

**ZEBRAFISH CARDIOVASCULAR cDNA MICROARRAYS:  
EXPRESSION PROFILING AND GENE DISCOVERY  
IN EMBRYOS EXPOSED TO 2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN**

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by

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submitted in partial fulfillment of the requirements for the degree of

**Doctor of Philosophy**

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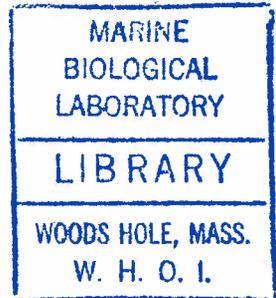
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*“Success is going from failure to failure without a loss of enthusiasm.”*

-Winston Churchill



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**Abstract**

2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is a potent teratogen that impacts the developing cardiovascular system. Hallmarks of embryonic exposure include cardiac malformation, impaired circulation, loss of erythrocytes, pericardial and yolk sac edema, and early life stage mortality. However, the mechanism of TCDD cardiovascular embryotoxicity is poorly understood. The primary goal of this thesis was to identify TCDD-responsive genes likely to be involved in processes of toxicity.

We constructed microarrays using cDNA libraries derived from zebrafish embryonic and adult heart tissue. Embryonic heart arrays were used for protocol development. The resulting workflow was employed in the production of adult heart microarrays containing ~2800 unique cardiovascular genes.

These arrays were used to establish gene expression profiles of zebrafish embryos exposed to  $1.84 \pm 0.42$  or  $10.74 \pm 0.138$  ng TCDD/g embryo. Alterations in cardiovascular gene expression were limited; 44 genes or ESTs were significantly differentially expressed  $\geq 1.8$ -fold ( $p$ -values  $\leq 5 \times 10^{-4}$ ), and only CYP1A and CYP1B1 were induced  $>4$ -fold. Transcriptional responses to TCDD were highly dose-dependent, and adaptive responses were a prevalent feature of TCDD-modulated gene expression.

Microarray analyses indicated induction of genes in three major functional classes – xenobiotic detoxification, sarcomere structure, and energy transfer. TCDD-modulation of selected genes was verified by RT-PCR. Induction of mitochondrial electron transfer genes was variable and modest; such induction provides a possible pathway to reactive oxygen generation and cardiac pathology. Sarcomere genes were generally robustly induced, but RT-PCR indicated suppression of cardiac troponin T2. The current data suggest that TCDD causes cardiomyopathy in zebrafish embryos.

Investigation of a TCDD-induced EST cluster led to the discovery of a novel retroelement, EZR1. EZR1 elements lack genes necessary for autonomous retrotransposition, but are highly expressed in normal and TCDD-exposed cardiac tissue. Putative regulatory elements in LTR sequences may account for observed expression patterns. The function, if any, of EZR1 remains open to speculation.

Thesis Supervisor: John J. Stegeman, Senior Scientist and Chairman of Biology, WHOI



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