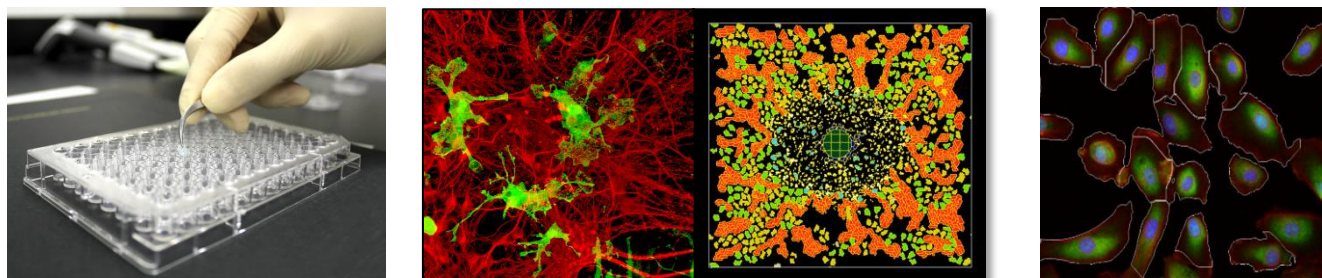


US EPA – A*STAR Partnership

*Accelerating Acceptance of Next-Generation Sciences and
Application to Regulatory Risk Assessment*



A*STAR Symposium

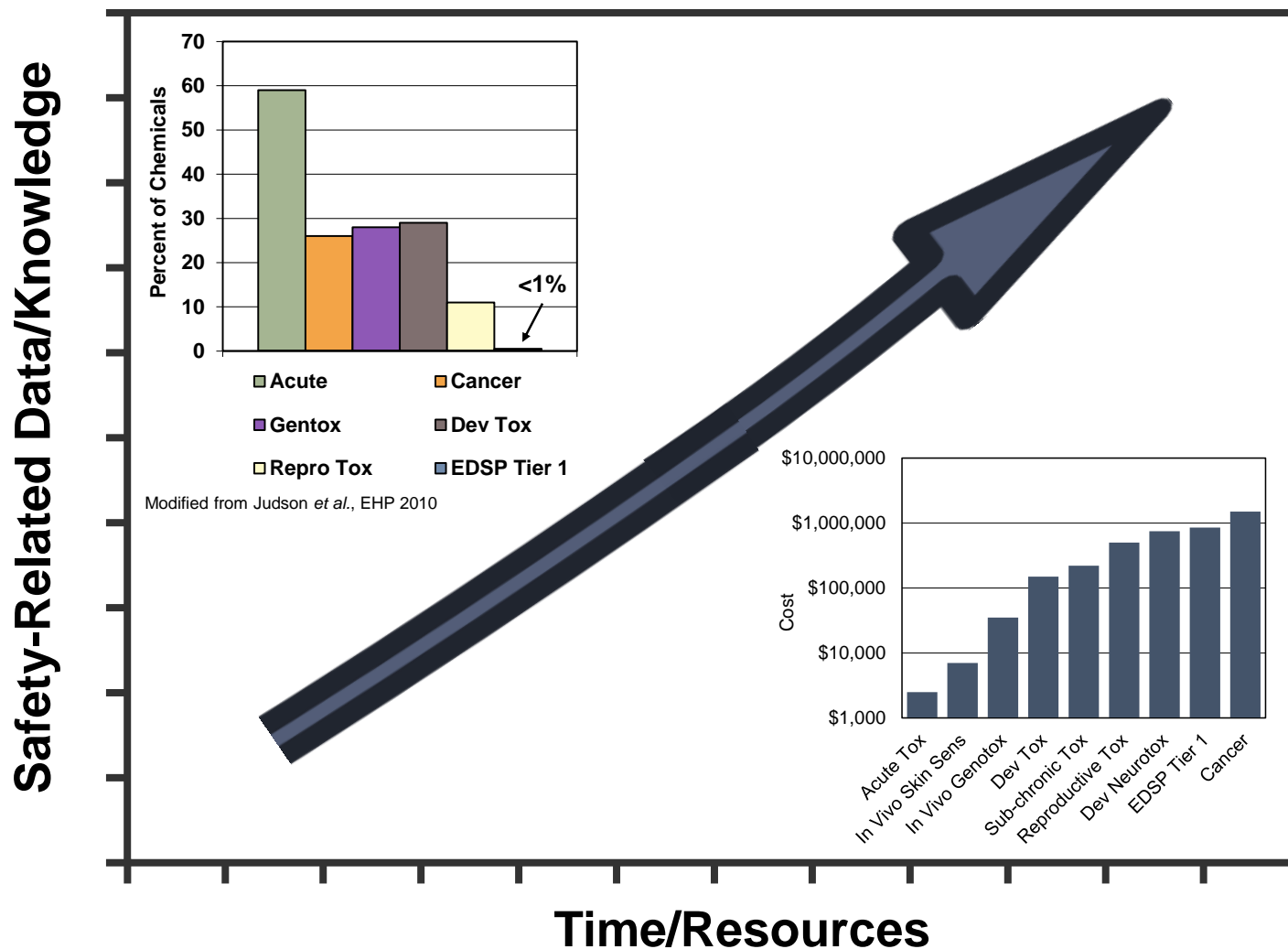
February 6, 2017

Russell S. Thomas*, Daniele Zink[†], Lit-Hsin Loo[†], Keith A. Houck*, Hanry Yu[†],
Imran Shah*, Florent Ginhoux[†], and Thomas B. Knudsen*

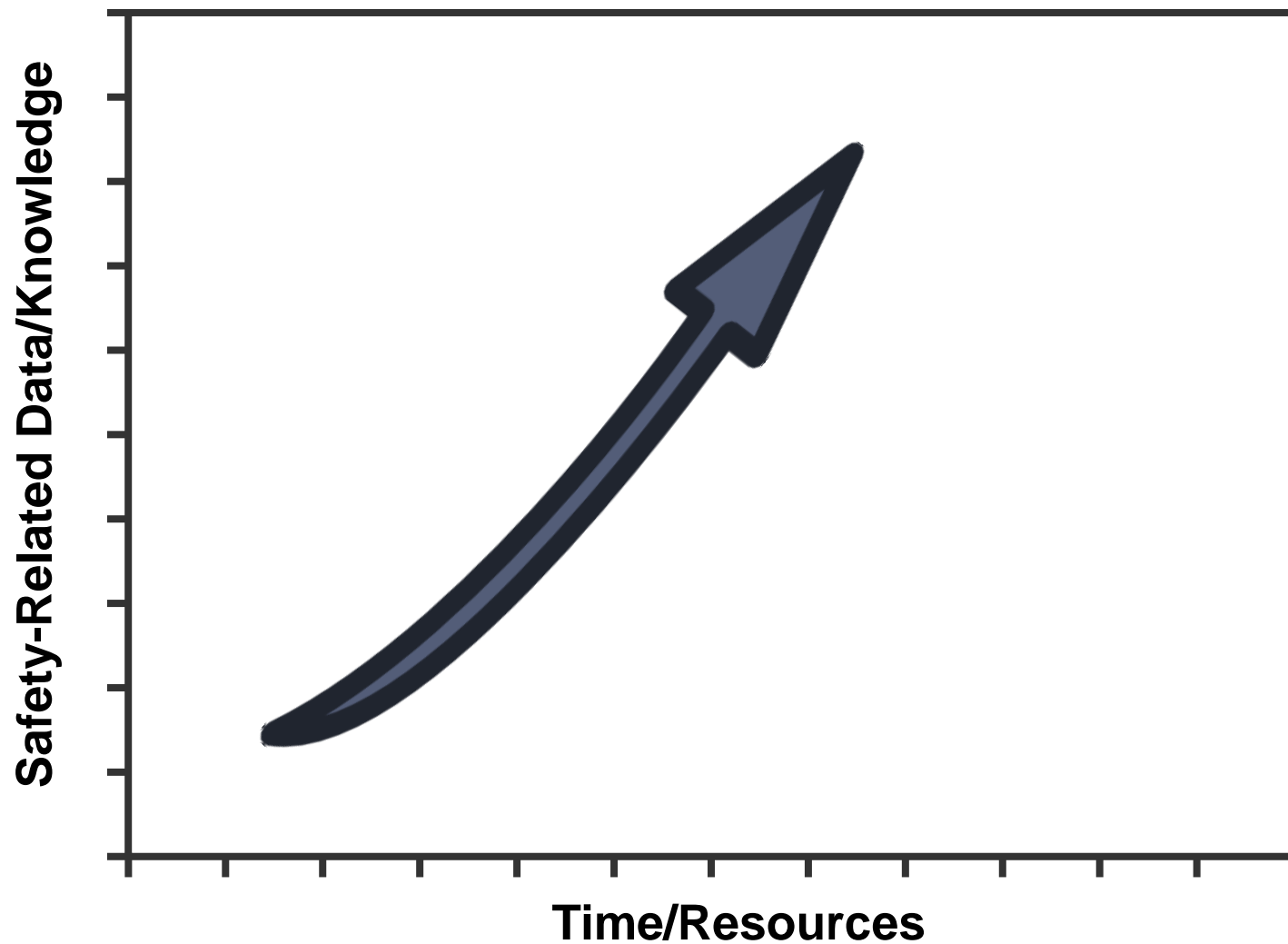
*U.S. Environmental Protection Agency

[†]Agency for Science, Technology, and Research (A*STAR)

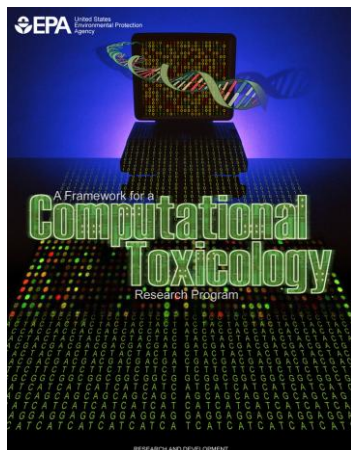
Evaluating Chemical Safety is a Difficult Balance...



New Strategies Required to Bend the Curve



U.S. National Center for Computational Toxicology



- National Center for Computational Toxicology established in 2005 to integrate and apply advanced technologies and computational methods to more efficiently and economically assess the potential toxicity and exposure to these tens of thousands of data poor chemicals
- Currently staffed by 32 federal and 35 non-federal employees
- Works within EPA's Chemical Safety for Sustainability National Program



Multi-Component Program to Address Challenge

ToxCast

Cheminformatics/
Chemical Library

ExpoCast

ToxRefDB

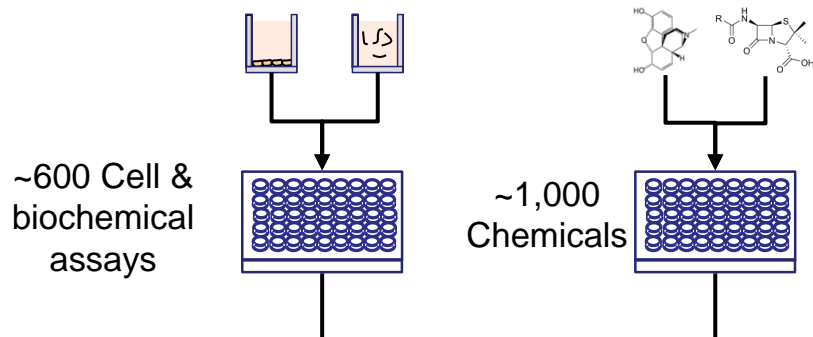
Virtual
Tissues

HT
Toxicokinetics

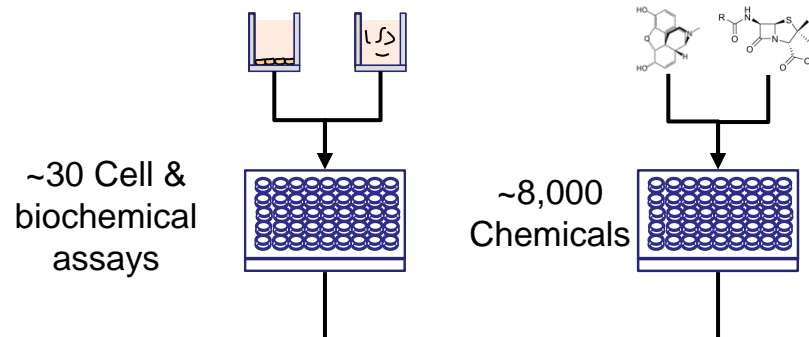


HTS to Identify Potential Mechanisms of Toxicity

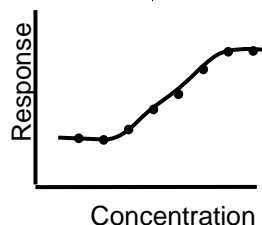
ToxCast



Tox21



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



ToxCast Contains Diverse Selection of Assays, But...

Assay Provider

ACEA
Aprelica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (PerkinElmer)
Odyssey Technologies
Vala Sciences

Biological Response

cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding

Target Family

response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases

Assay Design

viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Current assay portfolio has a limited number of
3D/organotypic assays or assays to predict kidney toxicity

Readout Type

single
multiplexed
multiparametric

Cell Format

cell free
cell lines
primary cells
complex cultures
free embryos

Species

human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

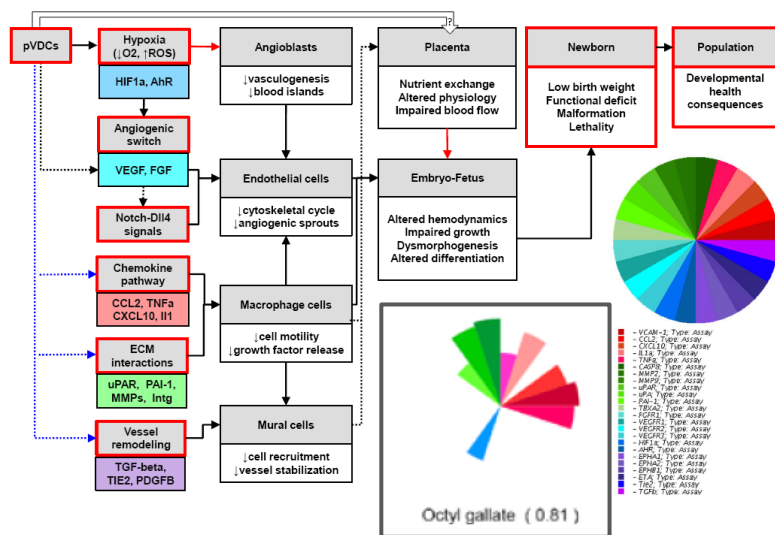
Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology

qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arraysan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

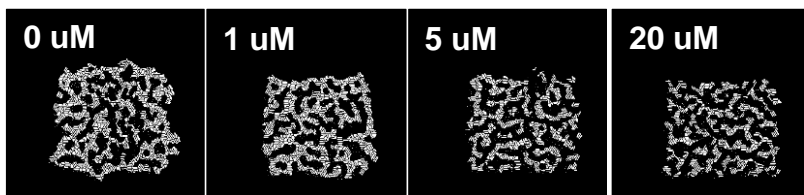
Virtual Tissue Modeling to Integrate Experimental Data

AOP for Developmental Vascular Disruption



Kleinstreuer *et al.*, PLoS Comp Bio, 2013

Model Simulations of Dev Vascular Disruption

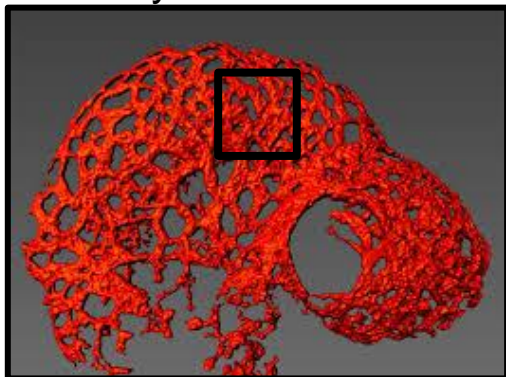


T. Knudsen

- Adverse outcome pathways (AOPs) developed for a broad range of endpoints (early focus on development)
- Computational models developed for each AOP using ToxCast data to parameterize models
- Validate model results with orthogonal organotypic assays and reference chemicals
- Computational modeling used to organize knowledge and test assumptions
- Evaluate impact of chemical perturbations on shape of the dose response curve and predict potential sensitive populations

Collaborating with A*STAR on Modeling BBB Formation

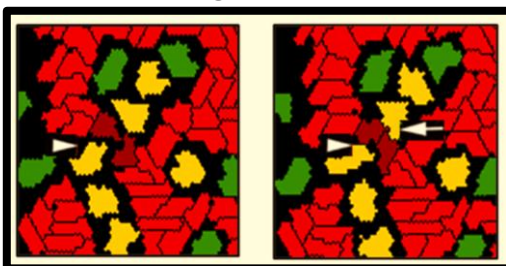
Embryonic vasculature








Cell field

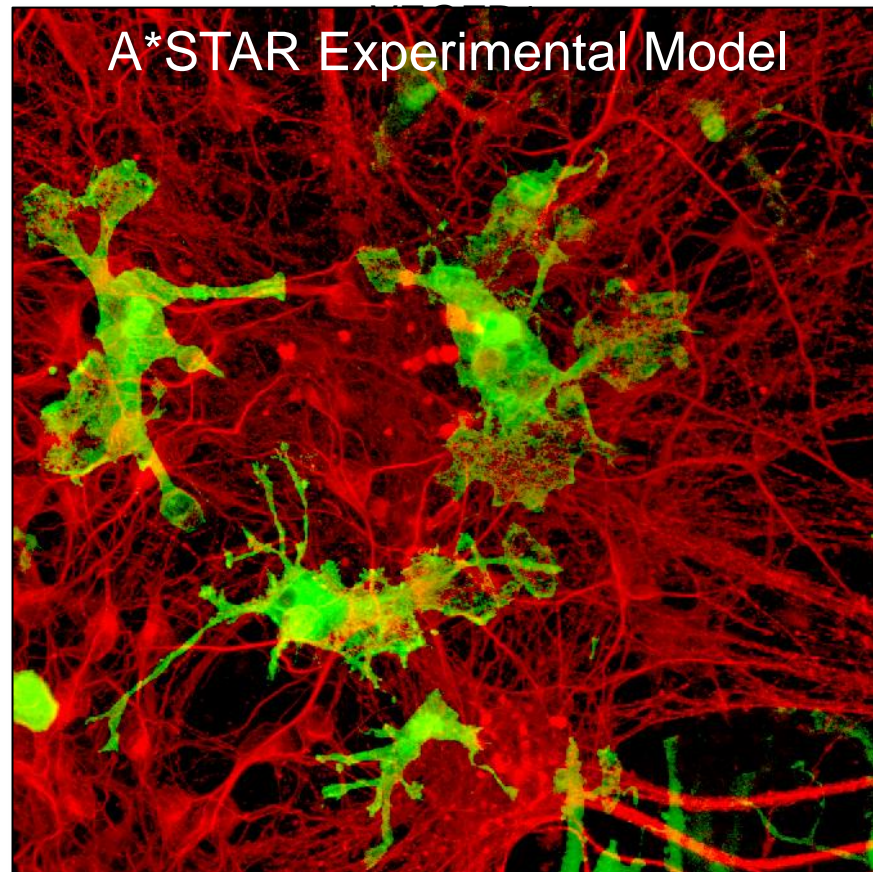


Stitching the network



- | | |
|--|---|
|  Endothelial (Tip) |  Pia |
|  Endothelial (Stalk) |  Ventricle |
|  Microglia | |

A*STAR Experimental Model



Tom Knudsen (EPA)
Todd Zurlinden (EPA)
Kate Saili (EPA)

*Florent Ginhoux (A*STAR)*
*Aymeric Silvain (A*STAR)*

Slides from Daniele Zink and Lit-Hsin Loo

Slides from Hanry Yu

An International Partnership for Predictive Toxicology

