EPA Public Access

Author manuscript

Curr Opin Toxicol. Author manuscript; available in PMC 2020 June 01.

About author manuscripts

Submit a manuscript

Published in final edited form as:

Curr Opin Toxicol. 2019 June 1; 15(1): 55–63. doi:10.1016/j.cotox.2019.04.004.

Systems Modeling of Developmental Vascular Toxicity

Katerine S. Saili^{1,a}, Jill A. Franzosa^{1,a,b}, Nancy C. Baker^{1,2}, Robert G. Ellis-Hutchings³, Raja S. Settivari³, Edward W. Carney^{3,t}, Richard Spencer⁴, Todd J. Zurlinden¹, Nicole C. Kleinstreuer⁵, Shuaizhang Li⁶, Menghang Xia⁶, Thomas B. Knudsen^{1,*}

¹National Center for Computational Toxicology (NCCT), Office of Research and Development (ORD), U.S. Environmental Protection Agency (USEPA) Research Triangle Park NC 27711

²Leidos, Research Triangle Park NC 27711

³Toxicology and Environmental Research and Consulting The Dow Chemical Company, Midland, MI 48674

⁴ARA, Research Triangle Park NC 27711

⁵National Toxicology Program (NTP), National Institute of Environmental Health Sciences (NIEHS) Research Triangle Park, NC 27711

⁶National Center for Advancing Translational Sciences (NCATS) National Institutes of Health, Bethesda, MD 20892

Abstract

The more than 80,000 chemicals in commerce present a challenge for hazard assessments that toxicity testing in the 21st century strives to address through high-throughput screening (HTS) assays. Assessing chemical effects on human development adds an additional layer of complexity to the screening, with a need to capture complex and dynamic events essential for proper embryo-fetal development. HTS data from ToxCast/Tox21 informs systems toxicology models, which incorporate molecular targets and biological pathways into mechanistic models describing the effects of chemicals on human cells, 3D organotypic culture models, and small model organisms. Adverse Outcome Pathways (AOPs) provide a useful framework for integrating the evidence derived from these *in silico* and *in vitro* systems to inform chemical hazard characterization. To illustrate this formulation, we have built an AOP for developmental toxicity through a mode of action linked to embryonic vascular disruption (Aop43). Here, we review the model for

***Corresponding Author:** Thomas B. Knudsen, PhD, National Center for Computational Toxicology (B205-01), U.S. Environmental Protection Agency Research Triangle Park, NC 27711, knudsen.thomas@epa.gov| Phone 919 541 9776.

^aThe first two authors contributed equally to this work.

^bPresent address: National Health and Environmental Effects Laboratory (NHEERL), Office of Research and Development (ORD), U.S. Environmental Protection Agency (USEPA), Research Triangle Park, NC 27711

^tdeceased

Publisher's Disclaimer: This is a PDF file of an unedited manuscript that has been accepted for publication. As a service to our customers we are providing this early version of the manuscript. The manuscript will undergo copyediting, typesetting, and review of the resulting proof before it is published in its final form. Please note that during the production process errors may be discovered which could affect the content, and all legal disclaimers that apply to the journal pertain.

Disclaimer: The views expressed in this article are those of the authors and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

quantitative prediction of developmental vascular toxicity from ToxCast HTS data and compare the HTS results to functional vascular development assays in complex cell systems, virtual tissues, and small model organisms. ToxCast HTS predictions from several published and unpublished assays covering different aspects of the angiogenic cycle were generated for a test set of 38 chemicals representing a range of putative vascular disrupting compounds (pVDCs). Results boost confidence in the capacity to predict adverse developmental outcomes from HTS *in vitro* data and model computational dynamics for *in silico* reconstruction of developmental systems biology. Finally, we demonstrate the integration of the AOP and developmental systems toxicology to investigate the unique modes of action of two angiogenesis inhibitors.

Keywords

Adverse outcome pathway; angiogenesis; 5HPP-33; TNP-470; ToxCast; Tox21

Introduction

The cardiovascular system is the first organ system to function in the vertebrate embryo reflecting its critical role during development and pregnancy [1–3]. Stepwise events in vascular patterning commence in the early embryo with nascent vessel assembly from angioblasts (vasculogenesis) and expand by sprouting new vessels from the pre-existing network (angiogenesis) into an arborized system of arteries, veins and lymphatics (angioadaptation) shaped by hemodynamic forces and microphysiological signals [4]. Vascular insufficiency is tied to many disease processes (stroke, diabetes, preeclampsia, neonatal respiratory distress, Alzheimer's, etc. [4]) and adverse pregnancy outcomes [5], including preterm labor [6], low birth weight [7], birth defects [8] and miscarriage [9]. The interplay between maternal exposure and fetal effects is reflected in the dynamics of vascular development. Thalidomide, for example, disrupts immature blood vessel development in the rudimentary limb-bud leading to altered mesenchymal outgrowth and, in turn, phocomelia [10]. Taken together, the evidence linking disruption of vascular development to adverse birth outcomes supports the hypothesis that gestational exposure to vascular disrupting chemicals is one of the major modes of action in teratogenesis [5].

A predictive signature for putative vascular disrupting compounds (pVDCs) was developed using information from genetic mouse models (Jackson Labs Mammalian Phenotype Browser) to map high-throughput screening (HTS) *in vitro* data (ToxCast™) to critical vascular developmental signaling pathways, and analyzed against the backdrop of *in vivo* legacy data from pregnant animal bioassays (ToxRefDB) [11, 12]. The U.S. Environmental Protection Agency (EPA) Toxicity Forecaster (ToxCast™) program was initiated to investigate the molecular targets of environmental contaminants using cell-free and cell-based HTS assays [13]. Computational models are built upon the results of this screening data to rapidly predict chemical hazards to human health [14, 15]. The *in vitro* data generated through the ToxCast™ program originally included 309 environmental chemicals tested across a diverse suite of >600 HTS assays [5, 11]. Many, although not all, chemicals associated with developmental toxicity *in vivo* had an *in vitro* bioactivity signature for vascular disruption [11]. For example, the anti-angiogenic thalidomide analogue, 5HPP-33,

scored high for predicted vascular disruption in the ToxCast™ dataset [11]. Its vascular disrupting potential was further demonstrated in a computational agent-based model with emergent features that recapitulated the *in vitro* effects of 5HPP-33 on human umbilical vein endothelial cell (HUVEC) vasculogenesis [16]. 5HPP-33 was subsequently confirmed to be embryolethal in rat whole embryo culture [17], consistent with evidence of embryotoxicity in rodents, and in contrast to its teratogenicity in rabbits when administered orally [8, 18, 19]. TNP-470 is an additional anti-angiogenic reference compound that is embryotoxic in rodents [20] and also scored high for predicted vascular disruption in the ToxCast™ dataset [11].

Adverse Outcome Pathways (AOPs) provide a useful framework to link molecular initiating events to key cellular- and tissue-level changes resulting in adverse outcomes, and to inform chemical prioritization for hazard characterization. We have built an AOP for developmental toxicity through a mode of action linked to embryonic vascular disruption for the Organization for Economic Cooperation and Development (OECD) Workplan. This AOP, referred to as Aop43 in the OECD Wiki [<https://aopwiki.org/aops/43>], centers on the ligand activation of vascular endothelial growth factor receptor 2 (VEGFR2) by vascular endothelial growth factor A (VEGFA), which initiates angiogenic sprouting, growth, and fusion during early developmental stages [21]. Inhibition of VEGFR2 activity is the molecular initiating event (MIE), which may occur through modification of local VEGFA levels, VEGFR2 expression or molecular function, or impacts on downstream signal transduction pathways [22–25]. Here, we review the construction of Aop43 and examine its usefulness as a framework to understand and predict developmental toxicity relevant to prioritization for hazard characterizations and human risk assessment decisions for cases that consider exposures and uses. To develop an AOP for embryonic vascular disruption (Figure 1), we searched the gene ontology (GO) and mammalian phenotype (MP) browsers of the Mouse Genome Informatics database [<http://www.informatics.jax.org/>] for terms affiliated with the disruption of vascular development. Terms for abnormal vasculogenesis [MP: 0001622] and abnormal angiogenesis [MP:0000260] were captured and linked to ToxCast™ assays. This list had 65 target genes with bona fide roles in vasculogenesis or angiogenesis, 50 of which had evidence of abnormal embryonic vascular development based on genetic mouse models [5]. The broader AOP framework links multiple, complementary studies that serve as weight of evidence supporting the AOP and from which developmental toxicity predictions are possible.

2. Assessing the weight of evidence in support of Aop43

First-generation predictive models for prenatal developmental toxicity built from the ToxCast™ data revealed a complex web of biological processes with many connections to vasculogenesis and angiogenesis [11, 26]. To assess the *in silico* pVDC predictions [5], 38 chemicals representing a range of pVDC scores were selected to represent roughly equal numbers of pVDCs and non-pVDCs ranging from known developmental toxicants to chemicals that have not demonstrated overt developmental outcomes [11]. These compounds were then tested across ten *in vitro* platforms from laboratories addressing different aspects of the vasculogenic/angiogenic cycle. Some of the platforms used traditional HUVECs while others used human induced pluripotent stem cells (iPSCs), rat fetal aortic explants [16, 27],

or transgenic reporter zebrafish lines [28, 29]. The platforms covered different stages of the vascular cycle, from angiogenic sprouting and network assembly in various matrices [30, 31] to co-culture models of tubulogenesis [32, 33], microvessel outgrowth and patterning [17, 28]. In several cases, a link between key event relationships (KERs) is evident through chemical toxicity profiles. For example, chemicals that disrupted angiogenic sprouting [30] also inhibited endothelial tubular network formation [31].

The weight of evidence for KERs in Aop43 is summarized in Figure 2, with ToxCast™ pVDC scores in the first column, followed by columns indicating chemical effects for each of the ten datasets on endothelial cell morphology [21, 28–34]. Shades of red indicate chemicals that were predicted positive (column A) or for which any *in vitro* assay measured a positive effect (column L), with higher scores indicated by darker shades of red. In contrast, a graded color scheme was not used for columns B through K (positive = red, negative = green, black = cytotoxic, white = not tested). While no single study confirmed all of the pVDC predictions, the combined vascular disrupting effects across all studies (column L) aligned well with the *in silico* predictions (column A). Based on this data, the *in silico* predictions included two false positives (tris(2-ethylhexyl) phosphate and PFOS) and three false negatives (1,2,4-Trichlorobenzene; diethanolamine, and tris(2-chloroethyl) phosphate) compared to column L. Overall, the *in silico* prediction sensitivity and specificity were 0.89 and 0.80, respectively; prediction accuracy was 87%, with greater predictivity of true positives (positive predictive value; PPV 93%) compared to predictivity of true negatives (negative predictive value; NPV 73%).

The Finnish Center for Alternative Methods (FICAM) assay (Figure 2; column B) [32] showed the greatest alignment with the pVDC predictions. This was one of six *in vitro* tubulogenesis assays represented. Briefly, the assay utilizes primary HUVECs and adipose-derived stromal cells to measure tubule formation [32]. In this study, the 38 chemicals tested were assigned tubulogenesis scores to derive a concentration response and predict half maximal activity (AC₅₀) values [33]. An additional tubulogenesis assay (NCATS; column C) that utilizes commercially available HUVECs in culture [34] was also highly correlated with the *in silico* predictions. A third tubulogenesis assay comparing growth of commercially available HUVECs in synthetic medium (column D) or Matrigel (column E) predicted several anti-angiogenic compounds [31], most of which aligned with FICAM predictions, albeit with less sensitivity. An additional tubulogenesis assay (column K) aligned poorly with the *in silico* pVDC predictions (only four of the 28 positive chemicals in column L were detected in this assay); however, endothelial cell migration assays (columns F and G) aligned better with the predictions (nine and ten of the 28 positive chemicals were detected in these assays, respectively) [33]. In addition to the *in vitro* assays, a VEGFR2 reporter assay using transgenic zebrafish picked up seven pVDC compounds (column I) [28], while an additional zebrafish screen assessing hyaloid bone development picked up 3 pVDCs (column J) [29]. Taken together, the cumulative results from the ten assays had a high correlation with the pVDC predictions. This example supports a weight of evidence approach based on an AOP framework that can better inform hazard evaluations than any study taken alone. While the growing evidence supporting key events in Aop43 is strong, gaps remain in our understanding of the molecular signaling events linking them (i.e., KERs). Integrated systems models (e.g., whole embryo cultures) are useful for investigating

the molecular biology underlying key event relationships and constructing AOP-based ontologies to elucidate the developmental toxicity of environmental contaminants.

3. Exploring key event relationships via transcriptomics analysis of integrated systems models

3.1. Vascular disrupting compounds, 5HPP-33 and TNP-470, cause lethality and dysmorphogenesis in rat whole embryo culture (WEC).

Embryonic zebrafish clearly show the quantitative linkages in Aop43 from molecular initiating event (VEGFR2 inhibition by PTK787) to dysmorphogenesis and viability later in life [21]. Because species differences may underlie some of the insensitivity of the zebrafish model towards the 38-chemical list in Figure 2, it is useful to identify mammalian developmental systems models for exploring key event relationships. Ellis-Hutchings et al. (2017) used rat whole embryo culture (WEC) [17] focused on two known angiogenesis inhibitors with high pVDC bioactivity scores from ToxCast™, yet differing *in vitro* activity scores (i.e., 5HPP-33, a synthetic thalidomide analog [35], had the highest *in vitro* activity score; while TNP-470, a synthetic fumagillin analog [17, 36], had the lowest positive *in vitro* activity score (Figure 2)). Endothelial cells are a primary target for both compounds based on the potency of 5HPP-33 (AC50 = 4.9 µM) and TNP-470 (AC50 = 0.3 µM) on inhibiting endothelial cell proliferation over any of the other ToxCast™ assays used for the vascular disruption signature [11, 17].

Ellis-Hutchings and colleagues next evaluated vascular aberrations in an intact conceptus (rat WEC) and *ex vivo* angiogenesis model (rat aortic explant assay) exposed to each of the two vasculogenesis / angiogenesis inhibitors [17]. Gestation day 10 (GD10) rat embryos isolated from the uterus and cultured in the presence of these inhibitors showed major effects at 48 h compared to DMSO-exposed vehicle controls: 5HPP-33 (15 µM) caused embryo lethality and TNP-470 (0.25 µM) caused dysmorphogenesis. These effects were also observed in a zebrafish embryotoxicity assay for 5HPP-33 (> 0.1 µM) and TNP-470 (> 0.01 µM) [17]. Both compounds also inhibited microvessel outgrowth from rat aortic explants. Taken together, both compounds consistently inhibited angiogenesis in all assays reported by Ellis-Hutchings and colleagues, with TNP-470 potency 10–100x greater than that of 5HPP-33, and with 5HPP-33 effects appearing at earlier stages of the angiogenic cycle [17].

3.2. The Aop43 framework guides exploration of key event relationships

Although modes of action for TNP-470 (non-canonical Wnt inhibition [37]) and 5HPP-33 (tubulin polymerization inhibition [38]) are known, the various entry portals to Aop43 by these and other structurally diverse compounds can be inferred with the Aop43 framework. For example, Ellis-Hutchings et al. (2017) referred to the HTS data to posit several molecular initiating events that may underlie the embryo lethality of 5HPP-33 and the dysmorphogenesis effects (e.g., abnormal caudal extension and somite patterning) of TNP-470 [17]. They noted that 5HPP-33's primary reported effects on microtubule destabilization could impact cell growth through PI3K inhibition, p53 accumulation, and estrogen receptor signaling, among others, based on the ToxCast™ bioactivity data. For TNP-470, they highlighted that the reported mode of action is methionine aminopeptidase II

(MetAP2) inhibition, possibly through the Wnt signaling pathway, among other ToxCast™ hits that were not discussed. While ToxCast™ bioactivity assay results may identify putative molecular targets for a given chemical, these data are based on cell-free or cell-based assays that cover a finite set of biological space and is not expected to capture the full suite of dynamic interactions among the numerous tissue types involved in embryonic development.

Another approach to identifying the signaling pathways and molecular events for an observed phenotype in intact organisms (*ex vivo* or *in vivo*) is to look at global gene expression at a time point at the beginning of the exposure period that may capture molecular initiating events or key events leading to embryotoxicity. Here, we describe previously unpublished results of a transcriptomics study connected to the embryology previously reported [17] to further understand how disruption of embryonic vascular development leads to developmental toxicity. The methods are described in detail in the Supplemental Materials, and the data have been deposited to the Gene Expression Omnibus (GEO) under accession number GSExref. Briefly, we used RNA sequencing analysis of whole, 10-day-old rat embryos at an early *ex vivo* culture time point (4 h) following exposure to three concentrations of 5HPP-33 or TNP-470, which were selected to cover *ex vivo* effects ranging from 0% to 100%. We next conducted differential gene expression analysis to identify molecular and cellular signaling pathways potentially underlying the *ex vivo* effects.

Pathway analysis of the 9006 differentially expressed genes (DEGs) for TNP-470 and 5HPP-33 identified several pathways as being unique to 5HPP-33 versus TNP-470 embryotoxicity, which could explain the higher potency at which TNP-470 causes vascular defects or the specific non-vascular effects such as somite defects and caudalization observed in WECs at 48 h. While differences in gene expression changes were expected based on different phenotypic outcomes, we also hypothesized that similarities in DEG patterns would be tied to disruption of vascular patterning. Unsupervised hierarchical clustering of embryonic DEGs indicated transcriptional responses to the highest concentration of 5HPP-33 (30 μ M) were similar to responses elicited by TNP-470 at any concentration (Figure 3). TNP-470 invoked concentration-dependent changes in the subset of DEGs at 4 h of exposure, consistent with the concentration-dependent dysmorphogenesis observed at 48 h. Three canonical pathways were consistently implicated in both TNP-470 and 5HPP-33 toxicity: FXR/RXR Activation, LXR/RXR Activation, and p53 signaling. FXR and LXR activation were among the top pathways for TNP-470 2.5 μ M and 25 μ M and 5HPP-33 30 μ M. Based on gene expression changes, inhibition of FXR and LXR pathways may be key events via RXR heterodimerization. Similarly, inhibition of p53 signaling was implicated as a shared mechanism for TNP-470 2.5 μ M and 5HPP-33 5 μ M versus DMSO controls, consistent with ToxCast™ predictions. P53 signaling was the top canonical pathway for TNP-470 at 2.5 μ M. Although it was not a top enriched pathway for 5HPP-33 at 5 μ M, TRP53COR1, a corepressor of p53, was implicated as a potential upstream regulator by the increased expression of three downstream genes: *Foxe1*, *Lhx5*, and *Ntn1*. Moreover, the highest concentrations that caused overt phenotypes (5HPP-33, 30 μ M; TNP-470 25 μ M) were associated with gene expression changes greater than 8-fold for p53-regulated genes such as *Ccng1*, *Cknd1*, and *Fas*. It is worth noting that systemic toxicity may mediate the higher concentration effects associated with p53 signaling; however, the lower concentration

effects are more likely linked to specific vascular genes involved in this pathway as they are below expected cytotoxicity thresholds identified from the ToxCast™ dataset. While p53 signaling is not currently incorporated into Aop43, literature evidence also supports a link between p53 signaling, VEGF repression [39] and TNP-mediated angiogenesis disruption [40]. Overall, the transcriptomics study demonstrates that whole genome transcriptome profiling of tissues from rat embryos exposed to known VDCs can be used to identify toxicity pathways and biological processes associated with, but not necessarily exclusive to, embryonic vascular disruption. Taken together, the assays described in this article provide some support that *in silico* chemical screening to predict *in vivo* bioactivity while reducing animal experimentation is becoming more realistic.

4. Application

The *in vivo* tests used for assessing prenatal developmental toxicity for regulatory purposes describe gross apical endpoints but are not designed for mechanistic insights. As such, a challenge for predictive toxicology is characterizing surrogate processes that can be monitored by new approach methodologies such as HTS and interpreted within the context of an adverse developmental outcome. Altered blood vessel formation and remodeling is one potential surrogate, substantiated by results from a large number of HTS and functional assays with strong correlations to *ex vivo* developmental effects. Through whole genome sequencing, we uncovered additional pathways that bolster our mechanistic understanding of the role of embryonic vascular disruption in developmental toxicity and help refine the vascular disruption AOP.

The WEC transcriptomics study identified molecular signaling pathways that are not currently incorporated into Aop43. These findings, in addition to new RNA-Seq results, demonstrate the importance of p53 signaling in developmental angiogenesis and support the hypothesis that chemical disruption of canonical vascular developmental pathways may invoke adverse developmental outcomes of a diverse nature, from embryoletality to morphological defects. These data also provide a more comprehensive list of the early transcriptomic responses following potential molecular initiating events that can be scaled to larger chemical libraries [41].

Since the publication of the pVDC manuscript [5], additional ToxCast™ chemical library phases were tested. We now have 1059 chemicals with HTS bioactivity data covering the pVDC signature that is publicly available in ToxCast™ (<https://www.epa.gov/chemical-research/exploring-toxcast-data-downloadable-data>). Applying the same pVDC signature using the latest ToxPi (v2.1) software [42] resulted in a new chemical ranking with 5HPP-33 and TNP-470 among the top 25% and the other 36 chemicals generally retaining their respective locations on the pVDC spectrum consistent with previous predictions (Figure 4). This analysis also identified several new pVDCs with ToxPi scores greater than that of 5HPP-33. For example, farglitazar (Figure 2, row 1, 9th from left) is a synthetic peroxisome proliferator-activated receptor-gamma (PPAR- γ) ligand [43], a group studied as angiogenesis inhibitors and developmental toxicants [44–46]. Additionally, tributyltin chloride (Figure 2, row 1, 2nd from left) is cytotoxic to endothelial cells [47, 48] and causes embryoletality when administered during organogenesis [49].

It is important to note that tributyltin chloride might inhibit angiogenesis through more specific modes of action beyond cytotoxicity. Moreover, this and other chemicals may target additional cell types (e.g., neurons [50]) and organ systems beyond the scope of Aop43. For example, tributyltin chloride is a neurobehavioral toxicant [51], implicating the central nervous system (CNS) as a target organ of toxicity. Thus, while Aop43 is useful for identifying potential vascular disruptors, this endpoint may not always be the most important from a regulatory decision-making perspective. Furthermore, for some chemicals the primary targets may not always be the vasculature, particularly in the case of exposures resulting in sublethal or sub-teratogenic adverse outcomes. These caveats support the need for follow-up *in vitro* and *in silico* studies to investigate the angiogenesis disruption potential, as well as other target organ effects, of the high-ranking pVDCs shown in Figure 4. Taken together, mapping HTS features to AOPs brings into context the weight of evidence for critical determinants that potentially invoke the altered phenotype in a self-organizing, integrated system. AOPs provide the necessary structure for quantitative prediction of cellular and tissue responses to molecular perturbation, and such frameworks provide mechanistic insight to chemical hazard assessments in a rapid and robust manner.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments:

The whole embryo culture research was conducted under a Cooperative Research and Development Agreement (CRADA) with U.S. Environmental Protection Agency and The Dow Chemical Company. RNA-seq analysis was conducted by IQVIA Institute for Human Data Science, formerly Expression Analysis, Inc., through a contract funded by the EPA/NCCT and the WEC research was funded by Strategic Research Project (SRP) at The Dow Chemical Company. The authors gratefully acknowledge the support of V.A. Marshall, A.J. Wood, L.K. Sosinski, D. DeLine in the conduct of the WEC experiments.

6. References

1. Chung AS and Ferrara N, Developmental and pathological angiogenesis. *Annu Rev Cell Dev Biol*, 2011 27: p. 563–84. [PubMed: 21756109]
2. Maltepe E and Simon MC, Oxygen, genes, and development: an analysis of the role of hypoxic gene regulation during murine vascular development. *J Mol Med (Berl)*, 1998 76(6): p. 391–401. [PubMed: 9625296]
3. Walls JR, et al., Three-dimensional analysis of vascular development in the mouse embryo. *PLoS One*, 2008 3(8): p. e2853. [PubMed: 18682734]
4. Carmeliet P, Angiogenesis in life, disease and medicine. *Nature*, 2005 438(7070): p. 932–6. [PubMed: 16355210]
5. (••) Knudsen TB and Kleinstreuer NC, Disruption of embryonic vascular development in predictive toxicology. *Birth Defects Res C Embryo Today*, 2011 93(4): p. 312–23. [PubMed: 22271680]
6. Goldenberg RL, Factors influencing perinatal outcomes. *Ann N Y Acad Sci*, 2004 1038: p. 227–279. [PubMed: 15838118]
7. Andraweera PH, et al., Single-nucleotide polymorphisms in the KDR gene in pregnancies complicated by gestational hypertensive disorders and small-for-gestational-age infants. *Reprod Sci*, 2012 19(5): p. 547–54. [PubMed: 22344734]
8. Vargesson N, Thalidomide-induced teratogenesis: history and mechanisms. *Birth Defects Res C Embryo Today*, 2015 105(2): p. 140–56. [PubMed: 26043938]

9. Li TC, et al., Recurrent miscarriage: aetiology, management and prognosis. *Hum Reprod Update*, 2002 8(5): p. 463–81. [PubMed: 12398226]
10. Therapontos C, et al., Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation. *Proc Natl Acad Sci U S A*, 2009 106(21): p. 8573–8. [PubMed: 19433787]
11. (••) Kleinstreuer NC, et al., Environmental impact on vascular development predicted by high-throughput screening. *Environ Health Perspect*, 2011 119(11): p. 1596–603. [PubMed: 21788198]
12. Knudsen TB, et al., Profiling the activity of environmental chemicals in prenatal developmental toxicity studies using the U.S. EPA's ToxRefDB. *Reprod Toxicol*, 2009 28(2): p. 209–19. [PubMed: 19446433]
13. Judson RS, et al., In vitro screening of environmental chemicals for targeted testing prioritization: the ToxCast project. *Environ Health Perspect*, 2010 118(4): p. 485–92. [PubMed: 20368123]
14. Kavlock R, et al., Update on EPA's ToxCast program: providing high throughput decision support tools for chemical risk management. *Chem Res Toxicol*, 2012 25(7): p. 1287–302. [PubMed: 22519603]
15. Richard AM, et al., ToxCast Chemical Landscape: Paving the Road to 21st Century Toxicology. *Chem Res Toxicol*, 2016 29(8): p. 1225–51. [PubMed: 27367298]
16. Kleinstreuer N, et al., A computational model predicting disruption of blood vessel development. *PLoS Comput Biol*, 2013 9(4): p. e1002996. [PubMed: 23592958]
17. (••) Ellis-Hutchings RG, et al., Embryonic vascular disruption adverse outcomes: Linking high throughput signaling signatures with functional consequences. *Reprod Toxicol*, 2017 70: p. 82–96. [PubMed: 28527947]
18. Fratta ID, Sigg EB, and Maiorana K, Teratogenic Effects of Thalidomide in Rabbits, Rats, Hamsters, and Mice. *Toxicol Appl Pharmacol*, 1965 7: p. 268–86. [PubMed: 14298018]
19. Schumacher H, et al., A comparison of the teratogenic activity of thalidomide in rabbits and rats. *J Pharmacol Exp Ther*, 1968 160(1): p. 189–200. [PubMed: 5639104]
20. Rutland CS, et al., Maternal administration of anti-angiogenic agents, TNP-470 and Angiostatin4.5, induces fetal microphthalmia. *Mol Vis*, 2009 15: p. 1260–9. [PubMed: 19572040]
21. (•) Tal TL, et al., Immediate and long-term consequences of vascular toxicity during zebrafish development. *Reprod Toxicol*, 2014 48: p. 51–61. [PubMed: 24907688]
22. Carmeliet P, et al., Abnormal blood vessel development and lethality in embryos lacking a single VEGF allele. *Nature*, 1996 380(6573): p. 435–9. [PubMed: 8602241]
23. Ferrara N, et al., Heterozygous embryonic lethality induced by targeted inactivation of the VEGF gene. *Nature*, 1996 380(6573): p. 439–42. [PubMed: 8602242]
24. Fong GH, et al., Role of the Flt-1 receptor tyrosine kinase in regulating the assembly of vascular endothelium. *Nature*, 1995 376(6535): p. 66–70. [PubMed: 7596436]
25. Shalaby F, et al., Failure of blood-island formation and vasculogenesis in Flk-1-deficient mice. *Nature*, 1995 376(6535): p. 62–6. [PubMed: 7596435]
26. Sipes NS, et al., Predictive models of prenatal developmental toxicity from ToxCast high-throughput screening data. *Toxicol Sci*, 2011 124(1): p. 109–27. [PubMed: 21873373]
27. Sarkanen JR, et al., Intra-Laboratory Pre-Validation of a Human Cell Based in vitro Angiogenesis Assay for Testing Angiogenesis Modulators. *Front Pharmacol*, 2010 1: p. 147. [PubMed: 21779245]
28. (•) Tal T, et al., Screening for angiogenic inhibitors in zebrafish to evaluate a predictive model for developmental vascular toxicity. *Reprod Toxicol*, 2017 70: p. 70–81. [PubMed: 28007540]
29. (•) McCollum CW, et al., Identification of vascular disruptor compounds by analysis in zebrafish embryos and mouse embryonic endothelial cells. *Reprod Toxicol*, 2017 70: p. 60–69. [PubMed: 27838387]
30. (•) Belair DG, et al., Human iPSC-Derived Endothelial Cell Sprouting Assay in Synthetic Hydrogel Arrays. (submitted) 2016.
31. (•) Nguyen EH, et al., Versatile synthetic alternatives to Matrigel for vascular toxicity screening and stem cell expansion. *Nat Biomed Eng*, 2017 1.

32. (•) Toimela T, et al., Intra-laboratory validated human cell-based in vitro vasculogenesis/angiogenesis test with serum-free medium. *Reprod Toxicol*, 2017 70: p. 116–125. [PubMed: 27915012]
33. Saili K, et al., An integrative systems toxicology model for neurovascular developmental toxicity. In: *The Toxicologist: Supplement to Toxicological Sciences*, [Volume(Issue)], Society of Toxicology Abstract no. 1943, 2018.
34. (•) Li S, et al., Identification of Angiogenesis Inhibitors Using a Co-culture Cell Model in a High-Content and High-Throughput Screening Platform. *SLAS Technol*, 2018 23(3): p. 217–225. [PubMed: 28922619]
35. Noguchi T, et al., Angiogenesis inhibitors derived from thalidomide. *Bioorg Med Chem Lett*, 2005 15(24): p. 5509–13. [PubMed: 16183272]
36. Ingber D, et al., Synthetic analogues of fumagillin that inhibit angiogenesis and suppress tumour growth. *Nature*, 1990 348(6301): p. 555–7. [PubMed: 1701033]
37. Knudsen TB, et al., Activity profiles of 309 ToxCast chemicals evaluated across 292 biochemical targets. *Toxicology*, 2011 282(1–2): p. 1–15. [PubMed: 21251949]
38. Inatsuki S, et al., Tubulin-polymerization inhibitors derived from thalidomide. *Bioorg Med Chem Lett*, 2005 15(2): p. 321–5. [PubMed: 15603947]
39. Pal S, Datta K, and Mukhopadhyay D, Central role of p53 on regulation of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) expression in mammary carcinoma. *Cancer Res*, 2001 61(18): p. 6952–7. [PubMed: 11559575]
40. Zhang Y, et al., Cell cycle inhibition by the anti-angiogenic agent TNP-470 is mediated by p53 and p21WAF1/CIP1. *Proc Natl Acad Sci U S A*, 2000 97(12): p. 6427–32. [PubMed: 10841547]
41. Thomas RS, et al., The US Federal Tox21 Program: A strategic and operational plan for continued leadership. *ALTEX*, 2018 35(2): p. 163–168. [PubMed: 29529324]
42. Marvel SW, et al., ToxPi Graphical User Interface 2.0: Dynamic exploration, visualization, and sharing of integrated data models. *BMC Bioinformatics*, 2018 19(1): p. 80. [PubMed: 29506467]
43. Henke BR, et al., N-(2-Benzoylphenyl)-L-tyrosine PPARgamma agonists. 1. Discovery of a novel series of potent antihyperglycemic and antihyperlipidemic agents. *J Med Chem*, 1998 41(25): p. 5020–36. [PubMed: 9836620]
44. Giaginis C, Tsantili-Kakoulidou A, and Theocharis S, Peroxisome Proliferator-Activated Receptor-gamma Ligands: Potential Pharmacological Agents for Targeting the Angiogenesis Signaling Cascade in Cancer. *PPAR Res*, 2008 2008: p. 431763. [PubMed: 18464916]
45. Lehmann JM, et al., An antidiabetic thiazolidinedione is a high affinity ligand for peroxisome proliferator-activated receptor gamma (PPAR gamma). *J Biol Chem*, 1995 270(22): p. 12953–6. [PubMed: 7768881]
46. Peraza MA, et al., The toxicology of ligands for peroxisome proliferator-activated receptors (PPAR). *Toxicol Sci*, 2006 90(2): p. 269–95. [PubMed: 16322072]
47. Bernardini C, et al., Deleterious effects of tributyltin on porcine vascular stem cells physiology. *Comp Biochem Physiol C Toxicol Pharmacol*, 2016 185–186: p. 38–44.
48. Botelho G, et al., Effect of tributyltin on mammalian endothelial cell integrity. *Comp Biochem Physiol C Toxicol Pharmacol*, 2015 176–177: p. 79–86.
49. Ema M, et al., Comparative developmental toxicity of butyltin trichloride, dibutyltin dichloride and tributyltin chloride in rats. *J Appl Toxicol*, 1995 15(4): p. 297–302. [PubMed: 7594199]
50. Yamada J, et al., Low-concentration tributyltin perturbs inhibitory synaptogenesis and induces neuronal death in immature but not mature neurons. *Toxicol Lett*, 2010 198(2): p. 282–8. [PubMed: 20659539]
51. Ema M, Itami T, and Kawasaki H, Changes of spontaneous motor activity of rats after acute exposure to tributyltin chloride. *Drug Chem Toxicol*, 1991 14(1–2): p. 161–71. [PubMed: 1889374]

Figure 1.
Aop43 for embryonic vascular disruption (reproduced with permission from Knudsen and Kleinstreuer (2011) [5]).

Figure 2.

Vascular disruption potential of 38 ToxCast™ chemicals selected to represent a range of pVDC scores from 0% (green) to 100% (red). ToxCast™ pVDC prediction scores are shown in column A, with 10 platform endpoints represented in columns B – K. Chemical endpoints in each of columns B – K were assigned a score of 1 (red) or zero (green), with cumulative *in vitro* scores (i.e., number of assay positives) indicated in column L. Gradient colors used in columns A and L correspond to pVDC scores (A) or cumulative *in vitro* scores (L). Black = cytotoxicity; white = not tested. Compared to total *in vitro* outcomes (L), the *in silico* (A) prediction sensitivity and specificity were 0.89 and 0.80, respectively; prediction accuracy was 87%

Figure 3.

Transcriptomic analysis of rat whole embryo culture (WEC) at 4 days after exposure to angiogenesis inhibitors, 5HPP-33 (0 to 30 μ M) or TNP-470 (0 to 25 μ M). A) 9006 total differentially expressed genes (DEGs) at any exposure concentration compared to DMSO controls included 2831 that were significantly affected by both compounds. B) Self-organizing map (SOM) comprising all 9006 DEGs ranked 5HPP-33 (HPP) and TNP-470 (TNP) treatments by similarities in gene expression changes. Each of the 12 boxes represents a comparison between two treatments. SOM analysis identified a group of 464 genes (black box) that were similarly differentially expressed following exposure to 2.5 μ M TNP-470 or 5 μ M 5HPP-33. C) Fold-change comparison of 464 genes from region of interest (ROI) in part B. D) Pathway analysis exploring the effects of 2.5 μ M TNP-470 and 5 μ M 5HPP-33 on p53 signaling.

Figure 4.

Updated pVDC signature ranking the 38 chemicals (circled; selected to represent a range of pVDC scores) among 1058 ToxCast™ Phase I and II chemicals. Each ToxPi slice represents a ToxCast™ molecular target included in the Aop43 model as described in Knudsen and Kleinstreuer (2011) [5]. 5HPP-33 and TNP-470 rank in the top 25% of chemicals.