**Application of AOPs to Developmental Outcomes** case study on developmental vascular toxicity

DISCLAIMER: The views expressed are those of the presenter and do not necessarily reflect Agency policy.

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Teratology Society's 59th Annual Meeting

June 22-26, 2019

## **Vascular Development**

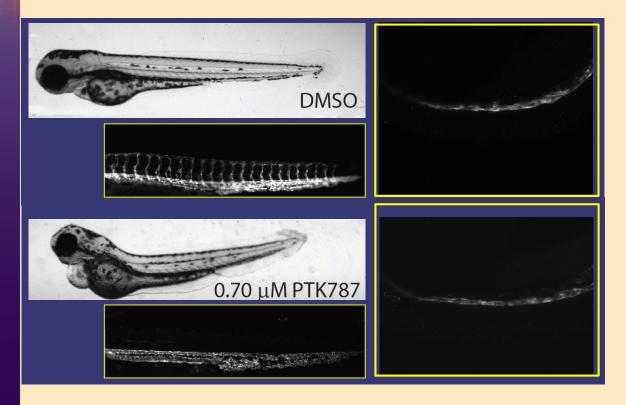
- Blood vessel formation is essential to embryogenesis (cardiovascular is first functioning organ system across *Vertebrate* species).
- Vascular insufficiency is tied to many disease processes (diabetes, preeclampsia, neonatal respiratory distress, osteoporosis, teratogenesis, ...).
- Aop43: one of 28 AOPs included in the OECD work plan with status 'open for citation & comment' [<u>https://aopwiki.org/wiki/index.php/Aop:43</u>].



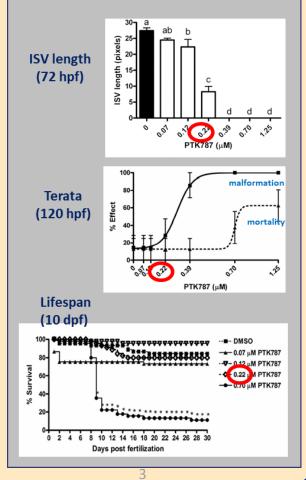


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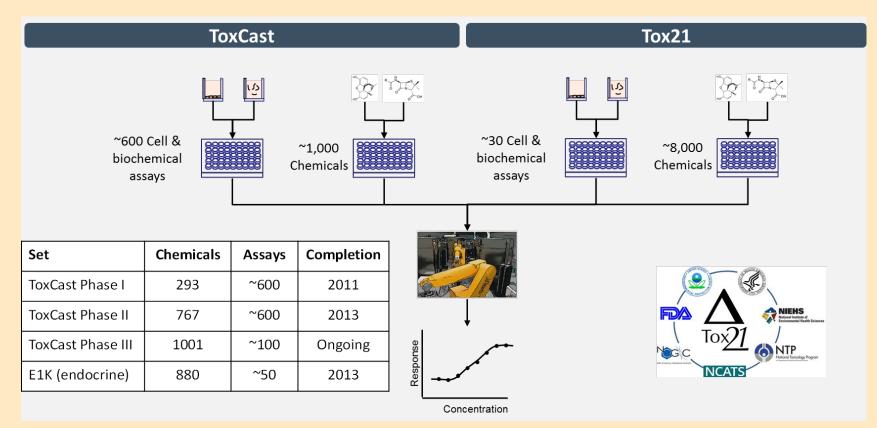
## **VEGFR2** inhibition (PTK787)



SOURCE: Tal et al. (2014) Reprod Toxicol



## Shifting toxicology to pathway-based approaches



### https://www.epa.gov/chemical-research/toxcast-dashboard

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#### United States Environmental Protection Agency

<b>Environmental Topics</b>	Laws & Regulations	About EPA	Se	arch EPA.gov		٩
Related Topics: Safer Chemicals Research			CONTACT US	SHARE <b>f</b>	<b>) ()</b>	

### ToxCast Dashboard

#### What is the ToxCast Dashboard?

The ToxCast Dashboard helps users examine high-throughput assay data to inform chemical safety decisions. To date, the ToxCast Dashboard has data on over 9,000 chemicals and information from more than 1,000 high-throughput assay endpoint components. Users of the ToxCast Dashboard can explore the data from a chemical or an assay viewpoint. Once the user selects the chemicals and assays of interest, they can then explore the biological activity for the chemical-assay combinations. Results from the selections are shown with tables, graphs and charts that can be downloaded by the user.

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#### Web Application

ToxCast Dashboard

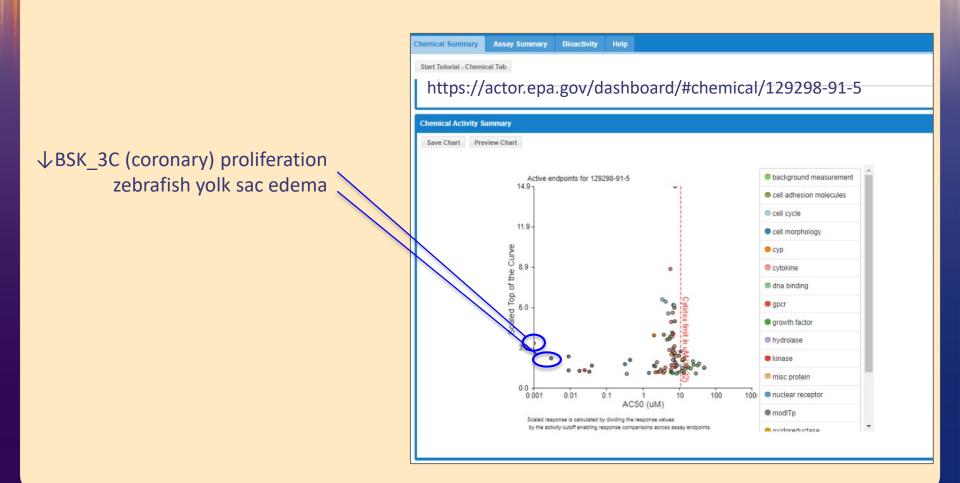
#### **Publications and Resources**

Journal Articles about ToxCast Factsheet about the ToxCast Dashboard Distributed Structure-Searchable Toxicity.(DSSTox) Database Information Download Computational Toxicology Data Download ToxCast Data

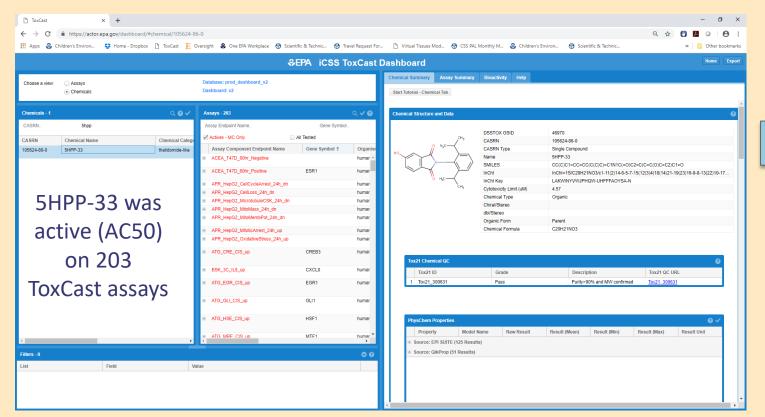
## **Example:** TNP-470, an anti-angiogenic pharma compound

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CASRN. TNP CASRN Chemical Name Chemical Catego 129299-91-5 TNP-470 Was active (AC50) on 82 ToxCast	Assay Endpoint Name Gene Symbol Assay Component Endpoint Name Gene Symbol TOX21_PPARg_BLA_Agonist_ratio TOX21_PPARg_BLA_Agonist_ratio TOX21_ARE_BLA_agonist_ratio TOX21_ARE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio TOX21_HSE_BLA_agonist_ratio	Organisr humar humar humar humar humar	$ \int_{0}^{\infty} \int_{0}^{\infty} \int_{0}^{\infty} \int_{0}^{\infty} \int_{0}^{\infty} \int_{0}^{0} \int_{$					
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### https://actor.epa.gov/dashboard/



## **Example:** 5HPP-33, an anti-angiogenic pharma compound

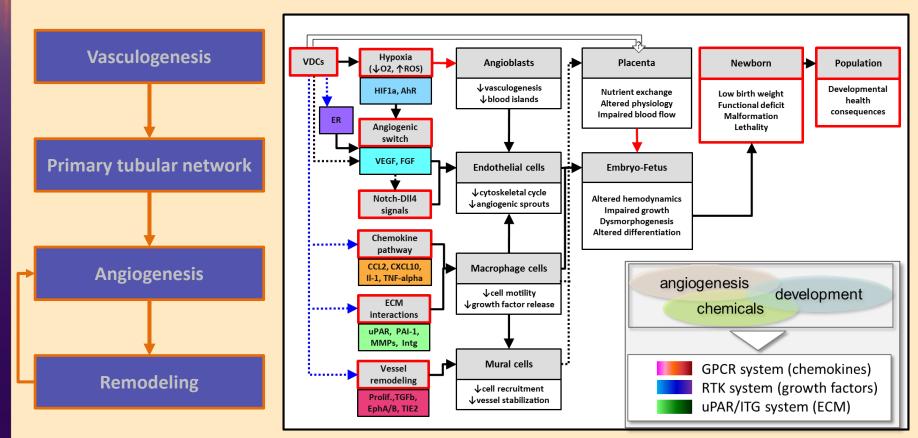


### https://actor.epa.gov/dashboard/

### 

Chemical Summary Assay Summary Bioactivity Help Start Tutorial - Chemical Tab Chemical Categories: thalidomide-like https://actor.epa.gov/dashboard/#chemical/105624-86-0 Chemical Activity Summary Save Chart Preview Chart background measurement Active endpoints for 105624-86-0 14.1-0 cell adhesion molecules cell cycle 11.3 modITp 0 0 INP cell morphology 0 😑 сур the cytokine Top dna binding 5.6 Scaled . gpcr growth factor 2.8 hvdrolase kinase 0.0 0.001 0.01 0.1 100 100 misc protein 10 AC50 (uM) nuclear receptor Scaled response is calculated by dividing the response values • by the activity cutoff enabling response comparisons across assay endpoints. ovidoraductasa

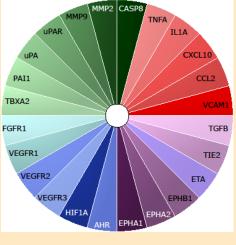
## AOP framework: developmental vascular toxicity (DVT)



SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res

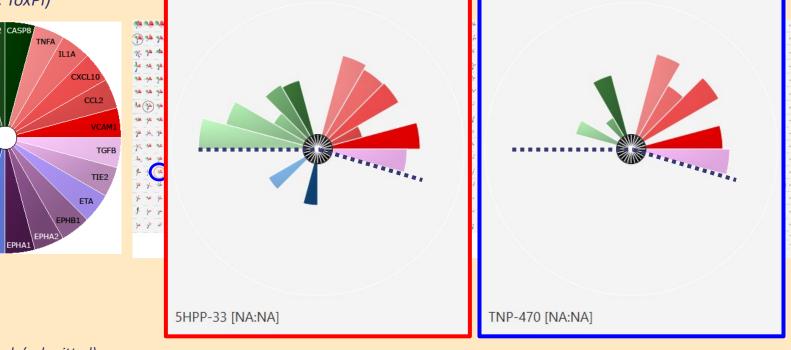
AOP-based ranking: predicted vascular disrupting chemicals (pVDCs)

24 ToxCast target assays (pVDC ToxPi)

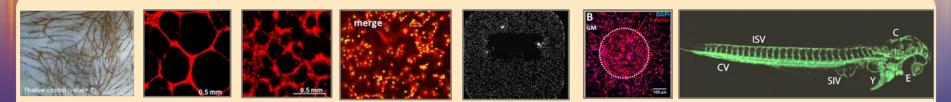


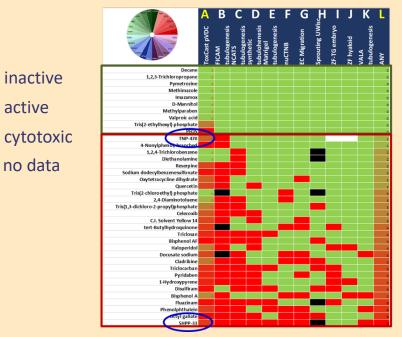
1058 ToxCast chemicals ranked by pVDC ToxPi

11



SOURCE: Saili et al. (submitted)





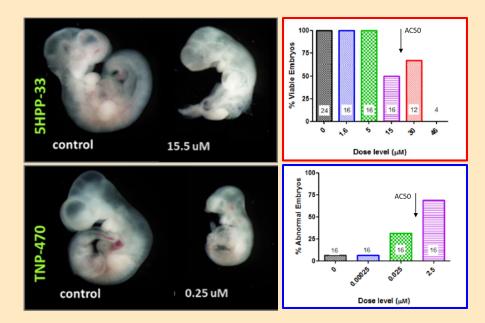
#### A pVDC ToxPi

- B HUVEC tubulogenesis (FICAM)
- C HUVEC tubulogenesis (NCATS)
- D tubulogenesis in synthetic matrices (HMAPS)
- E tubulogenesis in Matrigel (HMAPS)
- F nuCTNB biomarker (VALA)
- G endothelial cell migration (VALA)
- H iPSC endothelial sprouting (HMAPS)
- I ISV reporter zebrafish (NHEERL)
- J reporter zebrafish (UDUBLIN)
- K HUVEC tubulogenesis (VALA)

### L ANY (B to K)

sensitivity 0.89, specificity 0.80 balanced accuracy 87% (PPV 93%, NPV 73%)

## Embryotoxicity: rat whole embryo culture



### 5HPP-33

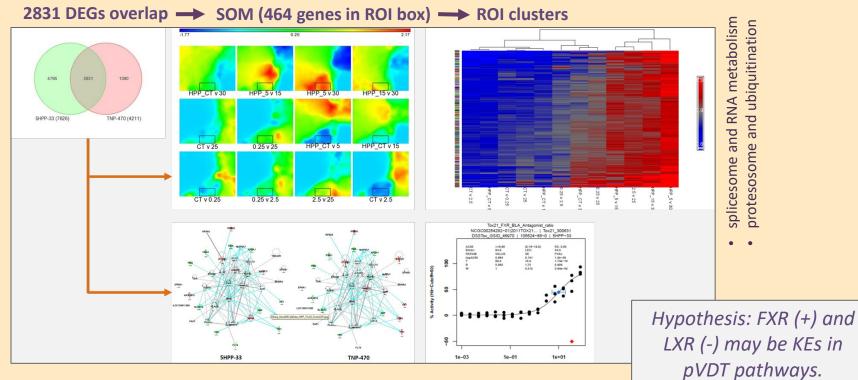
- synthetic thalidomide analog
- microtubule disruptor
- critical effect  $\downarrow$ embryo viability
- AC50 = 21.2 μM

### TNP-470

- synthetic fumagillan analog
- MetAP II inhibitor
- critical effect ↑dysmorphogenesis
- AC50 = 0.038 μM

SOURCE: Ellis-Hutchings et al. (2017) Reprod Toxicol

## **RNAseq profile:** 5HPP-33 vs TNP-470 (4 hr whole embryo culture)



SOURCE: Saili et al. (submitted)

## **Computer simulation:** cell agent based models (cABMs)

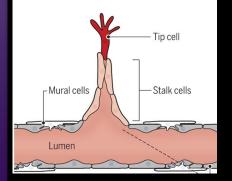
**Approach:** build and test self-organizing morphogenetic systems in silico using an open-source modeling environment (CompuCell3d.org).

**Input:** A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties to emulate developmental progression (embryogeny).

**Emergence:** simulation resolves into normal or perturbed phenotypes reading in vitro data input from specific ToxCast assays (cybermorphs).

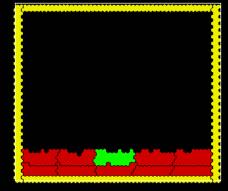
**Output:** probabilistic rendering of where, when and how a developmental defect might occur (critical phenomena).

## **Cell Agent-Based Models (cABMs)**



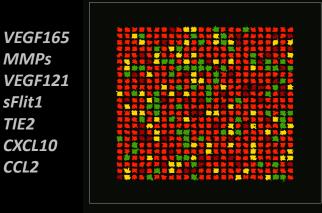
Li and Carmeliet (2018) Science

### **VEGF** corridors



Nicole Kleinstreuer

### Network assembly



**MMPs** 

sFlit1

TIE2

CCL2

Kleinstreuer et al. (2013) PLoS Comp Biol

- Endothelial Stalk
- Endothelial Tip
- Mural Cell
- Inflammatory Cell .

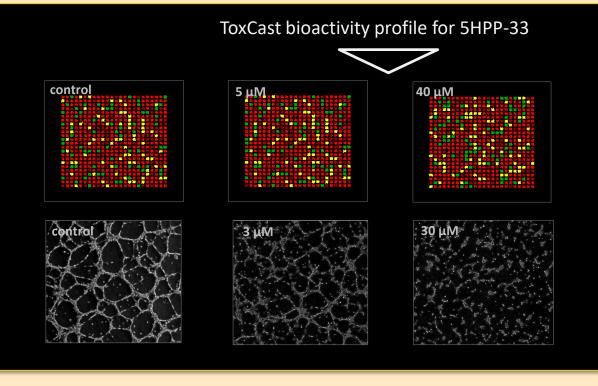
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BioComplexity Institute, Indiana U



## Angiogenesis disruption: in silico simulation of the pVDC ToxPi



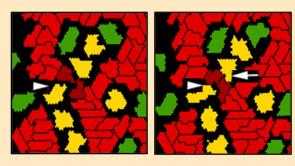
SOURCE: Kleinstreuer et al. (2013) PLoS Comp Biol 9(4): e1002996



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# Smart Models: emergence and self-organization

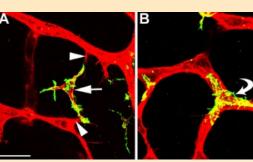
Time→



*in silico* model spontaneously displayed co-optingof tip cells (dark red) by inflammatory cells(yellow) during endothelial network formation.

<sup>-</sup>entin et al. (2010)

leinstreuer et al. (2013



 recapitulates the natural phenomenon referred to as 'macrophage bridging' co-opting of during endothelial network formation.



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## Placeholder for any references / rights for images

### **Summary:** *"decoding the toxicological blueprint of vascular development"*

- AOP-based ontologies provide the necessary structure for quantitative prediction of cellular and tissue responses to molecular perturbation(s).
- For DevTox, this can be demonstrated by an AOP framework for embryonic vascular disruption represented in the OECD AOP-KB (Aop43).
- Because the 'angiogenic cycle' is responsive to diverse genetic and physiological signals, Aop43 provides a kernel for co-opting larger AOP networks.
- Computer modeling and simulation puts all key events into motion enabling a new way to predictively model multicellular complexity in a self-organizing 'virtual' system.



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