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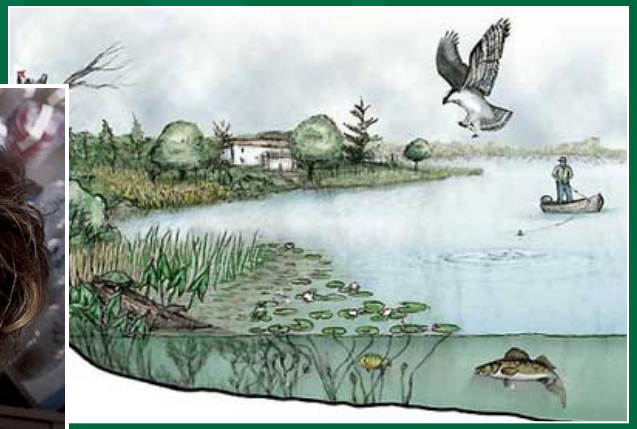
# Application of Computational Toxicology to Prospective and Diagnostic Ecological Risk Assessment

*Daniel L. Villeneuve, US EPA, Duluth, MN



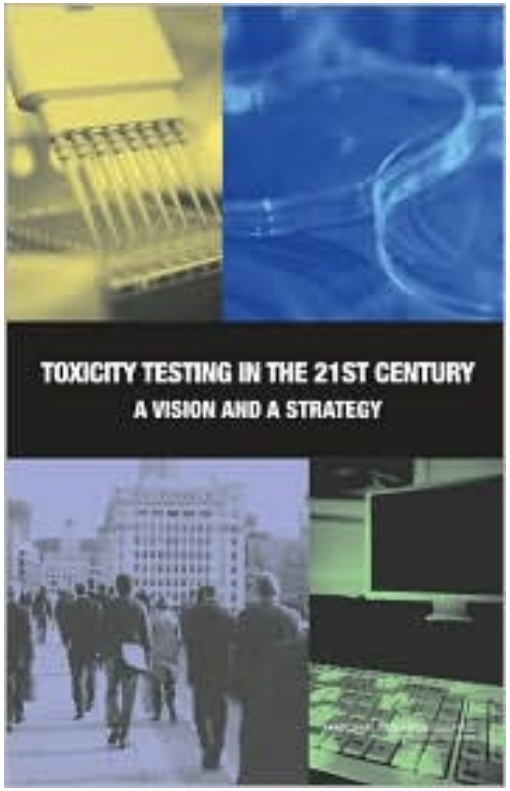
**Office of Research and Development**
National Health and Environmental Effects Research Laboratory, Mid-Continent Ecology Division

**** The contents of this presentation neither constitute nor, necessarily, reflect US EPA views or policies.***

# Toxicity Testing in the 20th Century


- Expensive
- Time-consuming
- Animal intensive
- Empirical
  - observation > understanding

# Toxicity Testing in the 21st Century




**Four competing objectives**

- **Depth – providing the most accurate, detailed, characterization possible.**
- **Breadth – providing data on the broadest universe of chemicals, endpoints, species, life-stages, etc.**
- **Animal welfare – using the fewest animals possible and minimizing suffering.**
- **Conservation – minimizing expenditure of money and time on testing and review.**

# Components of the Vision

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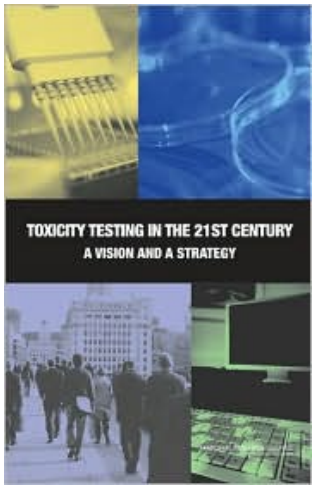
"Transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin"

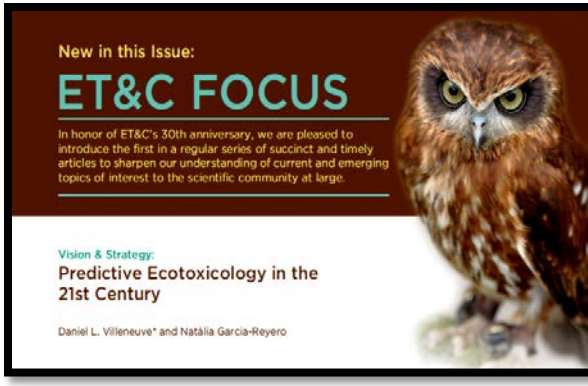
"The vision emphasizes the development of suites of predictive, high-throughput assays ….." (p. 7)

"The mix of tests in the vision include tests that assess critical mechanistic endpoints involved in the induction of overt toxic effects rather than the effects themselves." (p. 121)



# Toxicity Testing in the 21st Century

## How does the vision apply to Ecotoxicology?





- Ecotox is faced with the same competing objectives
- Need to generate useful hazard data cheaper, faster
- Need to prioritize what whole animal testing should be done

But….

- Human cell lines will not address species extrapolation
- Less willingness to apply precautionary approach – “show me the adversity”
- Units of concern are populations and ecosystem services/functions, not individuals.

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# *Chemical Safety for Sustainability (CSS)*

## Task 2.1.1: AOP Discovery and Definition

1. Developing AOP knowledge and populating an AOP knowledge-base.

2. Tools to evaluate conservation of molecular targets (i.e., molecular initiating events) as a basis for defining taxonomic susceptibility domains.

3. Supporting HTS assay development and application.

4. Supporting development of “virtual tissue” models.

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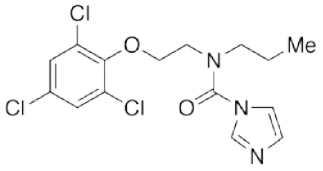
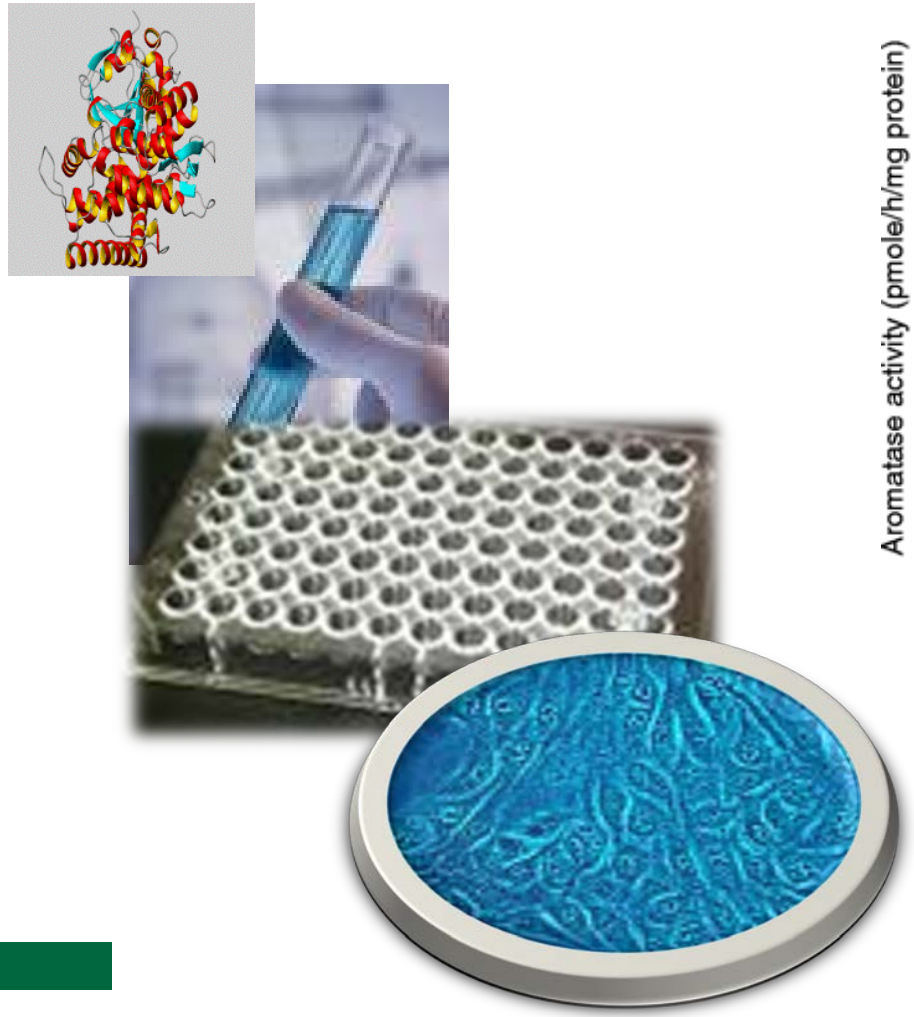
# Can we use in vitro data to predict eco hazards?

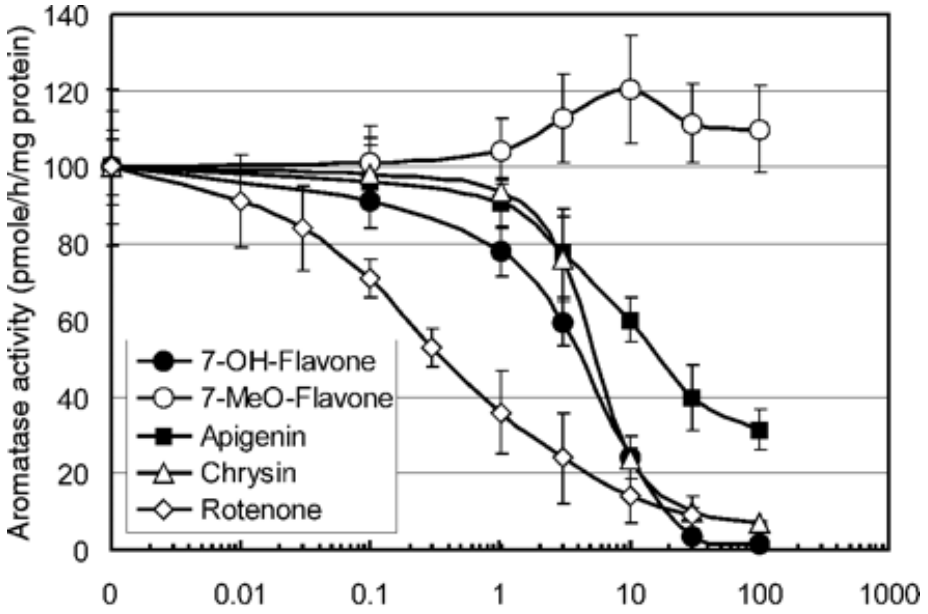
# Example




## Aromatase (cyp19) inhibition



**High throughput assay with human cell line**




**Sanderson et al. 2004, Toxicol. Sci. 82: 70-79**

# Aromatase Inhibition

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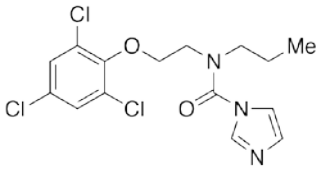
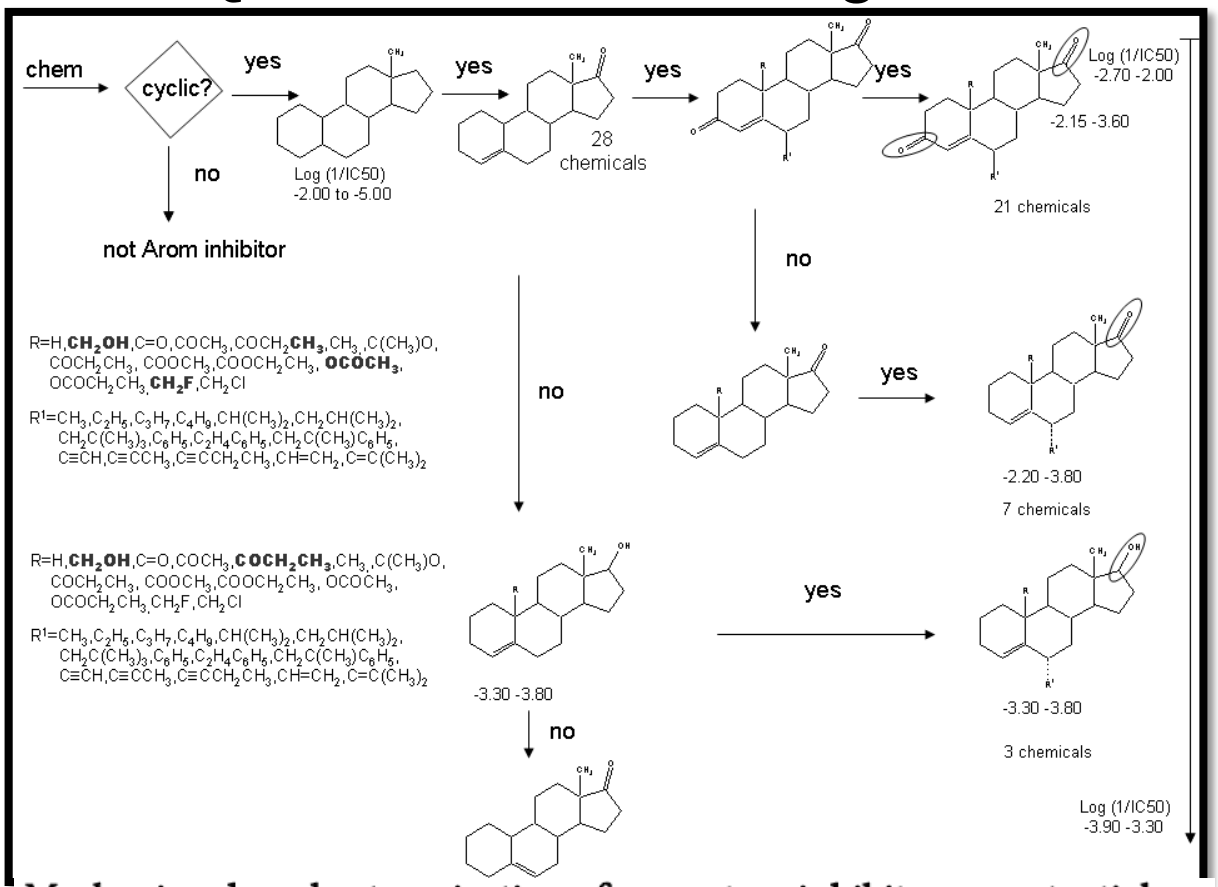



## QSAR, chemical categories




**Mechanism-based categorization of aromatase inhibitors: a potential discovery and screening tool**

P. I. Petkov [a]; S. Temelkov [a]; D. L. Villeneuve [b]; G. T. Ankley [b]; O. G. Mekenyan [a]

[a] Laboratory of Mathematical Chemistry, Bourgas As. Zlatarov University, Bourgas, Bulgaria [b] US Environmental Protection Agency, Mid-Continent Ecology Division, Minnesota, USA

*SAR and QSAR in Environmental Research, 20: 657-678.*

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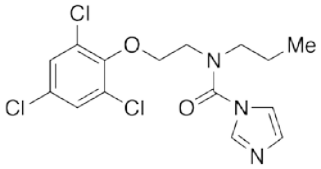
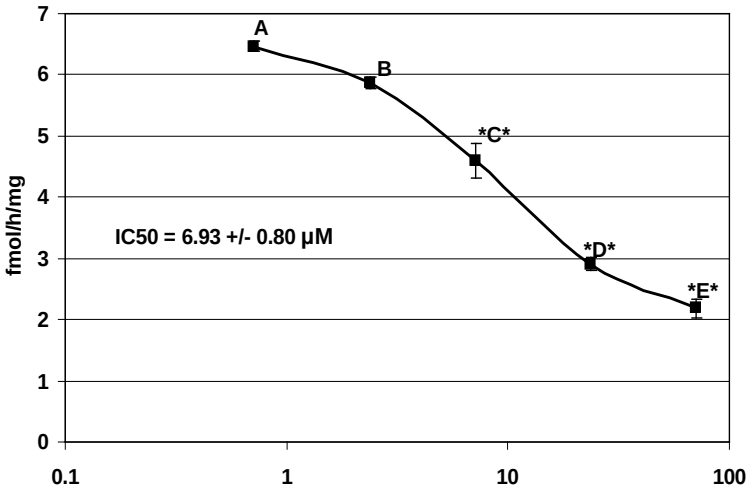



IC50 = 7.0 ± 0.8 µM




**Villeneuve et al. 2006. Aquat. Toxicol. 76:353-368**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# Population Impacts


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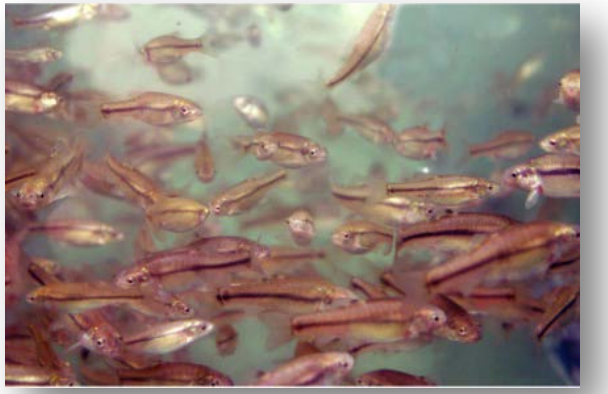
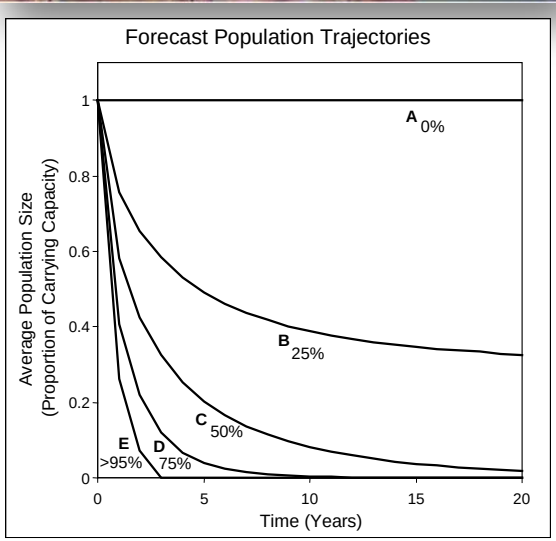
OPPT





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- •QSARs
- •Read across
- •High throughput screening
- •In vitro
- •Biomarkers
- •Genomics

Use in hazard/risk assessment has been limited by lack of well defined (predictive) linkages between these alternative types of data and adverse outcomes traditionally considered relevant to risk assessment.

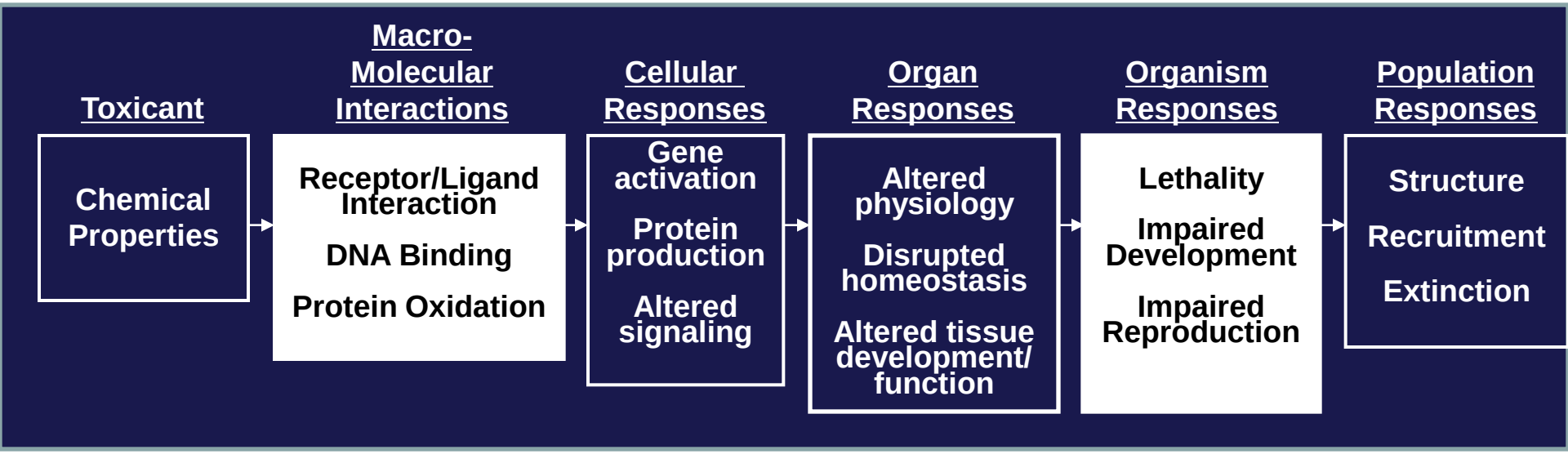
Molecular | Cellular | Tissue | Organ | Organ System | Individual | Population | Ecosystem

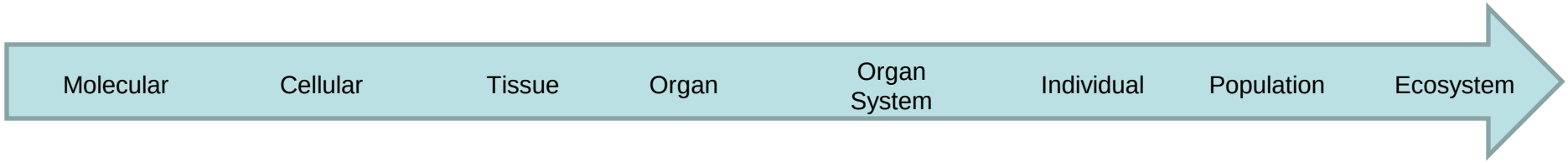

Human Health


Ecosystem Health

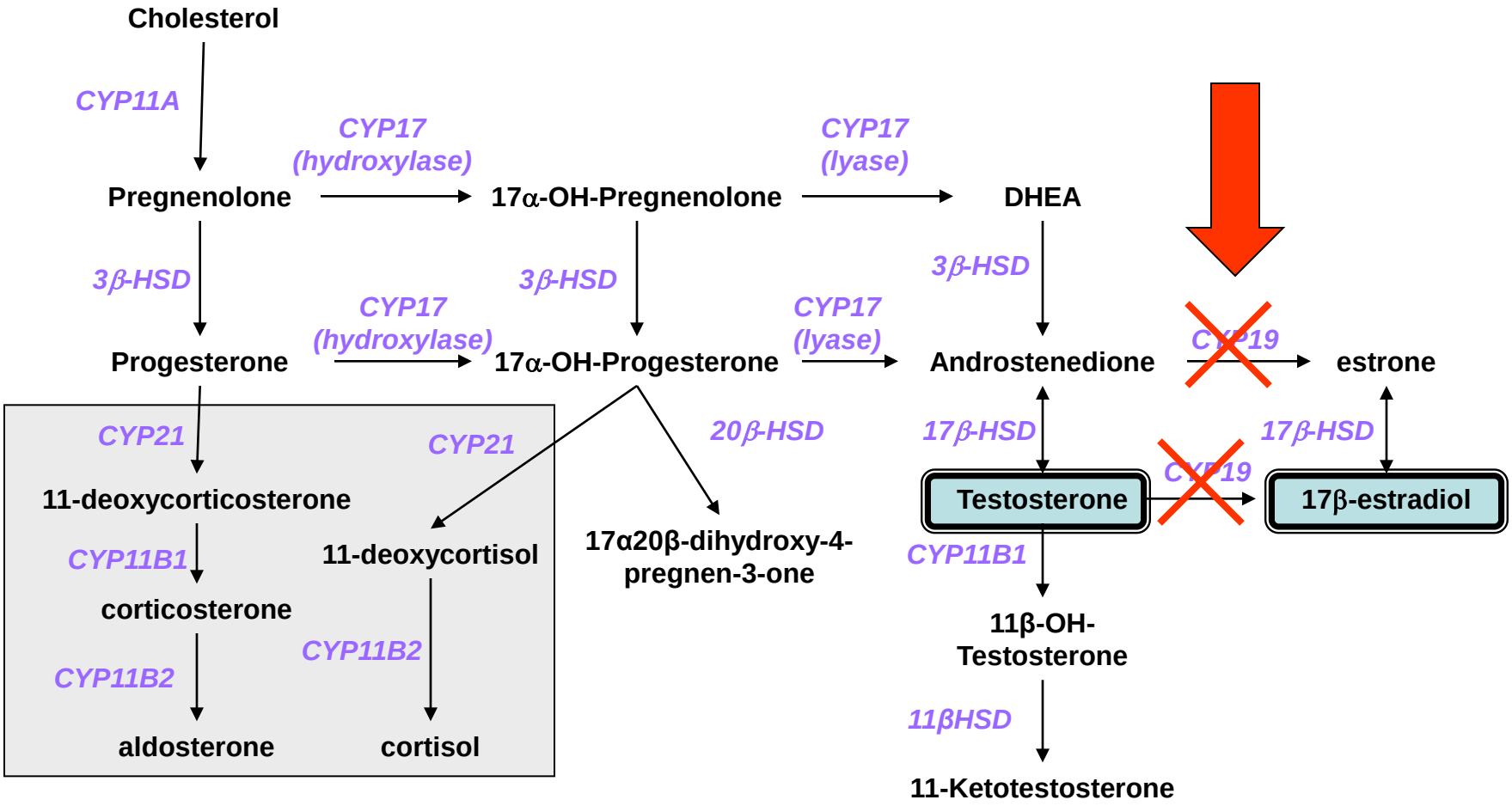
**An Adverse Outcome Pathway (AOP) is a conceptual framework that portrays existing knowledge concerning the linkage between a direct molecular initiating event and an adverse outcome, at a level of biological organization relevant to risk assessment.**

(Ankley et al. 2010, Environ. Toxicol. Chem., 29(3): 730-741.)



# Aromatase Inhibition



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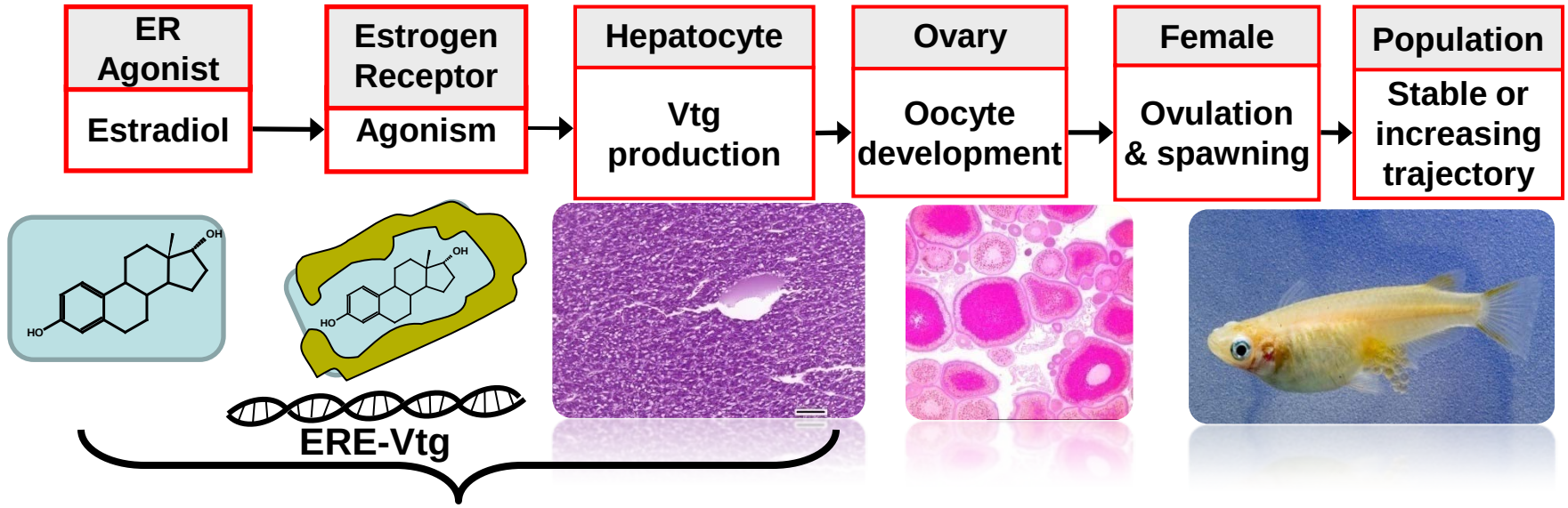
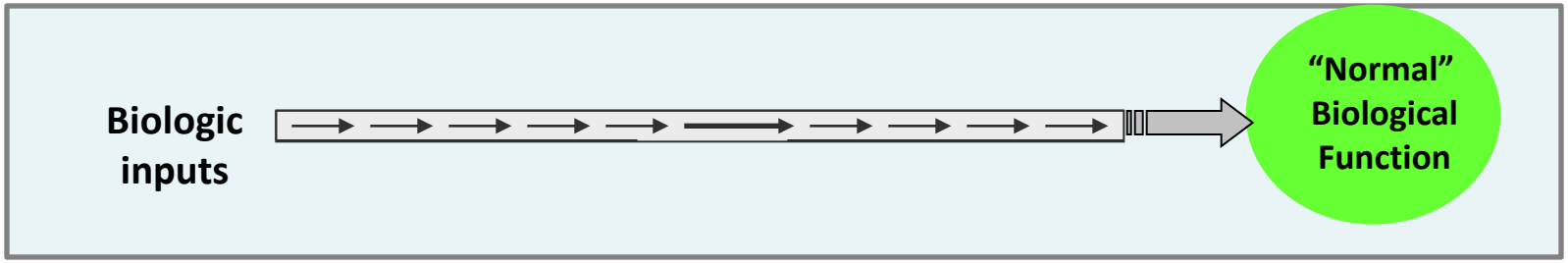
# A bit of fish reproductive biology

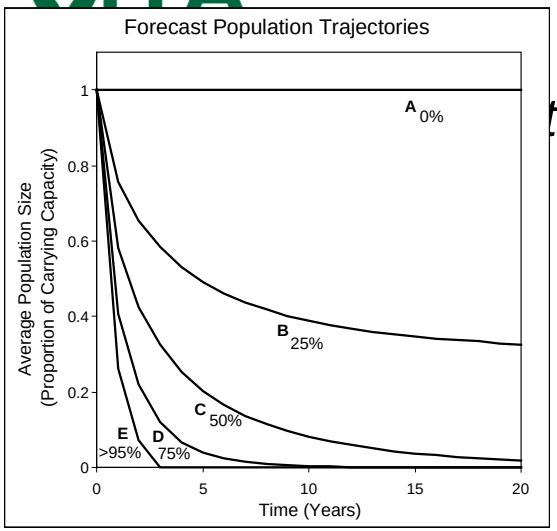


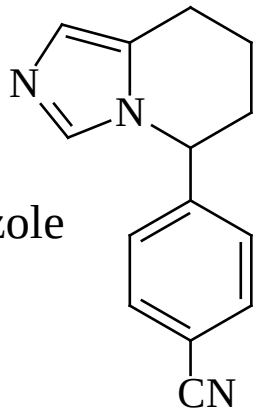

Cellular response pathway



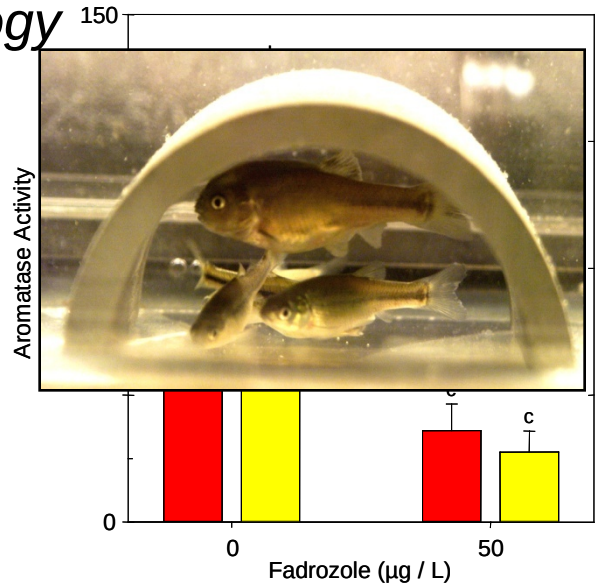
# Adverse Outcome Pathways – definition and example

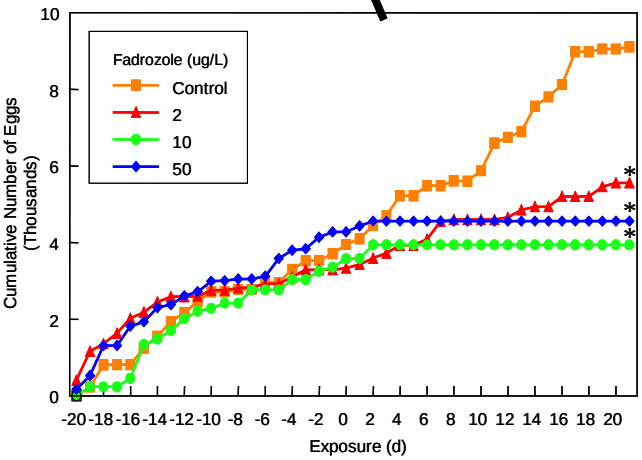
*...turbed fish reproductive biology*

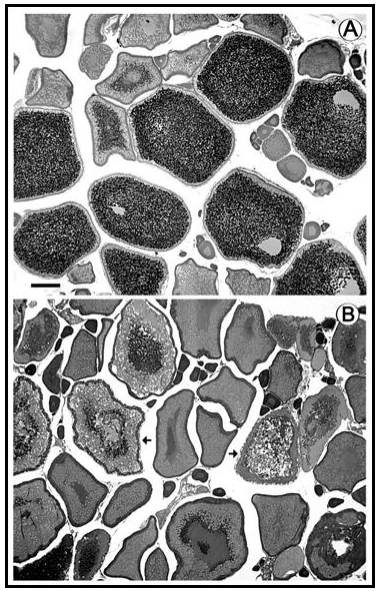


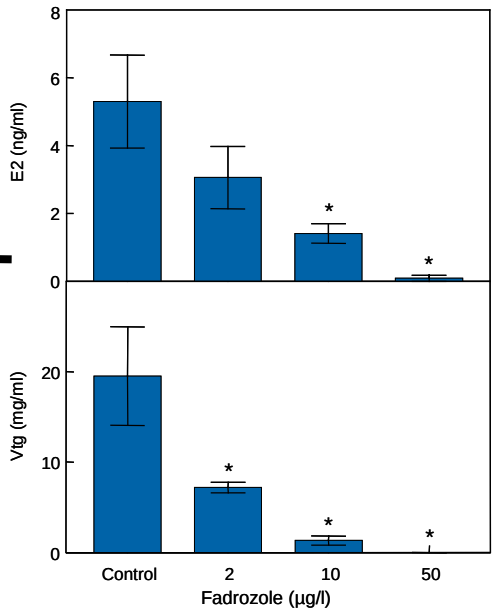




Fadrozole




*Ankley et al., 2002, Toxicol. Sci. 67:121-130*

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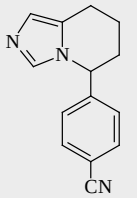
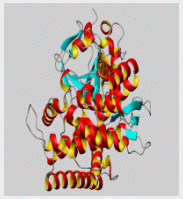
## Adverse Outcome Pathway

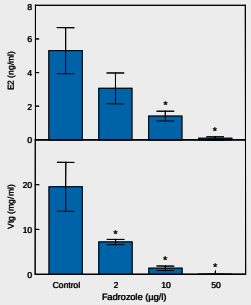
| Toxicant | Macro-Molecular Interactions | Cellular Responses | Organ Responses | Individual Responses | Population Responses |
|---|---|---|---|---|---|
| Chemical Property Profile | Receptor/Ligand Interaction<br>DNA Binding<br>Protein Oxidation | Gene Activation<br>Protein Production<br>Altered Signaling<br>Protein Depletion | Altered Physiology<br>Disrupted Homeostasis<br>Altered Tissue Development or Function | Lethality<br>Impaired Development<br>Impaired Reproduction<br>Cancer | Structure<br>Recruitment<br>Extinction |



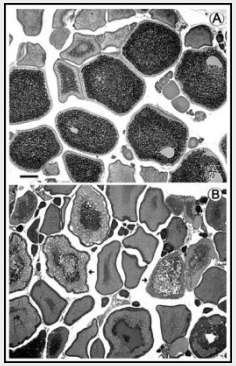

**Aromatase inhibition**



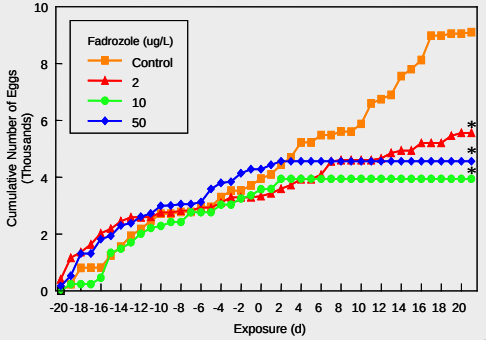
**Reduced E2, Vtg synthesis**




**Impaired vitellogenesis**




**Reduced fecundity**




| Molecular initiating event | Key events or predictive relationships spanning levels of biological organization | Adverse outcome relevant to risk assessment |
|---|---|---|

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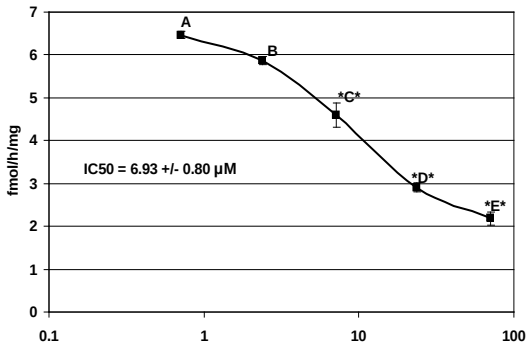
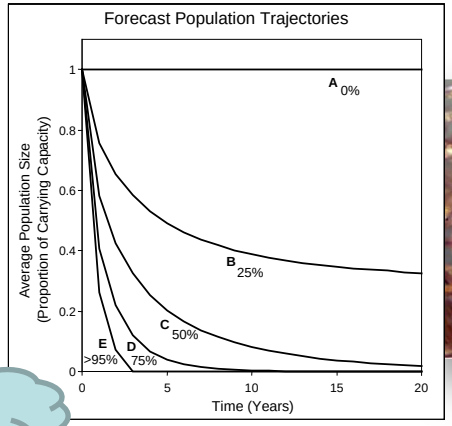
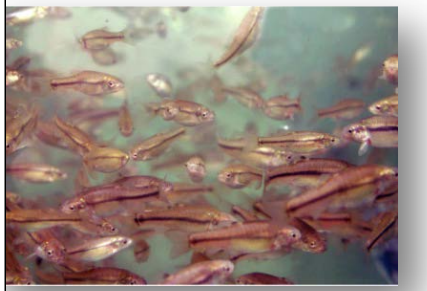
IC50 = 7.0 ± 0.8 µM




AOP

Population Impacts



Aha!

Hazard identification

Prioritization of testing

# Risk Assessment

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•**AOPs assume sufficient perturbation to cause the adverse outcome.**

• **Risk assessment – probability of an adverse outcome under defined circumstances.**

•Requires an understanding of adaptive/homeostatic capacity of biological systems and their limits, relative to concentration and duration of exposure.

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# Systems Biology Approach

## Integrated Genomic and Proteomic Analyses of a Systematically Perturbed Metabolic Network

Trey Ideker,[1,2]* Vesteinn Thorsson,[1,2] Jeffrey A. Ranish,[1,2] Rowan Christmas,[1] Jeremy Buhler,[3] Jimmy K. Eng,[1] Roger Bumgarner,[4] David R. Goodlett,[1] Ruedi Aebersold,[1,2] Leroy Hood[1,2]

www.sciencemag.org SCIENCE VOL 292 4 MAY 2001

(i)Define the subset of genes, proteins, and other small molecules constituting the pathway of interest.

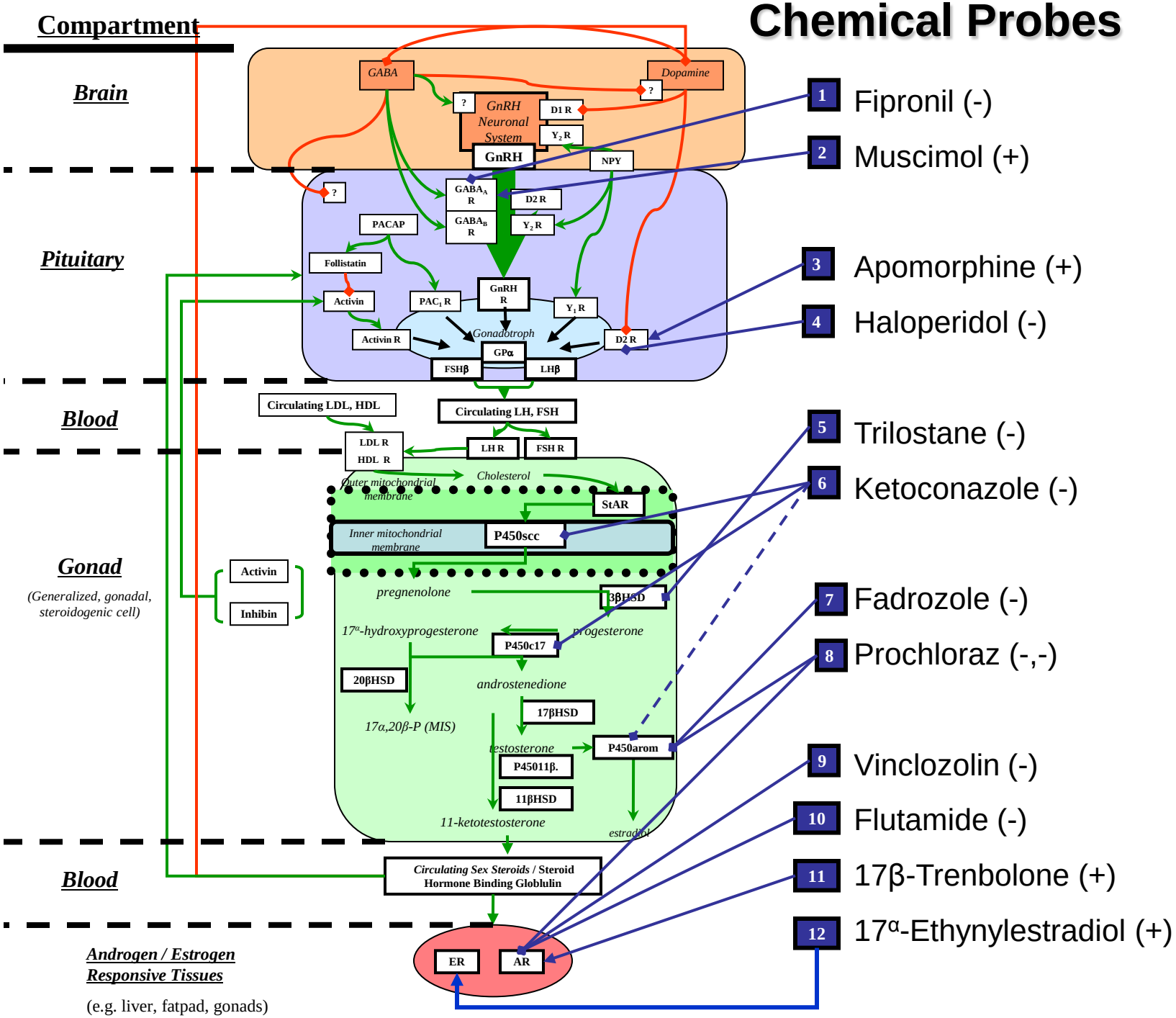
(ii) Perturb each pathway component Detect and quantify the corresponding global cellular response to each perturbation

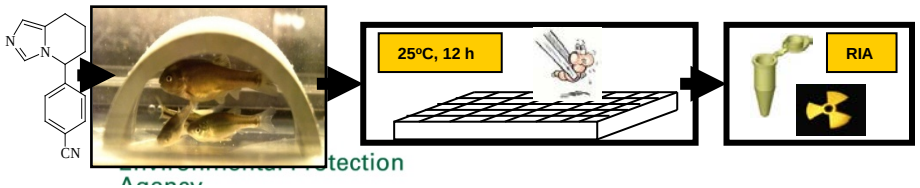
(iii) Integrate the observed responses with the current, pathway specific model

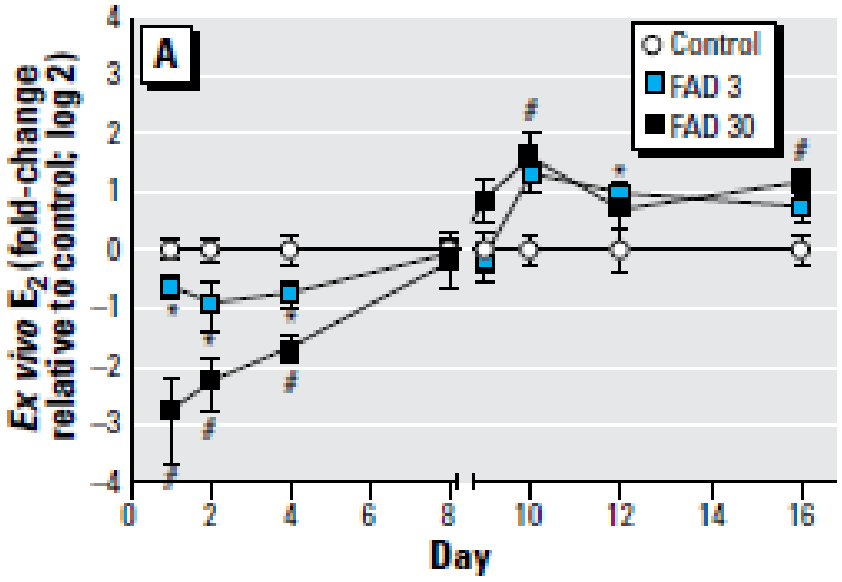
(iv) Formulate new hypotheses to explain observations not predicted by the model.

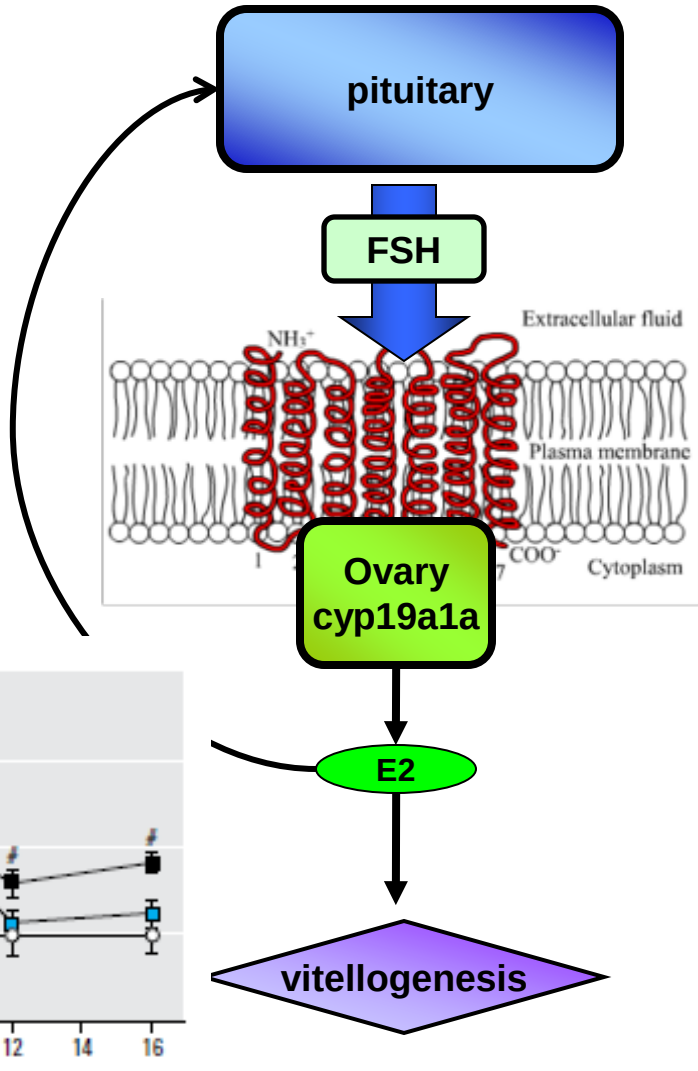
Design additional perturbation experiments to test these, and iteratively repeat steps (ii), (iii), and (iv).

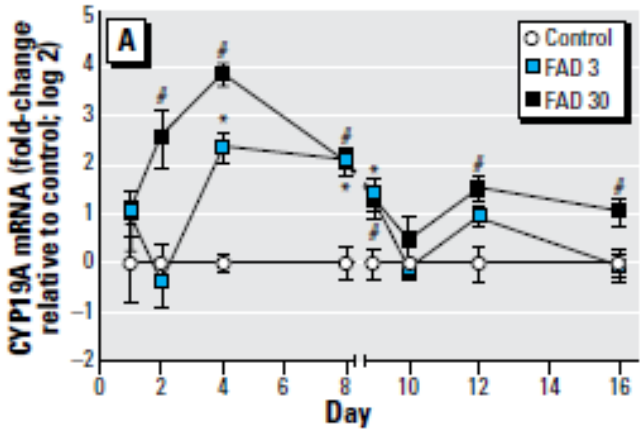
# Chemical Probes

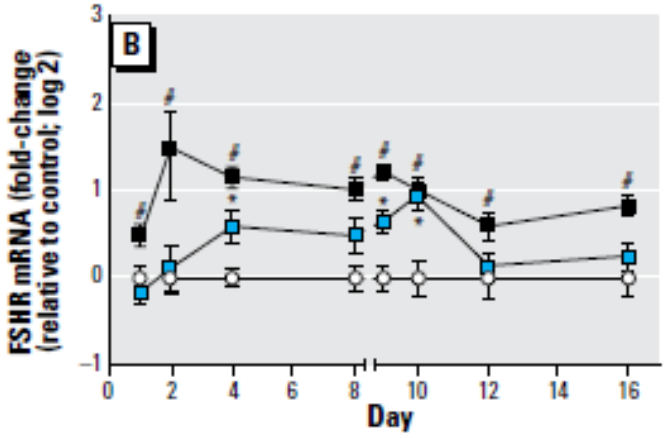




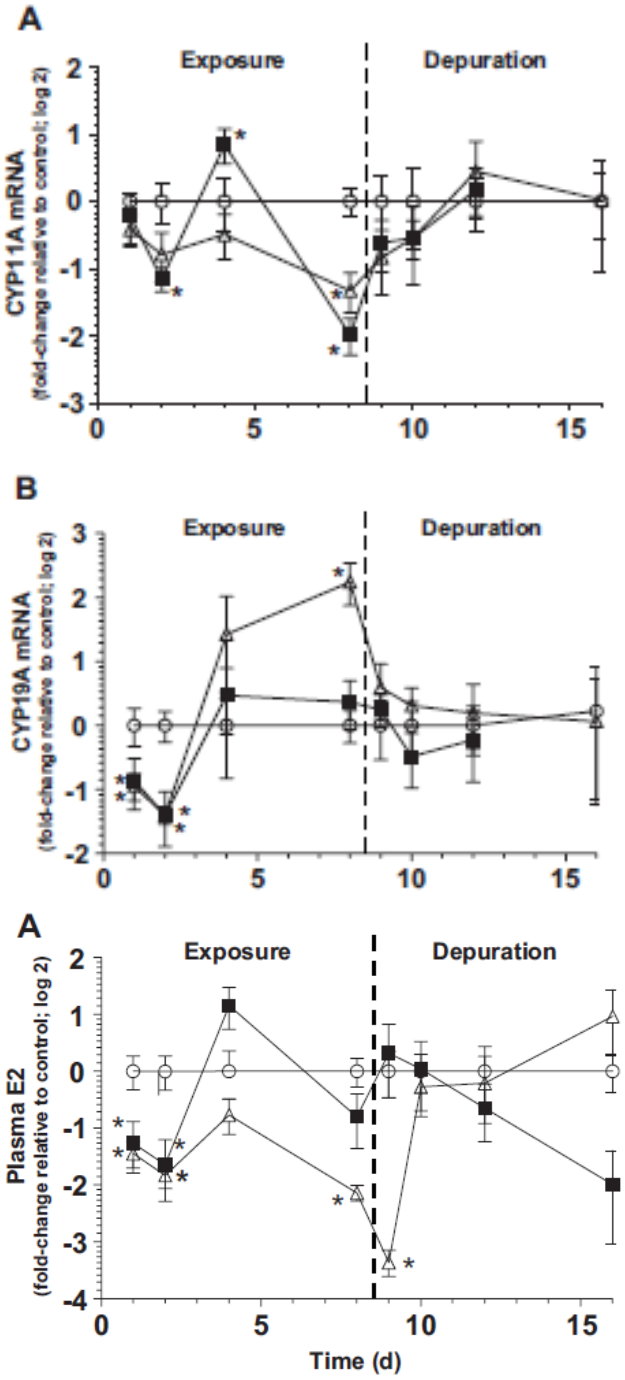



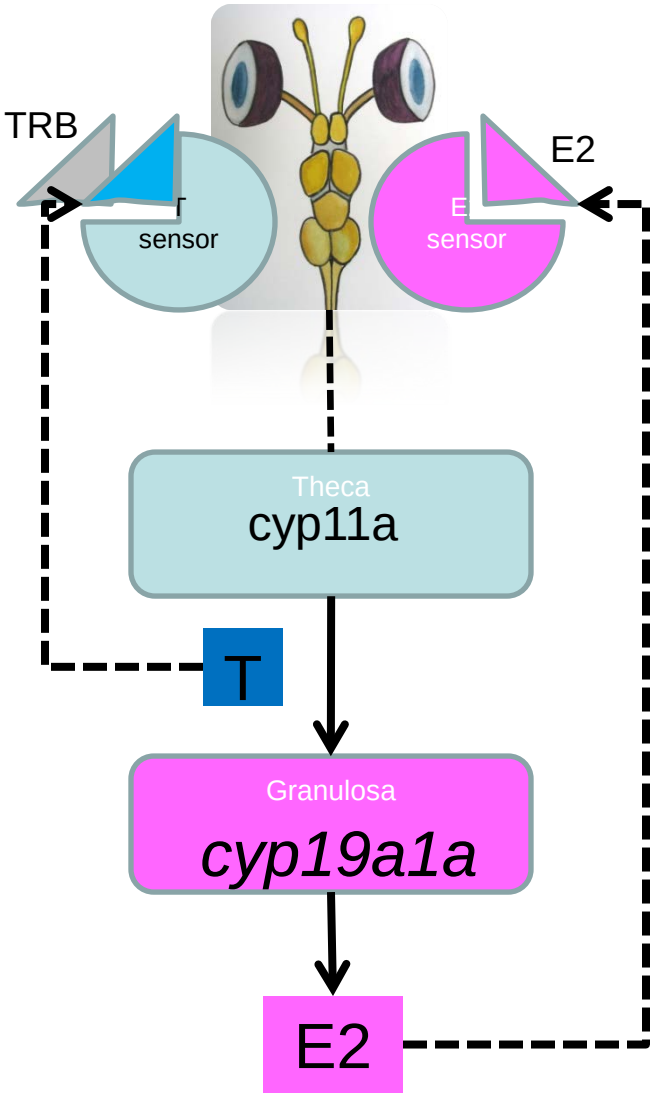




*Villeneuve et al. 2009 Environ. Health Perspect. 117:624-631*




*Ekman et al. 2011, ET&C 30:319-329*

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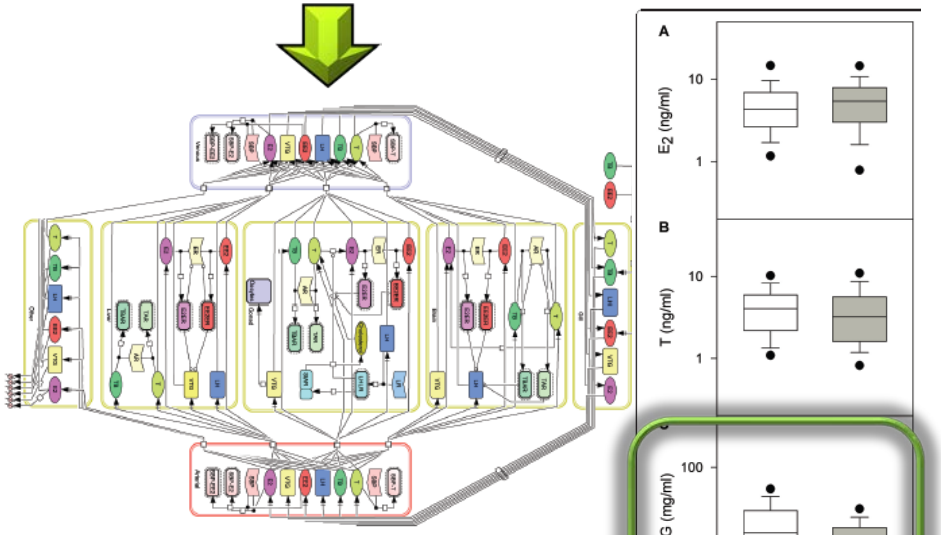
Measured Input:
EE2 or TRB conc. in water

Predicted Output






*Li et al. 2011. BMC Systems Biology 5:63*

**Predicted Output:**
Plasma VTG

## Mechanistic Modeling

- To aid extrapolation from molecular perturbations to outcomes.
- How much perturbation is too much?

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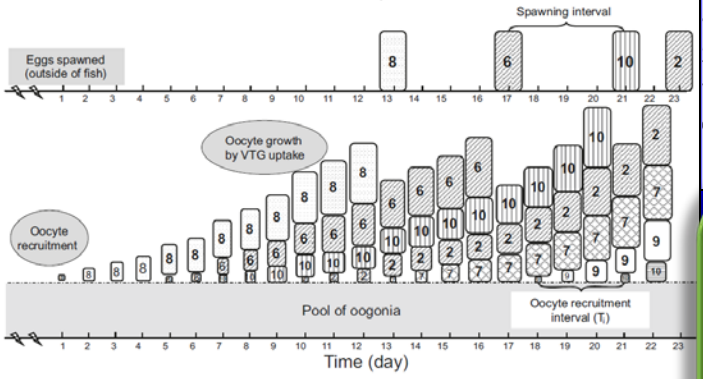
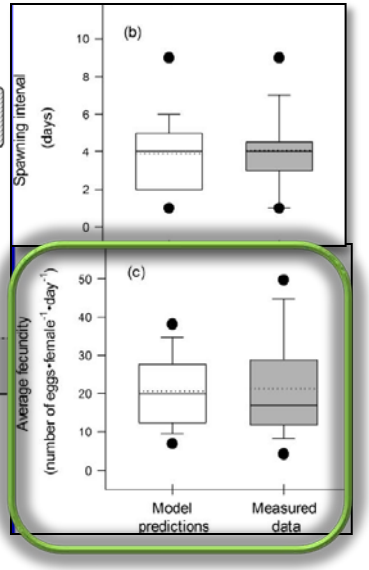
**Predicted Input:**
Plasma VTG




*Li et al. 2011. Can. J. Fish. Aquat. Sci. 68:1528-1538*

**Predicted Output:**
Average Fecundity

## Mechanistic Modeling

- To aid extrapolation from molecular perturbations to outcomes.
- How much perturbation is too much?
- Predictive toxicology

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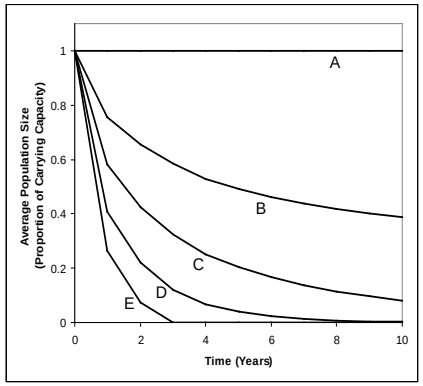
**Predicted Input:** Average Fecundity

Life table for the fathead minnow [illegible]al rates derived from populations (Westman, 1938; Zischke et al., 1983; Duda, 1989; Gleason and Nacci, 2001)

| Age (years) | Survival (per year) | Fecundity (eggs/year) |
|---|---|---|
| 0 | 1 | 0 |
| 1 | 0.001 | 750 |
| 2 | 0.00039 | 1500 |
| 3 | 0.0001521 | 3000 |
| 4 | 0 | N/A |

$$M_{\text{fathead minnow}} = \begin{bmatrix} 0.75 & 1.5 & 3 \\ 0.39 & 0 & 0 \\ 0 & 0.39 & 0 \end{bmatrix}$$

Fig. 1. Leslie matrix for the fathead minnow derived from field studies (Westman, 1938; Zischke et al., 1983; Duda, 1989; Gleason and Nacci, 2001) and developed using birth pulse survival and fertility rates and a prebreeding census (Gotelli, 1998; Caswell, 2001).




***Miller and Ankley, 2004. Ecotoxicol. Environ. Saf. 59:1-9***

**Predicted Output:** Population forecast

## Mechanistic Modeling

- To aid extrapolation from molecular perturbations to outcomes.
- How much perturbation is too much?
- Predictive toxicology

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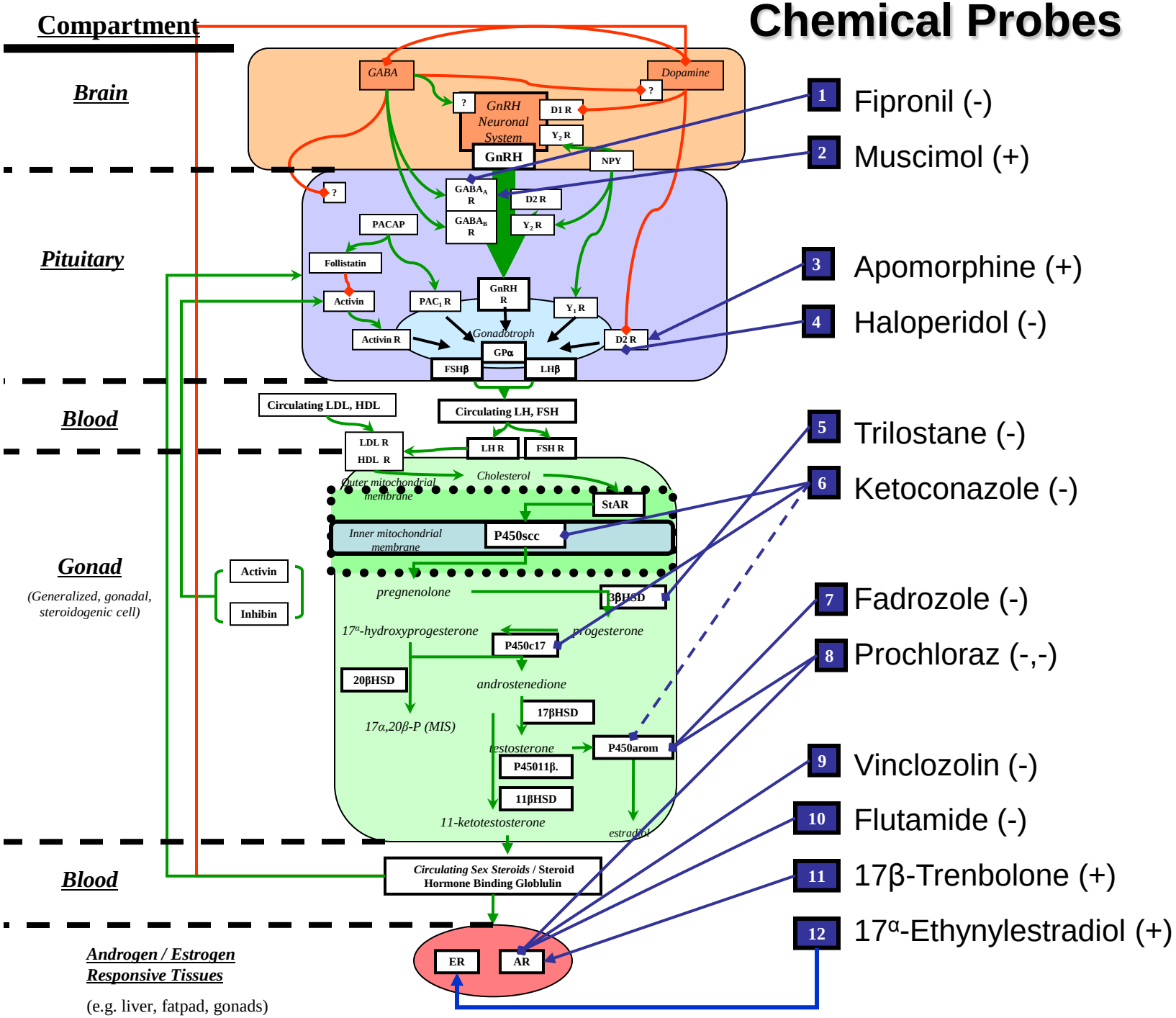
Agency

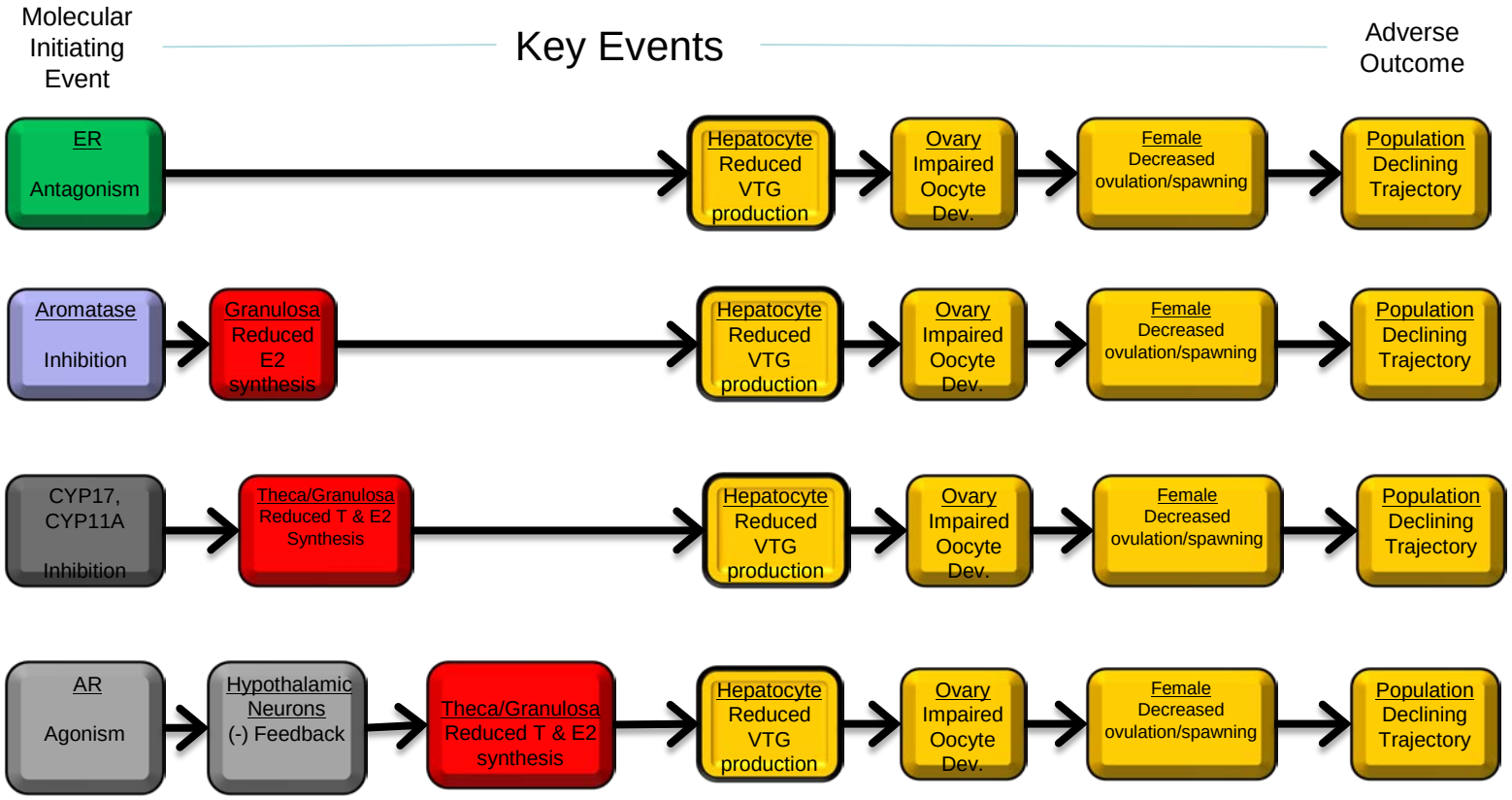
# Scaling it up:

## Can we use human-oriented HTS data to predict eco hazards?

# Chemical Probes



# Assemble AOPs into AOP Knowledge-base



UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

COMPUTATIONAL TOXICOLOGY

# ToxCast™ Assays

## Biochemical Assays

*(Primarily Human / Rat)*

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Receptor binding
  - Enzyme activity
  - Co-activator recruitment

**~600 Total Endpoints**

## Cellular Assays

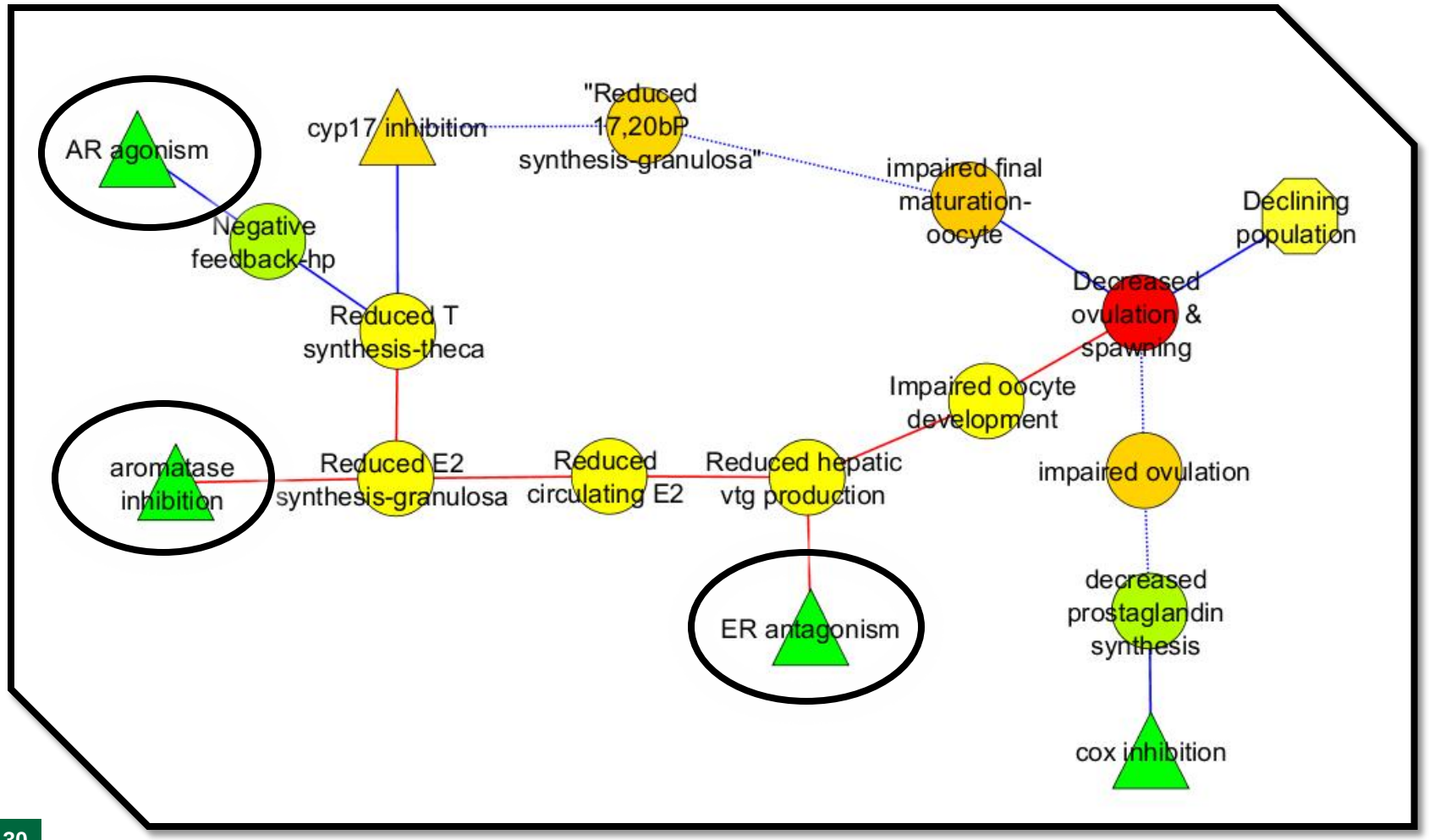
- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
  - T47D human breast carcinoma
  - PC12 rat neuronal
- Primary cells
  - Human vascular endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
  - Rat hepatocytes
  - Mouse embryonic stem cells
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Protein expression
  - High-content imaging

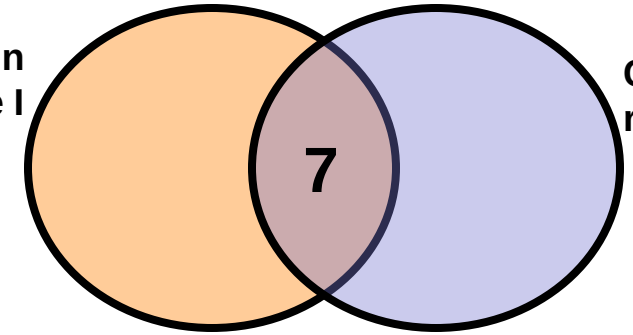
## Model Organisms

- Zebrafish embryo development
- *C. elegans* growth

# AOP Networks

- MIE (▲) and Key Events (●) aligned with molecular screening assays





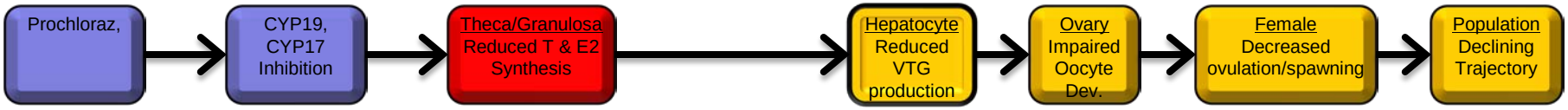

| Seven Test chemicals | Putative M.I.E. |
|---|---|
| **Prochloraz** | Cyp19, cyp17 inhib. |
| **Propiconazole** | Cyp inhib. |
| **Fenarimol** | ER antagonist |
| **Bisphenol A** | ER agonist, AR ant. |
| **Vinclozolin** | AR antagonist |
| **Prometon** | Photosystem II inhib. |
| **Fipronil** | GABA-A receptor, chloride channel blocker |

Post-hoc analysis

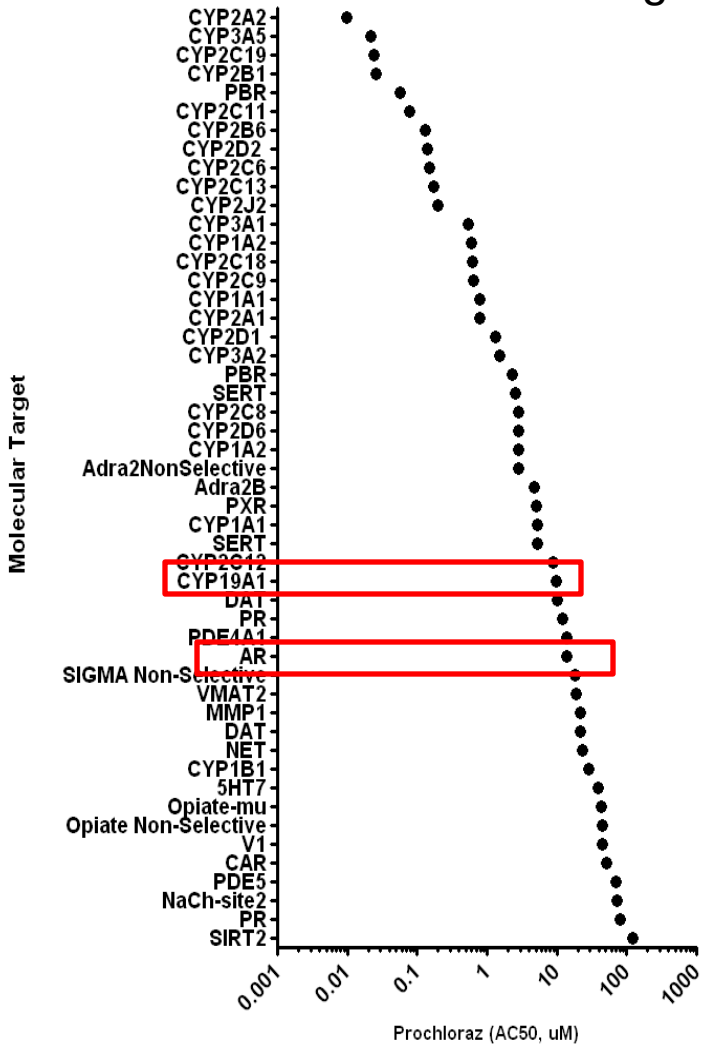
Did MS flag a MIE known to be relevant to fish reproduction?

Were the in vivo effects consistent with our AOP(s)?

# Prochloraz




## Molecular Screening AC50s



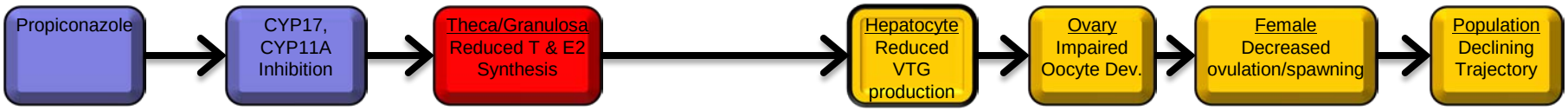

Derived from Knudsen et al. 2011, Toxicol. 282:1-15

## In vivo effects related to AOP

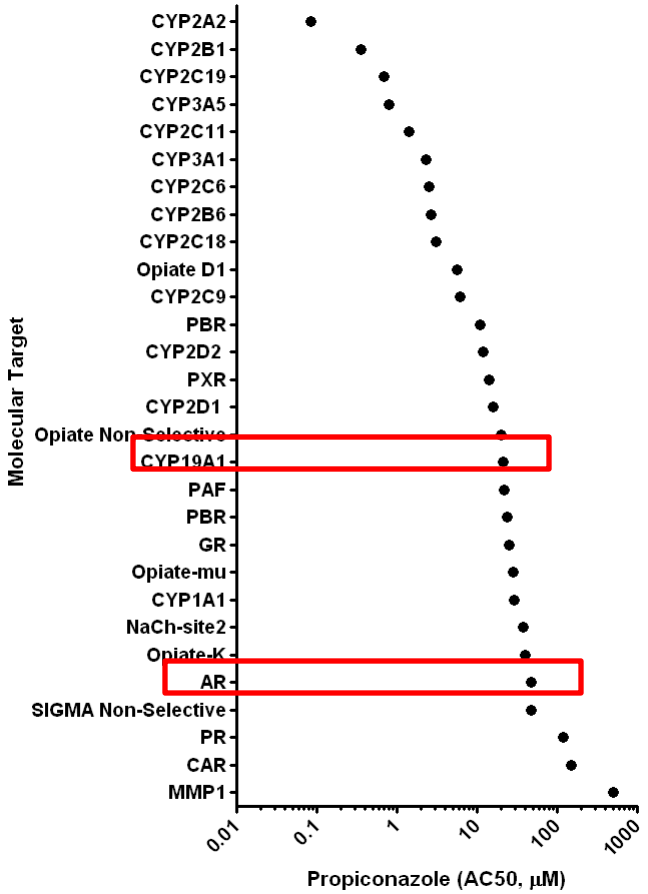
| Endpoint | Effects* |
|---|---|
| Plasma E2 | Sig ↓, 0.3 mg/L |
| Plasma Vtg | Sig ↓, 0.1 mg/L |
| Cumulative Fecundity | Sig ↓, 0.1 mg/L |

*All effects are after 21 d of continuous exposure
See Ankley et al. , 2005, Toxicol. Sci. 86:300-308

# Propiconazole




## Molecular Screening AC50s




Derived from Knudsen et al. 2011, Toxicol. 282:1-15

## In vivo effects related to AOP

| Endpoint | Effect |
|---|---|
| Plasma E2 | Sig ↓, 0.5 mg/L |
| Plasma Vtg | Sig ↓, 0.5 mg/L* |
| Cumulative Fecundity | Sig ↓, 0.5 mg/L |

* From 96 h range finding study, all other data are from a 21 d exposure. Vtg data for 21 d exposure not yet available.
From Skolness et al. (in press, Toxicol. Sci. 2012)

# Prometon, Fipronil

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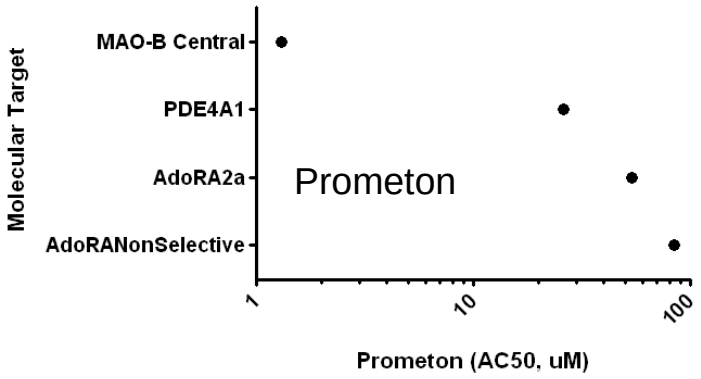
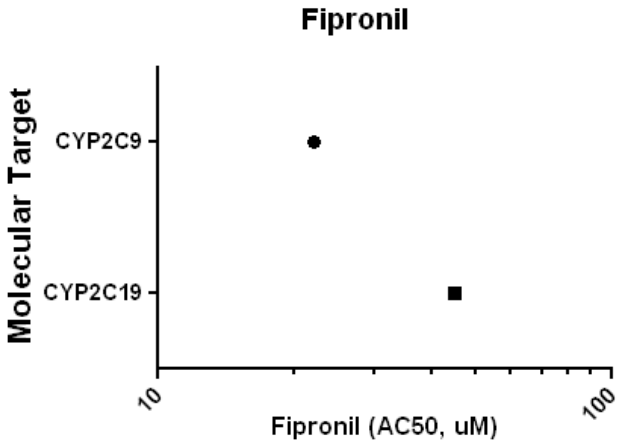
## Molecular Screening AC50s




## In vivo

| Endpoint | effects | Effects* |
|---|---|---|
| Plasma E2 | | No effect (up to 1 mg/L) |
| Plasma Vtg | | No effect (up to 1 mg/L) |
| Cumulative fecundity | | No effect (up to 1 mg/L) |

*Villeneuve et al. 2006, Environ. Toxicol. Chem. 25: 2143-2153

| Endpoint | Effects* |
|---|---|
| Plasma E2 | No effect (up to 5 ug/L) |
| Plasma Vtg | No effect (up to 5 ug/L) |
| Cumulative fecundity | No effect (up to 5 ug/L) |

*Bencic et al., in preparation

# Results

- Overall, when MS indicated perturbation of molecular target associated with AOP, adverse effects on reproduction and related key events were observed.

- Predicted pattern of effects observed, despite other targets being affected.

- Two chemicals with least impact on fish repro, also quite inactive in MS assays.

- Testing of *a priori* predictions based on MS and AOPs needed

# Prospective Assessments

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Data poor

Large Inventory

e.g., TSCA

## Screening

- **Efficient/cost effective methods to predict hazard.**
- **Get more information from "alternative" data that are available.**

## Prioritization

- **Conduct only the tests most likely to drive assessment**
- **More effective use of testing resources increase efficiency.**

Data-rich

Programs

e.g., FIFRA

# Diagnostic Assessments

Regulatory context

Environmental Monitoring

e.g., Regions, OW, GLNPO

- **Complex and undefined exposures**
- **If apical responses are observed, it's too late**

**Need**

- **Ability to cast a broad net**
- **Early warning signs**
- **Indication/elimination of cause(s) - diagnostic**


Early environmental risk assessors.

# Diagnostic Assessments

- Chemical monitoring strategies are effective for chemicals whose hazards are well understood and for which sensitive analytical methods are available.
- Biological effects monitoring can be a powerful complement to chemical monitoring
  - Can detect exposure to chemicals for which analytical methods are unavailable or impractical.
  - Can provide insight into the potential biological consequences of those exposures.

# Chemical Safety for Sustainability (CSS)

United States Environmental Protection Agency

## Task 2.1.2:
## AOP-Based Effects Monitoring and Exposure Reconstruction

1. Apply AOP knowledge to development of effects-based monitoring approaches.

2. Methods for collecting and preparing environmental samples for HTS.

3. AOPs to support identification/elimination of causes of biological responses [exposure reconstruction]

# One Common Approach to Effects-Based Monitoring

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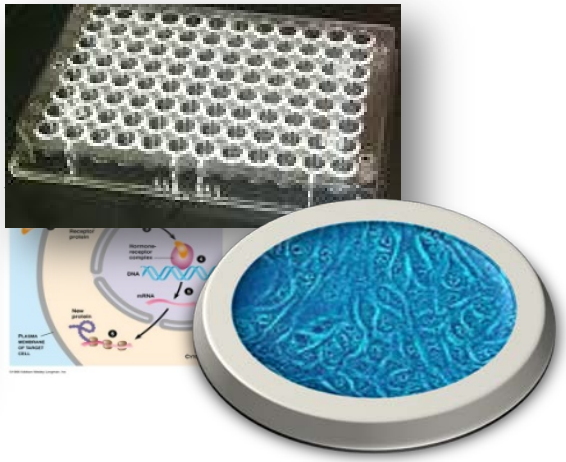
Environmental Sampling

Sample Extraction/Prep

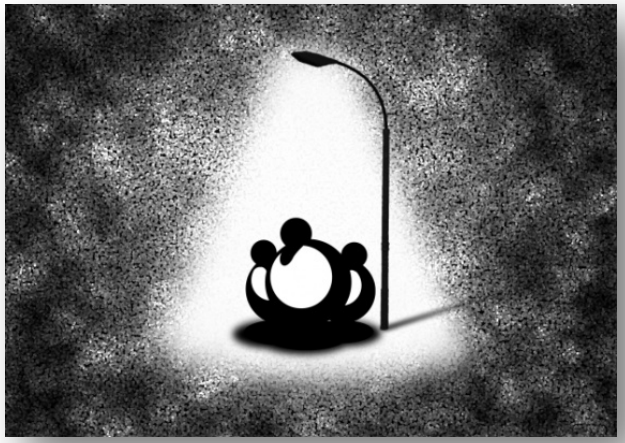
In vitro bioassay



# Supervised/Targeted Effects-based Monitoring

## Looking under the biological lamp-post

- Will only detect the biological activities we look for
- Effective once a hazard of concern has been identified
- Not ideal for surveillance
- May miss activities that influence in vivo biological/ecological outcomes



# Unsupervised Effects-based Monitoring

United States Environmental Protection Agency

## Ability to detect what we might not expect

- Take advantage of HTP to rapidly/cost effectively screen wide range of biological activities
- Ideal for surveillance
- Identification of targeted assays/endpoints for subsequent monitoring of status and trends
- More complete picture of how mixed biological activities influence in vivo biological/ecological outcomes

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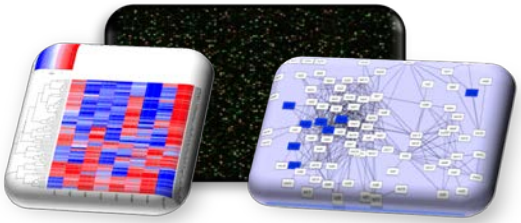
## Unsupervised

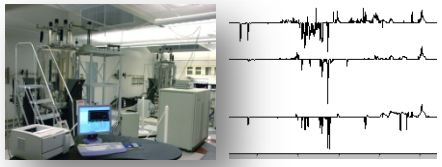
## Supervised

### Fish exposed in situ

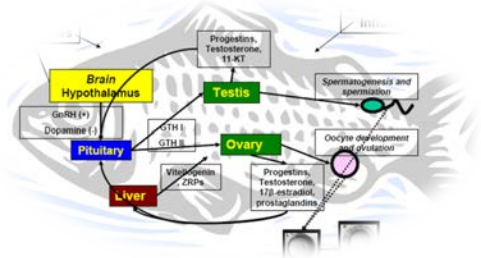
DNA-microarray
Transcriptomics



Metabolomics



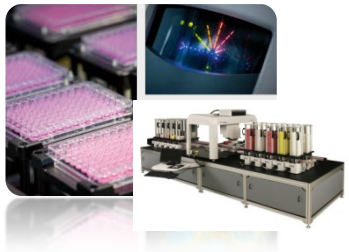
- Endpoints associated with established adverse outcome pathways:

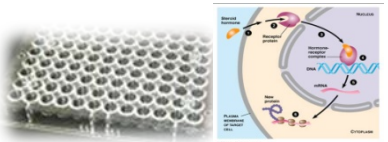



- E.g., Biochemical and molecular markers of endocrine disruption and adverse reproductive outcomes.

### Surface water samples/extracts

High throughput
in vitro screening





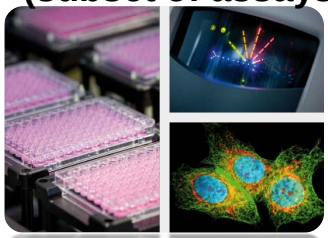
In vitro bioassays
- MDA-kb2: (anti)androgenic activity
- T47D: (anti)estrogenic activity
- H4IIE: dioxin-like contaminants
- H295R: steroidogenesis inhibitors
- Others ……

CSS 2.1.2

EPA United States Environmental Protection Agency

## Surveillance

Ambient water sample

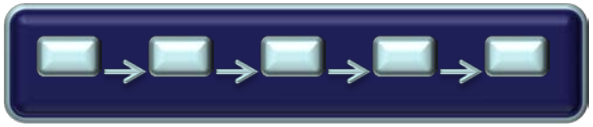


Toxcast (subset of assays)

Response profile

## Assay/Endpoint Selection

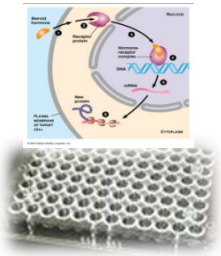
Response profile



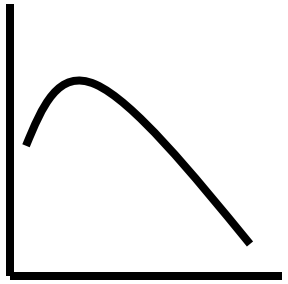
AOP knowledge

## Monitoring

Ambient water samples



Targeted assay/endpoint



status and trends

# Predicting effects of mixtures

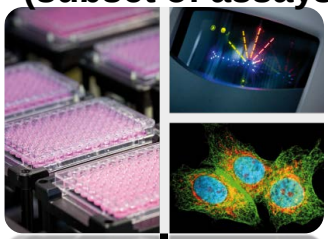
Ambient water sample

Analytes detected in sample

Chemicals analysed in Toxcast (I, II)

extract

**Toxcast Phase 3 (subset of assays)**



Empirical profile of "positive" repsonses

?

Predicted profile of "positive" responses

Compare and discuss

# Conclusions

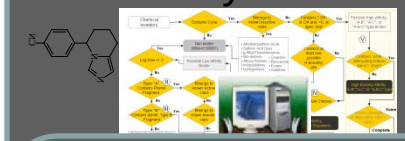
- Computational toxicology has important role to play in 21st C ecotoxicology

- AOPs are a critical foundation

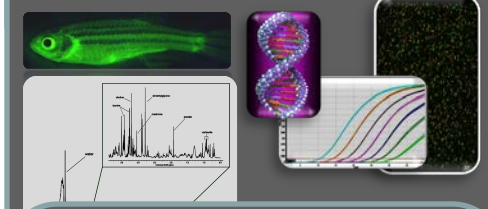
# Alternative Data for Prospective Assessments

Molecular | Cellular | Tissue | Organ | Organ System | Individual | Population | Ecosystem

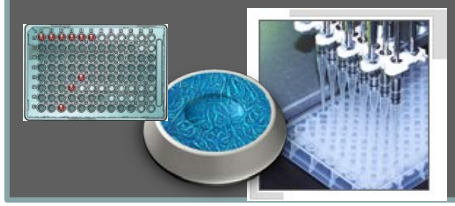
QSAR, expert systems



Biomarkers



Screening & Prioritization
Toxcast, Tox21, in vitro




AOP

Demographic significance
- Survival
- growth-development
- reproduction

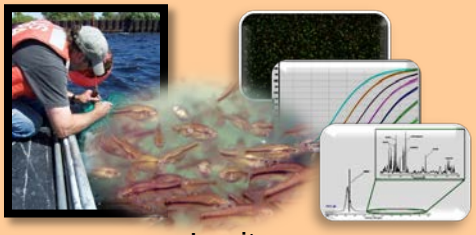
# Effects Data for Diagnostic Assessments

Molecular | Cellular | Tissue | Organ | Organ System | Individual | Population | Ecosystem

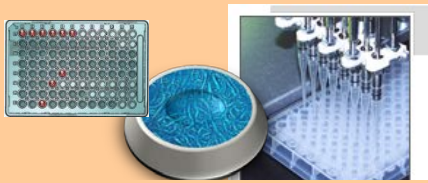
Endpoint selection for monitoring

- Remediation/restoration
- Effectiveness of regulation

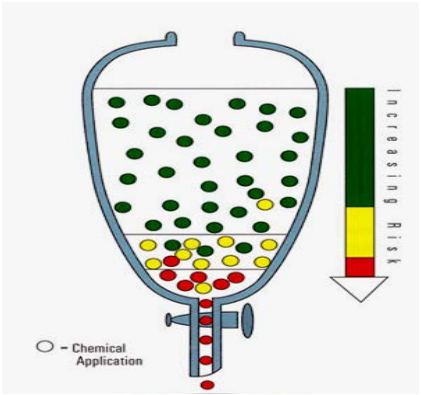
**AOP Knowledge**



In situ
Effects-based monitoring

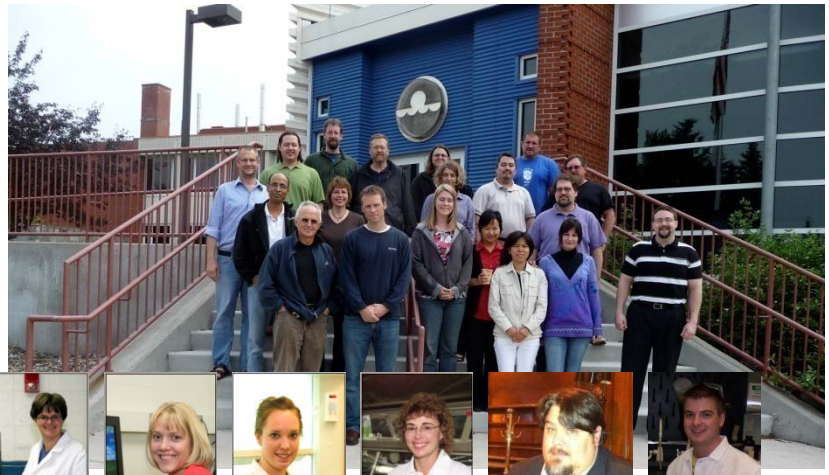


In vitro and HT screening
Environmental samples




Diagnostic assessments
High priority – chemical(s), class(es)

# Acknowledgements

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