Type</th>
<th>Min</th>
<th>Median</th>
<th>Max</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>17α-estradiol</td>
<td>Stream</td>
<td>Cattle</td>
<td>nd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>Run-off</td>
<td>Broiler chickens</td>
<td>50</td>
<td>150</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>Pond</td>
<td>Cattle</td>
<td>5</td>
<td>23</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>River</td>
<td>Dairy cows</td>
<td>nd</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>Run-off</td>
<td>Poultry</td>
<td></td>
<td>1,280</td>
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<td></td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>Run-off</td>
<td>Broiler chickens</td>
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<td>2,530</td>
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<td></td>
</tr>
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<td>17β-estradiol</td>
<td>Springs</td>
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<td>66</td>
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</tr>
<tr>
<td>17β-estradiol</td>
<td>Stream</td>
<td>Cattle</td>
<td>nd</td>
<td>nd</td>
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</tr>
<tr>
<td>Estradiol</td>
<td>Streams</td>
<td>Cattle</td>
<td>&lt;0.5</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>E2 equivalents</td>
<td>Streams</td>
<td>Dairy cows</td>
<td>0</td>
<td>2</td>
<td>26.5</td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>River</td>
<td>Dairy cows</td>
<td>nd</td>
<td>0.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estrone</td>
<td>Stream</td>
<td>Cattle</td>
<td>0.246</td>
<td>7.7</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Soto et al. 2004 (33), Finlay-Moore et al. 2000 (32), Kolodziej et al. 2004 (33), Nichols et al. 1997 (59), Petersen et al. 2000 (60), Shore et al. 1995 (61), Matthiessen et al. 2006 (62).*
Effects on Wildlife

Studies on the effects of natural estrogens on wildlife species have found a wide array of responses, including feminization, intersex, decreased sperm count, and vitellogenin induction (31). Synthetic hormones are often created to be more potent than natural estrogens, which combined with their increased persistence in the environment, makes them especially of great concern. Most studies performed to assess the effects of concentrated animal feedlot effluent on wildlife species have focused on fish. Orlando et al. (32) found that female fathead minnows downstream of a cattle feedlot effluent were defeminized, with decreased estrogen to androgen ratios, while male fathead minnows were demasculated, with lowered testosterone synthesis and smaller testis. The authors suggest that either there are potent androgenic substances in the FLE [feedlot effluent], and/or there is complex mixture of androgenic and estrogenic substances that alter the hypothalamic-pituitary-gonadal axis, inhibiting the release of gonadotropin-releasing hormone or gonadotropins (32). A companion study (33) detected estrogenic activity in the surface water using E-SCREEN bioassays. Estrone and 17β-estradiol were all detected at least once, although only estrone concentrations were high enough to account for a substantial component (3-46%) of the measured hormonal activity of the water (33).

Effects on Humans

Hormones from livestock may affect humans either through dietary intake of hormones remaining in meat and milk products or through contamination of drinking water. Most of the information regarding the effects of human exposure to livestock hormones has been obtained by investigating the effects on surrogate mammal species. For example, zarenol and its metabolites cause accelerated puberty and vaginal opening, abnormal estrus, and anovulatory ovary in prepubertal mice fed 10 mg/kg of zarenol daily (34-35). In utero exposure to zerenol by feeding pregnant mice 10 mg/kg of zerenol had earlier and abnormal testicular differentiation in offspring (36). The no observed effect concentration of zerenol in monkey species was determined to be 0.225 mg/kg (37). More information is needed on the occurrence and concentrations of zerenol and other hormones in meat to begin to determine the effects of human consumption.

Case Studies of the Occurrence of Estrogenic Compounds in the Environment

Long term studies on temporal and spatial scales of hormone contamination are needed. Thus, we undertook two monitoring studies with the objective of quantifying concentrations of hormones and other organic chemicals in surface waters. The first study was performed in an agricultural area of northern Missouri, USA, and the second study was conducted in a relatively pristine recreational area in southern Missouri.
Occurrence of Organic Chemicals in the Grand River Watershed, Northern Missouri

The Grand River Watershed is located in a glaciated plain in northern Missouri (Figure 1). Approximately 60 to 70% of the area is covered by native grasses. The major soil types are GARA-22-E2, Armstrong-24C2, Lamoni-15C2, and Adair-14C2. Constant soil erosion is characteristic of the region, particularly during spring and late autumn. Row crops include soybean and corn. Pastured cattle farms are spread over the region (Table 2), and there are approximately one million hogs raised by a corporate hog farm and by individual farmers. Streams within the watershed may be affected by runoff from confined hog operations and subsequent land application of manure, as well as run-off from pastured cattle and corn and soybean fields. This has led to concern over the potential for endocrine disruption of aquatic species via natural or synthetic hormones, as well as industrial chemicals and pesticides. Therefore, a monitoring study was undertaken between June 2003 and September 2005 to determine occurrence and concentrations of estrogens and other organic chemicals that might be estrogenic. Water samples were collected monthly during the growing season from two streams. Two sampling sites were established on each of the streams. Automatic sampling devices (ISCO model 3710, Teledyne ISCO, Lincoln, Nebraska, USA) were used to collect 24-h composite samples in a time-proportional mode. Eight liters of water sampled from each site were collected monthly from June to November 2003, May to November 2004, and June to September 2005. The samples were filtered through 1.0 μm glass fiber filters (Whatman, NJ, USA). Solid phase extraction was then performed using C-18 cartridges. The extract was dried and reacted with Sylon BFT (Supelco, Bellefonte, PA, USA) to produce silyl derivatives. Concentrations of selected organic chemicals were determined by gas chromatography/mass spectrometry (Figure 2) (38). Mean recoveries of all analytes in the laboratory spike from GC-MS analysis generally exceeded 70% at all spike levels. Percent recoveries of the steroids were between 56% and 79% with recoveries of the phenolic compounds between 70% and 92%. The linearity of calibration curves (R^2) was greater than 0.97, with the exception of simazine at 0.87. Limits of detections (LODs) ranged from 0.1 to 3.4 ng/L. Nutrients and several physicochemical parameters of the water were determined by Hach methods (Hach Company, Loveland, Colorado) that are endorsed by USEPA.

During the three year study period, temperature ranged from 5.5 to 32.7 °C and pH values ranged from 4.5 to 8.9. Median turbidity was 29 NTU. Median total nitrogen and total phosphorus concentrations were 0.78 mg/L N and 0.14 mg/L, respectively, and median total organic carbon was 12.8 mg/L. Thirteen of the nineteen organic chemicals were detected at least once between June 2003 and September 2005 (Table 3). 17α-Estradiol was detected three times, with a maximum concentration of 54 ng/L. Estrone was detected twice with a maximum concentration of 29 ng/L. Both cattle and swine farming activities are likely to contribute the source of 17α-estradiol and estrone. Studies have shown that cattle mainly excrete 17α-estradiol instead of 17β-estradiol (16). The most commonly detected chemicals were dibutyl phthalate, metolachlor, nonylphenol,
bisphenol A, atrazine, and benzyl butyl phthalate. All these chemicals have been shown to be estrogenic to various biological endpoints (7) with the exception of metolachlor, which has not been tested. Concentrations of metolachlor and atrazine decreased throughout the growing season, while concentrations of industrial chemicals showed no clear pattern. Currently we are conducting a field enclosure study of amphibian tadpoles to evaluate total estrogenic effects of these compounds.

Table 2. Land-use upstream of the four sampling sites in Little Medicine Creek (LMC) and West Locust Creek (WLC) (data obtained from MoRAP GIS data set). Zero percent of the land-use is classified as urban and less than or equal to 0.1% is classified as water.

<table>
<thead>
<tr>
<th>Site</th>
<th>Latitude</th>
<th>Longitude</th>
<th>Drainage Area (ha)</th>
<th>% Row Crop</th>
<th>% Forest</th>
<th>% Grassland</th>
</tr>
</thead>
<tbody>
<tr>
<td>LMC1</td>
<td>N 40° 29'</td>
<td>W 93° 24'</td>
<td>4,278</td>
<td>23</td>
<td>9</td>
<td>67</td>
</tr>
<tr>
<td>LMC2</td>
<td>N 40° 20'</td>
<td>W 93° 23'</td>
<td>16,023</td>
<td>23</td>
<td>17</td>
<td>60</td>
</tr>
<tr>
<td>WLC1</td>
<td>N 40° 29'</td>
<td>W 93° 11'</td>
<td>8,699</td>
<td>17</td>
<td>11</td>
<td>72</td>
</tr>
<tr>
<td>WLC2</td>
<td>N 40° 25'</td>
<td>W 93° 10'</td>
<td>12,320</td>
<td>15</td>
<td>12</td>
<td>73</td>
</tr>
</tbody>
</table>

LMC1: north site in LMC; LMC2: south site in LMC; WLC1: north site in WLC; WLC2: south site in WLC.

Table 3. Percent detection, median, and maximum concentrations of 19 selected chemicals found in filtered streamwater samples from Little Medicine Creek and West Locust Creek during 2003-5. Data from both creeks were pooled (n = 65). “nd”= not detected; “-” = not applicable.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Detection Limit (ng/L)</th>
<th>3-yr Ave Detection (%)</th>
<th>Median conc. (ng/L)</th>
<th>Maximum conc. (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dibutyl phthalate</td>
<td>0.3</td>
<td>78</td>
<td>27</td>
<td>1,127</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>0.4</td>
<td>75</td>
<td>9</td>
<td>472</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>1.7</td>
<td>72</td>
<td>43</td>
<td>725</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.1</td>
<td>66</td>
<td>6</td>
<td>1,986</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.9</td>
<td>65</td>
<td>12</td>
<td>536</td>
</tr>
<tr>
<td>Benzyl butyl phthalate</td>
<td>0.8</td>
<td>63</td>
<td>5</td>
<td>145</td>
</tr>
<tr>
<td>Simazine</td>
<td>2.4</td>
<td>34</td>
<td>&lt;DL</td>
<td>375</td>
</tr>
<tr>
<td>p,p'-DDE</td>
<td>0.6</td>
<td>23</td>
<td>&lt;DL</td>
<td>2,247</td>
</tr>
<tr>
<td>4-Octylphenol</td>
<td>2.0</td>
<td>22</td>
<td>&lt;DL</td>
<td>812</td>
</tr>
<tr>
<td>Tebuthiuron</td>
<td>3.4</td>
<td>15</td>
<td>&lt;DL</td>
<td>373</td>
</tr>
<tr>
<td>Bioallethrin</td>
<td>0.8</td>
<td>3</td>
<td>&lt;DL</td>
<td>28</td>
</tr>
<tr>
<td>17a-Estradiol</td>
<td>1.3</td>
<td>3</td>
<td>&lt;DL</td>
<td>54</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.9</td>
<td>2</td>
<td>&lt;DL</td>
<td>29</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>0.6</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Estriol</td>
<td>0.8</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17a-Ethynyl estradiol</td>
<td>0.8</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>1.3</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.9</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>1.1</td>
<td>nd</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Figure 1. Study sites in northern and southern Missouri. Two study sites are located in Little Medicine Creek and West Locust Creek in northern Missouri. Another two study sites are located in North Fork of the White River and Eleven Point River in southern Missouri.

Figure 2. Total ion chromatogram from GC/MS analysis of trimethylsilyl (TMS) derivatives of environmental contaminants monitored in river waters. 1 = Tebuthiuron, 2 = 4-tert-octyl phenol, 3 = simazine, 4 = nonyl phenol, 5 = d₅-atrazine (internal std), 6 = atrazine, 7 = 4-octyl phenol, 8 = d₁₀-anthracene (internal std), 9 = dibutyl phthalate, 10 = metolachlor, 11 = bioallethrin, 12 = p,p'-DDE, 13 = bisphenol A, 14 = benzyl butyl phthalate, 15 = diethylstilbestrol, 16 = estrone, 17 = 17α-estradiol, 18 = d₅-estradiol (internal standard), 19 = 17β-estradiol, 20 = permethrin, 21 = tamoxifen, 22 = 17α-ethinyl estradiol, 23 = cholestane (internal std), 24 = estriol, 25 = β-sitosterol.
Occurrence of Estrogens in Two Rivers in Southern Missouri

A two-year monitoring study was conducted in two relatively pristine rivers in southern Missouri to identify possible estrogenic chemicals in the rivers. Study sites were established in the lower sections of the Eleven Point and the North Fork of the White Rivers (Figure 1). The stretches of both rivers are fast moving and spring fed with numerous pools and riffles. The land use adjacent to the rivers is deciduous forest with pasture and grassland in the upland areas. Traditional family-run cattle farms are located throughout the watersheds. Most of the areas adjacent to the riparian zones of the two rivers were intact without much crop cultivation. Canoeing in the summertime drew large crowds, particularly during weekends and holidays. Twenty-four hour composite water samples were collected monthly from August 2003 to November 2004. Water samples were analyzed with the same protocols used in the northern Missouri study.

Data from both rivers were very similar. For regional comparison with northern Missouri, data from Eleven Point River and North Fork of the White River are pooled. Temperature ranged from 7.4 to 23.3°C and pH values ranged from 5.7 to 8.6. Median turbidity was 1.9 NTU. Median total nitrogen and total phosphorus concentrations were 0.68 mg/L N and 0.03 mg/L, respectively. Median total organic carbon concentration was 1.6 mg/L. No natural estrogens from humans or livestock were detected throughout the study period (Table 4). However, several estrogenic chemicals were present. For instance, benzyl butyl phthalate, dibutyl phthalate, and bisphenol A were all detected in the majority of samples (87 to 97%) with median concentrations ranging from 18 to 164 ng/L. Occurrences of dibutyl phthalate, benzyl butyl phthalate, and nonylphenol were all highly correlated, which may indicate a common source or process controlling their concentrations in the rivers, but further study would be needed to determine such mechanisms. Additionally, the correlation between nonylphenol and metolachlor is consistent with nonylphenol being used as an inert ingredient in herbicides (39). Dibutyl phthalate and bisphenol A have both been shown to be estrogenic in amphibians, causing deformities and impaired spermatogenesis (40), and altered sex ratios (41), although at concentrations ranging from 100-10,000 µg/L for dibutyl phthalate and 2.3 to 228 µg/L for bisphenol A. Nonylphenol has been shown to alter sex ratios in Rana pipiens and R. sylvatica tadpoles at concentrations as low as 10 µg/L (42). The plant sterol β-sitosterol, a weak binder of estrogen receptors, was detected in the Eleven Point and North Fork of the White River (38). Metolachlor, an herbicide generally used in soybean fields and in mixtures with atrazine on corn fields, was the most commonly detected herbicide. Little evidence is available related to its estrogenicity. Tebuthiuron, an herbicide typically applied to roadsides and other non-cropland areas, was also detected. Its estrogenicity is also unknown. Atrazine, an herbicide commonly applied to corn fields, was not detected. Though atrazine is considered estrogenic, its effects and potency on wildlife are still under vigorous debate.

Despite the prevalence of livestock in the Grand River Watershed in northern Missouri, few occurrences of estrogens were found. Our findings of low frequency of presence of estrogens are in agreement with those of other
studies in areas downstream of cattle, hog, and poultry operations (Table 1). In our study, the rare detection of natural and synthetic estrogens in may be attributed to several factors such as the efficiency of the waste management system, rapid breakdown, and sorption to soils and sediments. Additionally, it should be noted that bed and suspended sediments in the streams were not measured. Due to the high turbidity, it is also likely that most of estrogenic chemicals are adsorbed in the organic matrix of the water. A special analytical protocol needs to be developed and validated to analyze the water with such high turbidity.

In contrast to the Grand River Watershed, the Eleven Point River and the North Fork of the White Rivers in southern Missouri are located in relatively isolated areas with much fewer livestock operations. It is thus not a surprise that estrogens were below detection limits.

Table 4. Detection frequency, median, and maximum concentrations of organic chemicals in Eleven Point and North Fork of the White Rivers during 2003-4. Data were pooled from two rivers (n=31).

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Detection Frequenty (%)</th>
<th>Median (ng/L)</th>
<th>Maximum (ng/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Benzyl butyl phthalate</td>
<td>0.8</td>
<td>97</td>
<td>56</td>
</tr>
<tr>
<td>Dibutyl phthalate</td>
<td>0.3</td>
<td>97</td>
<td>154</td>
</tr>
<tr>
<td>β-Sitosterol</td>
<td>1.0</td>
<td>88</td>
<td>190</td>
</tr>
<tr>
<td>Bisphenol A</td>
<td>0.1</td>
<td>87</td>
<td>21</td>
</tr>
<tr>
<td>Nonylphenol</td>
<td>1.7</td>
<td>46</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Metolachlor</td>
<td>0.4</td>
<td>36</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>Tebuthiuron</td>
<td>3.4</td>
<td>30</td>
<td>&lt;DL</td>
</tr>
<tr>
<td>4-Octylphenol</td>
<td>0.3</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>17α-Ethinylestradiol</td>
<td>0.8</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>17α-Estradiol</td>
<td>1.3</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>17β-Estradiol</td>
<td>1.3</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Estradiol</td>
<td>0.8</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Estrone</td>
<td>0.9</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Diethylstilbestrol</td>
<td>0.6</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Tamoxifen</td>
<td>1.1</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Atrazine</td>
<td>0.9</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>p,p′-DDE</td>
<td>0.6</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Bioallethrin</td>
<td>0.8</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Permethrin</td>
<td>0.9</td>
<td>nd</td>
<td>-</td>
</tr>
<tr>
<td>Simazine</td>
<td>2.4</td>
<td>nd</td>
<td>-</td>
</tr>
</tbody>
</table>

*DL = detection limit

Levels of Human Exposure to Environmental, Dietary, and Medicinal Estrogens

We have discussed environmental concentrations of natural and synthetic estrogens as well as exposure of aquatic organisms to them. Estimation of
human exposure to estrogenic compounds would be informative to the general public. In addition to being potentially exposed to estrogenic chemicals in drinking water and contaminants in the diet (categorically "environmental"), humans may be exposed to medicinal estrogens and dietary phytoestrogens. To understand contributions from these three sources, we estimate possible maximum exposure to each source. Exposure levels and potency factors of each source of estrogens were adopted from several studies. Clinical data show that medicinal estrogens in female serum/plasma range between 0.032 and 12.3 ng/mL (Table 5) (43-48). The Third National Health and Nutrition Examination Survey from 1988-1994 showed that the phytoestrogen levels in human serum/plasma ranged from non-detectable to 373.2 ng/mL (Table 6) (49). Many studies have investigated serum levels of estrogenic organic chemicals from the environment. However, the data were normalized by fat contents and thus were not comparable here. To our knowledge, only one study estimated serum/plasma levels of DDT and metabolites, aldrin, dieldrin, and p,p'-methoxychlor in the control subjects of the Long Island Breast Cancer Study Project. The concentration range was between 2.39 and 6.85 ng/mL (Table 6) (50). The serum/plasma estrogen levels acquired from consuming the livestock with administered estrogens were unknown and difficult to estimate.

The potency factors for medicinal estrogens and livestock estrogens were assumed to be 1.0 because those estrogens have binding affinities and elicit biological effects similar to 17β-estradiol. According to the available receptor binding affinity and cell proliferation data, the potency factors of phytoestrogens range from 10⁻³ to 10⁻², and the highest number was adopted (7). The potency factors of environmental estrogens (e.g., DDT and its metabolites, certain PCBs) range between 10⁻⁶ and 10⁻³ (Table 7) (7).

Table 7 summarizes human exposure levels to medicinal estrogens, dietary phytoestrogens, environmental estrogens, and estrogens from the release of livestock. 17β-Estradiol equivalent concentrations are the product of estrogens in serum/plasma and their potency factors. Accordingly, the ranking order of 17β-estradiol equivalent concentrations is medicinal estrogens > phytoestrogens >> environmental estrogens. Unfortunately, the 17β-estradiol equivalent concentrations from exposure to the release of livestock estrogens can not be calculated and ranked because of the lack of adequate exposure data. It should be noted that the calculation of 17β-estradiol equivalent concentrations can be much improved when more chemicals are tested for additional critical toxicological endpoints.
Table 5. Steady-state concentrations of medicinal estrogens in plasma or serum.

<table>
<thead>
<tr>
<th>Brand Name (form)</th>
<th>Types of estrogens</th>
<th>Serum level (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Premarin® (tablet)</td>
<td>Estrone, equilin, 17α- and 17β-dihydroequilin, 17α- and 17β-estradiol</td>
<td>12.3</td>
</tr>
<tr>
<td>Prempro® (tablets)</td>
<td>Conjugated estrogens found in Premarin, medroxyprogesterone</td>
<td>11.7</td>
</tr>
<tr>
<td>Cenestin® (tablets)</td>
<td>Conjugated estrogens found in Premarin tablets, 17β-estradiol, equilenin, 17α- and 17β-dihydroequilin</td>
<td>7.7</td>
</tr>
<tr>
<td>Alora® (transdermal patch)</td>
<td>17β-Estradiol</td>
<td>0.092 - 0.144</td>
</tr>
<tr>
<td>Climastr® (tablet)</td>
<td>17β-Estradiol</td>
<td>0.032 - 0.147</td>
</tr>
<tr>
<td>Vivelle® (transdermal patch)</td>
<td>17α-Ethyl estradiol</td>
<td>0.046 - 0.133</td>
</tr>
</tbody>
</table>

Table 6. Levels (ng/ mL⁻¹) of phytoestrogens and environmental estrogens detected in human serum or plasma. Ranges are given followed by means in parentheses. n.d. = not detectable.

<table>
<thead>
<tr>
<th>Chemicals</th>
<th>Levels¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daidzein</td>
<td>n.d. – 162 (3.9)</td>
</tr>
<tr>
<td>Equol</td>
<td>n.d. – 8.2 (LOD)</td>
</tr>
<tr>
<td>Genistein</td>
<td>n.d. – 203 (4.7)</td>
</tr>
<tr>
<td>Total isoflavones</td>
<td>n.d. – 373.2 (8.6)</td>
</tr>
<tr>
<td>Enterolactone</td>
<td>n.d. – 112 (3.6)</td>
</tr>
<tr>
<td>Matairesinol</td>
<td>n.d. – 3.3 (LOD)</td>
</tr>
<tr>
<td>Enterodiol</td>
<td>n.d. – 19 (1.8)</td>
</tr>
<tr>
<td>Total lignans</td>
<td>n.d. – 134.3 (5.4)</td>
</tr>
<tr>
<td>Total environmental estrogenic chemicals</td>
<td>2.39 – 6.85⁵</td>
</tr>
</tbody>
</table>

¹ The Third National Health and Nutrition Examination Survey from 1988-1994 (49).
⁵ DDT and metabolites, aldrin, dieldrin, and p,p'-methoxychlor in the control subjects of the Long Island Breast Cancer Study Project (50).

Other Livestock Hormones

In addition to estrogens, the levels and effects of progesterone, 17α-trenbolone, testosterone, and trendione were reported in several studies (Table 8). One study found that progesterone was below the detection limit (0.2 ng/mL) in any of the groundwater samples (51). Levels of testosterone ranged
from not detected to 1,830 ng/L, with the highest concentration in run-off from land-applied broiler chicken manure (52). The most commonly measured synthetic hormones were 17α- and 17β-trenbolone, which ranged in concentration from not detected to 120 ng/L. Studies focusing on fish and the effects of trenbolone acetate metabolites have found that concentrations ranging from 9-193 ng/L cause reduced fecundity and plasma vitellogenin and steroid concentrations (53-56). In male fish, concentrations of trenbolone acetate metabolites ranging from 50-41,000 ng/L caused decreased 11-ketotestosterone concentrations and increased testicular area and sperm percentage (53, 55). Holbech et al. (57), in a study using juvenile zebra fish, found that concentrations of trenbolone of 9.7 ng/L led to the formation of all male populations. In a modeling study of population viability of fathead minnows, concentrations of 27 ng/L trenbolone acetate metabolites caused a 50% reduction in population size after two years of exposure. (58).

**Table 7. Human exposure levels of estrogens (ng/mL) via three different sources.** Estrogens from the environment can range from estrogenic chemicals with very low potency (e.g., DDT and its metabolites, certain PCBs) to those with very high potency like livestock estrogens. Data sources were stated in the text.

<table>
<thead>
<tr>
<th>Category</th>
<th>Estrogens in serum/plasma</th>
<th>Potency factor</th>
<th>17β-estradiol equivalent concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Medicinal estrogens (female)</td>
<td>0.032 – 12.3</td>
<td>1.0</td>
<td>0.032 – 12.3</td>
</tr>
<tr>
<td>Phytoestrogens</td>
<td>n.d. – 373.2</td>
<td>10^3</td>
<td>n.d. – 3.73</td>
</tr>
<tr>
<td>Environmental estrogens</td>
<td>2.39 – 6.85</td>
<td>10^3</td>
<td>0.00239 – 0.00685</td>
</tr>
<tr>
<td>Estrogens from livestock areas</td>
<td>N/A</td>
<td>10^6 – 1.0</td>
<td>N/A</td>
</tr>
</tbody>
</table>

**Table 8. Concentrations of natural and synthetic estrogens in streams from different livestock types (ng/L).** nd is not detected. Mean or median was not available.

<table>
<thead>
<tr>
<th>Chemical</th>
<th>Source</th>
<th>Livestock Type</th>
<th>Min</th>
<th>Max</th>
<th>Citation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Progesterone</td>
<td>River</td>
<td>Dairy cows</td>
<td>nd</td>
<td>nd</td>
<td>a</td>
</tr>
<tr>
<td>17α trenbolone</td>
<td>Discharge</td>
<td>Cattle</td>
<td>nd</td>
<td>120</td>
<td>b</td>
</tr>
<tr>
<td>17α trenbolone</td>
<td>Stream</td>
<td>Cattle</td>
<td>0.0016</td>
<td>0.035</td>
<td>c</td>
</tr>
<tr>
<td>17α trenbolone</td>
<td>Stream</td>
<td>Cattle</td>
<td>nd</td>
<td>50</td>
<td>b</td>
</tr>
<tr>
<td>17β trenbolone</td>
<td>Discharge</td>
<td>Cattle</td>
<td>nd</td>
<td>20</td>
<td>b</td>
</tr>
<tr>
<td>17β trenbolone</td>
<td>Stream</td>
<td>Cattle</td>
<td>&lt;0.0004</td>
<td>0.0015</td>
<td>c</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Run-off</td>
<td>Broiler chickens</td>
<td>15</td>
<td>125</td>
<td>d</td>
</tr>
<tr>
<td>Testosterone</td>
<td>River</td>
<td>Dairy cows</td>
<td>nd</td>
<td>0.6</td>
<td>a</td>
</tr>
<tr>
<td>Testosterone</td>
<td>River</td>
<td>Cattle and fish</td>
<td>nd</td>
<td>6</td>
<td>e</td>
</tr>
<tr>
<td>Testosterone</td>
<td>Run-off</td>
<td>Broiler chickens</td>
<td>10</td>
<td>1,830</td>
<td>d</td>
</tr>
</tbody>
</table>

^Kolodka et al. 2004 (51), ^Durham et al. 2006 (63), ^Soto et al. 2004 (33), ^Finlay-Moore et al. 2000 (52), ^Shore et al. 2004 (64).
Conclusions

The natural hormones 17α- and 17β-estradiol, estrone, estriol, testosterone, and progesterone, as well as the growth hormones 17β-estradiol, testosterone, progesterone, zeranol, trenbolone acetate, and melengestrol acetate, are all excreted in large quantities by livestock. These hormones have the potential to reach the environment through run-off from confined animal feedlots and land-applied manure. Natural hormones are rapidly degraded under aerobic conditions and sorb strongly to soils and sediments, which may decrease their tendency to accumulate in the environment. Synthetic hormones are more persistent in the environment. Overall, the detection frequencies and levels of estrogens in aquatic ecosystems seem to be low. However, their effects on aquatic organisms cannot be overlooked due to their high estrogenic potency.

There are numerous knowledge gaps that need to be filled to make informed decisions about the effects of current livestock production practices on environmental quality. Research needs include, but are not limited to: 1) determination of acute toxicity to aquatic organisms due to overland flow, especially during storm events, as higher concentrations of hormones were observed in many studies, 2) assessment of the effects of hormones on soil organisms, given that both natural and synthetic growth hormones bind strongly to soils, 3) study of sorption to sediments in surface waters as an environmental sink for hormones, of which toxic effects to benthic organisms are unknown, 4) quantification of the amount of livestock hormones in meat to get a better understanding of human exposure levels, and 5) study of effects of testosterone, which is understudied compared to estrogens.

Acknowledgements

The authors thank the Missouri Department of Natural Resources, the Missouri Department of Conservation, the U.S. Department of the Interior Fish and Wildlife Service, and the Saint Louis Zoo for financial support. Our results have not been subjected to agency review and therefore do not necessarily reflect the views of our funding agencies and no official endorsement should be inferred.

References


