Assessing Potential for Ground and Surface Water Impacts from Hormones in CAFOs

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NRMRL – National Risk Management Research Laboratory

- **LRPCD** – Land Remediation and Pollution Control Division - fate of EDCs in municipal wastewater treatment plants, adapting EDC bioassays to monitor performance of risk management processes, land application of biosolids containing EDCs

- **APPCD** – Air Pollution Prevention & Control Division - emission of EDCs from biomass & diesel fuel composition

- **WSWRD** – Water Supply & Water Resources Division - evaluation of drinking water treatment technologies for removal of EDCs

- **GWERD** – Ground Water & Ecosystems Restoration Division - ground water impacts from hormones in CAFOs
Ground Water Issues

- Ground Water Monitoring – required for lagoons, but generally not for land application, even though...

Farm Size: 160 acres

4,000 Hogs = 20,000 People

- CAFO wastes generally undergo minimal treatment (lagoon only)
Potential Ground Water Stressors

- Nutrients (NH4, NO3, PO4)
- Metals (As, Cu, Se, Zn)
- Pathogens (bacteria, viruses)
- Antibiotics (tetracyclines, sulfonamides, macrolides)
- Endocrine disrupting chemicals (natural hormones, synthetics)
Why Focus on Ground Water Impacts?

- **Ground Water as a Resource**
  
  - Ground water accounts for approximately 40% of the nation’s drinking water*
  
  - Ground water accounts for approximately 36% of the nation’s irrigation water*

* USGS Circular 1186 (1999)
Nationally, approximately 40% of average annual streamflow is from ground water*

* USGS Circular 1186 (1999)
Endocrine Disrupting Chemicals (EDCs)
Initial Focus on Natural Estrogens

- Estrone
- Estriol
- β-Estradiol
Direct Analysis for Estrogens by GC/MS/MS

Ground Water

Filtered + Form.

Shipped on Ice

RSKERC

500 mL

25 mL

Add Isotope Dilution Standards

SPE (Oasis HLB)

Derivatization

1) PFB Deriv.

2) TMS Deriv.

Centrifuge

Filter

Lagoon

500 mL

25 mL

Add Isotope Dilution Standards

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Derivatization

1) PFB Deriv.

2) TMS Deriv.

Centrifuge

Filter
Estrogens vs Estrogen Conjugates

These are all “free estrogens”
Estrogen Conjugates

- Estradiol-3-glucuronide-17-sulfate
- Estriol-17-glucuronide
- Estradiol-3-glucuronide-17-sulfate

Three of many possible sulfate and glucuronide conjugates of estrone, estradiol, and estriol.
Pathways for transformation of estrogens and estrogen conjugates

17β-Estradiol Conjugates

17β-Estradiol

Estrone Conjugates

Estrone

16α-hydroxy-Estrone

Estriol Conjugates

Estriol

17α-Estradiol Conjugates

17α-Estradiol
**Direct Analysis for Estrogen Conjugates**

- **Ground Water**: Shipped on Ice
- **Lagoon**: Filtered

**Procedure Steps**:
- Add Isotope
- Dilution Standard
- **SPE (Carbopack X)**
- LC/MS/MS

- **1** 500 mL
- **2** 25 mL
- **3** + Form.
- **4** + Form.
**Indirect Analysis for Estrogen Conjugates**

1. **Filtered Ground Water**
   - Add Isotope Dilution Standards
   - β-gluc/sulf enzyme 37°C for 16 hr

2. **Filtered Lagoon**
   - Enzyme Treatment
   - Ground Water + Form.
   - Lagoon + Form.

3. **SPE (Oasis HLB)**
   - Derivatization
   - 1) PFB Deriv.
   - 2) TMS Deriv.

4. **GC/MS/MS**
CAFO Lagoon Survey

- Eight different lagoons used for land application
- Swine, poultry, dairy, beef operations
- Three sample locations per lagoon
- Analyses include $\alpha$-estradiol and estrogen conjugates
Aqueous Free Estrogens in Lagoons

- Estrone
- 17α-Estradiol
- 17β-Estradiol
- Estriol

Concentration (ng/L)

Swine Sow
Swine Finisher
Swine Nursery
Poultry Secondary
Poultry Primary
Dairy
Beef
Poultry Tertiary
### Estrogen Conjugates Detected by LC/MS/MS

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<thead>
<tr>
<th>Standards Available</th>
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<tbody>
<tr>
<td>Estrone-3-sulfate</td>
<td>Estriol-3-sulfate-17-glucuronide</td>
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<tr>
<td>Estrone-3-glucuronide</td>
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<td>Estriol-3-sulfate-16-glucuronide</td>
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Contribution to Total Estrogen Load

Percent distribution of estrogen groups for total estrogen equivalents. Number at the top of each bar is the total estrogen equivalent for that lagoon.
GWERD’s Current Analytical Suite for Hormones

- Estrogens – direct analysis by GC/MS/MS
- Estrogen conjugates – direct analysis by LC/MS/MS
- Estrogen conjugates – indirect analysis by enzyme treatment followed by estrogen analysis by GC/MS/MS
Direct Analysis for Trenbolone by GC/MS/MS – Method Development

1. CMOA Deriv.
2. PFB Deriv.
3. TMS Deriv.

RO/Nanopure Water

1000 mL

Add $\alpha,\beta$-Trenbolone and Isotope Dilution Standards

100 mL

SPE (Oasis HLB)

Centrifuge

Lagoon

Filter

Derivatization

GC/MS/MS
CAFO EDC Research – Future Directions

- **GWERD**
  - Field study focus on hormones in land application
  - Continued work with estrogen conjugates
  - Inclusion of androgens into analytical suite
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