Welcome to Manure Du Jour – Season II

Serving Pennsylvania’s Best Practices for Animal Ag-, Air- and Water Quality Protection

Movement of Estrogen and Other Emerging Contaminants through the Water Environment

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Penn State Center - PITTSBURGH
Emerging Contaminants in the Water Environment

Objectives:

- Frame the Issue
- Share an Unwanted Medication Collection Event
Caution!

- Parts Per Trillion
- 1 drop in 16 Olympic size swimming pools
- 2 liters/day for 274,000 years to consume a dose of one ibuprofen tablet (200 mg)
The Beginning!
Headwater Importance!

- Water Quality
- Water Supplies
- Flood Control
- Trap Sediments
- Sustain Downstream Ecosystems
- Maintain Biological Diversity

WHERE RIVERS ARE BORN:
The Scientific Imperative for Defending Small Streams and Wetlands

Our nation's network of rivers, lakes, and surface waters emerges from a myriad of small streams and wetlands. The topographic maps most commonly used to view stream networks do not show most of the nation's small or headwater streams and wetlands but, based upon local and regional studies, scientists know that headwater streams make up at least 86 percent of the nation's stream network. At least one out of five wetlands does not have a visible connection to a waterways end, in some cases, more than half of the wetlands fall into the category. Despite the abundance of such wetlands, the United States has no national inventory of their numbers or locations.
There are many sources of contaminated wastewater: municipal wastewater treatment plants, on-site waste disposal facilities, hospitals, livestock, poultry and fish production facilities. Contamination continues in smaller concentrations via runoff from impervious surfaces (e.g., roads), and from contaminated sediment, including runoff from areas where treated water and/or residual biosolids from wastewater treatment are applied.

Source: EPA. Symbols courtesy of the Integration and Application Network (ian.umces.edu/symbols/), University of Maryland Center for Environmental Science.
Pharmaceutical Inputs

- Medicines are produced and used in very large volumes
- Nearly 50% use at least 1 prescription drug daily
- 4 of every 5 patients leave doctor with a prescription
- Almost 800 million prescription items were dispensed in 2007 - 59.2 per cent more than in 1997
- It has been estimated that hospitals and long-term care centers intentionally discard an estimated 250 million pounds a year
- 40% of antibiotics manufactured are fed to livestock
Environmental Concerns

- Aquatic Organisms (Fish and Frogs)
  - Sensitive to Low Levels of Exposure
    - Developmental Sensitive Times
  - Gender Ratio Imbalance
  - Intersex Conditions
  - Poor Egg Hatching
  - Decreased Fertility and Growth
  - Altered Behavior
Environmental Concerns
More ‘s than Answers

- Antibiotic Resistance
- Endocrine Disruption

- Developing fetuses and those with suppressed immunity may be particularly vulnerable to human health effects

- Potential risks to public health and safety have yet to be determined
Motivation

- USGS “Reconnaissance” study in 1999-2000 was 1st nationwide investigation of pharms, hormones, & other organic contaminants in 139 streams in 30 states:
  - 82 of 95 antibiotics, prescription & non-prescrip drugs, steroids, & hormones were found in at least 1 sample
  - 80% streams had 1 or more contaminant
  - 75% streams contained 2 or more
  - 54% had more than 5
  - 34% had more than 10
  - 13% tested positive for more than 20 targeted contaminants

DEP Project Overview

Phase I

Purpose:

- To document the occurrence and distribution of selected pharmaceuticals and antibiotics in streams and well water in South Central PA.
**DEP Project Overview**

**Phase I**

- Screen for pharmaceutical and antibiotic compounds in South-Central PA
  - 6 streams—effluent dominated
  - 6 streams—agricultural areas
    - Samples were collected at locations upstream and downstream of the municipal effluents or animal feeding operations
- 6 wells
Results

Streams Receiving Wastewater Effluent

- **Pharmaceuticals:**
  - Caffeine (4.75 μg/L) (micrograms per liter)
  - Para-xanthine (0.853 μg/L)
  - Carbamazepine (0.516 μg/L)
  - Ibuprofen (0.227 μg/L)

- **Antibiotics:**
  - Azithromycin (1.65 μg/L)
  - Sulfamethoxazole (1.34 μg/L)
  - Ofloxacin (0.329 μg/L)
  - Trimethoprim (0.256 μg/L)
Results

- Streams receiving runoff from AFOs
  - Pharmaceuticals: (max. conc. of 0.053 μg/L)
    - Acetaminophen
    - Caffeine
    - Cotinine
    - Diphenhydramine
    - Carbamazepine
  - Antibiotics: (max. conc. of 0.157 μg/L)
    - Oxxytetracycline
    - Sulfadimethoxine
    - Sulfamethoxazole
    - Tylosin
Results

- The average number of compounds detected downstream from municipal-wastewater effluents was 13.

- The average number of compounds (pharmaceuticals and antibiotics) detected in sites downstream from animal-feeding operations was 3.
Results

- Well-water samples
  - 5 detections total
    - Tylosin was detected 2 times
    - Cotinine
    - Sulfamethoxazole
    - Diphenhydramine
Concentrations of Selected Pharmaceuticals and Antibiotics in South-Central Pennsylvania Waters, March through September 2006

http://pubs.usgs.gov/ds/300/

U.S. Geological Survey Data Series 300

Why Can’t We Take the Compounds out of the Water?

- POTWs are not designed to remove emerging contaminants

- Promising technologies include:
  - Oxidation
  - Ozonation
  - Ultrasound
  - Activated carbon
  - Reverse osmosis

- Focus on controlling disposal at source
  - Pollution Prevention
Bradford Regional Medical Center
May 16, 2009
Unwanted Medication Collection
Intake
71 Participants
42 (59%) Mine
51 (72%) Household
9 (13%) Friend
3 (4%) Pet
37 (52%) Newspaper
34 (48%) Had flushed in the past

Intake Form 5/16/09
McKean Unwanted Medication Collection

Town/County you live in?

Whose medication was it?

_____ Mine
_____ Family or Household Member
_____ Friend
_____ Pet

How did you find out about this event?

Age Demographics:

_____ Under 20 _____ 20 to 30 _____ 31 to 40
_____ 41 to 50 _____ 51 to 60 _____ 61 to 70
_____ 71 to 80 _____ 81 and up

Have you flushed or washed medications down the drain in the past?

_____ Yes _____ No

Comments and Recommendations:
Inventory
Law Enforcement and Control Drugs
Environmental Enterprises
Uncontrolled
The Good
The Bad – Outdated Free Samples
The Ugly
Federal Disposal Guidelines for Individuals

- Take unwanted meds out of original containers and place in trash
- Mix prescription drugs with undesirable substance (e.g., used coffee grounds or kitty litter) and put in impermeable, nondescript containers and place in the trash
- Flush only if label says to do so
  - FDA lists 13 pharms that should be flushed
- Use community return programs
Office of National Drug Policy
Feb 07 FDA 13 Toilet Disposal

- Actiq (fentanyl citrate)
- Daytrana Transdermal Patch (methylphenidate)
- Duragesic Transdermal System (fentanyl)
- Oxycontin Tablets (oxycodone)
- Meperidine HCL Tablets
- Percocet (Oxycodone and Acetaminophen)
- Xyrem (Sodium Oxybate)

- Avinza Capsules (morphine sulfate)
- Baraclude Tablets (entecavir)
- Reyataz Capsules (atazanavir sulfate)
- Tequin Tablets (gatifloxacin)
- Zerit for Oral Solution (stavudine)
- Fentora (fentanyl buccal tablet)
Probe Sought Of Septic Systems' Role In Pharmaceutical Pollution

Chris Wood, President of the Pennsylvania Association of Sewage Enforcement Officers (PASBO) has called upon the General Assembly to initiate and oversee a study exploring the effect pharmaceutical waste discharged through on-lot sewage treatment systems is having on Pennsylvania's groundwater resources. In a letter to each member, Wood wrote, "PASBO believes that the General Assembly must play a major role in protecting our water resources. We call upon the General Assembly to direct and fund a study regarding the ability of on-lot systems to renovate (treat and remove) pharmaceutical waste."

"It is well documented that sewage treatment plants discharge pharmaceutical laden effluent into rivers, lakes, and streams. Around the world and within the Commonwealth, there is virtually no place scientists have looked that they have failed to find these chemicals." On-lot systems usually process the waste anaerobically, in a septic tank. The effluent is usually then discharged directly into the ground. This creates a potential for inadequately treated pharmaceutical waste to enter into the groundwater, the major source of drinking water throughout the Commonwealth. PASBO is concerned that the lack of knowledge about this potential transfer may result in the problem being overlooked until it is too late, or conversely, regulations being enacted without the proper scientific study to determine their need or effectiveness. The association believes the Legislative Budget and Finance Committee is best suited for the tasks of commissioning and administering the study and publication of the final report."

The Pennsylvania Association of Sewage Enforcement Officers (PASBO) represents the nearly 1,000 men and women who daily protect our water resources through the implementation of the Pennsylvania Sewage Facilities Act. There are roughly 1.3 million septic systems in use in the Commonwealth releasing up to 520 million gallons of treated effluent daily.
District Attorney Issues Prescription Drug Warning

BY KIMBERLY HOAK
STAFF REPORTER

Pottawatomie County District Attorney Dawn Fink recently issued a press release asking residents to “lock up your prescription drugs” as a result of a number of recent deaths in the county which are directly related to prescription drug overdoses.

"An increased number of our teens and young adults in Pottawatomie are abusing prescription drugs. These typically include painkillers such as Vicodin, Oxycontin, Percocet, Dilaudid; and, Fentanyl; sleeping/anti-anxiety medication such as Alprazolam, Valium, and Xanax; and, amphetamines such as Ritalin and Dexedrine," the district attorney wrote.

"What many people don’t understand is that when theses drugs are used recreationally, they are not taken in the prescribed amounts or even in the usual manner. For instance, kids are grinding up oxycodone and snorting it or snorting on fentanyl patches to get a more concentrated high," she wrote in the press release.

Fink continued, "Misuse of these prescription drugs can easily lead to addiction. When taken inappropriately, painkillers such as vicodin and oxycodone can have the same effect on the brain as heroin. Worse yet, abuse of these prescription drugs can even lead to death. Tragically, Pottawatomie has lost several young adults to prescription drug overdoses in the last few years."

"We can help curb this destructive trend by keeping all prescription medications locked up. Buy a key lock to place on your medicine cabinet or kitchen cupboard or purchase a small metal box with a lock. It’s best to keep them under lock and key at all times, even if you don’t have teens living with you."

"Home break-ins are occurring in an effort to find these drugs. Also, be sure to dispose of all unused prescriptions, even if you think that you may need them again someday," the district attorney warned residents.

DA Fink said she will be issuing more statements and interviews about this “serious prescription drug abuse problem” in the near future.

"I just wanted folks to be aware of what is currently happening in our communities. It is a big problem — much bigger than most people realize. We have to take action now — hopefully we can save some lives," district attorney Fink told the Leader-Enterprise earlier this week.
Change is Happening

- Safe Pharmaceutical Disposal Act in Illinois
- Effective January 1, 2010
- Bans health care institutions in the state from flushing unused meds into a public wastewater system or septic system.
Assessing antibiotic breakdown in manure

SUBMITTED
Agricultural Research Service, USDA

Scott Yates, ARS, Riverside CA

Agricultural Research Service (ARS) scientist Scott Yates is studying how oxytetracycline (OTC), an antibiotic that is administered to animals, breaks down in cattle manure.

Livestock producers in the United States often use antibiotics to control disease in their animals, and confined U.S. livestock and poultry generate about 63.8 million tons of manure every year. The drugs are often only partially absorbed by the digestive tract, and the rest are excreted with their pharmaceutical activity intact.

Yates, who works at the ARS Contaminant Fate and Transport Research Unit in Riverside, Calif., found that in controlled laboratory conditions, OTC in cattle manure was degraded more quickly as temperatures increased and as the moisture content in the manure increased. But the OTC breakdown slowed as water saturation levels neared 100 percent. Yates concluded that this slowdown resulted when oxygen levels were not high enough to fuel the OTC biodegradation.

Yates also noted that OTC breaks down more quickly in manure than in soil. Compared to soil, manure has higher levels of organic material and moisture, which support the microorganisms that break down this pharmaceutical.

This laboratory research may be useful in designing studies that evaluate the potential effects of lagoons, holding ponds and manure pits on bacteria and antimicrobial resistance.

Livestock producers also might use the results from this study to maximize the breakdown of organic materials and potential antibiotics in manure by designing storage environments with optimum temperatures and moisture levels.

Results from this study were published in the Journal of Agricultural and Food Chemistry.

ARS is the chief intramural scientific research agency of the U.S. Department of Agriculture.
Dr Rachel Brennan, PSU

- Removal of endocrine disruptors from wastewater using ecological processes.
- Mycoremediation – Bioremediation using fungi mycellia (vegetative part)
- White Rot Fungi
- [http://live.libraries.psu.edu/mediasite/Viewer/?peid=24ac93f831bc4d0f937ea33892764438](http://live.libraries.psu.edu/mediasite/Viewer/?peid=24ac93f831bc4d0f937ea33892764438)
References

- The Groundwater Foundation
  PO Box 22558
  Lincoln, NE 68542-2558
  www.groundwater.org

- Arianne Proctor, PA Department of Environmental Protection

- Dr. Conrad Volz, University of Pittsburgh

- Medication Disposal PowerPoint

- Sea Grant – Illinois-Indiana

- Canada Information Resources and Services:
Contacts

- Jim Clark, Penn State Extension Educator, 814-887-5613 or jac20@psu.edu
- http://water.cas.psu.edu
Thank You

Penn State is committed to affirmative action, equal opportunity, and the diversity of its workforce.
Movement of Estrogen and Other Emerging Contaminants through the Water Environment

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Male Bass Across Region Found to Be Bearing Eggs
Pollution Concerns Arise In Drinking-Water Source

By David A. Fahrenthold
Washington Post Staff Writer
Wednesday, September 6, 2006; Page A01

Sunday, August 14, 2005

Estrogen found in waters alters sex organs of fish
Pharmaceuticals, other substances from sewage plants end up in lakes, cause sexual mutations.
By Gene Schabath / The Detroit News

Hormonal chemicals may be imperiling fish
By Warren Cornwall and Keith Ervin
Seattle Times staff reporters

Male fish becoming female?
Researchers worry about estrogen and pollutants in the water
By Tom Costello
Correspondent
NBC News

Think Again
It impacts, or may impact, numerous functions

Female reproduction
Sexual development
Male reproduction
Bone structure and density
Liver protein production
Brain antioxidants and mood
Blood vessels – imp. blood flow
Oncogene – breast and uterine cancer
Pregnancy
Role in sex differentiation of the brain
Sources of Estrogen in the Environment

“Livestock manure would account for at least 90% of the total estrogen in the environment.” (Maier et al, 2000. Cited in Khanal et al, 2006)

Other sources would include: wastewater treatment plants, plastics, etc.
## Different Forms of Estrogen Hormones

<table>
<thead>
<tr>
<th>Estrogen Hormone</th>
<th>Acronyms</th>
<th>Chemical Structure</th>
<th>MW $\text{g/mole}$</th>
<th>$S_w$ (mg/L)</th>
<th>$\text{Log } K_{ow}$</th>
<th>$VP^0$ (kPa)</th>
<th>E2 Equivalent</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Estrone</td>
<td>E1</td>
<td><img src="image" alt="Estrone Structure" /></td>
<td>270.37</td>
<td>0.8 - 12.4</td>
<td>3.1 - 3.4</td>
<td>$3 \times 10^{-8}$</td>
<td>0.1 - 0.2</td>
<td>(1)</td>
</tr>
<tr>
<td>17β-estradiol</td>
<td>E2</td>
<td><img src="image" alt="17β-estradiol Structure" /></td>
<td>272.38</td>
<td>5.4 - 13.3</td>
<td>3.8 - 4.0</td>
<td>$3 \times 10^{-8}$</td>
<td>1</td>
<td>(14)</td>
</tr>
<tr>
<td>Estriol</td>
<td>E3</td>
<td><img src="image" alt="Estriol Structure" /></td>
<td>288.38</td>
<td>3.2 - 13.3</td>
<td>2.6 - 2.8</td>
<td>$9 \times 10^{-13}$</td>
<td>0.02</td>
<td>(14)</td>
</tr>
<tr>
<td>17α-estradiol</td>
<td>17α</td>
<td><img src="image" alt="17α-estradiol Structure" /></td>
<td>272.38</td>
<td>3.2 - 13.3</td>
<td>3.4 - 4.0</td>
<td>$3 \times 10^{-8}$</td>
<td>1-2</td>
<td>(15)</td>
</tr>
</tbody>
</table>

* MW: molecular weight; $S_w$: solubility in water; $K_{ow}$: octanol–water partition coefficient; $VP^0$: vapor pressure.

Adapted from Khanal et al, (2006)
Concentrations of Estrogen in Human and Livestock wastes.

<table>
<thead>
<tr>
<th>Steroidal Hormone Origin</th>
<th>Excretion Amount in µg/day per 1,000 kg Live Animal Weight (LAW)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Estrone (E1)</td>
</tr>
<tr>
<td>Human being</td>
<td></td>
</tr>
<tr>
<td>Male (pre-pubertal)</td>
<td>35</td>
</tr>
<tr>
<td>Male (adult)</td>
<td>88</td>
</tr>
<tr>
<td>Female (pre-pubertal)</td>
<td>41</td>
</tr>
<tr>
<td>Female (adult, cycling)</td>
<td>110 – 497</td>
</tr>
<tr>
<td>Female (adult, pregnant)</td>
<td>N/A</td>
</tr>
<tr>
<td>Female (post cycling)</td>
<td>N/A</td>
</tr>
<tr>
<td>Cattle</td>
<td></td>
</tr>
<tr>
<td>Bulls (slurry)</td>
<td>&lt;2</td>
</tr>
<tr>
<td>Milk cows (slurry)</td>
<td>255 – 640</td>
</tr>
<tr>
<td>Milk cows (10 days before parturition)</td>
<td>840 – 1,000</td>
</tr>
<tr>
<td>Swine</td>
<td></td>
</tr>
<tr>
<td>Swine (slurry)</td>
<td>&lt;2 – 84</td>
</tr>
<tr>
<td>Sow (feces, late gestation)</td>
<td>15 – 28</td>
</tr>
<tr>
<td>Poultry</td>
<td></td>
</tr>
<tr>
<td>Immature broilers (males and female)</td>
<td>Total: 65 – 133</td>
</tr>
<tr>
<td>Hens (laying)</td>
<td>254</td>
</tr>
<tr>
<td>Roosters</td>
<td>670</td>
</tr>
</tbody>
</table>

N/A = not available
The Needle in a Haystack

- Low concentrations, nearly immeasurable, can cause serious physiological changes to aquatic species
  - Trout, turtles and minnows
    - Sexually inhibited or reversed at concentrations in the tens of ng/L
  - 5 ng/L induced production of female proteins in a male fish

1 ng/L is equivalent to 1 drop of pure estrogen in enough water to fill a 10 mile train of tankers
How can we measure such a low concentration? *It's not easy....*

1 Liter of Sample

HPLC + PDA
Sensitivity = 10 ug/L (on a really really good day)

Solid Phase Extraction
Condense 1 liter into 1 mL

Tandem Mass Spectrometer
Sensitivity = 1 ug/L (on a good day)

10 ng/L

1 ng/L
How is Estrogen Transformed to Our Streams and Groundwater?

- **Point Discharge: WWTPs normally discharge into the stream.**
  - WWTPs do remove some of the hormones, but they do not remove all.
  - Easy to measure effects of discharge: above and below stream samples
  - Land application of wastewater: An alternative?

- **Non-Point Discharge: Agriculture**
  - Surface runoff
    - Sorption to soil particles
  - Leaching
    - Macropore flow
How do estrogens and organic pharmaceutical compounds interact with soil?
“Batch” sorption studies

Estimate the relationship between the concentration of estrone in the soil solution and the estrone that is adsorbed to the soil
Organic chemicals are adsorbed to organic matter in soil. The more organic matter, the stronger the adsorption of organic chemicals.
Soil Organic Matter – Humic Acid
Soil Organic Matter – Saccharides
Soil Organic Matter – Proteinaceous Material
Soil Organic Matter – Water Molecules
Positively charged inorganic chemicals are adsorbed to negatively charged soil clay particles. The more negatively charged clay, the stronger the adsorption of positively charged inorganic chemicals.
“Batch” sorption studies

\[ S = K_d \, C_e \]

\[ \text{OR} \]

\[ S = K_f \, C_e^N \]

\( S \) = amount of chemical adsorbed onto the soil
\( C_e \) = concentration of chemical in the soil solution
\( K_d \) = Linear sorption coefficient
\( K_f \) = Freundlich sorption coefficient
\( N \) = “fitting” exponent
For organic chemicals,

\[ S = K_d \, C_e \]

\[ K_d = K_{oc} \times \text{O.C. (as a decimal)} \]

(if Soil Organic Carbon content is 1%, the O.C. is 0.01)
$K_d = K_{oc} \times O.C.$

So $K_{oc}$ provides a standard procedure to obtain sorption coefficients for organic chemicals, and transfer the data to a large number of soils if the organic carbon content is known.
Amount of Estrone adsorbed to the soil

Amount of Estrone remaining in the soil solution

Sorption Study – 24 hrs

$y = 66.9413x^{0.9316}$

$R^2 = 0.9985$

$K_f = 66.9413$

$N = 0.9316$
Suppose we have a 1 kg sample of soil, which has a Bulk Density of 1.45 g/cm³

The soil would be able to contain 0.45 cm³ water per cm³ soil when saturated

Suppose the Kd of estrone was measured to be 50 for this soil

How much would reside in the soil water and how much would reside on the soil organic matter?
If BD = 1.45; theta sat = 0.45

<table>
<thead>
<tr>
<th>Ce (ng/mL)</th>
<th>S (ng/g)</th>
<th>Amt of estrone in soil solution (ng)</th>
<th>Amt of estrone adsorbed on soil (ng)</th>
<th>Amt on soil vs amt in solution</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>50</td>
<td>0.45</td>
<td>72.5</td>
<td>160</td>
</tr>
<tr>
<td>10</td>
<td>500</td>
<td>4.5</td>
<td>725</td>
<td>160</td>
</tr>
<tr>
<td>100</td>
<td>5000</td>
<td>45</td>
<td>7250</td>
<td>160</td>
</tr>
</tbody>
</table>
Changes in sorption coefficients over time implies that how tightly estrone is adsorbed to the soil depends upon how long it has been in contact with the soil.
Summary and Possible Implications

- Over short times (e.g. less than 4 days) the sorption of estrone to soil appears to be highly time dependent.
  - Implies that if it rains within 4 days after a manure application, estrone is more likely to move offsite with rainfall running off site than would occur later.
  - Implies that injection of manure may help reduce the likelihood of estrone runoff, especially if heavy rainfall occurs within 4 days of manure application.
Summary

- Sorption of estrone to soil will slow the transport of estrone through soil with a high amount of organic matter compared with its movement through a sand with low organic matter content.

  - Implies that maintaining soil organic matter can help reduce the loss of estrone from a cultivated field
Wastewater Irrigation at Penn State

- 1950’s: Population Growth = “major pollution problem” in Spring Creek, the main water course of the area.

- 6 year drought led to reduced water supplies

- Several accidental chemical spills caused fish kills.

- May 16, 1963: Start of wastewater application to the “Living Filter”.

- 1983: Research application turned into a full scale operation.

- 2009: Irrigation system still in full operation
Penn State currently irrigates all of its wastewater
• Research since the 1960’s
• Full-scale since 1983

Approximately 2.7 million gallons/day
• 365 days a year

Permitted to add 102 inches/year
• Similar to a tropical climate

2 Sites
• 208 ha
• 3100 sprinklers
• 96 km of pipe

3 Types of Land Cover
• Cropped Fields
• Grass Fields
• Forested Areas
Objective 3: Quantify estrogen in wastewater

Methods

- Chlorine Contact Tank
- Triplicate Samples
  - 1 Liter
- Amber Glass Bottles
- Glass Fiber Filter
- Solid phase extraction
  - Within 2 hours of sampling
Literature has shown concentrations of estrone to be between 1 and 10 ng L\(^{-1}\)

Limits of quantification are usually between 1 and 10 ug L\(^{-1}\)

Solid phase extraction allows for concentration of samples
Objective 3: Quantify estrogen in wastewater

Methods

- LC/MS/MS analysis
  - HPLC not sensitive enough

- Column: Waters XTerra MS C18 2.5 μm (2.1mm x 50mm)

- Mobile Phase: ACN + NH₄OH & H2O + NH₄OH @ 0.2 mL min⁻¹

- 20 uL sample
Objective 3: Quantify estrogen in wastewater

Results

![Graph showing estrogen levels in wastewater]

- Estrone
- 17 Beta
- 17 Alpha

17 α ethynylestradiol

Just under 2 ngL⁻¹
Objective 4: Determine estrogen concentrations in the soil profile

**Background**

- Few studies have investigated estrogen sorption to soils at the field scale
- Extraction procedures vary widely
- Goal: to extract estrogens and leave everything else behind
  - Not realistic
Objective 4: Determine estrogen concentrations in the soil profile

Sample Site
Objective 4: Determine estrogen concentrations in the soil profile

**Extractions**

- 20 gram soil samples
- 40 mL 250 mg/L Sodium Azide & water
- 60 mL ethyl acetate
- Shake overnight
- Remove 50 mL EA concentrate to 3 mL
- LC/MS/MS analysis
- 60% efficient
Objective 4: Determine estrogen concentrations in the soil profile

**Results**

- Estrone concentration in the wastewater: $1.74 \pm 0.41 \text{ ng L}^{-1}$
- Extractions performed 3 weeks after irrigation event
- 2 inches of precipitation

1 ng/g = 1000 ng/kg total estrone in soil ~ 20 ng/L in the soil water;
Objective 4: Determine estrogen concentrations in the soil profile

**Results**

- $17\alpha$ ethynylestradiol levels were consistently lower in soils when compared to estrone

- Despite high levels detected in the wastewater ($72 \pm 16 \text{ ng L}^{-1}$)

- Degradation
A Different Example: Carbamazepine, an anti-epileptic drug

- Carbamazepine concentrations were higher at the soil surface than at depth.
- The amount of carbamazepine accumulated in the soil was greater than the amount of estrone.
The Wastewater Irrigation Facility at Penn State:
- Irrigated over 100,000 gallons of wastewater
- Provided more than 80,000 gallons of aquifer recharge
- Provided additional tertiary treatment without huge energy inputs
Does Manure Application Methodology Play a Role in Transport?

Are there differences between surface and injection applications?

- Surface application = high concentrations in runoff?
- Injected application = leaching potential?
  - Will the injected application “buy some time” for degradation?

What are the effects of repeated applications?
Question and Answers

• Recordings of this session will be posted by Friday, March 26
• www.aec.cas.psu.edu
What’s ahead for season II?

• Next week: **DEMYSTIFYING THE TMDL: THE STATE WAY, THE BAY WAY**
  
  – **Brian Benham**, Virginia Tech Center for TMDLs and Watershed Studies, with introduction by **Denice Wardrop**, PSU Geography and Penn State Institutes of Energy & Environment (and Chair, Bay Program Science & Technical Advisory Committee – STAC)
  
  – Full schedule for the Manure du jour program: [www.aec.cas.psu.edu](http://www.aec.cas.psu.edu)

• Nutrient Management Continuing Education Credits ARE AVAILABLE for Apr 15, Apr 22, Apr 29 programs.