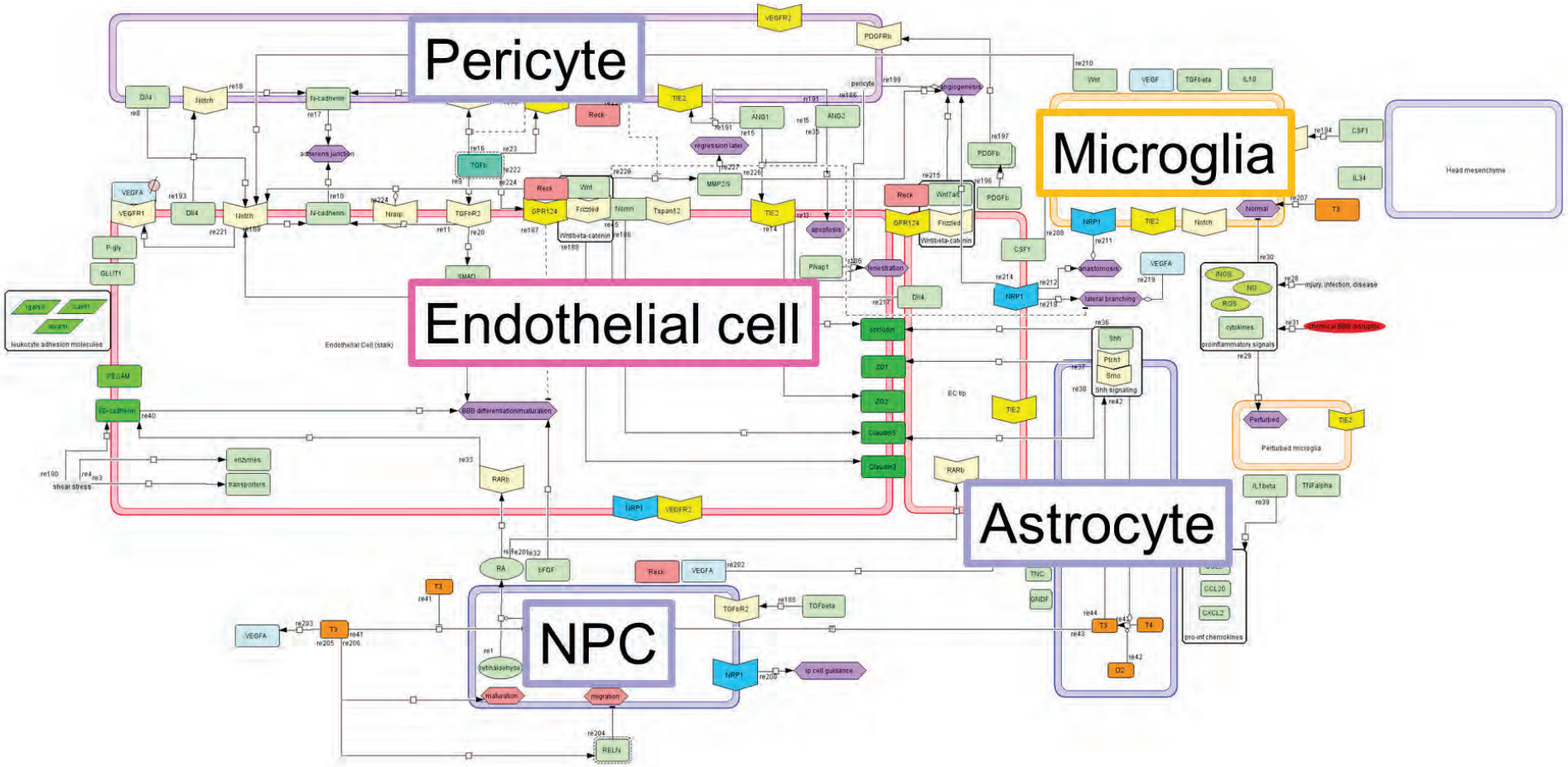
European Partnership for
Alternative Approaches to Animal Testing
Brussels – October 1-2, 2019

Computational systems biology/pharmacology approaches:
Translating cellular lesions into quantitative phenotypes

Thomas B. Knudsen, PhD
Developmental Systems Biologist
National Center for Computational Toxicology
US Environmental Protection Agency
Research Triangle Park, NC 27711

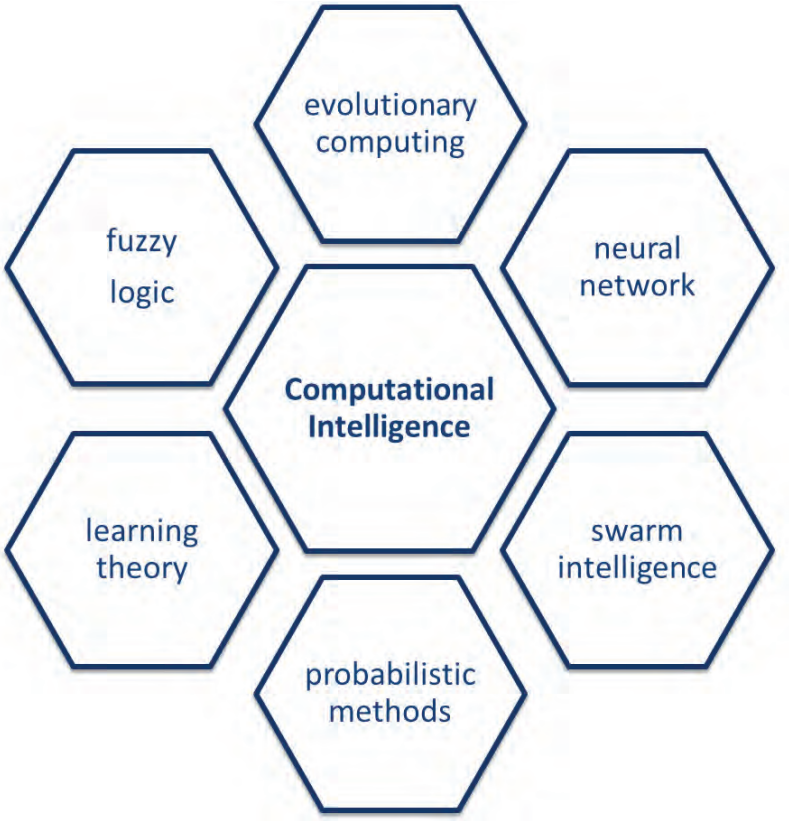
DISCLAIMER: The views expressed in this presentation are those of the presenter and do not necessarily reflect the views or policies of the US EPA


Pericyte
Endothelial cell
Microglia
Astrocyte
NPC
VEGFR2
PDGFRb
Dll4
Notch
N-cadherin
TGFb
TGFbR2
Reck
Wnt
Frizzled
Norrin
Tspan12
GPR124
Wnt/beta-catenin
MMP2/9
ANG1
ANG2
TIE2
VEGFA
VEGFR1
Nrarp
apoptosis
Plvap1
fenestration
occludin
ZO1
ZO2
Claudin5
Claudin3
PECAM
VE-cadherin
BBB differentiation/maturation
enzymes
transporters
shear stress
RARb
RA
bFGF
TGFbeta
VEGFA
T3
T4
D2
retinaldehyde
maturation
migration
RELN
NRP1
tip cell guidance
pericyte
angiogenesis
regression later
PDGFb
Wnt7a/b
CSF1
IL34
Head mesenchyme
Normal
anastomosis
lateral branching
Shh
Ptch1
Smo
Shh signaling
EC tip
iNOS
NO
ROS
cytokines
proinflammatory signals
injury, infection, disease
chemical BBB disruptor
Perturbed
Perturbed microglia
IL1beta
TNFalpha
TNC
GNDF
CCL20
CXCL2
pro-inf chemokines
leukocyte adhesion molecules
Endothelial Cell (stalk)
adherens junction

Computational Intelligence



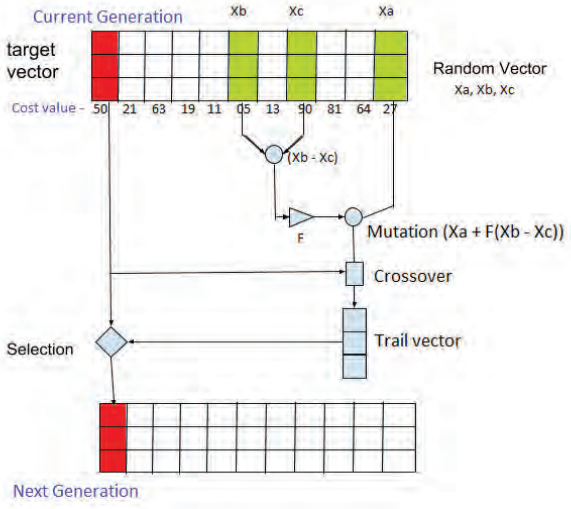
https://www.thescientist.com May, 2019

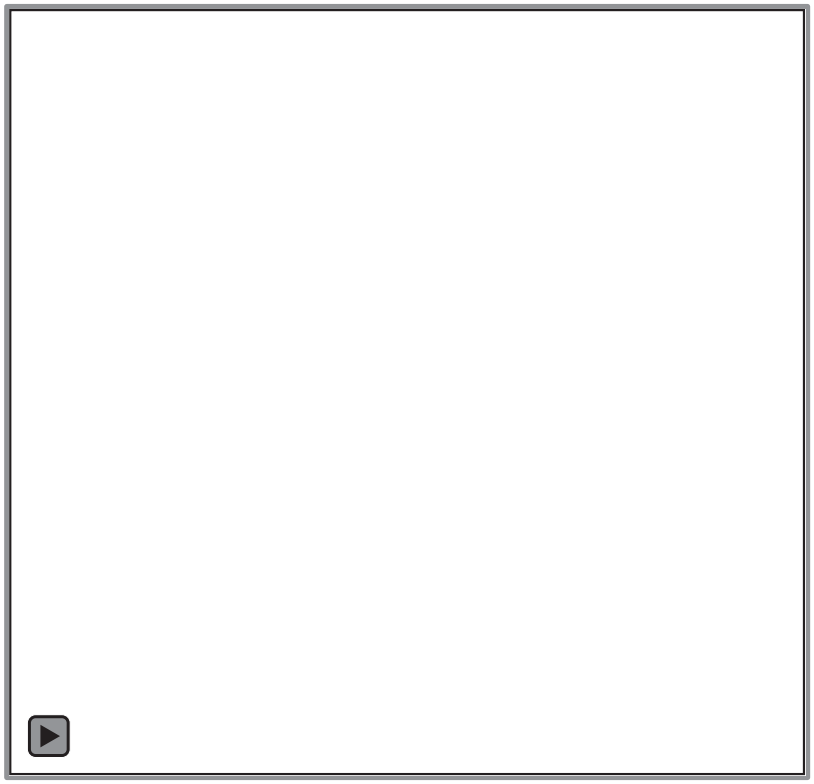


Anatomical homeostasis in a self-regulating ‘Virtual Embryo’

- EA for self-regulation (fitness measure) - simulation executes randomly paired agents (parent cells) that generate daughter cells mutated in their rules.

- You only need to specify the goal of the computation; EA searches rule-space using ‘survival of the fittest’ (good solutions propagate, poor solutions discarded).

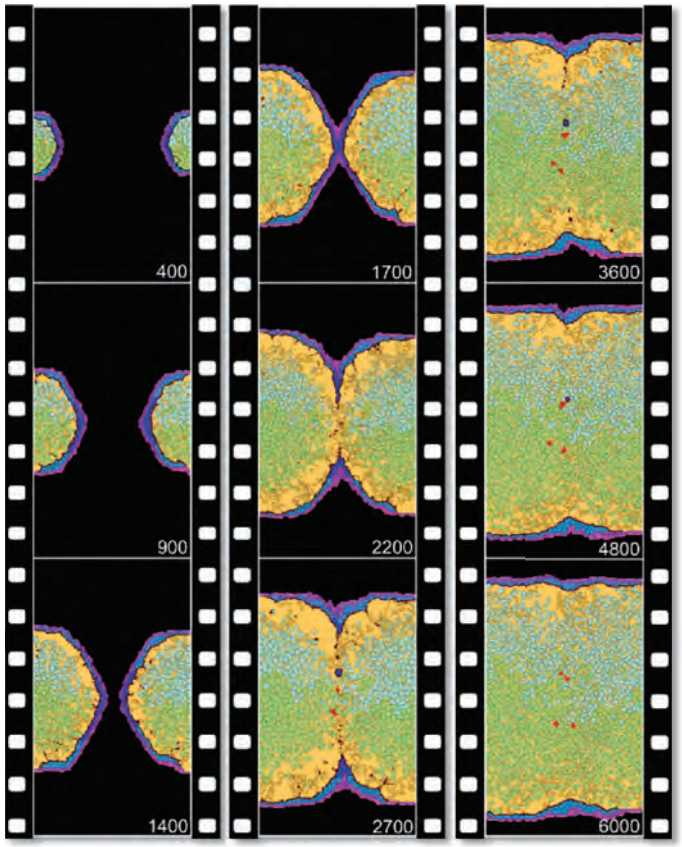




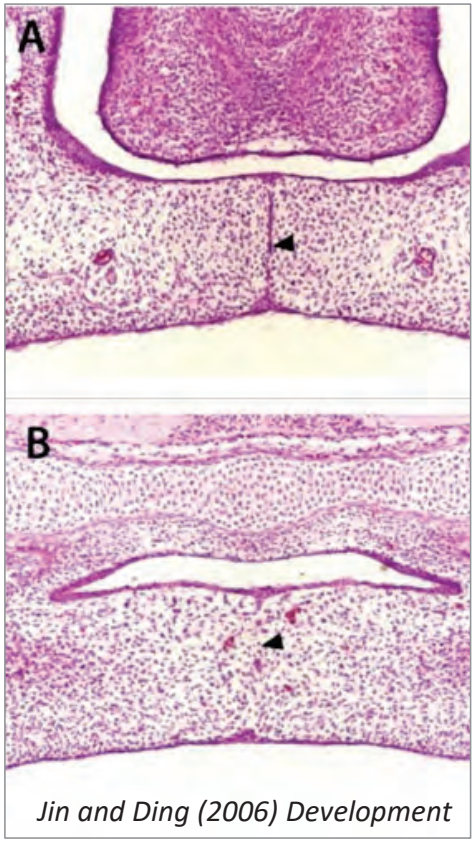
SOURCE: Andersen, Newman and Otter (2006) Am. Assoc. Artif. Intel.

Morphogenetic fusion (palate)

in silico

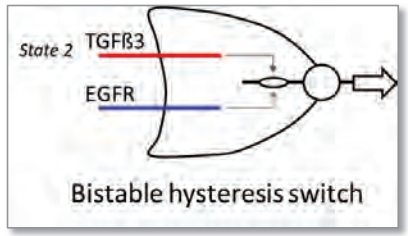


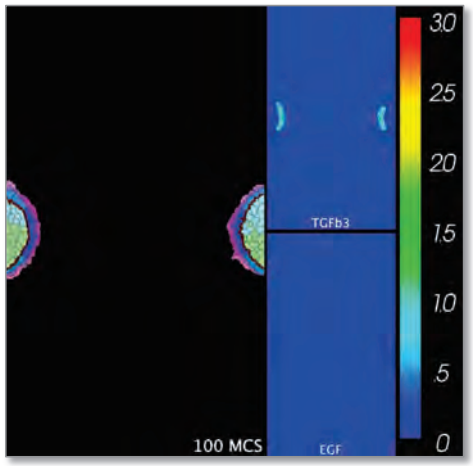
in vivo



Jin and Ding (2006) Development







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

Smart model …



Chemical Research in Toxicology

Article

pubs.acs.org/crt

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*[,∥]

[†]Department of Physics & Astronomy, Department of Biological Sciences and Vanderbilt Institute for Integrative Biosystem Research & Education, Vanderbilt University, Nashville, Tennessee 37235, United States

[‡]Oak Ridge Institute for Science & Education, Oak Ridge, Tennessee 37832, United States

[§]Leidos, Research Triangle Park, Durham, North Carolina 27711 United States

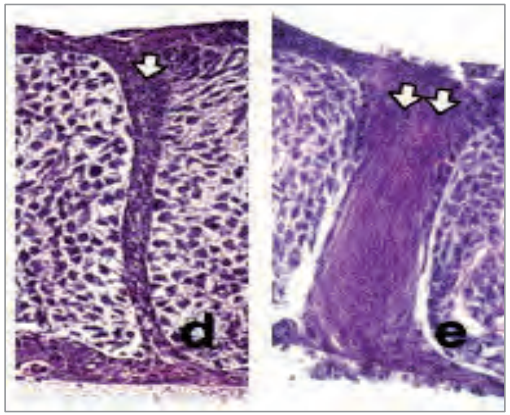
[∥]National Center for Computational Toxicology, Office of Research & Development, U.S. Environmental Protection Agency, Research Triangle Park, Durham, North Carolina 27711, United States

Supporting Information

ABSTRACT: Morphogenetic events are driven by cell-generated physical forces and complex cellular dynamics. To improve our capacity to predict developmental effects from chemical-induced cellular alterations, we built a multicellular agent-based model in CompuCell3D that recapitulates the cellular networks and collective cell behavior underlying growth and fusion of the mammalian secondary palate. The model incorporated multiple signaling pathways (TGFβ, BMP, FGF, EGF, and SHH) in a biological framework to recapitulate morphogenetic events from palatal outgrowth through midline fusion. It effectively simulated higher-level phenotypes (e.g., midline contact, medial edge seam (MES) breakdown, mesenchymal confluence, and fusion defects) in response to genetic or environmental perturbations. Perturbation analysis of various control features revealed model functionality with respect to cell signaling systems and feedback loops for growth and fusion, diverse individual cell behaviors and collective cellular behavior leading to physical contact and midline fusion, and quantitative analysis of the TGF/EGF switch that controls MES breakdown—a key event in morphogenetic fusion. The virtual palate model was then executed with theoretical chemical perturbation scenarios to simulate switch behavior leading to a disruption of fusion following chronic (e.g., dioxin) and acute (e.g., retinoic acid) chemical exposures. This computer model adds to similar systems models toward an integrative "virtual embryo" for simulation and quantitative prediction of adverse

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.*"

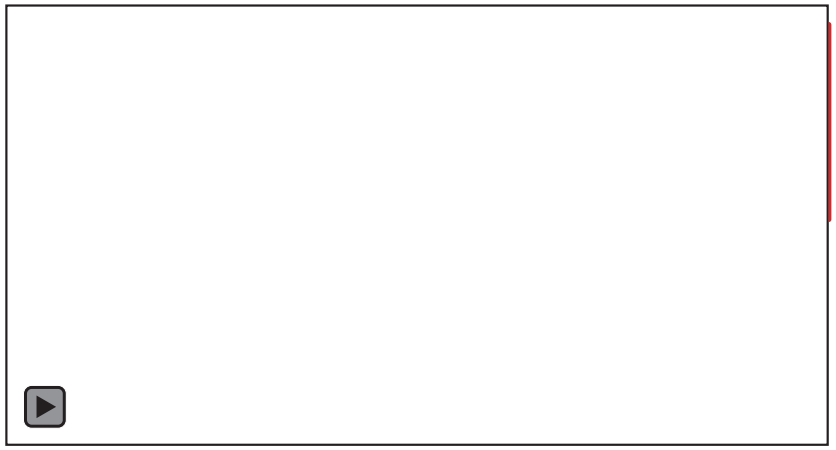
Our Response: TGF-b3 knockout mouse palates transduced with ALK vectors *in vitro. (from Dudas et al. 2004).*

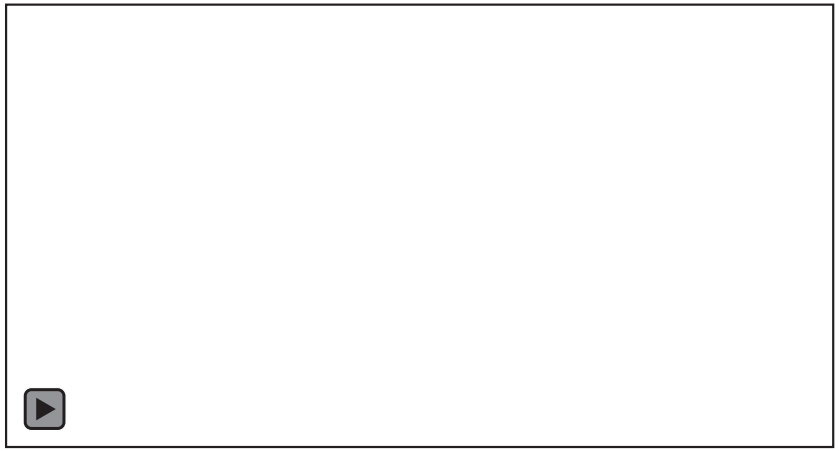


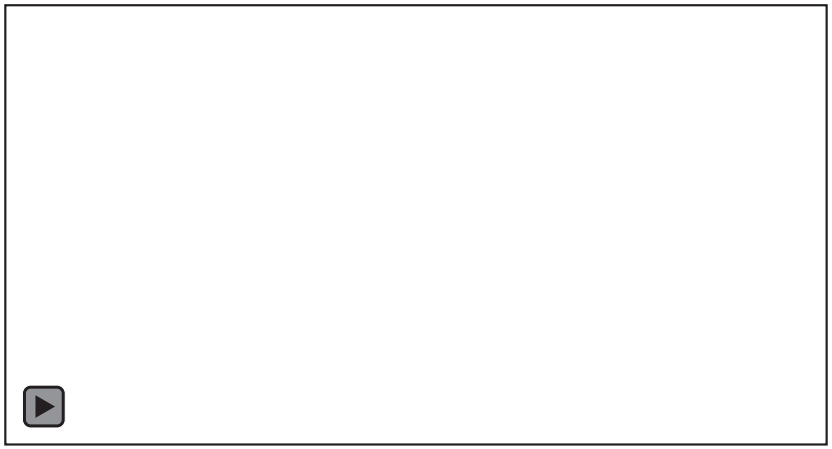


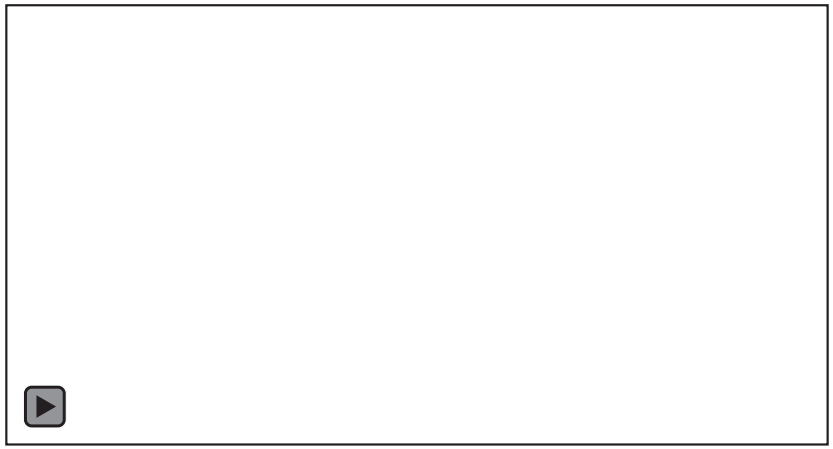
SOURCE: Hutson et al. (2017) Chem Res Toxicol

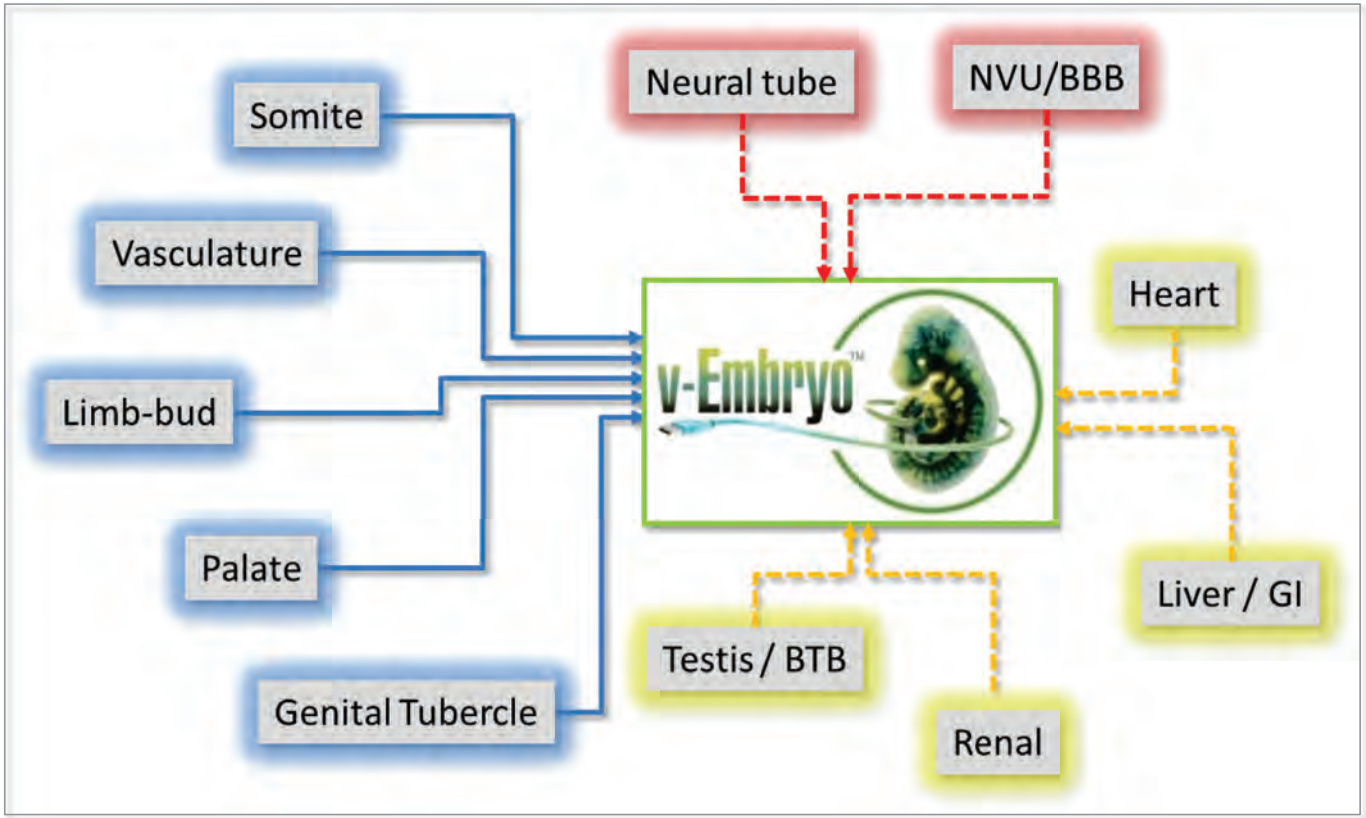
In silico dose-response: *translating ↑EGF/TGFb in vitro profile into a critical effect*







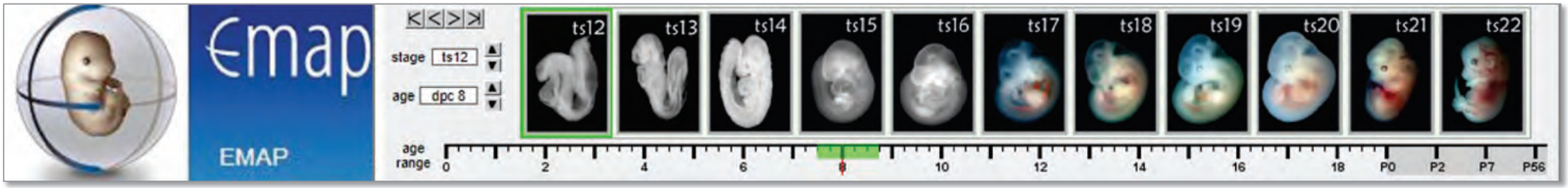




VTLS

- *access to models & simulations*
- *VT-KB (knowledgebase)*
- *Literature mining*
- *tied to ToxCastDB*
- *high-performance computing*

vtls.epa.gov/



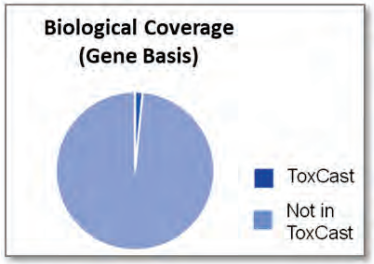
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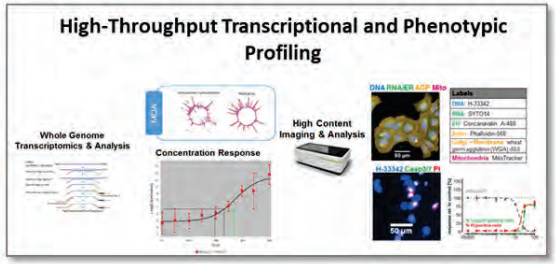
Take Home Messages from Rusty …

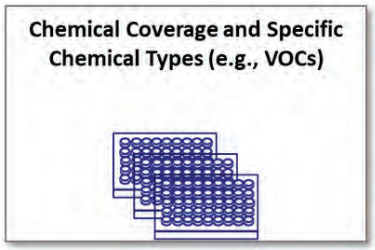


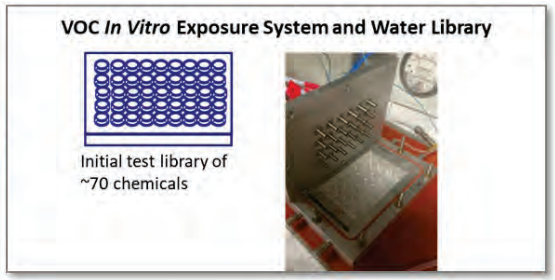
- Multiple opportunities exist for using high-throughput and computational approaches to address challenges in toxicology and risk assessment.
- Using high-throughput approaches will require systematically addressing key technical and data analysis challenges.
- Enabling application of high-throughput data to chemical safety decisions will require delivery and integration using a broad range of IT tools.
- Partnering with regulators on case studies will increase confidence and acceleration application to chemical risk assessment.

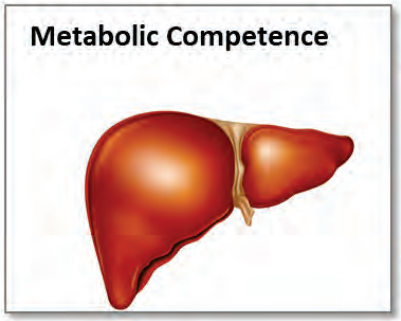
Some Existing Limitations in HTS and *In Vitro* Test Systems

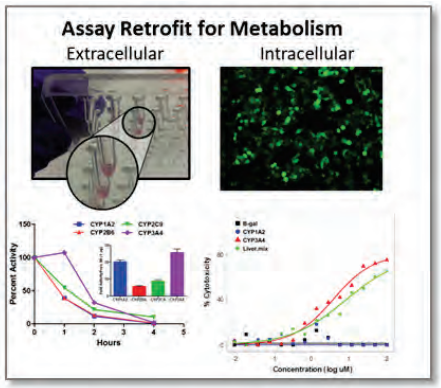




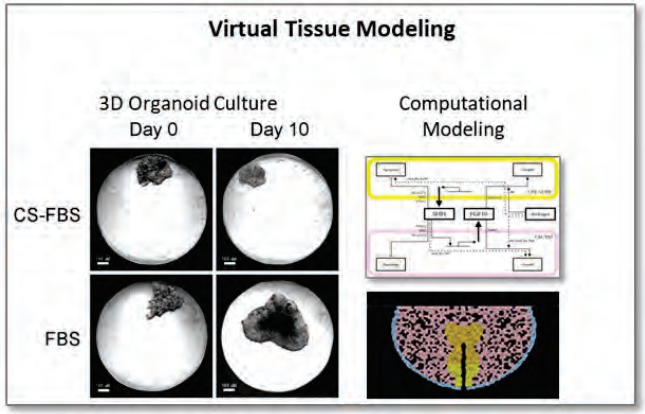




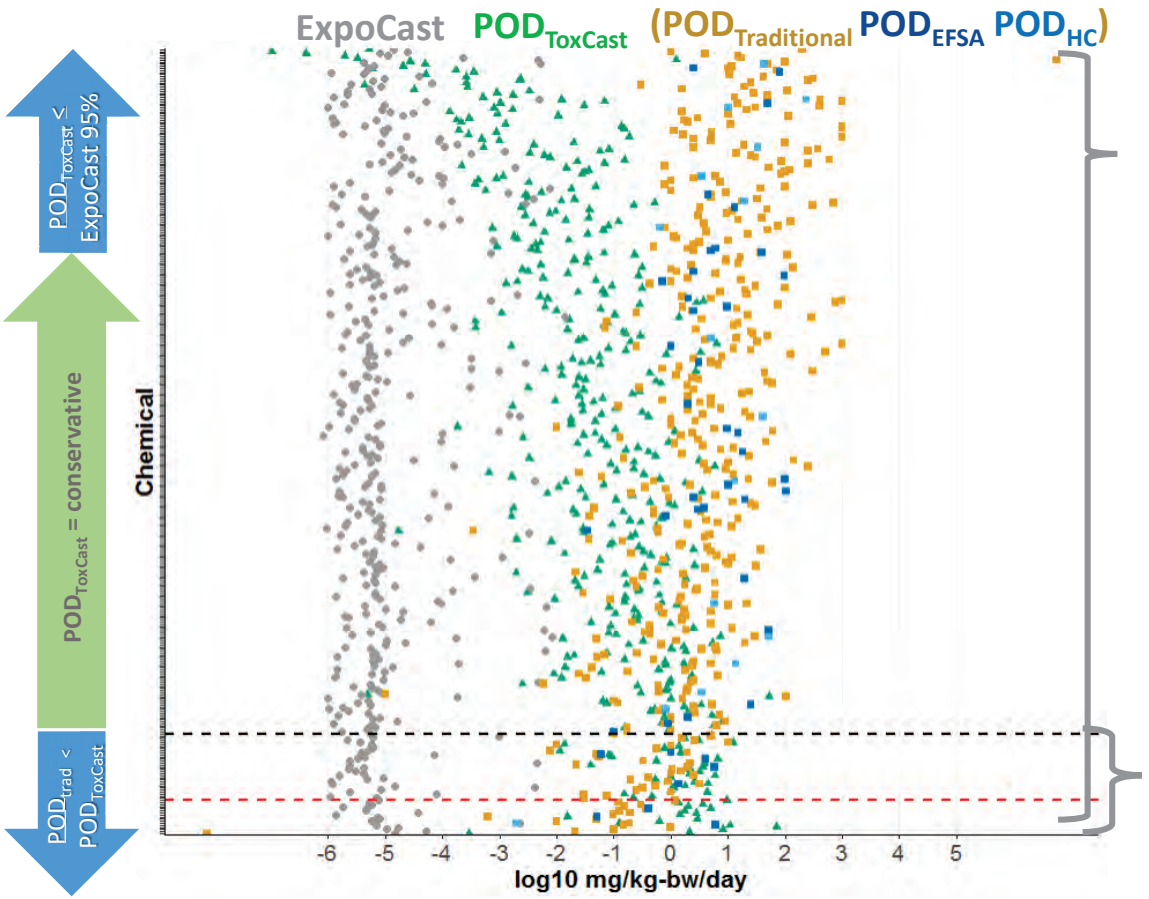








Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL



Total =

380 chemicals

httk, ToxCast data, and POD value(s) currently available

For ~91.3% of the chemicals, $POD_{ToxCast}$ was conservative.

(~130-fold with human HTTK

~40-fold with rat HTTK)

Missing an important component of biology?