

## Session 1: Cardiovascular Toxicology

### Screening for Vascular Disruptor Compounds *In Vivo* and *In Vitro*

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Vascular disruptor compounds (VDCs) can cause birth defects, as exemplified by the vascular disrupting drug thalidomide that induced severe malformations in babies after prenatal exposure. Only a few out of the tens of thousands of registered industrial chemicals in the U.S. market have been tested for vascular disrupting capacity, mostly due to the limited availability of appropriate screening models. Here, we present the development of two VDC screening models, an *in vivo* model based on zebrafish and an *in vitro* model of endothelial tube formation using the murine yolk sac-derived endothelial cell line C166. We first used transgenic *kdr1:eGFP* fish line to visualize the development of the vascular system under normal and perturbed conditions using the VEGFR2 inhibitor PTK787. Next, we screened about 200 compounds from the ToxCast Phase I library for vascular disrupting capacity *in vivo*. Thirty-seven VDCs were identified to cause a plethora of vascular perturbations in the zebrafish embryo, including malformed intersegmental vessels, abnormal caudal vein plexus, brain vessel malformation and hemorrhages. The effects of the identified VDCs on endothelial tube formation were analyzed in C166 cells. Twenty-four hits were identified that perturbed both vascular development in zebrafish *in vivo* and endothelial tube formation *in vitro*. In addition to the ToxCast chemicals, we show that arsenic acts as a VDC in both zebrafish and C166 cells. To study the mechanism of vascular disruption we mapped changes in expression of key regulators of angiogenesis and vasculogenesis after arsenite exposure to zebrafish embryos, and found that the expression of eight key factors was significantly altered. In conclusion, our results show that several environmental pollutants have vascular disrupting capacity and that the developing zebrafish embryo is an efficient *in vivo* model that in combination with *in vitro* models can be used for identification of VDCs.

## TCDD and AHR in the Zebrafish Heart

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The organisms most sensitive to dioxin (TCDD) toxicity are developing teleost fish, so we have used the zebrafish embryo as a model, focusing on TCDD-induced developmental heart failure. We find that TCDD prevents both the emergence of epicardial progenitors to form the proepicardium and the migration of epicardial cells across the developing heart. Because of the known importance of the epicardial layer in heart formation, the loss of the epicardium can account for TCDD-induced heart failure during development. To examine the contribution of AHR receptor (AHR) activation in the cells of the heart itself, we developed a constitutively active form of the zebrafish AHR2 (caAHR) driven by the cardiomyocyte-specific *cmlc2* promoter. We find that AHR activation solely within cardiomyocytes recapitulates almost all of TCDD toxicity, indicating that AHR activation in the heart by TCDD can explain the toxic effects. Although the caAHR prevented epicardium formation, it did not replicate the effect of TCDD on the proepicardium: the proepicardium formed on the pericardial mesothelium in spite of caAHR expression in the nearby cardiomyocytes. We have developed an *in vitro* culture model to better understand the effects of TCDD on epicardium and myocardium. Although the acute exposure model is useful, we are also interested in effects of sublethal exposure. Juveniles were exposed to 50 parts per trillion TCDD in two 1 h treatments prior to sexual maturation and raised to adulthood. While non-lethal, these exposures produced defects in the axial skeleton and in reproduction that were also seen in subsequent unexposed generations. Adult hearts removed from fish exposed as early juveniles have substantial lesions in the heart muscle. These lesions show alterations in the normal relationship between myocardium and epicardium, suggesting that processes affected in the embryo by acute lethal doses may also be altered by sublethal exposure.

*All procedures involving animals were approved by the Animal Care and Use Committee of the University of Wisconsin–Madison, and adhered to the National Institutes of Health's "Guide for the Care and Use of Laboratory Animals."*

## **High-Content Screening Assay for Identification of Chemicals Impacting Cardiovascular Function in Zebrafish Embryos**

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Zebrafish offer one of the most promising alternative and cost-effective vertebrate models for predicting prenatal developmental toxicity and fish early life-stage toxicity, particularly since zebrafish embryos are nonprotected life stages and, as such, considered alternative testing models within the European Union and United States. Unlike cell lines, zebrafish embryos offer an intact, multicellular system that models integrative physiological processes and, at the same time, is amenable to noninvasive, whole-animal imaging. Therefore, combined with rapid, *ex utero* development, high fecundity, transparency, and small size, zebrafish are ideal model systems for supporting chemical toxicity screening and prioritization efforts within the United States and abroad. Despite significant advancements over the last 10–15 years, targeted assays using zebrafish are needed to better evaluate effects of chemicals on organogenesis and begin classification of chemicals by toxicologically relevant modes-of-action. To this end, using transgenic zebrafish (*fli1:egfp*) that stably express eGFP within vascular endothelial cells, we have developed and optimized a 384-well-based high-content screening (HCS) assay that enables us to screen and identify chemicals affecting cardiovascular function at sublethal, nonteratogenic concentrations. Following static exposure of one embryo per well from 5–72 hours post-fertilization (hpf), automated image acquisition procedures and custom image analysis protocols are used to quantify body length, circulation, heart rate, pericardial area (a biomarker for cardiac looping defects), and intersegmental vessel area within freshly hatched live embryos. After optimizing 72-hpf anesthetization procedures, we evaluated each endpoint across four independent control plates containing 384 initial embryos per plate. Survival and imaging success rates across these plates ranged from 93–99% and 42–74%, respectively. Criteria were then defined for assay success and analysis of treatments, and 10 chemicals were screened for targeted effects on cardiovascular function. Compared to existing zebrafish-based assays, this method provides a comprehensive discovery platform with (1) increased sample sizes, (2) broad concentration-response format, and (3) the ability to identify chemicals that target cardiovascular function at nonteratogenic concentrations.

## **Epicardial Cells and Heart Regeneration**

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Adult mammals show limited muscle regeneration after cardiac injury. By contrast, adult zebrafish have a high natural capacity for heart regeneration. This regeneration occurs through activating proliferation of pre-existing cardiomyocytes at sites of injury, rather than *de novo* creation of new cardiomyocytes from stem cells. Nonmuscle epicardial and endocardial cells help stimulate regeneration by these cardiomyocytes, by aiding neovascularization and/or releasing mitogens. Here, we have used new technologies to examine the cellular and molecular mechanisms by which the epicardium responds to injury and influences heart regeneration in zebrafish.