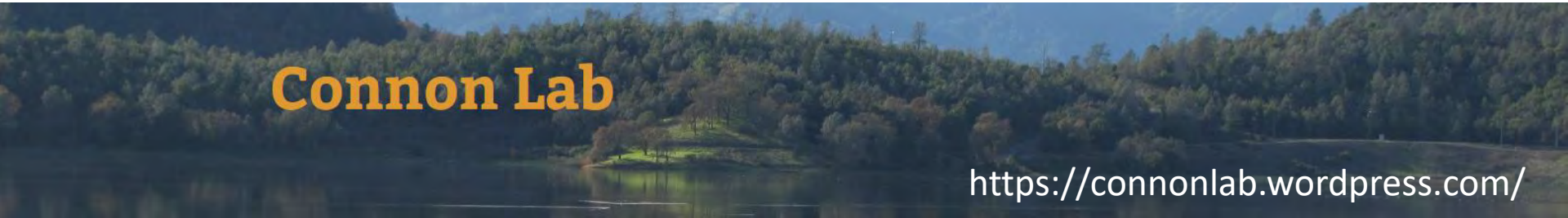
Bioanalytical techniques and thresholds (*In vivo*)

Richard E. Connon
Associate Adjunct Professor
School of Veterinary Medicine
Department of Anatomy, Physiology and Cell Biology
University of California, Davis

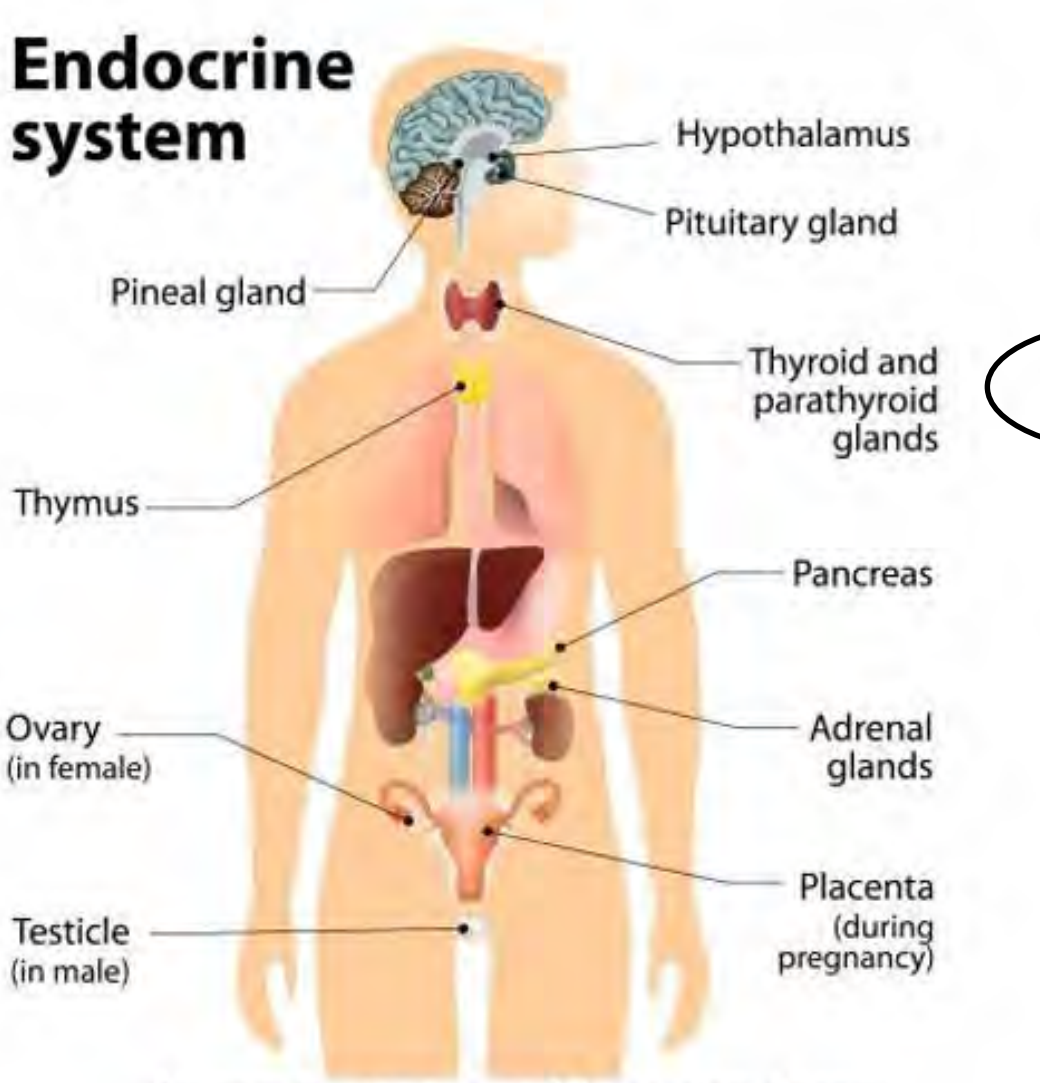
reconnon@ucdavis.edu

UCDAVIS VETERINARY MEDICINE

CEC Workshop May 1, 2017

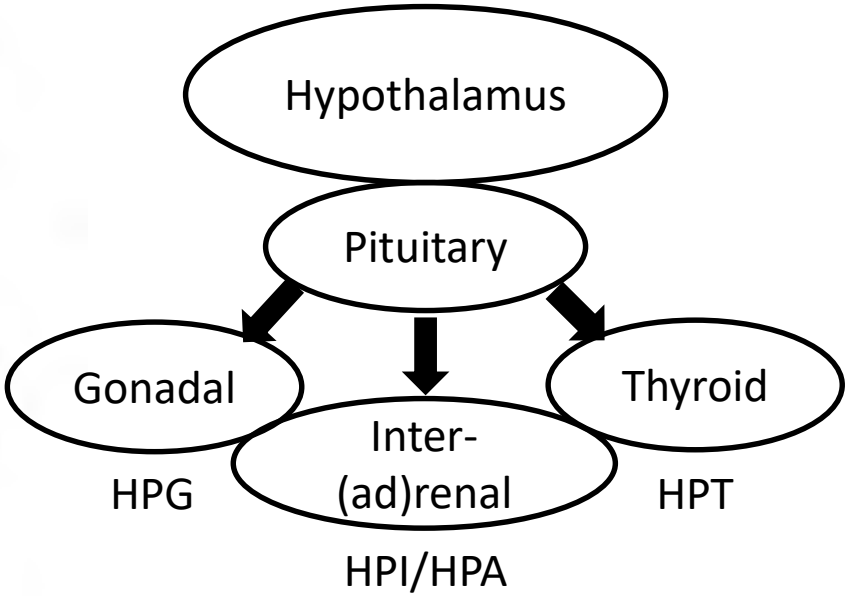


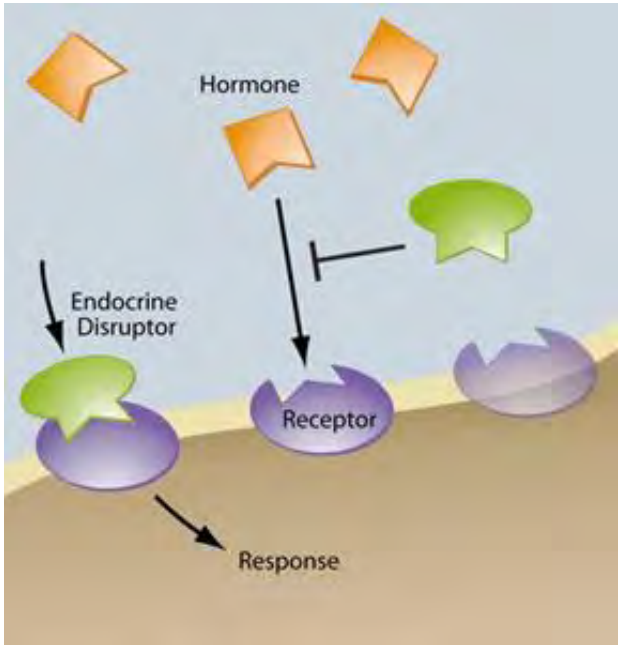
REPRODUCTIVE VS. NON-REPRODUCTIVE

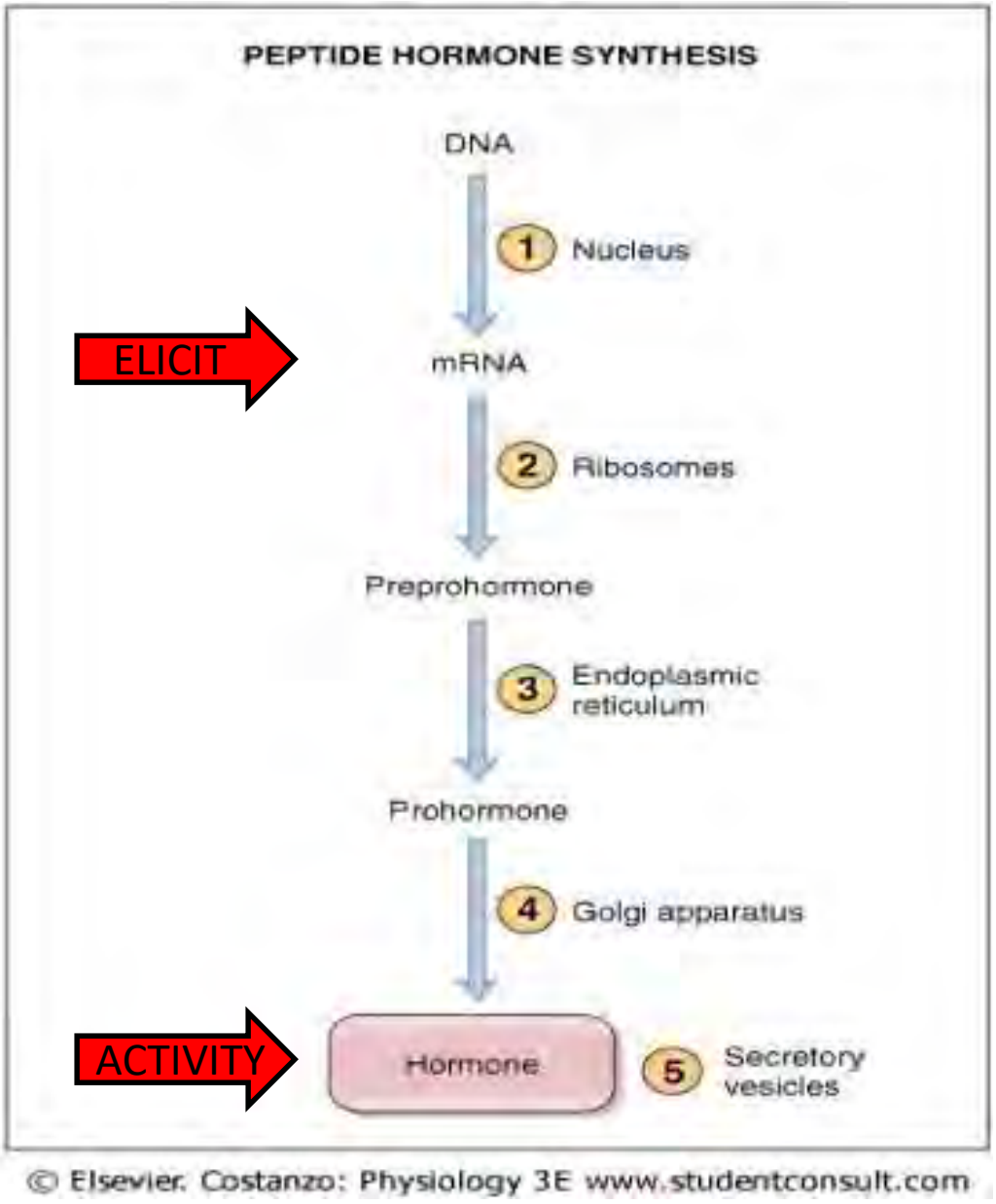


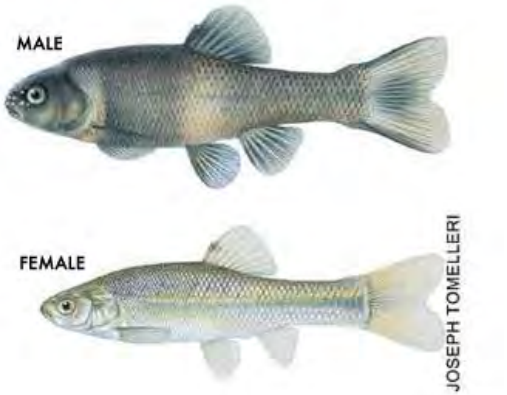
Source: Environmental Protection Agency

www.epa.gov/endocrine-disruption/what-endocrine-system





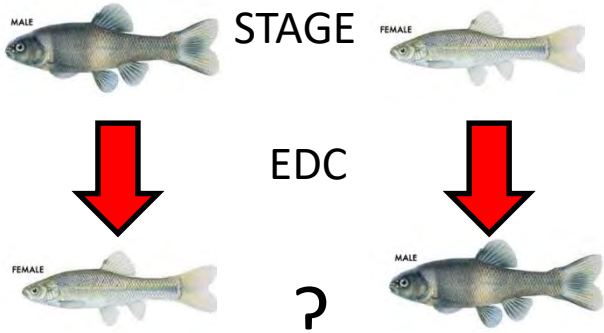




EXPOSED AS ADULTS

Reproductive output?

EXPOSED AT EARLY LIFE STAGE



Application of Research to Levels of Organization Based on Source to Outcome

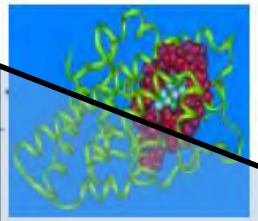
Source

Community

Population

Environmental Contaminant

Exposure



Key Event

Cellular Effects

Individual

Toxicity Pathway

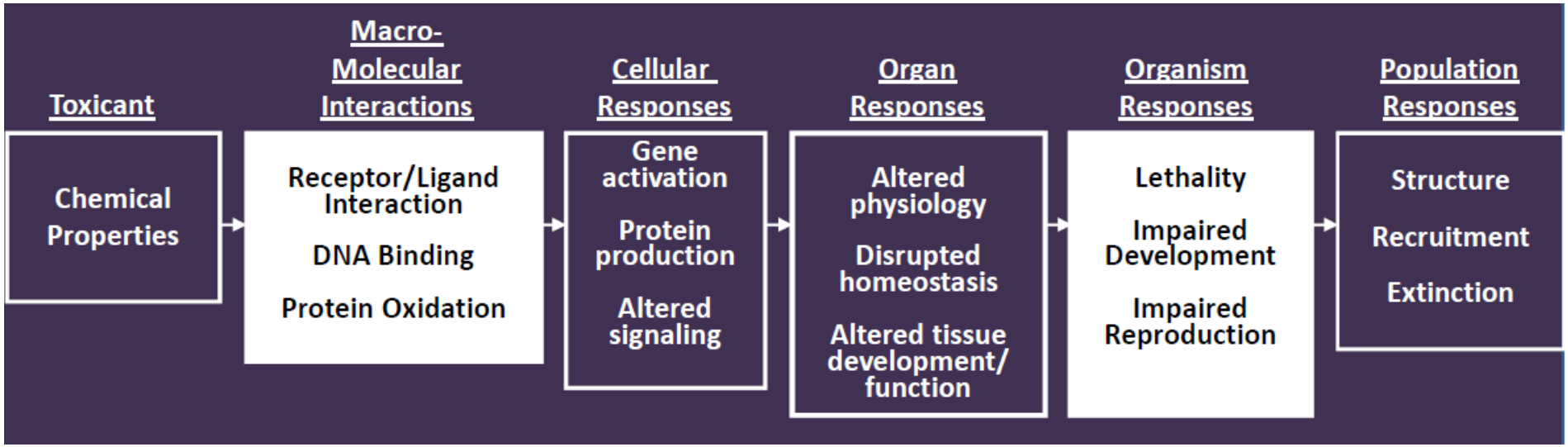
Mode of Action

Adverse Outcome Pathway

Source to Outcome Pathway

Source: Ed. Perkins; http://nas-sites.org/emergingscience/files/2011/08/Perkins.pdf

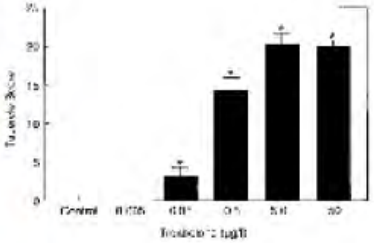
ADVERSE OUTCOME PATHWAY FRAMEWORK



Source: An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment. (Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)

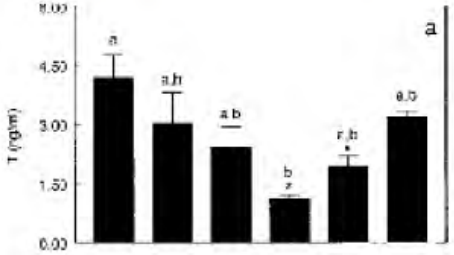
Example: Potent AR Agonists

Adverse Outcome Pathway: Fathead Minnow

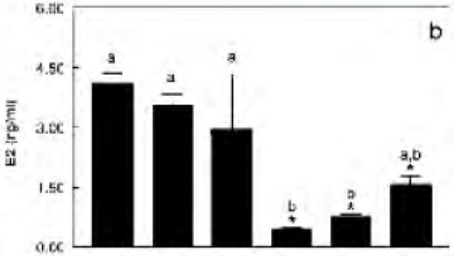


17β-trenbolone (trb-acetate) → AR binding → AR-dependent somatic cell proliferation → Tubercle and fatpad formation in females

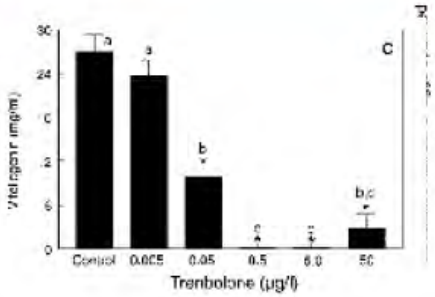
AR binding → Negative feedback, hypothalamic neurons → Reduced steroidogenesis, vitellogenesis → Reduced fecundity

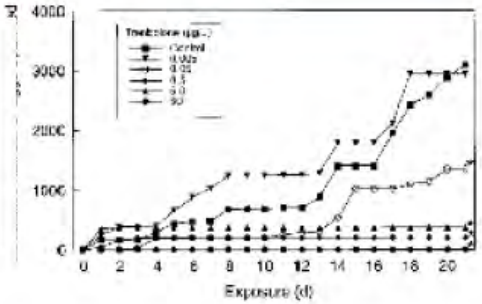


testosterone



estradiol





vitellogenin

Adverse Outcome Pathway: Human

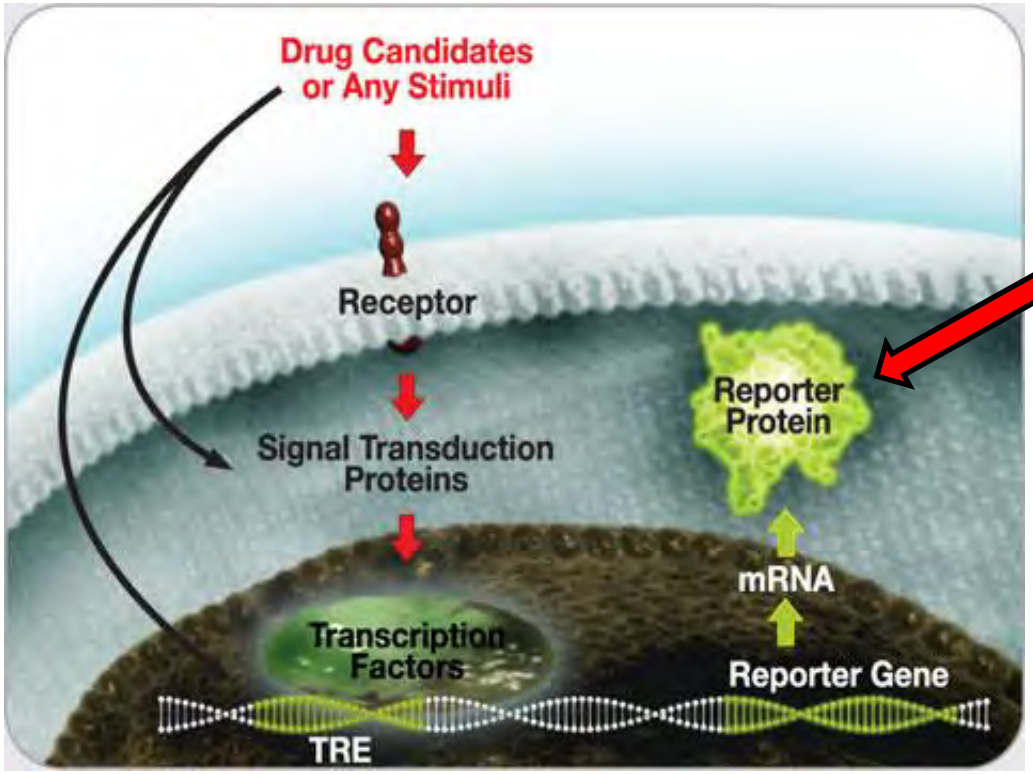
17β-trenbolone (trb-acetate) → AR binding → AR-dependent somatic cell proliferation → Increased body hair in females

AR binding → Negative feedback, hypothalamic neurons → Testicular atrophy, impaired sperm production → Infertility

In vivo Approaches

- Gene Expression: CEC (or metabolite) activates mRNA production to generate Hormones, i.e. Initiation of hormone synthesis mimic.
  - Targeted Quantitative PCR: receptor and/or HPX axis.
- Hormone quantitation/activity: mRNA has led to hormone production
  - Enzyme linked immunosorbent assay (ELISA)/Binding assays: e.g., vitellogenin, choriogenin, testosterone, T3, T4…

Zebrafish model transgenic ER reporter
Live determination of EDC activity

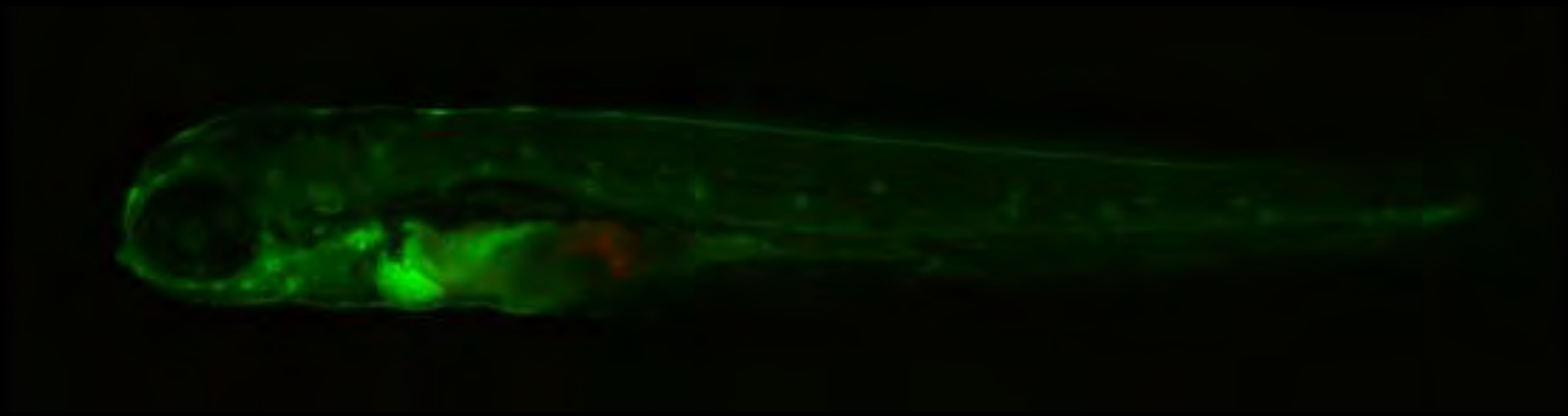


Transgenic line:
cyp19a1a (-/-);Tg(5xERE:egfp)

FLUORESCENCE REPORTER: glows if receptor is activated

NO ENDOGENOUS HORMONE:
Only external "mimics" activate reporter

Zebrafish model transgenic ER reporter
Live determination of EDC activity



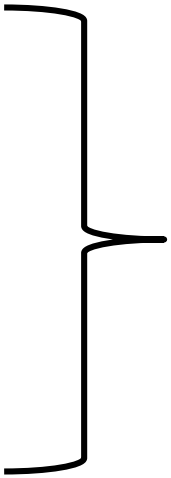
In vivo Approaches

Males expressing of:

female hormones

Females expressing

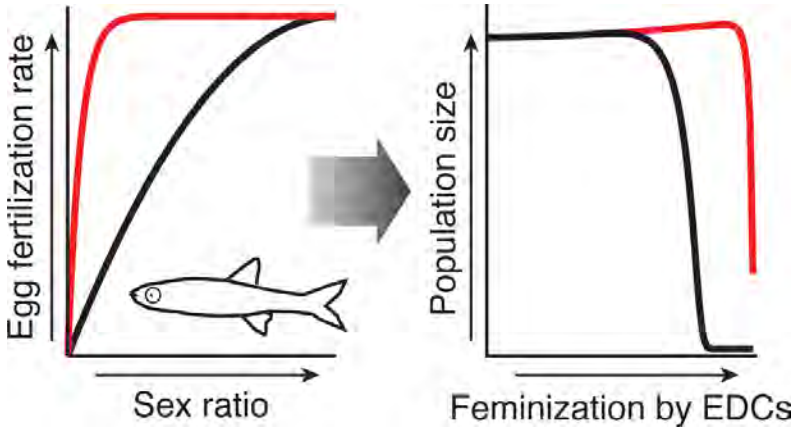
male hormones



Impacts contribution of the individual to the population

Population effects

- Fecundity: emergence/number of offspring
- Sex ratios: male:female skewness
- Epigenetics: parental transfer.
  - MethylSeq – DNA methylation



White J.W., Cole B., Cherr G., Connon R.E. and Brander S. (2017). Scaling up the individual-level effects of endocrine disruptors: how many males does a population need? Environmental Science and Technology, 51(3): 1802–1810.

In vivo methods are crucial in identifying the connection between exposure and biological effects

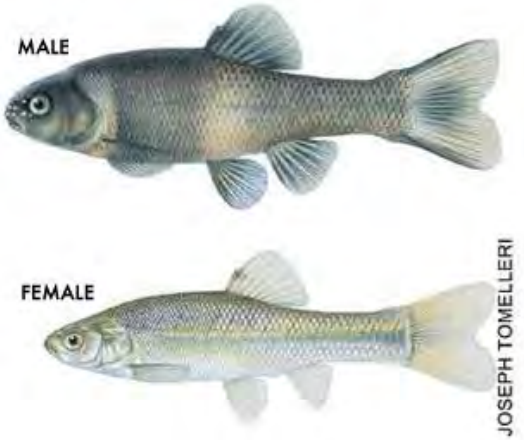
Pros:

- cross-talk between biological pathways,
- environmental influence,
- integration of action through different mechanisms at different tissues
- metabolic transformations, bioaccumulation, and homeostatic controls

However (Cons):

- inter-individual, seasonal, and temporal variability
- expensive, cannot accommodate high throughput screening.

Screening with *in-vitro*



Verification with *in-vivo*

THANK YOU!

Questions?

https://ntp.niehs.nih.gov/iccvam/docs/endo_docs/expertpanfinalrpt/panelrpt1102.pdf

- The proposed EDSP consists of a Tier 1 screening battery of tests that is designed to identify substances capable of interacting with the endocrine system, and different Tier 2 testing assays that are designed to confirm and extend the Tier 1 results. If, based on a weight of evidence evaluation of the results from the Tier l screening battery, the test substance is identified as a potential endocrine disruptor, Tier 2 *in vivo* tests are conducted to provide detailed information on concentration response relationships and specific abnormal effects that may result. The proposed Tier 1 *in vitro* assays include estrogen receptor (ER) and androgen receptor (AR) assays. Currently, the U.S. EPA proposes that either a binding assay or a transcriptional activation (TA) assay be used. These *in vitro* assays are relevant for screening purposes because they might identify substances that alter natural endocrine processes by binding with estrogen and/or androgen receptors, resulting in agonist and/or antagonist activity.
- The Panel recommended that a sequential testing strategy be evaluated for utility during the pre-validation of *in vitro* ER/AR binding and TA agonism/antagonism assays. In this approach, if a substance induces a positive response in any assay, then testing in any of the other binding/TA assays would not need to be conducted. In support of this strategy, the Panel concluded that further classification of the activity of a positive test substance using additional binding/TA endpoints would provide little additional information that would assist with prioritization and the design of subsequent *in vivo* studies.
- Panel recommended determination of the predictive value of these assays for estimating *in vivo* responses. Therefore, the Panel recommended that substances proposed for validation of the *in vivo* test methods should also be evaluated in the *in vitro* assays included in the screening battery and, to the extent possible, vice-versa.
- the *in vivo* endocrine disrupting activity of a chemical would most likely be tissue-, cell-, and promoter-specific. Therefore, the intrinsic responsiveness of a cell line cannot be generalized based on the result of a single assay system, due to the potential differences in co-activator populations, cross-talk with other receptors, and other signal transduction pathways between cell types.
- There is a need to assess the ability of these *in vitro* screens to predict *in vivo* responses. One way to accomplish this is to make sure that substances to be tested in the *in vitro* screens are also tested in the *in vivo* screens and tests so that information and the "weight of the evidence" can be assessed for particular chemicals.
- If a substance induces a positive effect in any of these assays, testing in additional *in vitro* ER and AR binding or TA agonism/antagonism assays should not be conducted before proceeding to short term Tier 1 *in vivo* studies.
- It is recognized that agonists working through this *in vitro* mechanism may be false positives compared to *in vivo* results. Ideally, the *in vitro* assays should predict *in vivo* activity.