UNC Advanced Toxicology course: reproductive toxicology section
April 24, 2019 – Rosenau Hall, room 228, 1:25 – 2:15 pm

Computational Approaches to Developmental and Reproductive Toxicology

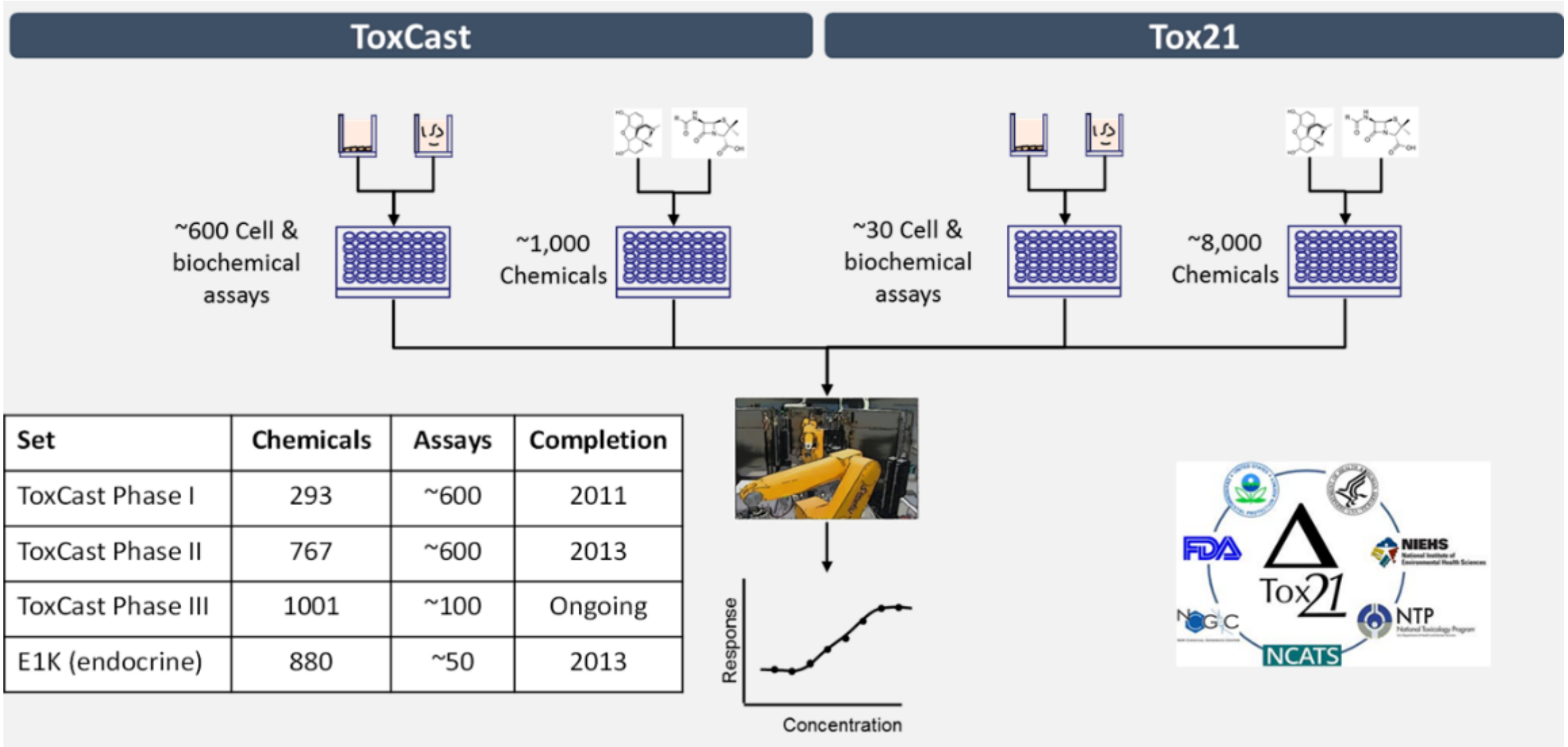
Thomas B. Knudsen, PhD
National Center for Computational Toxicology (NCCT)
US Environmental Protection Agency
Research Triangle Park, North Carolina
(919) 541-9776
knudsen.thomas@epa.gov
ORCID 0000-0002-5036-596x

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

Problem statement

- Chemical regulation is challenged by >80K chemicals on EPA's inventory under the Frank R. Lautenberg Chemical Safety for the 21st Century Act of 2016.
- Requires regulatory affirmation of "low" and "high" priority substances based on unreasonable risk utilizing New Approach Methods (NAMs) where possible.
- Automated *in vitro* assays enable high-throughput screening (HTS) to 'decode the toxicological blueprint of active substances' that interact with pregnancy.
- Vast HTS data (ToxCast/Tox21) in hand [https://comptox.epa.gov/dashboard], the need arises for predictive models of developmental toxicity.
- Key challenge: model 'critical phenomena' in self-organizing embryonic systems that compute with complex genetic circuits and multi-cellular networks.

Shifting toxicology to pathway-based approaches



Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013

EPA United States Environmental Protection Agency

Environmental Topics | Laws & Regulations | About EPA | Search EPA.gov

Related Topics: Safer Chemicals Research

CONTACT US SHARE

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.

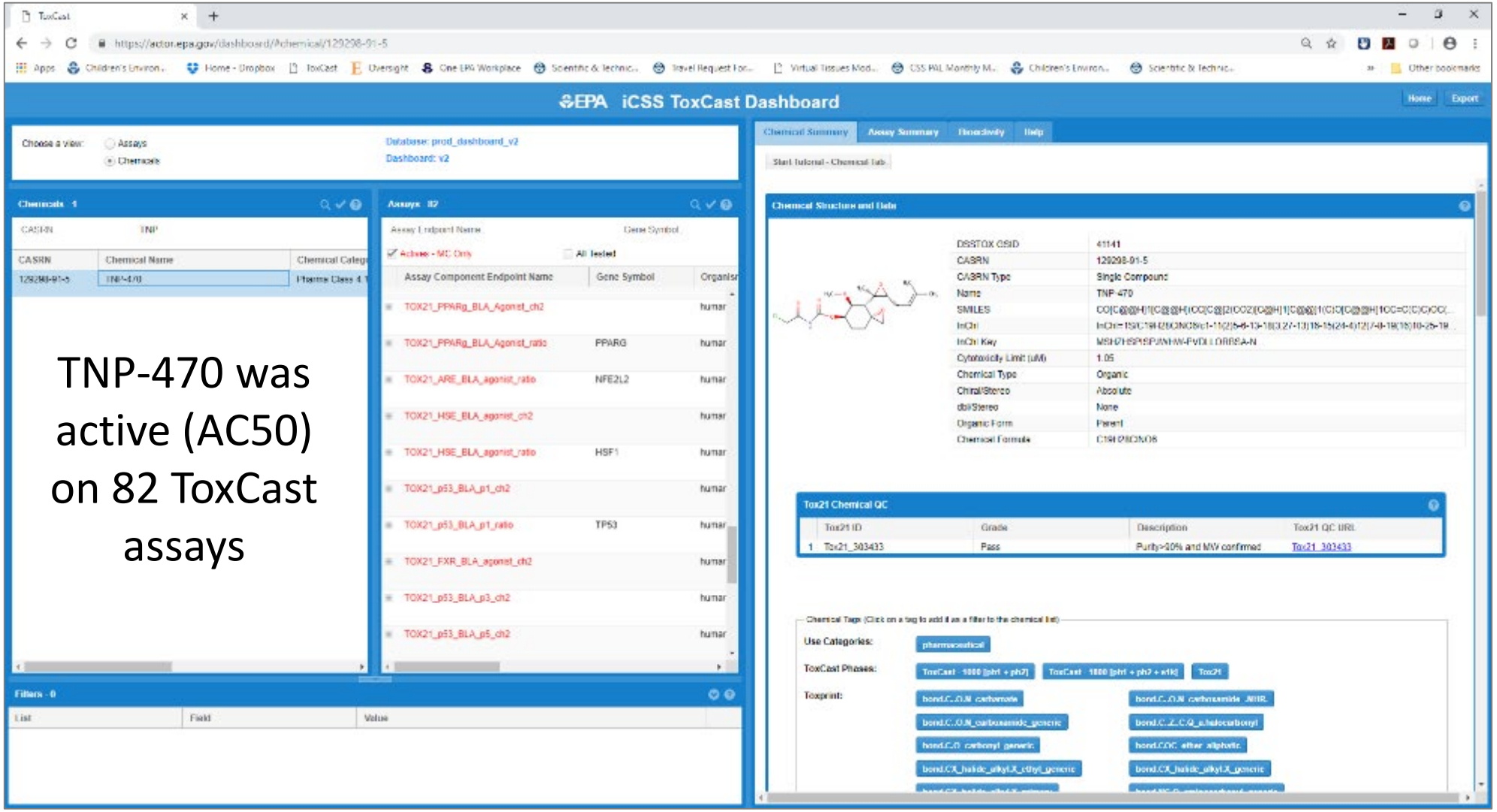
Web Application

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

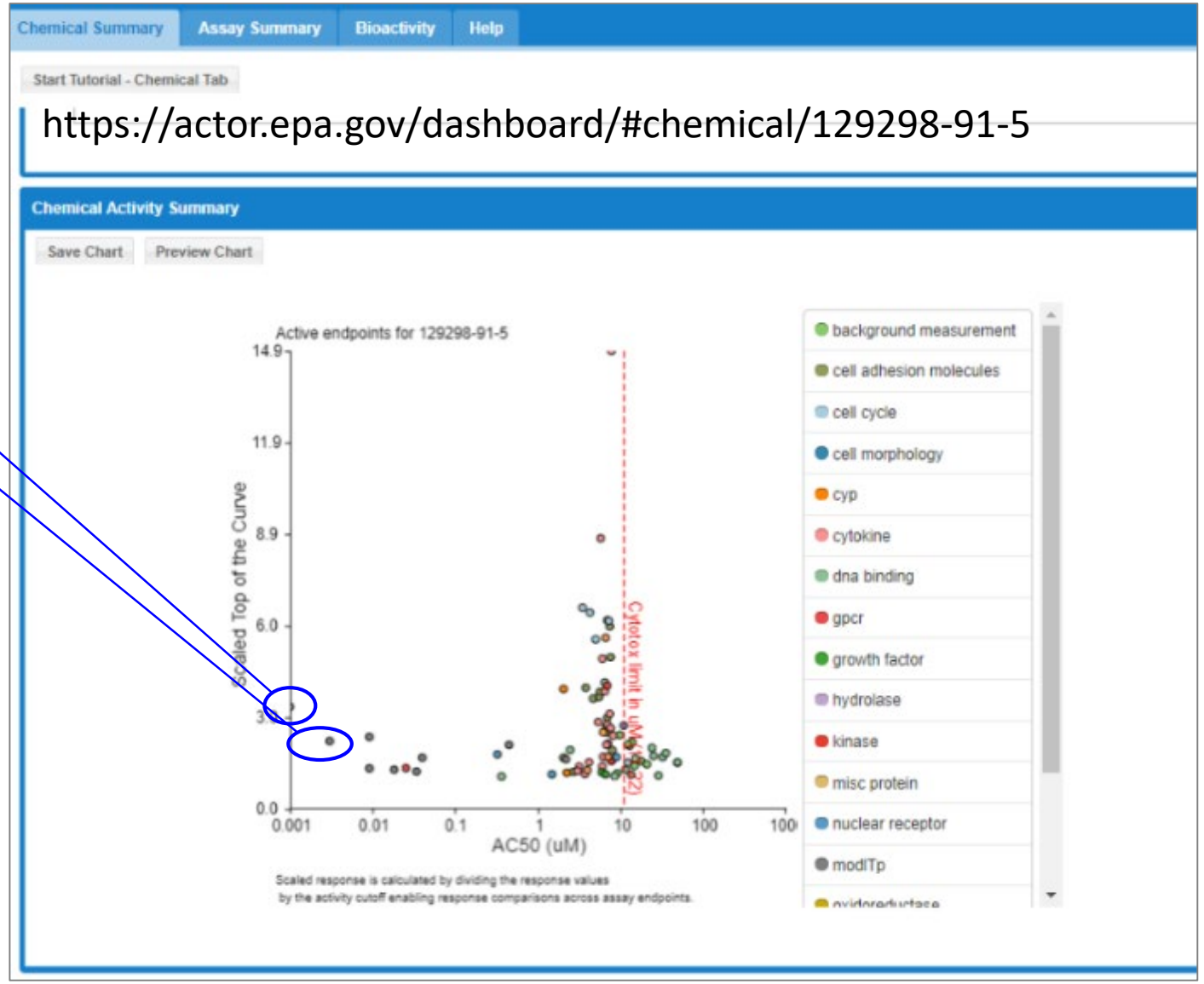
Example: TNP-470, a synthetic anti-angiogenic fumagillin analog



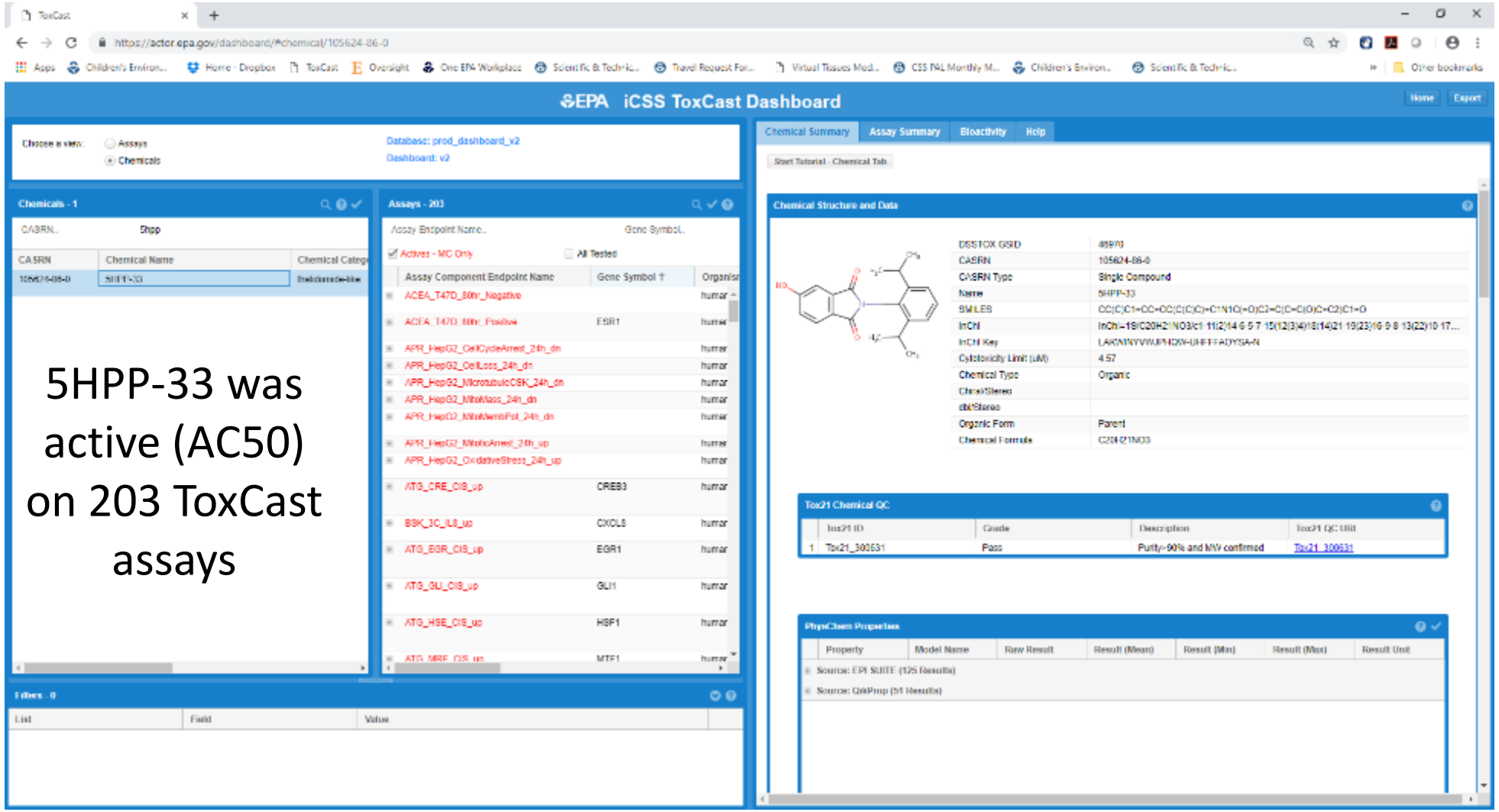
TNP-470 was active (AC50) on 82 ToxCast assays

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

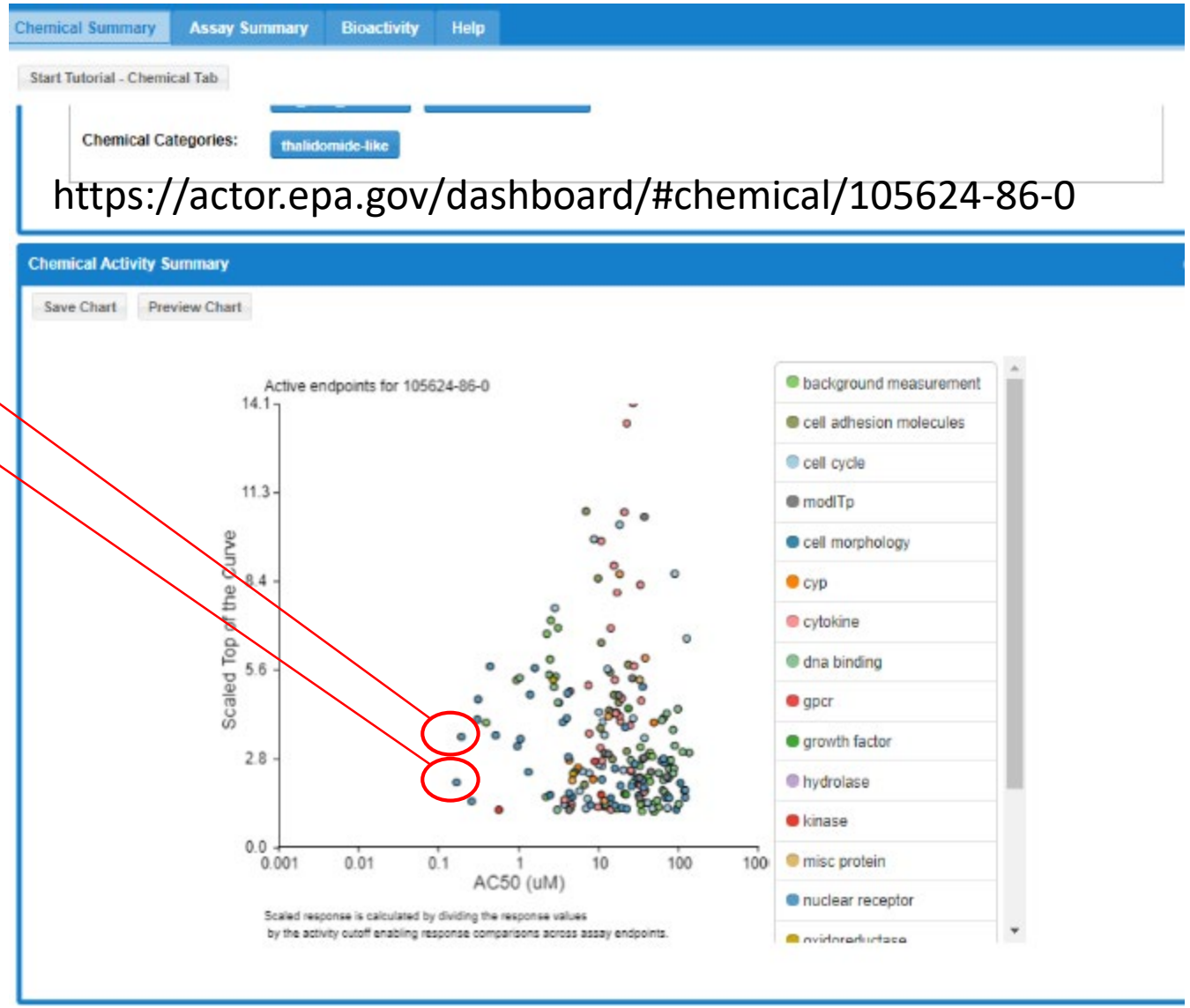
↓BSK_3C (coronary) proliferation
zebrafish yolk sac edema



Example: 5HPP-33, a synthetic anti-angiogenic thalidomide analog



↑TOX21_ERa agonist
↑ATG_ERa transactivation



How can we integrate the biomolecular data into pathways and processes that are relevant to developmental toxicity?

1. adverse outcome pathways (AOPs)

2. agent-based models (ABMs)

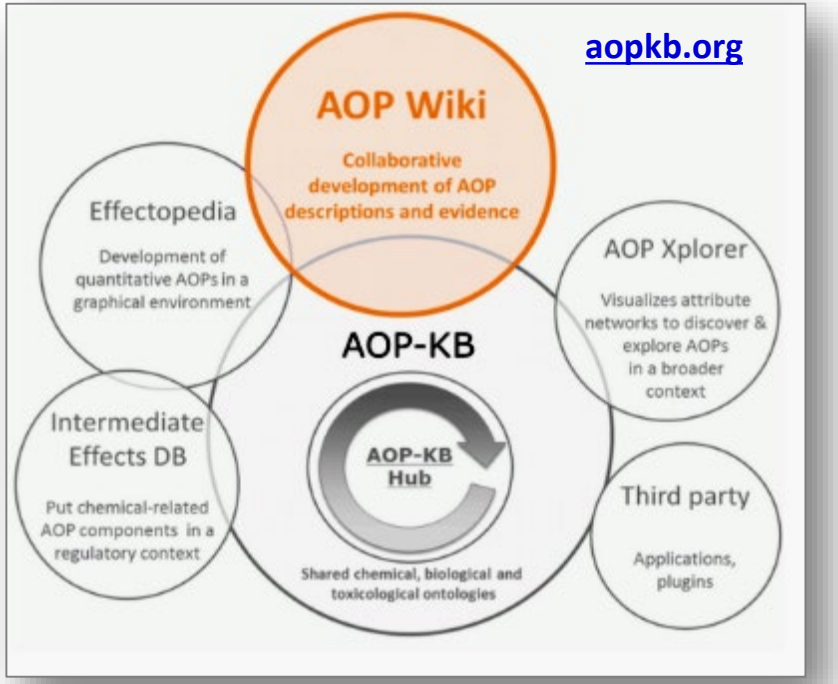
1. Adverse Outcome Pathways (AOPs)

- With HTS we can test the majority of chemicals in commerce within the decade, but using the NAMs for *in vitro* profiling and assessing toxicity is a challenge.

- Considerable mechanistic data exists in the literature (QSAR and Read-Across, 'omics, high content imaging, small model organisms) but is under-utilized for regulatory toxicology.

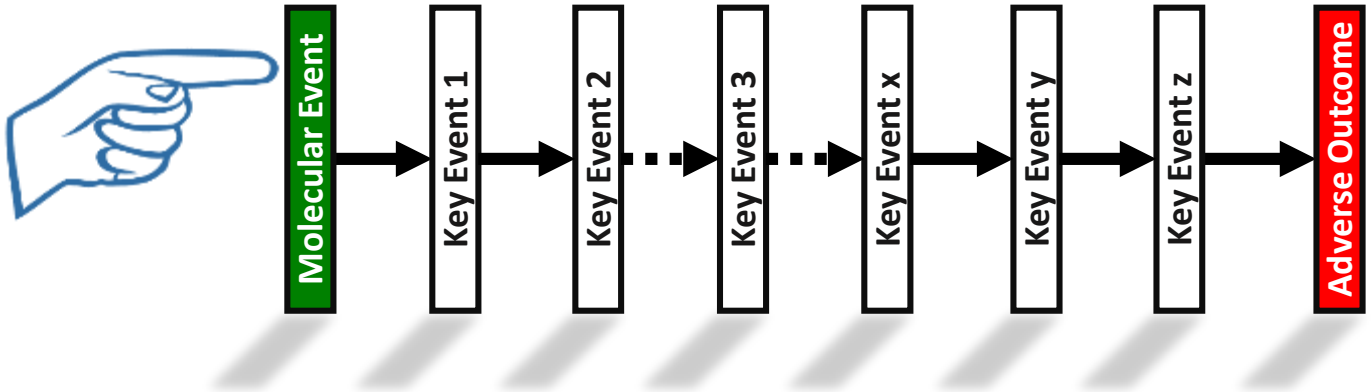
- **AOP:** says "*here is a biological perturbation that can lead to a specific adverse outcome, and here is how we think it happens*".

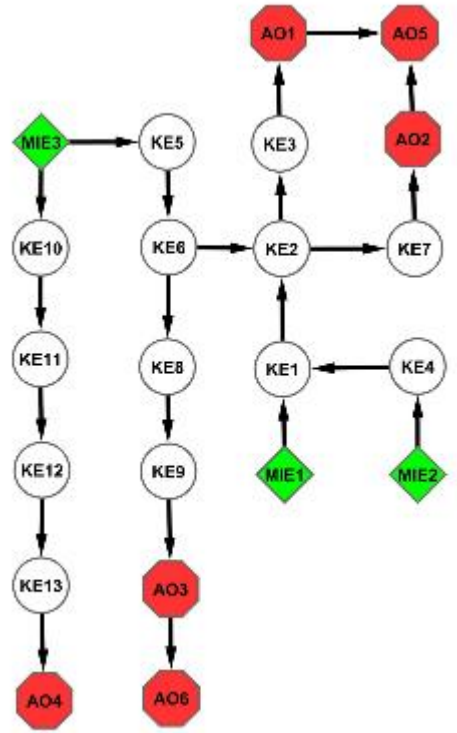
- **AOP-KB:** compendium of curated AOPs with demonstrated relevance connecting a molecular perturbation to adverse outcome.



SOURCE: Dan Villeneuve, USEPA

Principles for Building an AOP

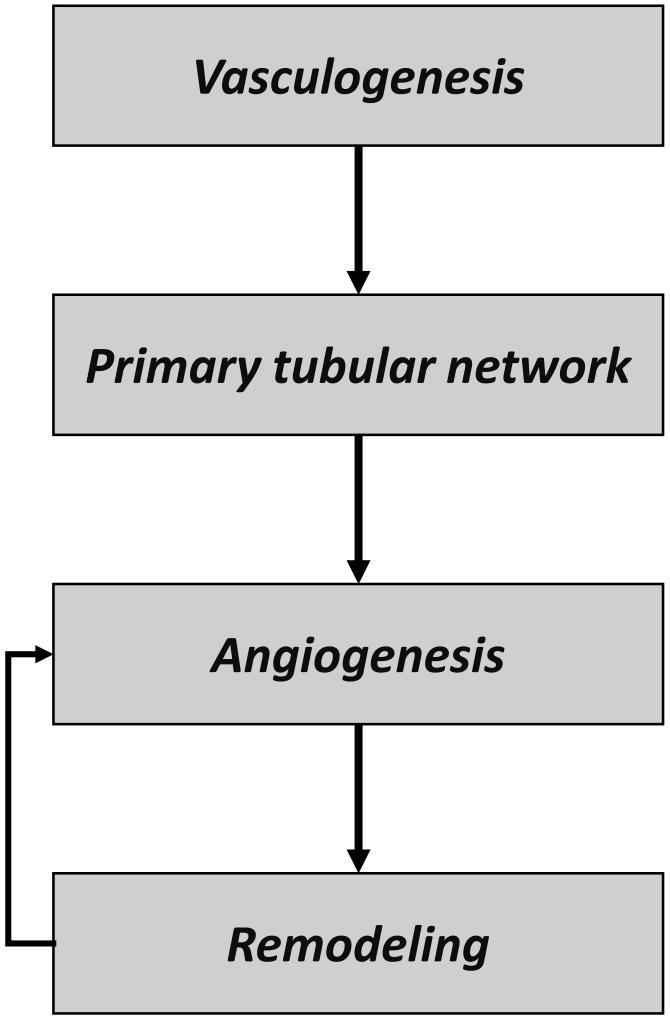


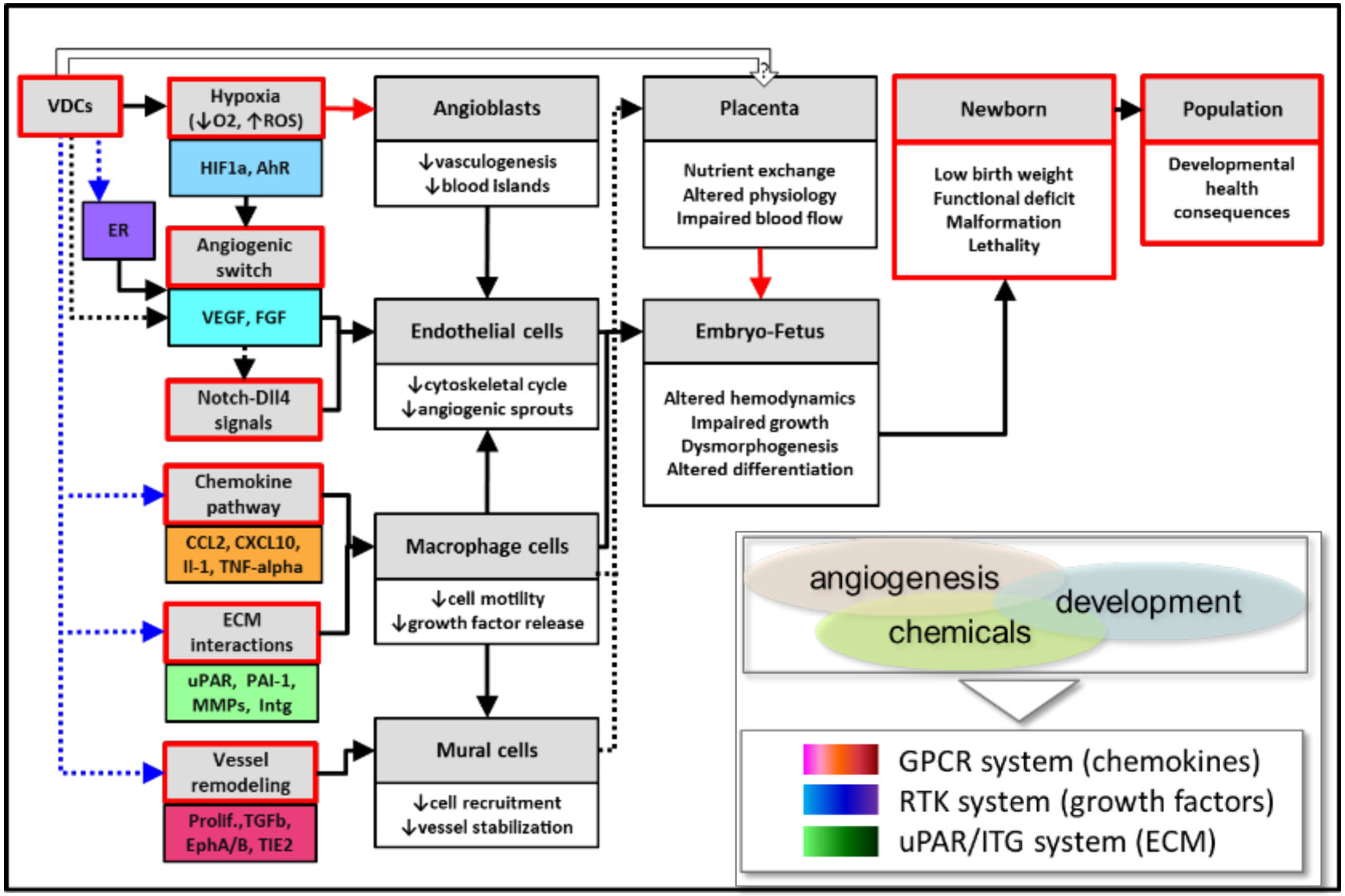


1. ***AOPs are not chemical-specific*** (based on biological motifs of failure)
2. ***AOPs are modular*** (individual relationships based on weight of evidence)
3. ***Individual AOPs are a pragmatic simplification*** (linearized sequence of biology)
4. ***AOP networks are the functional unit of prediction*** (in most cases)
5. ***AOPs are living documents*** (evolve as knowledge grows)

SOURCE: Villeneuve et al. (2014) Toxicol Sci

AOP framework: developmental vascular toxicity (DVT)

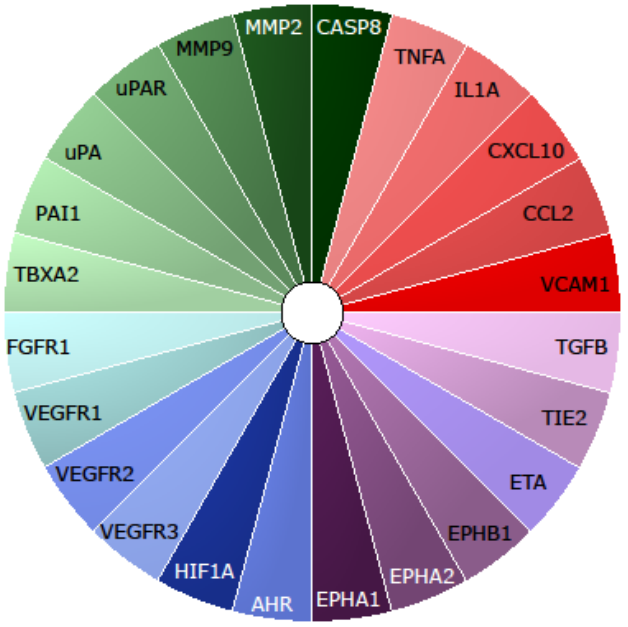




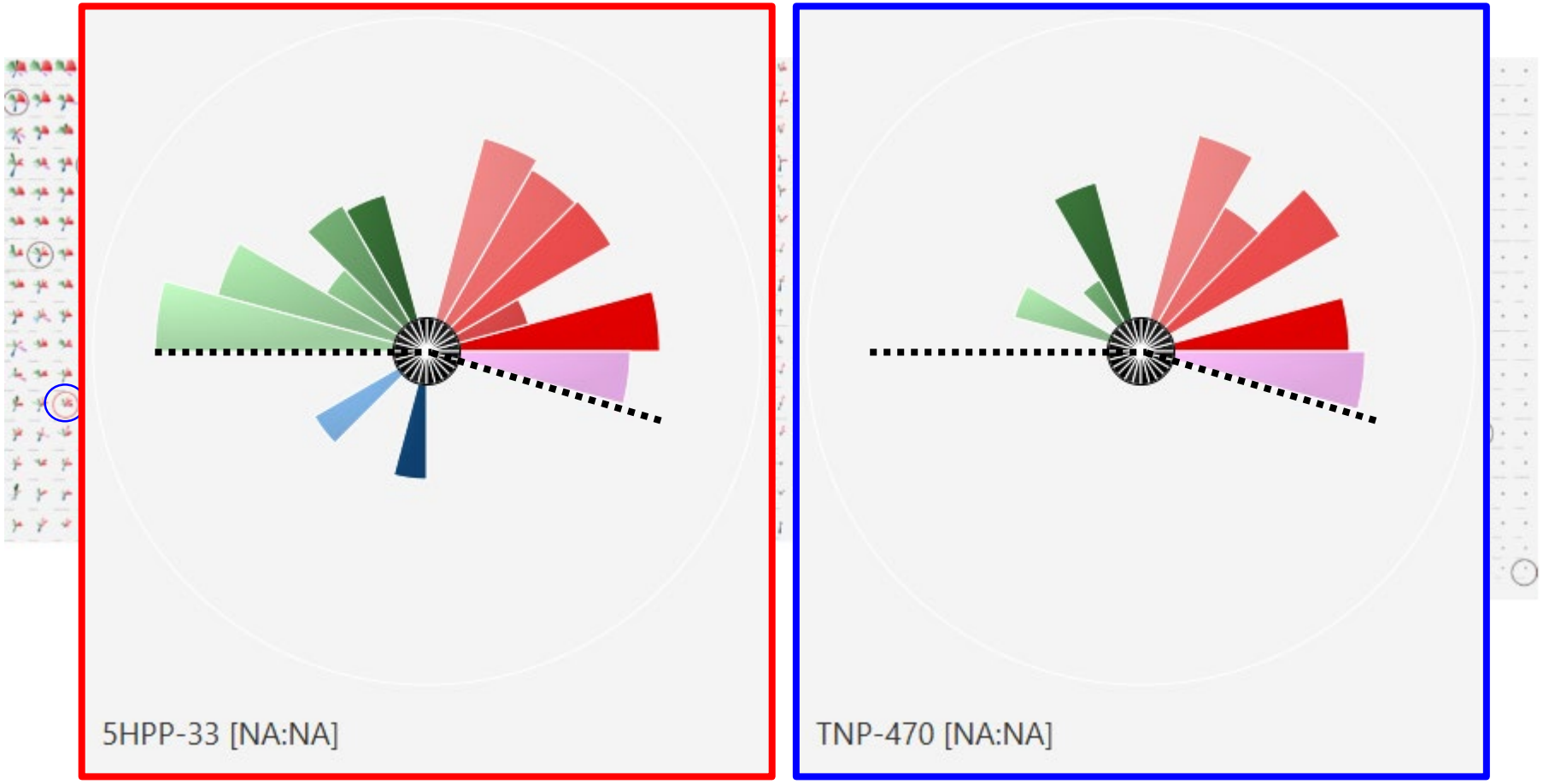
SOURCE: Knudsen and Kleinstreuer (2011) Birth Defects Res

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

24 ToxCast target assays (pVDC ToxPi)

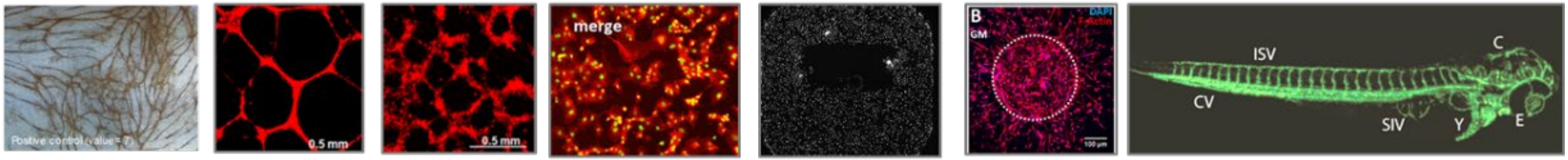


1058 ToxCast chemicals ranked by pVDC ToxPi (38 circled for validation)

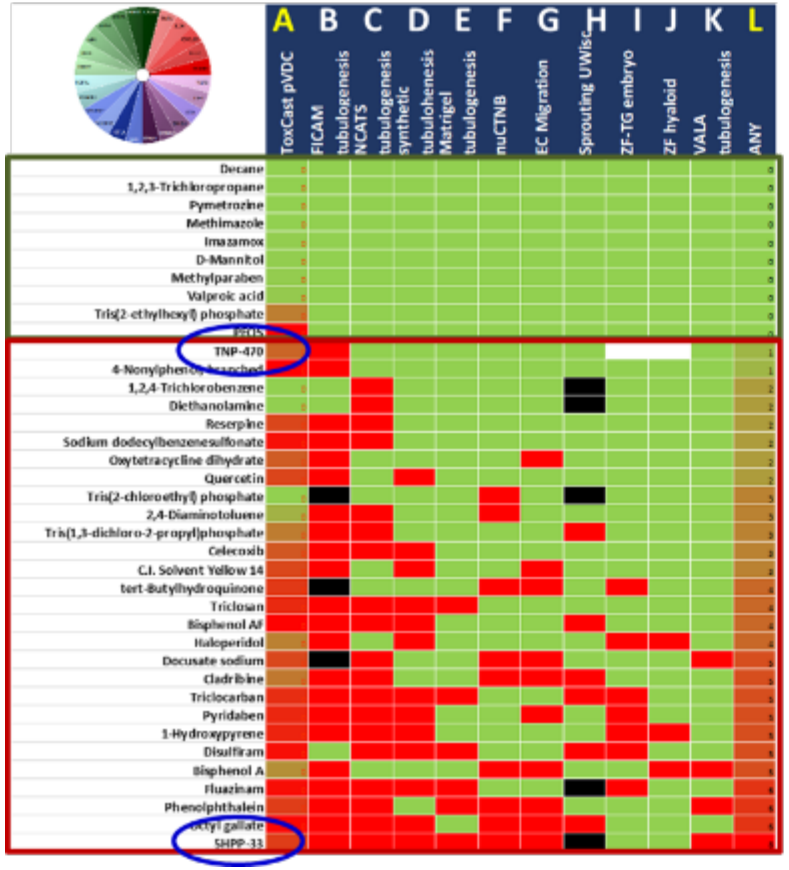


SOURCE: Saili et al. (2019) Current Opinion Toxicology (under review)

13



- inactive
- active
- cytotoxic
- no data



A pVDC ToxPi ⟵ ····· ***PREDICTED***

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) ····· ⟶ ***OBSERVED***

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

SOURCE: Saili et al. (2019) Current Opinion Toxicology (under review)

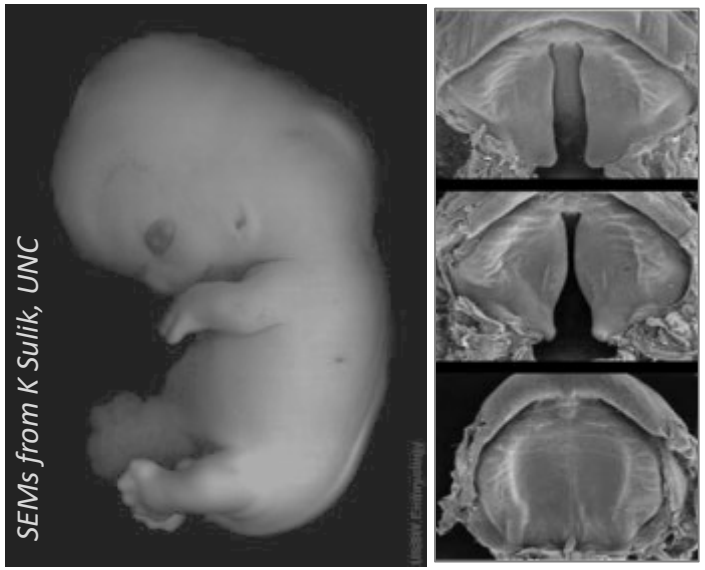
2. Agent-based models (ABMs)

- **Approach:** build and test self-organizing morphogenetic systems *in silico* using CC3D modeling environment (www.compucell3d.org).
- **Input:** A.I. cast into mathematically-defined cells (agents), synthetic gene circuits, and viscoelastic properties to emulate developmental progression.
- **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).

Anatomical homeostasis in a self-regulating ‘Virtual Embryo’

SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

AOP framework: *cleft palate as an example*



ToxCast Chemicals

500 chemicals summarized by ToxCast gene score and chemotype for machine-learning

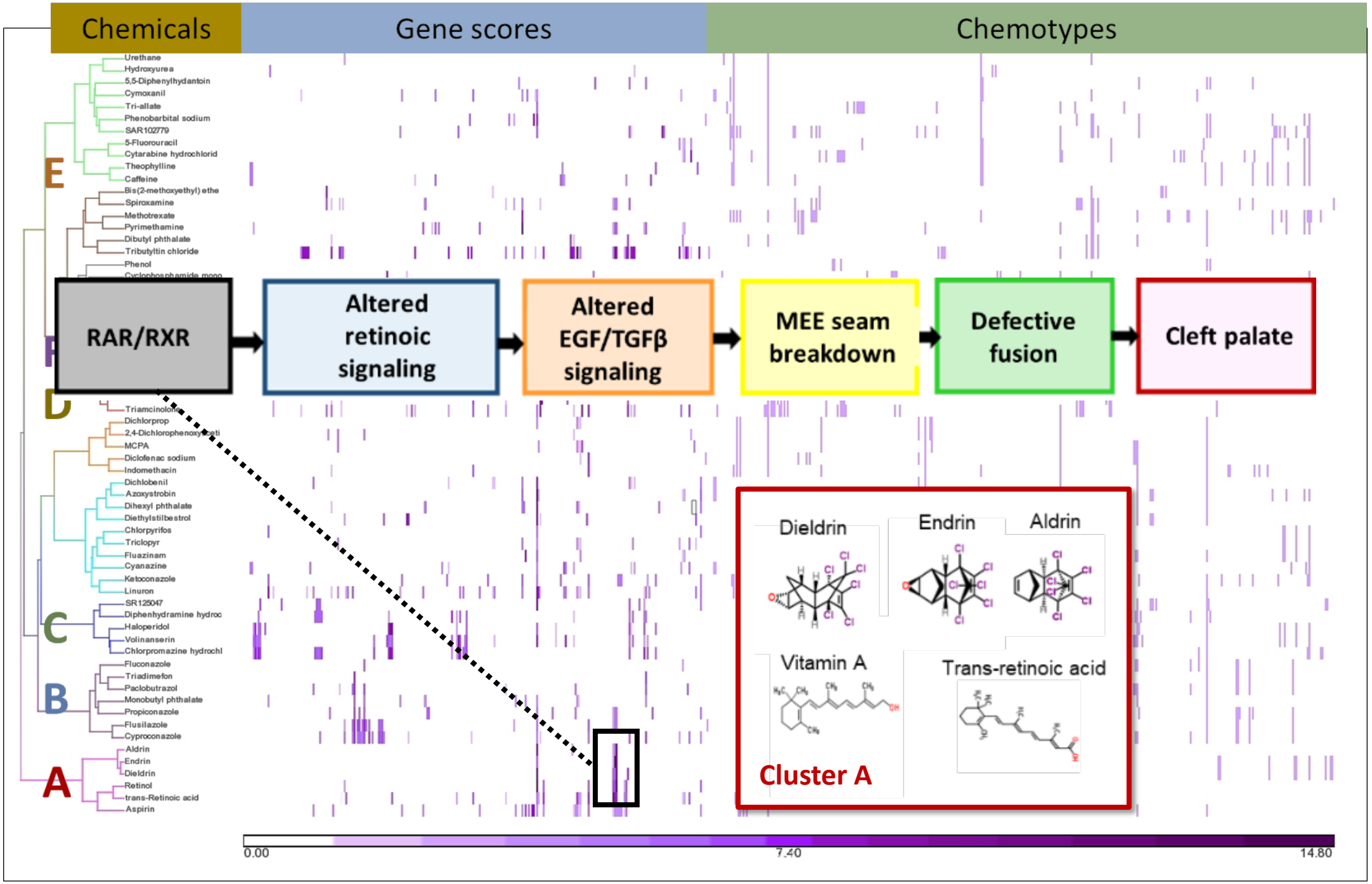
Animal studies

63 chemicals associated with cleft palate in ToxRefDB or open literature

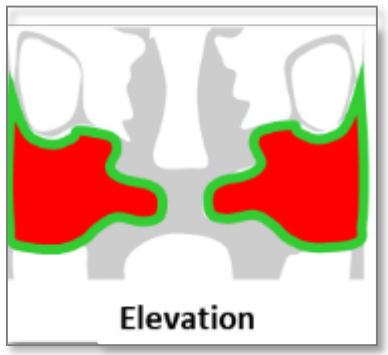
AOP clusters

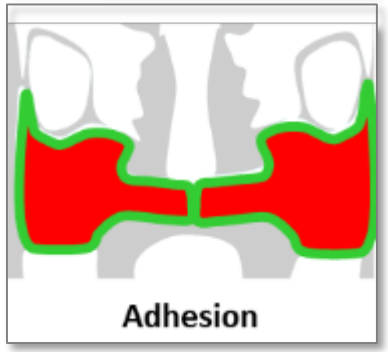
6 mechanistic pathways inferred from integration of HTS data with chemical structure.

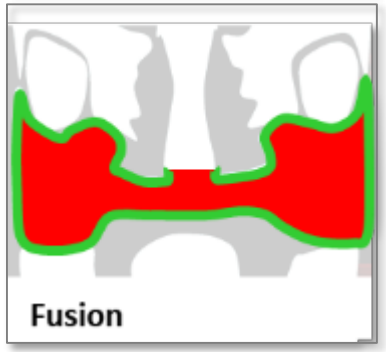
Baker et al. (manuscript submitted)

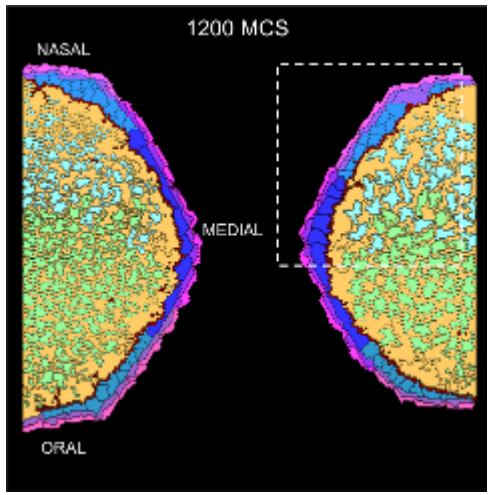

Chemicals
Gene scores
Chemotypes
Urethane
Hydroxyurea
5,5-Diphenylhydantoin
Cymoxanil
Tri-allate
Phenobarbital sodium
SAR102779
5-Fluorouracil
Cytarabine hydrochlorid
Theophylline
Caffeine
Bis(2-methoxyethyl) ethe
Spiroxamine
Methotrexate
Pyrimethamine
Dibutyl phthalate
Tributyltin chloride
Phenol
E
RAR/RXR
Altered retinoic signaling
Altered EGF/TGFβ signaling
MEE seam breakdown
Defective fusion
Cleft palate
Triamcinolone
Dichlorprop
2,4-Dichlorophenoxyacti
MCPA
Diclofenac sodium
Indomethacin
Dichlobenil
Azoxystrobin
Dihexyl phthalate
Diethylstilbestrol
Chlorpyrifos
Triclopyr
Fluazinam
Cyanazine
Ketoconazole
Linuron
SR125047
Diphenhydramine hydroc
Haloperidol
Volinanserin
Chlorpromazine hydrochl
C
Fluconazole
Triadimefon
Paclobutrazol
Monobutyl phthalate
Propiconazole
Flusilazole
Cyproconazole
B
Aldrin
Endrin
Dieldrin
Retinol
trans-Retinoic acid
Aspirin
A
Dieldrin
Endrin
Aldrin
Vitamin A
Trans-retinoic acid
Cluster A
0.00
7.40
14.80

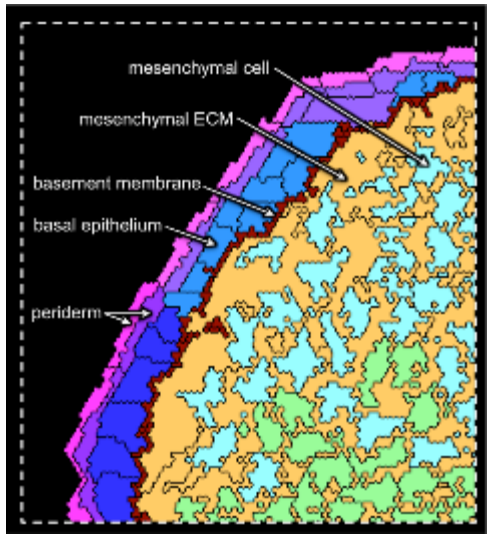
Palatal fusion in silico (CompuCell3d.org)

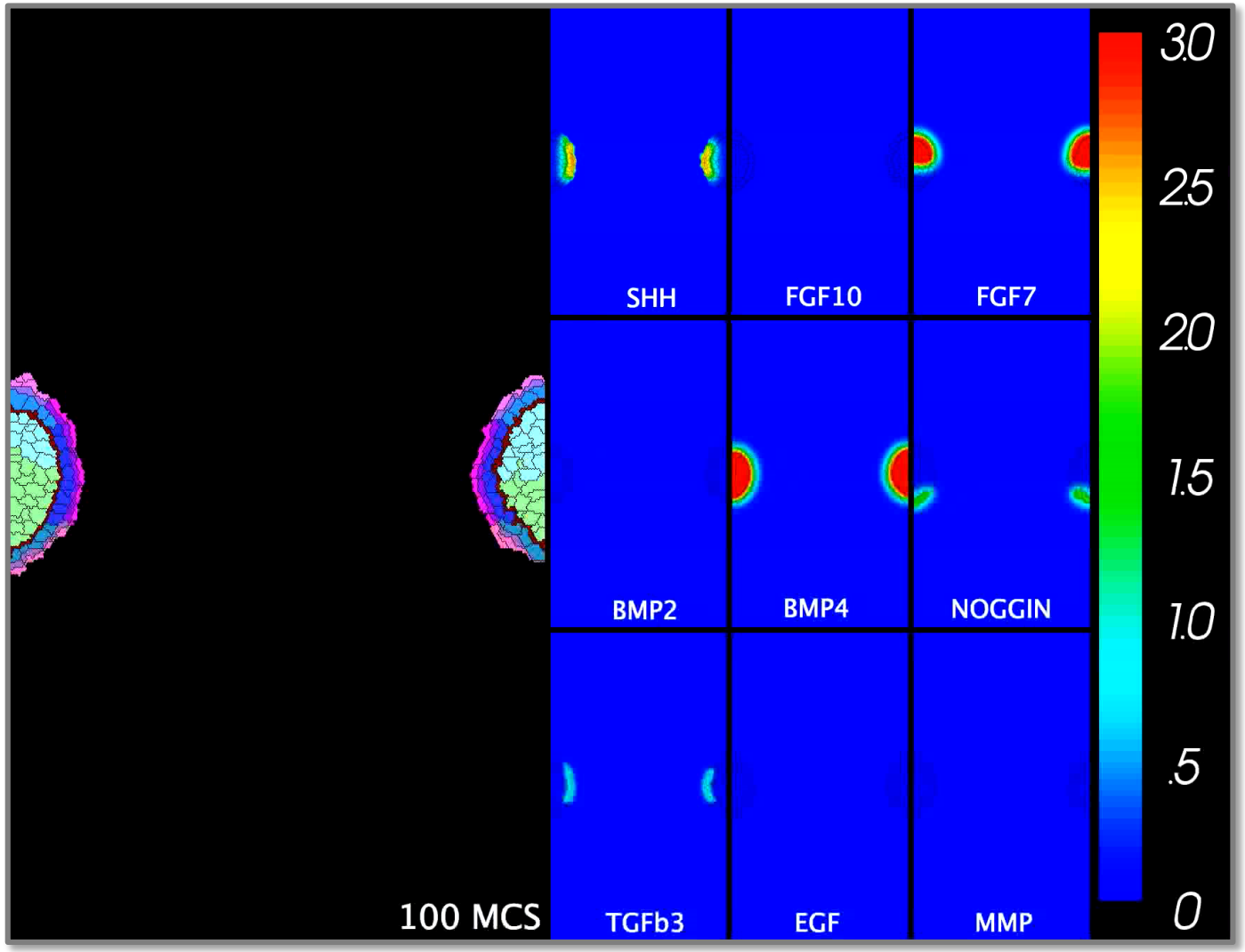






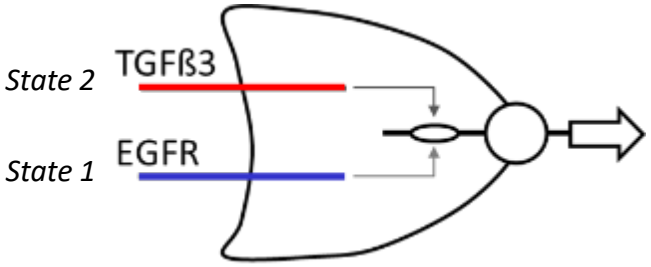




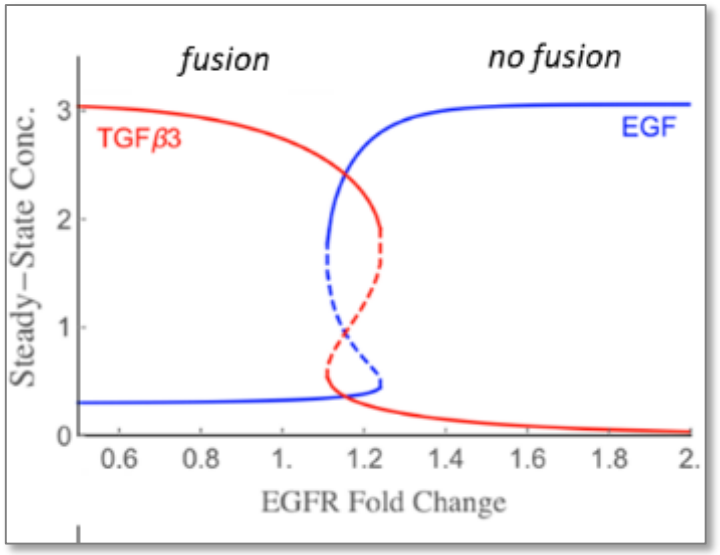


Hutson et al. (2017) Chem Res Toxicol

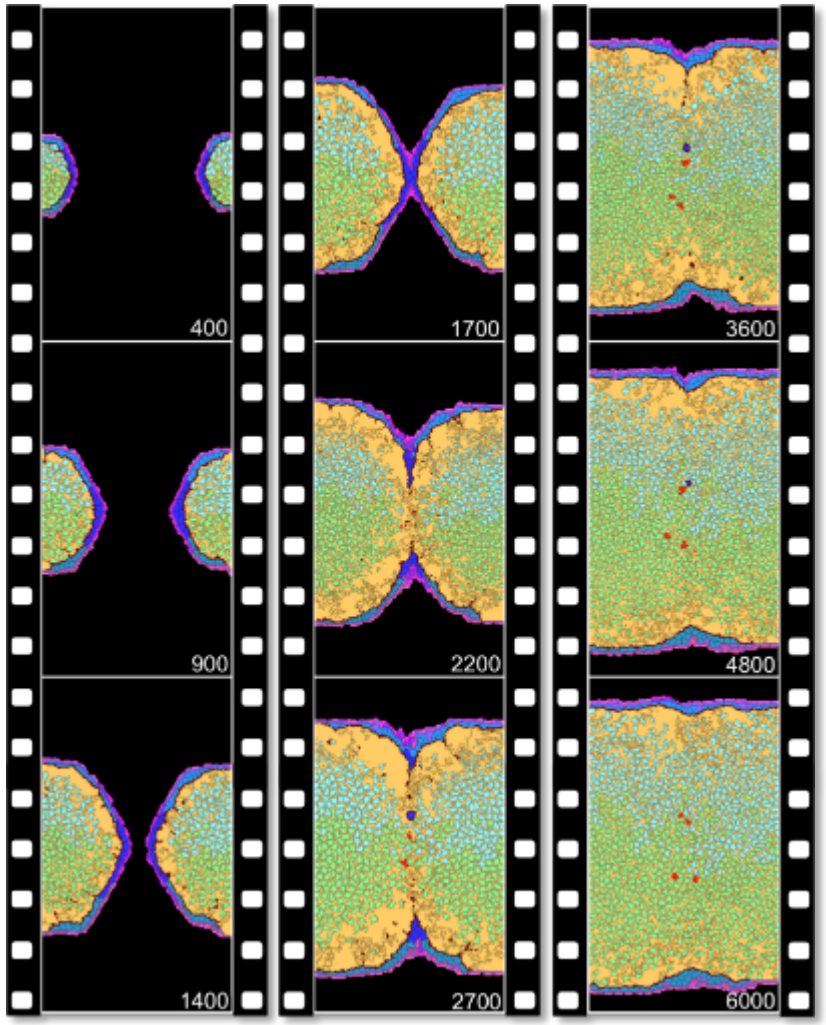
Key event: *involution of the Medial Edge Seam (MES)*



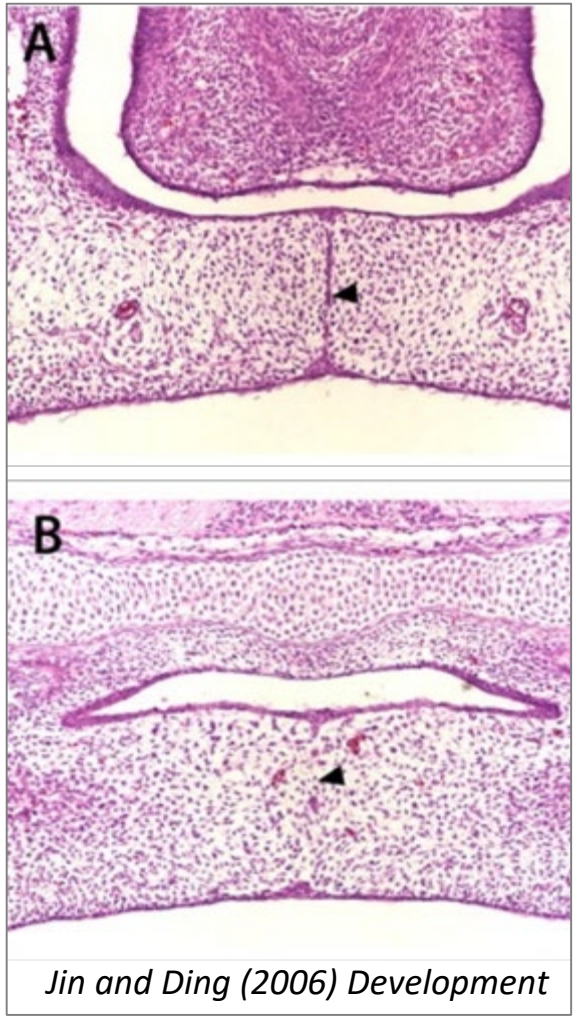
Bistable hysteresis switch



in silico

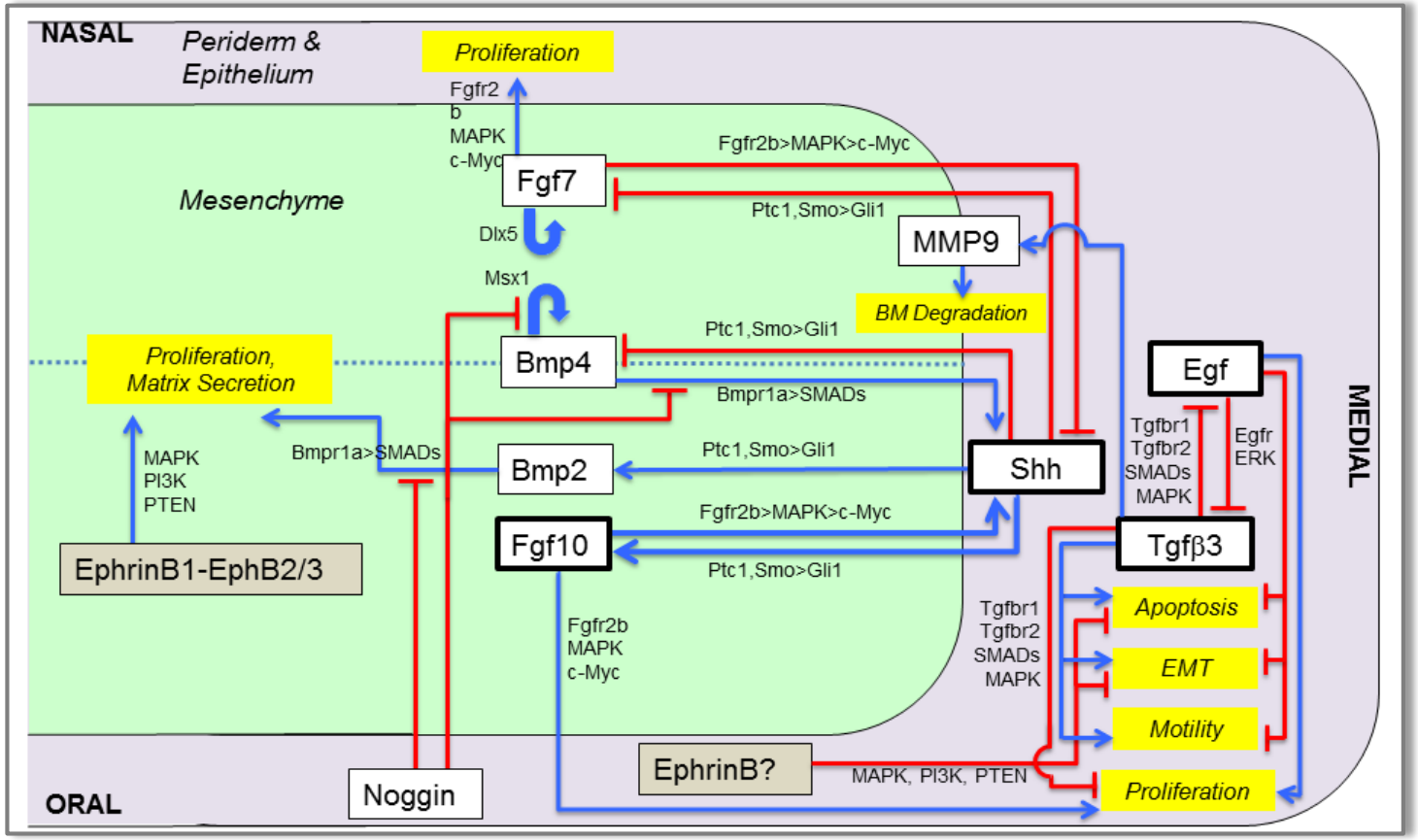


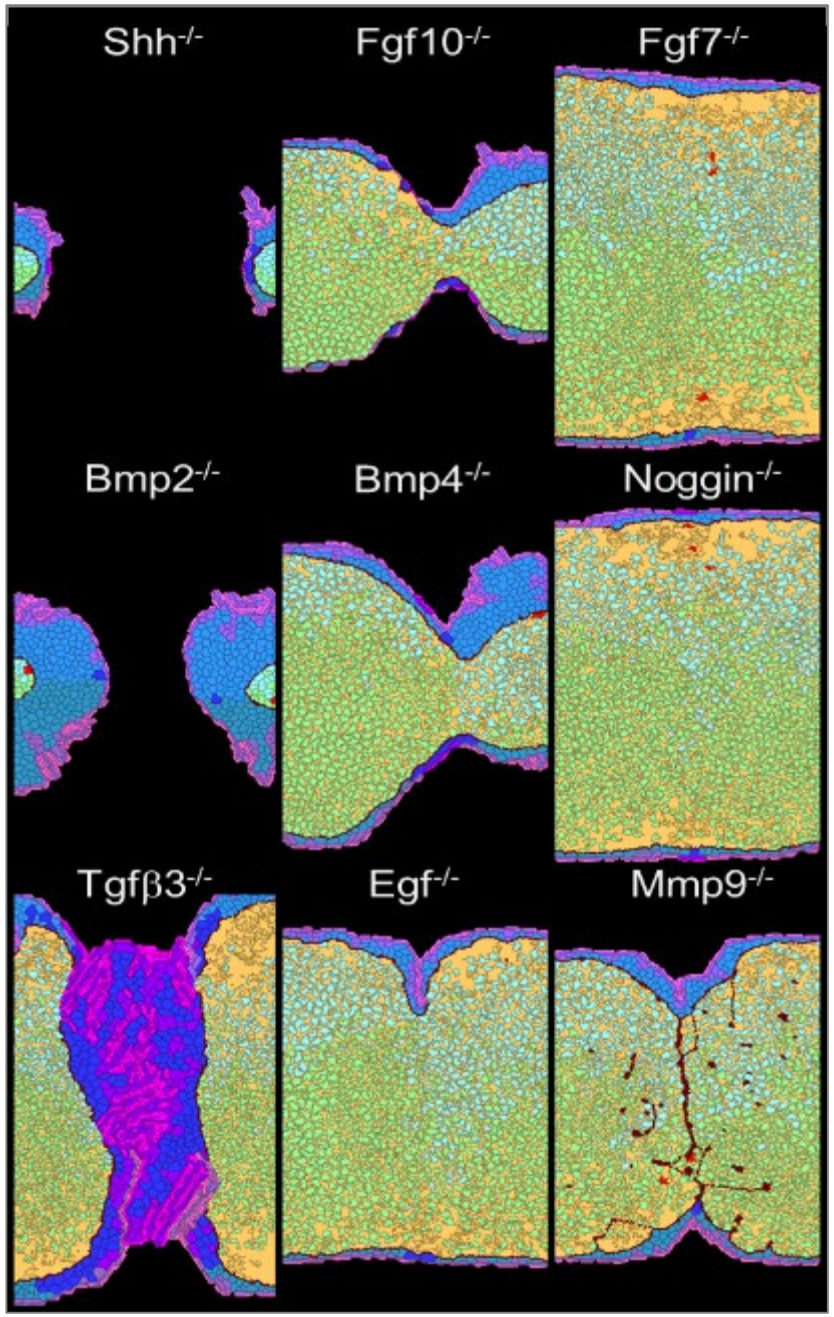
in vivo



Jin and Ding (2006) Development

Hacking the control network





A.I. = synthetic cell signaling networks

Cybermorphs = simulated loss of function

SOURCE: Hutson et al. (2017) Chem Res Toxicol

Smart model …

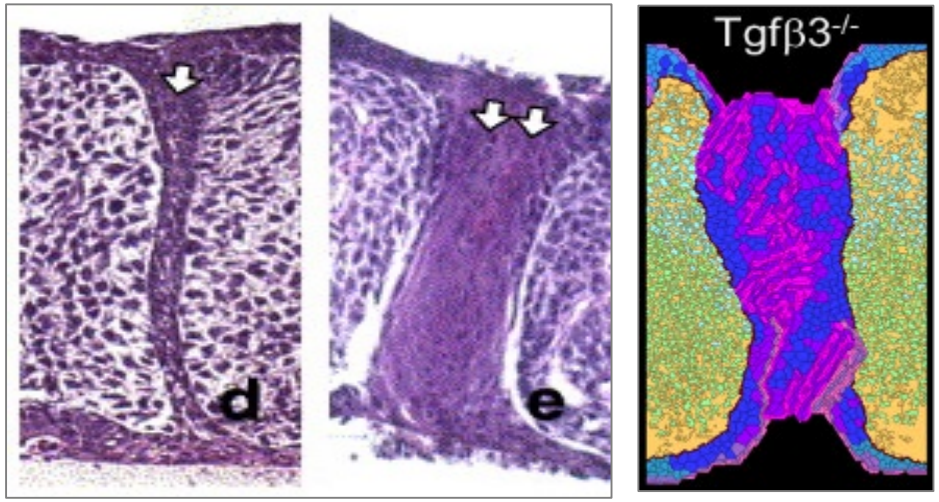
Chemical Research in Toxicology

Computational Model of Secondary Palate Fusion and Disruption

M. Shane Hutson, Maxwell C. K. Leung, Nancy C. Baker, Richard M. Spencer, and Thomas B. Knudsen

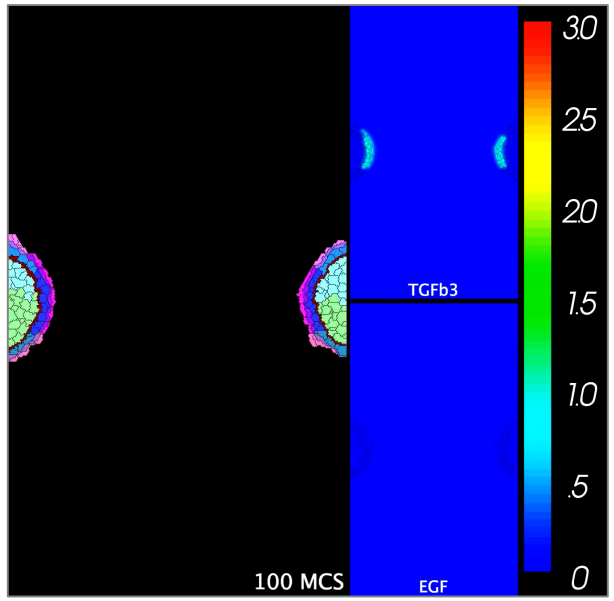
Reviewer Comment: *"Crucial mechanisms occurring during palate fusion, especially opposing palatal shelf adhesion, are not considered in the model. In fact, the main reason why Tgf-b3 KO mice have cleft palate is a failure of opposing MEE adhesion, leading to separation of palatal shelves after their initial contact. Even in those strains in which palatal shelves adhere partially, I have never seen a MES as the one shown in Fig. 5."*

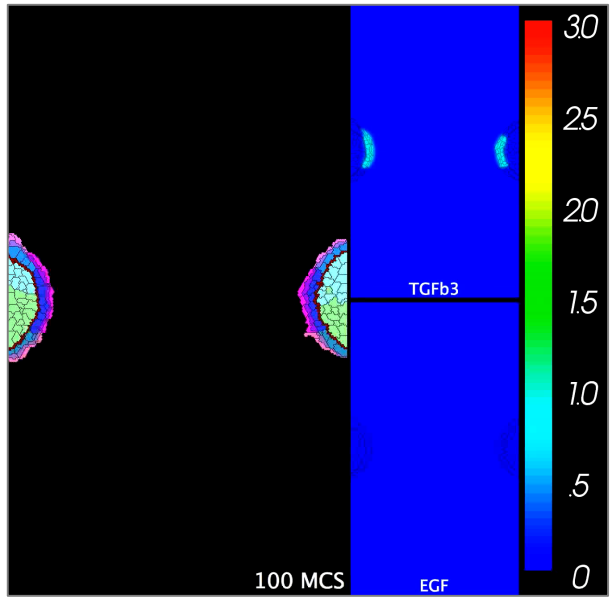
Our Response: see TGF-b3 knockout mouse palates transduced with ALK vectors *in vitro. (from Dudas et al. 2004).*

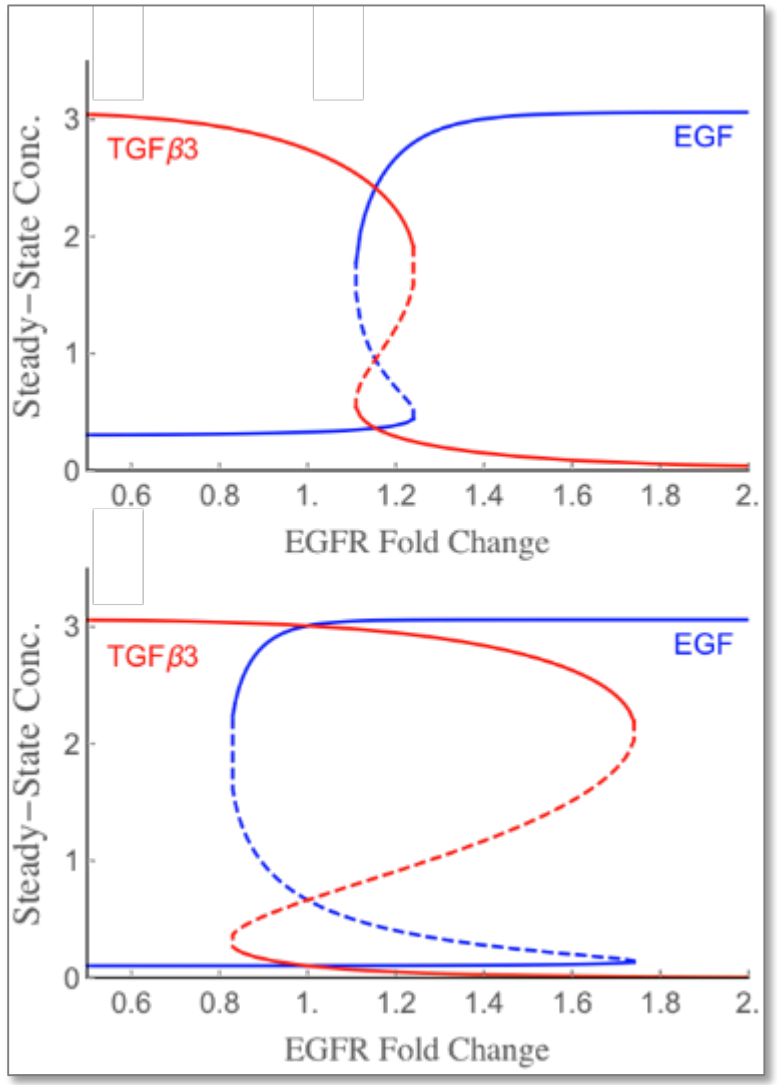


Hutson et al. (2017) Chem Res Toxicol

Messin' with the switch: *two scenarios for bistable dynamics*







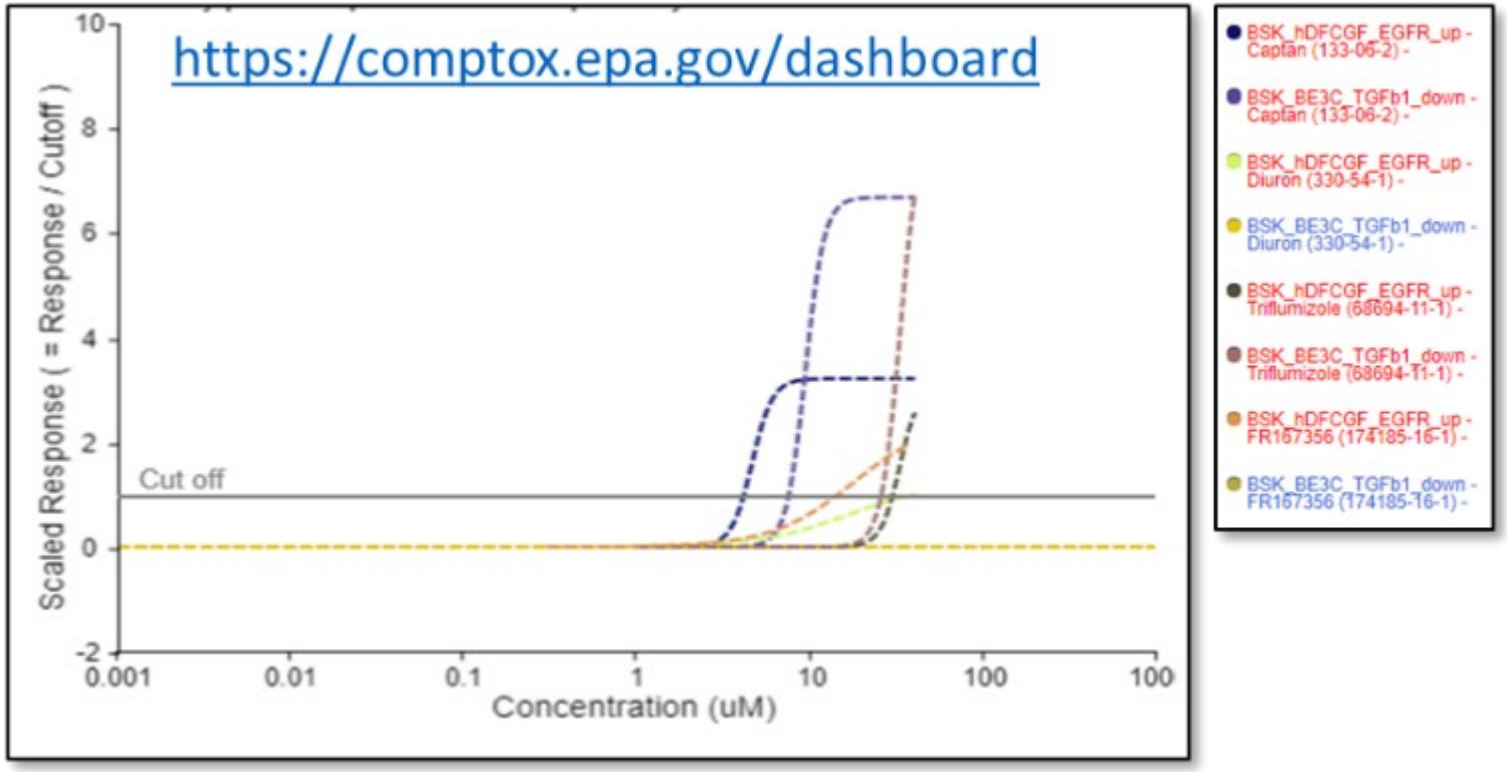
Narrow hysteresis:
less resilient but reversible

Broad hysteresis:
more resilient but irreversible

ToxCast dataset: *39 ↑EGF-signaling; some also ↓TGF-beta signaling*

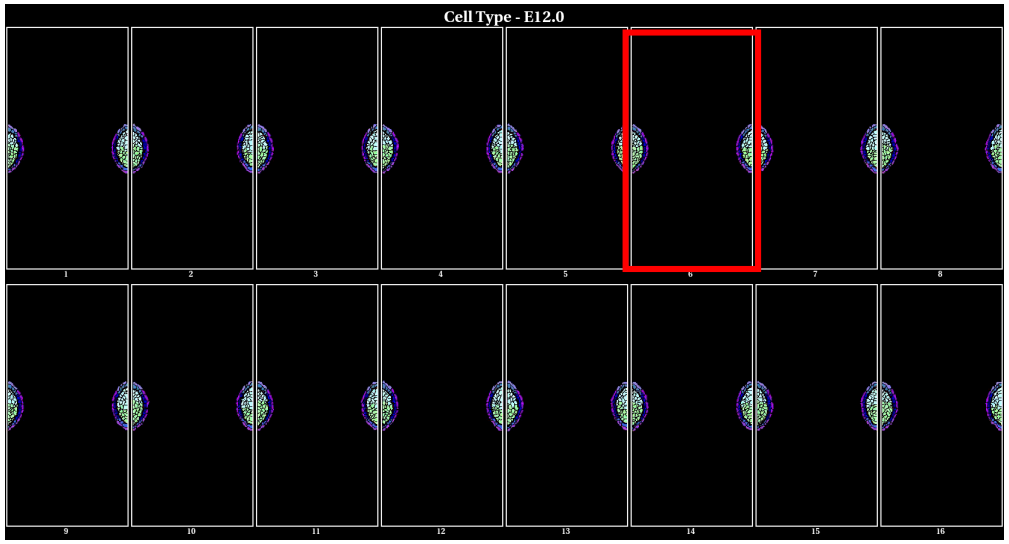
ChemicalName	EGFR_up (uM AC50)	TGFb1_down (uM AC50)	STM (uM TI)	ToxRefDB (low)
Carbaryl	0.07	1000.00	2.92	POS
Captafol	1.02	3.76	0.35	POS
Fipronil	1.18	1000.00	66.01	POS
Fluazinam	2.39	2.48	10.75	POS
Thiram	4.45	6.95	8.26	POS
Linuron	10.91	1000.00	30.94	POS
Maneb	0.01	1000.00	NEG	POS
Propoxur	1.67	1000.00	NEG	POS
Captan	4.59	7.15	NEG	POS
Bendiocarb	8.75	1000.00	NEG	POS
Raloxifene hydrochloride	12.40	15.94	NEG	POS
Tri-allate	19.19	x	NEG	POS
Triflumizole	32.71	19.88	NEG	POS
Butachlor	32.71	17.85	NEG	POS
Rotenone	0.82	1000.00	0.05	NEG
Zoxamide	14.22	17.37	16.13	NEG
Diuron	16.51	1000.00	68.06	NEG
Forchlorfenuron	0.02	1000.00	NEG	NEG
Azamethiphos	0.89	1000.00	NEG	NEG
Methylene bis(thiocyanate)	1.14	5.93	NEG	NEG
2-(Thiocyanomethylthio)benzothiazole	2.28	6.48	NEG	NEG
Methyl isothiocyanate	4.60	1000.00	NEG	NEG
Bromacil	20.50	1000.00	NEG	NEG
Diphenylamine	32.71	5.95	NEG	NEG
TNP-470	7.78	3.97	0.02	x
PharmaGSID_48511	12.19	11.22	0.02	x
4-Pentylaniline	0.00	x	NEG	x
Monobutyl phthalate	0.01	1000.00	NEG	x
Estrone	0.03	1000.00	NEG	x
SAR102779	0.05	12.95	NEG	x
Niclosamide	0.58	1000.00	NEG	x
CP-457920	3.50	1000.00	NEG	x
Perfluoroundecanoic acid	6.81	4.76	NEG	x
1,2-Benzisothiazolin-3-one	8.22	11.91	NEG	x
SB243213A	10.24	x	NEG	x
Phenolphthalein	16.26	x	NEG	x
FR167356	17.65	1000.00	NEG	x
SB281832	34.72	1000.00	NEG	x
p,p'-DDT	38.17	x	NEG	x

ChemicalName	EGFR_up (uM AC50)	TGFb1_down (uM AC50)	STM (uM TI)	ToxRefDB (low)
Fluazinam	2.39	2.48	10.75	POS
Captan	4.59	7.15	NEG	POS
Diuron	16.51	1000.00	68.06	NEG
FR167356	17.65	1000.00	NEG	x

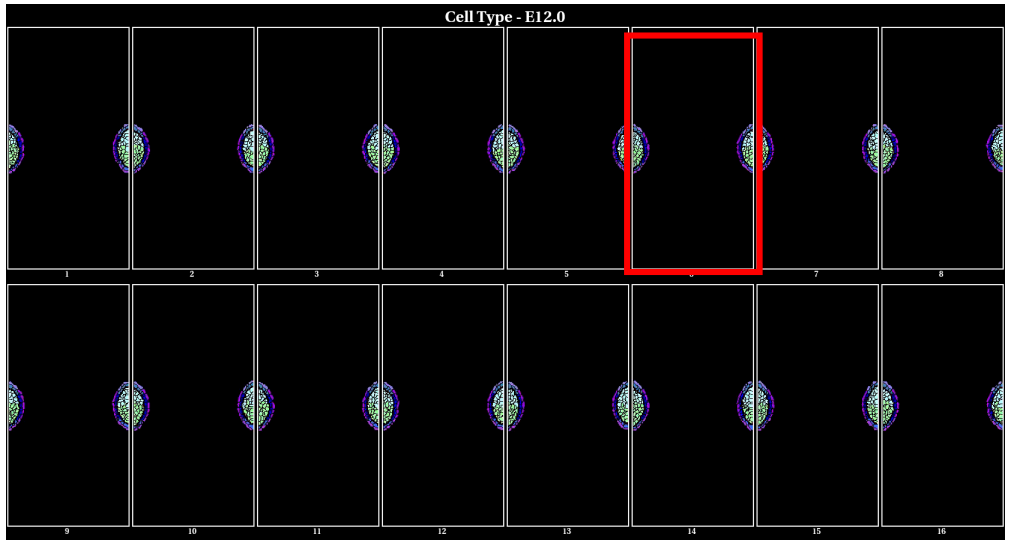


***In silico* dose-response:** *translating ↑EGFR conc. profile into a critical dose*

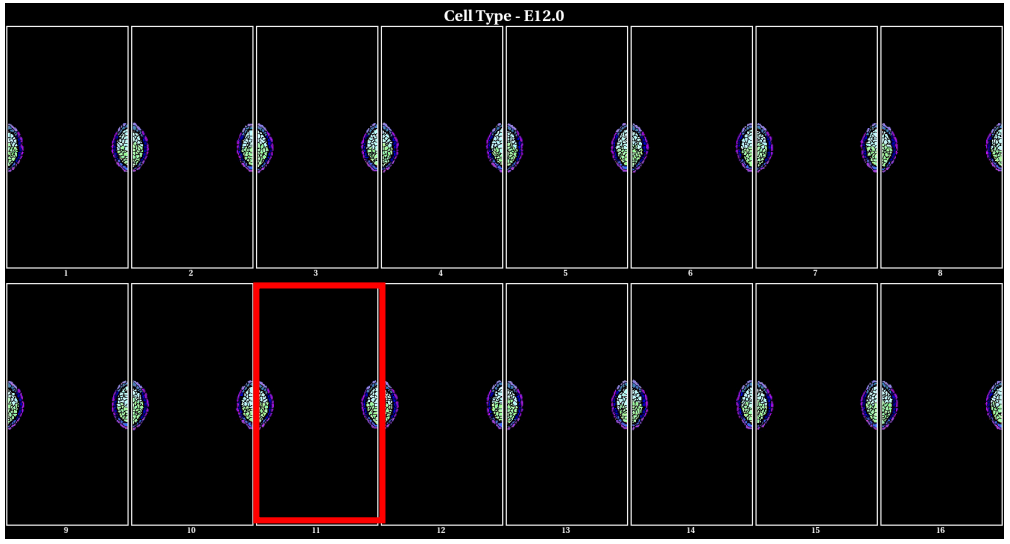
Fluazinam



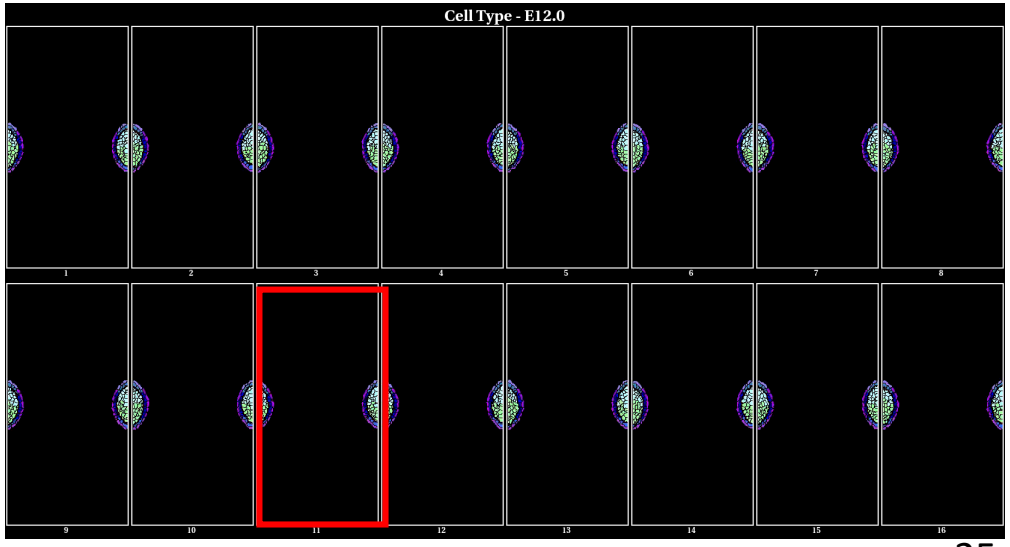
Captan



Diuron

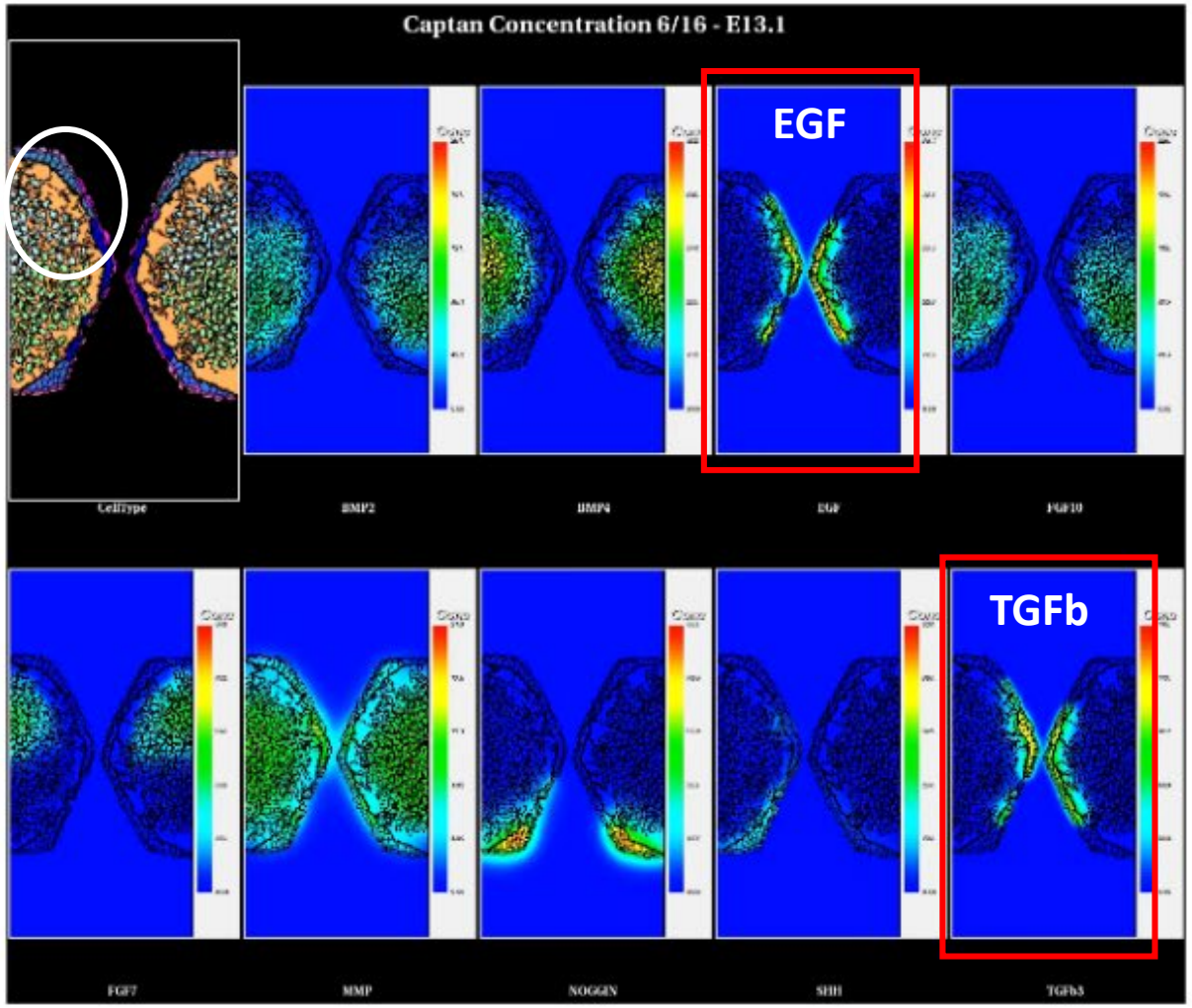


FR167356

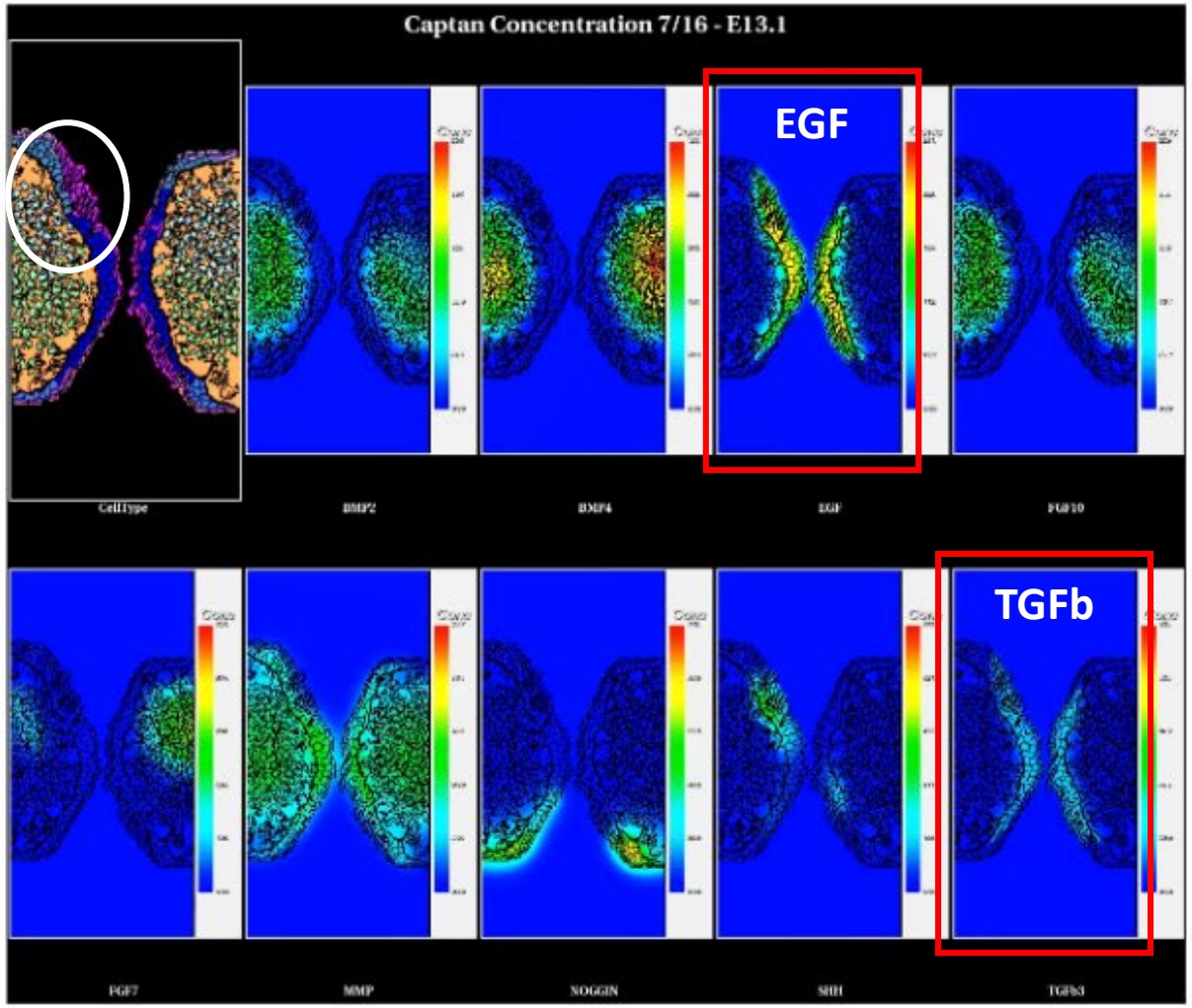


Pathogenesis: *simulating the prefusion alterations*

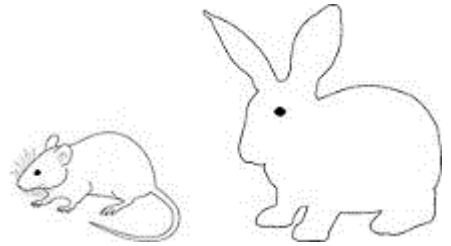
pre-critical dose



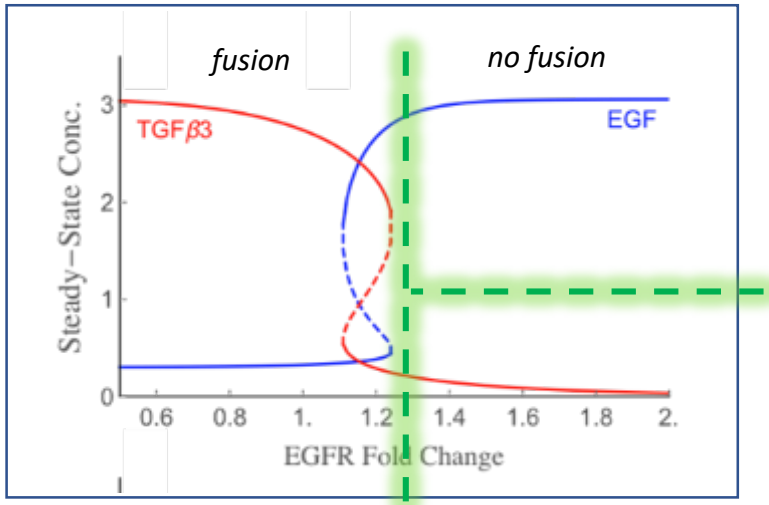
post-critical dose



Predictive model: *modeling the critical phenomenon*

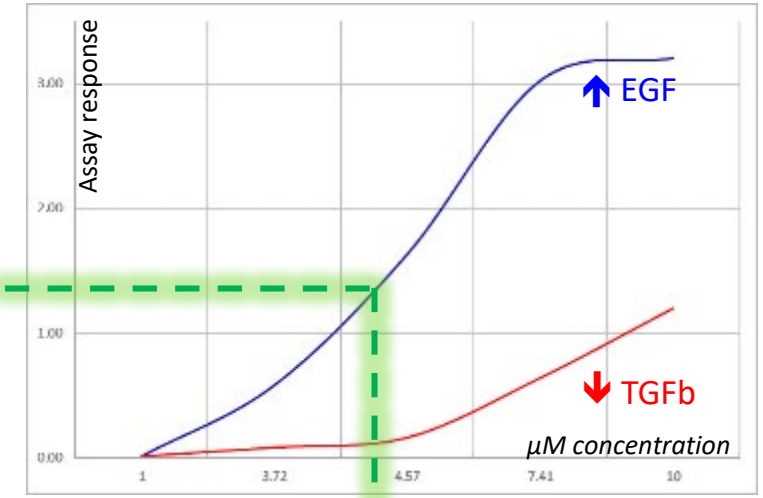


INPUT: switch dynamics



tipping point predicted by computational dynamics (hysteresis switch)

Captan in ToxCast



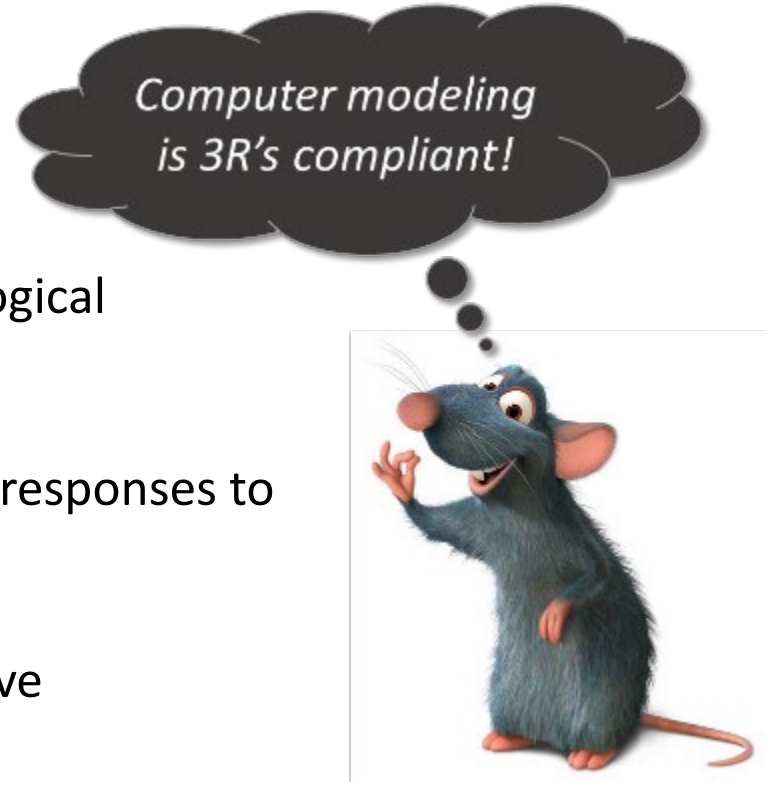
OUTPUT: tipping point mapped to concentration response (4 μM)

Captan in ToxRefDB
NEL = 10 mg/kg/day
LEL = 30 mg/kg/day

human HTTK model
2.39 mg/kg/day would achieve a steady state of 4 μM in fetal plasma

CompTox Chemicals Dashboard
exposure prediction
0.88×10^{-7} mg/kg/day

Summary and Conclusions



- NAMs are available for HTS chemical inventories for profiling chemical-biological interactions *in vitro*.
- AOPs provide a framework for quantitative prediction of cellular and tissue responses to molecular perturbation(s).
- Integrative models are needed to 'decode the toxicological blueprint of active substances' that interact with developing systems.
- Computational biology is uniquely positioned to capture this connectivity and help shift decision-making to mechanistic prediction.
- Cell ABMs recapitulate morphogenesis cell-by-cell and interaction-by-interaction as an embryonic system advances in time.
- 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.

Special Thanks

Todd Zurlinden – NCCT
Kate Saili – NCCT
Nancy Baker – Leidos / NCCT
Richard Spencer – ASA / EMVL
John Wambaugh – NCCT
John Cowden – NCCT/CSS
Shane Hutson – Vanderbilt U
Barbara Abbott – NHEERL
Nicole Kleinstreuer - NICEATM
William Murphy – U Wisconsin
William Daly – U Wisconsin
Gaurav Kaushik – U Wisconsin
Rob Ellis-Hutchings – DOW Chemical
James Glazier – Indiana University
Sid Hunter – NHEERL / ISTD
Tuula Heinonen – U Tampere
Your name here

National Center for Computational Toxicology

v-Embryo™

EPA science in ACTION
www.epa.gov/research
INNOVATIVE RESEARCH FOR A SUSTAINABLE FUTURE
Virtual Tissue Models: Predicting How Chemicals Impact Human Development

CSS


EPA
Post-Doctoral Research Program
Multiscale modeling and simulation for predictive developmental toxicity

https://cfpub.epa.gov/ordpd/PostDoc_Position.cfm?pos_id=1100

Pondering the way forward …

Translational: what do synthetic models of human development - both computational and organoids - bring to future of DART testing?

Investigational: how smart must these models be (A.I.) to support decision-making in the animal-free (3Rs) zone?

Operational: what best practices are needed to implement synthetic models into an integrative decision framework (eg, AOP-based IATAs)?

Communication: what are the practical considerations for science, engineering, and stakeholder engagement (academics, government, industry, NGOs, policy, …)?