Emerging Contaminants (Hormones and Antibiotics) in Agricultural Watershed

Presenter: Sudarshan (Shan) Dutta
Lecture Outline

1. Overview of Emerging Contaminants
2. Focus on Agricultural Watershed: Hormones and Antibiotics
   - Sources
   - Environmental Significance
   - Fate and Transport Mechanisms
3. Analytical Techniques
4. Laboratory Illustration of ELISA
Overview
What are Emerging Contaminants?

“…unregulated contaminants, which may be candidates for future regulation…..”


e.g. Steroidal Hormones,
Antimicrobial disinfectant (Antibiotics),
Insect repellents,
Detergent metabolites, etc.
Why they are Important?

- Toxic at a very low concentrations (parts per trillions or ng/L)
- Develop female characteristics in male species resulting ecological disturbance. Also known as Endocrine Disruption.
- Promote antibiotic resistance in pathogenic microorganisms
Environmental Significance

- This is considered as “gender bending” pollution.

- In 2004, 3 Colorado rivers have 50% of male fish with both male and female characteristics dominant. (MSNBC, 2004)

- Every third fish in English Rivers are changing sex. (Daily Mail, 2006)
Where in US?

Location of 139 stream sampling sites.

Koplin et al., 2002
Focus on Agricultural Watershed: Hormones and Antibiotics
Hormones in Agriculture Landscape

- Types of hormones
- Sources
- Environmental Significance
- Fate and Transport
- Analytical techniques for measurement
Types of Hormones
Different Steroidal Hormones include:

- Estrone (E1)
- Estradiol (E2β and E2α)
- Estriol (E3)
- Testosterone (T) (Androgen)
- Progesterone (P)
Sources of Hormones in Agricultural Landscape
- Farm animals (e.g.: Cattle, Swine, Poultry) secrete different steroidal hormones through their feces and urine.

- Therefore, animal manure produced from these animals naturally contain steroidal hormones; like Estrogens, Testosterone, etc.
Concentration of hormones in manure depends on:

- Animal and Species types, Sex (male vs. female), and Age of the animals
- Concentrations vary between feces and urine
- Storage condition
  - Aeration, Dryness, Temperature, Sunlight
- Storage period
  - Hormone concentrations in manure decrease with time
Estrogen concentrations at Cattle manure:

(Hanselman et al., 2003)

**TABLE 2. Estimated Rates of Fecal and Urinary Estrogen Excretion from Dairy Cattle**

<table>
<thead>
<tr>
<th>Reproductive stage</th>
<th>N</th>
<th>Excretion rate/1000 kg LAM (µg d⁻¹)</th>
<th>Estrogens measured</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Fecal Excretion</td>
<td></td>
</tr>
<tr>
<td>nonpregnant</td>
<td>21</td>
<td>600 ± 200</td>
<td>E2α,</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>7</td>
<td>400 ± 10</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>0–80 d pregnant</td>
<td>10</td>
<td>300 ± nd</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>0–84 d pregnant</td>
<td>7</td>
<td>400 ± 20</td>
<td>E1, E2α, E2β</td>
</tr>
<tr>
<td>80–210 d pregnant</td>
<td>10</td>
<td>1500 ± nd</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>140–200 d pregnant</td>
<td>7</td>
<td>11400 ± 1200</td>
<td>E1, E2α, E2β, Eβ</td>
</tr>
<tr>
<td>210–240 d pregnant</td>
<td>10</td>
<td>5400 ± nd</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Urinary Excretion</td>
<td></td>
</tr>
<tr>
<td>nonpregnant</td>
<td>7</td>
<td>500 ± 40</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>55–81 d pregnant</td>
<td>5</td>
<td>700 ± 60</td>
<td>E1, E2α, E2β</td>
</tr>
<tr>
<td>101–123 d pregnant</td>
<td>13</td>
<td>14400 ± nd</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td>111 d pregnant</td>
<td>3</td>
<td>34300 ± nd</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td>107–145 d pregnant</td>
<td>4</td>
<td>3400 ± 1200</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>165–175 d pregnant</td>
<td>5</td>
<td>28800 ± nd</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td>205–209 d pregnant</td>
<td>4</td>
<td>22300 ± 2500</td>
<td>E1, E2α, E2β,</td>
</tr>
<tr>
<td>250–254 d pregnant</td>
<td>5</td>
<td>86800 ± 28000</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td>271–285 d pregnant</td>
<td>13</td>
<td>163000 ± 20000</td>
<td>E1, E2α, E2β, E3</td>
</tr>
</tbody>
</table>
### TABLE 3. Estimated Rates of Fecal and Urinary Estrogen Excretion from Sows

<table>
<thead>
<tr>
<th>Reproductive stage</th>
<th>N</th>
<th>excretion rate/1000 kg LAM (\mu g \text{ d}^{-1})</th>
<th>Estrogens measured</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Fecal Excretion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonpregnant</td>
<td>4</td>
<td>800 ± nd</td>
<td>E1, E2β, E3</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>69</td>
<td>100 ± 70</td>
<td>E1</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>6</td>
<td>600 ± 250</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>27</td>
<td>900 ± nd</td>
<td>not specified</td>
</tr>
<tr>
<td>14–34 d pregnant</td>
<td>6</td>
<td>1500 ± nd</td>
<td>E1, E2β, E3</td>
</tr>
<tr>
<td>25–33 d pregnant</td>
<td>466</td>
<td>1000 ± 680</td>
<td>E1</td>
</tr>
<tr>
<td>0–35 d pregnant</td>
<td>30</td>
<td>1600 ± nd</td>
<td>E1, E2α, E2β, E3</td>
</tr>
<tr>
<td><strong>Urinary Excretion</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>nonpregnant</td>
<td>4</td>
<td>600 ± 350</td>
<td>E1</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>2</td>
<td>500 ± 600</td>
<td>E1</td>
</tr>
<tr>
<td>nonpregnant</td>
<td>2</td>
<td>400 ± 300</td>
<td>E1</td>
</tr>
<tr>
<td>0–42 d pregnant</td>
<td>2</td>
<td>4400 ± 6200</td>
<td>E1</td>
</tr>
<tr>
<td>42–77 d pregnant</td>
<td>2</td>
<td>5000 ± 6200</td>
<td>E1</td>
</tr>
<tr>
<td>77–105 d pregnant</td>
<td>2</td>
<td>108000 ± 106000</td>
<td>E1</td>
</tr>
</tbody>
</table>

(Seifert et al., 2003)
### Estrogen concentrations at Poultry manure:

<table>
<thead>
<tr>
<th>Type of Litter</th>
<th>Types of Hormones</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chicken Litter (Dry)</td>
<td>Estrogen (Estrone + Estradiol): 133 µg/kg</td>
</tr>
<tr>
<td>Laying Hens (Dry)</td>
<td>Estrogen (Estrone + Estradiol): 533 µg/kg</td>
</tr>
<tr>
<td>Immature Broilers Female</td>
<td>Estrogen (Estrone + Estradiol): 65 µg/kg</td>
</tr>
<tr>
<td>Immature Broilers Male</td>
<td>Estrogen (Estrone + Estradiol): 14 µg/kg</td>
</tr>
<tr>
<td>Raw Poultry Litter</td>
<td>17β-estradiol: 14–904 µg kg-1, Estrone: ND</td>
</tr>
</tbody>
</table>

**We found (in LC/MS):**

- E1: 1.98µg/kg;
- E2β-17S: 11.42µg/kg;
- E2β: 0.47µg/kg
- E1-3S: 0.52µg/kg
Storage Condition
Application of Manure in Agricultural Landscape

Agriculture field receiving animal manure also receive steroidal hormones.
Why should we be concerned about Hormones?
Environmental Significance

- Animal Manure contain naturally producing steroidal hormones; e.g. Estrogens, Androgens, etc.

- Steroidal hormones are also known as Endocrine Disrupting Chemicals (EDCs)

- Endocrine disruption results abnormal reproductive and physiological behaviors; e.g. Fish feminization.
Threshold Hormone Concentrations

Predicted No-effect Concentration:

17β-Estradiol (E2β or E2): 1 ng/L !!

Estrone (E1): 3 – 5 ng/L !!

1 ng/L = 0.0000000001g in 1 L.

(Yong, 2005)
**Toxicity Levels of Hormones on Exposure Assays**

<table>
<thead>
<tr>
<th>Effect of hormones</th>
<th>Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endocrine disruption in male fathead minnow at 21 day exposure</td>
<td>$E_2 \beta = 40 \text{ ng/L}$</td>
</tr>
<tr>
<td>Predicted No-effects Concentration (PNEC)</td>
<td>$E_2 \beta = 1 \text{ ng/L}$</td>
</tr>
<tr>
<td>Induction of vitellogenin (VTG) in juvenile female rainbow trout on 14 day exposure</td>
<td>$E_2 \beta = 19-26 \text{ ng/L};$</td>
</tr>
<tr>
<td></td>
<td>$E_1 = 60 \text{ ng/L}$</td>
</tr>
<tr>
<td>Feminization of 84 to 100% of the masu salmon and chum salmon.</td>
<td>500 to 1000 ng/l of E2</td>
</tr>
<tr>
<td>40% of female fish showing male characteristics.</td>
<td>10 ng/L Methyl Testosterone</td>
</tr>
</tbody>
</table>

**Toxicity depends on species and period of exposure**
## Observed Concentrations of Estrogens in Agricultural Surface Runoff

<table>
<thead>
<tr>
<th>Management Practices</th>
<th>Hormone Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Raw &amp; Pelletized Poultry Litter</td>
<td>E2β: 200 – 500 ng/L</td>
</tr>
<tr>
<td>(Haggard et al., 2005)</td>
<td></td>
</tr>
<tr>
<td>Raw Poultry Litter</td>
<td>E2β: 42 – 350 ng/L</td>
</tr>
<tr>
<td>(Yonkos, 2005)</td>
<td></td>
</tr>
<tr>
<td>Raw Poultry Litter</td>
<td>E2β: 20 – 2530 ng/L</td>
</tr>
<tr>
<td>(Finlay-Moore et al., 2000)</td>
<td>T: 10-1830 ng/L</td>
</tr>
</tbody>
</table>

Hormone concentrations in surface runoff were much higher than the concentrations required to create endocrine disruption.
What happens to the hormones applied to agricultural landscape?
Fate and Transport of Hormones in Agricultural Landscape

- Sorption on the soil
- Degradation by temperature, sunlight, and microbes
- Surface Runoff
- Movement with sediments
- Leaching through preferential flow-path

Lee, 2008
Sorption on Soil

“Sorption”

The affinity of compounds such as hormones and antimicrobials for soil particles.

Soil Solution

Lee, 2008
Sorption on Soil Depends on

- Soil organic matter content
- Soil texture, especially clay content

The affinity of hormones for soil is primarily controlled by the organic matter in soil.

Soil-water Distribution Coefficient: $K_d = \frac{\text{Concentration in Soil}}{\text{Concentration in Water}}$

Hormone sorption increases with increasing soil organic matter content.
Degradation in Soil

- Increases with increase in temperature
  - Colucci et al., 2001
- Photo degradation (Sunlight)
- Microbial degradation (Microbes)
  - Hanselman et al., 2003
## Movement with Surface Runoff

<table>
<thead>
<tr>
<th>Management Practices</th>
<th>Hormone Concentrations</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Raw Poultry Litter</strong></td>
<td><strong>E2β: 30 – 300 ng/L</strong></td>
</tr>
<tr>
<td>(Jenkins et al., 2009)</td>
<td></td>
</tr>
<tr>
<td><strong>Raw Poultry Litter</strong></td>
<td><strong>E2β: 25 – 2530 ng/L</strong></td>
</tr>
<tr>
<td>(Finlay-Moore et al., 2000)</td>
<td></td>
</tr>
</tbody>
</table>
Movement with sediments:

- Large fractions of estrogens, received in soil, move with the sediments

Mechanism:

- Estrogens are sorbed with soil particles and particulate organic matter (POM) which control the movements with sediments

Kuster et al., 2004
Lee et al., 2007
Downward Movements:

- Estrogens can move with preferential flow
- Therefore, have potential to contaminate groundwater

Figure: Weston and Seelig, 1994
To Date, Environmentally Significant Transport Mechanism:

Surface Runoff. It ends up in streams
Estrogen have different forms !!!!

However, to date, most previous studies have analyzed only E2β
Different forms of Estrogens

Estrogens are obtained in two different forms

- i) **Free Forms**
  Estrone (E1); 17β-Estradiol (E2β / E2); Estriol (E3)

- ii) **Conjugated forms**
  Sulfate and Glucuronide conjugates of free estrogens

Toxicity of free forms >>>>> conjugated forms

Conjugates can be converted to free forms
Free and Conjugated Estrogens:

Hutchins et al., 2007
My Research:

Objective:

- To investigate various forms of estrogens in surface runoff
- Receiving different litter treatments
Site Description and Experimental Design:

Experimental Field (Plot Size: 12m X 5m)

Experimental Design with Treatments

<table>
<thead>
<tr>
<th>Pelletized Poultry Litter (PPL)</th>
<th>Control Plot</th>
<th>Raw Poultry Litter 1 (RPL1)</th>
<th>Raw Poultry Litter 2 (RPL2)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Farm Lane</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Reduced Tillage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No-Tillage</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Raw Poultry Litter 1 (RPL1)
Raw Poultry Litter 2 (RPL2)
Flow-weighted concentrations of E1, E2β and E2β-17S in surface runoff

Dutta et al., Submitted
Temporal pattern of mass exports (E1).

- Rainfall intensity played important role in mass export.
- Exports were high for some late events.
Estrogens in Surface Runoff

Mainly controlled by:

- Amount of surface runoff
- Timing of the event
- Dissolved organic matter (DOM)
Key Questions
Remain Unanswered

- Fate and Transport of all free and conjugated forms

- Downward movements of different forms of Estrogens through preferential flowpaths

- Watershed scale study:
  - changes in forms and toxicity of estrogens at different watershed compartments
Estrogens at Agricultural Watershed

Inamdar et al., 2009

BOX 1: Conceptual model illustrating sources, sinks, and processes influencing hormone and antibiotic fate and transport in the watershed

- Litter application on cropland
- Transport of chemicals with surface runoff & sediment
- Preferential vertical drainage
- Aerobic oxidation & degradation
- Deposition of sediment
- Adsorption/desorption from deposited sediment
- Anaerobic conversion of E2β to E1

Cropland
Riparian forest
Riparian wetland
Stream

Groundwater
Subsurface flow
Anoxic conditions
Antibiotics in Agriculture Watershed

- Sources in Agriculture Watershed
- Environmental Significance
- Fate and Transport
- Analytical techniques for measurement
Major antibiotics used in agriculture farm
- Tetracycline; Sulfonamides; Quinolones
- Practice of feeding to poultry, swine and cattle
- Used to treat illness
- Feed additive to promote growth
- An estimated 60-80% of all livestock and poultry receive antibiotics
  - Some are metabolized
  - Some are excreted with the manure

Beatty, 2004
Why we should know about Antibiotics?
Environmental Significance

→ Promote evolution of antimicrobial resistant bacteria.

→ Compromising the efficacy of important human medicines.

→ Reported concentrations in environment are lower than Lowest Observable Adverse Effect Level (LOAEL).
Fate and Transport of antimicrobials

- Can be sorbed with soils.
- Can move with surface runoff.
- Sorbed fraction can move with sediments.

Tetracycline mainly moves with sediment as it gets sorbed strongly with soil. Sulfonamide sorption is less than Tetracycline.
Sorption on Soil Depends on:

- Soil organic matter content
- Clay Content
- Soil pH

Sorption is pH-dependent for almost all antimicrobials.

Sorption correlates well to Soil Cation Exchange Capacity from clay & organic matter.

Sorption correlates well to Soil Organic Carbon.

Lee, 2008
Yes, for some antibiotics and some plant species. However,

Estimated potential daily intake for a human consuming these plants is in the order of a few μg/day - not an amount expected to pose a threat to human health.
Transport of Antimicrobials

- DOM-Facilitated Transport
  - Through Surface Runoff (Major Process)
  - Through Soil Profile

Mechanism:
Antimicrobials bind to DOM by
- Cation Exchange
- Cation Bridging

Lee et al., 2007
Our new Watershed scale study funded by USDA AFRI (2010 - 2012)

We will monitor

☞ How do watershed landscape positions influence the amounts and forms of hormones and antibiotics?

☞ How do the seasonal changes over the year influence the persistence and exports of hormones and antibiotics
Analytical Techniques
Two Major Techniques

- Immunoassays
- Chromatography
Immunoassays

- Enzyme Linked Immunosorbent Assay (ELISA)
- Bioassays
- Radio-immunoassays
ELISA

- Competitive Immunoassay technique.

- Binding between specific antibody with specific antigen is measured

- Competition for antibody binding sites between antigen enzyme conjugates and the samples (specific hormones)

- The binding between enzyme conjugates and antibody is measured by coloring agent
Pros and Cons of ELISA

- **Pros.**
  - Easy and Quick to use.
  - Less costlier

- **Cons.**
  - Overestimation due to cross reactivity with organic substances
  - Can not measure conjugates

Can be used as an **initial screening tool** followed by chromatographic techniques.
Over estimation by ELISA our study:

<table>
<thead>
<tr>
<th>Method</th>
<th>Reduced Tillage</th>
<th></th>
<th>No Tillage</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PPL#</td>
<td>RPL1</td>
<td>RPL2</td>
<td>PPL</td>
</tr>
<tr>
<td>ELISA (ng/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>77.15</td>
<td>53.71</td>
<td>49.97</td>
<td>41.03</td>
<td>39.67</td>
</tr>
<tr>
<td>LC/MS/MS (ng/L)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.30</td>
<td>0.84</td>
<td>0.76</td>
<td>0.11</td>
<td>0.86</td>
</tr>
</tbody>
</table>

Dutta et al., submitted
Bioassays (YES: Yeast Estrogen Screening)

- These work based on the mechanism of activation of the estrogen receptor
- These are used for determining the estrogenic potencies of individual compounds
- Total estrogenic activity of complex mixtures of compounds can be determined
Chromatographic Techniques

- Gas Chromatography – Mass Spectrometry (GC-MS)
- Liquid Chromatography – Mass Spectrometry (LC-MS)
- Detection in mass spectrometry.

LC-MS is preferred over GC-MS due to less requirement of samples.
Questions?
Illustration of ELISA Technique

304 Worilow Hall
(The next building on the north)

3rd Floor