Systems Biology Modeling of Fathead Minnow Response to Endocrine Disruptors

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research & development

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Science Question

The objective of this study is to develop a computational model to evaluate molecular and protein biomarkers in relation to reproductive dysfunction in fathead minnows exposed to environmental estrogens. The model will incorporate a number of biochemical endpoints along the hypothalamic-pituitary-gonadal axis, direct evaluation of physiological changes and reproductive endpoints and the pharmacokinetics of the contaminants.

We are testing the hypotheses that (1) genomic and proteomic biomarkers will be diagnostic of the biological effects of environmental estrogens and (2) that they will provide insightful understanding of the mechanisms of action that will relate specifically to reproductive endpoints in FHM.

Specific Questions:

- Is an exposure to a strong estrogenic compound the same as to an equivalent dose of a weak estrogenic compound? Are the same biochemical pathways activated?
- Can we use gene expression patterns to determine the class of compound to which an animal is exposed?
- Do individual compounds have specific gene expression patterns?
- Can we develop a computational model to integrate gene expression pathways, synthesis of specific proteins and their activities, and specific reproductive endpoints?

Research Goals

Research goals:

- We will determine dose-response characteristics in the low-dose region and determine sensitive in vivo end-points.
- We will determine gene expression and proteomics profiles for chemical classes of interest – including estrogens, androgens and their antagonists – and determine their potencies.
- We will develop a computational model to integrate the data.

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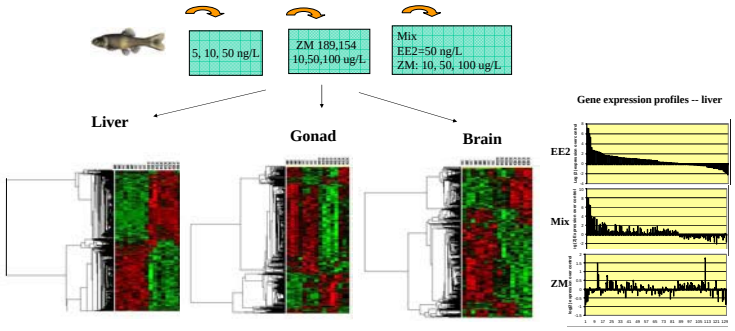
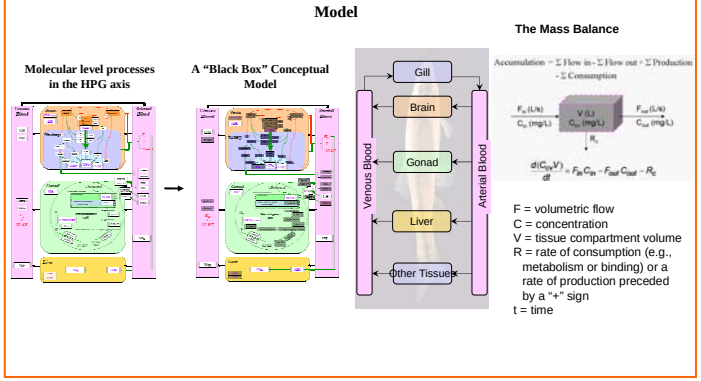
Methods/Approach

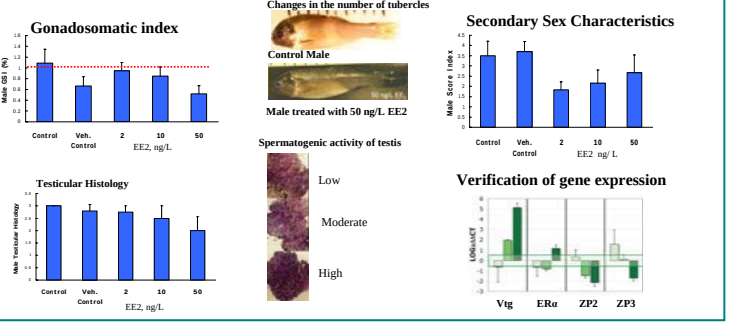
Gene Expression Studies: Exposure with EE2 and ZM and a mix of EE2 + ZM: 48 h exposure



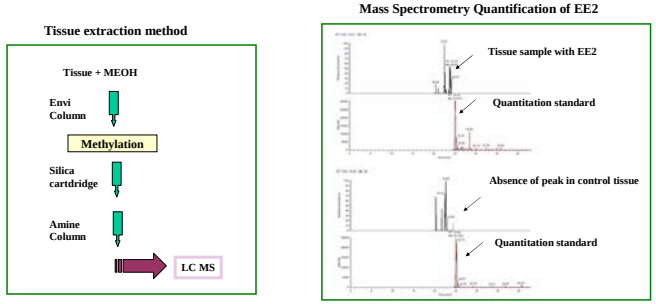
Model



Phenotypic Anchoring Experiments: 21 day exposures



EE2 measurements for model



Results/Conclusions

1. We have developed profiles for model compounds E2, and ZM 189,154 (estrogen and estrogen antagonist) and a mix of the two.
2. Exposure of male FHM to EE2 and ZM 189,154 for 21 days showed significant changes in GSI, testicular morphology and secondary sex characteristics but only at the highest dose – 50 ng/L.
3. We have developed a simplified physiologically based (PB) model to describe the pharmacokinetics of EE2, steroid hormones (e.g., E2 and T), and vitellogenin along with other intermediates in the hypothalamic-pituitary-gonadal axis pathways.

Impact and Outcomes

1. Use of a molecular approach to gain insight into processes occurring at the level of individual fish (gene expression, protein synthesis and physiological endpoints) and those occurring at the population level (reproductive outcome).
2. This work is highly relevant to recent and ongoing decisions and activities regarding remediation at superfund sites contaminated by EDCs.
3. Risk assessment requires an understanding of the long-term impacts of exposure to EDCs and related chemicals on fish inhabiting rivers and lakes.

Future Directions

1. We continue to analyze data for trenbolone and flutamide exposures and then apply information to exposure to zearalenone.
2. We continue to perform 21-day exposures to obtain data on phenotypic changes.
3. We continue to refine the PB model with new data from our project – getting input from various measurements including plasma E2, and 11-KT.
4. Work is in progress to integrate gene and protein expression into the model .

References

1. D. Villeneuve, et al. Conceptual HPG axis model, manuscript in preparation.
2. K. H. Watanabe, et al. A physiologically based model of ethinylestradiol exposed fathead minnows (*Pimephales promelas*) and the prediction of steroid hormone concentrations. (Planned submission to General and Comparative Endocrinology).
3. N. Garcia-Reyero, et al. "Expression signature for exposure to ethinylestradiol in fathead minnows (*Pimephales promelas*). (Planned submission to Environmental **Science and Technology).**

Long Term Goal I