

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

**Stephen R. Hutchins, Ph.D.
Research Environmental Scientist, ORD/NRMRL/GWERD
ORD CAFO Workshop, Chicago, IL
August 21, 2007**

NRMRL EDC Research ***(<http://www.epa.gov/ORD/NRMRL/EDC>)***

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

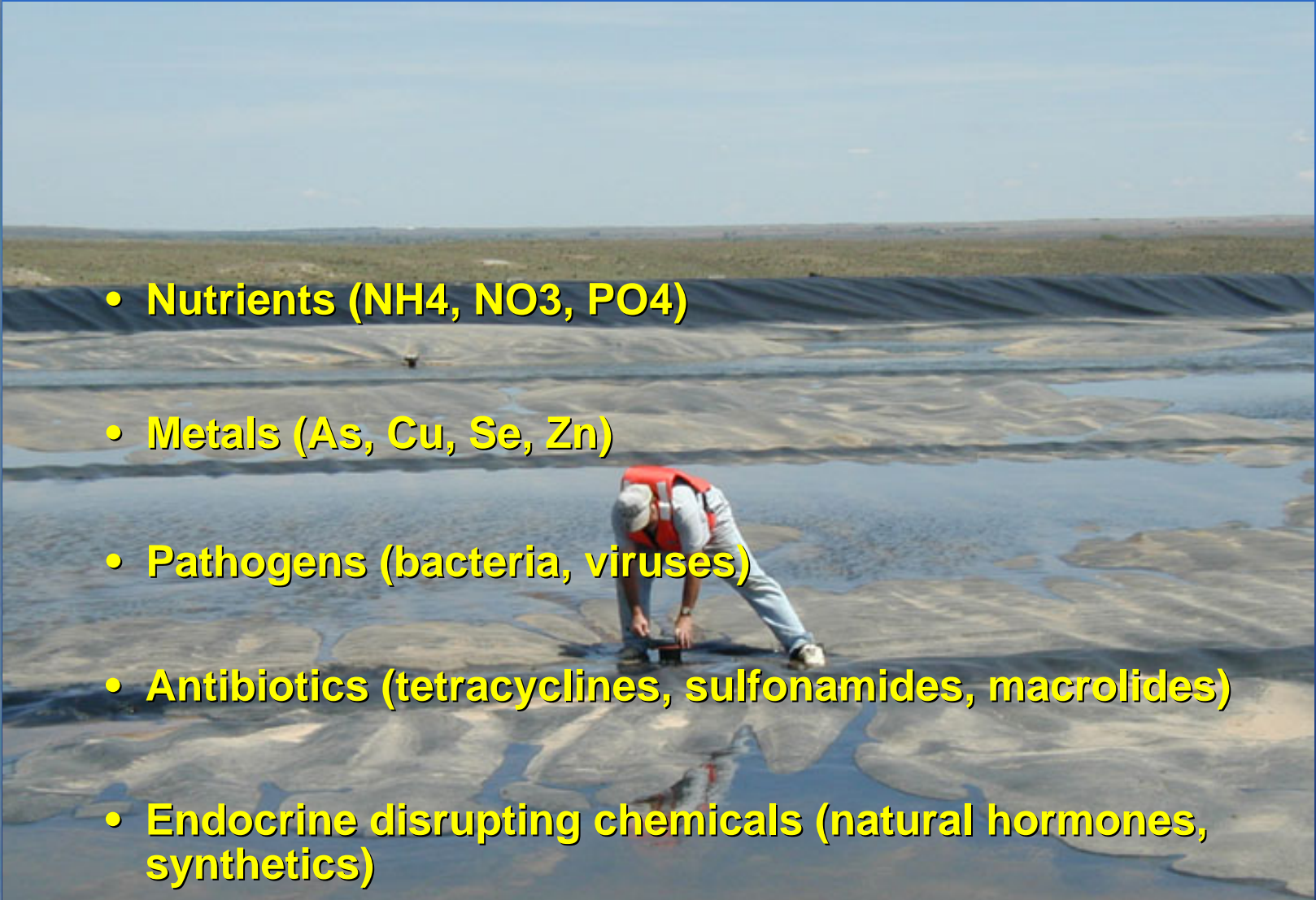
=



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***



Ground Water as a Conduit



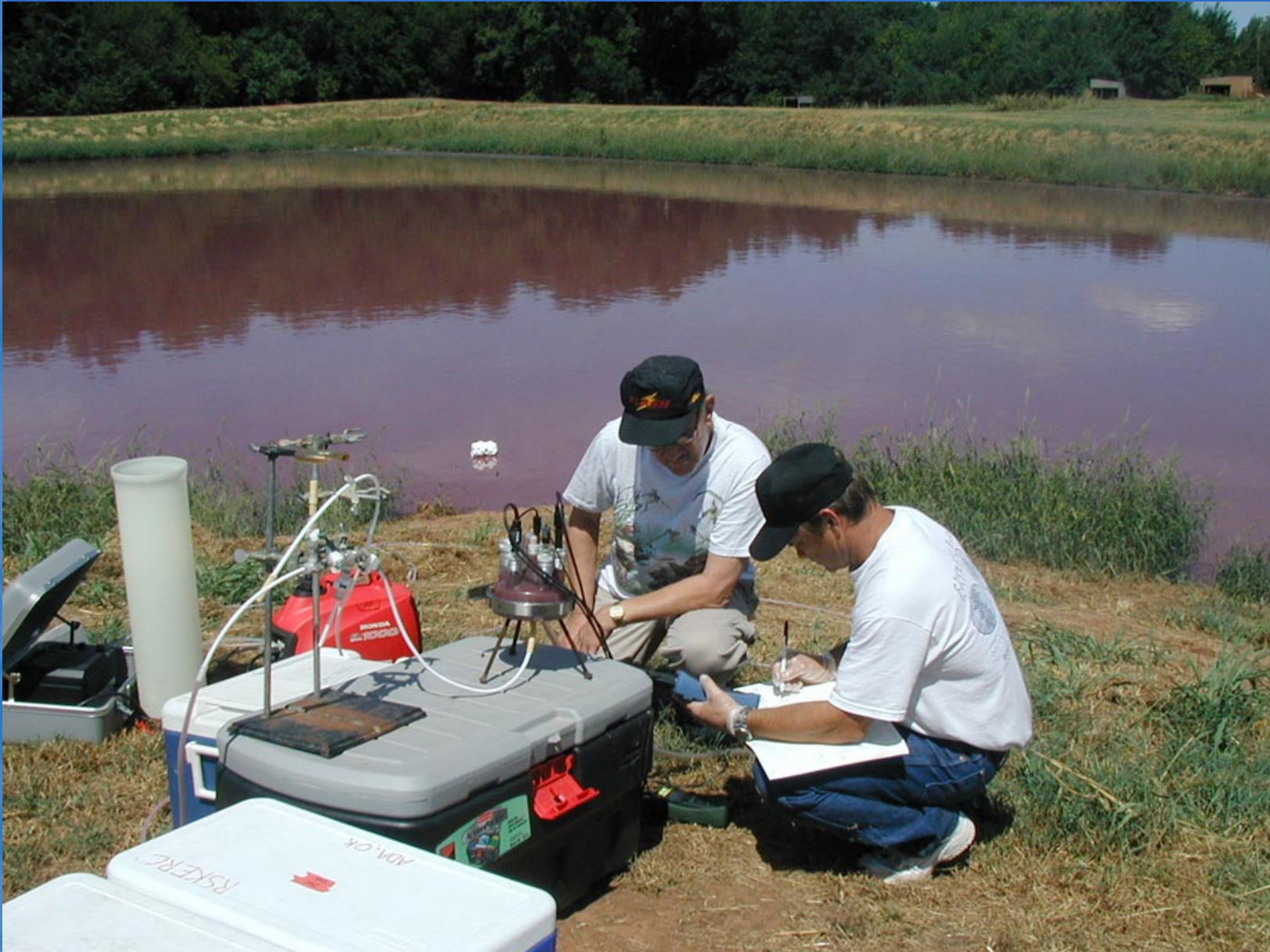
- **Nationally, approximately 40% of average annual streamflow is from ground water***

* USGS Circular 1186 (1999)

RESEARCH &
DEVELOPMENT

*Building a
scientific
foundation
for sound
environmental
decisions*

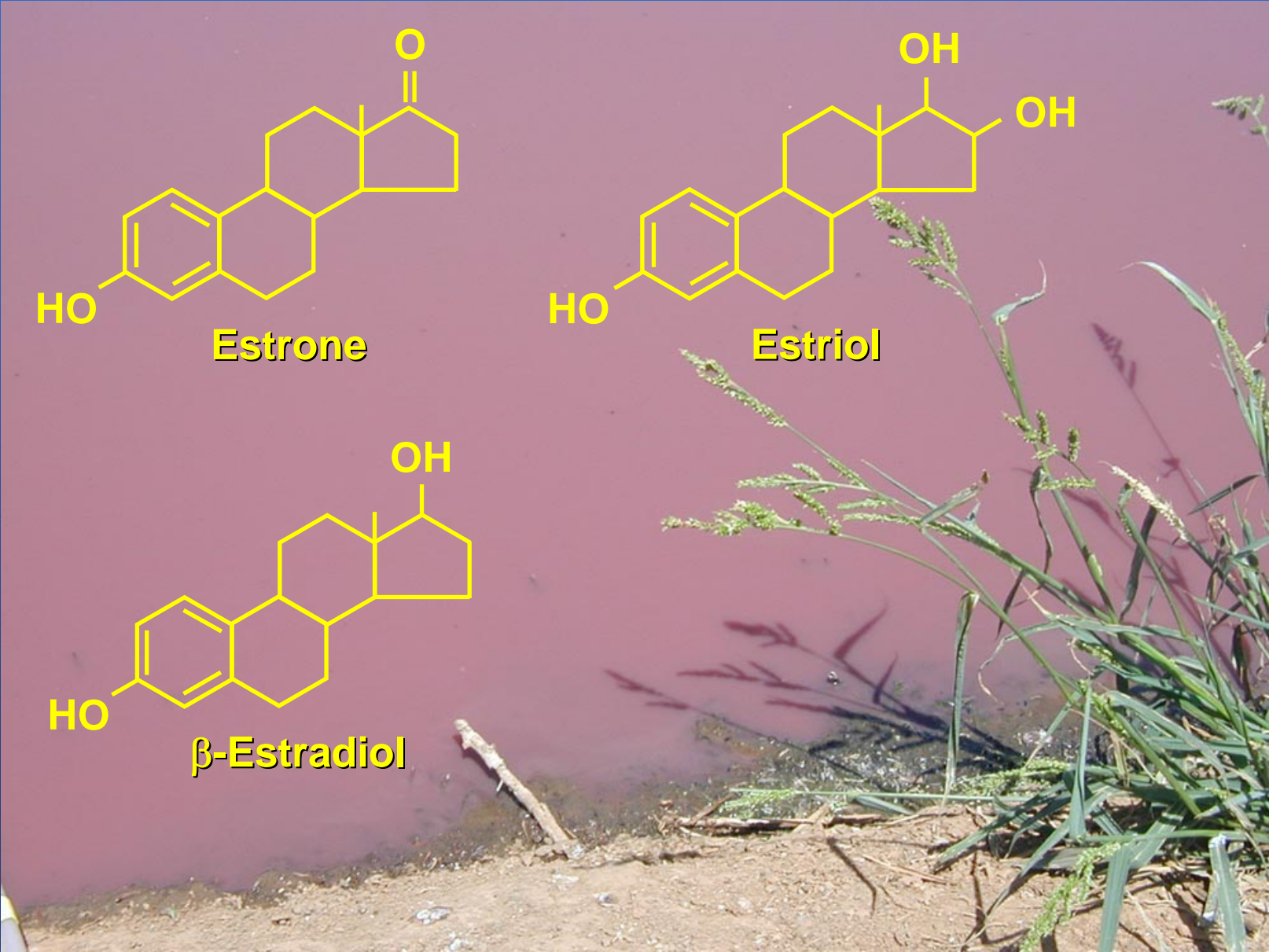
Endocrine Disrupting Chemicals (EDCs)



NRGO

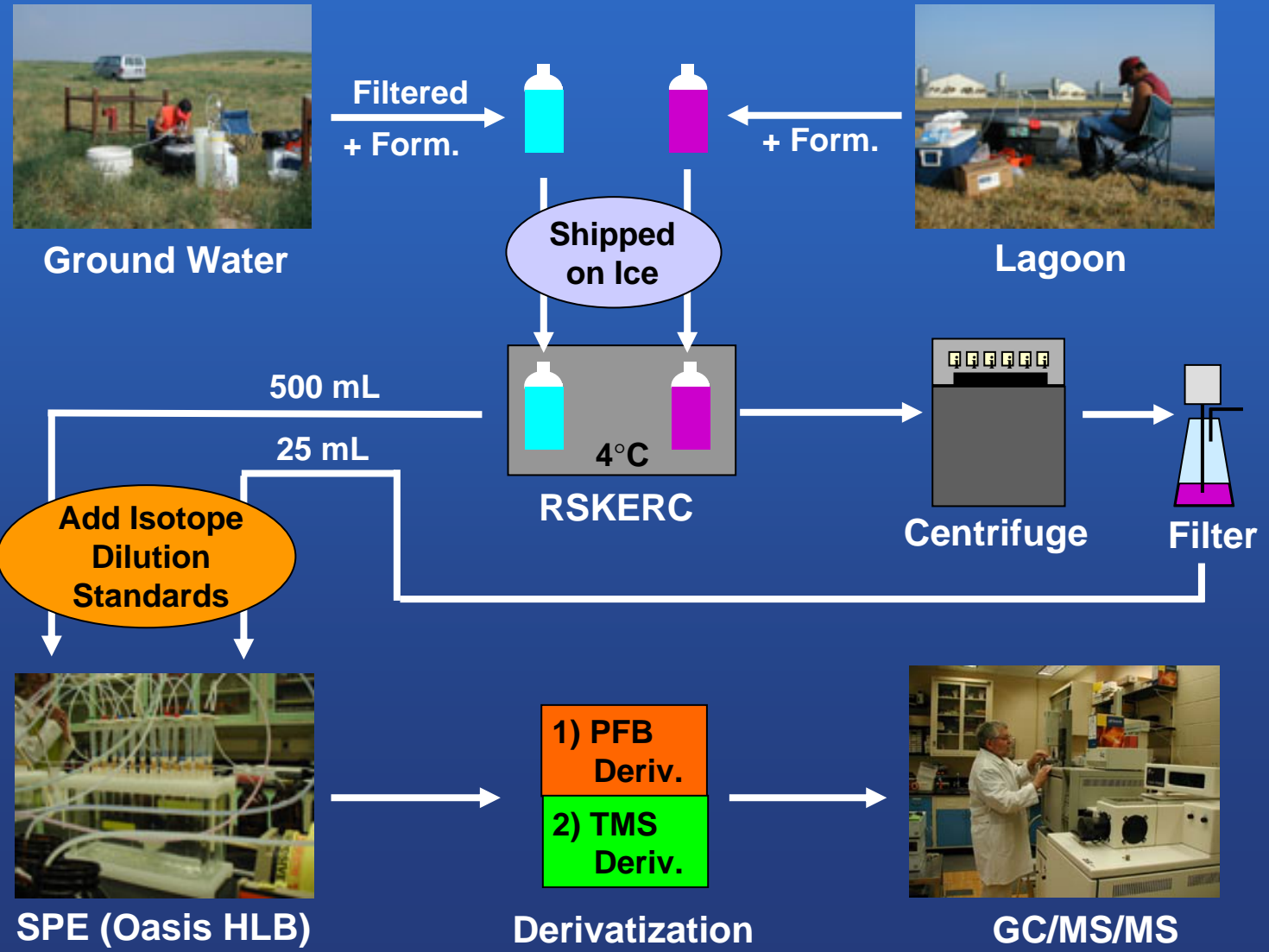
Building a
scientific
foundation
for sound
environmental
decisions

Initial Focus on Natural Estrogens



Building a scientific foundation for sound environmental decisions

Direct Analysis for Estrogens by GC/MS/MS



Estrogens vs Estrogen Conjugates

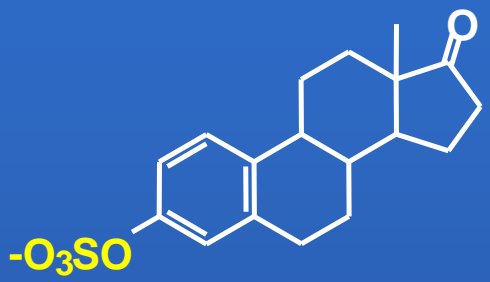


These are all “free estrogens”

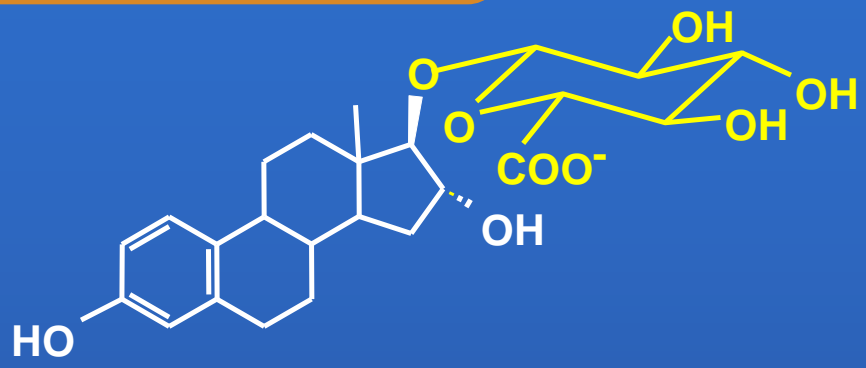


Building a scientific foundation for sound environmental decisions

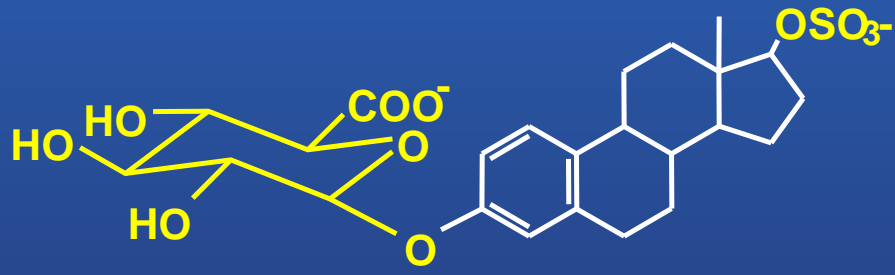
Estrogen Conjugates



Estrone-3-sulfate



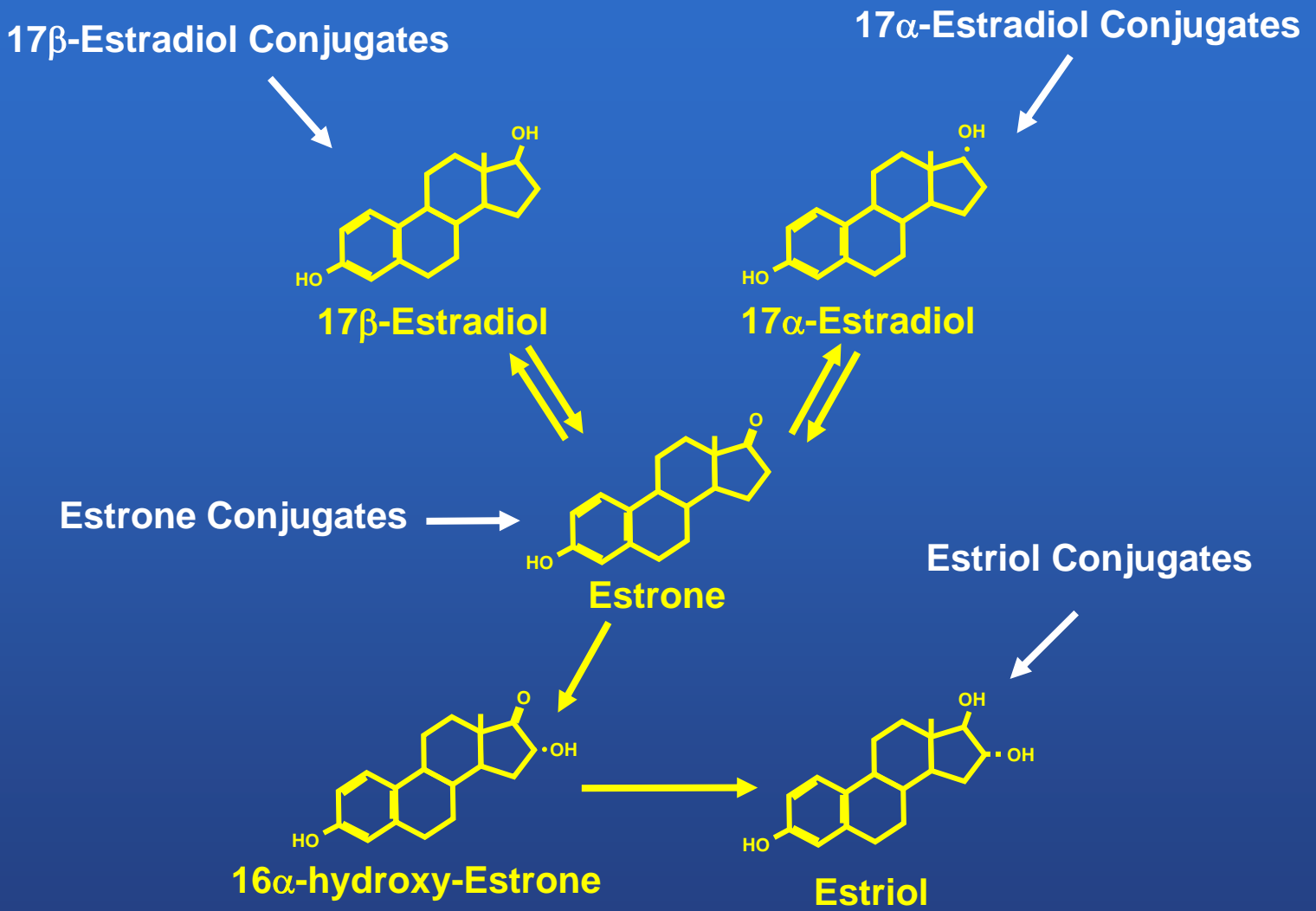
Estriol-17-glucuronide



Estradiol-3-glucuronide-17-sulfate

Three of many possible sulfate and glucuronide conjugates of estrone, estradiol and estriol

Building a scientific foundation for sound environmental decisions



Pathways for transformation of estrogens and estrogen conjugates

Building a scientific foundation for sound environmental decisions

Direct Analysis for Estrogen Conjugates



Ground Water

Filtered + Form.

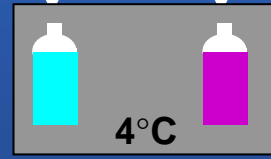


+ Form.



Lagoon

Shipped on Ice

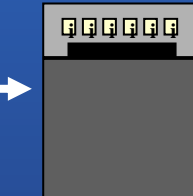


RSKERC

500 mL

25 mL

Add Isotope Dilution Standard



Centrifuge



Filter



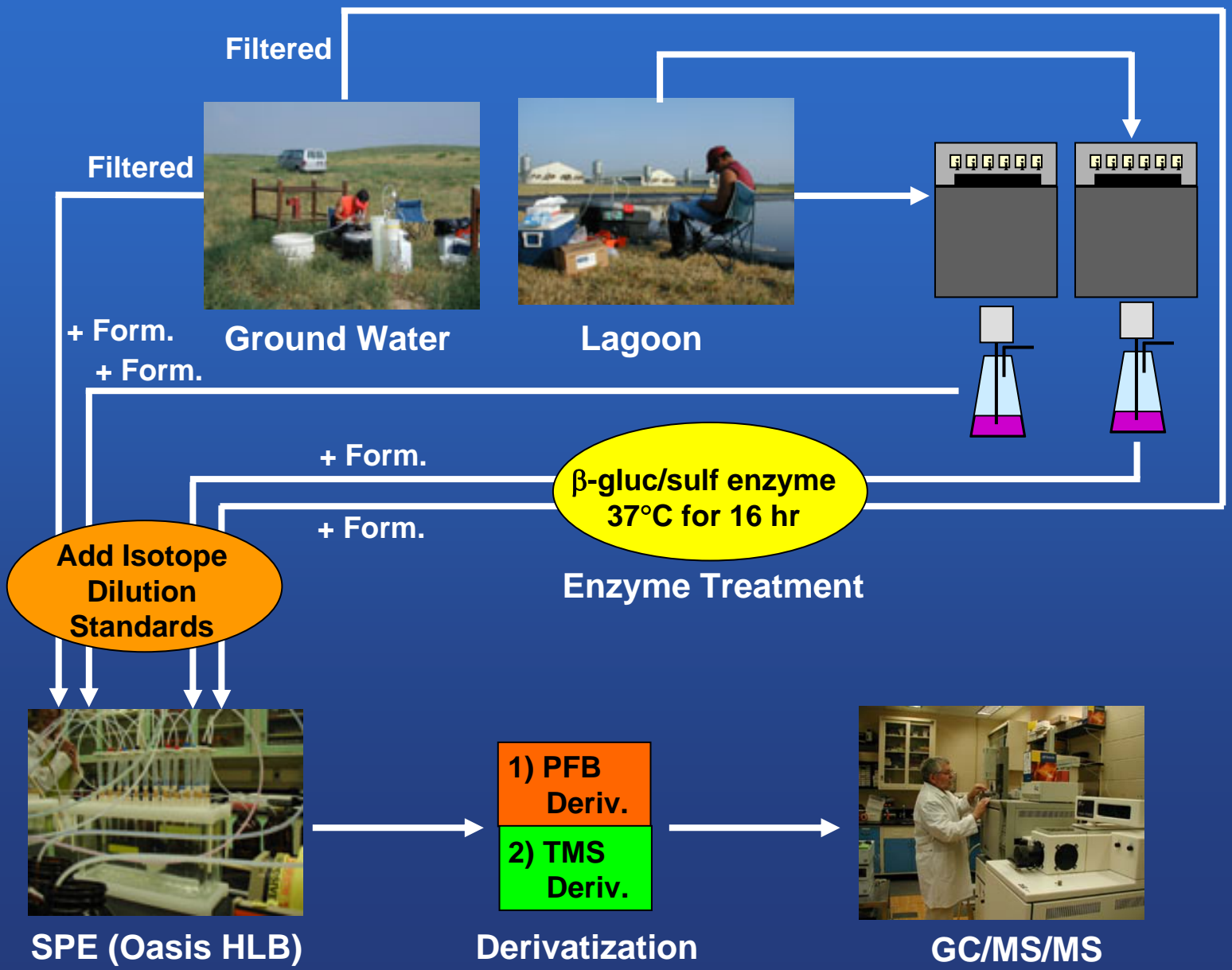
SPE (Carbopack X)



LC/MS/MS

Building a scientific foundation for sound environmental decisions

Indirect Analysis for Estrogen Conjugates



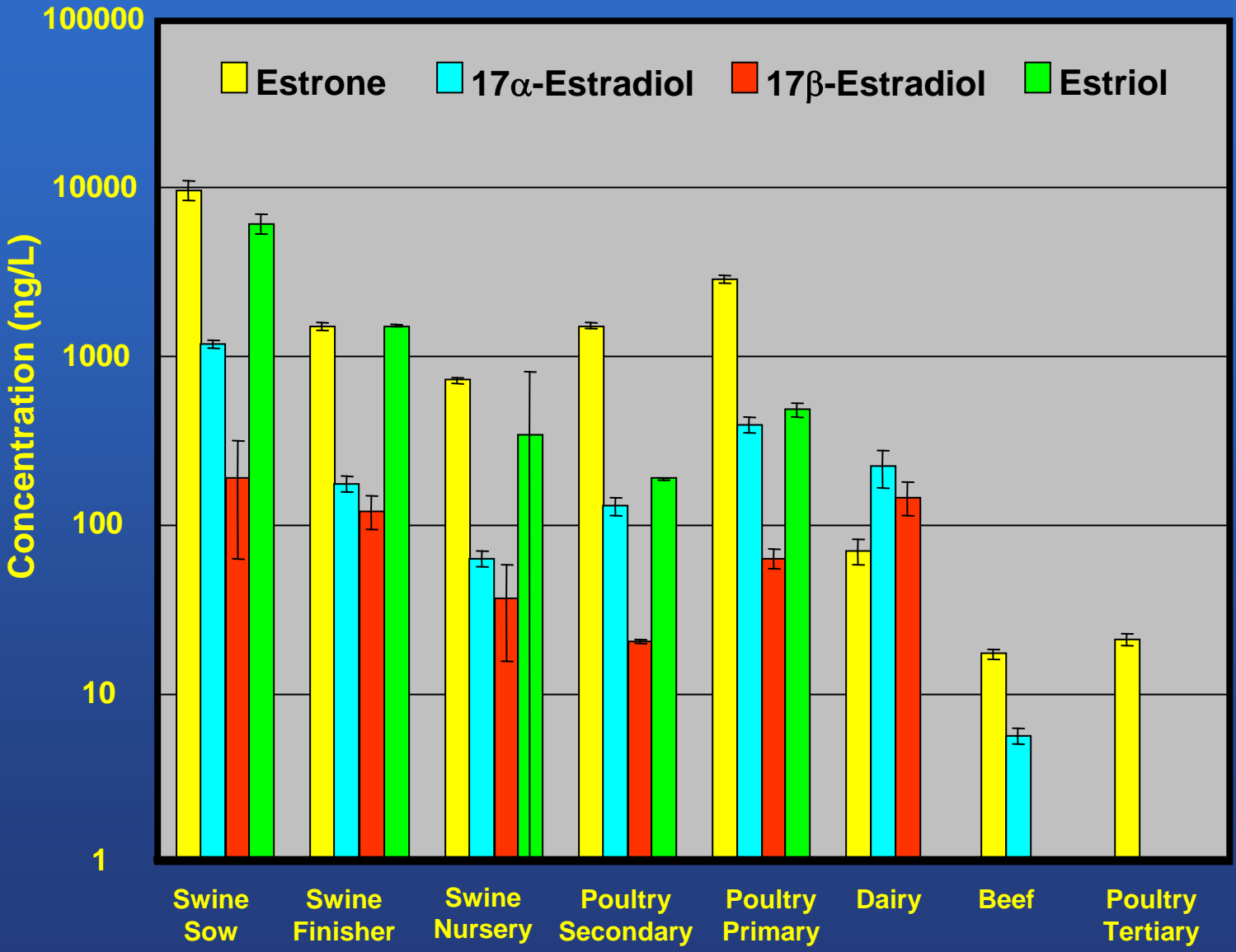
CAFO Lagoon Survey

- Eight different lagoons used for land application
- Swine, poultry, dairy, beef operations
- Three sample locations per lagoon
- Analyses include α -estradiol and estrogen conjugates



Building a scientific foundation for sound environmental decisions

Aqueous Free Estrogens in Lagoons



Estrogen Conjugates Detected by LC/MS/MS

Standards Available

Estrone-3-sulfate
Estrone-3-glucuronide
 α , β -Estradiol-3-sulfate
 β -Estradiol-3-glucuronide
 β -Estradiol-17-glucuronide
 β -Estradiol-3-glucuronide-17-sulfate
 β -Estradiol-3,17-disulfate
Estriol-3-sulfate
Estriol-3-glucuronide
 β -Estradiol-17-sulfate
 β -Estradiol-3-sulfate-17-glucuronide
 β -Estradiol-3,17-diglucuronide
Estriol-16-sulfate
Estriol-17-sulfate
Estriol-16-glucuronide

Estriol-17-glucuronide
Estriol-3-sulfate-16-glucuronide

No Standards Available

Estriol-3-sulfate-17-glucuronide
Estriol-16-sulfate-3-glucuronide
Estriol-16-sulfate-17-glucuronide
Estriol-17-sulfate-3-glucuronide
Estriol-17-sulfate-16-glucuronide
Estriol-3,16-disulfate,17-glucuronide
Estriol-3,17-disulfate,16-glucuronide
Estriol-16,17-disulfate,3-glucuronide
Estriol-3,16-diglucuronide,17-sulfate
Estriol-3,17-diglucuronide,16-sulfate
Estriol-16,17-diglucuronide,3-sulfate
Estriol-3,16-disulfate
Estriol-3,17-disulfate
Estriol-16,17-disulfate
Estriol-3,16-diglucuronide
Estriol-3,17-diglucuronide
Estriol-16,17-diglucuronide
Estriol-3,16,17-trisulfate
Estriol-3,16,17-triglucuronide

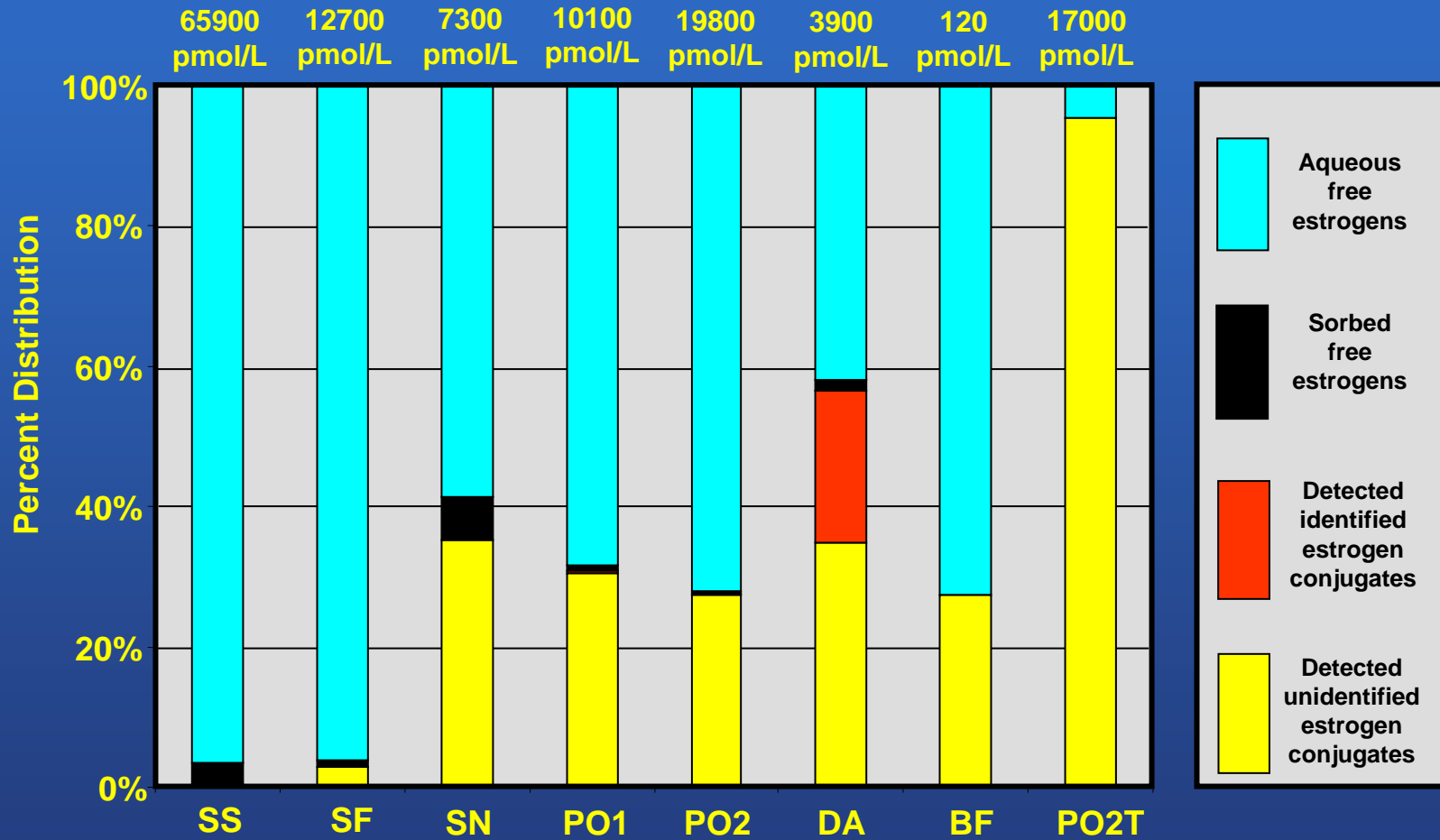
Found

Not Found

Undetermined

Building a scientific foundation for sound environmental decisions

Contribution to Total Estrogen Load



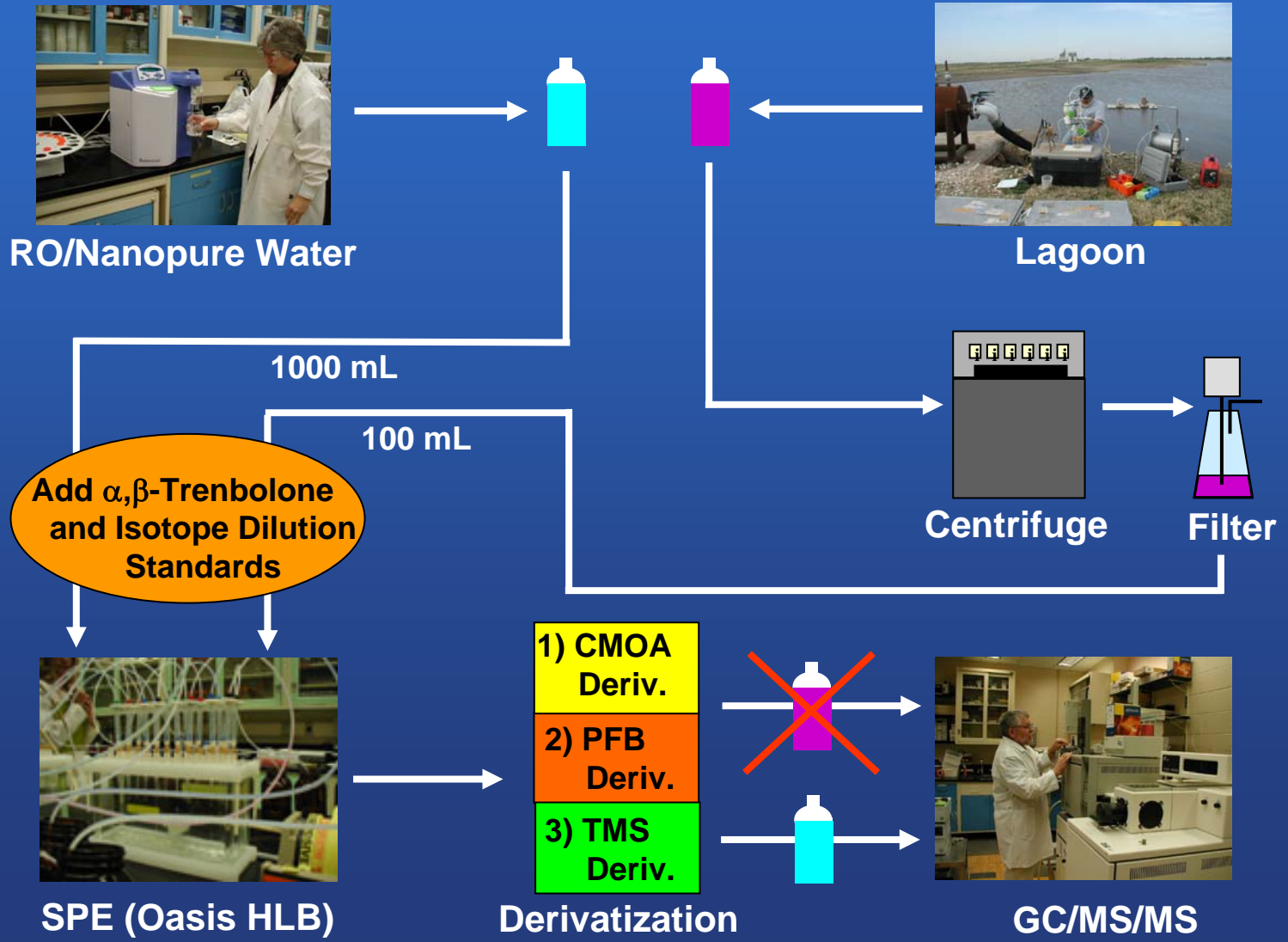
Percent distribution of estrogen groups for total estrogen equivalents. Number at the top of each bar is the total estrogen equivalent for that lagoon.

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



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**



GWERD CAFO EDC Research Personnel



**Mark V. White, Environ. Scientist
U.S. Environ. Protection Agency
Robert S. Kerr Environmental
Research Center, P.O. Box 1198,
Ada, OK 74820
Phone: (580) 436-8709
email: white.mark@epa.gov**



**Dennis D. Fine, Analytical Chemist
Shaw Environmental, Inc.
Robert S. Kerr Environmental
Research Center, P.O. Box 1198,
Ada, OK 74820
Phone: (580) 436-8669
email: fine.dennis@epa.gov**