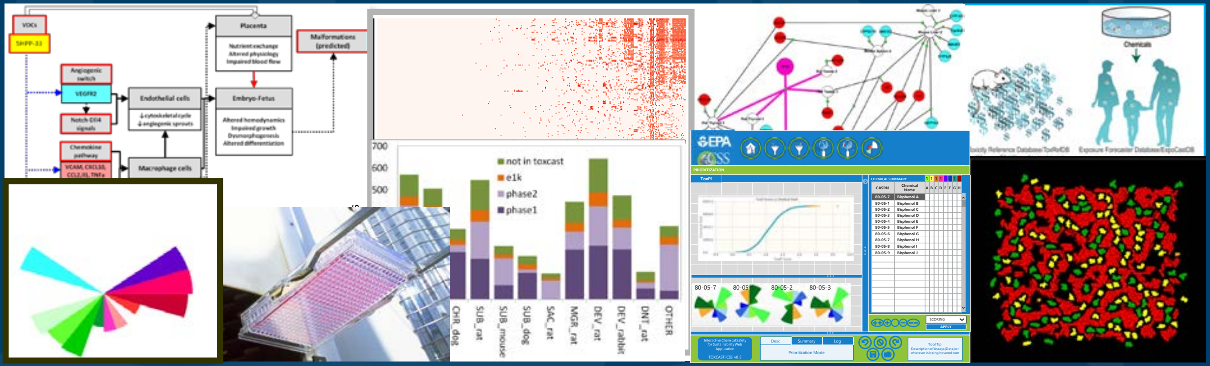
Filling in the Gaps: The role functional genomics can play in 21st century toxicology for environmental risk assessment

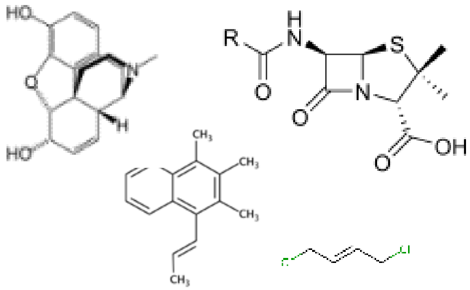


NAS Workshop: The Promise of Genome Editing Tools to Advance Environmental Health Research
January 10-11, 2018

Keith Houck
National Center for Computational Toxicology

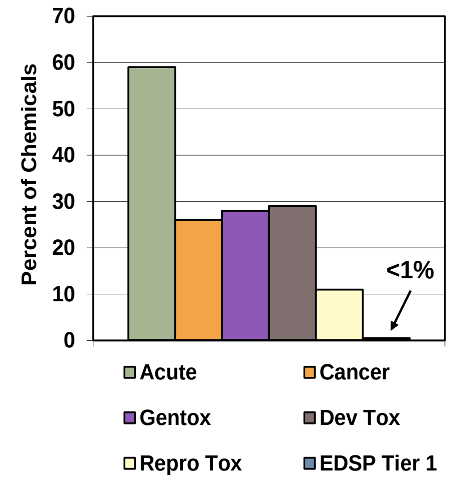
All Understand Why We Need to Innovate In This Space…

Number of Chemicals/Combinations



Why?

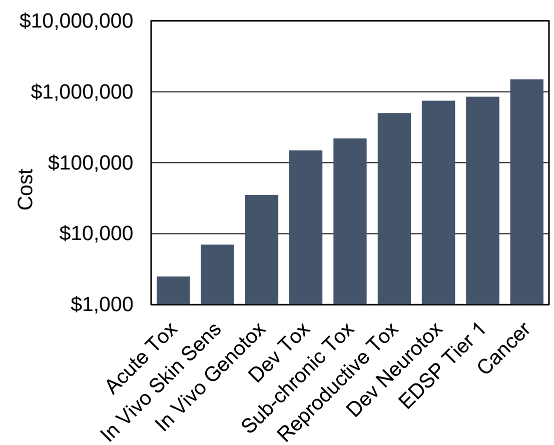
Lack of Data



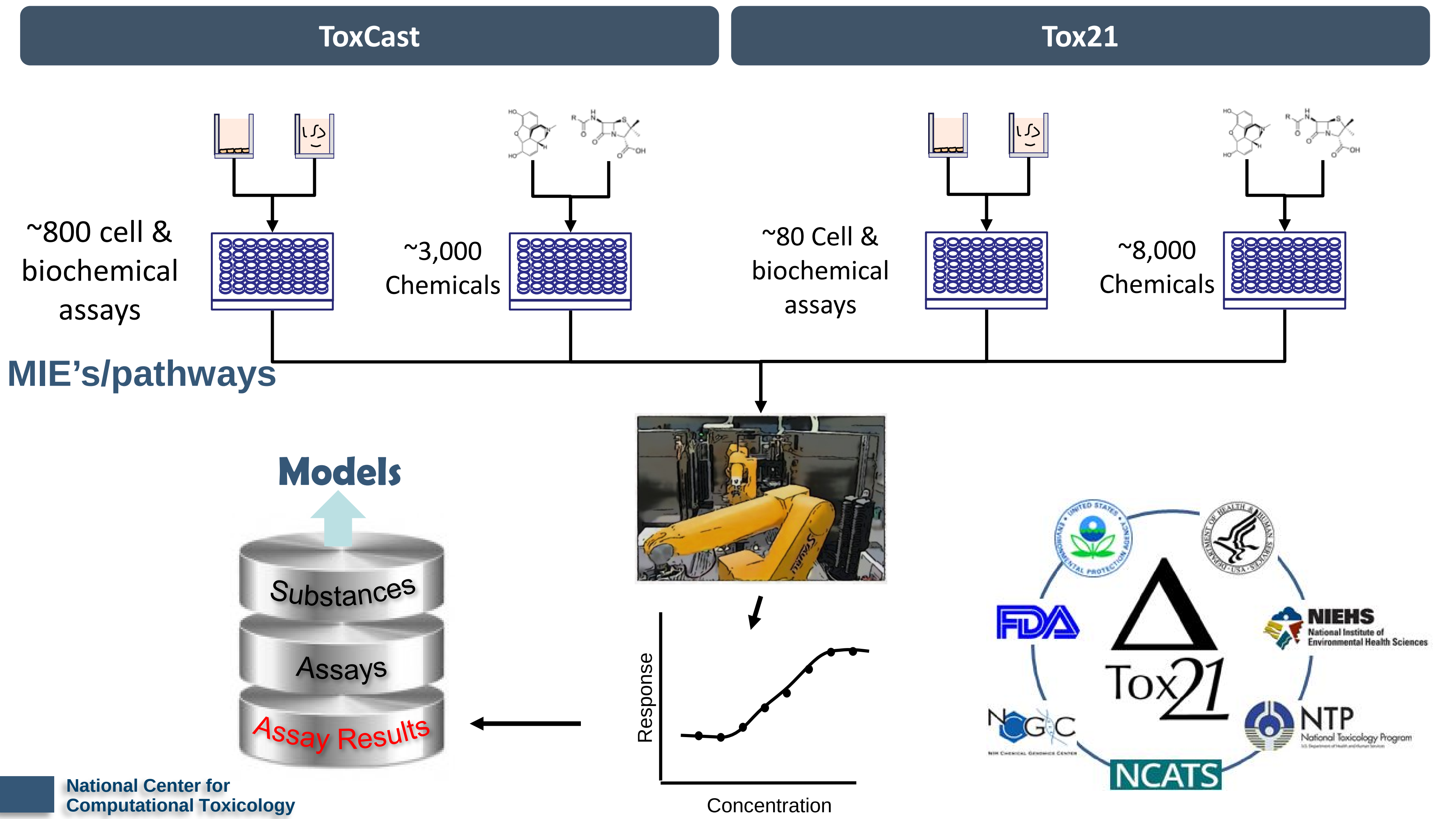
Modified from Judson *et al.*, EHP 2010

Ethical Concerns

Economics

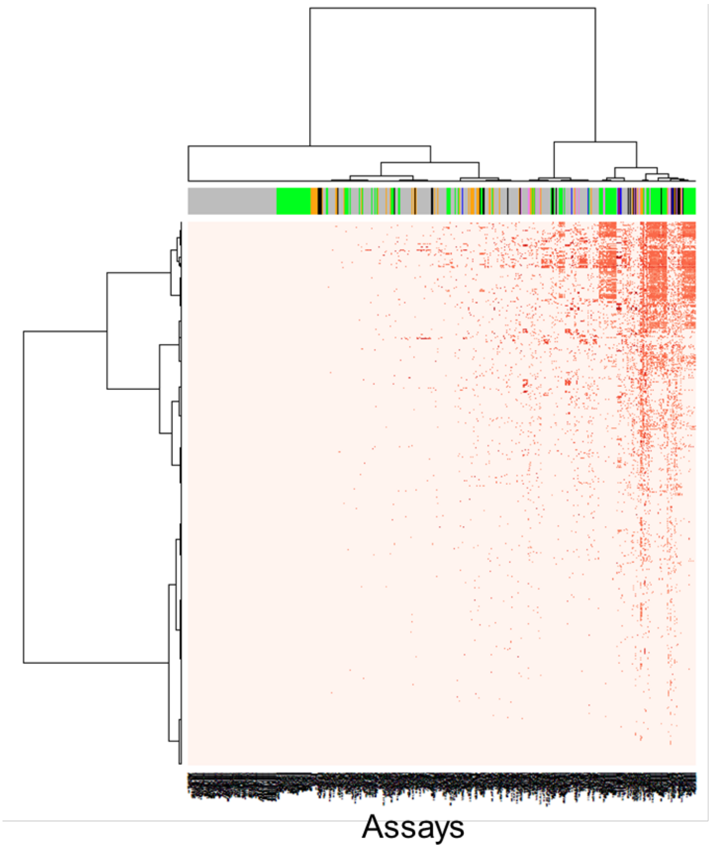


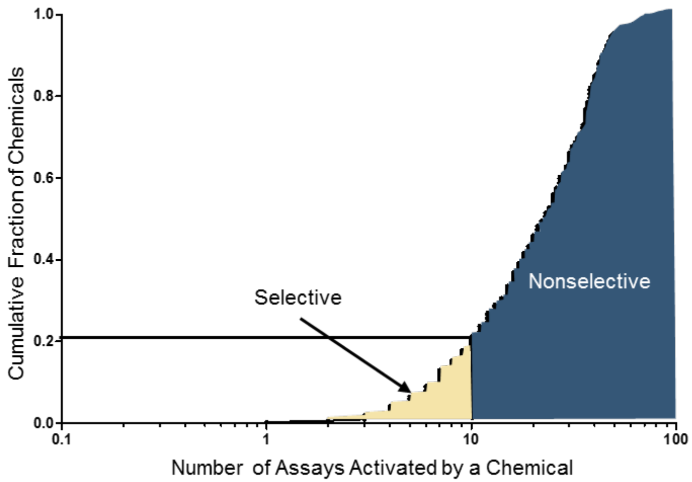
One Approach: High-Throughput Hazard Screening



Promiscuous Chemical Response is the Rule

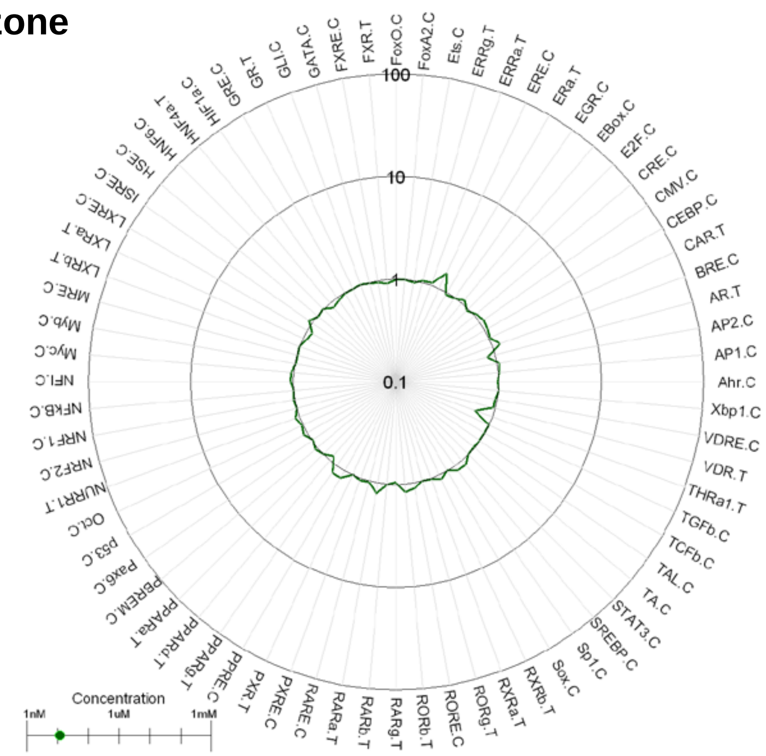
1000 chemicals/
800 assay endpoints





Thomas et al., 2013

Troglitazone



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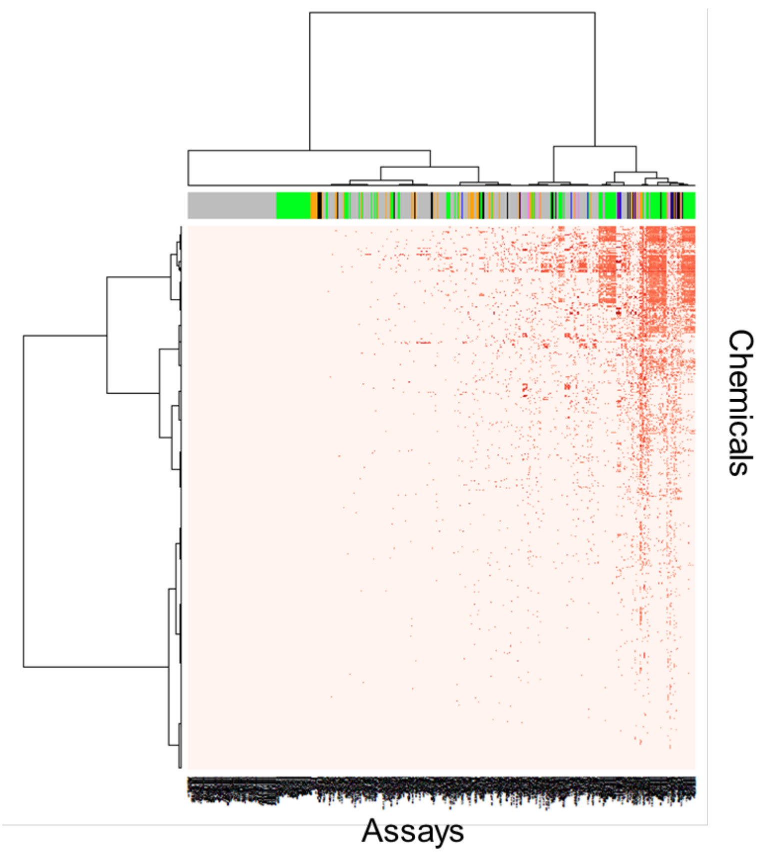
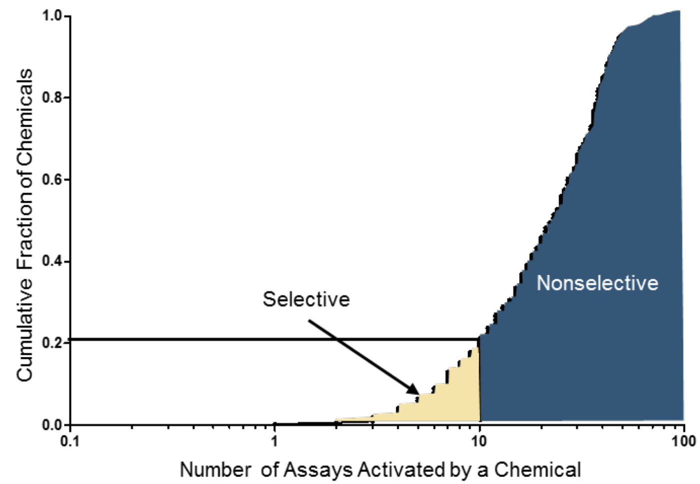
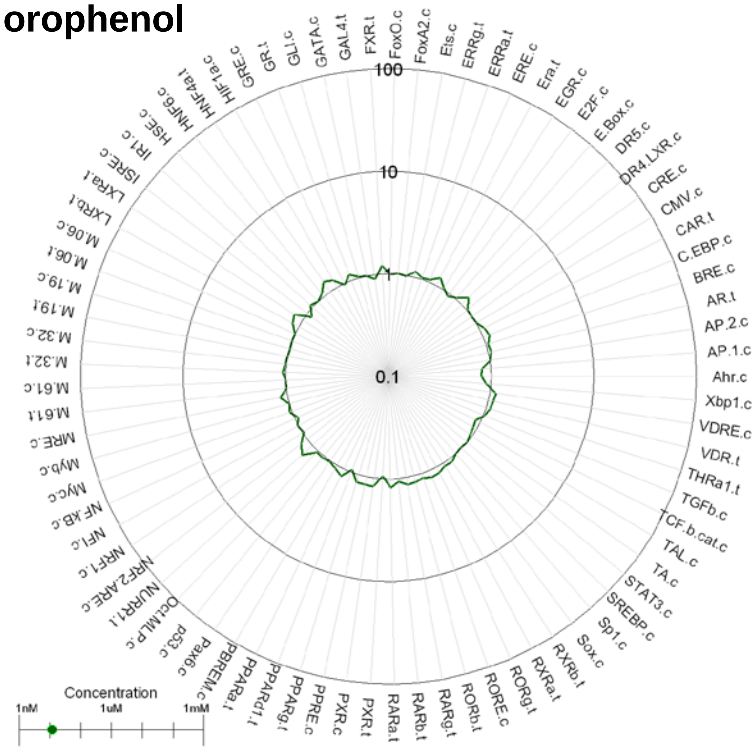


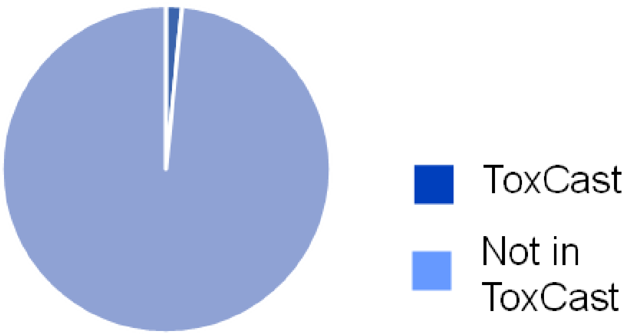


Pentachlorophenol

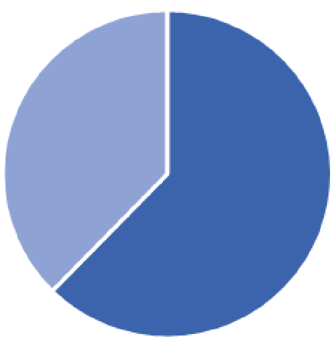


Beginning to Address Concerns for Increased Biological Coverage

Gene Coverage

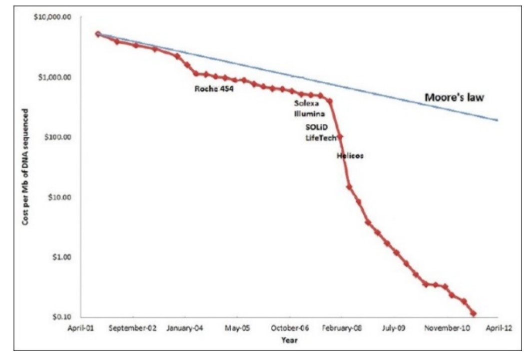


Pathway Coverage*

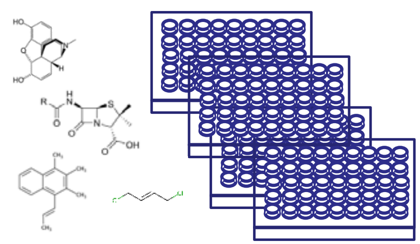


*At least one gene from pathway represented

High-throughput Genomics (HTTr)

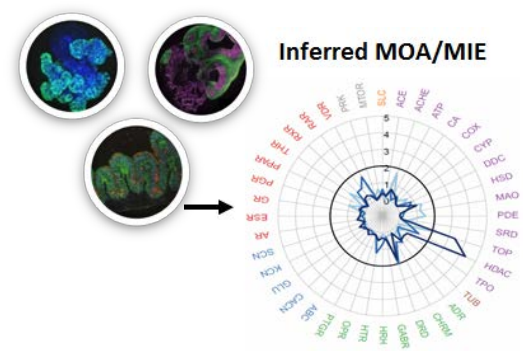


Thousands of chemicals



X

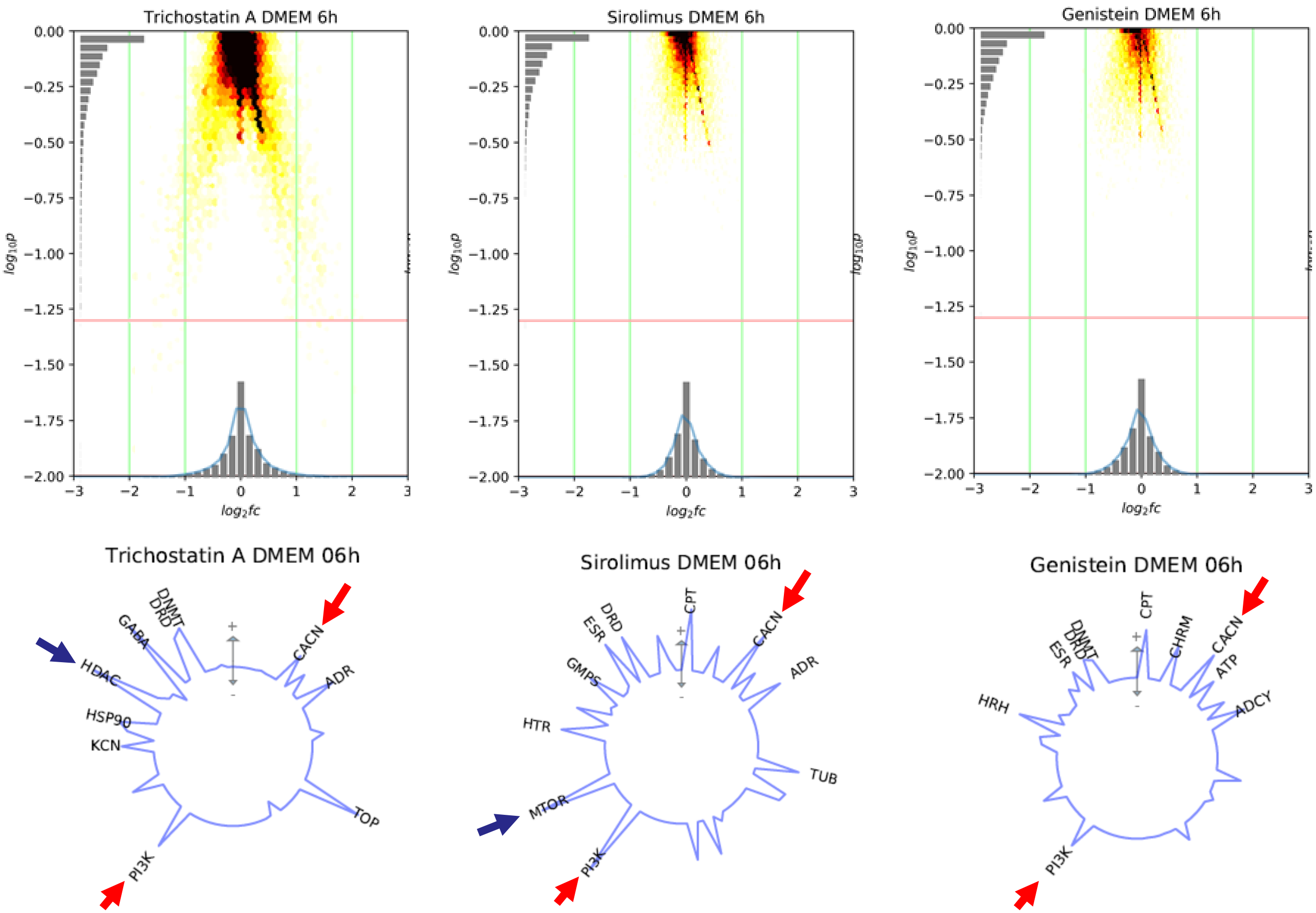
Multiple Cell Types



Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Connectivity Mapping Demonstrates Multiple Pathway Matches



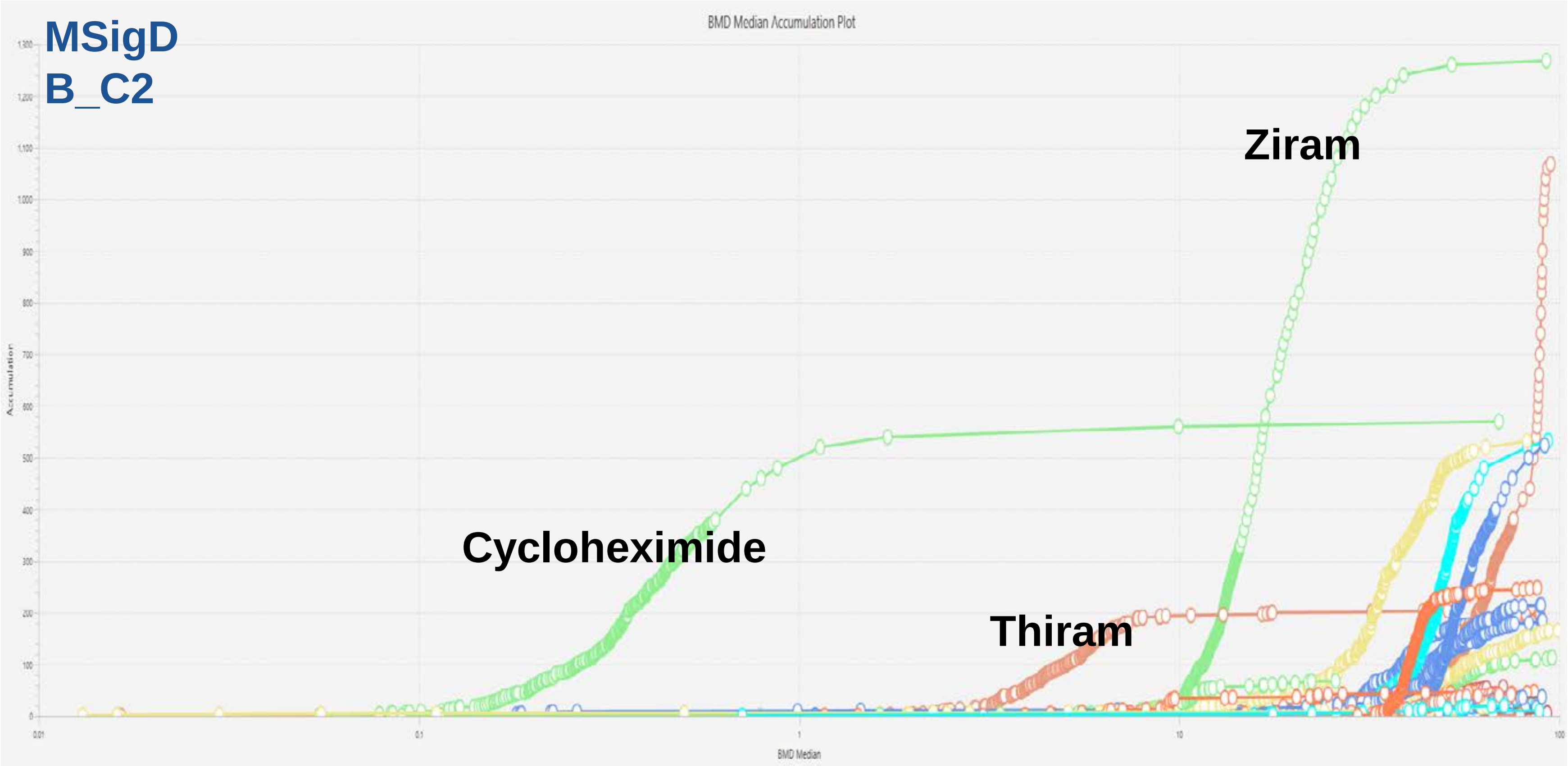
Putative target

Promiscuous Target Mapping

- Differential gene expression observed with reference chemicals
- Putative targets identified using Connectivity Mapping
- Large degree of promiscuity of predicted targets observed
- Currently evaluating additional methods for MIE prediction

Imran Shah, NCCT, unpublished

Pathway Potencies by BMD Analysis

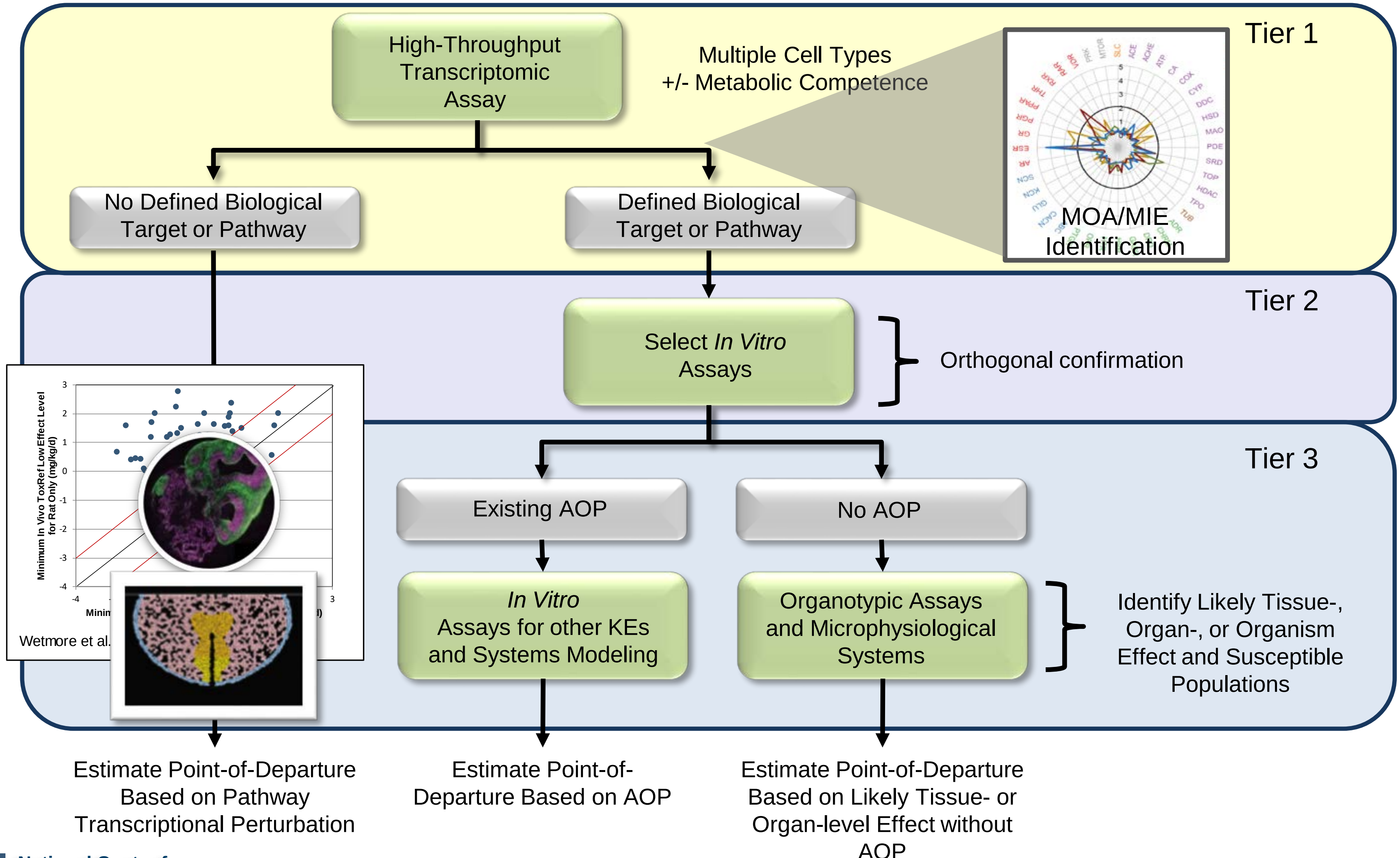


- Broad range of pathway level potency estimates and number of pathways affected across chemicals.

Josh Harrill, NCCT, unpublished

EPA United States Environmental Protection Agency

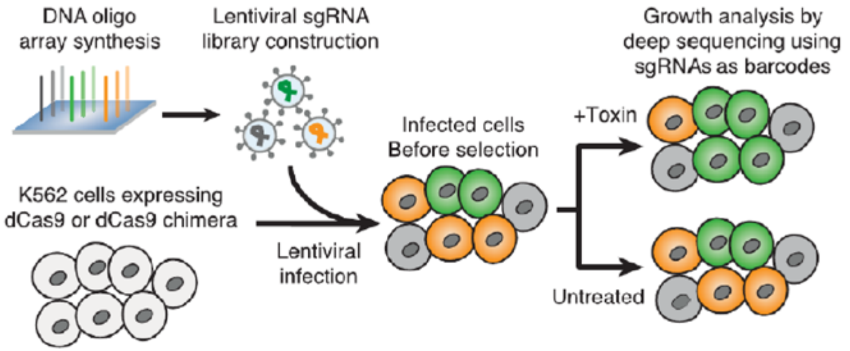
Framework for Integrating Hazard Components…



Functional genomics: Defining Relevancy

- Most chemicals have apparent polypharmacology—what is the critical/relevant MOA?
  - Could use potency to define but this may not be linked to adversity
  - Transcriptomics is high content but function is generally inferred

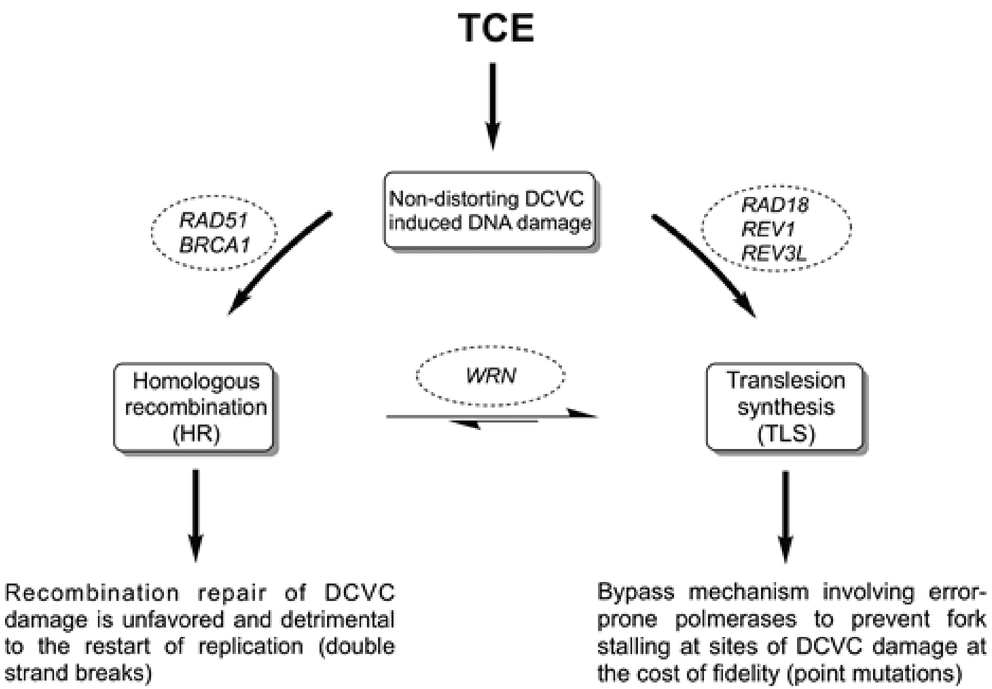
- Functional genomics allows for bridging between genotype and phenotype
- Previously mostly used in prokaryotic systems such as *S. cerevisiae*
- Advent of CRISPR-Cas9 opens door for higher throughput applications in mammalian cells



Gilbert et al., Cell, 2014

Environmental Application of Functional Genomics

- Trichloroethylene (TCE) metabolite study in yeast mutants (n=4607)
- Genome-wide profiling of yeast mutants (n=4607) identified the error-prone translesion synthesis (TLS) pathway conferring sensitivity to TCE metabolite DCVC
- Results were confirmed in a eukaryotic system using DT40 avian kidney cells



De la Rosa et al., Toxicological Sciences, 2017

Pilot Project

- Collaboration between University of Florida (Chris Vulpe) and USEPA (NCCT, Keith Houck)
- Funded by USEPA SMARTi award to Keith Houck and Audrey Bone
- **Goal of the project is to test the feasibility of using CRISPR-Cas9 genome editing in human cells for screening environmental chemicals in a functional genomics toxicology format**

Experimental Design

Use CRISPR-Cas9 genome editing technology with a targeted, short guide RNA library (n=3675 genes) in X cells

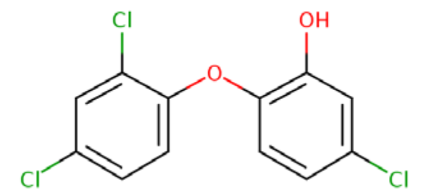
Expose cells to curated set of ~10 chemicals

Measure cell viability and identify genes that conferred sensitivity or resistance

Evaluate results in context of putative mechanisms of cytotoxicity for each chemical

EPA United States Environmental Protection Agency

Chemical Selection



Triclosan

- Criteria
  - Mix of uses (pharmaceutical, pesticide, consumer, industrial)
  - Well-characterized mechanisms of cytotoxicity
    - Mitochondrial toxicity
    - DNA damage
    - Oxidative stress
    - Microtubule disruption
    - Proteosome inhibition
  - Known cytotoxic in Tox21/ToxCast assays without metabolic activation

- 11 chemicals
  - Colchicine
  - Triphenyltin chloride
  - Triglycidyl isocyanurate
  - Cytembena
  - Propargite
  - Octhilinone
  - Triclosan
  - Tralopyril
  - Dibutyltin dichloride
  - Malachite green
  - Bisphenol A glycidyl methacrylate

United States Environmental Protection Agency

Weaknesses

- Current approach limited to identifying genes that lead to reduction in cell viability
- Problems inherent to CRISPR tech
  - Unintended mutations
- “Pathway” analysis
- Larger perspective- same problems as other HTT approaches
  - Metabolic capacity
  - Toxicokinetic integration
- BUT functional aspects increases confidence in relevance of specific genes to adverse effects

Future Needs for Pathway to Integration of Functional Genomics into Environmental Risk Assessment

- More proof-of-concept and pilot studies with well-characterized chemicals
- Development of assay technology that facilitates expansion of functional endpoints beyond cell viability
  - *in vivo* assays in organisms suited to HTT such as *C. elegans* or *Danio rerio*
  - *cell line engineering of pathway reporter lethality assays*
- Dependent on continued integration of HTT into risk assessment paradigms

Other Uses of Genome Editing Tools

- Validation of HTTr signatures
- Validation of AOPs
  - What is the effect of knockdown/activation of MIE's/KE's to the adverse endpoint?
  - Can this help with the development of **quantitative** AOP's?
- Rapid generation of specific animal model MOAs
  - Sensitivity/resistance to specific MOA's
  - Modulation of critical ADME parameters
- Generation of sensitive population models *in vivo* and *in vitro*
  - Single genetic mutation disease models
  - Engineered ADME genetic variability
  - SNP recapitulation of sensitive populations from GWAS-type studies (complex)
  - Validation of sensitivities identified through GWAS-type analysis

Thank You for Your Attention!

Acknowledgements

EPA Colleagues:

NCCT

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NIEHS

- Allison Harrill

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- Chris Vulpe
- Abderrahmane Tagmount

EPA's National Center for Computational Toxicology