Fate and Transport of Estrogenic Hormones in Subsurface Waters

Francis X.M. Casey
Professor and Director, School of Natural Resource Sciences, NDSU
What the Frogs Are Trying to Tell Us
BY BRIAN LAVENDEL

ESTROGEN IN LAKE BENDS THE GENDER OF MALE FISH IN TEST
Source: SETH BORENSTEIN

Declining male fertility

THE FIGHT OVER FOOD/HORMONES ...WORRY ABOUT THE SAFETY OF RESIDUES IN THEIR FOOD.
Lee Egerstrom, Staff Writer

‘Gender-bender’ threat to marine life
By Helen Briggs, science reporter
Endocrine Disrupting Compounds (EDCs)

“chemicals that can induce adverse health effects by disruption of an organism’s endocrine system or normal development”

(Ashby et al., 1997)
Exogenous Hormones are EDCs

- Exogenous Hormones mimic an endogenous hormone and bind strongly to hormone receptors.
- Fish are especially susceptible to EDCs b/c of their special sexual adaptive strategies.
Panter et al. (ES&T 2002) intermittent exposure (alternate days, 1 day in 4, or 3 days in 6) to 0.120 $\text{g L}^{-1}$ E2 = significant vitellogenin induction in fathead minnows (*Pimephales promelas*).
Structural differences occur in the arrangement of functional groups at the C-16 and C-17 on the D-ring structure.

Majority of excreted estrogens are conjugated and considered to be biologically inactive.

Natural Hormones

17β-Estradiol (E2)

Estrone (E1)

Estriol (E3)

Sulfate or Glucuronide
Animal Estrogen Excretion Dwarfs Humans

- 6.7 billion humans = 26.8 metric tons/year estrogen
- US+EU Animals = 82 metric tons/year
Potential for Contamination

- Shore et al. (1998) measured 30 ng g\(^{-1}\) 17\(\beta\)-estradiol in poultry litter.
- 1997 U.S. poultry produced over ten billion kg of broiler litter
  - 90% applied to fields
  - Potentially contained 270 kg 17\(\beta\)-estradiol.
- Worse case, 27,000,000,000,000 L of water contaminated at 10 ng L\(^{-1}\).
  - Enough to cover North Dakota in nearly 3” of rain.
Analyzing the Ignored Environmental Contaminants

As analytical chemists develop new tools for detecting organic wastewater contaminants, the number of compounds they find in the environment continues to grow. Low levels of reproductive hormones, steroids, antibiotics, and numerous other prescription and nonprescription drugs, as well as some of their metabolites, have been detected in European waters and, more recently, in U.S. streams. Along with pharmaceuticals, products used in everyday life, such as detergents, disinfectants, fragrances, insect repellants, fire retardants, and plasticizers, are turning up in aquatic environments.

The U.S. Geological Survey reports some of the first monitoring data on pharmaceuticals and other emerging organic wastewater contaminants in U.S. streams.

BRITT R. BRICKSON
A National Reconnaissance by Koplin et al. (2002)
Reference 777 times since 2002

139 streams in 38 states were tested for pharmaceuticals, reproductive hormones, and other organic compounds.
Chemical sources include agricultural, industrial, and residential.

**Organic wastewater contaminants by general use category**

Emerging organic contaminants in U.S. streams, as reported by the U.S. Geological Survey, can be broken down into 15 categories. Orange bars show frequency of detection, and yellow bars show the percent of the total measured concentration. The number of compounds in each category is shown above the orange bars.
How does it get into the Environment?

- Evidence of hormone movement from manured lands into surface and ground water in concentrations that may affect wildlife (e.g., Peterson et al., 2000; Bushee et al., 1998; Nichols et al., 1997)
- Hog waste sprayed onto crop fields contains high levels of natural estrogens (Servos et al., 1998).
- On-farm measurements were enough to cause premature utter development in heifers (Shore et al., 1998).
Fate and Transport

Laboratory vs. Field
Field Setting - Hog Farm
Hecla–Hamar Series/loamy fine sand:
17β-Estradiol Identification and Quantification

Liquid chromatography Mass spec / Mass spec

- (First MS identifies molecular ion)/(2nd MS identifies & quantifies on fragment ions)
- This is coupled with the retention times that have a drift error of +/- 5% retention time
- 25 pg mass on-column reliably detected using selected ion monitoring

Analytical Methods

- Waters Q-TOF Ultima API-US
- Negative ion mode (ES-)
- Capillary voltage = 2.33
- Cone voltages = 55
- Source temp = 120 °C
- Desolvation temp = 400 °C
- Cone gas flow= 0 L h⁻¹
- Desolvation gas flows = 500 L h⁻¹
- Injection volumes = 10 µL
Effects of field-manure applications on stratified 17β-estradiol concentrations

Mary C. Schuh a, Francis X.M. Casey b,*, Heldur Hakk c, Thomas M. DeSutter b, Karl G. Richards d, Eakalak Khan e, Peter G. Oduor f
Mean(lnE2) = -2.51 + 1.81 \times \text{Mean(rainfall)}

r^2 = 0.91

p = 0.03^*$

24 May 2007

13 Nov 2006

14 June 2006

5 June 2006

Shallow piezometer field samples
Fate and Transport

Laboratory vs. Field
- Trapped $^{14}\text{CO}_2$
- Inject HgCl$_2$ to sterilize at end of experiment
17β-estradiol

~80% recovery

~80% recovery
\[ \frac{\partial C_1}{\partial t} + \rho_b \frac{\partial S_{r,1}}{\partial t} = \theta v \lambda \frac{\partial^2 C_1}{\partial x^2} - \theta v \frac{\partial C_1}{\partial x} - (\omega_{irr,1} + \omega_{s,1}) \rho_b S_{r,1} - (\omega_{w,1} + \omega_{w,2}) C_1 \]

\[ \frac{\partial C_2}{\partial t} + \rho_b \frac{\partial S_{r,2}}{\partial t} = \theta v \lambda \frac{\partial^2 C_2}{\partial x^2} - \theta v \frac{\partial C_2}{\partial x} + \omega_{w,1} \theta C_1 - \omega_{irr,2} \rho_b S_{r,2} + \omega_{s,1} \rho_b S_{r,1} \]

\[ \frac{\partial C_3}{\partial t} + \rho_b \frac{\partial S_{r,3}}{\partial t} = \theta v \lambda \frac{\partial^2 C_3}{\partial x^2} - \theta v \frac{\partial C_3}{\partial x} + \omega_{w,2} \theta C_1 - \omega_{irr,3} \rho_b S_{r,3} \]
17ß-estradiol
- Immobile – 40% irreversibly bound
- Readily degrades – < 0.5% of original E2 was recovered in soil and none in the effluent
Research Questions

If estrogens are short lived (<1day) and bind readily and strongly to soil, they why are they detected so frequently in the environment?
Explaining Movement and Persistence

1. What is the role of dissolve and colloidal fractions?
   - Holbrook et al. (2004) indicated that up to 60% of aqueous E2 and EE2 concentrations may be associated with organic colloids in wastewater.

2. What is the role of hormone conjugates?
Batch studies

topsoil vs subsoil
natural vs sterile
Batch Soil Experiment Comparing
- DOC/COC (derived from liquid swine manure) spiked with $^{14}$C E2
- CaCl$_2$ (control) spiked with $^{14}$C E2

**Results**
- E2 is gone in CaCl$_2$ solution by 7 days
- E2 persists in DOC/COC solution at least to 14 days

**Conclusions**
- DOC/COC causes E2 to remain suspended and inhibits E2 from binding to soil
- DOC/COC causes E2 to persist longer in solution compared to the CaCl$_2$ (control)

Kim Zitnick-Anderson’s Master study, 2010
General Conclusions for DOC/COC

1. Manure DOC/COC solution caused E2 to persist in aqueous phase longer
   1. Reduced degradation transformation
   2. Reduced sorption

2. Likely contributed to greater than expected mobility in the field
Explaining Movement and Persistence

2. Conjugate transport

\[ R = -O_3SO \quad \text{or} \quad O - \text{COO}^{-} - \text{OH} \]

\[ \text{Sulfate or Glucuronide} \]

\[ \text{C}_{18}\text{H}_{24}\text{O}_{2} \]
\[ 17\beta\text{- Estradiol} \]
Batch studies

- Determine parent (i.e. E2, E2-3S, E2-3G) and metabolite concentrations through time in aqueous and bound phases

  topsoil vs subsoil
  natural vs sterile
Estrogen conjugates synthesis

Sulfate or Glucuronide

R = O₃SO or O COO⁻ OH OH

OR

RO

17β-Estradiol

C₁₈H₂₄O₂

17β-Estradiol

Research Article

Synthesis and characterization of radiolabeled 17β-estradiol conjugates

Suman L. Shrestha,¹ Xuelian Bai,¹ David J. Smith,³ Heldur Hakk,³,* Francis X. M. Casey,¹ Gerald L. Larsen,³ and G. Padmanabhan¹
Fate and Transformation of an Estrogen Conjugate and Its Metabolites in Agricultural Soils
Suman L. Shrestha,† Francis X. M. Casey,*† Heldur Hakk,† David J. Smith,‡ and G. Padmanabhan§
Sorbed phase-E2-3G
Freundlich Sorption isotherms of E2-17S

\[ S = K_f C^N \]

- Topsoil
- Subsoil

Aqueous concentration, log C (mg L\(^{-1}\))
Sorbed concentration, log S (mg Kg\(^{-1}\))

\[ \log K_{OC} \text{ for E2} = 3.12^1 \]
\[ \log K_{OC} \text{ for E1-3S} = 1.73 \text{ to } 2.08^2 \]

\(^1\)Sarmah et al. (2008); \(^2\)Scherr et al. (2009)
Field conjugate detection (E2-3G)

- E2-3G detected in well 6.5–8.1 m deep
- Average conc = 425 (± 63) ng L⁻¹ (ppt), which if hydrolyzed, would produce 258 (± 38) ppt E2.
Estrogen Research Highlights

- **Field Results**
  - Detected frequently in the environment

- **Laboratory Results**
  - Degrade readily
  - Bind strongly and irreversibly
  - Immobile

- **Colloidal and Dissolved Fractions**

- **Sulfate/Glucuronide Conjugates**
  - Enhanced persistence
  - Enhanced mobility