

A Computational Approach to Predict Endocrine Disrupting Activity in Water Using a Medaka Fish Model

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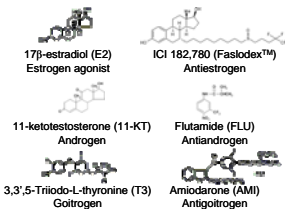
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Background

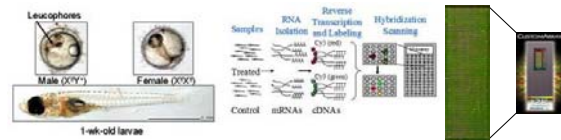
Endocrine disrupting chemicals (EDCs) occur within a suite of surface water, groundwater, and wastewater contaminants that could be compromising human and environmental health. Aquatic EDCs from natural and anthropogenic origins including agricultural runoff and industrial effluents significantly reduce the quality of water resources. In many cases, potential EDCs exist in the environment as mixtures that could act synergistically (Falconer et al. 2006).

A novel computational approach was utilized to characterize the patterns of genomic response of Qurt strain Japanese medaka (*Oryzias latipes*) larvae after short term exposures to biologically effective concentrations of three receptor agonist/antagonist EDCs and their mixtures.

Three pairs of prototypic agonist/antagonist EDCs



Methodology



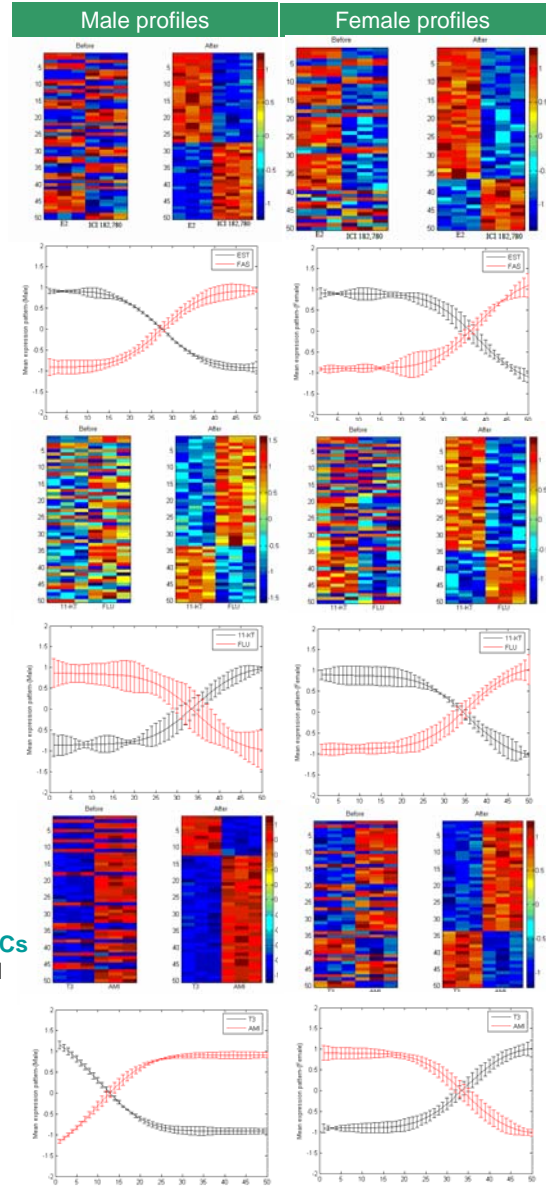
Custom oligonucleotide array = ~9.4K unique probes
Sample size = 200 larvae per gender per three replicates
Static 6h exposure of males and females
RNA isolation and purification
Microarray hybridization and scanning

Data/Analysis

Identification of unique gene response patterns of EDCs
Six Prototypical EDCs: E2, ICI, 11-KT, FLU, T3 and AMI
Three EDC mixtures: E2+ICI, 11-KT+FLU, and T3+AMI
Clustering/classification analysis:

Microarray feature extraction with ScanAlyze™ software
Loess normalization and functional embedding for high dimension data analyses (Muller and Wu 2007)
Latent ordering of genes for pattern analysis and identification
Annotation of unique EDC probes in Qurt medaka

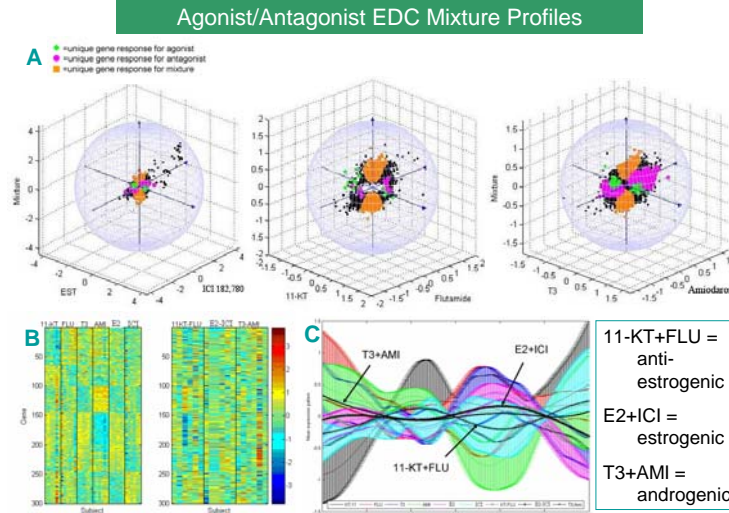
Figure 1. Gender-specific expression profiles of EDCs: Heat map images before and after application of a functional embedding technique and corresponding scattered smoothed mean curves.



Results

Figure 2. Synergistic effects of EDC mixtures:

(A) Vector Analysis results for genomic responses to prototypic EDCs single and paired (B) Heat map and (C) smoothed curves comparison of single and mixed genomic profiles.



Agonist/Antagonist EDC Mixture Profiles

Potential Biomarkers for prototypic EDCs in medaka

Gene name and rank	E-value	Regulation in ♂	
		11KT	FLU
Regulation in ♂			
cytP450 (18)	0	↑	↑
Regulation in ♀			
putative:ferritin(7)	0	↑	↓
putative:ER-binding cyclophilin(22)	0	↑	↓
putative:GSH-T gene(23)	0	↑	↓
cyclin B1(36)	0	↓	↑
Regulation in ♂			
Regulation in ♀			
Regulation in ♂			
Regulation in ♀			
Regulation in ♂			
Regulation in ♀			
Regulation in ♂			
Regulation in ♀			

Discussion and Conclusion

- Diagnostic probes identified in this study are a powerful means to discriminate between different types of endocrine disrupting activity at the transcription level.
- Annotation of diagnostic probes revealed alterations of potential biomarkers associated to metabolism, stress responses, molecular repair, neural networks, and reproductive physiology in male and female medaka.
- Critics of EDC screening and testing programs have indicated that the current approach of regulatory agencies does not allow a thorough examination of synergistic interactions among chemicals (Bienen 2006). Our computational approach could be an effective tool for the assessment of EDC mixture activity and their synergistic effects.

Future Directions

To utilize the microarray assay in rapid screening and prioritizing chemicals for EDC activity.

To utilize the medaka microarray and computational approach to test for the activity of endogenous and synthetic hormones, pharmaceuticals and personal care products in ambient water.

Evaluate how EDC gene patterns change over time and dose.

References

Bienen, L. (2006) *Frontiers Ecol. And the Environ.* 4(4):174.
Falconer et al. (2006) *Environ. Toxicol.* 21(2):181-191.
Muller and Wu (2007) In preparation.

Partners

