# *Integrated EPA/ORD Studies on EDCs in CAFOs: Past, Present and Future*

# Estrogens in the Environment: Effects on Fish

- Early 90s in the UK biologists collected fish from several locations below WWTP outfalls that appeared (externally) to be males, but had female gonads
- "Caged" male fish held in effluents developed functional characteristics of females-ovatestes and production of vitellogenin (egg yolk protein)
- Further studies indicated that natural and synthetic steroids, excreted by humans, were likely culprits
- Common problem throughout Europe, Asia and North America

# CAFOs vs WWTPs: Similarities and Differences

- Many animals in limited space
- Variety of natural and synthetic steroids present
- Often adjacent to waterbodies
- As opposed to WWTP, CAFO wastes often receive minimal treatment

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# ENDOCRINE DISRUPTING EFFECTS OF CATTLE FEEDLOT EFFLUENT ON AN AQUATIC SENTINEL SPECIES, THE FATHEAD MINNOW

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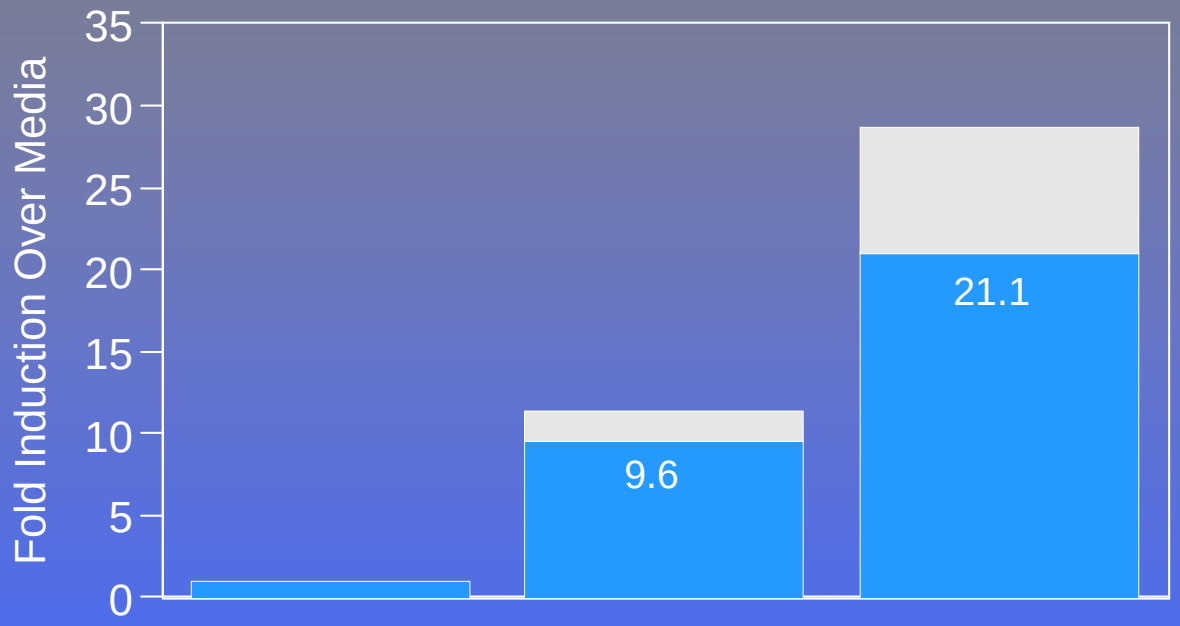
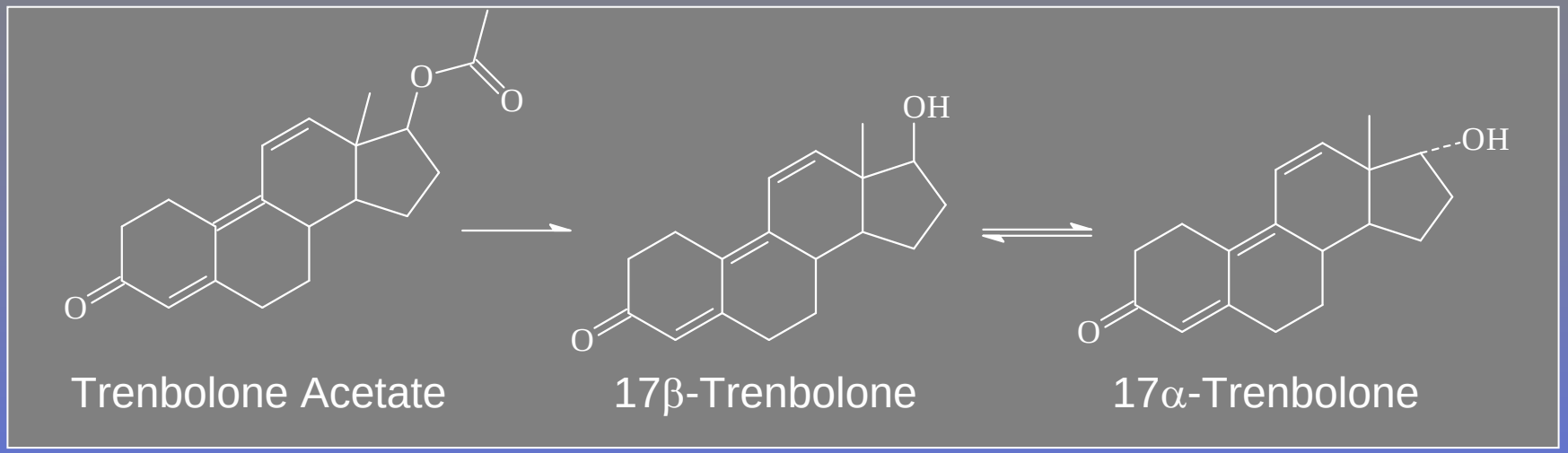
Department of Health and Human Services

***Androgenic activity of a Nebraska feedlot effluent (FLE) relative to dihyrotestosterone (DHT) in transiently transfected CV-1 cells***

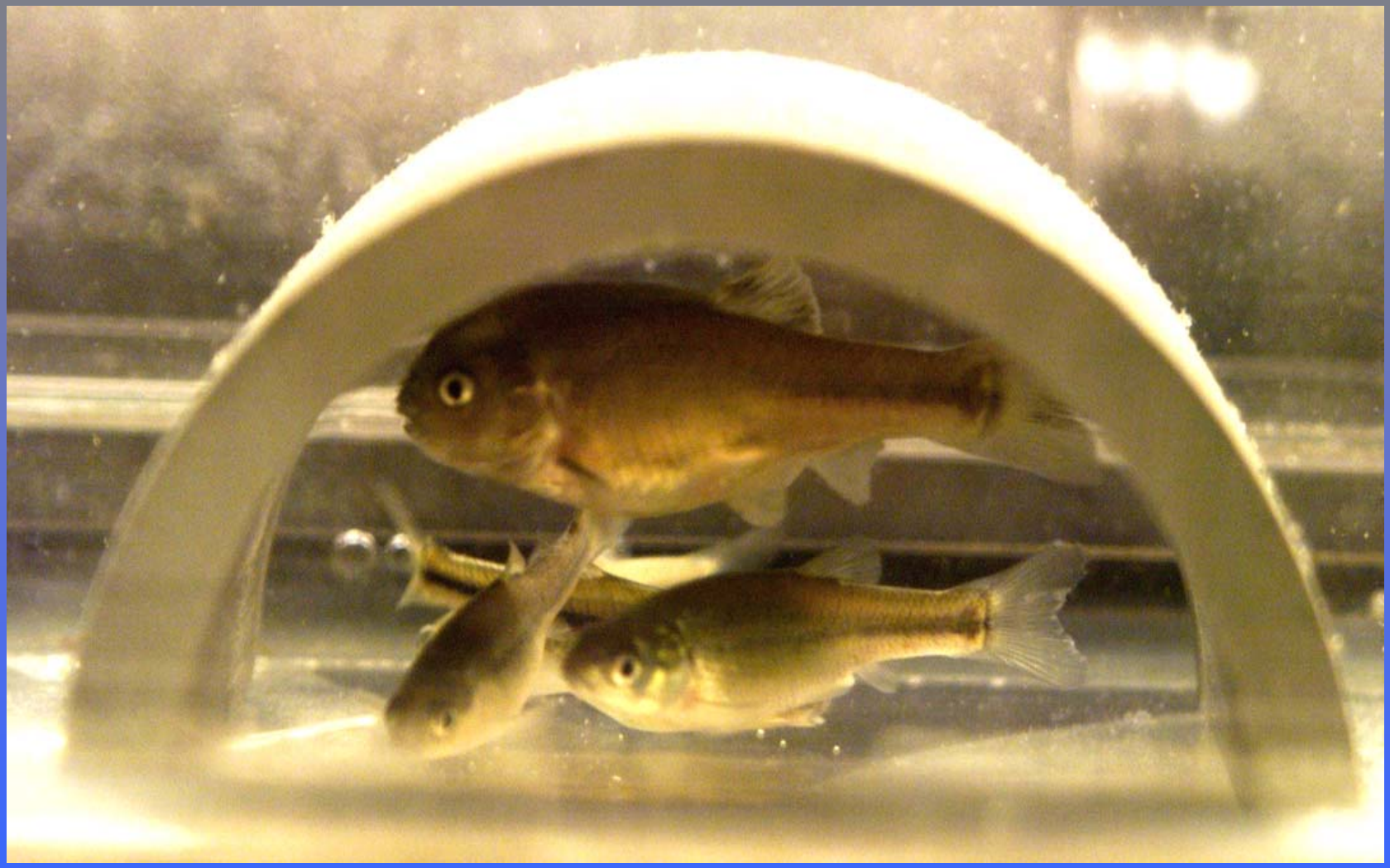



| | Media | DHT 1nM | FLE |
|---|---|---|---|
| X | 1 | 9.6 | 21.1 |
| SE | | 1.8 | 7.5 |

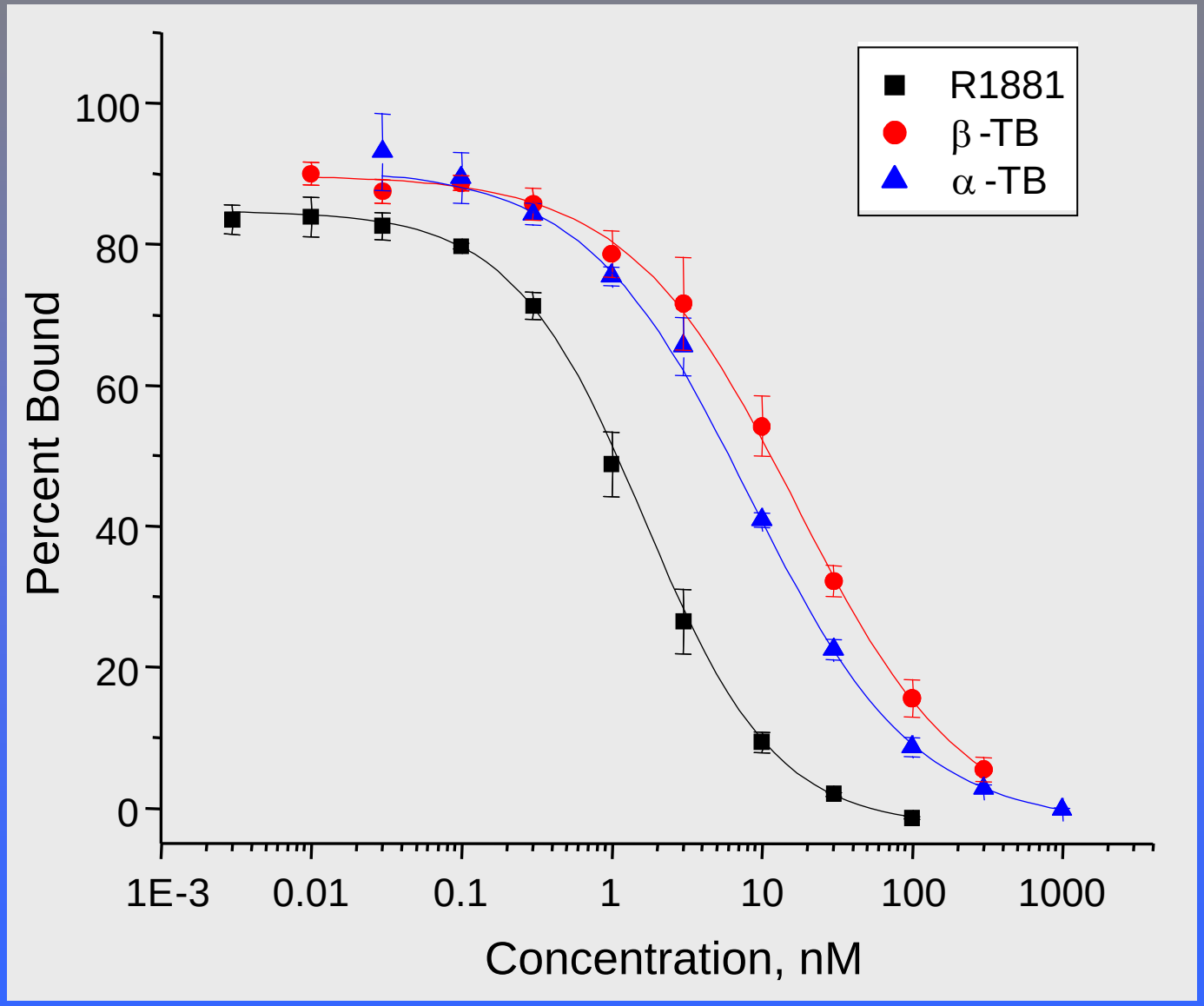
Orlando et al. (2004)




- Used as growth promoter for beef production
- Greater than 90% of production in the US utilize trenbolone implants, often in CAFOs



# Fathead Minnow Androgen Receptor Binding*



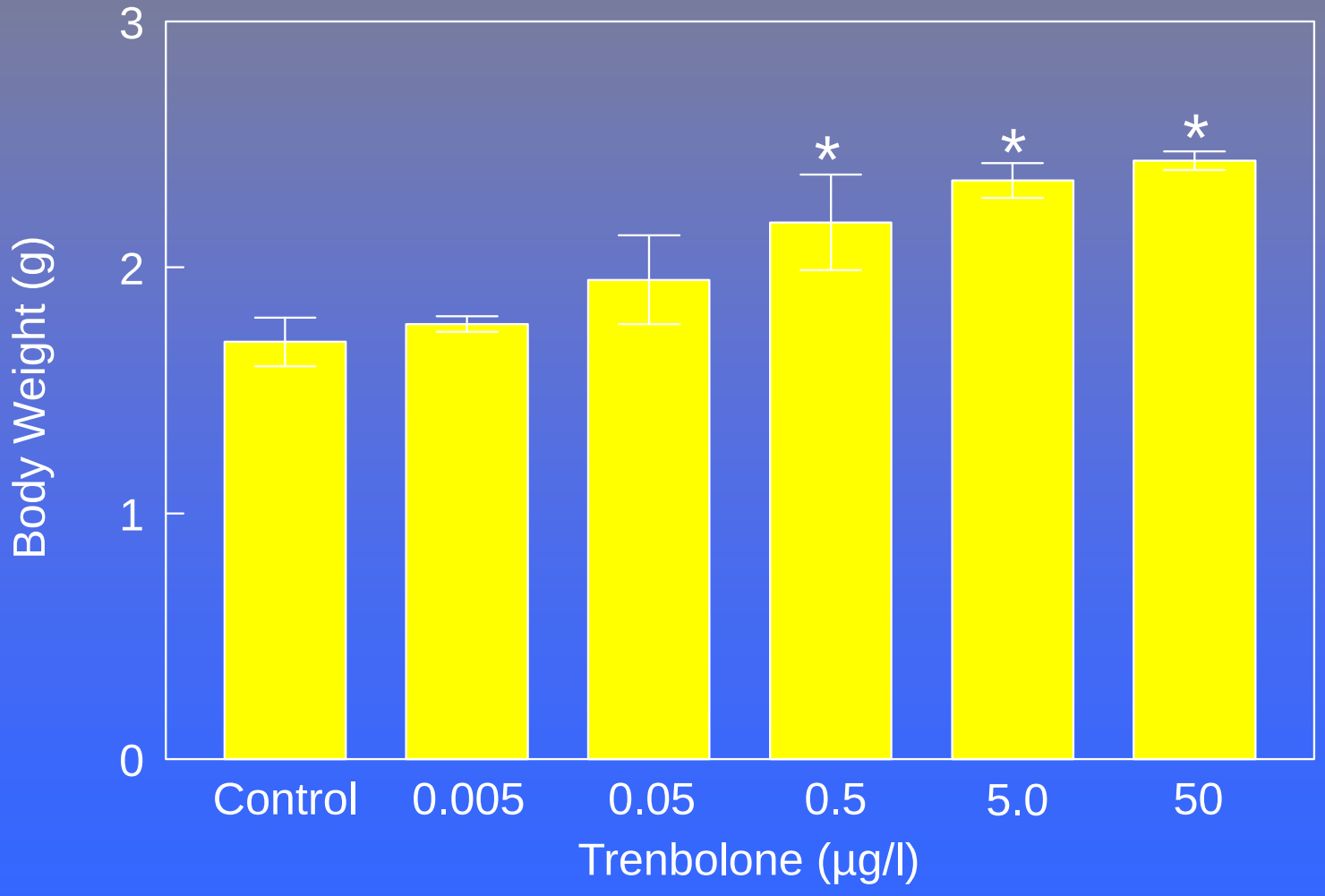

*Wilson et al. (2004)

# Test Overview

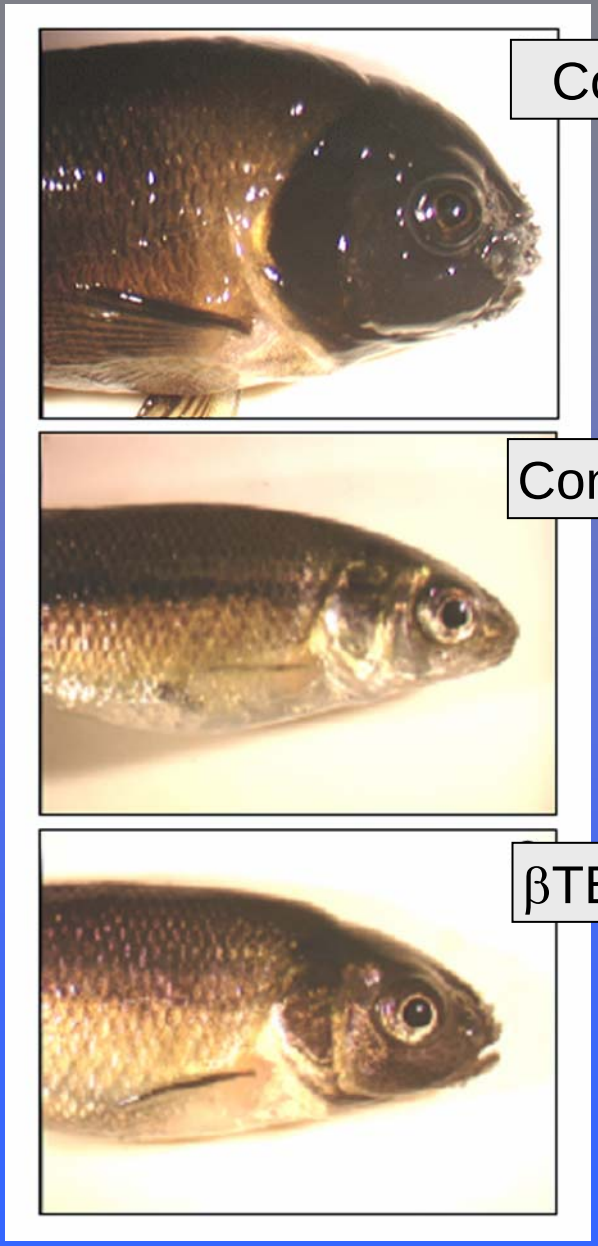
- Initiated with mature, spawning fish
- 14-21 day pre-exposure followed by ≥ 21 day chemical exposure
  - Behavior
  - Fecundity
  - Fertility
  - Hatch
  - Secondary sex characteristics
  - Gonadal status (GSI, histology)
  - Plasma vitellogenin
  - Plasma steroids (E2, T, KT)


High Concentration

# βTB Enhances Growth of Female Fathead Minnows



# Masculinization by βTB

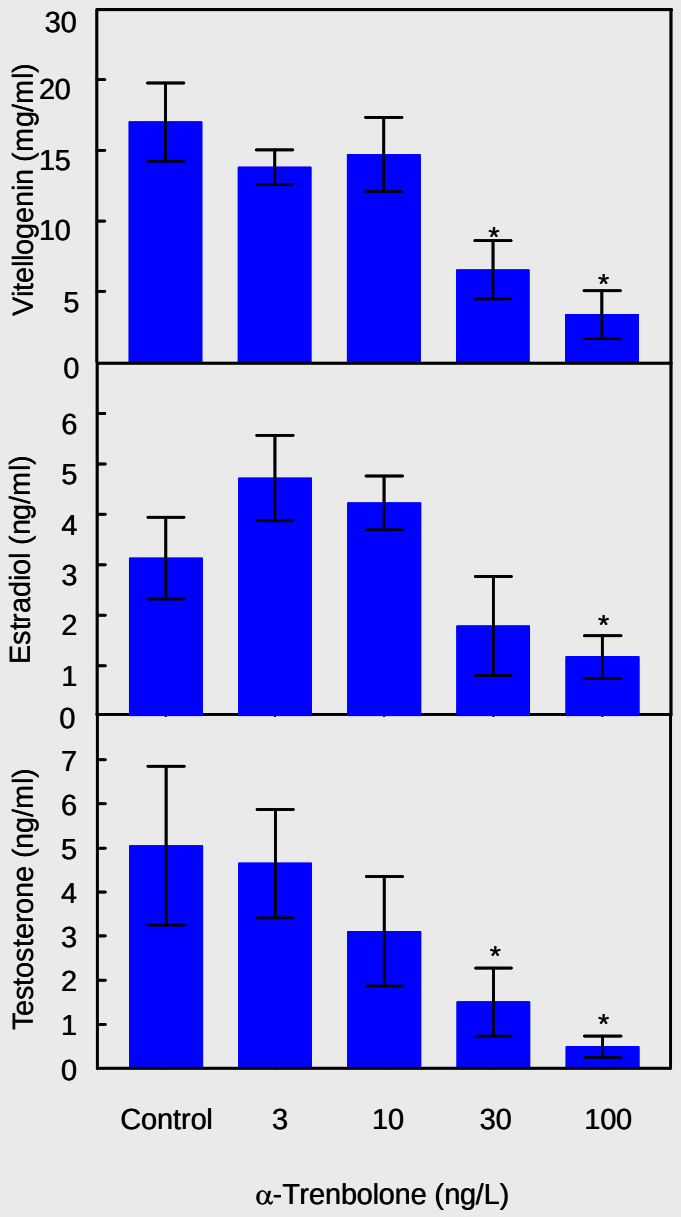


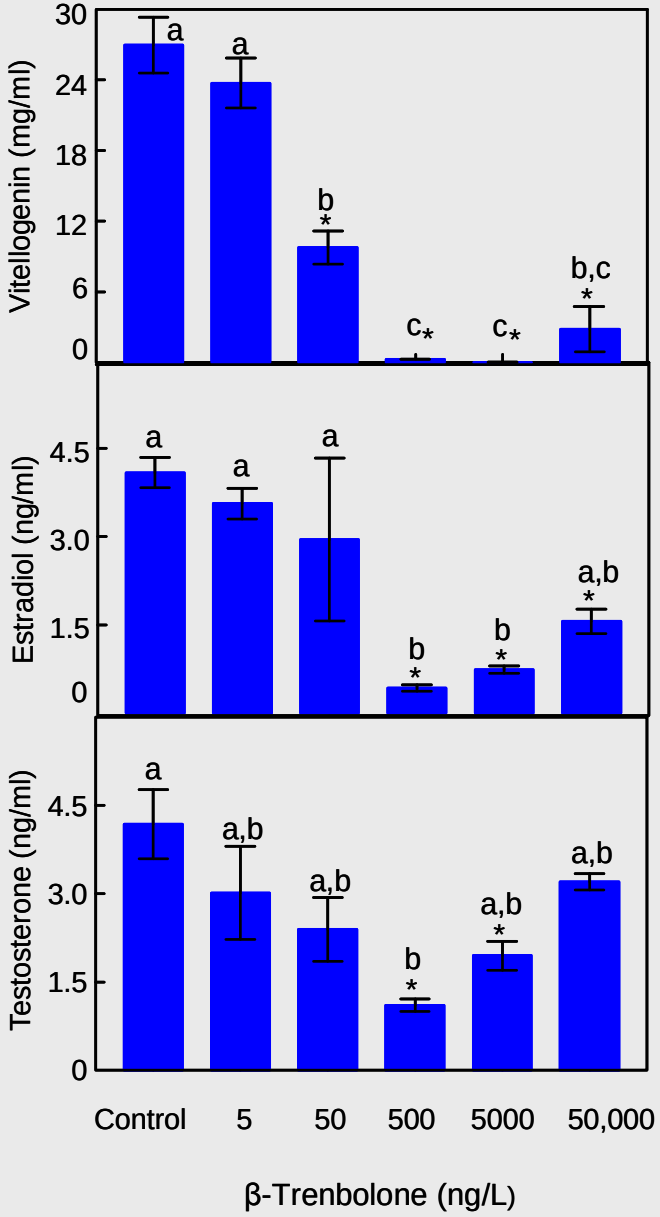
Control Male

Control Female

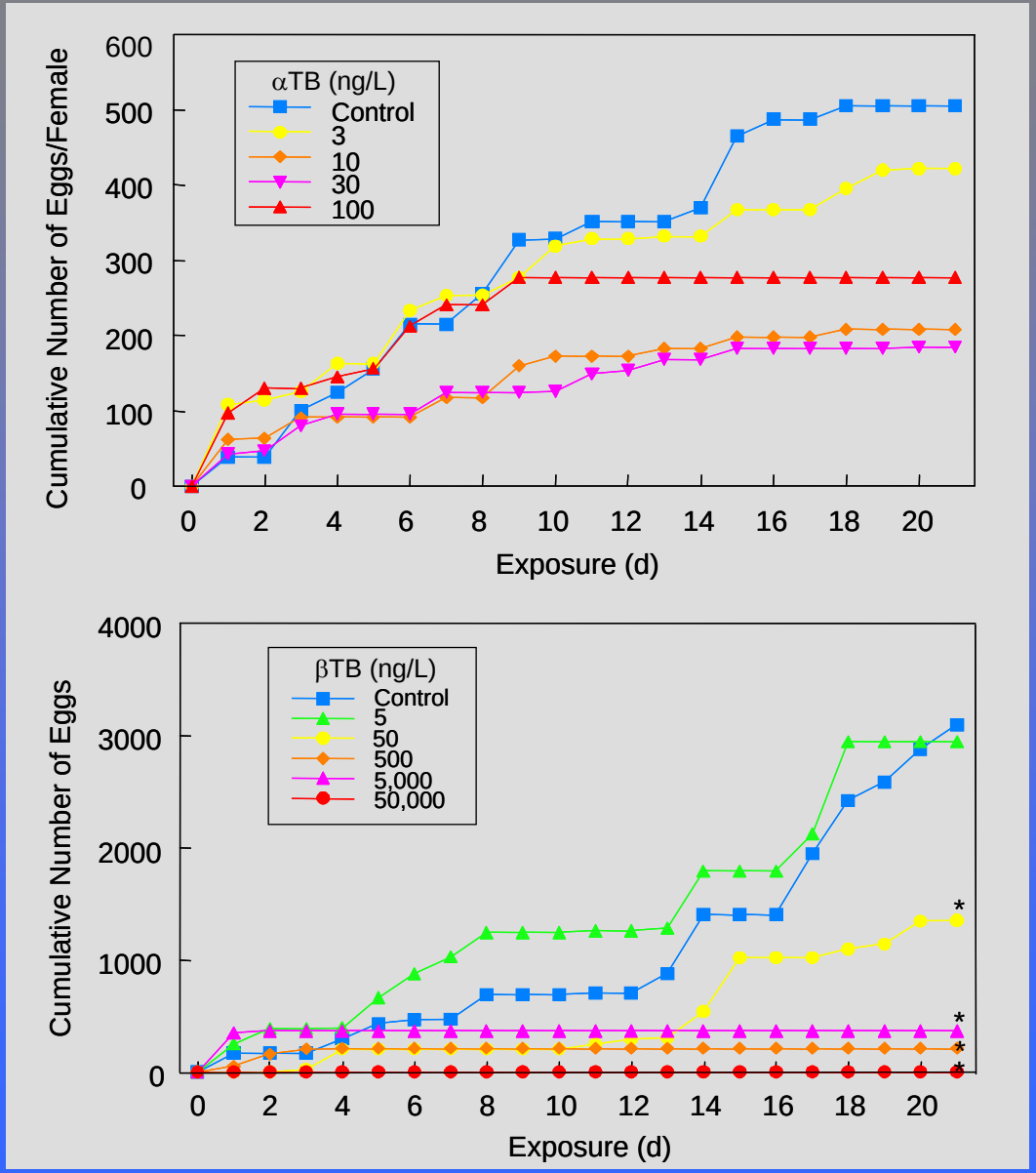
βTB - Exposed Female

# αTB, βTB Effects on Females



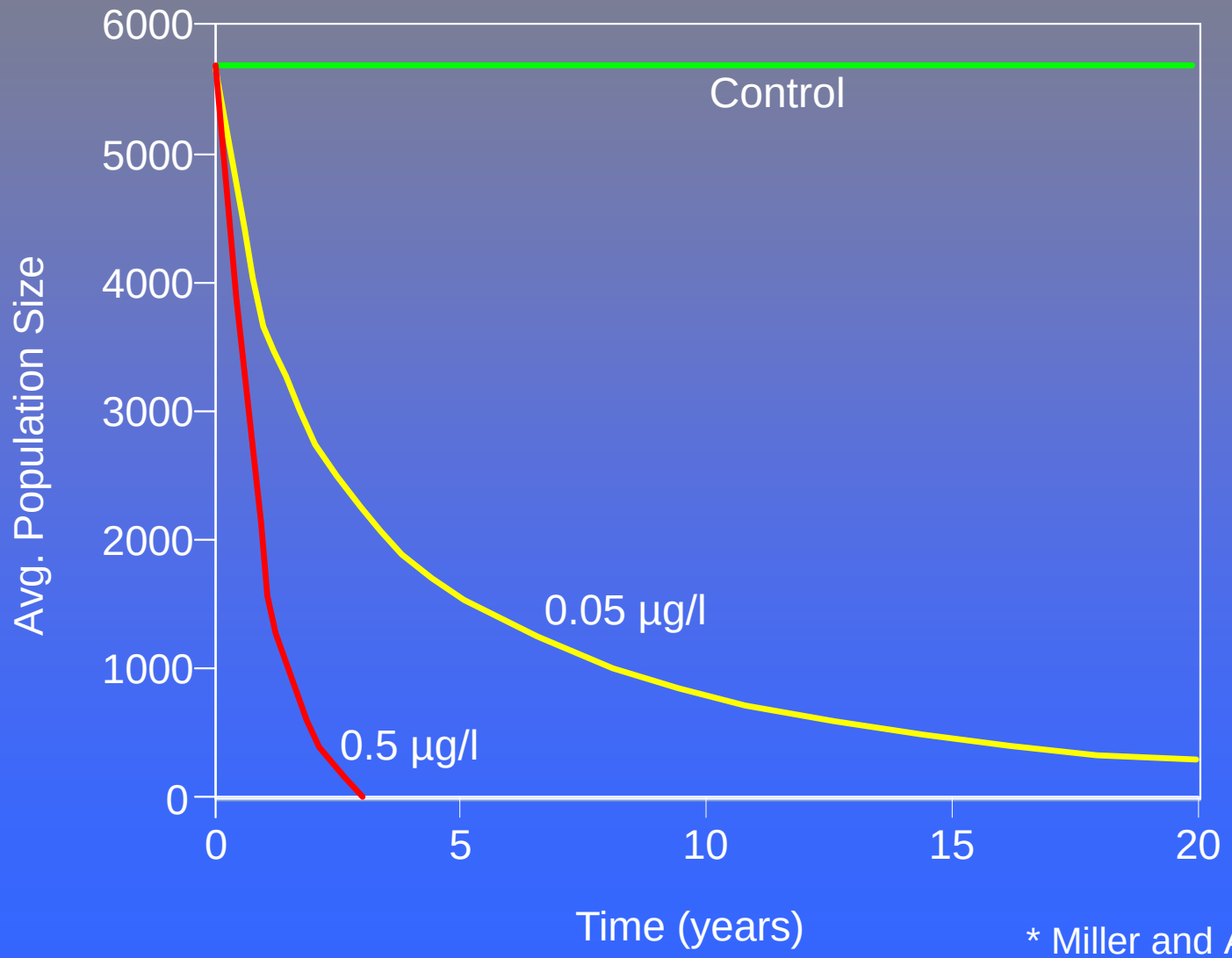



# Effects of αTB and βTH on Fathead Minnow Reproduction*




*Ankley et al. (2003; βTB), Jensen et al. (2006; αTB)

# *Trenbolone Effects on Fathead Minnow Population Dynamics**




* Miller and Ankley (2004)

# Toxicity Summary

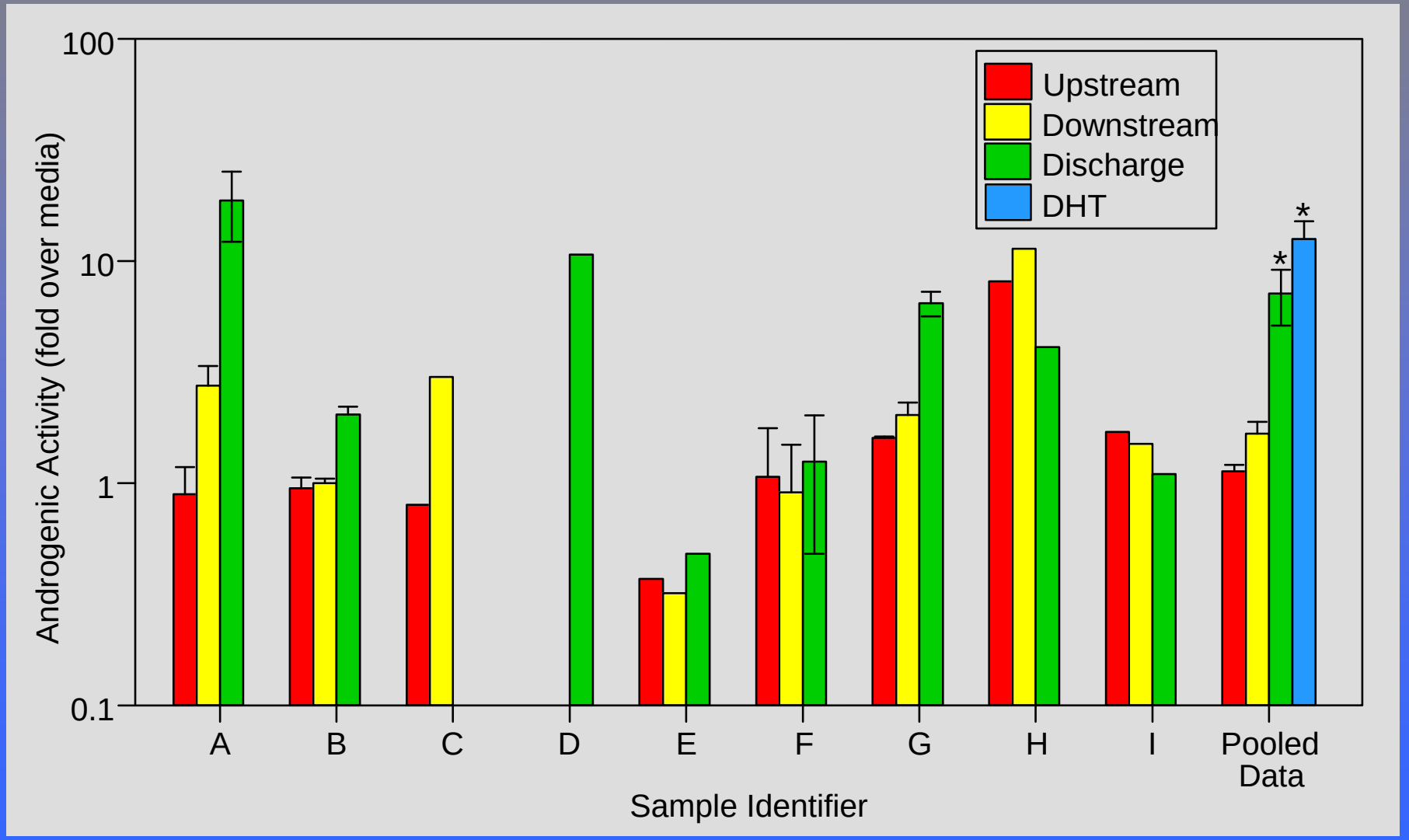
- αTB and βTB bind with high affinity for fathead AR
- Both isomers masculinize females and produce similar response profiles in plasma steroids and vitellogenin
- Similar potency for fecundity effects
  - αTB EC50 = 11 ng/L
  - βTB EC50 = 18 ng/L
- Provides concentration-response data for risk estimates ***and*** theoretical basis for additive toxicity model for mixtures
- If concentrations of this magnitude occur, substantial population effects possible

# So what does this mean on the field?

# Study Objectives

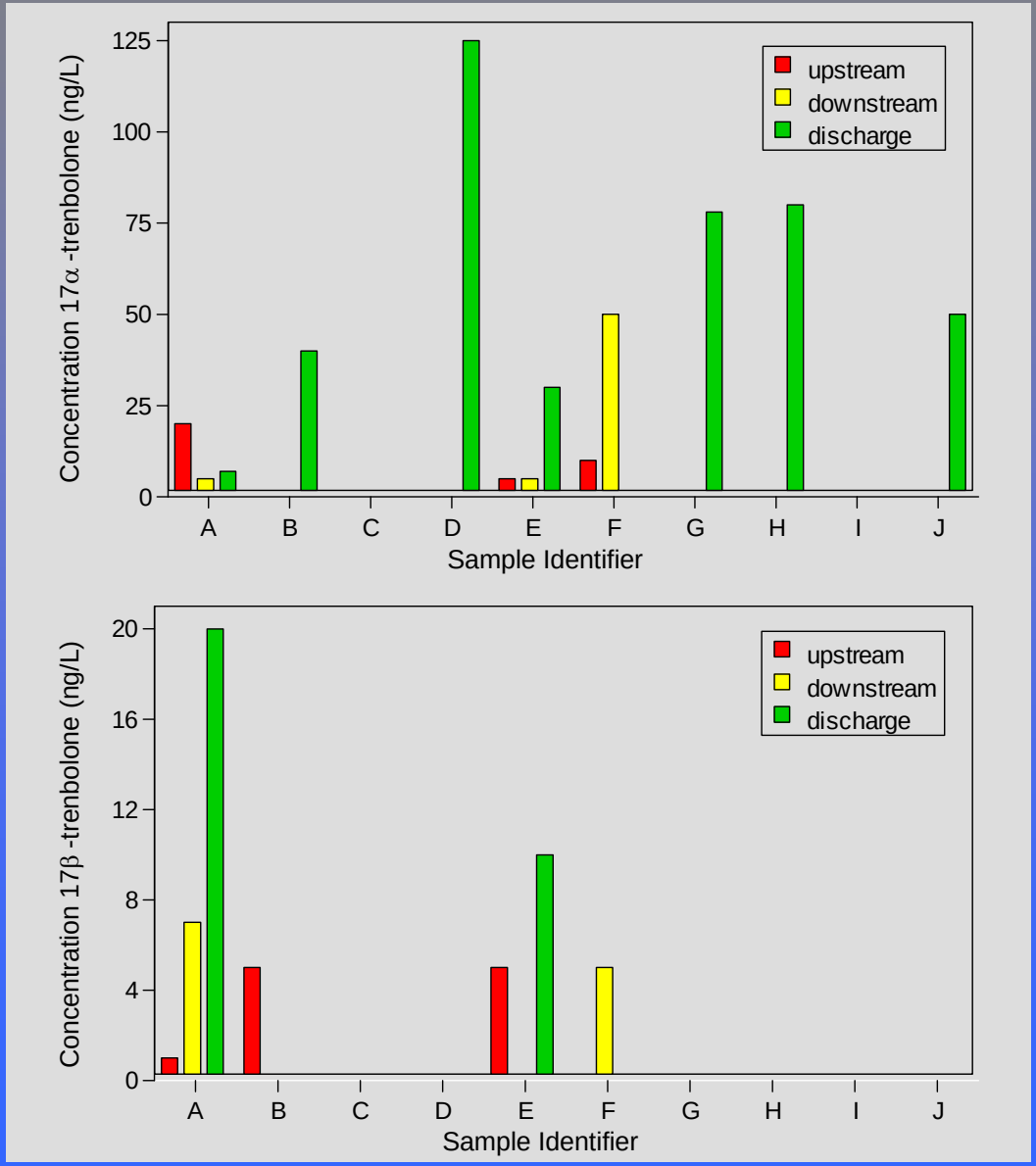
- Evaluation of possible exposure scenarios both spatially and temporally
  - Analytical (GC/LC MS) measurements
  - Androgenic activity determination (CV-1 cells)

# Androgenic Activity of Water Associated with Beef CAFO



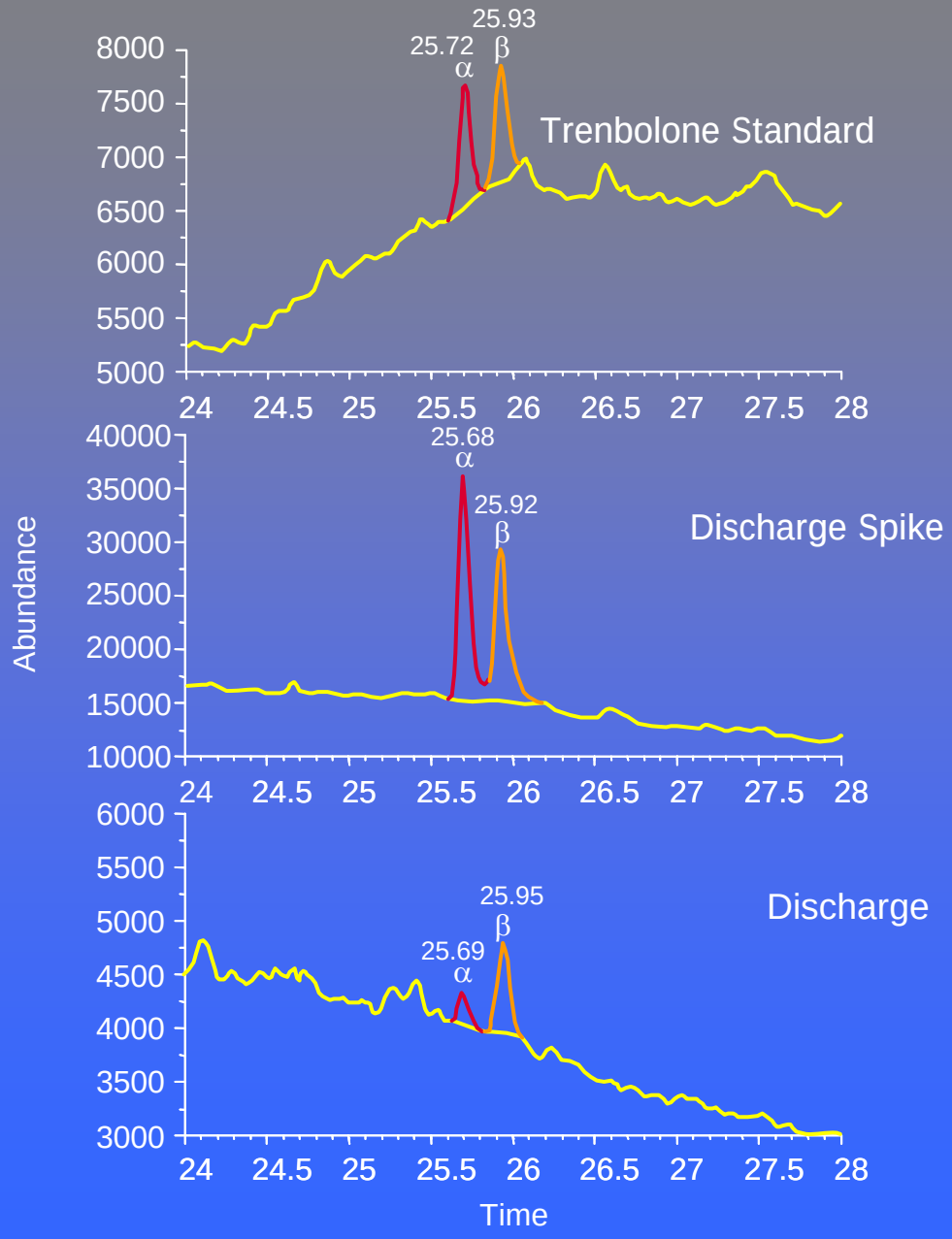

Durhan et al. (2006)

# TB Concentrations in Water Associated with Beef CAFO




Durhan et al. (2006)

# MS Confirmation of Trenbolone Metabolites




Durhan et al. (2006)

# Field Summary

- Water samples associated with a typical, operating beef CAFO consistently exhibited androgenic activity
- Both $\alpha$TB and $\beta$TB consistently measured (and confirmed) in samples
- Simple estimate of risk ratio (exposure/effects) indicates sufficient TB to adversely affect egg production in fish

# ORD CAFO/EDC Research Team

- Assembled as a follow-up to initial observations of EDCs (primarily androgens) associated with CAFOs
- Features novel integration of scientists from four ORD labs/centers
  - NERL (exposure)
  - NHEERL (effects)
  - NRMRL (management)
  - NCER (extramural grants/co-ops)

## Goal:

- Characterize occurrence and ecological impacts of estrogenic and androgenic chemicals from CAFO waste

## Approach:

- Develop *in vitro* and analytical methods to identify and quantify compounds responsible for endocrine activity
- Assess ecological impacts using a combination of laboratory and field studies
- Identify ecologically-relevant biomarkers in aquatic species using genomic approaches
- Evaluate the environmental fate, transport and metabolism of CAFO-derived EDCs in surface and ground waters
- Evaluate capability of existing risk management technologies for CAFOs to reduce exposure to EDCs

# ORD Expertise Areas

- Development of MOA-specific biomarkers (NERL)
- Field sample collection and *in situ* testing (NERL)
- Cell-based assays for estrogenicity and androgenicity (RTP, NHEERL)
- Toxicity-based fractionation (TIE; MED & RTD, NHEERL)
- Fish reproductive toxicity testing and population modeling (MED)
- Steroid analysis (including conjugates) in manure, water (NRMRL)
- Ground water monitoring (NRMRL)
- Risk mediation/remediation (NRMRL)

# Partnerships

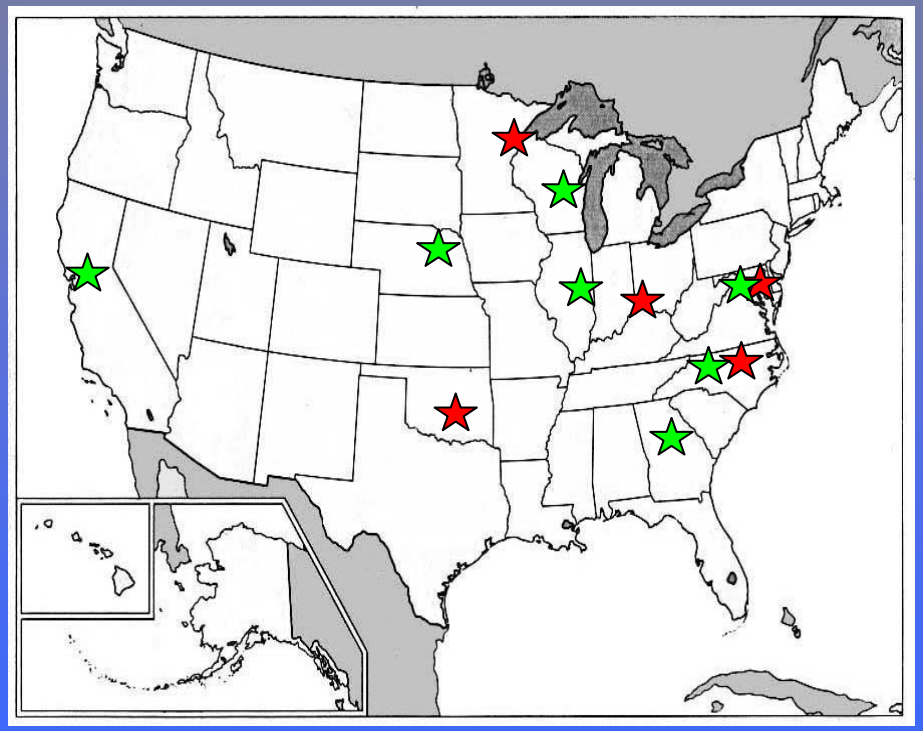
- CAFO USEPA-ORD workgroup member

**NHEERL-** National Health and Environmental Effects Research Laboratory
MED: Gary Ankley, Elizabeth Durhan, Dalma Martinović
RTD: Earl Gray, Phillip Hartig, Christy Lambright, Vickie Wilson

**NERL-** National Exposure Research Laboratory
EERD: Jim Lazorchak, Dave Bencic

**NRMRL-** National Risk Management Research Laboratory
LRPCD: Marc Mills
GWERD: Steve Hutchins

**NCER-** National Center for Environmental Research
Susan Laessig, Chris Saint



- The CAFO workgroup has been instrumental in awarding seven STAR grants for further CAFO studies, several of which will be cooperative agreements.

# Impacts

- Support site-specific risk assessments and development of risk management options for hormones in waste from CAFOs
- Increase knowledge of risks associated with CAFOs as potential sources for hormones in surface and groundwater, waste, and soil to support the current activities of the EPA Office of Water (OW) and Regional Offices with respect to the regulation of CAFOs

# Impacts continued

- Several chemicals to be assessed are regulated as veterinary pharmaceuticals. Currently pharmaceuticals in the environment, both human and veterinary, are an emerging topic of environmental concern by EPA and other Federal Agencies. This work will contribute directly to developing methods for addressing pharmaceuticals used for livestock production.
- In addition to steroidal EDCs, there are a number of other materials used in livestock production of potential environmental concern, such as pesticides and antibiotics. The types of studies/approaches used in our studies could be expanded to consider potential risks of these