Toxicological Tipping Points & Cellular Stress

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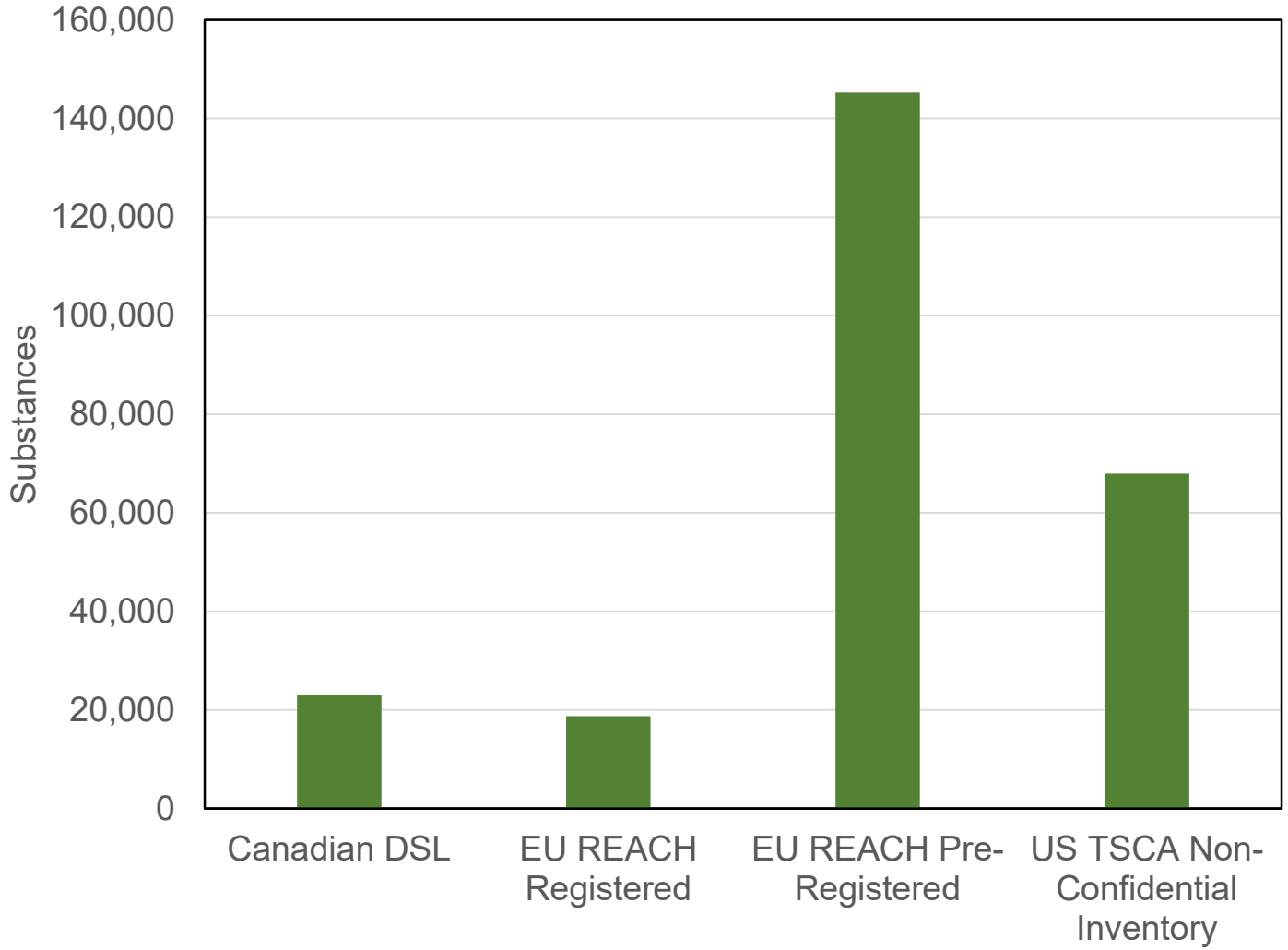
Imran Shah
National Center for Computational Toxicology

Large number of chemicals in commerce



Lack of Toxicity Data

Toxicity Testing

Strategies to Determine Needs and Priorities

Steering Committee on Identification of Toxic and Potentially Toxic Chemicals for Consideration by the National Toxicology Program

Board on Toxicology and Environmental Health Hazards

Commission on Life Sciences

National Research Council

- Major challenge is too many chemicals and not enough data
- Total # chemicals = 65,725
- Chemicals with no toxicity data of any kind = ~46,000

NATIONAL ACADEMY PRESS
Washington, D.C. 1984

The Toxicity Data Landscape for Environmental Chemicals

Richard Judson,[1] Ann Richard,[1] David J. Dix,[1] Keith Houck,[1] Matthew Martin,[1] Robert Kavlock,[1] Vicki Dellarco,[2] Tala Henry,[3] Todd Holderman,[3] Philip Sayre,[3] Shirlee Tan,[4] Thomas Carpenter,[5] and Edwin Smith[6]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Office of Pesticide Programs, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Arlington, Virginia, USA; [3]Office of Pollution Prevention and Toxics and [4]Office of Science Coordination and Policy, Office of Prevention, Pesticides, and Toxic Substances, U.S. Environmental Protection Agency, Washington, DC, USA; [5]Office of Water, Office of Ground Water and Drinking Water, U.S. Environmental Protection Agency, Washington, DC, USA; [6]Great Lakes National Program Office, U.S. Environmental Protection Agency, Chicago, Illinois, USA

OBJECTIVE: Thousands of chemicals are in common use, but only a portion of them have undergone significant toxicologic evaluation, leading to the need to prioritize the remainder for targeted testing. To address this issue, the U.S. Environmental Protection Agency (EPA) and other organizations are developing chemical screening and prioritization programs. As part of these efforts, it is important to catalog, from widely dispersed sources, the toxicology information that is available. The main objective of this analysis is to define a list of environmental chemicals that are candidates for the U.S. EPA screening and prioritization process, and to catalog the available toxicology information.

DATA SOURCES: We are developing ACToR (Aggregated Computational Toxicology Resource), which combines information for hundreds of thousands of chemicals from > 200 public sources, including the U.S. EPA, National Institutes of Health, Food and Drug Administration, corresponding agencies in Canada, Europe, and Japan, and academic sources.

DATA EXTRACTION: ACToR contains chemical structure information; physical–chemical properties; *in vitro* assay data; tabular *in vivo* data; summary toxicology calls (e.g., a statement that a chemical is considered to be a human carcinogen); and links to online toxicology summaries. Here, we use data from ACToR to assess the toxicity data landscape for environmental chemicals.

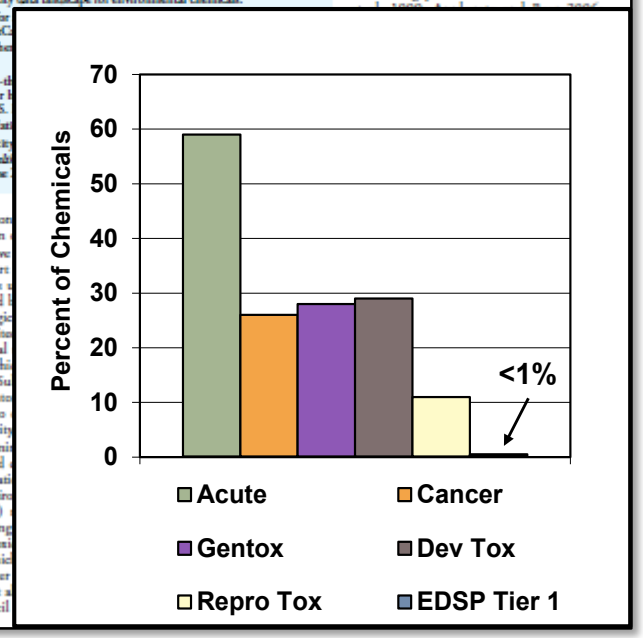
DATA SYNTHESIS: We show results for analysis as part of the U.S. EPA ToxC and medium-production-volume che water contaminants.

CONCLUSIONS: Approximately two-th maries available. About one-quarter h evaluation database such as the U.S. Risk Information System, and the Nati

KEY WORDS: ACToR, carcinogenicity reproductive, toxicity. *Environ Healt* available via *http://dx.doi.org/* [Online

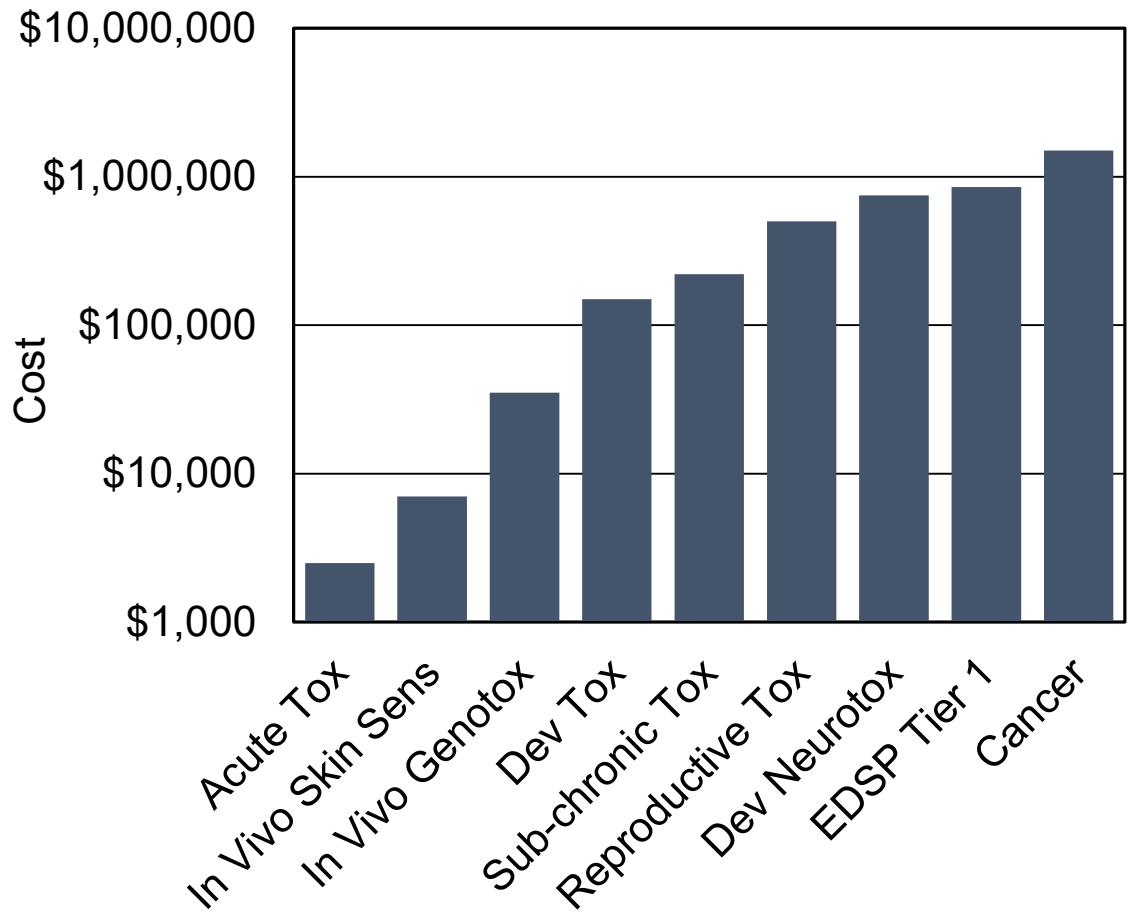
The U.S. Environmental Protection (EPA) has a significant interest in ing more efficient and informative determination approaches in part of the large number of chemicals jurisdiction. Ultimately, it would b ficial to characterize the toxicologic of all chemicals in use in the Unite However, the size of this chemical [in excess of 75,000 chemicals, whi estimated number in the Toxic Su Control Act (TSCA 1976) invento EPA 2004b) makes this goal too using current approaches to toxicity terization that rely on extensive ani ing, cost millions of dollars, and 2–3 years per chemical. The Internati Sciences Institute/Health and Enviro Sciences Institute (ILSI/HESI) released several reports describing focused, tier-based approach for toxi ing of agricultural chemicals, whic ultimately lead to the use of fewer (Barton et al. 2006; Carmichael et a The National Research Council

Howard 2006). The European Union's Registration, Evaluation, and Authorization of Chemicals (REACH) program has recently released its first set of registered substances, which contains > 140,000 entries (REACH 2008). The exact number of chemicals in use is, in a sense, unknowable because it depends on where one sets the threshold of use and because use changes over time. The major point is that the number is relatively large and that only a relatively small subset of these chemicals have been sufficiently well characterized for their potential to cause human or ecologic toxicity to support regulatory action. This "data gap" is well documented (Allanou



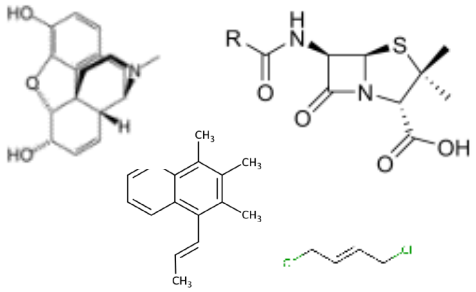
Modified from Judson *et al.*, EHP 2009

Costs of Traditional Toxicity Testing



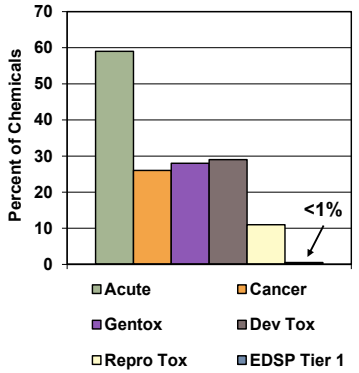
How can high-throughput approaches help?

Number of Chemicals /Combinations



Comprehensive Tox Evaluation

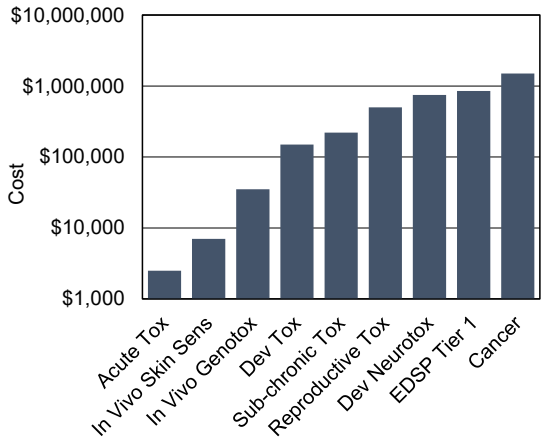
Limited Data



Modified from Judson *et al.*, EHP 2009

High-throughput Screening

Economics

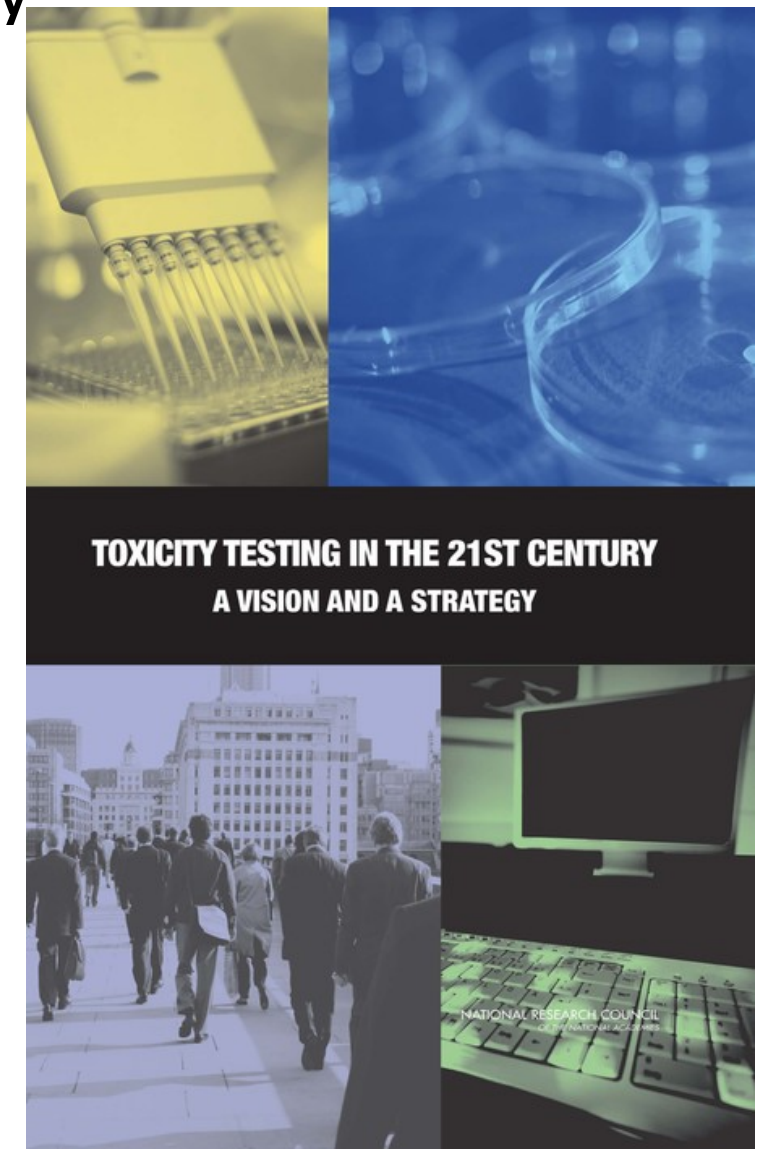


Toxicity Testing in the 21st Century

"Toxicity testing is approaching such a scientific pivot point. It is poised to take advantage of the revolutions in biology and biotechnology. Advances in toxicogenomics, bioinformatics, systems biology, epigenetics, and ***computational toxicology*** *could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods …."*

NRC 2007

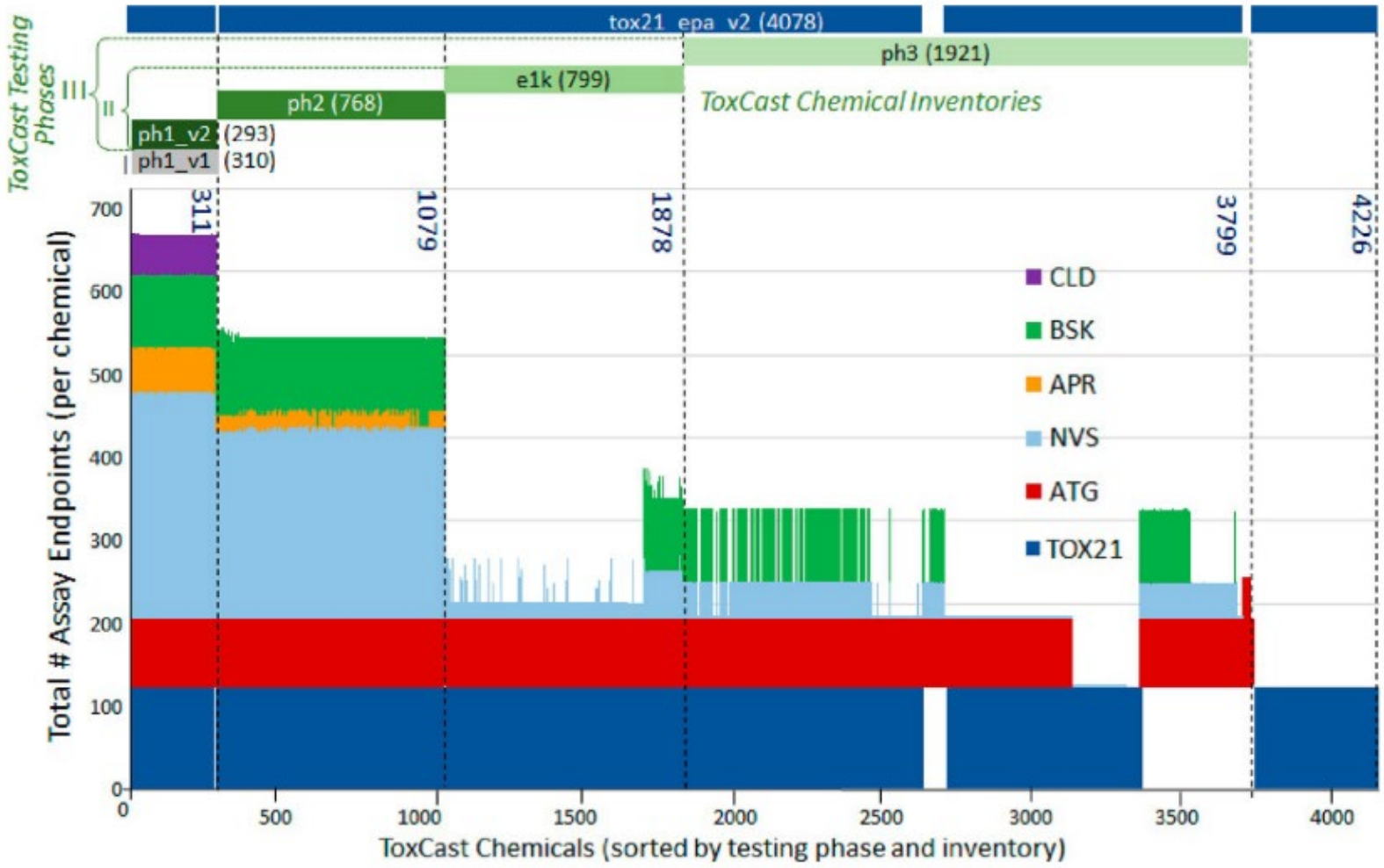
- Three key components:
  - **High-throughput screening (HTS)** using *in vitro* assays to evaluate the molecular and cellular effects of thousands of chemicals
  - **Toxicity pathways**, when sufficiently perturbed or beyond adaptive capacity, lead to adverse health outcomes
  - **Dose-response modeling and *in vitro* to *in vivo* extrapolation** to estimate risk



Computational Toxicology

Data Generation	**HTTK** ADME data for chemicals	**ToxCast** High-throughput screening (HTS), high-content imaging (HCI), transcriptomics	**OCM** Organotypic culture models	**ToxRefDB** Legacy animal testing data on all guideline testing studies used in risk assessment
Dose-response Modeling		**R/tcpl** Software tools and database to support diverse data sets produced by ToxCast		
Pathways		**Adverse Outcome Pathways (AOP)** Curated database of AOPs covering diverse MIEs and adverse outcomes		
Predictive Tools	**QSAR ADME** Machine learning to predict ADME	**QSAR & Bioactivity** Machine learning tools lever to predict molecular initiating events (MIEs), cellular effects and adverse outcomes		
	R/httk Cross-species/pop. PBTK modeling	**Virtual Tissue Models** Multi-scale agent-based and models of networks, cells, organs across predict dose-dependent effects of chemicals across life-stages		
Public Access	**CompTox Chemicals Dashboard comptox.epa.gov**			

ToxCast: 10 years later …



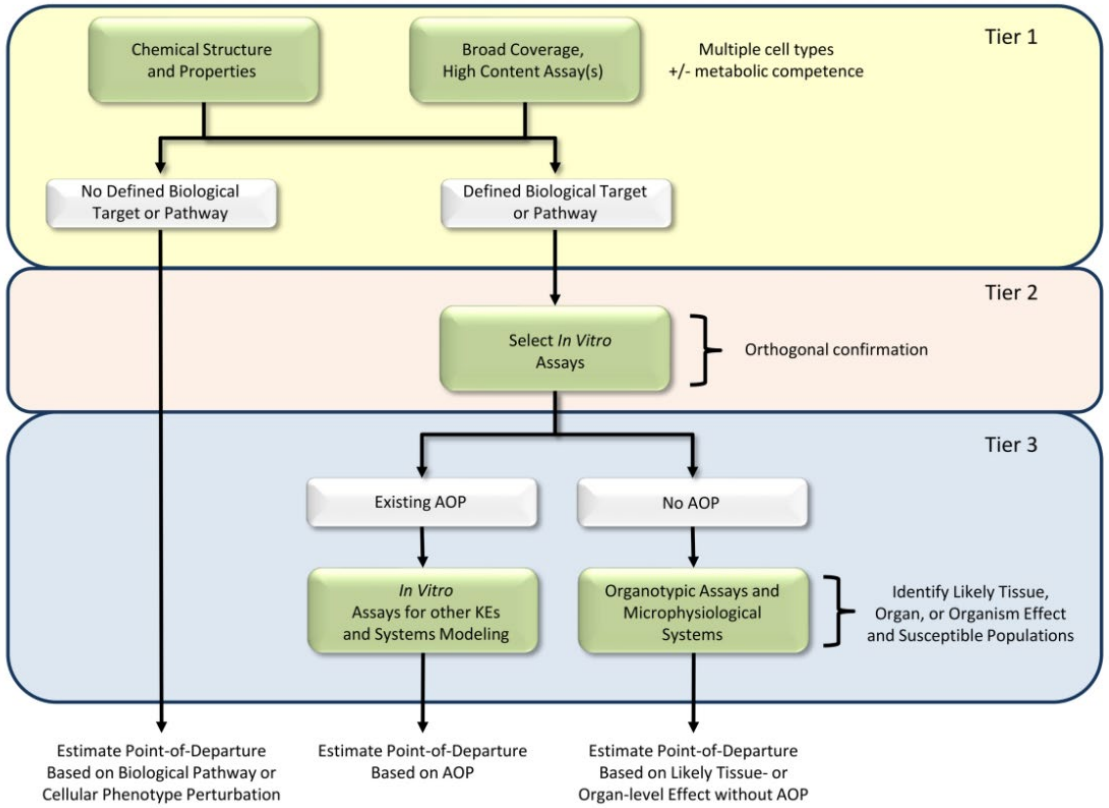
X chemicals
Y assays
Z genes
A cells
119 Publications

Richard et al, 2016

Computational Toxicology: Future

- Expanded biological coverage to screen thousands of additional chemicals
  - High-content imaging (HCI)
  - High-throughput transcriptomics (HTTr)
- Tiered-testing to determine whether …
  I. Chemicals are potent activators of specific molecular initiating events (MIEs). Use adverse outcome pathway (AOP) framework identify adverse outcomes (AO) associated with MIE. Estimate point of departure (POD) using additional *in vitro* data and systems modeling
  II. Chemicals are not potent but promiscuous. Estimate POD using pathway, cellular phenotype perturbations or other approach.
  III. Chemicals are inactive.
- Chemical potency values derived from *in vitro* studies are quantitatively extrapolated to *in vivo* doses using toxicokinetic modeling

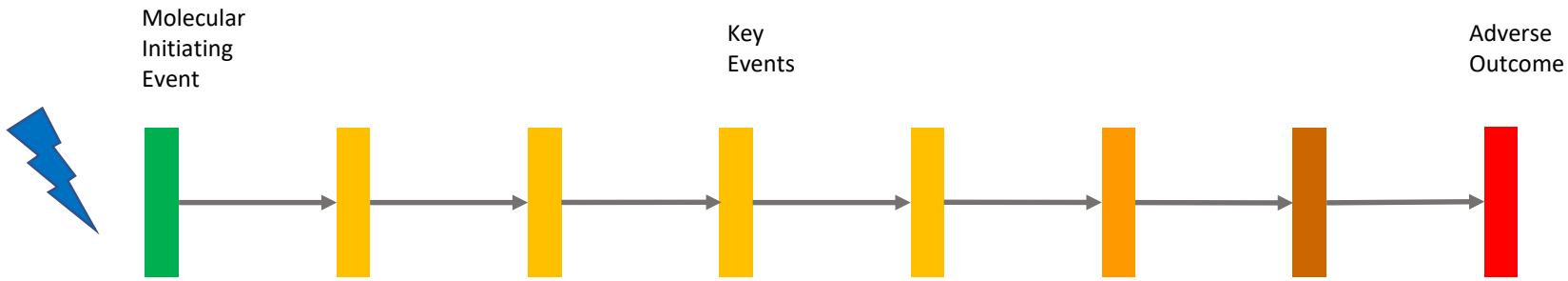
The next generation blueprint of computational toxicology at the U.S. Environmental Protection Agency



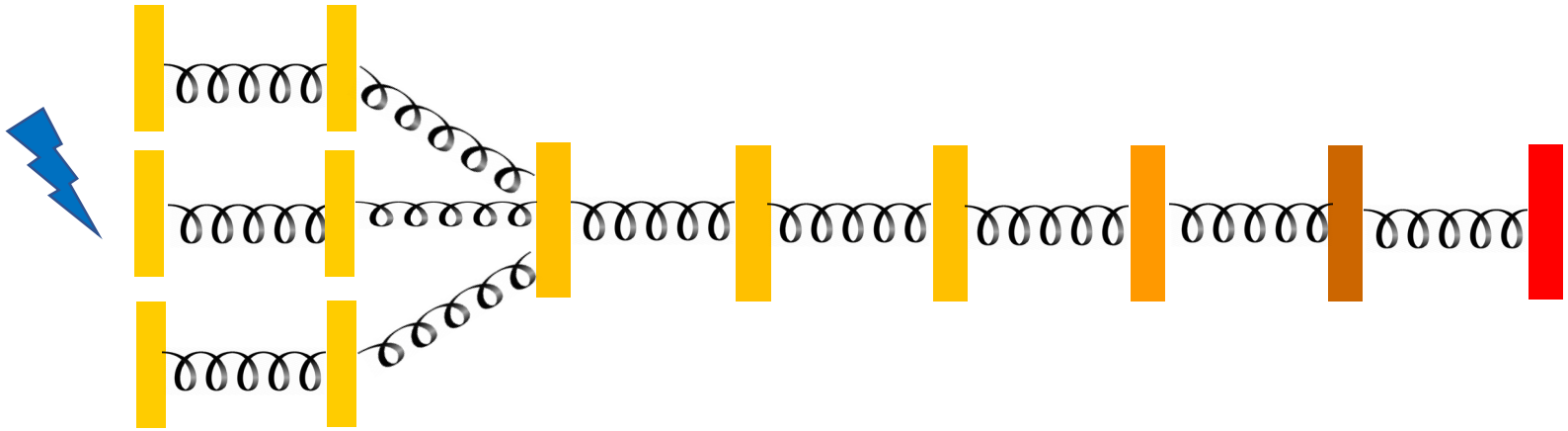
Thomas *et al*, 2019

How do chemical-induced perturbations propagate to adverse outcomes?

A. Activating MIE produces domino effect that results in adverse outcome (AOP)

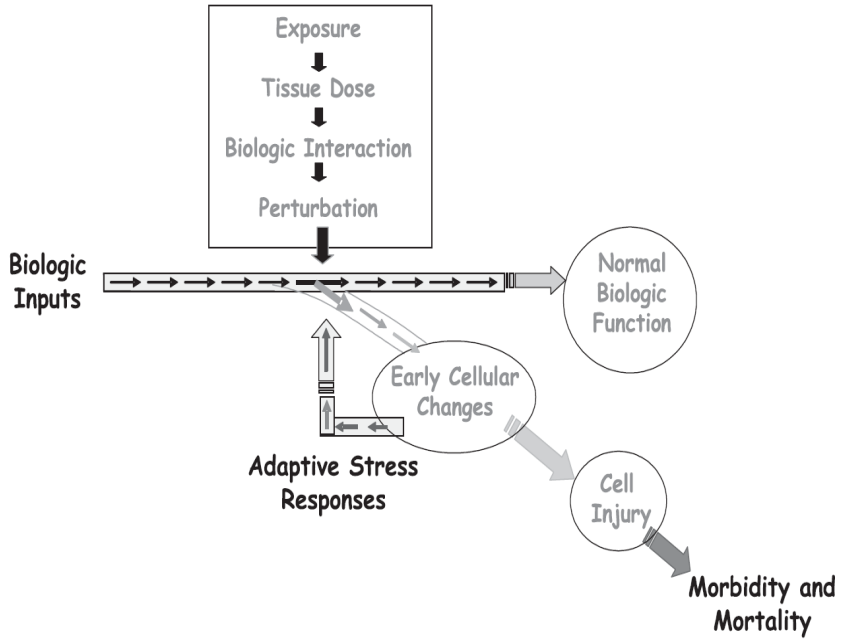


B. Multiple stress-response pathways are activated resulting in homeostatic adaptation. If perturbation exceeds “tipping point” then adverse outcome produced



Toxicological Tipping Points

- Biological systems are resilient and adapt to environmental perturbations
- Tipping points are dose-dependent transitions in the system from normal to abnormal functions
- Key questions:-
  1. What type of *in vitro* data are suitable for identifying tipping points?
  2. How can we used these data to define dose-dependent transitions?
  3. Can we use tipping points as POD for risk assessment?

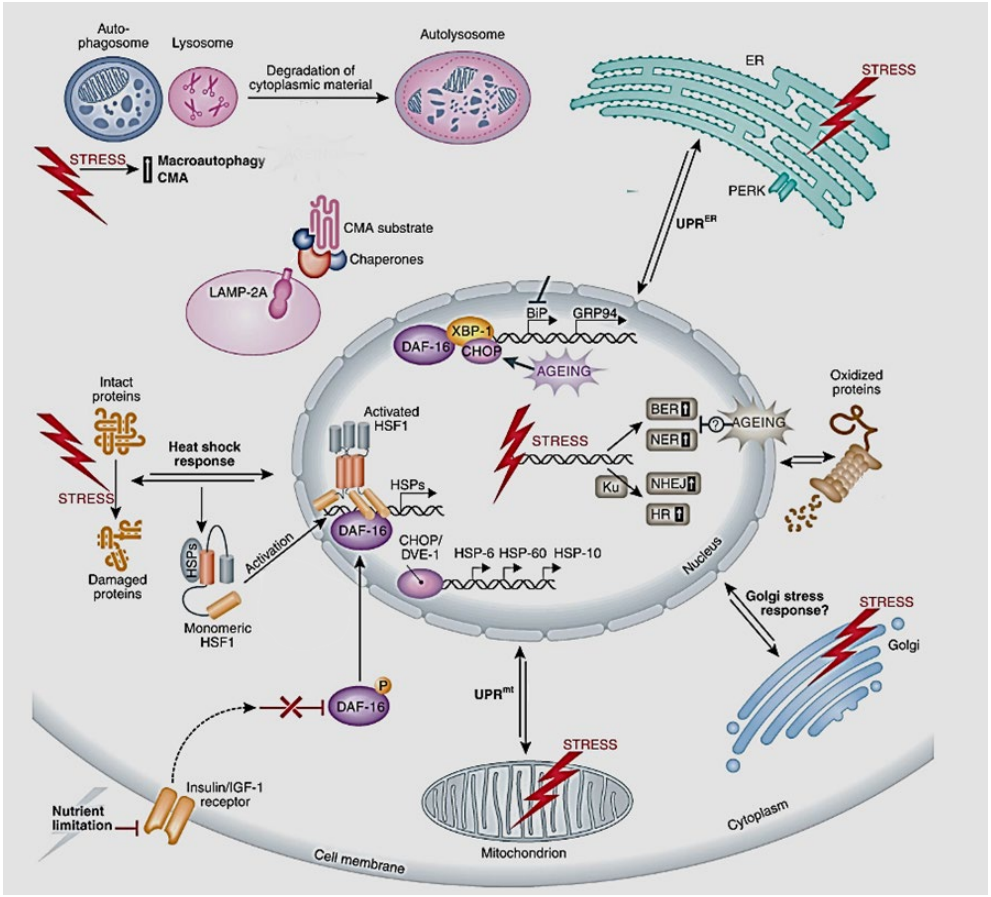


Krewski, Daniel, Daniel Acosta Jr, Melvin Andersen, Henry Anderson, John C Bailar 3rd, Kim Boekelheide, Robert Brent, et al. "Toxicity Testing in the 21st Century: a Vision and a Strategy." Journal of Toxicology and Environmental Health. Part B, Critical Reviews 13, no. 2–4 (February 2010): 51–138.

What type of data are suitable for analyzing tipping points?

Cellular stress responses

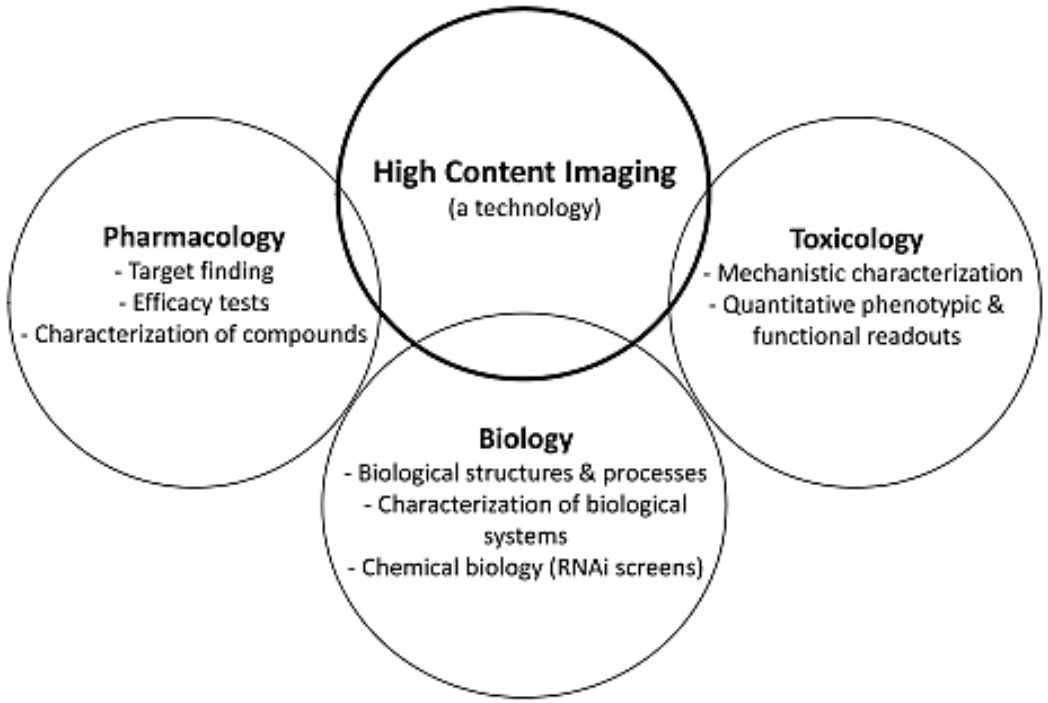
- Multiple stress-activated pathways are activated to counter chemical effects to maintain homeostasis:-
  - Heat-shock response
  - Ubiquitin-proteasome system
  - Endoplastmic reticulum (ER) stress
  - Mitochondrial stress
  - Lysosomal stress
  - DNA damage response
- There is cross-talk between stress-response pathways
- Overwhelming cellular adaptive capacity leads to cell death / autophagy

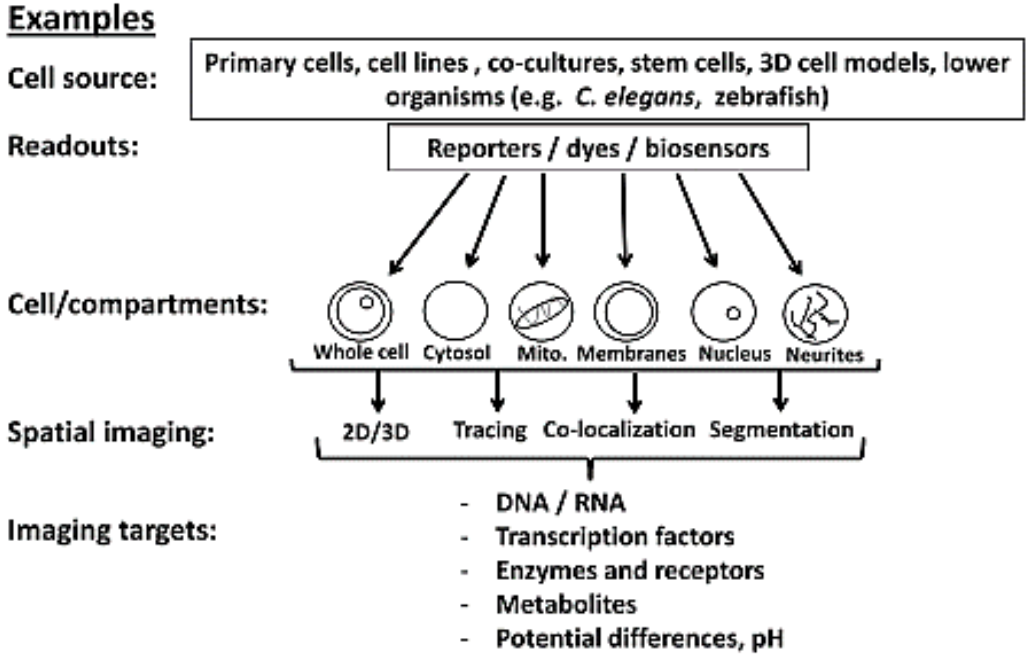


Kourtis & Tavernarakis, 2011

High Content Imaging (HCI)

A powerful technology to interrogate the phenotypic state of cells by analyzing single cell data





Vliet et al, 2014

Study Design: Stress responses using HCI

Cell model: Human hepatoma HepG2

Treatments:

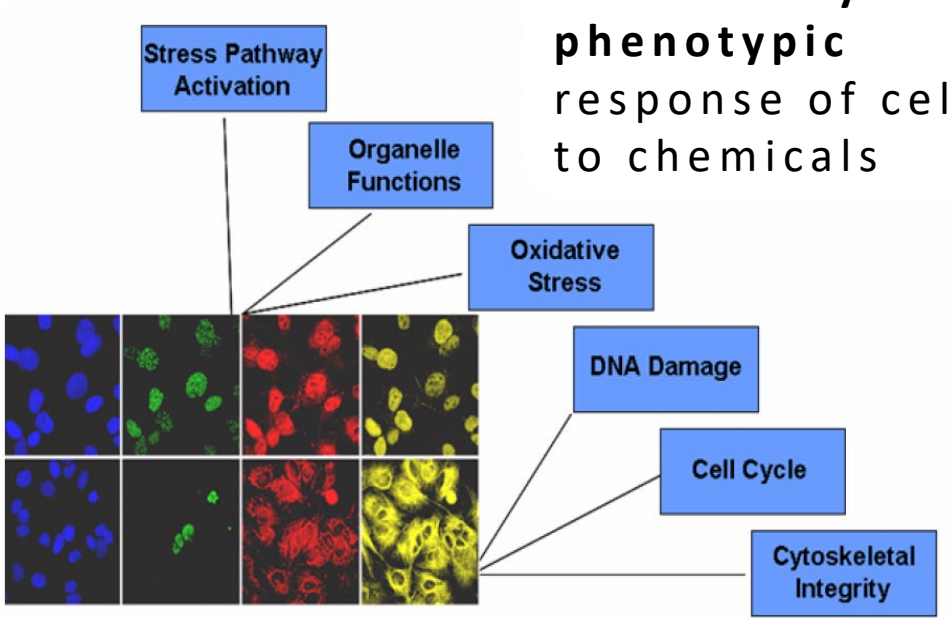
- Test chemicals: 978
- Controls: (-) DMSO; (+) CCCP, Taxol
- Conc: 0.39, 0.78, 1.56, 3.12, 6.24, 12.5, 25, 50, 100, 200 µM
- Duration: 1, 24 and 72 h.
- Reps: 2 on plate

Assay: High-content imaging (HCI)

- Stress Kinase (SK): c-Jun
- Oxidative Stress (OS): H2AX
- Mitochondrial function (MMP): MitoTracker Red
- Mitochondrial mass (MM): MitoTracker Red
- Mitotic arrest (MA): PH3
- Cytoskeletal stability (Mt): Tubulin
- Cell cycle arrest (CCA): Hoechst 33342
- Nuclear size (NS): Hoechst 33342
- Cell number (CN): Hoechst 33342

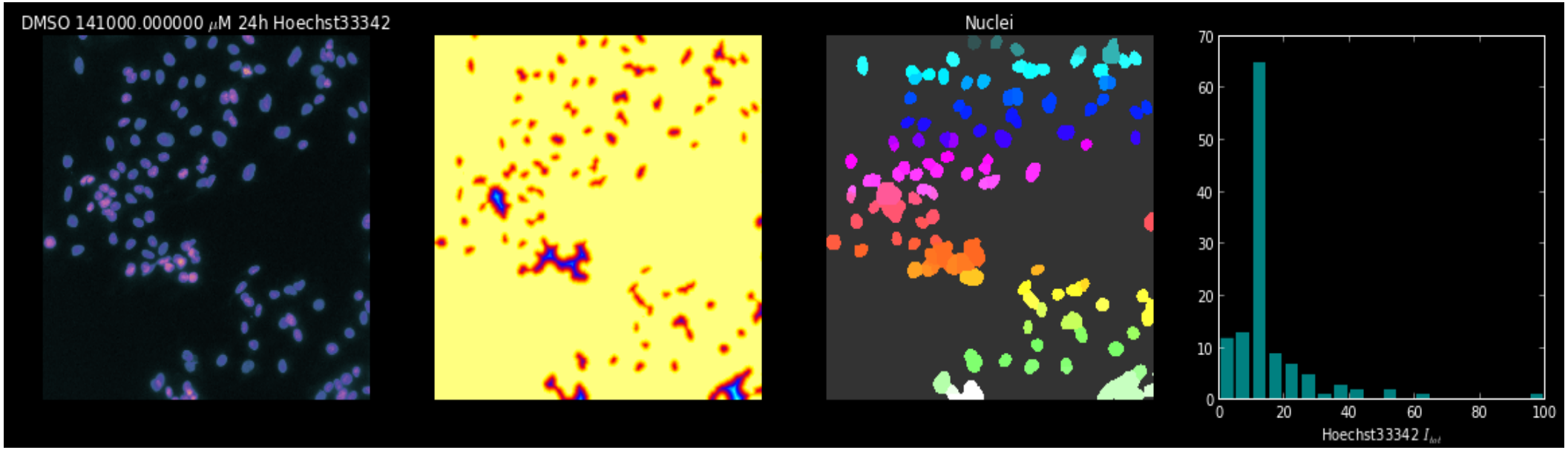
Data:

- ~400 plates
- ~100,000 wells
- ~2,400,000 images
- ~30,000 chemical-conc-time-response points



Measure Dynamic phenotypic response of cells to chemicals

Processing HCI data



Raw Image (Hoechst) → Intensity Analysis → Object Identification → Nuclear intensity distribution

Image analysis and cell level feature feature extraction conducting by Cyprotex Inc. (proprietary software)

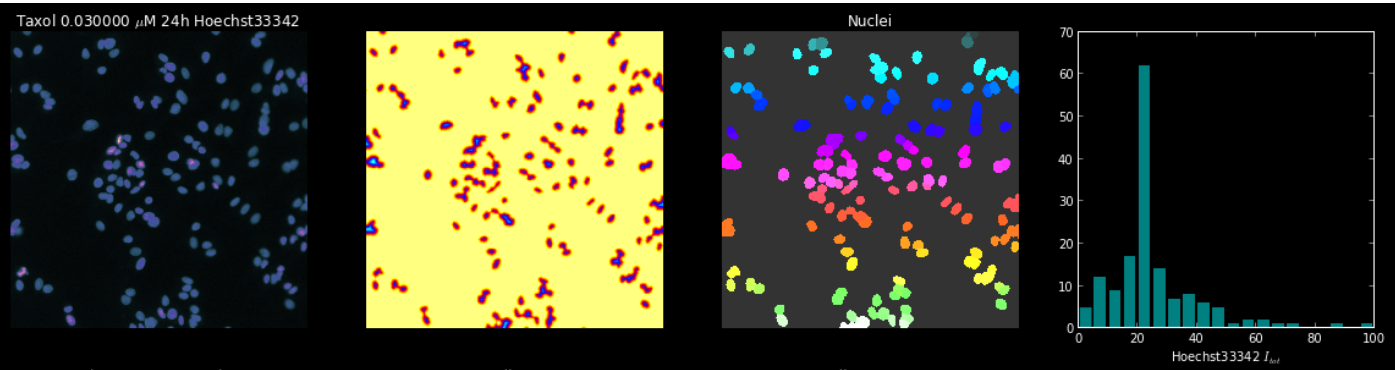
Taxol 0.03uM

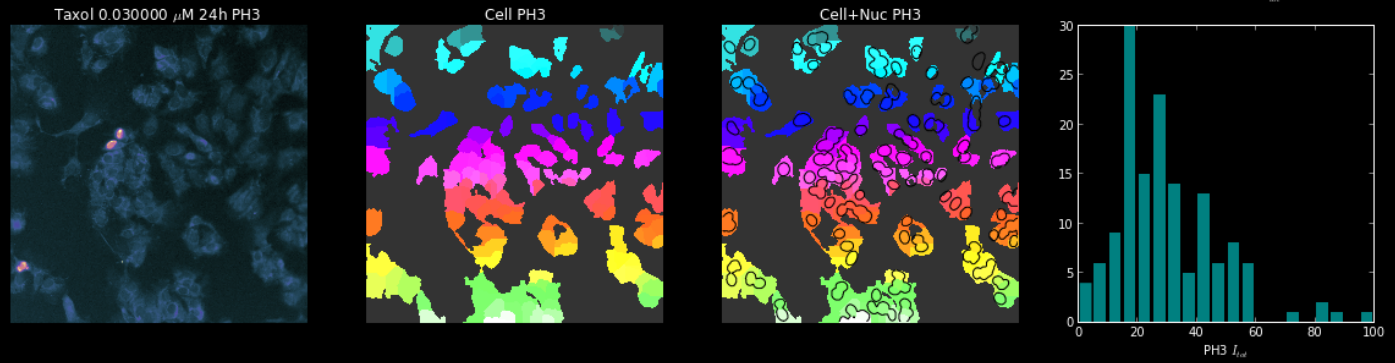
Hoechst33342

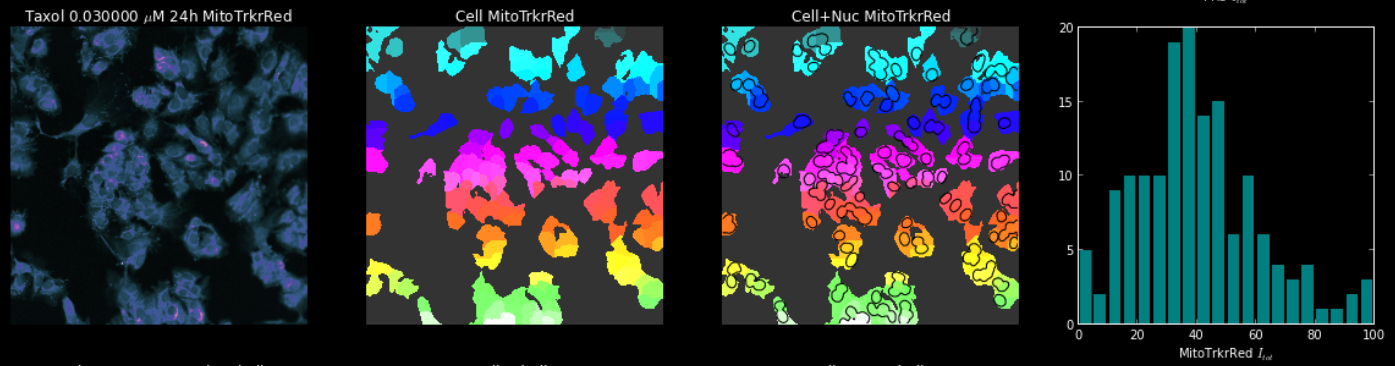
Phospho-Histone3

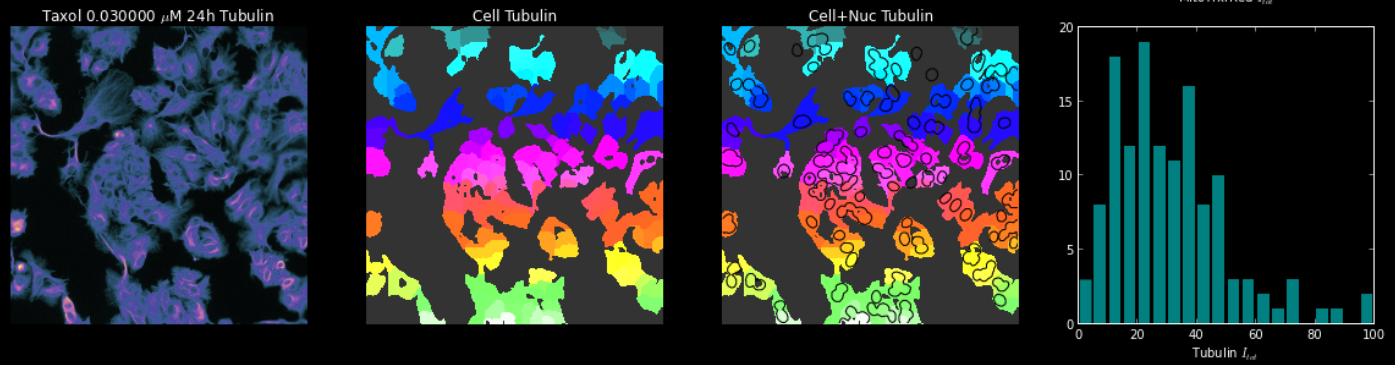
MitoTracker Red

Phospho-Tubulin









nuclear size (**NS**)
cell cycle arrest (**CCA**)
cell number (**CN**)

mitotic arrest (**MA**)

mitochondrial mass (**MM**)
mitochondrial membrane potential (**MMP**)

microtubules (Mt)

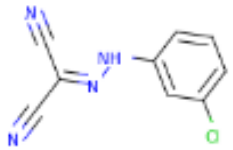
HCI to bioactivity profiles

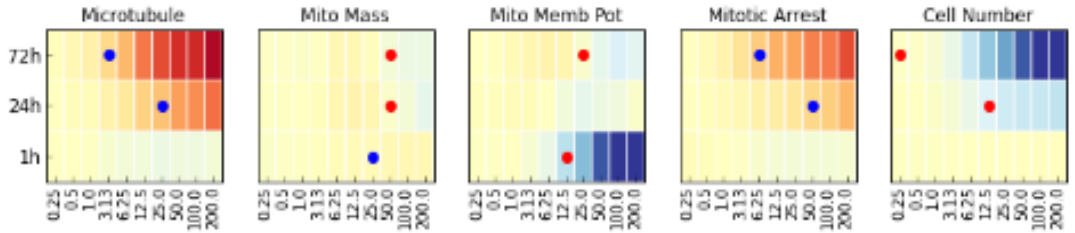
- Plate-level median-normalization – fold-change from background
- Report log2(fold change): decrease (BLUE), increase (RED) or no effect (YELLOW)
- Multi-dimensional bioactivity profile – “deviation from normal state” of HepG2 cells

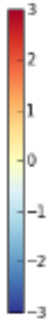
Shah et al, 2016

Mitochondrial disruptors

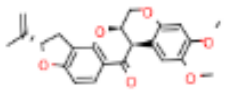
CCCP

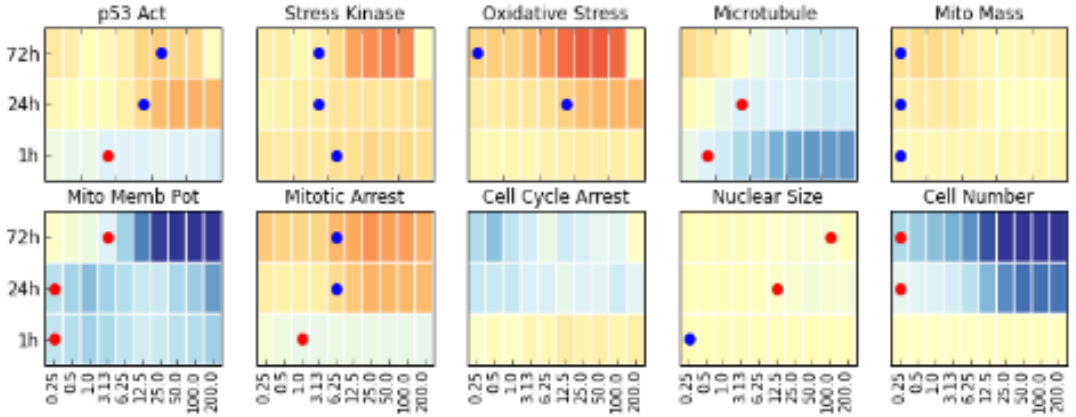






Rotenone





- log2(fold change): decrease (BLUE), increase (RED) or no effect (YELLOW)
- Bioactivity profile: “deviation from normal state” of HepG2 cells

Complex perturbations

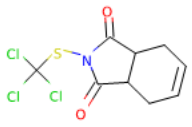
Fluazinam
79622-59-6
TX000777

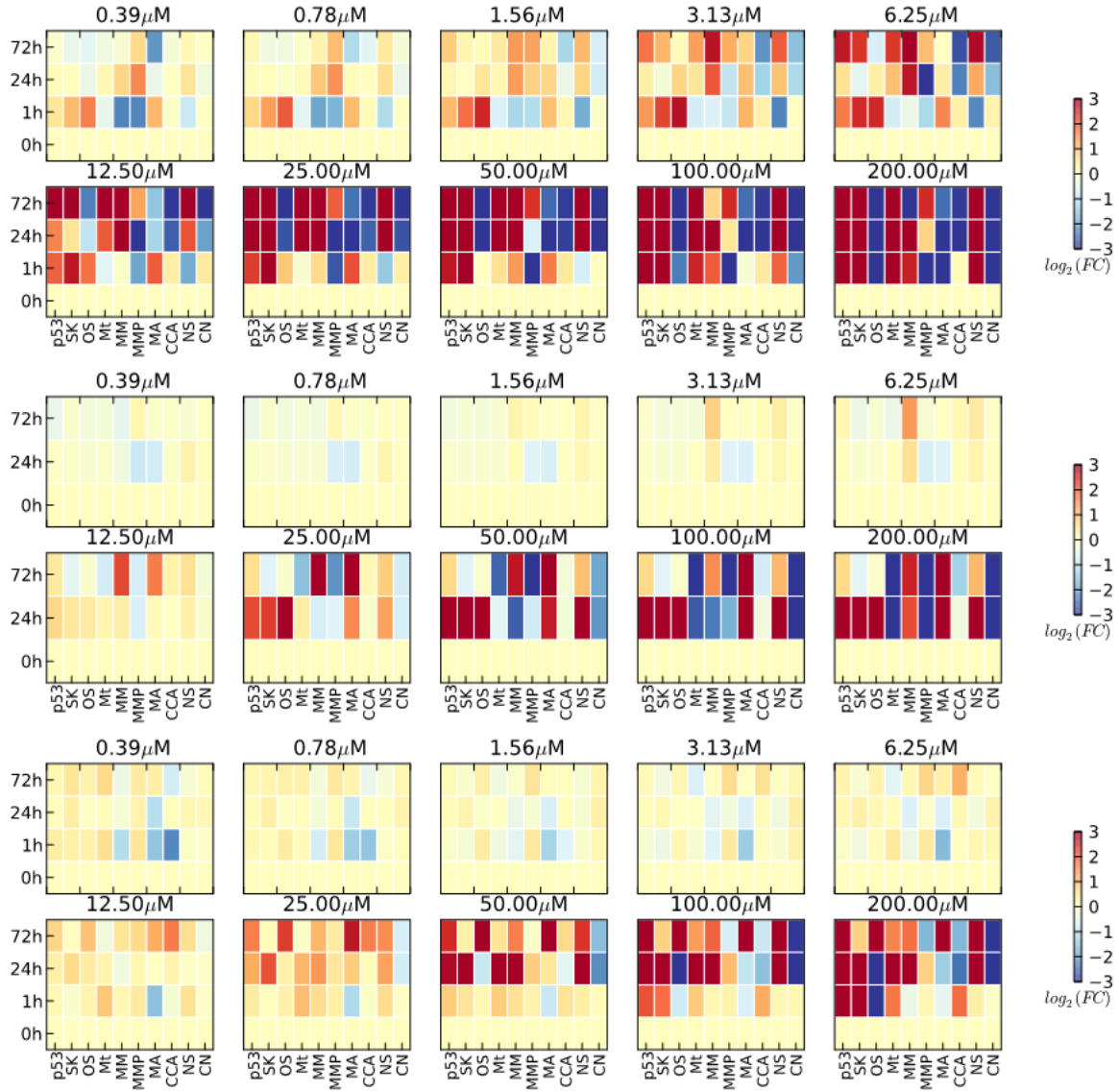
Mercuric chloride
7487-94-7
TX007182

Cl- Cl-

Hg+2

Captan
133-06-2
TX001434



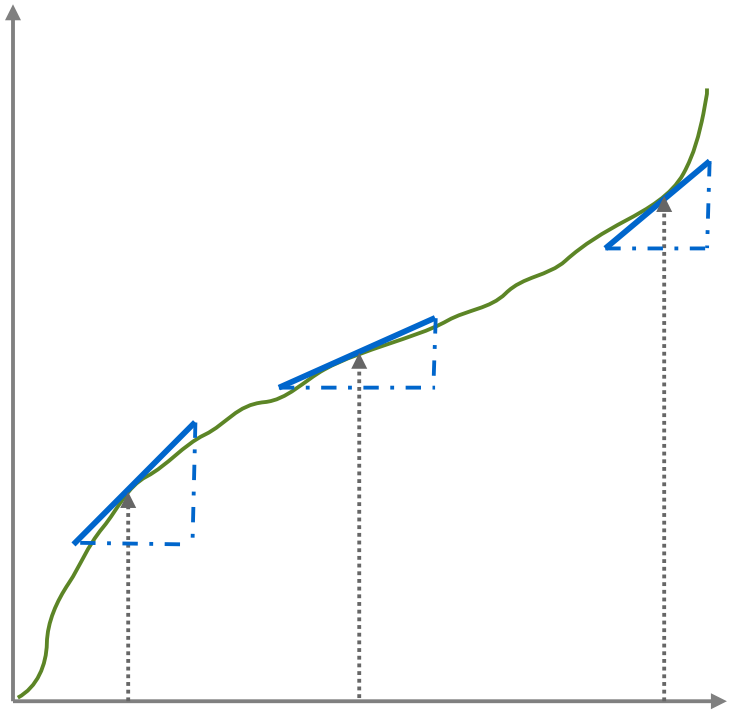


How can we use HCI data to identify tipping points?

Trajectories: system perturbation, |X|

Shah et al, 2016

Trajectories and pert. “velocity”



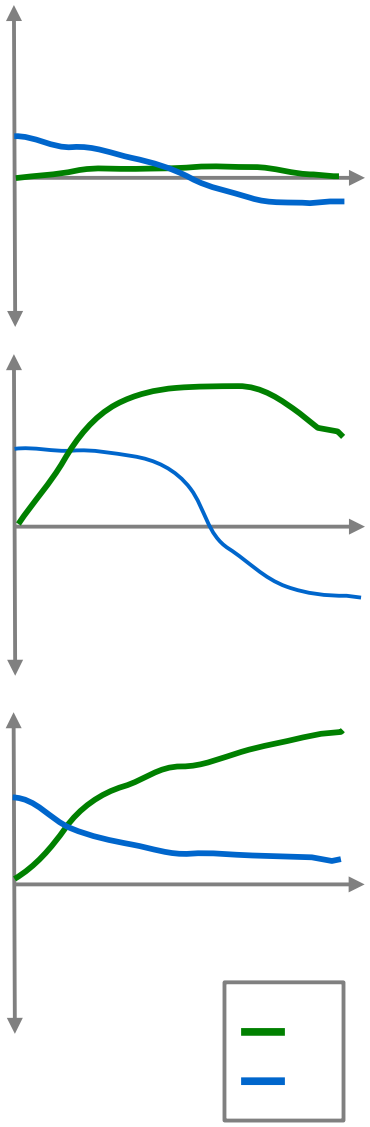
Velocity – rate of change of aggregate system perturbation

Trajectories: perturbation & velocity

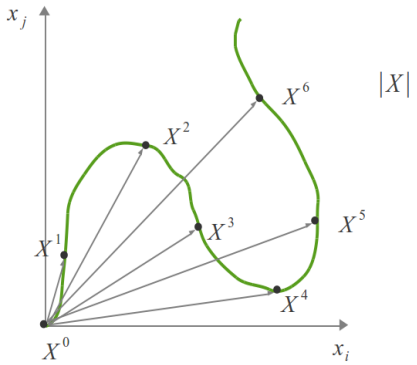
Some perturbation
Recovery

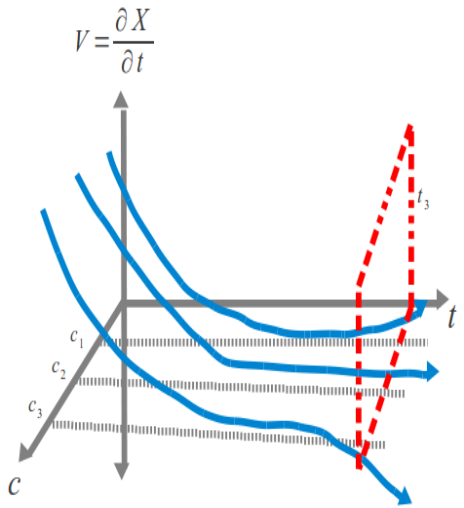
Adaptive stress response?
Partial recovery

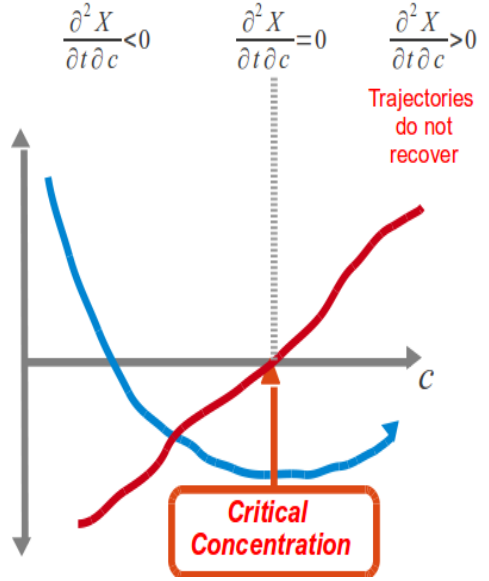
Adaptive capacity exceeded/
Cell injury?
No recovery



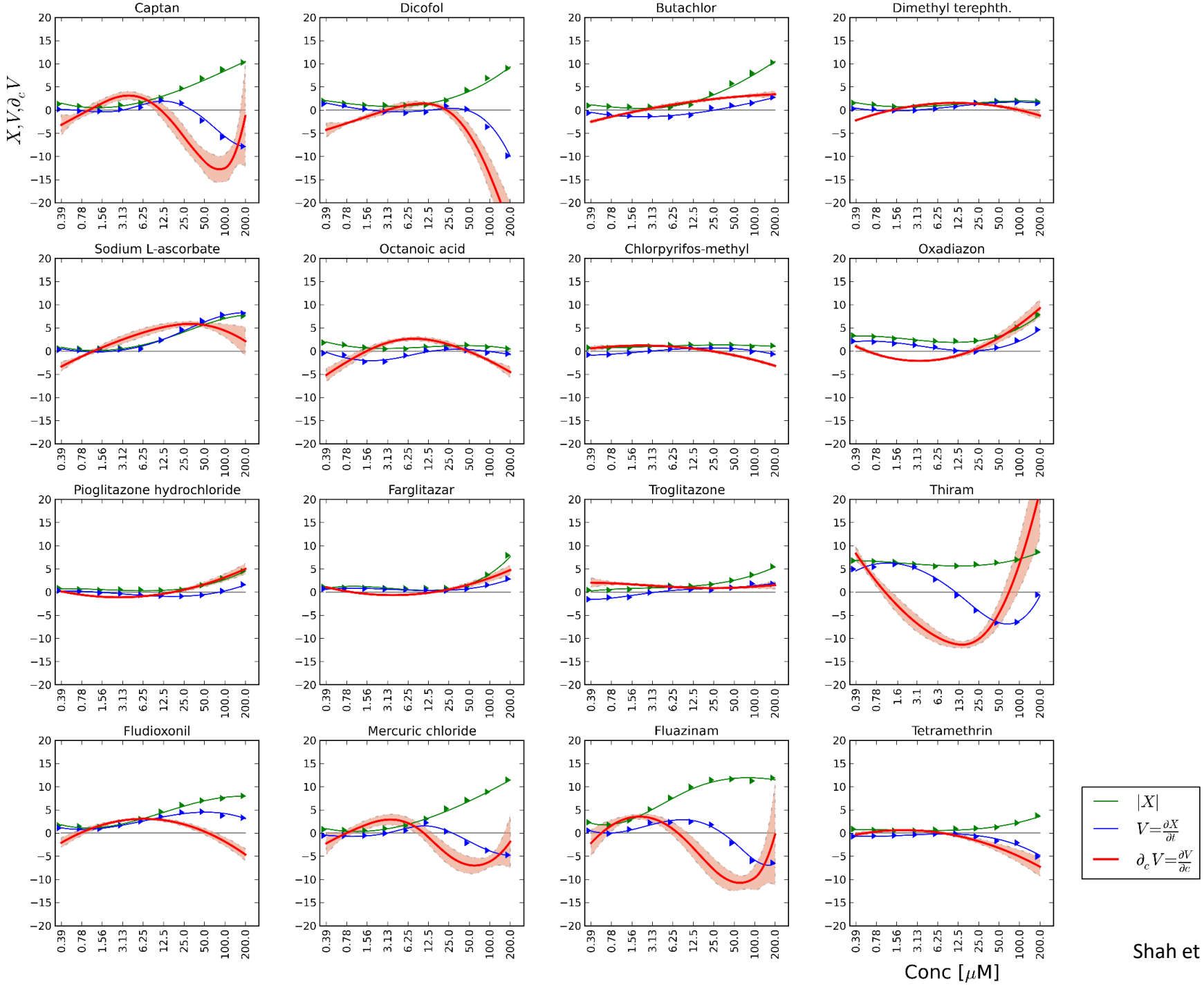
Tipping point for system recovery





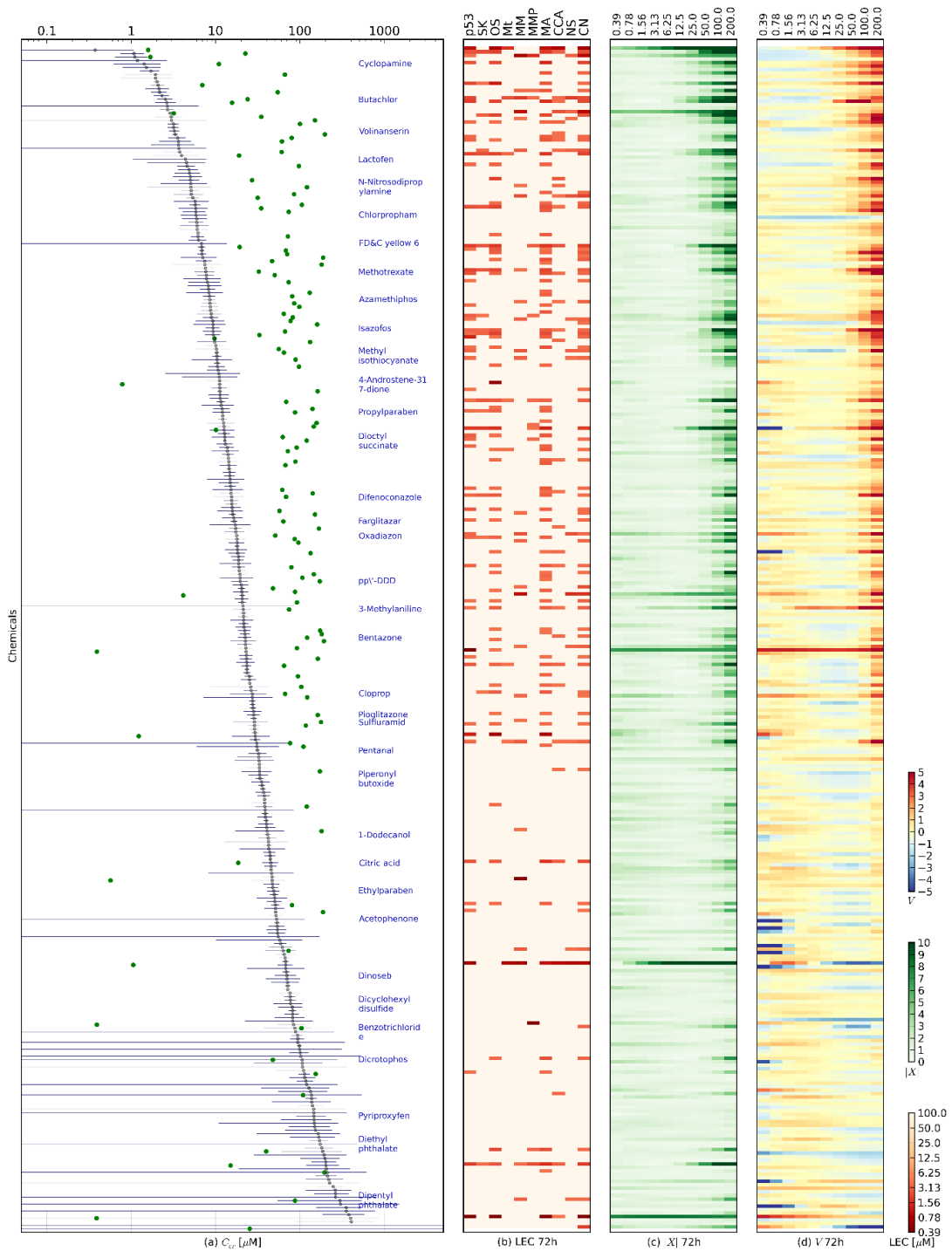


Shah et al, 2016



Shah et al, 2016

- ~340/967 chemicals had critical concentration
- ~170/967 chemicals always produced recovery
- Critical concentration ● more sensitive than cell loss



Shah et al, 2016

Research

A Section 508–conformant HTML version of this article is available at http://dx.doi.org/10.1289/ehp.1409029.

Using ToxCast™ Data to Reconstruct Dynamic Cell State Trajectories and Estimate Toxicological Points of Departure

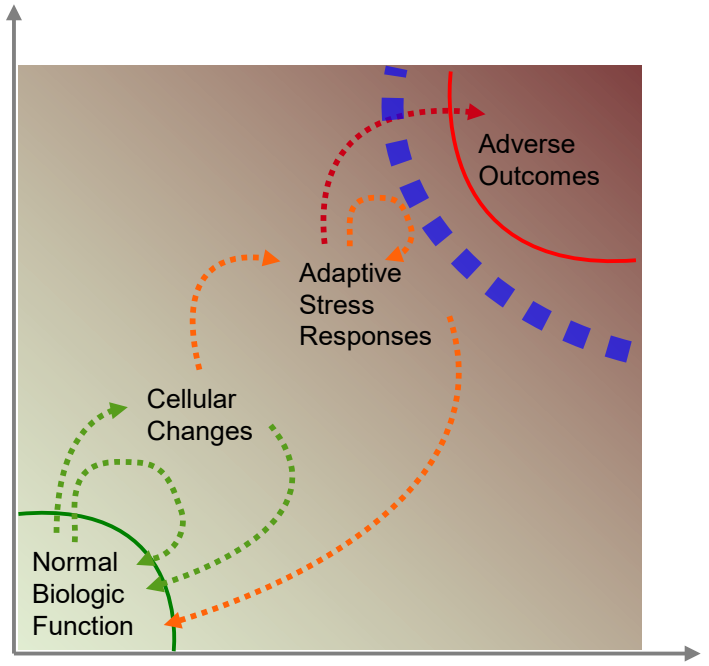
Imran Shah,[1] R. Woodrow Setzer,[1] John Jack,[2] Keith A. Houck,[1] Richard S. Judson,[1] Thomas B. Knudsen,[1] Jie Liu,[3] Matthew T. Martin,[1] David M. Reif,[4] Ann M. Richard,[1] Russell S. Thomas,[1] Kevin M. Crofton,[1] David J. Dix,[1] and Robert J. Kavlock[1]

[1]National Center for Computational Toxicology, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina, USA; [2]Department of Statistics, North Carolina State University, Raleigh, North Carolina, USA; [3]Oak Ridge Institute for Science Education (ORISE), U.S. Department of Energy, Oak Ridge, Tennessee, USA; [4]Department of Biological Sciences, North Carolina State University, Raleigh, North Carolina, USA

Shah et al, 2016

Summary

- Biological systems are resilient
- The boundary between adaptation and adversity is complex !
- System trajectory analysis can reveal the tipping point between adaptation and adversity
- Tipping points can be calculated from HTS data to estimate critical concentrations of chemicals
- Additional work underway to evaluate utility of critical concentrations for risk assessment



Biologic Pertubations:

System Trajectories:

- Some perturbation/ Recovery
- Adaptive stress response/ Recovery
- Adaptive capacity exceeded/ Cell injury/ No recovery

Acknowledgements and Questions

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