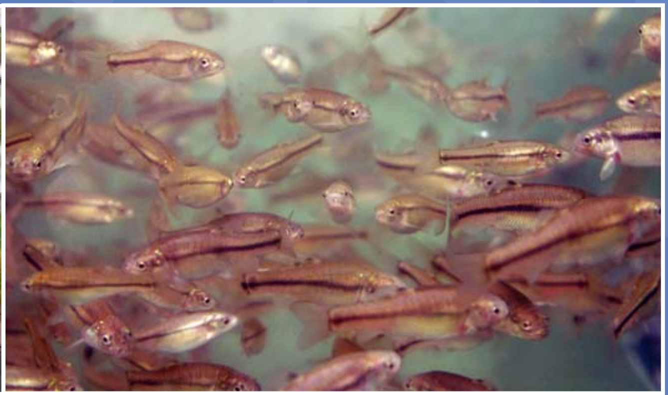
United States
Environmental Protection
Agency

Endocrine Disrupting Chemicals: New Tools for Assessing Ecological Risk

G. Ankley



Office of Research and Development
NHEERL, Mid-Continent Ecology Division, Duluth, MN

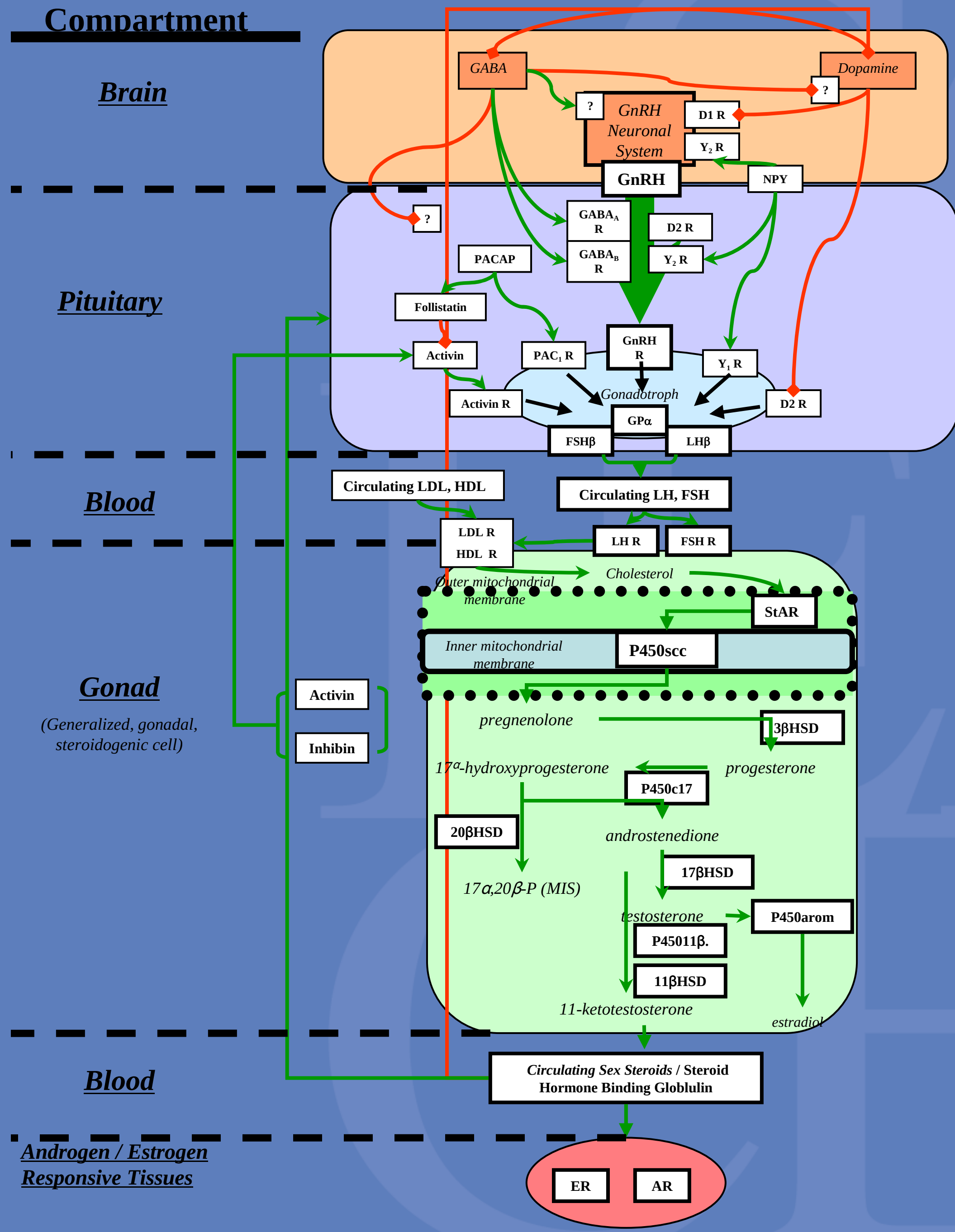
February 25, 2009

What are Endocrine-Disrupting Chemicals (EDCs)?

"Exogenous agents that interfere with the production, release, transport, metabolism, binding, action or elimination of natural hormones in the body responsible for the maintenance of homeostasis and the regulation of developmental process (Kavlock et al. 2007)"

Emphasis, to date, on mechanisms affecting reproduction and development via the hypothalamic-pituitary-gonadal (HPG) and HPT (thyroidal) axes

Generalized Vertebrate HPG Axis



United States Environmental Protection Agency

Postulated EDC Effects: Humans

- Cervical cancer (DES daughters)
- Breast Cancer
- Prostate/testicular cancers
- Learning/neurological deficits
- Decreases in sperm quality
- Precocious maturation

United States Environmental Protection Agency

Postulated EDC Effects: Wildlife

- Hermaphrodism in gastropods
- Developmental abnormalities in GL fish and birds
- Malformations in amphibians
- Feminization of reptiles
- Behavioral changes in birds
- Reproductive abnormalities in fish-eating mammals
- Feminization/masculinization of fish

United States Environmental Protection Agency

Assessing Ecological Risks of EDCs

Emphasis has been on predicting possible effects of new chemicals rather than diagnosing impacts in field, where several very challenging questions exist

-Analytical (What to measure at what detection?)

-Evaluation of mixtures (Endocrine MOA of concern? Interactive effects?)

-Biological endpoints (Biomarker vs. apical?)

-Population-level responses (How to discern?)

Tools Needed for Assessing Risk of EDCs in the Field

- Short-term *in vitro* and *in vivo* assays suitable for complex mixtures
- Analytical/fractionation approaches to identify specific chemicals causing biological effects
- Diagnostic endpoints (biomarkers) indicative of chemical MOA
- Approaches to link diagnostic responses to alterations in individuals and populations

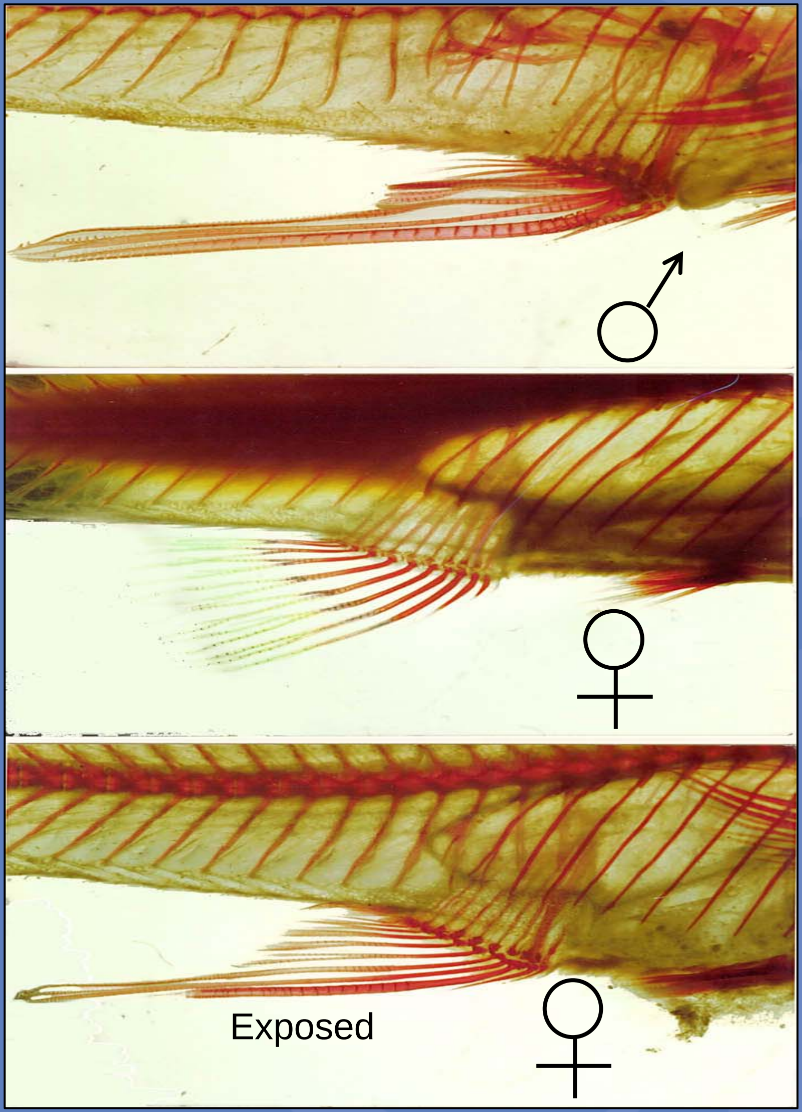
Assessing EDC Mixtures: A Pulp Mill Case Study

Buckeye Plant, Fenhalloway River, FL

Collaborators: G. Ankley, L. Durhan (EPA, MED); E. Gray, P. Hartig, C. Lambright, L. Parks, V. Wilson (EPA, RTD); L. Guillette (Univ. Florida)

United States Environmental Protection Agency

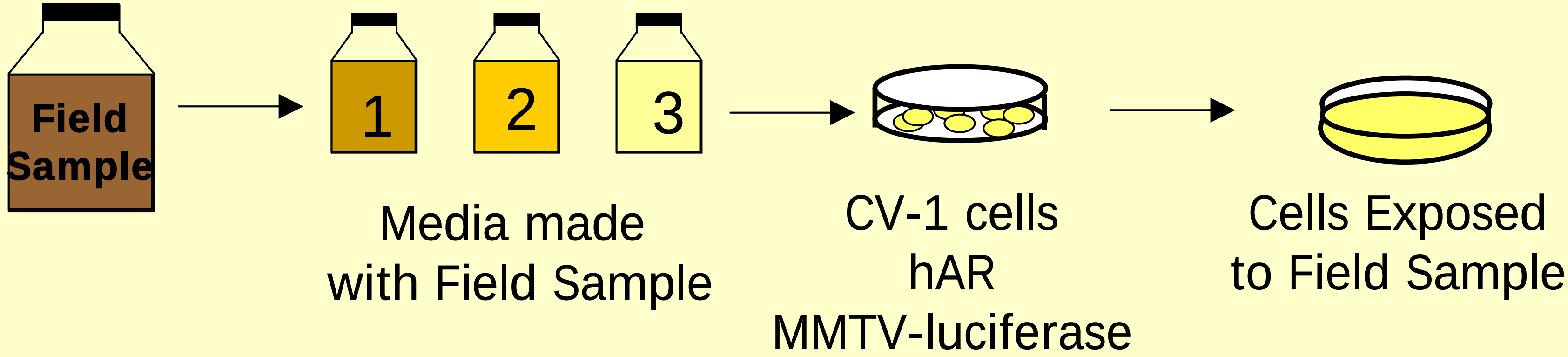
PME Masculinizes Fish in the Field



EPA United States Environmental Protection Agency

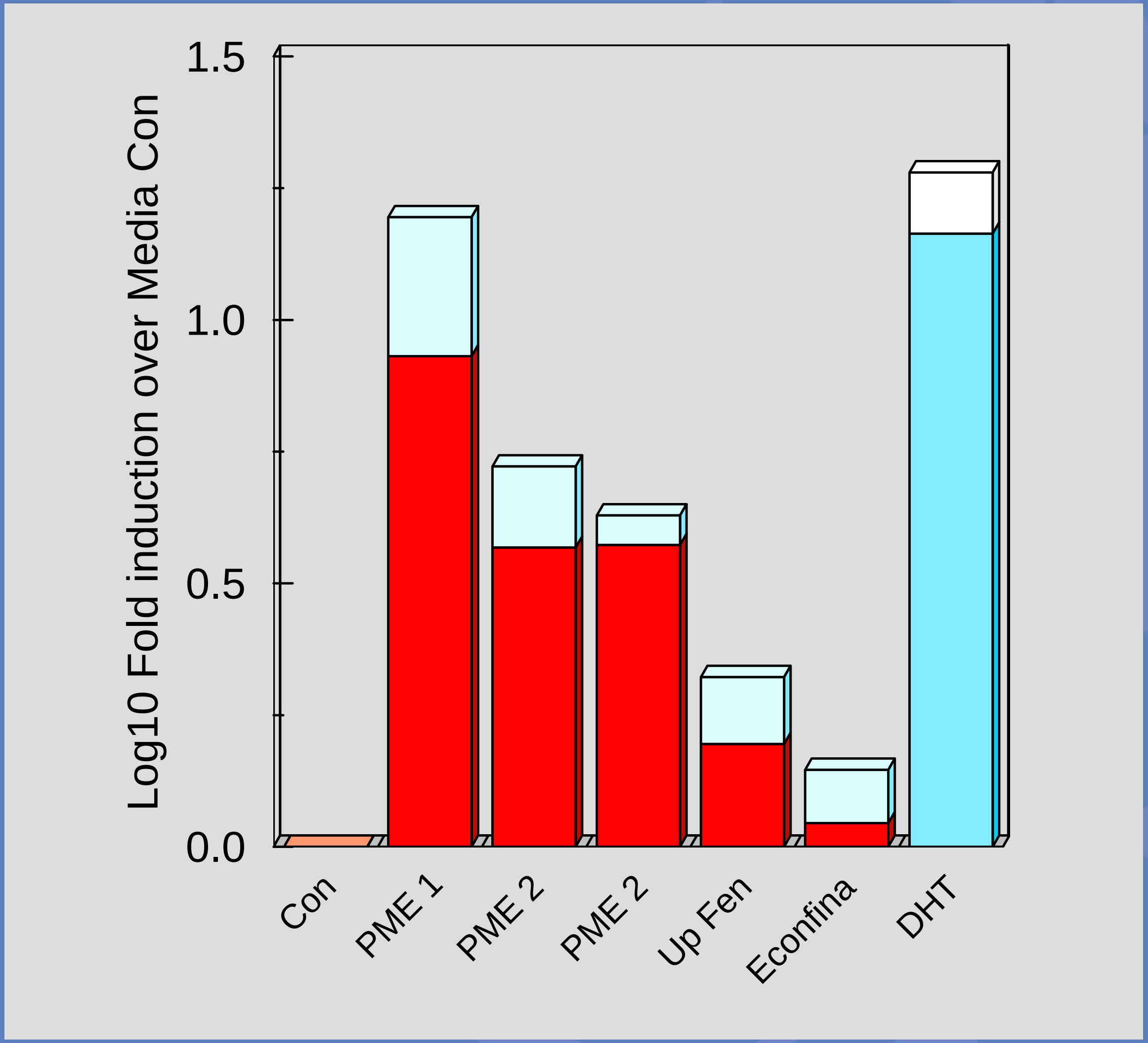
Cell-Based Assays for Detecting EDCs in Mixtures

I.

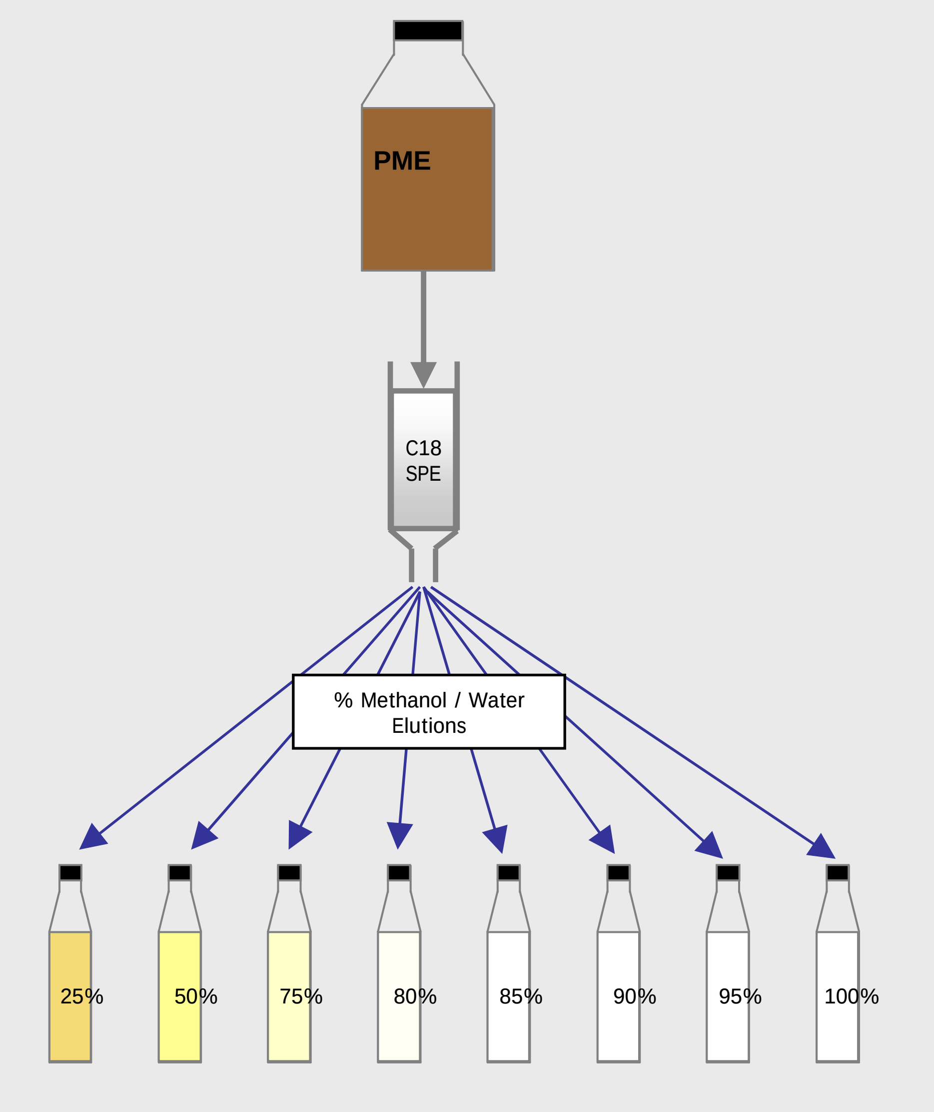


II. Measure Luciferase Activity

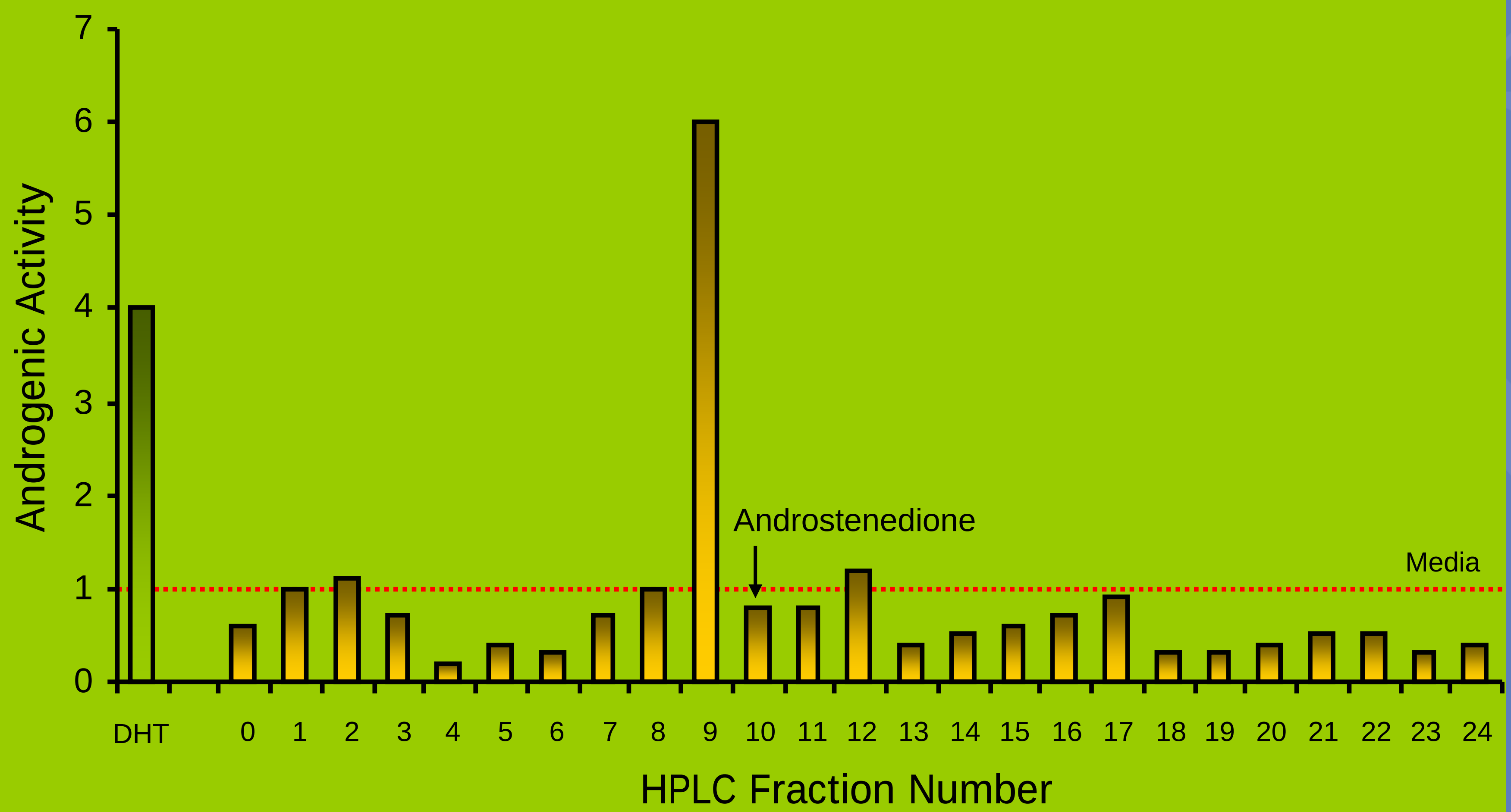
PME Androgenic Activity



Fractionation (TIE) Analysis to Identify EDCs in Complex PME



Linking EDC Activity in Cells to Fractionation of PME



United States Environmental Protection Agency

A Short-Term *in vivo* Test for Detecting Reproductive Toxicity of EDCs

Conducted with fathead minnow, a model species used for research and regulatory work in labs throughout the world

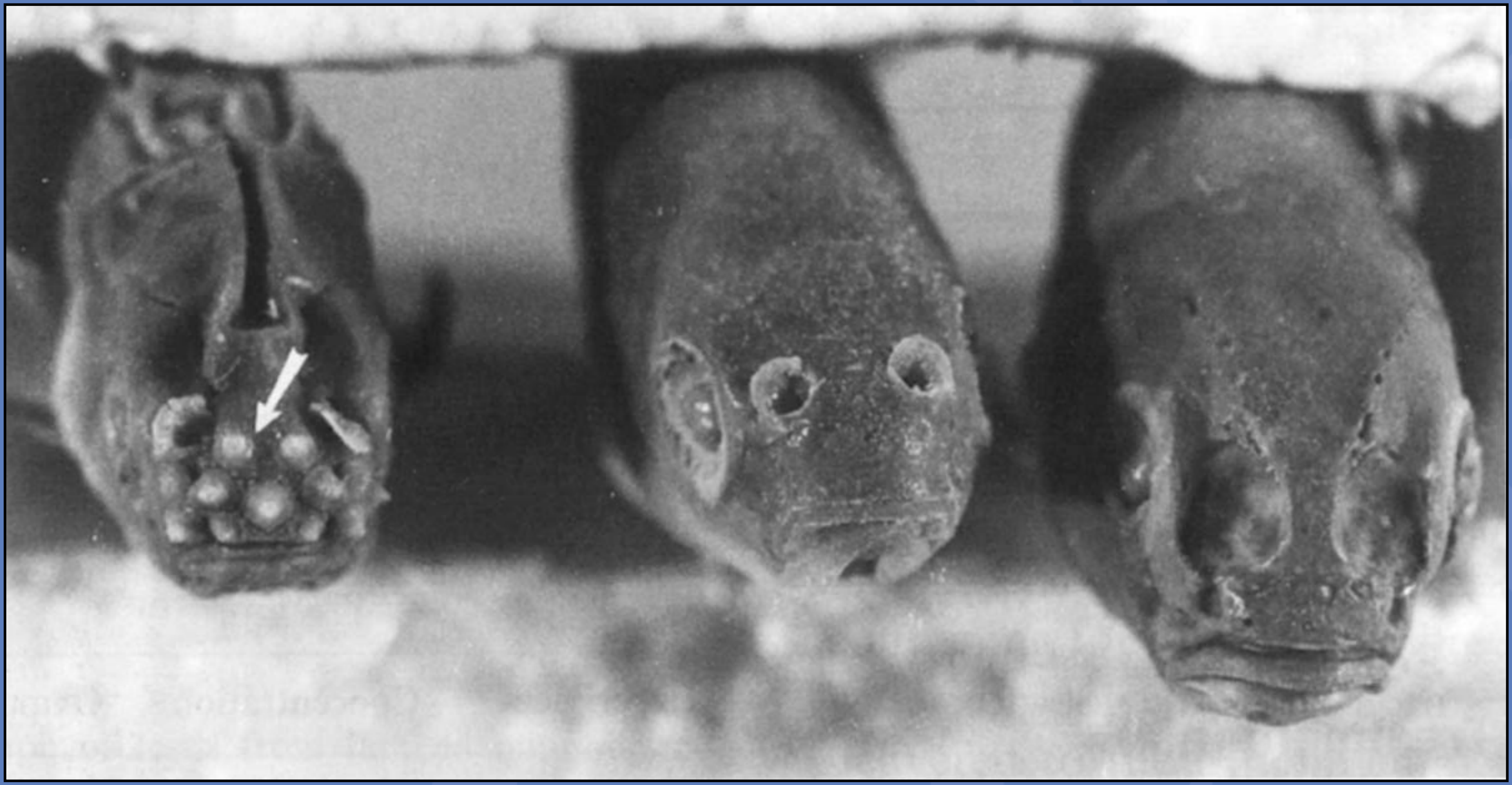
Three-week test includes endpoints reflective of specific classes of EDCs *and* apical responses useful to risk assessment

-Vitellogenin, steroids, SSC, histology

-Egg production, fertility, hatch

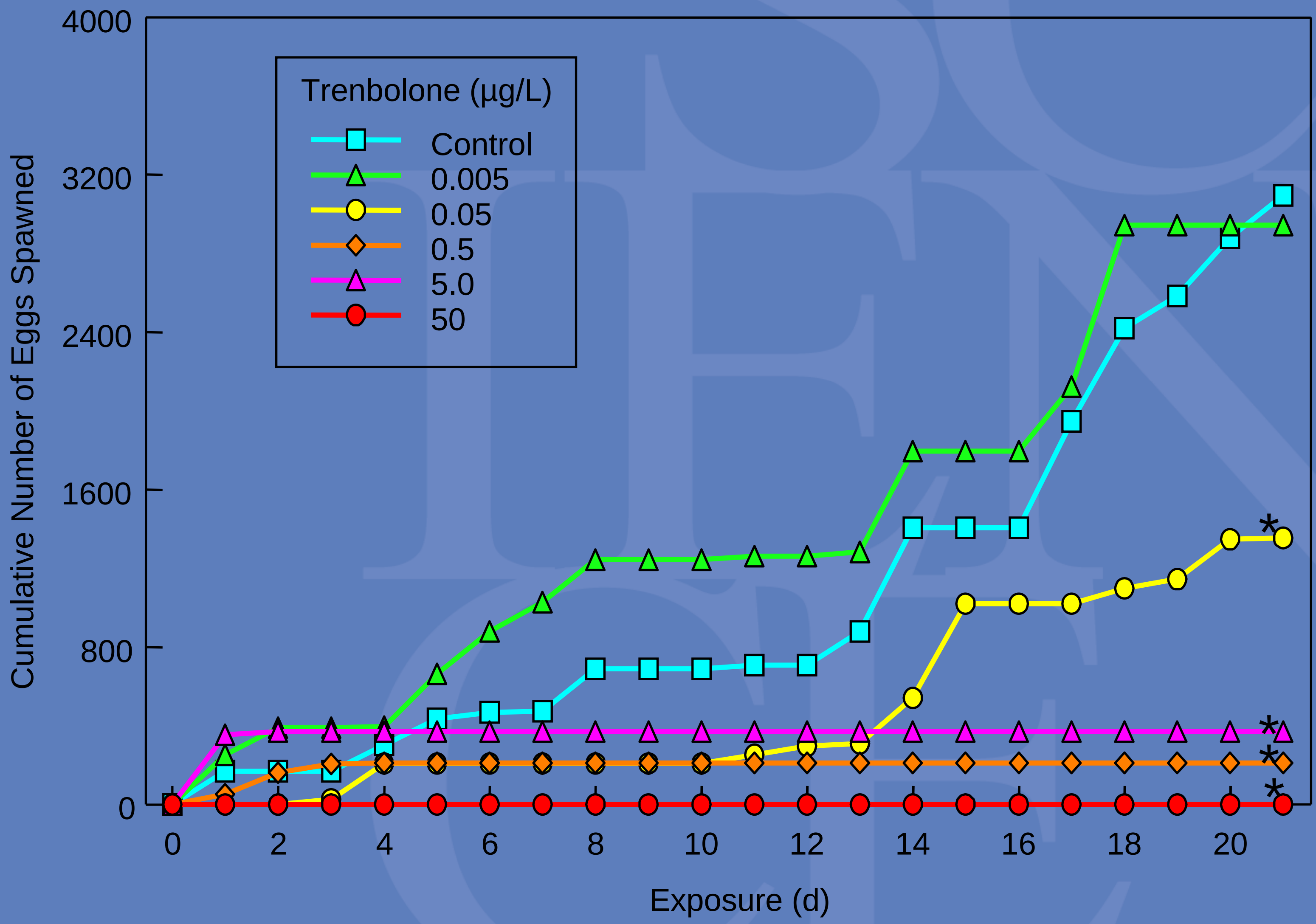
Developed for EPA's EDSP (Endocrine Disruptor Screening and Testing Program), but applicable to assessment of complex mixtures as well

Effects of an Estrogen on Male Secondary Sex Characteristics



Miles-Richardson et al. (1999) Aquat. Toxicol.

Effects of 17β-Trenbolone on Fathead Minnow Fecundity



United States Environmental Protection Agency

Example Applications to Diagnostic Assessments/Monitoring

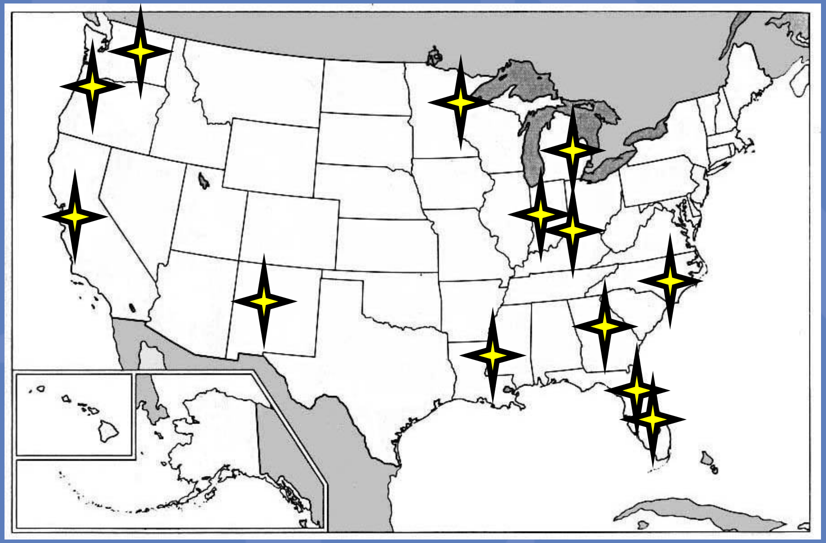
Monitoring Nebraska CAFO (Concentrated Animal Feeding Operation) samples for effects on reproduction and endocrine function (Kolok, Sellin et al.)

Evaluating UK municipal effluents for estrogenicity (Thorpe, Tyler et al.)

Monitoring Canadian pulp/paper mill effluents for reproductive and endocrine impacts (Kovacs, Parrott et al.)

Linkage of Exposure and Effects Using Genomics, Proteomics, and Metabolomics in Small Fish Models

- USEPA – Cincinnati, OH
  - D. Bencic, M. Kostich, D. Lattier, J. Lazorchak, G. Toth, R. Wang,
- USEPA – Duluth, MN, and Grosse Isle, MI
  - G. Ankley, E Durhan, M Kahl, K Jensen, E Makynen, D. Martinovic, D. Miller, D. Villeneuve
- USEPA – Athens, GA
  - T. Collette, D. Ekman, M. Henderson, Q. Teng
- USEPA-RTP, NC
  - M. Breen, R. Conolly
- USEPA STAR Program
  - N. Denslow (Univ. of Florida), E. Orlando, (Florida Atlantic University), K. Watanabe (Oregon Health Sciences Univ.), M. Sepulveda (Purdue Univ.)
- USACE – Vicksburg, MS
  - E. Perkins, N. Garcia-Reyero
- Other partners
  - Joint Genome Institute, DOE (Walnut Creek, CA)
  - Sandia, DOE (Albuquerque, NM)
  - Pacific Northwest National Laboratory (Richland, WA)
  - O. Mekenyan (University of Bourgas)



EPA
United States
Environmental Protection
Agency

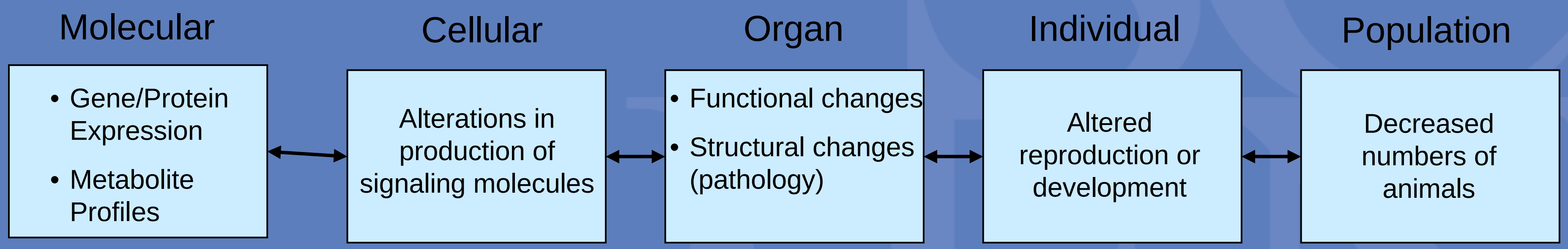
Project Objectives

Investigate effects of EDCs with different MOA using the fathead minnow 21-d test to establish toxicity pathways and ecologically- relevant responses

Identify the genomic responses to the same chemicals using shorter-term exposures to support development of specific biomarkers indicative both toxic MOA and adverse outcome

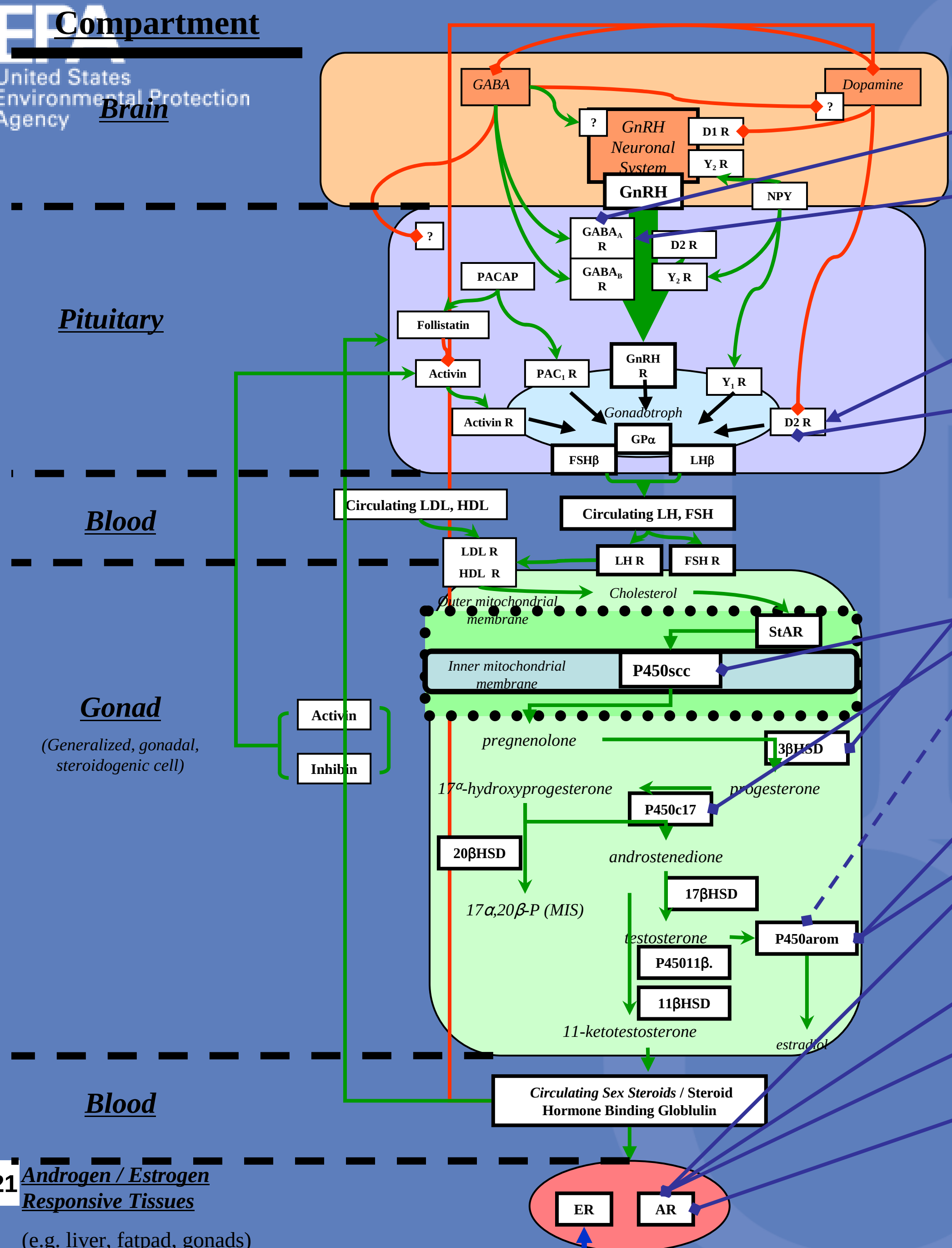
Concurrently integrate data in a systems and network modeling contexts, as well as relevant population modeling, as a basis for prediction

EPA United States Environmental Protection Agency



Conceptual Toxicity (Adverse Outcome) Pathway

Chemical Probes



1 Fipronil (-)

2 Muscimol (+)

3 Apomorphine (+)

4 Haloperidol (-)

5 Trilostane (-)

6 Ketoconazole (-)

7 Fadrozole (-)

8 Prochloraz (-,-)

9 Vinclozolin (-)

10 Flutamide (-)

11 β-Trenbolone (+)

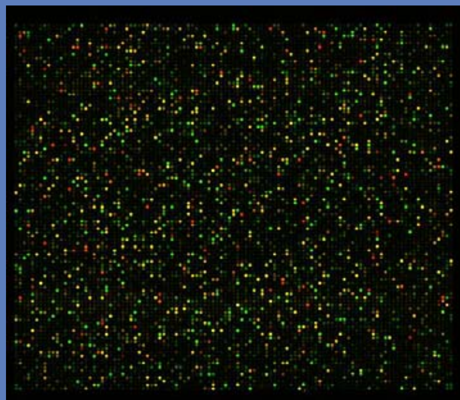
12 Ethynyl estradiol (+)

United States Environmental Protection Agency

Types of Genomic Data

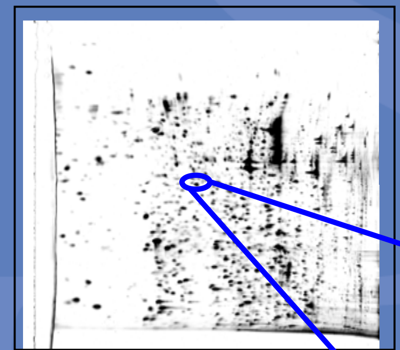
Transcriptomics

Fathead Minnow Microarray



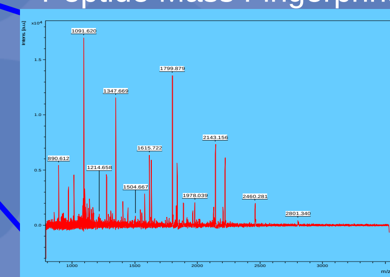
Data from EPA/ EcoArray© CRADA

Proteomics



Representative protein expression profile in testes of control zebrafish

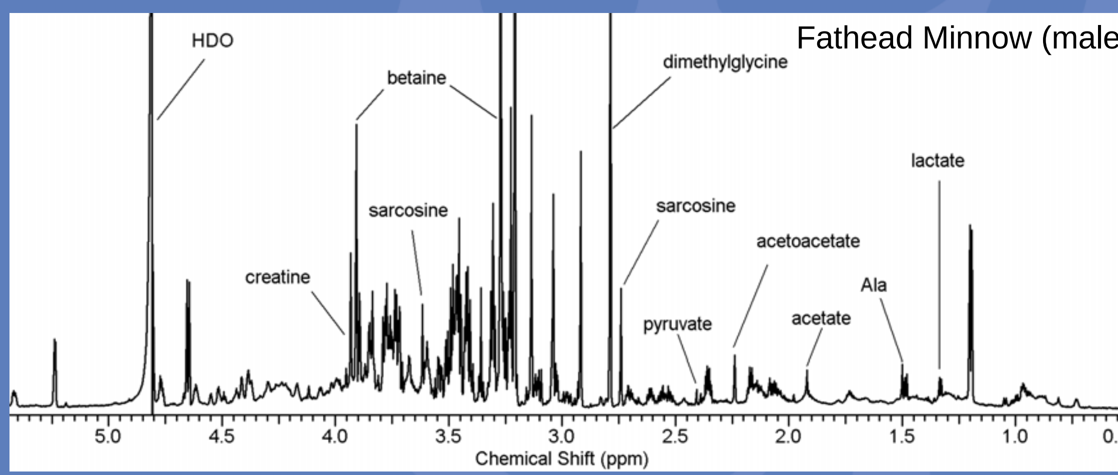
Peptide Mass Fingerprinting



Data from EPA-Cincinnati

Metabolomics

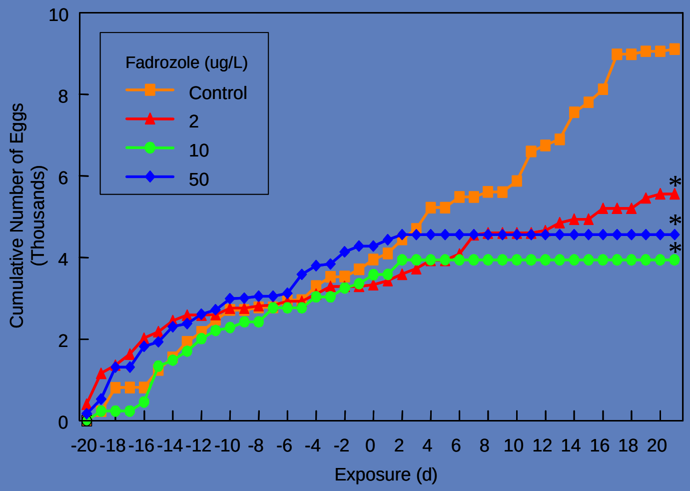
Fathead Minnow Liver NMR Scan

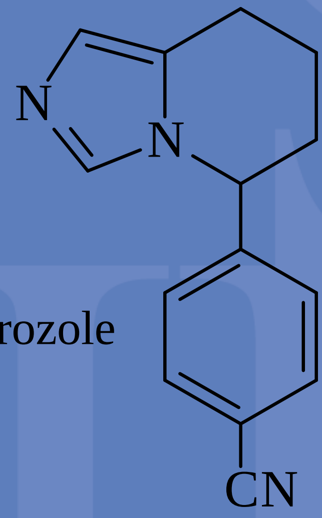


Data from EPA-Athens

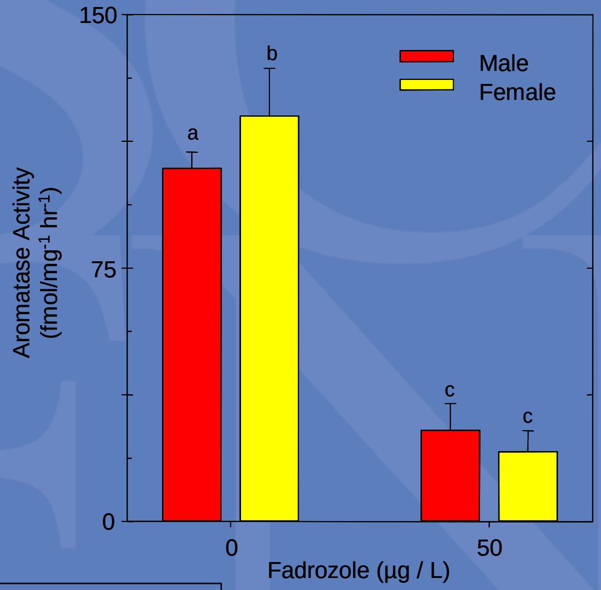
United States Environmental Protection Agency

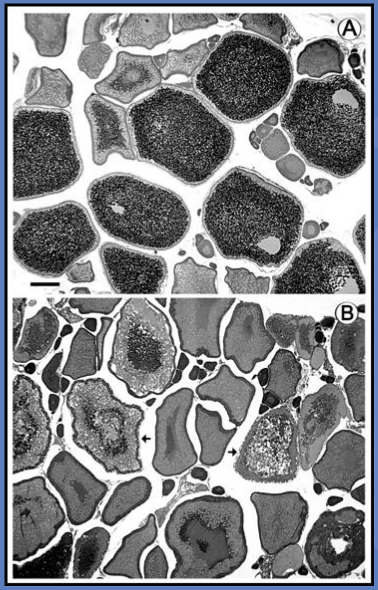
Effects of Aromatase Inhibition on Reproduction in the Fathead Minnow

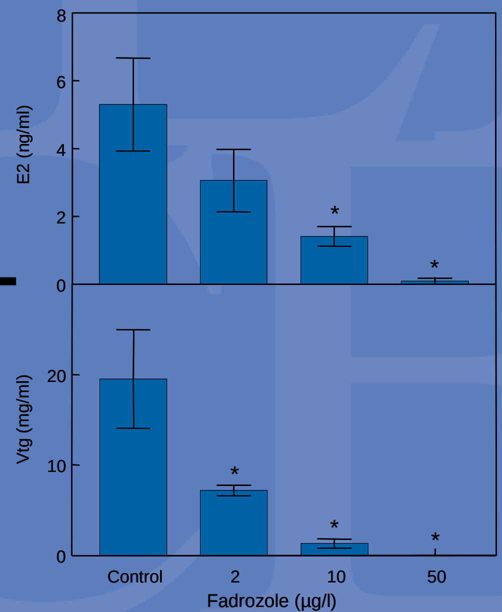




Fadrozole







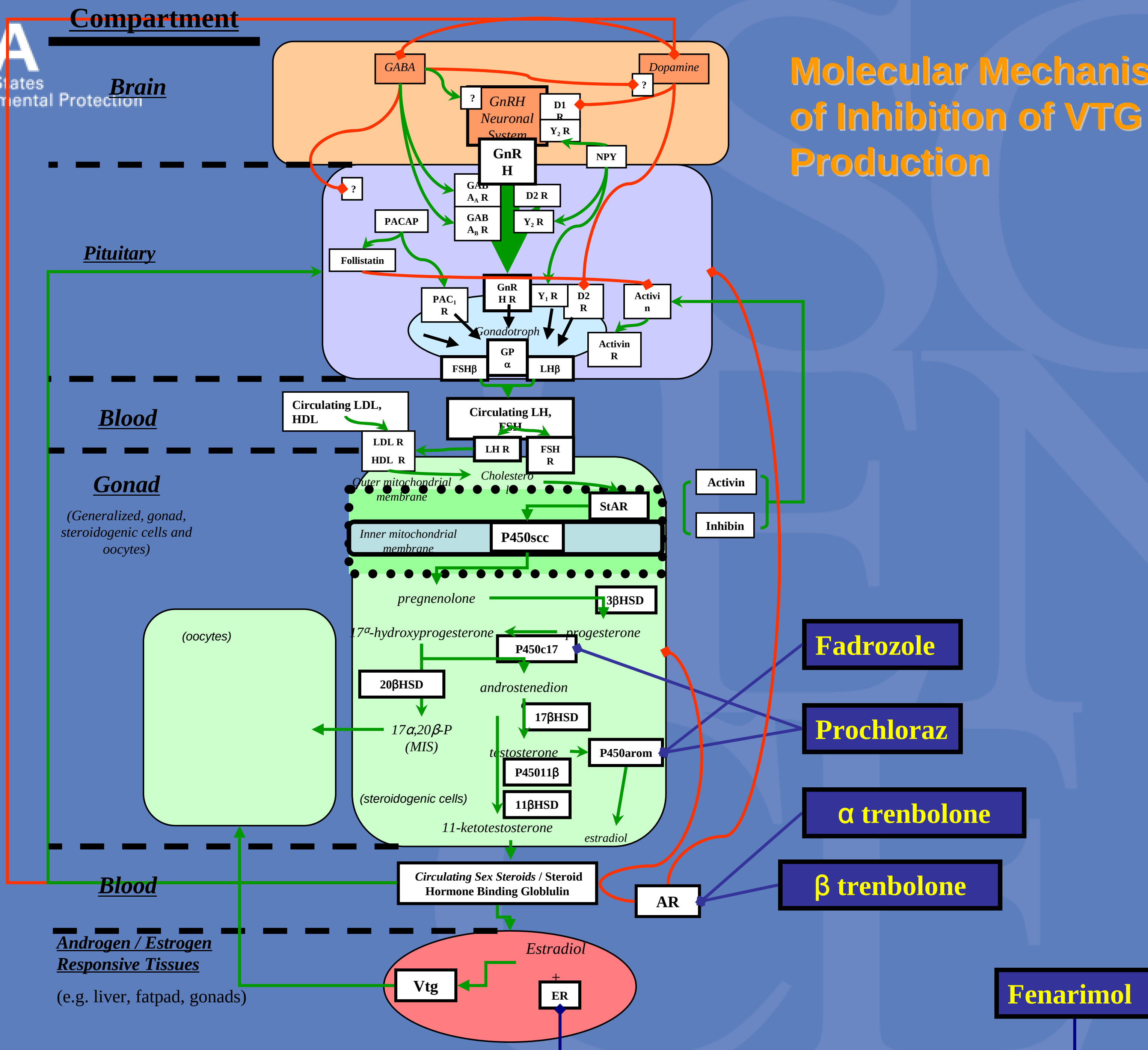
United States Environmental Protection Agency

Key Nodes in Toxicity Pathways: Illustration from the HPG Axis in Fish

- Vitellogenein (vtg), egg yolk protein, is produced normally by oviparous female vertebrates in response to stimulation of the ER by 17β-estradiol
- Commonly used exposure biomarker in males for exposure to exogenous estrogens
- Effective production of vtg in females critical to successful egg production
- Vtg production in females can hypothetically be decreased via several discreet mechanisms within the HPG axis

United States Environmental Protection Agency

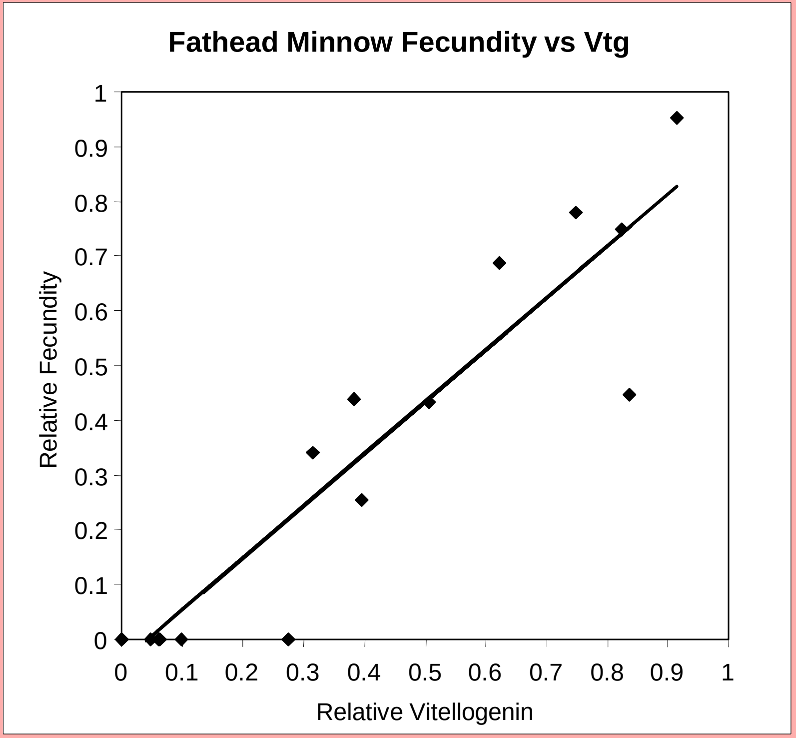
Molecular Mechanisms of Inhibition of VTG Production



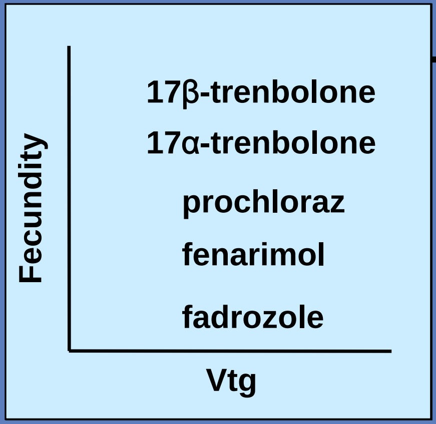
Linking Molecular Responses to Apical Effects: VTG and Fecundity

Chemical	Exposure Concentrations
17β-trenbolone	0.005µg/l, 0.05µg/l, 0.5µg/l, 5µg/l, and 50µg/l
17α-trenbolone	0.003µg/l, 0.01µg/l, 0.03µg/l, and 0.1µg/l
Prochloraz	0.03mg/l, 0.1mg/l, and 0.3mg/l
Fenarimol	0.1mg/l and 1mg/l
Fadrozole	2µg/l, 10µg/l, and 50µg/l

Fecundity = -0.042 + 0.95 * Vtg (R^2 = 0.88)



Population Forecasts Based on Molecular Responses



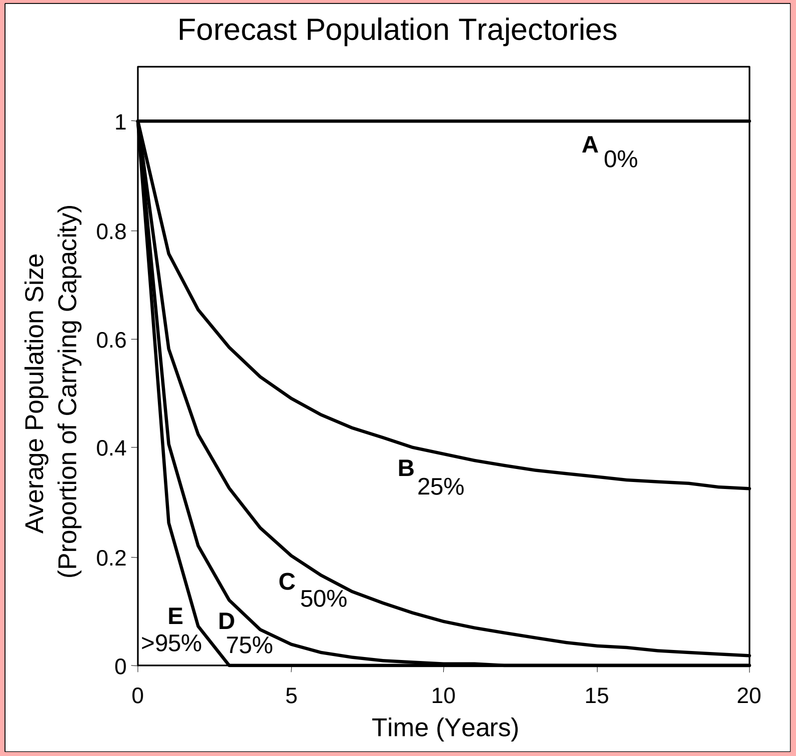
Measurement of vtg concentrations and fecundity for female fathead minnows

Life table with age specific vital rates of survival and fecundity for the fathead minnow population

Carrying capacity for the fathead minnow population

Projection of density dependent logistic population trajectories for the fathead minnow population based upon change in vtg

Population projection for populations at carrying capacity exposed to stressors that depress vitellogenin production



Tools Needed for Assessing Risk of EDCs in the Field

- Short-term *in vitro* and *in vivo* assays suitable for complex mixtures
- Analytical/fractionation approaches to identify specific chemicals causing biological effects
- Diagnostic endpoints (biomarkers) indicative of chemical MOA
- Approaches to link diagnostic responses to alterations in individuals and populations