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

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research & development

Project IA-1

## Agency Needs

1. Improved understanding of the linkages in the continuum between the source of a chemical in the environment and adverse outcomes
2. Predictive models for screening and testing
3. Improvements in quantitative risk assessment

## Research Goals

1. Identify new molecular markers of effects of exposure to chemicals representing different mechanisms of action (MOA) within the hypothalamic-pituitary-gonadal (HPG) axis of small fish models
2. Link these biomarkers to responses that are relevant to ecological risk assessments
3. Support development of integrated modeling approaches that utilize MOA as a basis for prediction of adverse outcomes

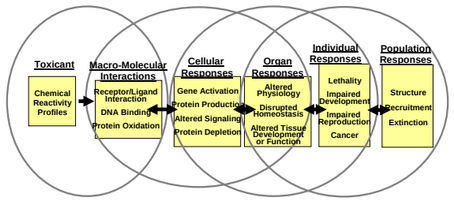
## Relevance to Client Offices

1. OSCP – Furthers validation of fathead minnow assay for testing endocrine disrupting chemicals. Impact on international programs for testing EDCs via relationship between OSCP and OECD.
2. OPPTS – a) Testing pesticides of direct interest to program office with respect to human health and/or ecological effects; b) helps develop linkages between mechanism-specific data and population level effects.
3. Contribution to EPA programs involved in monitoring and/or diagnostic (retrospective) risk assessments [e.g. NPDES program through OW and EPA Regions].

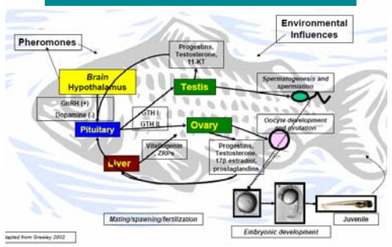
## Specific Approach

1. **Investigate effects of chemicals on the HPG axis using definitive tests in the fathead minnow to establish initial toxicity pathways and ecologically- relevant responses (Phase 1)**
2. **Identify the genomic, proteomic, metabolomic, and other whole-animal responses to the same chemicals using short-term zebrafish exposures (Phase 2)**
3. **Validate zebrafish molecular markers in the fathead minnow using extrapolation of relevant gene and protein markers with a subset of definitive endpoint tests and metabolite profiles for the HPG-active chemicals (Phase 3)**
4. **Concurrently integrate data from above in a systems modeling context, as well as relevant population modeling**

## Understanding Toxicity Pathways



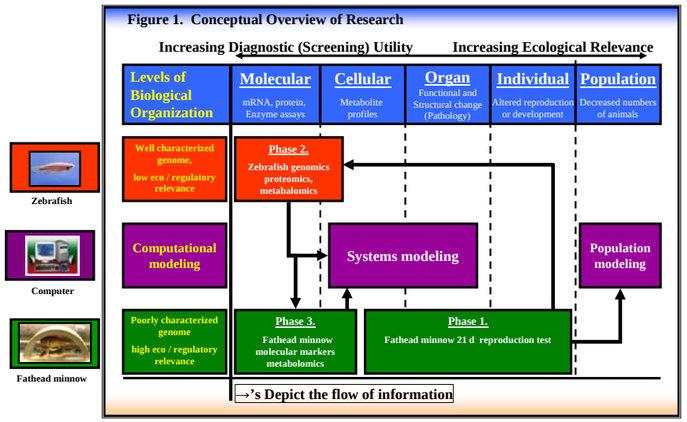

## Overview of Fish Reproduction

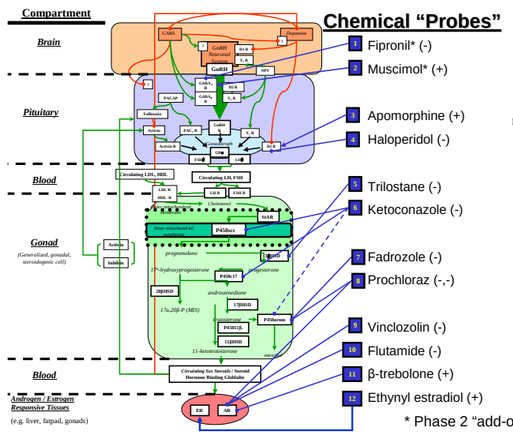



## Embracing Collaboration

## Methods/Approach

**Figure 1. Conceptual Overview of Research**



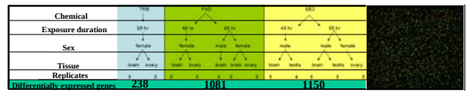




## Progress to Date

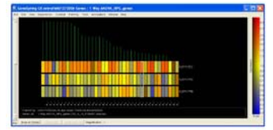
Phase 1 (definitive fathead minnow) assays completed with ketoconazole, fadrozole, prochloraz, flutamide, trenbolone, haloperidol, vinclozolin, apomorphine (preliminary). (EPA-MED)

Phase 2 (short-term zebrafish) assays completed with trenbolone, fadrozole, ethynyl estradiol (EE2), flutamide, and fipronil. (EPA-MED)

Zebrafish array work completed (Icoria) for EE2, fadrozole, trenbolone, and in progress for flutamide and fipronil (EPA-EERD).

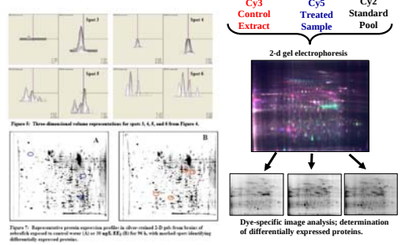



EE2 96 hr Male Brain: Differentially Expressed Genes



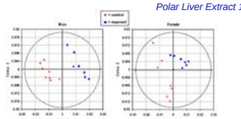
Relative expression of known HPG axis-related genes for the EE2, fadrozole, and trenbolone exposures.

Effects on the proteome of selected zebrafish tissues are being evaluated by Ettan™ DIGE 3-dye system (EPA-EERD)

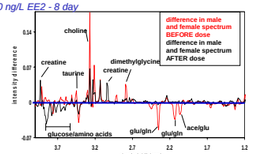



Proof-of-Concept Study for Eco-metabolomics Applied to EDCs:
Analysis of Male and Female Fathead Minnows Exposed to EE2 (EPA-ERD)

Polar Liver Extract 100 ng/L EE2 - 8 day

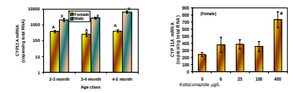


Exposure to EE2 produces a greater change in the metabolite profiles of males (as shown by a larger separation of classes in the scores plots) than females.
Exposure also reduces the similarity in profiles among males as compared to controls. The opposite is observed for the females.

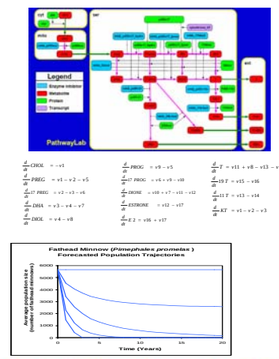



Results suggest feminization of males
Metabolites associated with this effect can be identified

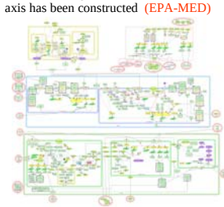
Over a dozen fathead minnow genes sequenced and quantitative RT-PCR assays developed for > 20 key HPG axis genes. Transcript levels measured under "baseline" conditions and after exposure to test chemicals. (EPA-MED)



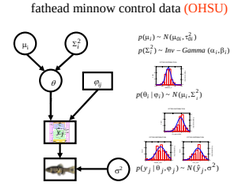

Deterministic computational model of vertebrate steroidogenesis (EPA – NCCT)



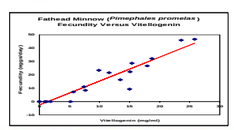

SBML compatible graphical systems model of the generalized teleost HPG axis has been constructed (EPA-MED)



Physiologically-based computational systems model being calibrated with fathead minnow control data (OHSU)



Population model linking from a biomarker, plasma Vtg levels, to predicted population outcomes.
(EPA-MED Grosse Ile)







Long Term Goal I

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY
COMPUTATIONAL TOXICOLOGY