Transport/fate/ecological Effects of Natural Steroids from Poultry Litter & Evaluations of Existing/novel Management Strategies

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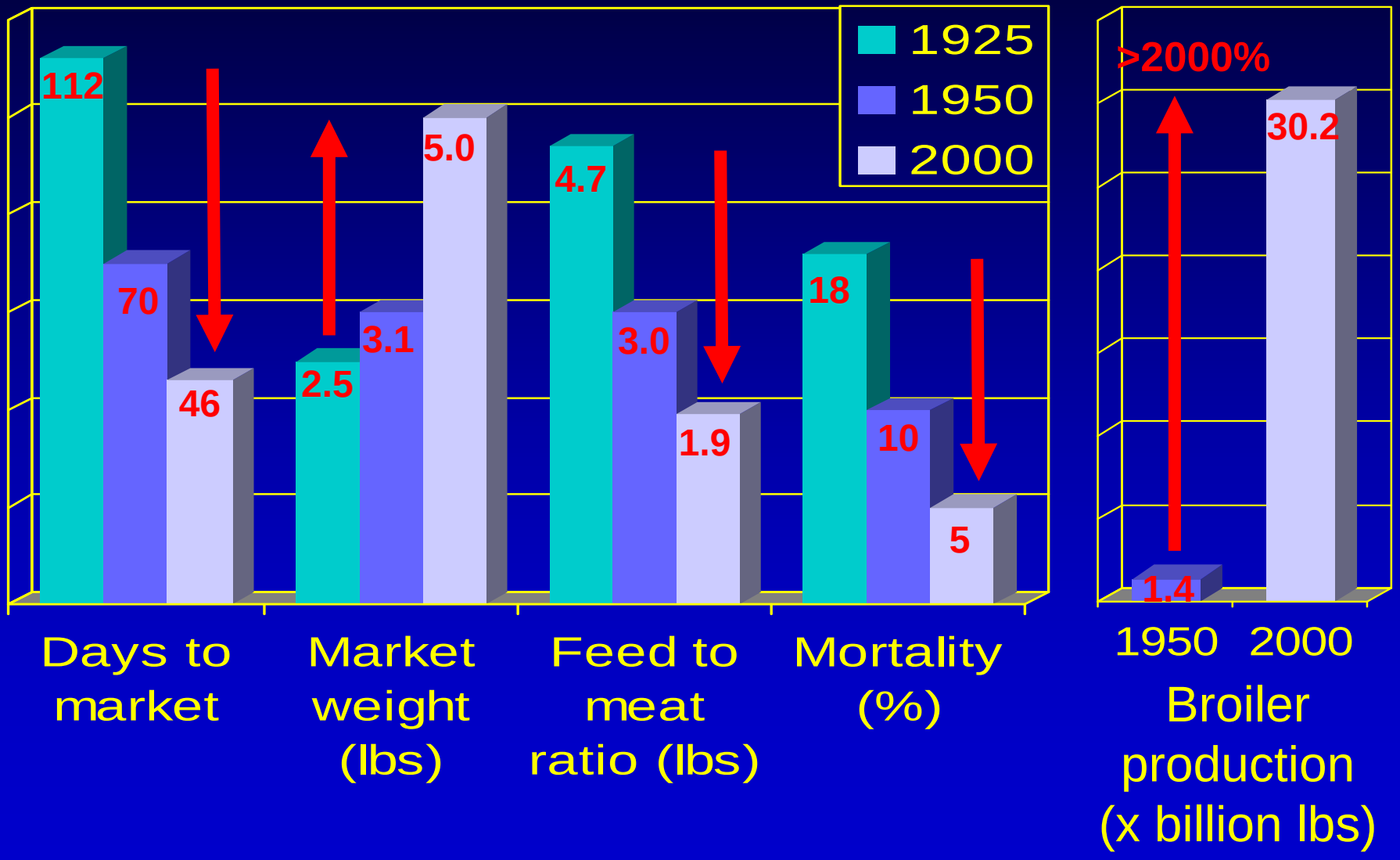
US Poultry Facts



Delmarva Poultry Facts

- ~600 millions birds are produced annually (~7% of US total)
- Litter is predominately land applied (primarily to satisfy nutrient requirements of corn production)
- Excessive land application can have impacts on regional surface and ground water quality

  Excessive nutrient impacts are most frequently cited

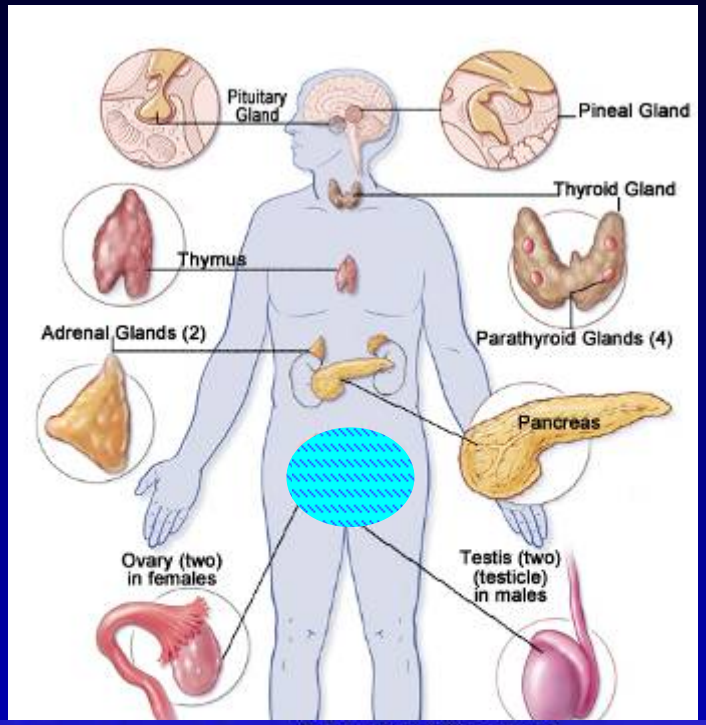
SOURCE OF STEROIDS IN POULTRY LITTER

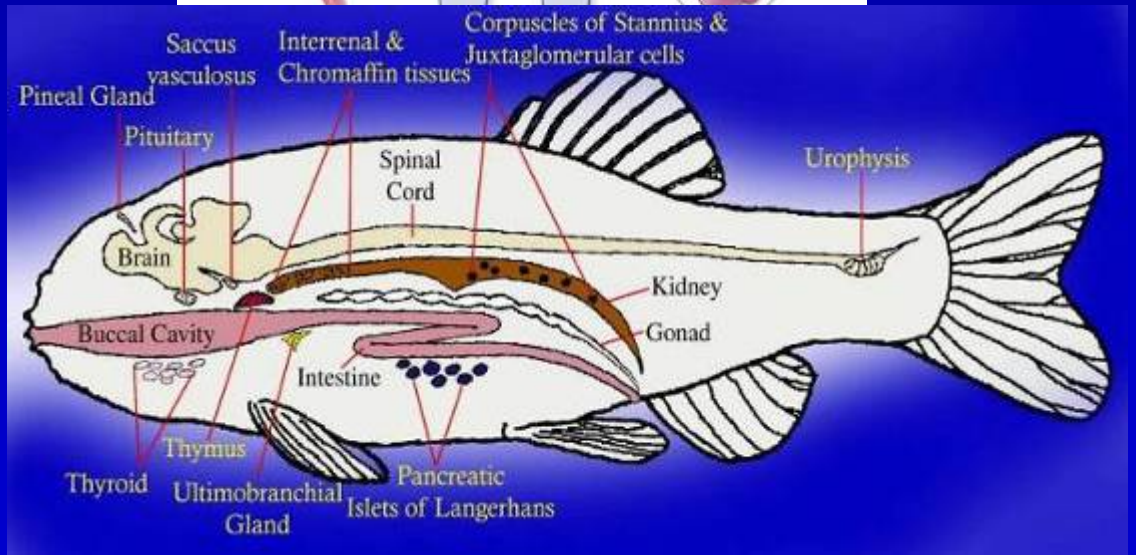
These are ***NATURAL BYPRODUCTS*** of chickens, and most other animals, including humans, fish and amphibians!!!!! They end up in the litter through excretion.

Steroids are not added to chicken feed in any form, unlike beef production that adds synthetic male hormones to the cattle (Trenbolone). This steroid increases muscle growth and appetite.

Male hormones result in bigger and more muscular cattle! Same thing happens in humans.

The Vertebrate Endocrine System





- Growth
- Development
- Reproduction
- Metabolism
- Osmoregulation
- Behavior
  - Breeding
  - Predator avoidance
- and much more

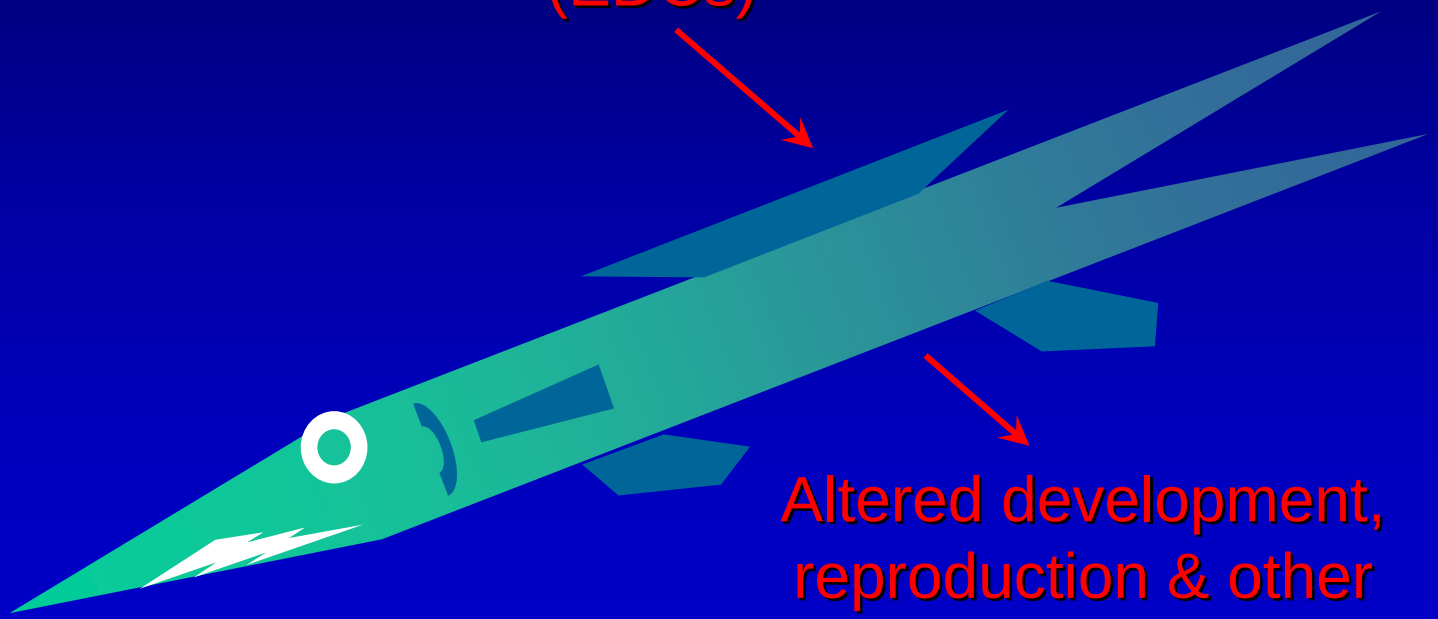
What are endocrine disruptors?

"An endocrine disruptor is an exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, its progeny, or population."

Damstra et al., 2002

What are endocrine disruptors?

Endocrine Disrupting Chemicals (EDCs)



Altered development, reproduction & other endocrine-mediated functions

Endocrine Disruption: Modes of Action

- Mimic activity of endogenous hormone (agonist)
- Inhibit activity of endogenous hormone (antagonist)
- Alter hormone synthesis, metabolism or transport
- Modify hormone receptor levels

» Endogenous hormones function at ppt concentrations

» Exogenous EDCs may act at similarly low concentrations

!! **IMPORTANT** !! – ENDOCRINE DISRUPTION CAN BE CAUSED BY MAN MADE CHEMICALS OR FROM EXCESS AMOUNTS OF NATURAL STEROIDS SUCH AS ESTRADIOL

Chemical Analysis of Poultry Litter

- Nutrients ~ Nitrogen (4% by weight) and Phosphorous (1.5% by weight)
- Bacterial and viral pathogens
- Historic & Current-Use Pesticides

  DDT & metabolites (~0.4 – 2.9 ng/g)
  Lindane (0.6 – 4.6 ng/g)
  Chlordanes (0.5 – 13 ng/g)
  Metolachlor (3.1 – 14 ng/g)
  λ-Cyhalothrin (3.7 – 28 ng/g)
  Atrazine (0.3 – 1.3 µg/g)

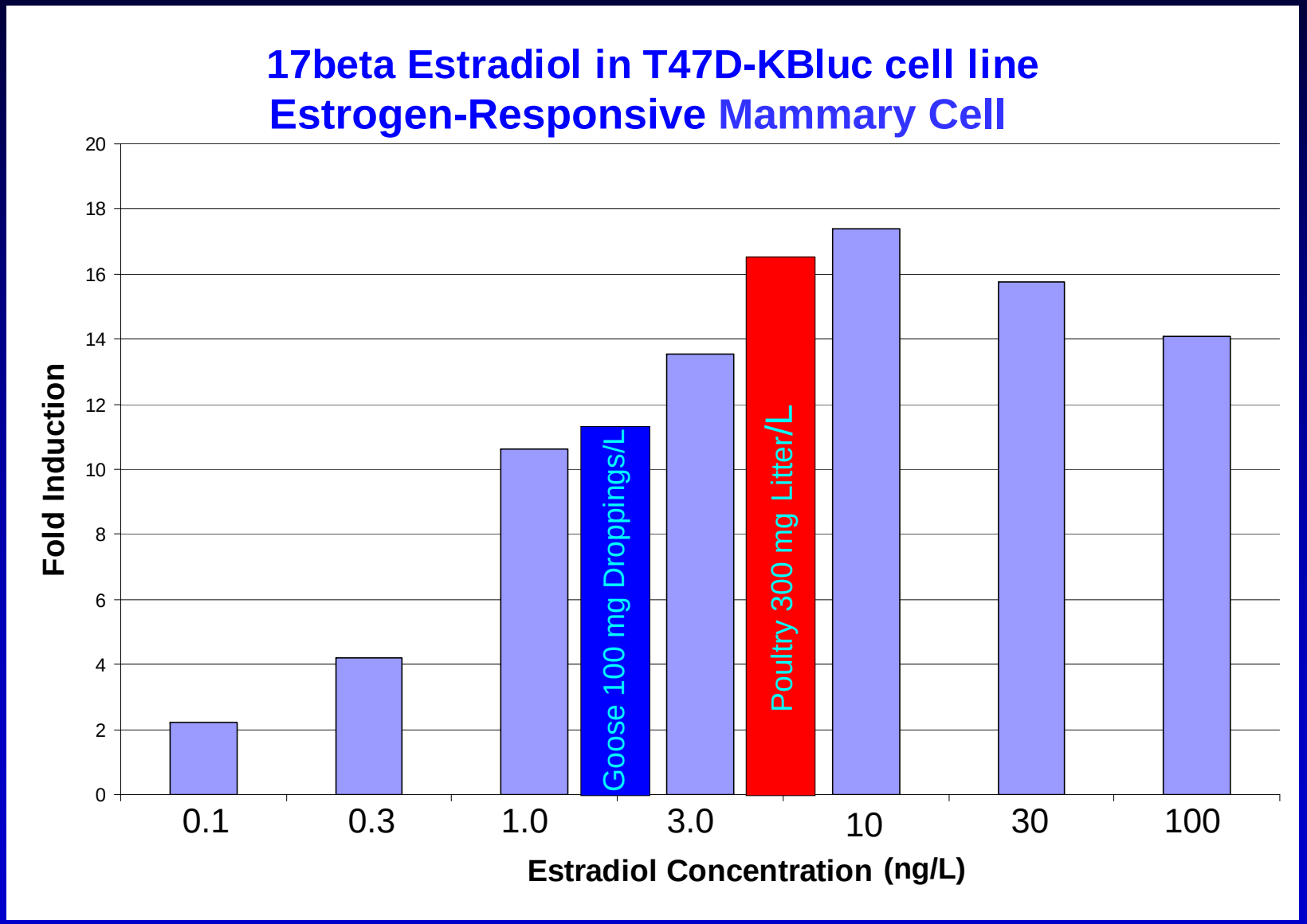
- PAHs ~ Benz[a]anthracene (0.21 – 0.27 µg/g)
- Antibiotics ~ Chlortetracycline (1.2 – 1.6 µg/g)
- Metals

  Copper (400 µg/g) Zinc (430 µg/g) Arsenic (29 µg/g)

- Steroids (from 8 litter sources)

  17 β-Estradiol (~ 125 ng/g dry litter) Testosterone (~50 ng/g dry litter)

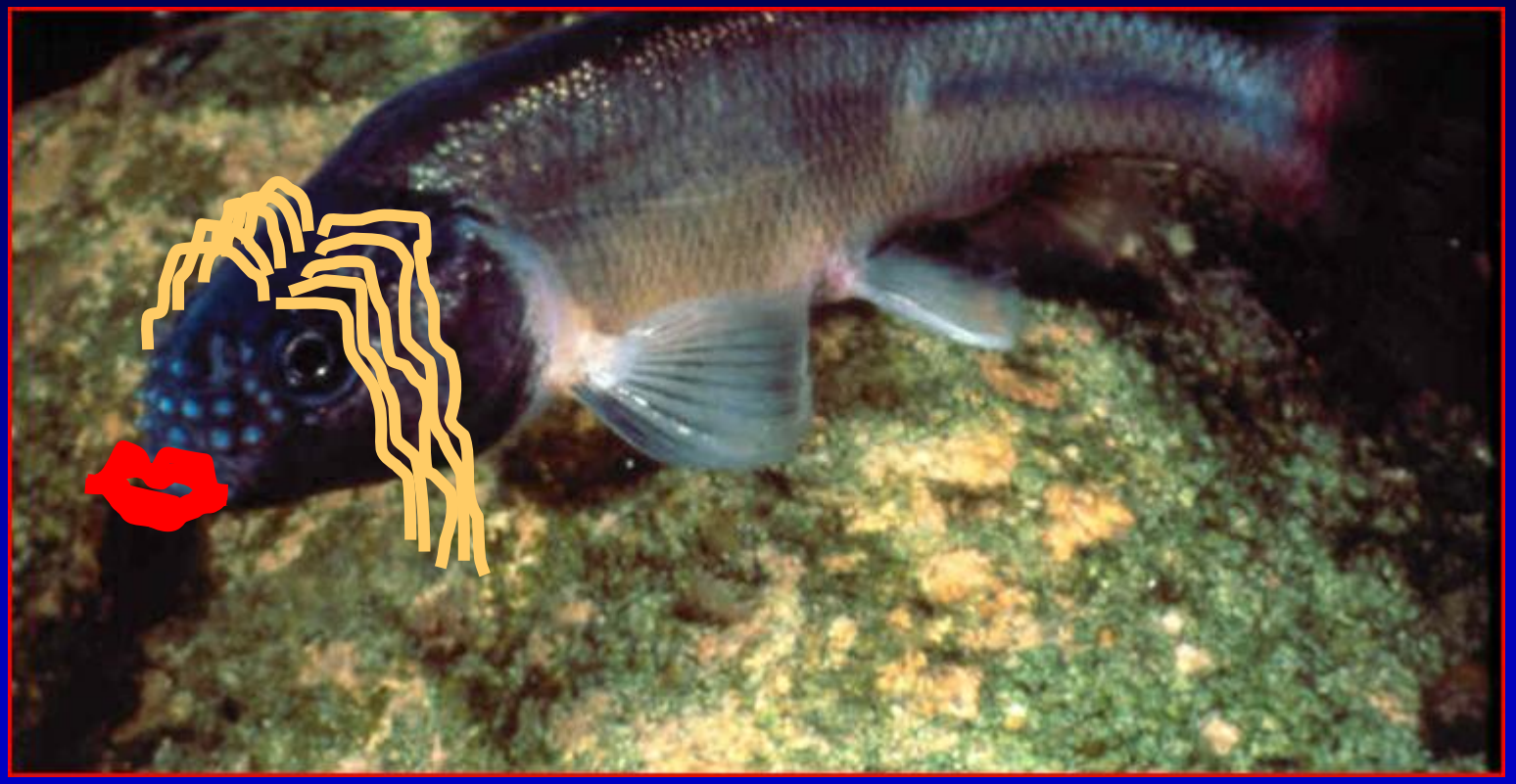
Poultry Litter Estrogenicity
Vickie Wilson – US EPA/NHEERL Research Triangle Park



Generation of Water-Soluble PLACs Treatments

- PLACS - Poultry Litter-Associated Contaminants
- Litter is a mixture of contaminants so when you do an exposure you are exposing test organisms to a mixture of contaminants.
- Litter introduced to test water at a rate of 2.5 g dry weight/L, then mixed for ~20 h
- Exposure treatments were produced by dilution of this mixture
- Litter treatments designated according to ratio of dry litter (mg) to diluent water (L)
- PLAC-833 = 833 mg poultry litter per 1 L of test water

Biological Indicators

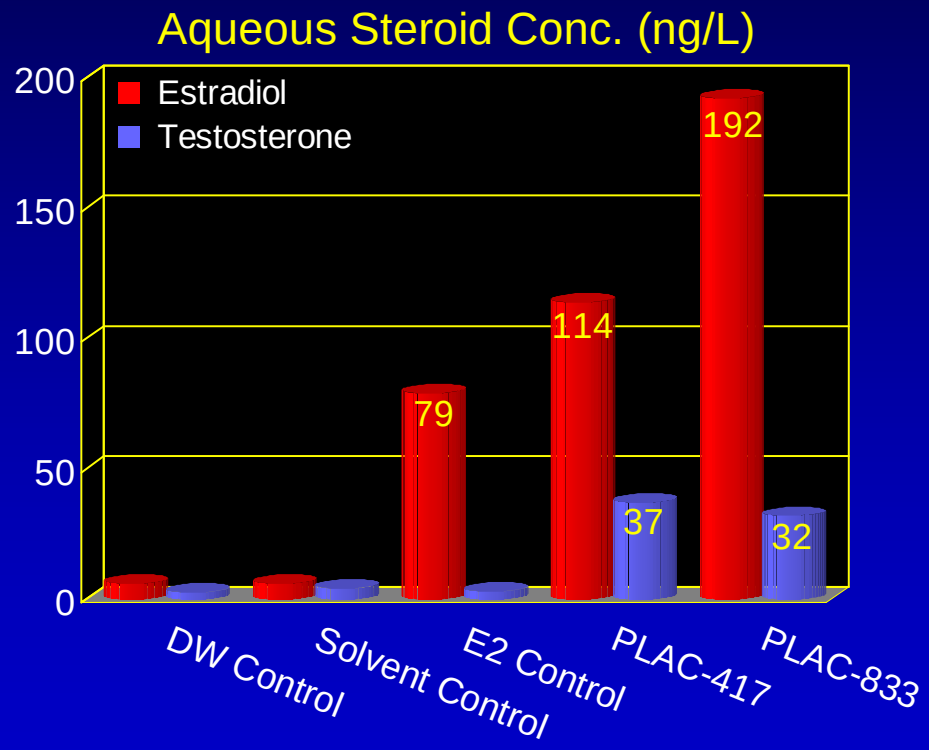


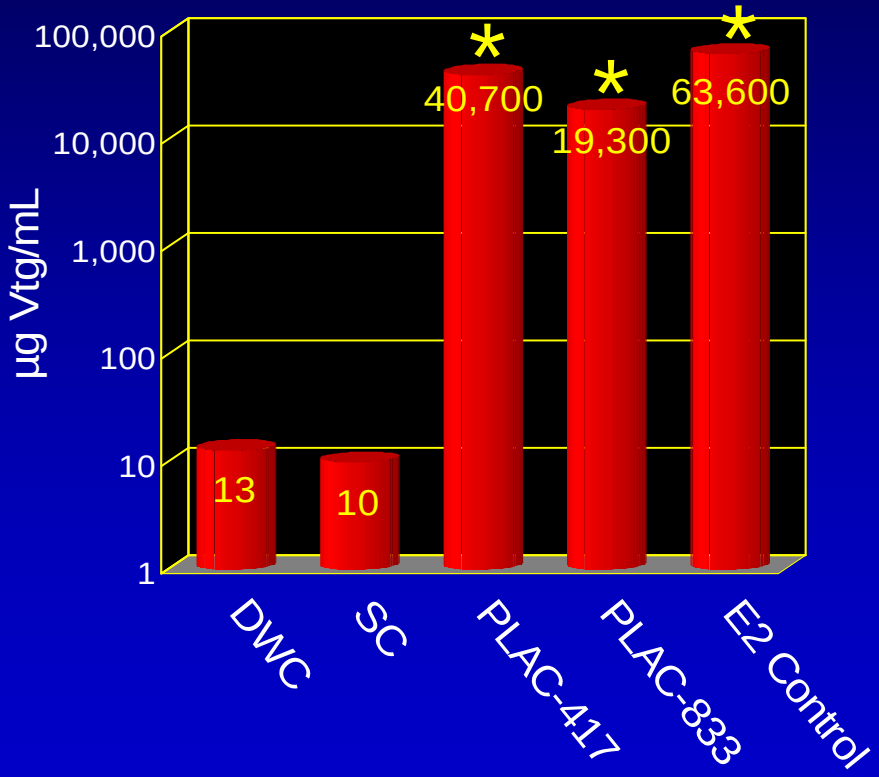
Biological Indicators

Vitellogenin (Vtg) Induction ~ lipoprotein precursor to egg yolk protein in mature females; males can produce Vtg in response to stimulation from external source of estrogens or estrogen agonists. Vtg is not found normally in male fish.

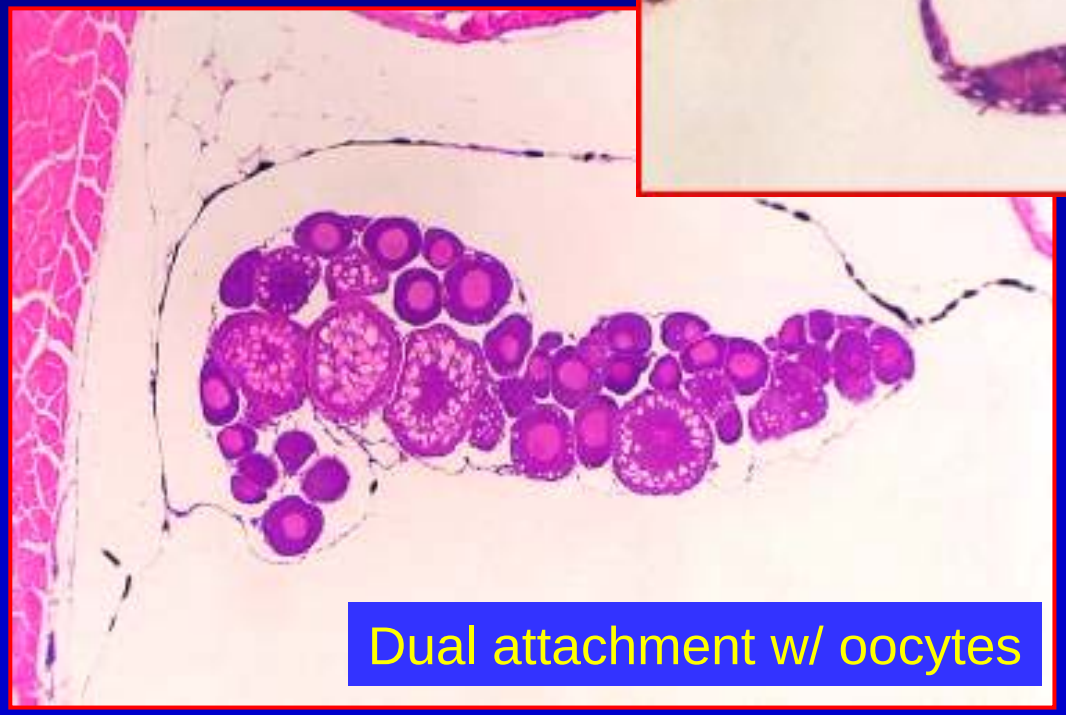
This is a broadly accepted bioindicator of estrogen or estrogen mimic exposure.

Plasma Vtg in Mature Male Fathead Minnows
(21-d lab exposure)



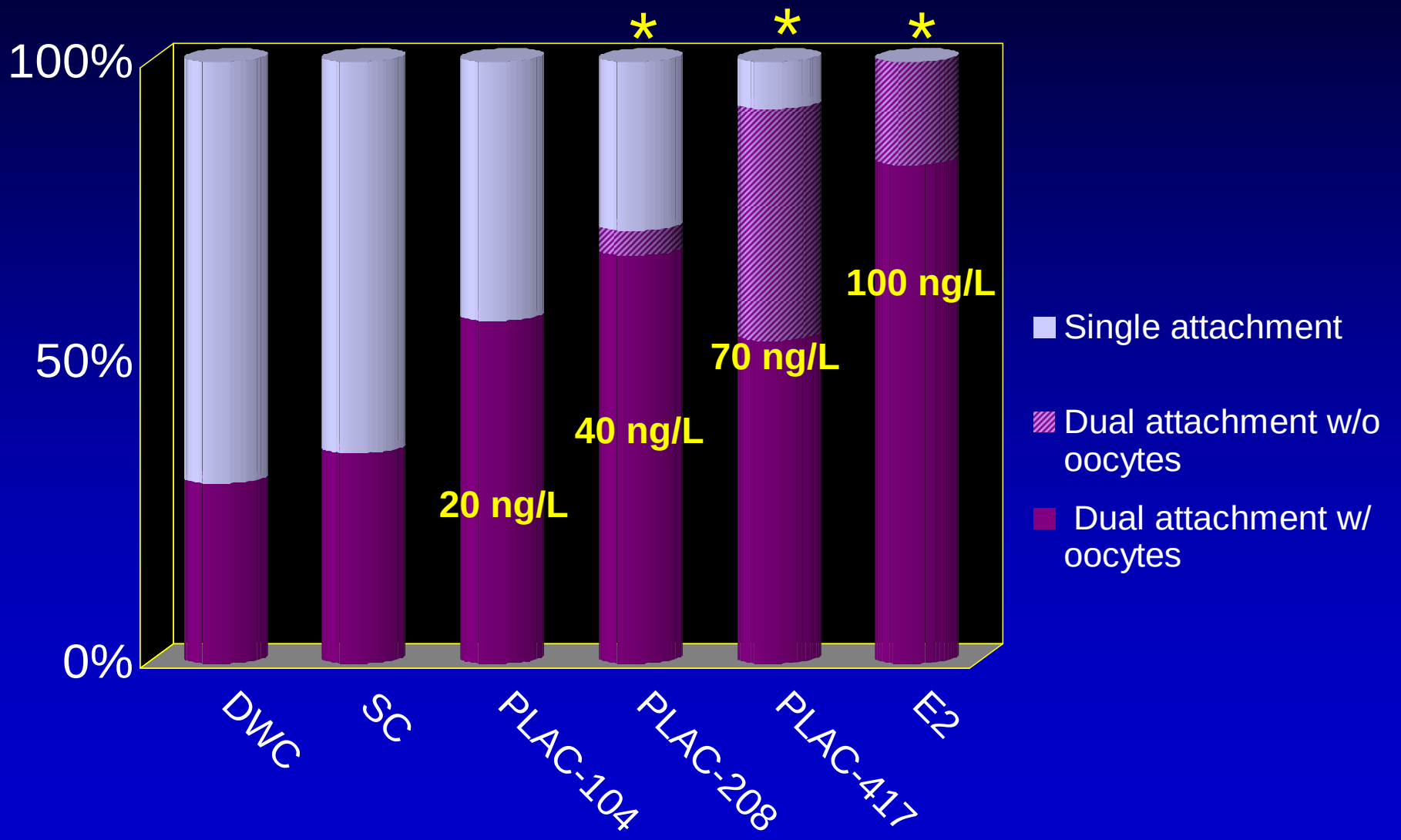


Gonad Development and Gender Differentiation



Gonad Development and Gender Differentiation

(Larval fatheads exposed 21-d and held to 60-dph)



Yellow = average E2 exposure concentration

Poultry Litter

Whole-house “scrape-out” of manure, feathers and bedding material (e.g., sawdust, wood chips) accumulated over ~ 2 years from a standard broiler operations.

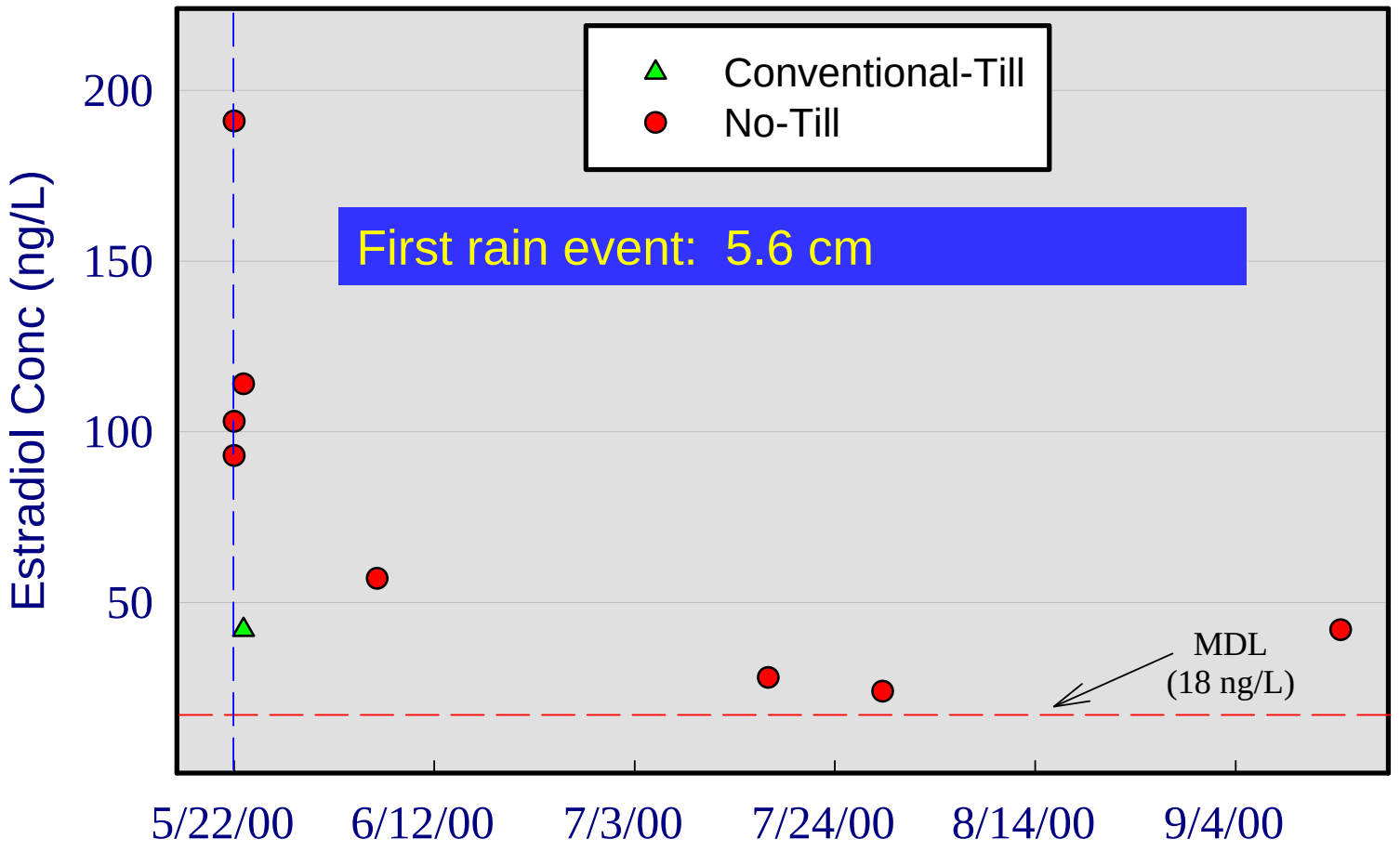
Wye Research and Education Center

Controlled Field Investigation

Controlled Field Investigation

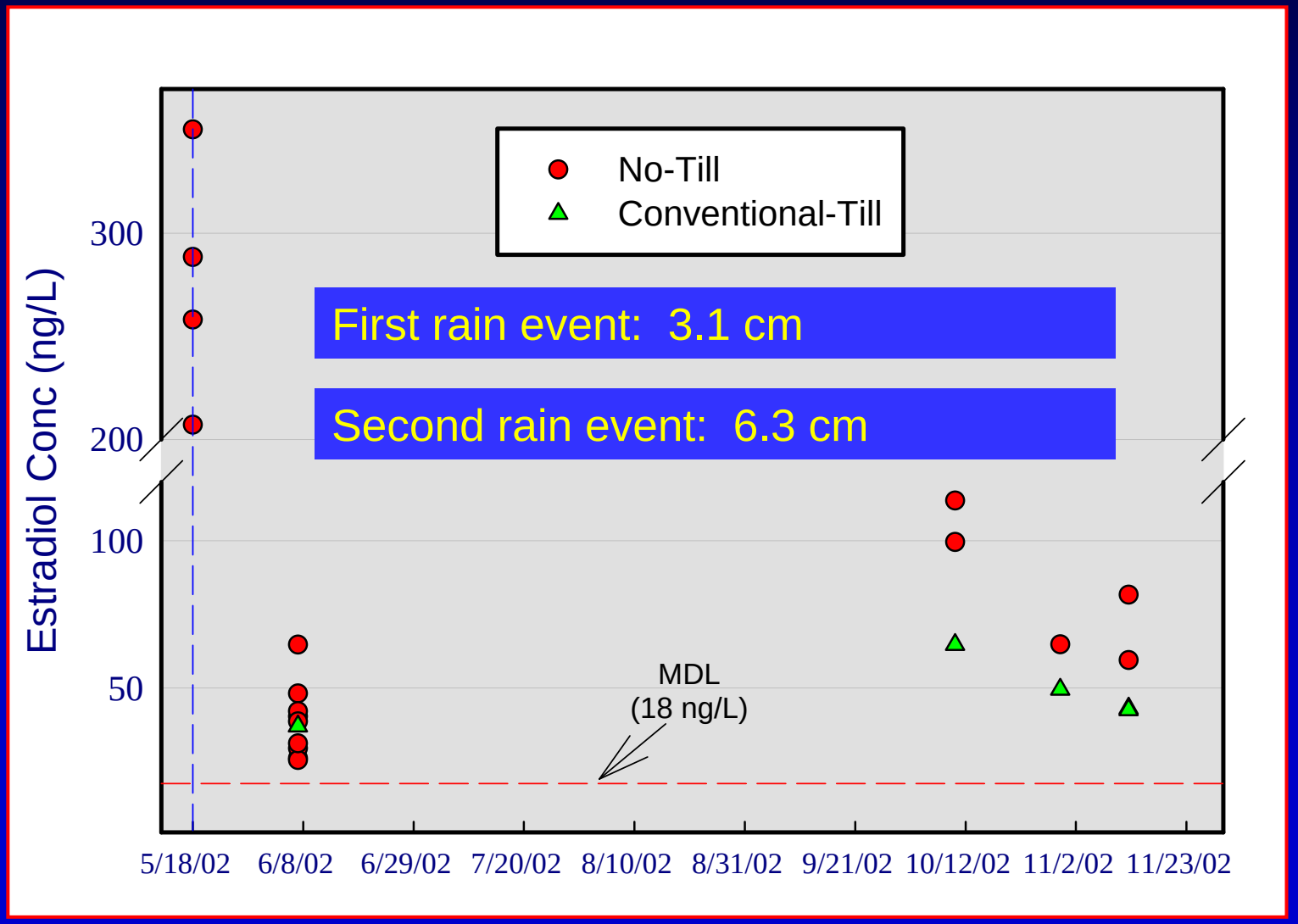
Controlled Field Investigation

Estradiol in runoff following *Spring 2000* litter application



Controlled Field Investigation

Estradiol in runoff following *Spring 2002* litter application



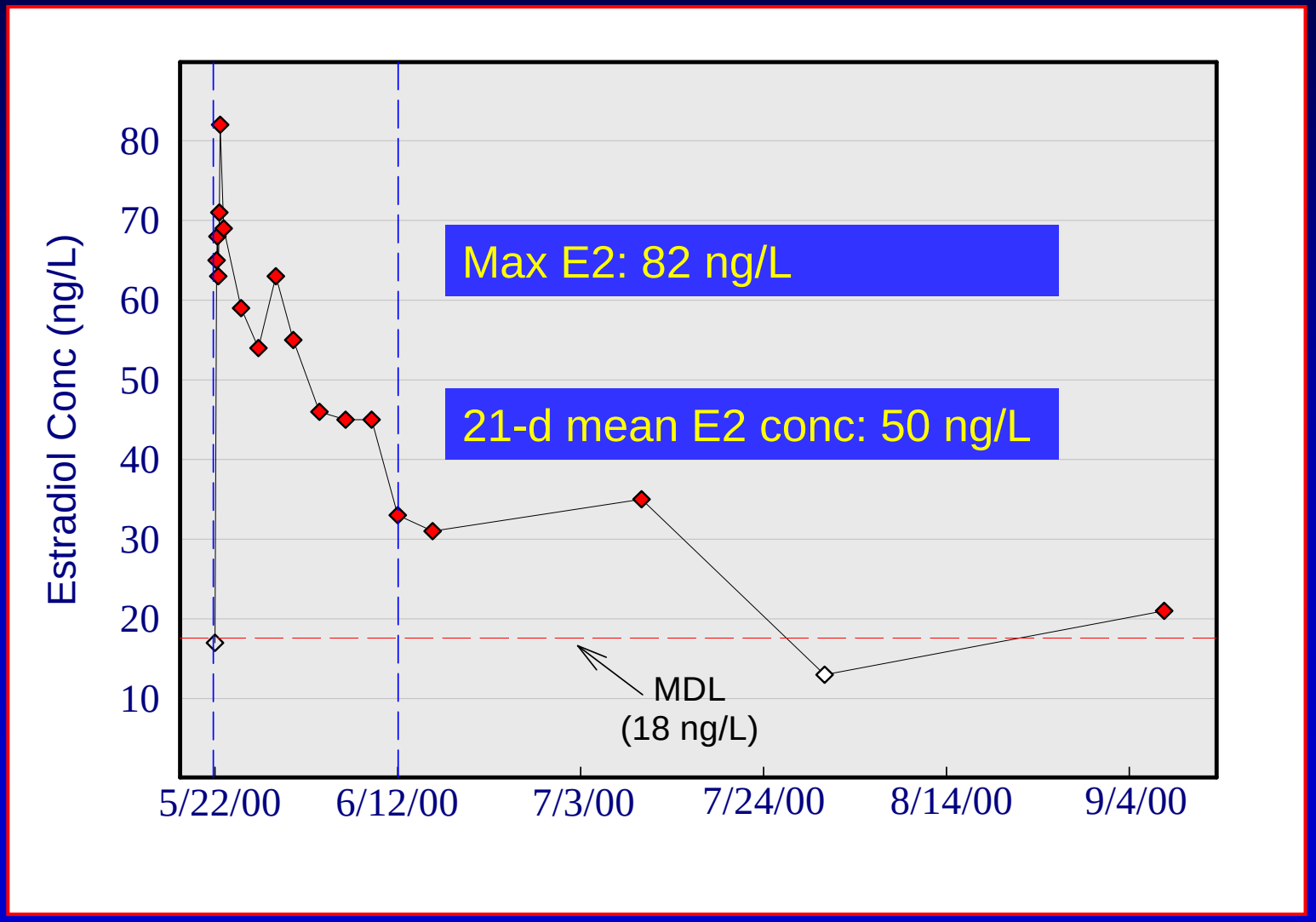
Controlled Field Investigation

- Fecal steroids readily transport from litter-amended fields to surface waters via runoff
- Amount transported was a function of precipitation frequency/intensity and of tillage practice employed
- Steroid transport under NT practices was 2x - 10x greater than under CT
- Additionally we have found, with Ken Staver, that the same trend holds for N, P, As, and Cu. That is, greater transport from field from No-till than Conventional till, especially after the first heavy rain event.

Controlled Field Investigation

Controlled Field Investigation

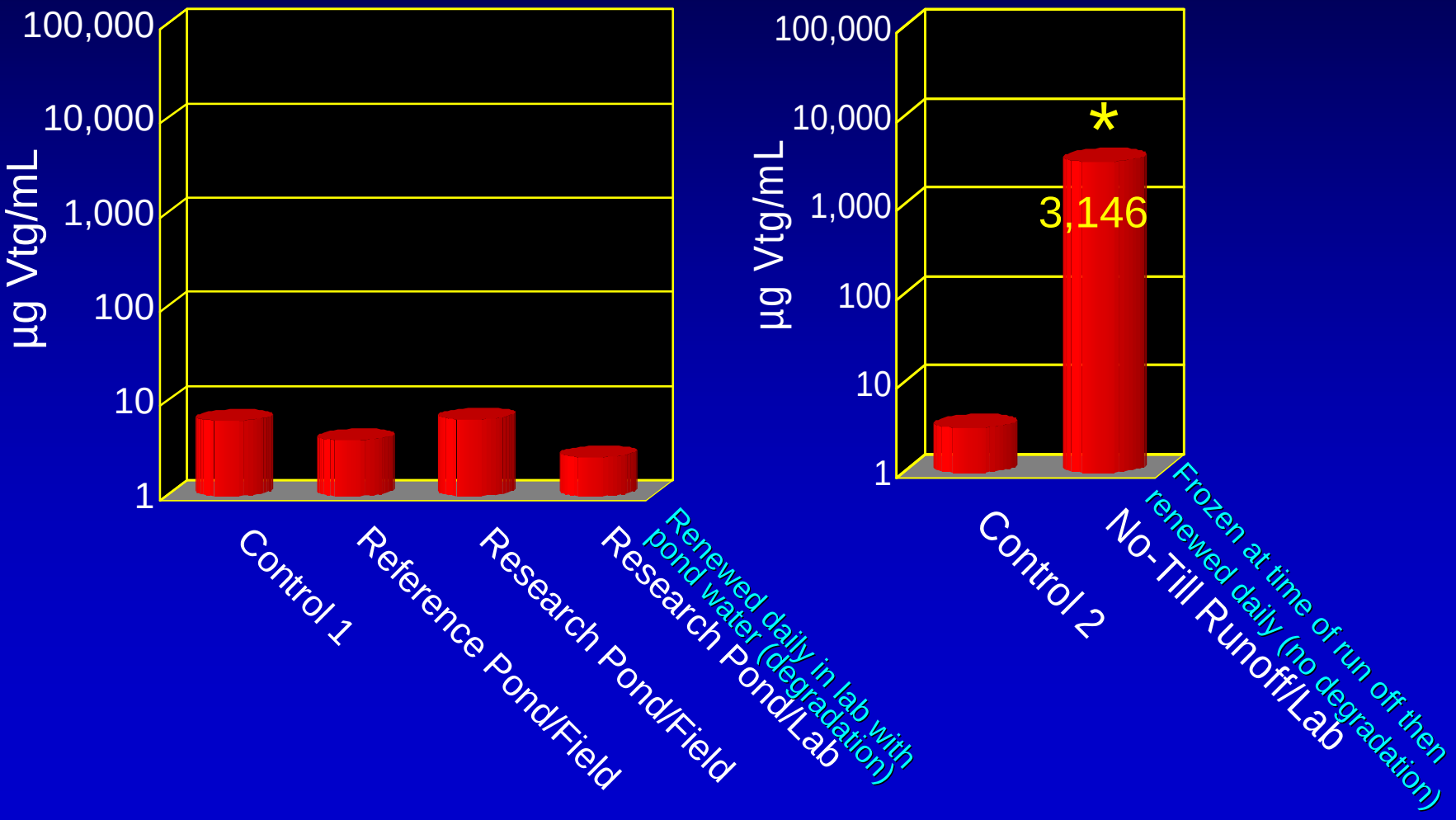
Estradiol in the retention pond following *Spring 2000* litter application



Runoff / Retention Pond Exposures

(Exposed for 21-d either in receiving ponds or lab)

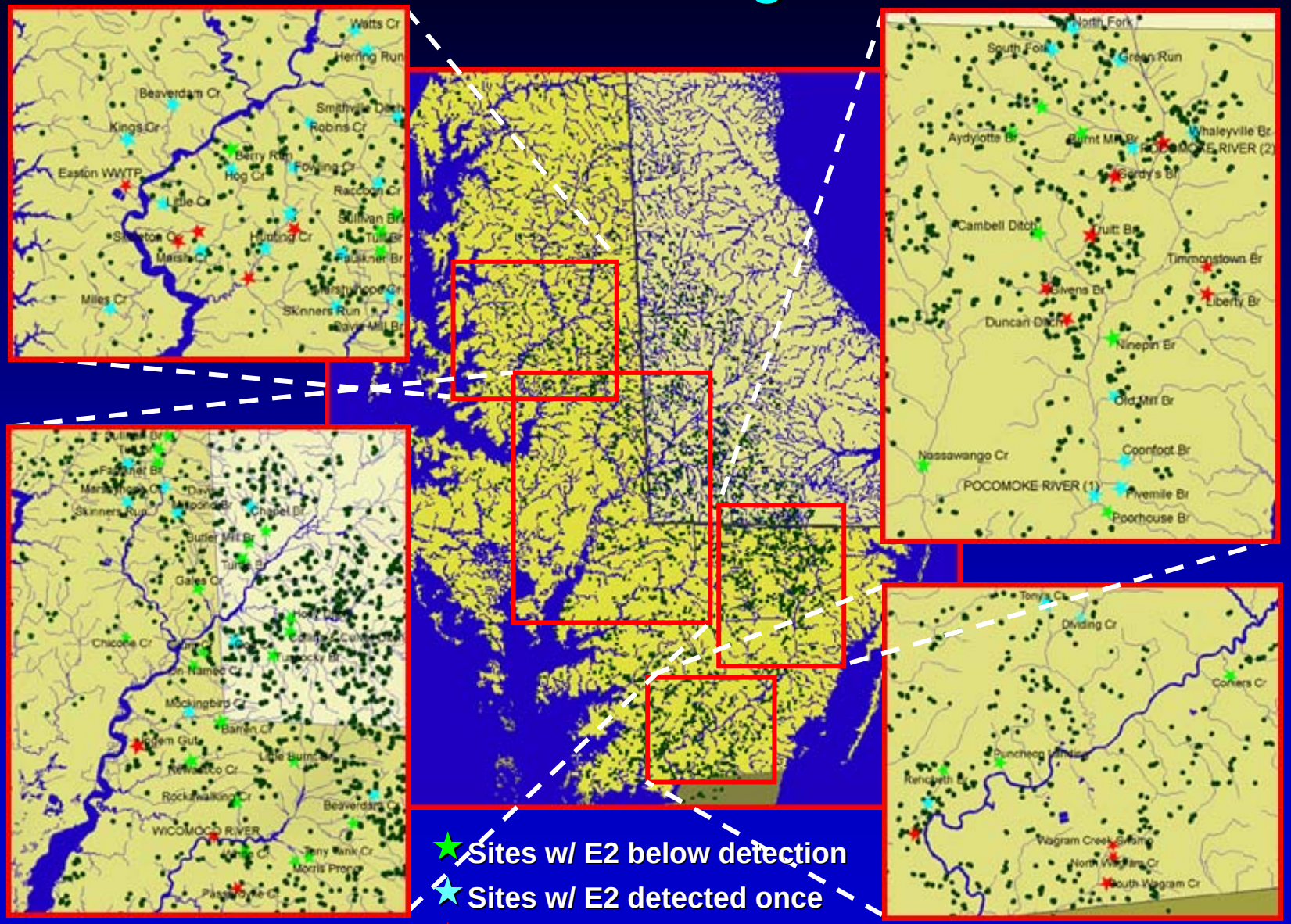
Plasma Vtg in Adult Males



Controlled Field Investigation

- Persistence of litter-derived E2 in surface waters on the order of weeks to months
- Sustained E2 levels greater than 40 ng/L definitely possible
- Runoff from litter-amended fields capable of inducing ED in fish (frozen sample)
- However, dilution and natural “aging” reduce estrogenicity

Field Investigation



★ Sites w/ E2 below detection

★ Sites w/ E2 detected once

★ Sites w/ E2 detected more than once

● Poultry houses (within Chesapeake

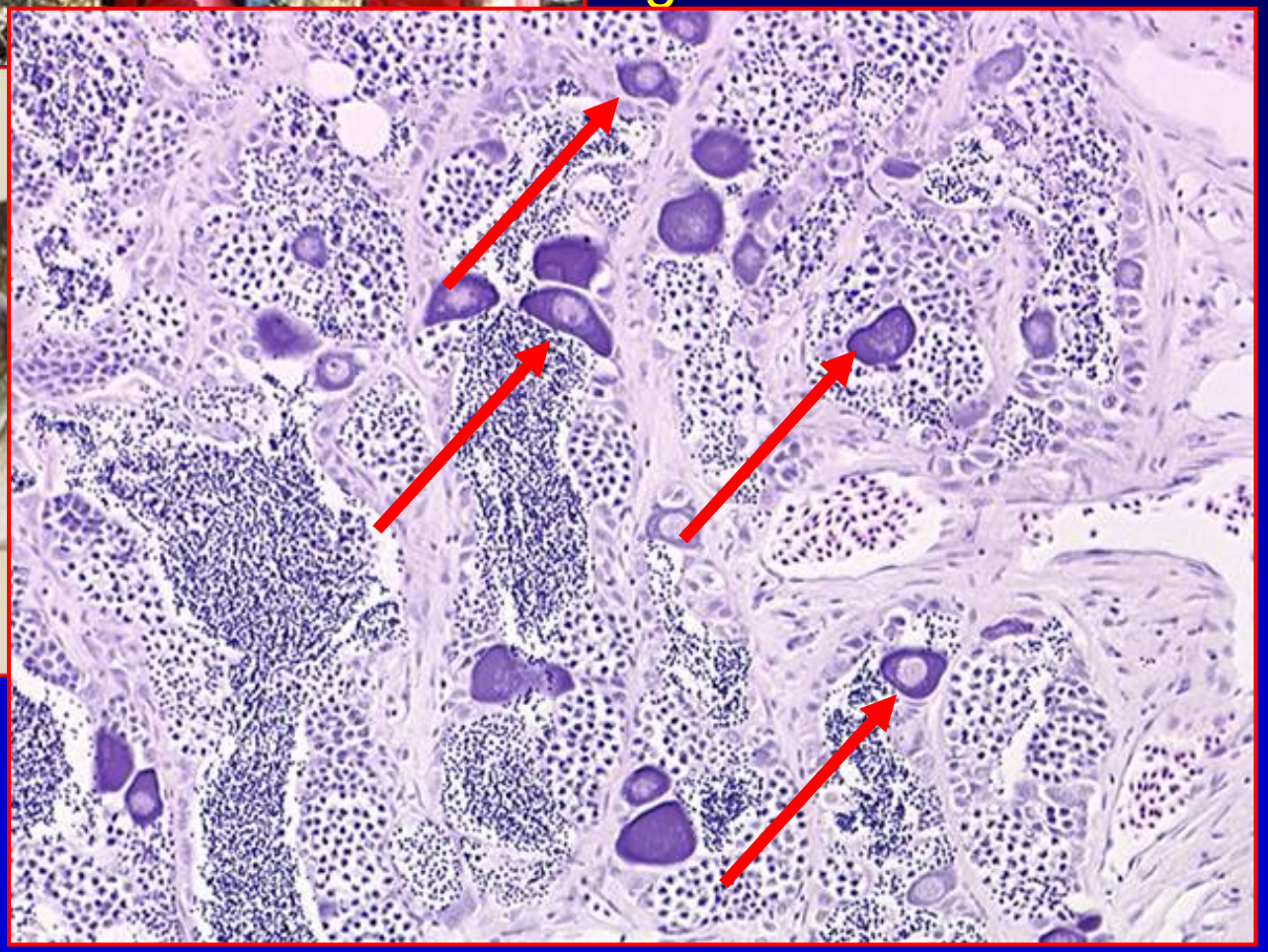
Field Investigation

Field Investigation

- Of sites sampled during SPRING RAIN EVENTS shortly after poultry litter application:
  - 60% had detectable E2 (≥18 ng/L)
  - Flowing streams: 18 – 45 ng/L
  - Ephemeral ponds: <245 ng/L
- Only 15% of sites sampled during LATE SUMMER LOW-FLOW CONDITIONS had detectable E2
- WWTP receiving waters had high E2 concentrations during both high- and low-flow periods
  - Range: 70 -112 ng/L

Field Investigation

n 15 sites in
eds during the
ring and summer



Slide showing intersex in largemouth bass taken from Potomac River by Vicki Blazer of USGS

Field Investigation

- Fish and frogs were collected from 15 sites in Pocomoke and Choptank watersheds during the fall of 2005 / 8 sites during the spring and summer of 2006
- Gonads (♂ & ♀) were examined histologically for ED-related pathology
- Plasma samples (♂ only) were analyzed for Vtg
- One of problems is that we don't get smallmouth bass in shore streams (not natural habitat) and we were able to only sample small numbers of largemouth bass because streams are so small.

Unanswered Questions from earlier work

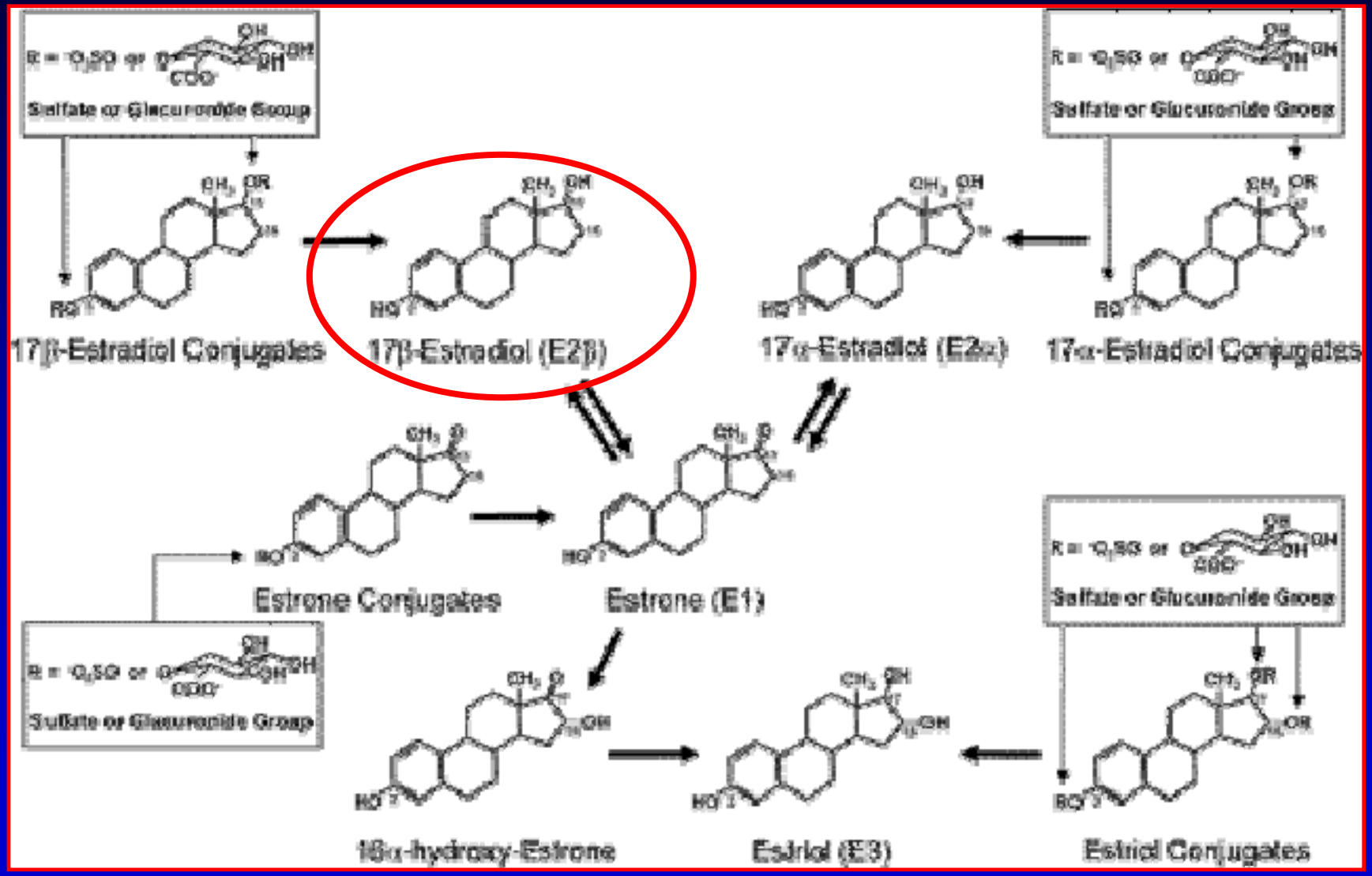
- Why are fish feminized in lab exposures but not in controlled field exposures?

- What are we actually measuring?

Ongoing Investigations
(EPA-STAR Grant: 2007-2010)

- Fully characterize sex steroid constituents
  - Poultry litter prior to field application (broilers and hens)
  - Runoff from litter-amended fields under various cropping strategies
  - Agricultural receiving waters
- Determine the fate / degradation pathways of fecal sex steroids
  - Laboratory generated aqueous mixtures
  - Runoff from litter-amended fields
  - Agricultural receiving waters

EPA-STAR Grant: 2007-2010



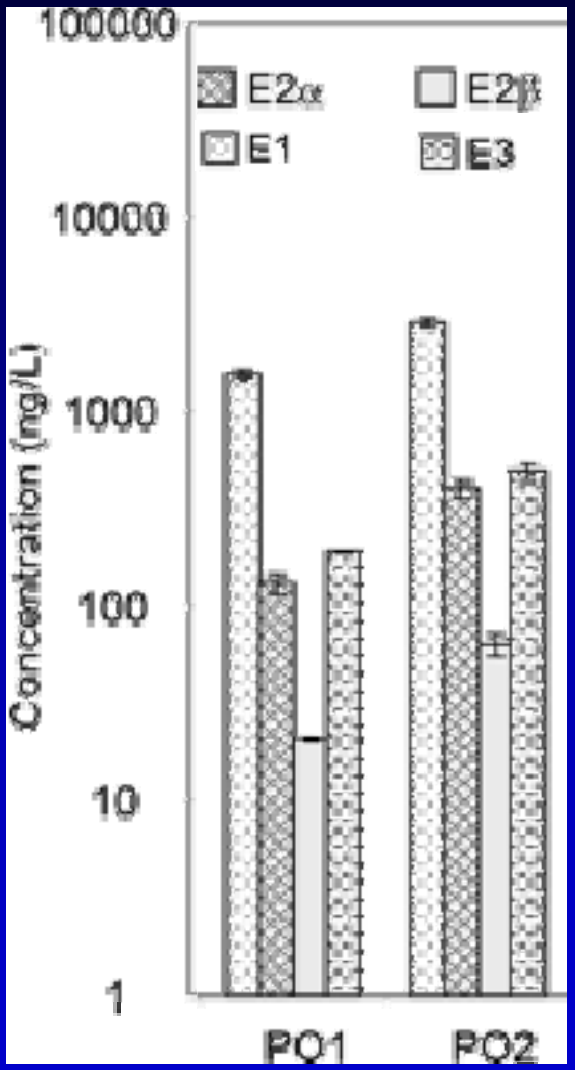
Ongoing Investigations –Chemistry always seems to be the big stumbling block ($$$$)

Estradiol Radioimmune Assay (RIA) Analysis Method

Specificity

- Measure Estrone
- Quantify free and conjugated steroids by solid phase extraction of aqueous samples before and after B-glucuronidase / sulfatase deconjugation
- Validate RIA results by analyzing a subset of laboratory and field collected samples using LC/MS/MS

	100%
[illegible]	17%
	4%
	0.57%
	0.1%
	0.1%
	0.07%
	0.03%
Aldosterone	<0.01%
	<0.01%
	<0.01%
	<0.01%
	<0.01%
	<0.01%
	<0.01%
17α-Hydroxyprogesterone	<0.01%



EPA STAR agreement has money available for extensive chemistry

EPA-STAR Grant: 2007-2010

- Investigate changes in steroidal activity in aqueous poultry litter mixtures over time
  - In vivo laboratory adult (Vtg) and larval (gonad histology) fish assays
  - Controlled field and in situ cage exposures
  - In vitro mammary cell estrogenicity assays
- Investigate relative steroidal activity of free vs. conjugated estrogens with fathead minnows
- Investigate the influence of testosterone at mitigating estrogenic exposure to fish
  - As mentioned earlier poultry litter has both T and E2 with E2 present at higher concentrations. We will use mixtures of E2 and T to investigate these interactions. We have noticed that different litters vary in the E2 to T ratio.

EPA-STAR Grant: 2007-2010

- Investigate individual / population / community level effects of poultry litter-associated steroids in aquatic habitats of the Eastern Shore of MD
  - Collection of additional fish and frogs from ag impacted waters for Vtg and histology (redbreast sunfish)
  - Coordinate with ongoing Maryland Biological Stream Survey (MBSS) conducted by MD DNR - fish IBI, invertebrate IBI, physical habitat condition indices, water quality, and land use patterns to determine stream health.
  - Compare MBSS scores from streams with high E2 concentrations to “reference” streams
- Investigate the utility of sub-surface poultry litter application as a means of reducing contaminants in runoff (**steroids**, **nutrients**, and possibly others if collaborators can be found (arsenic would be nice!)

EPA-STAR Grant: 2007-2010

Prototype Sub-Surface Poultry Litter Applicator

Post emergent

EPA-STAR Grant: 2007-2010

Cuts trench, applies litter, closes trench and compacts

- We will also work with a researcher at the Wye Research and Education Center who is looking at various cropping strategies to reduce N and P runoff on a small plot and whole field basis.
  - They will collect runoff samples from their plots for us for steroid analyses (if it ever rains!!!!)
  - They have money in their grant to pay for these analyses.
  - Cropping strategies – broadcast poultry litter with different tilling practices:
    - Disk/chisel/disc CT
    - Zone till – Till 15” around corn and 50% NT
    - Vertical (Turbo Till) – fluted disk with spikes – mixes litter 2 to 3” deep with soil. Appears like NT.
    - NT

Thanks for your attention