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Short communication

Dose–response relationships in gene expression profiles in rainbow trout, *Oncorhyncus mykiss*, exposed to ethynylestradiol

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Abstract

Determining how gene expression profiles change with toxicant dose will improve the utility of arrays in identifying biomarkers and modes of toxic action. Isogenic rainbow trout, *Oncorhyncus mykiss*,were exposed to 10, 50 or 100 ng/L ethynylestradiol (a xeno-estrogen) for 7 days. Following exposure hepatic RNA was extracted. Fluorescently labeled cDNA were generated and hybridized against a commercially available Atlantic Salmon/Trout array (GRASP project, University of Victoria) spotted with 16,000 cDNAs. Transcript expression in treated vs control fish was analyzed via Genespring (Silicon Genetics) to identify genes with altered expression, as well as to determine gene clustering patterns that can be used as "expression signatures". Array results were confirmed via qRT PCR. Our analysis indicates that gene expression profiles varied somewhat with dose. Established biomarkers of exposure to estrogenic chemicals, such as vitellogenin, vitelline envelope proteins, and the estrogen receptor alpha, were induced at every dose. Other genes were dose specific, suggesting that different doses induce distinct physiological responses. These findings demonstrate that cDNA microarrays could be used to identify both toxicant class and relative dose.

Keywords: Rainbow trout; cDNA microarray; Estrogens

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The field of toxicogenomics holds great promise for environmental and aquatic toxicology. Specific applications of microarrays in ecotoxicological studies include the identification of contaminants through analysis of expression patterns measured via microarrays in test organism exposed to previously uncharacterized contaminant mixtures, the identification of chemical mode of action based on the mRNA expression patterns, and the development of tools to assess endpoints for chronic exposure to mixtures that are tied to population-level consequences (Miracle and Ankley, 2005). However, the baseline transcriptomic response in organisms exposed to model toxicants must be characterized before this technology can be meaningfully applied to contaminated field sites. The objective of this study is to determine the relationship between gene expression profile and toxicant dose for the synthetic estrogen, ethynylestradiol (EE2).

Methods for exposure and EE2 kinetics are described in more detail in Skillman et al. (in press), while RNA preparation and labeling, microarray hybridization and data analysis are described in more detail in Hook et al. (2006). We used isogenic fish in this study to minimize the confounding effects of individual variability. Male isogenic Rainbow Trout (*Oncorhynchus mykiss*) were exposed to EE2 at nominal concentrations of 10, 50 and 100 ng/L EE2 (actual concentrations were 8, 33, and 115 ng/L EE2) via methanol drip (methanol concentration was less than 0.02%) for seven days and compared to methanol only controls. Fish were euthanized, then RNA was harvested from the livers of exposed and control trout using TRIzol reagent (Invitrogen) and the manufacturer's protocol. RNA was DNAase treated using TURBO – DNA free clean up procedure (Ambion). Total RNA (2 μg) was labeled with Cy 3 and Cy 5 fluors (Amersham) using Invitrogen's superscript indirect cDNA labeling system following the manufacturer's protocol. Three replicates of RNA from three different individual fish were labeled (3×3 design). High density microarrays (16,000 cDNA spots per array) were obtained from GRASP project at the University of Victoria and were prepared as described in Rise et al. (2004a). Fluorescently labeled cDNA was hybridized to the arrays overnight at 45° in a humidified

Table 1
Primers and Probes used for qPCR analysis

Gene	Forward	Reverse	Fluorescently labeled
Beta actin	ACGGCCAGAG-GCGTACAG	TTCAACCCT-GCCATGT	ACAACACGGCCT-GGATGGCCA
Estrogen receptor	GCAGGACCAAA-CTCCGTAGTG	TGGCCAACG-CGAGGTA	TACCCAGAGGCAA-AGTCGCTGCAGA
GnRH-1	AATGGTCCTCAG-TACGCCTATGTT	GCATCCCCCT-ATCCACATCA	ATATTCCATAACAAATC-TGCCATTTCCAGCTACAA
Haptoglobin	CACGGCACAGGA-CTTATCGA	CAGTCCAGGA-CCCCAAAGAC	ACAGGATCCCTGCAGC-ATACACTCT
Pentraxin	GCTGGGTCAGAC-CCGTTTTA	CAGTTTGCCAG-ATTCAAAAAATGA	AGTCCCAGGTCCAGCA-GATGGAGATCC
Serum albumin 2	TCTTCCCAGAGAG-CAGCAGATC	GATGCCAGCA-GCTTCCACAT	TTGCTGTCCTTGGTGC-AGAGCTCG
Vitellogenin	CTTGTGAACCCT-GAGATC	GCAGCTGGGA-CGAAAGG	TTGAGTACAGTGGTGT-GTGGCCCAAAGA
Vitelline envelope	GCCGGTTCCTCC-TCCAAAT	TCCGCTGCCC-AGTCTGA	CTGATATAGCTCCTGG-GCCCCTCATAGTTG

All primers are listed from 5′ to 3′. Fluorescently labeled primers have a 6-FAM fluor on the 5′ end and a TAMRA fluor on the 3′ end.