Identification of chemical vascular disruptors during development using an integrative predictive toxicity model and zebrafish and *in vitro* functional angiogenesis assays

Tamara Tal[1], Nicole Kleinstreuer[2], Tarja Toimela[3], Riina Sarkanen[3], Tuula Heinonen[3], Thomas Knudsen[4], and Stephanie Padilla[1].

[1]U.S. EPA/ORD/ISTD, RTP, NC; [2]ILS, inc./NICETUM, RTP, NC; [3]FICAM, University of Tampere, Tampere, Finland; and [4]U.S. EPA/ORD/NCCT RTP, NC

Chemically-induced vascular toxicity during embryonic development can result in a wide range of adverse prenatal outcomes. Previously, we constructed an embryonic vascular disruption adverse outcome pathway (AOP) based on molecular initiating events corresponding to genetic models with phenotypic evidence of abnormal embryonic vascular development in the Mouse Genome Informatics Database. Here we used ToxCast high throughput screening data for 25 assays mapping to targets in the Vascular Disruption AOP to prioritize 1060 chemicals for their potential to disrupt vascular development. A subset of 37 predicted vascular disrupting chemicals (pVDCs) or non-pVDCs, including pesticides, flame retardants, and endocrine active compounds, were selected for targeted testing in zebrafish (*D. rerio*). To test computational predictions, TG(flk1:GFP) zebrafish embryos were used to visualize and quantify blood vessel formation during development. Manual and automated methods of vessel quantification were developed, and the assay was evaluated with anti-angiogenic reference compounds PTK787 and AG1478, small molecule inhibitors of VEGFR2 and EGFR, respectively. The zebrafish assay was then used to test the effects of 37 chemicals in combination with a functional angiogenesis assay comprised of a human endothelial cell and fibroblast co-culture system. Chemical rankings were well correlated among the predictive signature and zebrafish and *in vitro* tubulogenesis assays. Taken together, the zebrafish assay meets a critical need for an *in vivo* platform that can assess predictions generated by computational models of developmental vascular toxicity. *This abstract does not necessarily reflect EPA policy.*

Keywords: Zebrafish, Vascular Toxicity, Predictive Toxicity