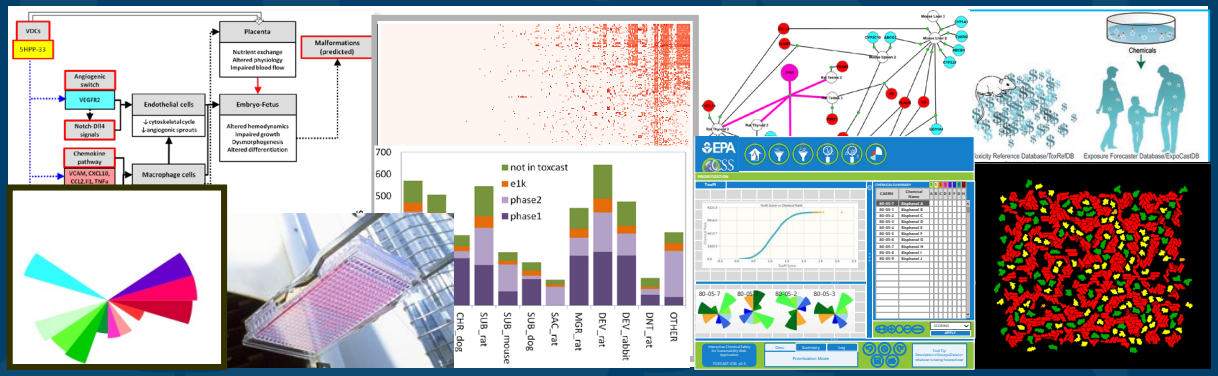
United States Environmental Protection Agency

Overview of NCCT Activities in the Chemical Safety for Sustainability National Program



LRI Meeting

October 3, 2018

Rusty Thomas
Director
National Center for Computational Toxicology

EPA United States Environmental Protection Agency

NCCT Activities in CSS Projects

- Chemical Evaluation:
  Project areas: **High-throughput Toxicology (HTT), Rapid Exposure and Dosimetry (RED).**

- Lifecycle Analytics:
  Project areas: Lifecycle-Human Exposure Modeling (LCHEM), **Sustainable Chemistry,** Emerging Materials (Nanomaterials), Ecological Modeling.

- Complex Systems Science:
  Project areas: Adverse Outcome Pathways Discovery and Development (AOPDD), **Virtual Tissue Modeling (VTM).**

  .

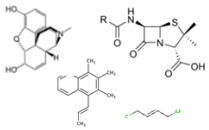
- Solutions-based Translation and Knowledge Delivery*:*
  Project area: **Demonstration and Evaluation for Risk-Based Decisions.**

EPA United States Environmental Protection Agency

Research Focus Areas

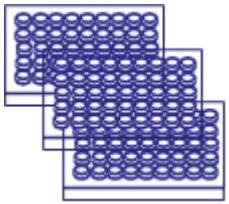
- Increasing biological coverage in high-throughput *in vitro* test systems
- Systematically addressing technical limitations of *in vitro* test systems
- Continued integration of high-throughput results into tiered testing
- Characterization of uncertainty and variability
- Delivery of data and models through decision support tools
- Building confidence through regulatory focused case studies

High-Throughput Screening in ToxCast



Thousands of Chemicals

Concentration Response Screening

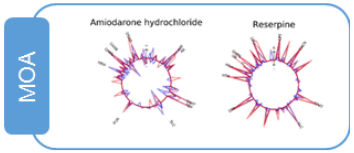


ToxCast Assays

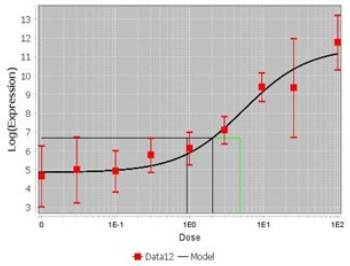
Transcription Factors
Transporter
Cytokines
Kinases
Nuclear Receptors
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion channels

~700 Assay Endpoints

Mode-of-Action Identification

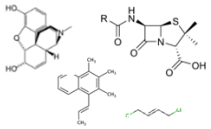


Concentration Response Modeling

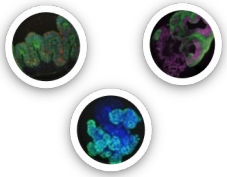


- 96, 384, and 1536-well, laboratory automation compatible
- Relatively expensive (~$20,000 - $30,000 / chemical)
- Coverage of molecular and phenotypic responses
- Multiple assay vendors/labs

Efforts to Expand Biological Coverage Using High Content Technologies

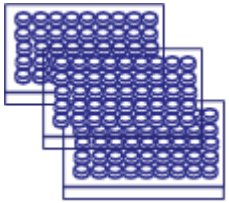


Thousands of Chemicals

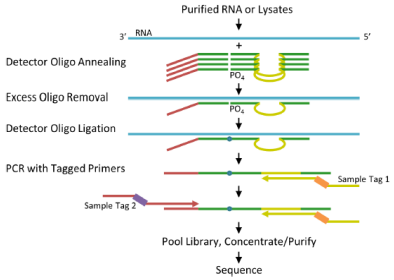


Multiple Cell Types

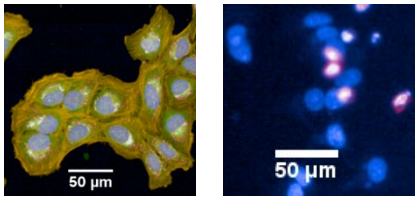
Concentration Response Screening



Whole Genome Transcriptomics

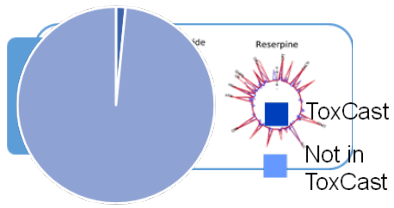


Multi-Parameter Cellular Phenotypic Profiling



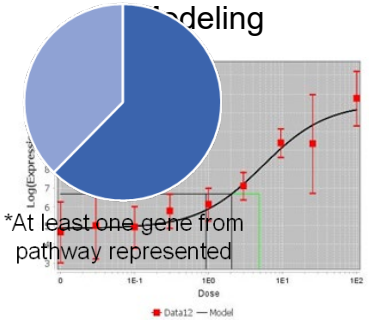
Gene Coverage

Mode-of-Action Identification



Pathway Coverage*

Concentration Response Modeling



*At least one gene from pathway represented

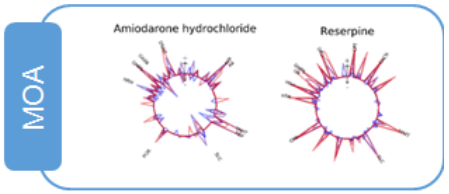
- 384-well, laboratory automation compatible
- Relatively inexpensive ($2.50 - $1,500 per chemical)
- Broad complementary coverage of molecular and phenotypic responses
- Integration of reference materials and controls for performance standards
- Increased portability

Initial Application of High-Throughput Transcriptomic Screening

Parameter	Description
Cell Type(s)	MCF7
Chemicals	2,112
Time Points:	6 hours
Concentrations:	8
Biological Replicates:	3

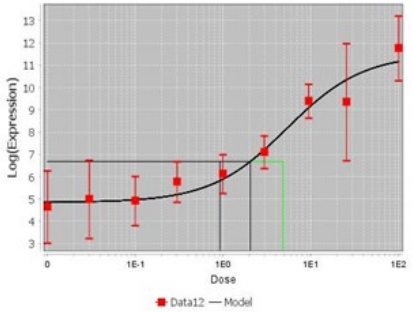
- **Number of samples:** 54,432
- **Number of endpoints:** 1.15×10^9
- **Total amount of data:** ~50 TB

Mode-of-Action Identification



Currently comparing a range of approaches…
Cmap, ML, Pathway

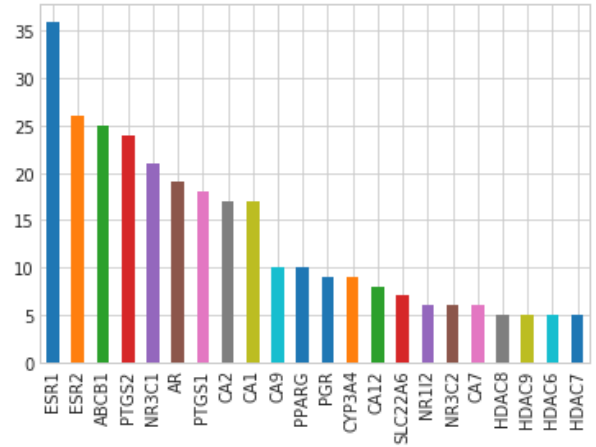
Concentration Response Modeling



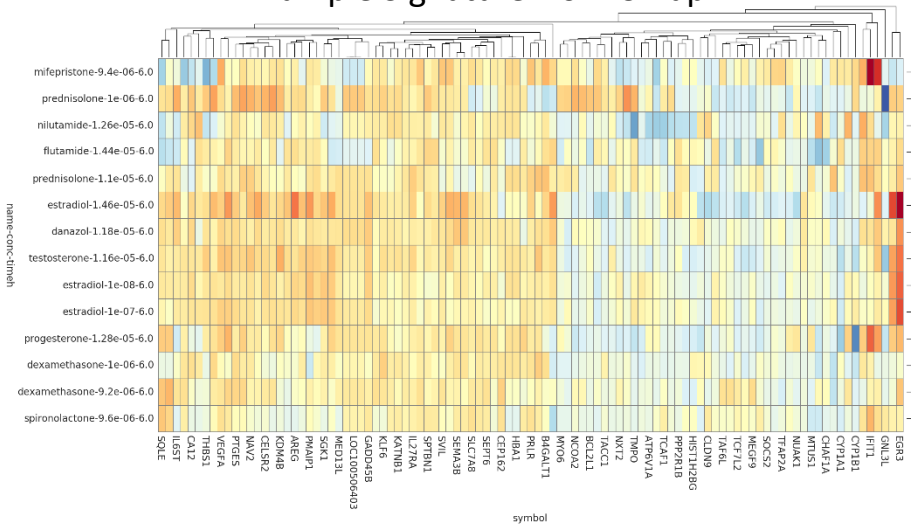
Currently comparing a range of approaches…
BMDExpress, Proast, tcpl, and new NB model

Identifying Potential Biological Targets

Annotated Targets in CMap v2 and RefChem



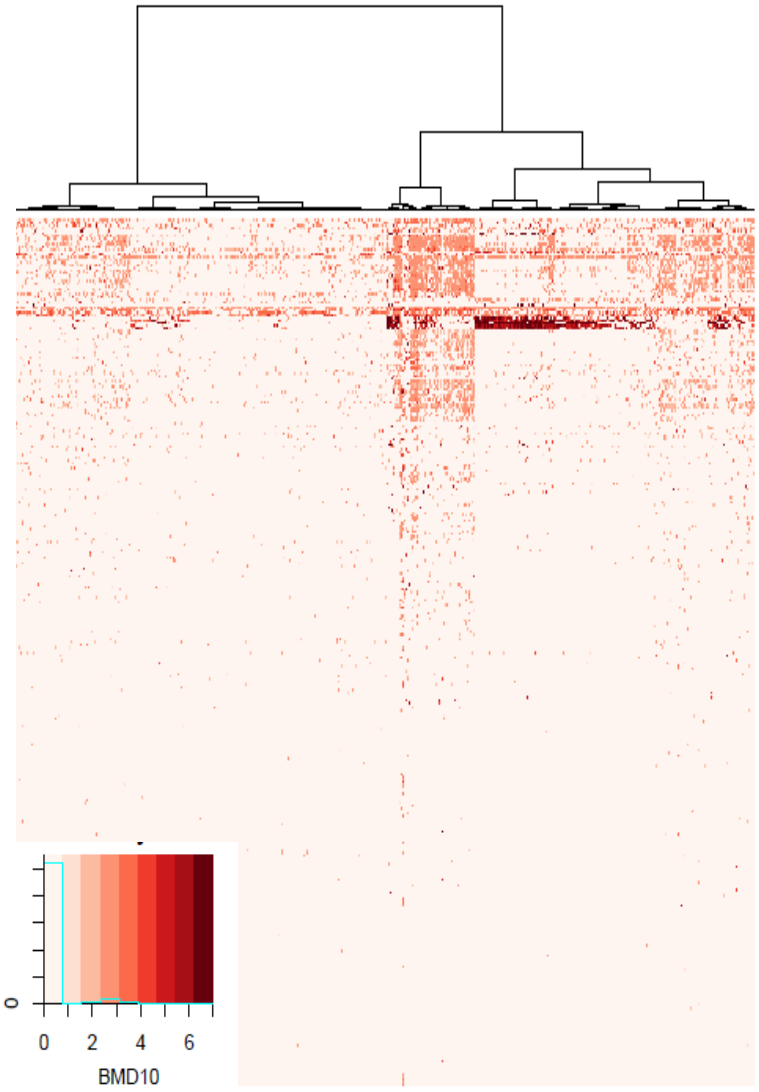
Example Signature from CMap v2

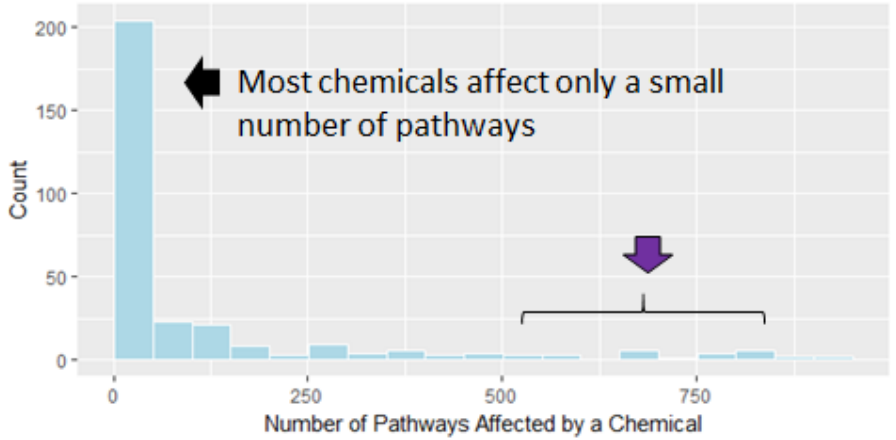


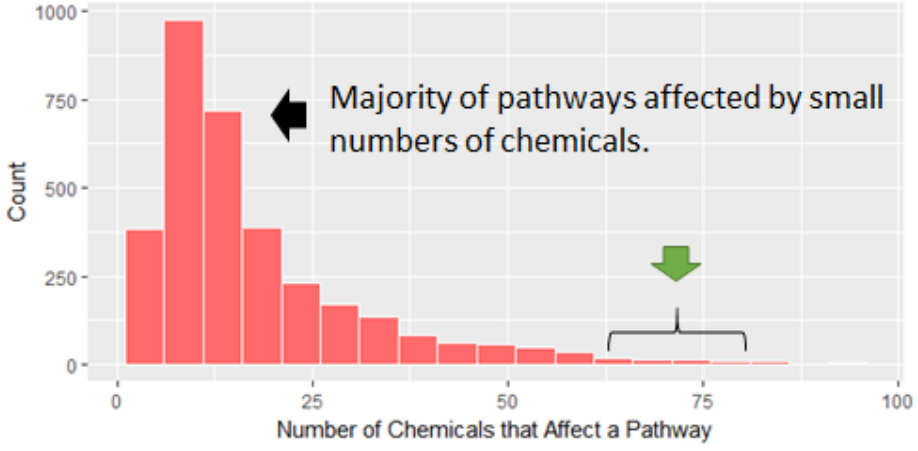
	CMap v2 / Affymetrix	HTTr-Phase I RefChem Hits	
Target	Signature size	Sensitivity	Positives
CYP2C9	131	1	1
ESR1	257	1	11
HDAC1	124	1	2
DHFR	215	1	2
NR1I2	139	1	2
PGR	115	1	1
HMGCR	236	1	1
ABCC2	357	1	1
TYMS	329	1	1
ESR2	281	0.86	7
AR	261	0.78	9
NR3C2	352	0.5	2
ABCB1	117	0.5	2
NR3C1	148	0.5	4
CA1	176	0.5	4
CA2	341	0.5	4
PTGS1	307	0.25	4

*In process of curating/testing hits to determine specificity

Characterizing Concentration Response







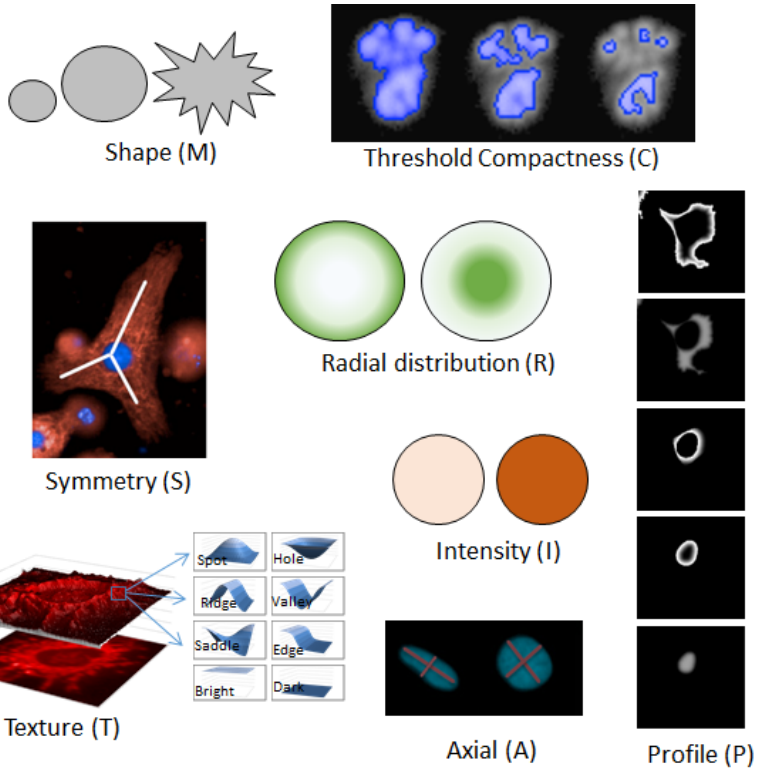
J. Harrill, I. Shah, W. Setzer, Judson Unpublished

Development of High-Throughput Phenotypic Profiling

United States Environmental Protection Agency

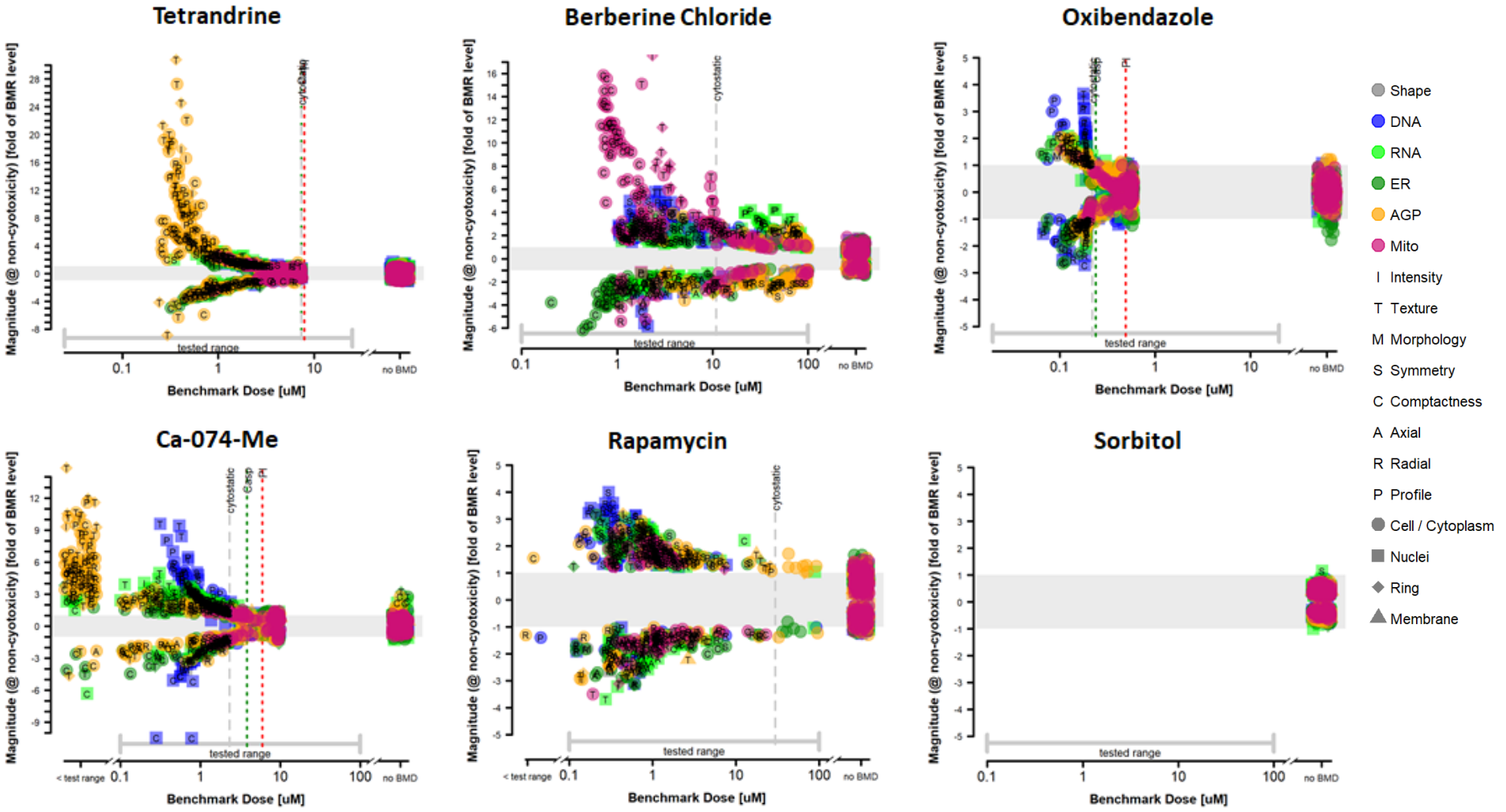
Cell Compartments

Non-Ab Dyes	NUCLEUS	RING	CYTOPLASM	MEMBRANE	CELL
DNA	S,C,A,R, P,I,T,M	--	--	--	S,C,A,R, P,M
RNA	S,C,A,R, P,I,T	--	--	--	S,C,A,R, P
ER	S,C,A,R, P,I,T	I,T	I,T	I	S,C,A,R, P
AGP	S,C,A,R, P,I,T	I,T	I,T	I,T	S,C,A,R, P
MITO	S,C,A,R, P,I,T	I,T	I,T	I	S,C,A,R, P



~1,300 total phenotypic endpoints

Unique Phenotypic Responses Associated with Different MOAs

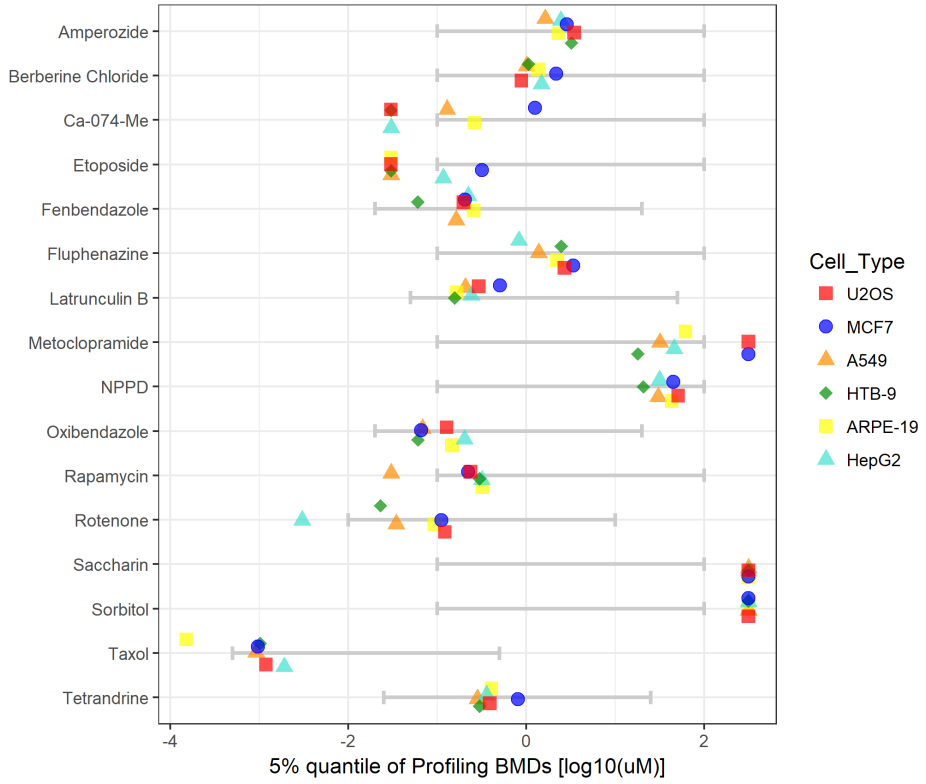


J. Nyffeler, J. Harrill, Unpublished

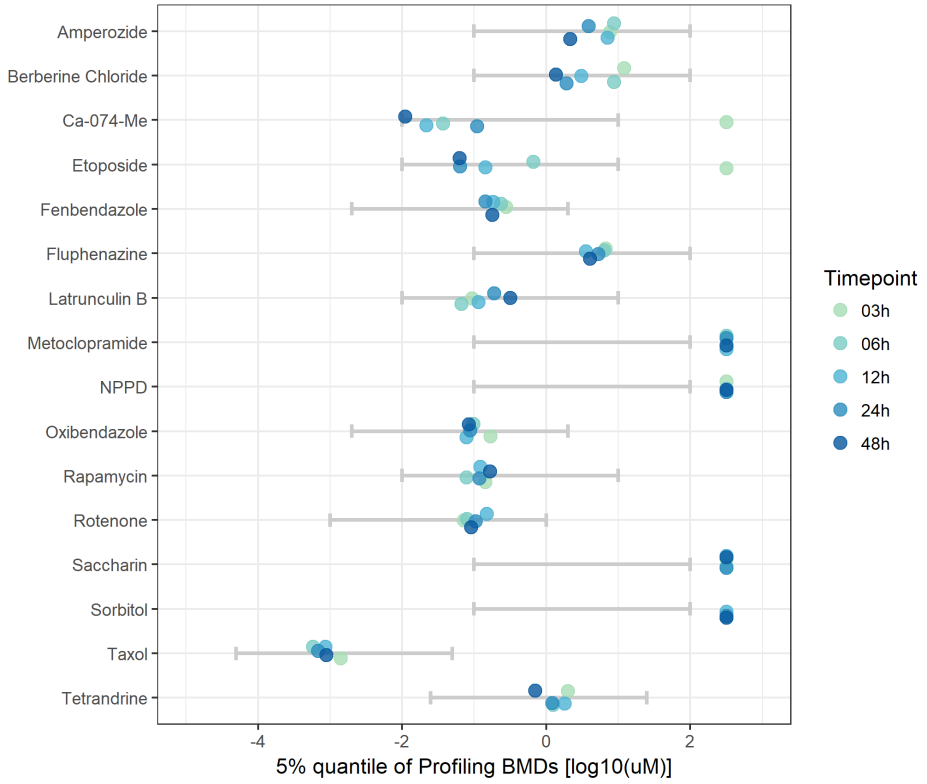
Variation in Phenotypic Potencies Across Cell Type and Time

United States Environmental Protection Agency

Cell Type Differences (48 hr)



Time Point Differences (U2OS cells)



*Data points represent 5th percentile of phenotypic BMDs

Tested range

J. Nyffeler, J. Harrill, Unpublished

United States Environmental Protection Agency

Research Focus Areas

- Increasing biological coverage in high-throughput *in vitro* test systems
- Systematically addressing technical limitations of *in vitro* test systems
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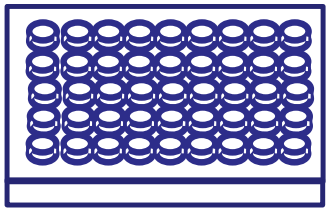
Expanding Chemical Coverage of High Throughput *In Vitro* Systems

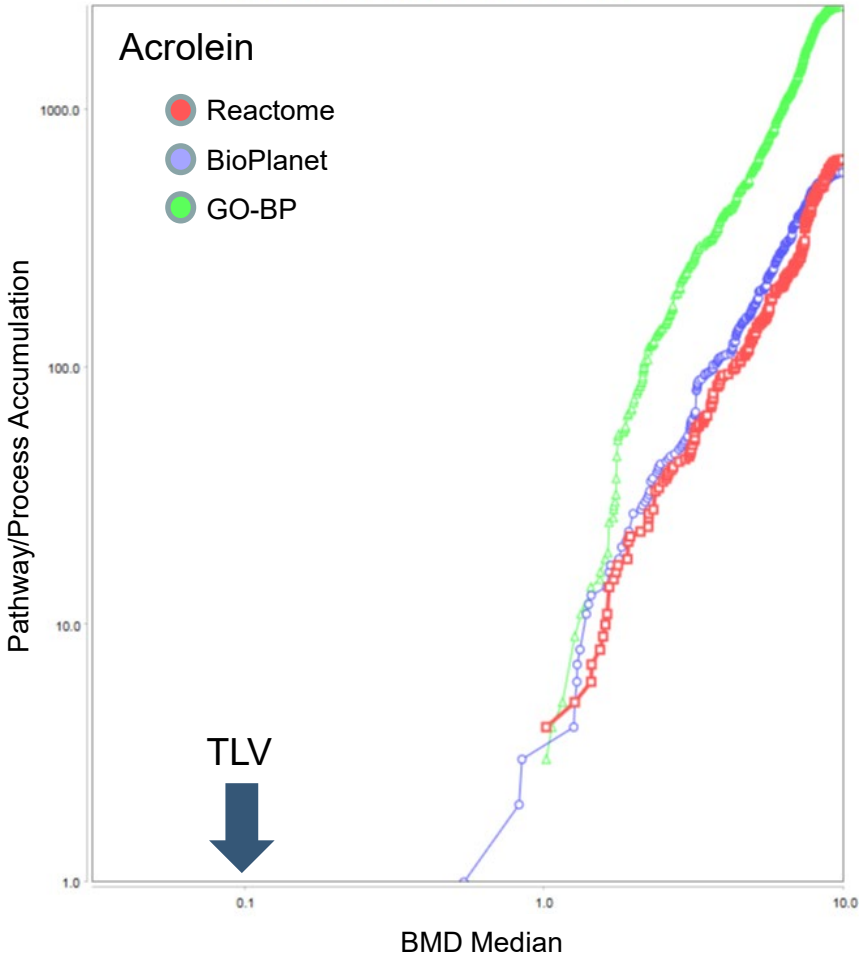
Pilot Scale Water Soluble Library



VOC *In Vitro* Exposure System

M. Higuchi (EPA-NHEERL)

Transcriptional BMDs from HTTR Analysis

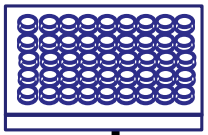


J. Harrill, M. Higuchi, and J. Zavala-Mendez, Unpublished

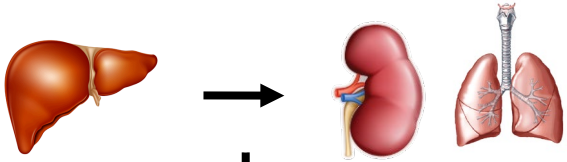
Addressing Limitations in Xenobiotic Metabolism

"Extracellular" Approach

Chemical metabolism in the media or buffer of cell-based and cell-free assays

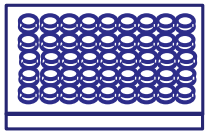


More closely models effects of hepatic metabolism and generation of circulating metabolites

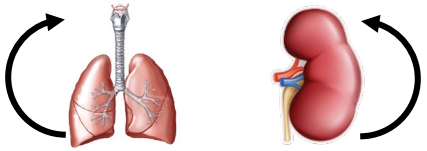


"Intracellular" Approach

Chemical metabolism inside the cell in cell-based assays



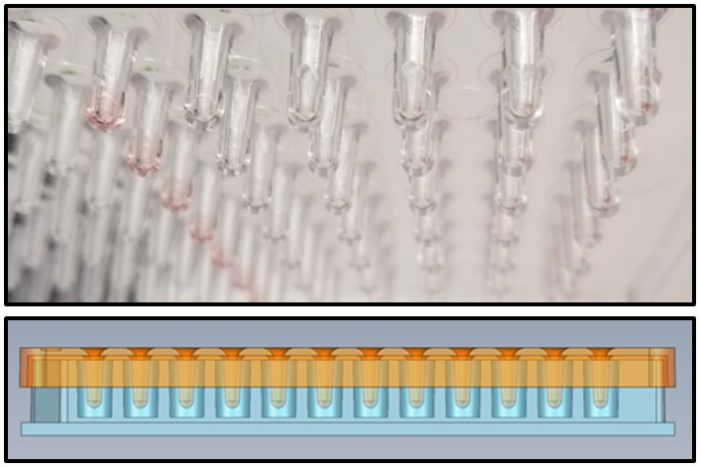
More closely models effects of target tissue metabolism



Integrated strategy to model *in vivo* metabolic bioactivation and detoxification

Application of Extracellular Strategy to Identify Estrogenic Metabolites

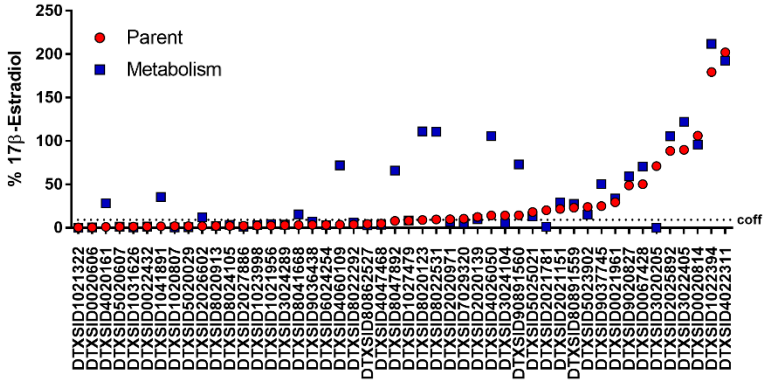
AIME Method: S9 Fraction Immobilization in Alginate Microspheres on 96- or 384-well peg



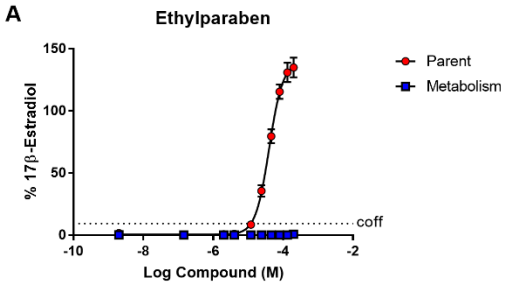
Screening Window of VM7 (formerly BG1) ER Transactivation Assay

		Metabolism	
		Neg	Pos
NRS	Neg	0.91	0.89
	Pos	0.91	0.71
		Z'	

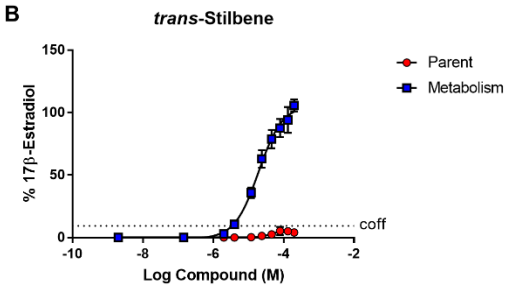
Pilot Screening Results of Pinto et al., 2016 Library



Example Detoxification

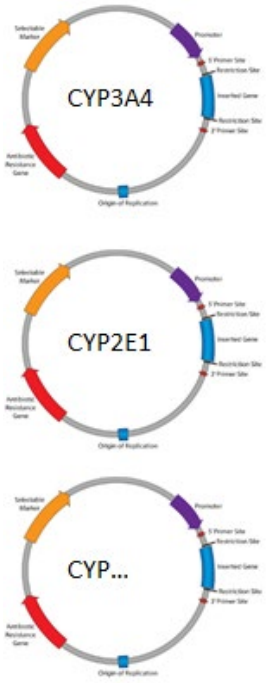


Example Bioactivation

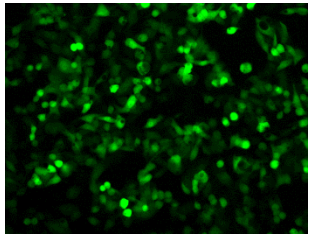


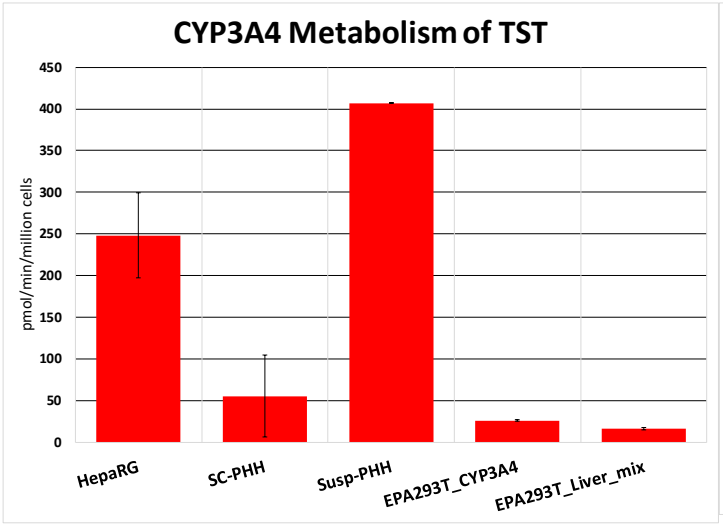
D. DeGroot, C. Deisenroth, Unpublished

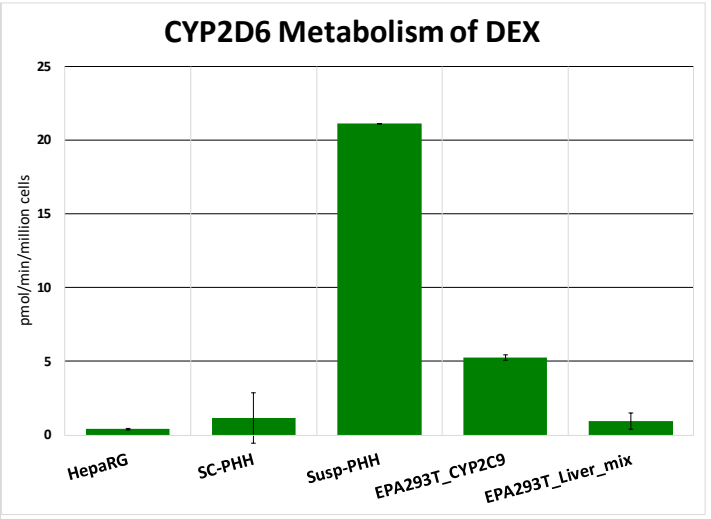
Application of Intracellular Strategy to Identify Cytotoxic Metabolites

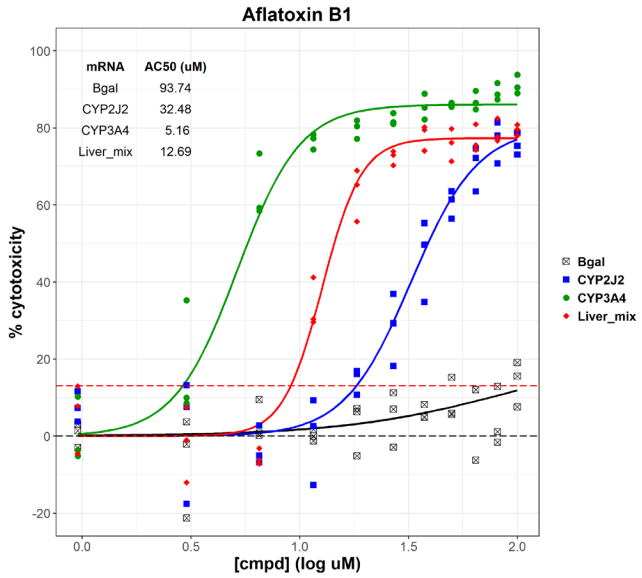


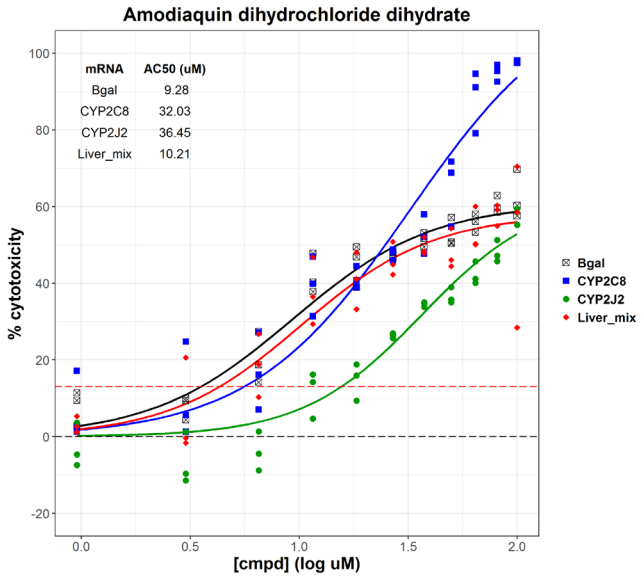
mRNA









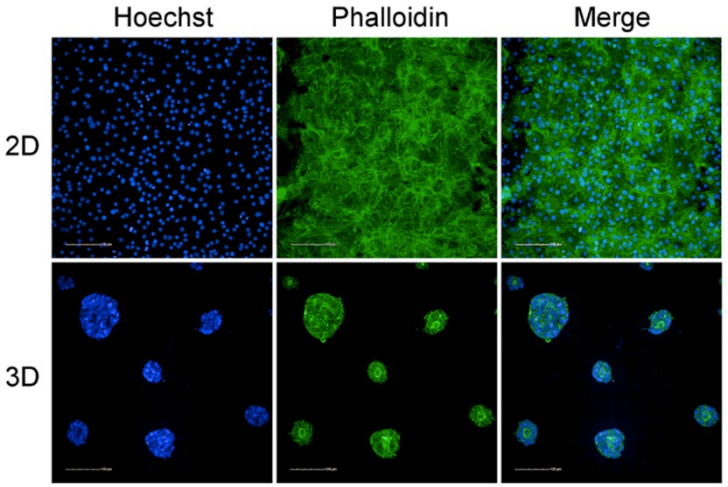


S. Simmons, Unpublished

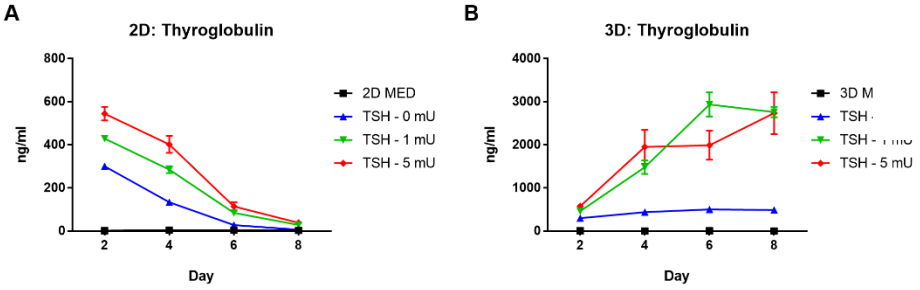
Developing Targeted Organotypic Models that Predict Tissue Effects

United States Environmental Protection Agency

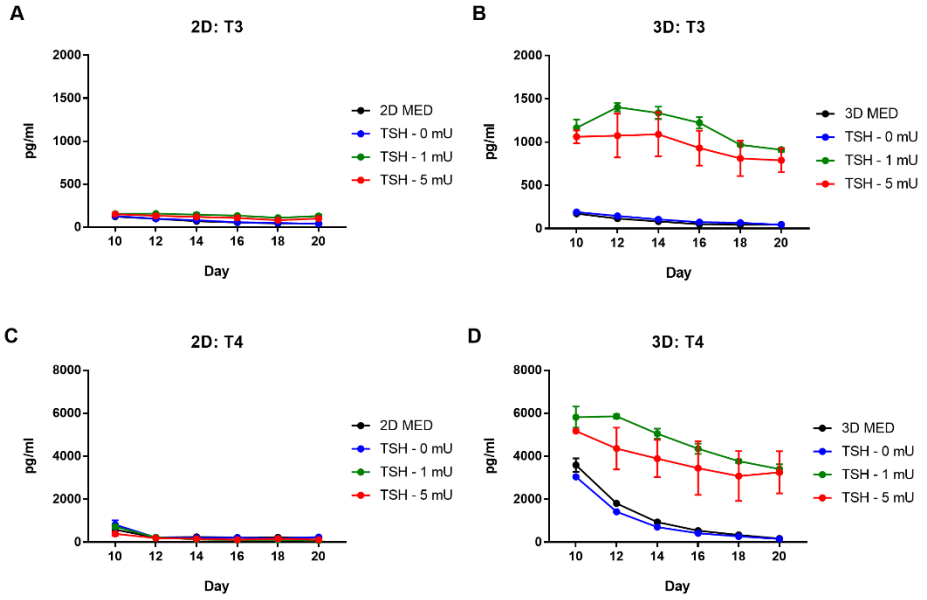
3D Microtissue Model of Primary Human Thyrocytes



Thyroglobulin secretion is enhanced over time in a 3D culture model



Thyroid hormone is synthesized and secreted over time in a 3D culture model



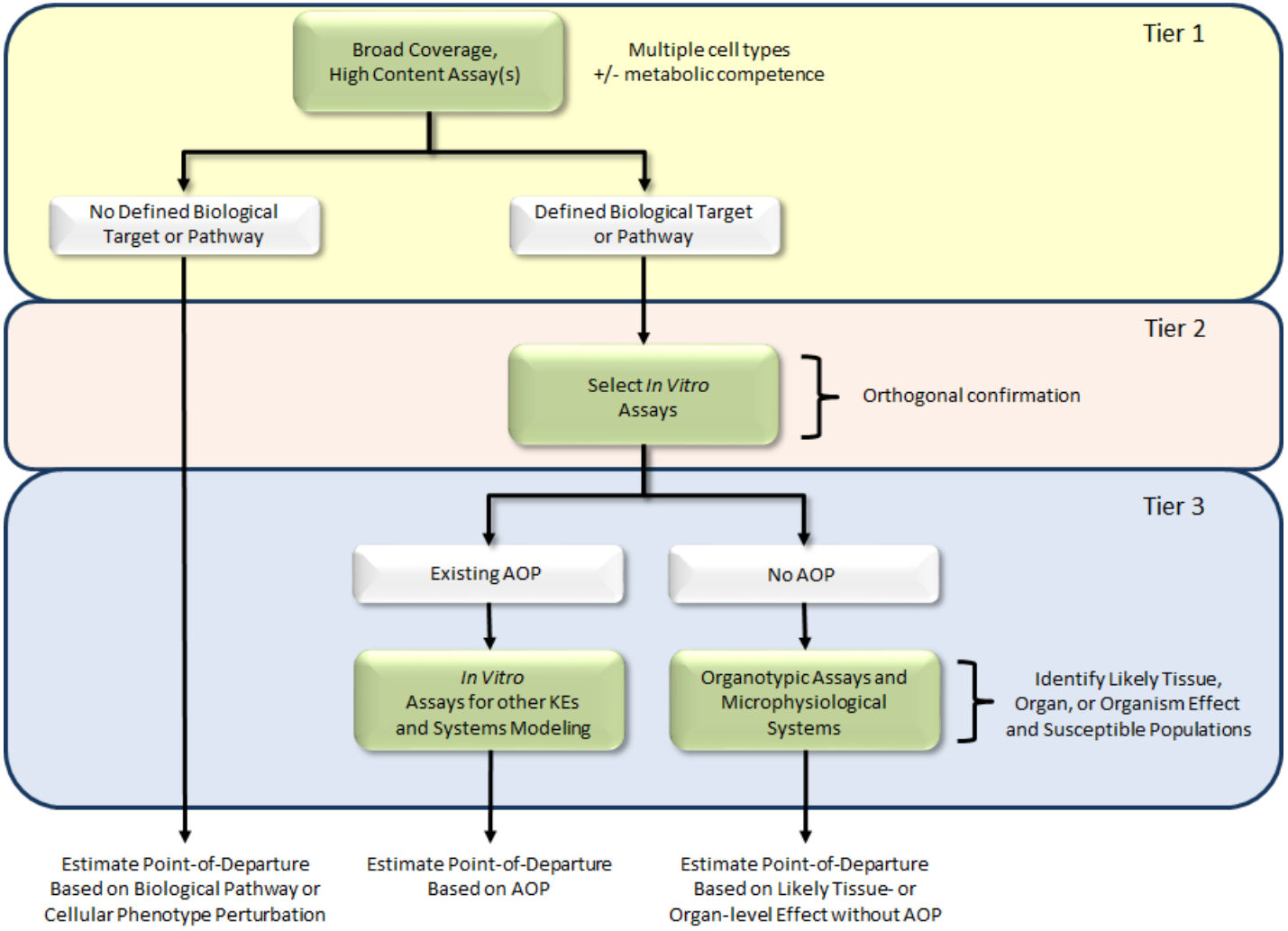
C. Deisenroth, Unpublished

EPA United States Environmental Protection Agency

Research Focus Areas

- Increasing biological coverage in high-throughput *in vitro* test systems
- Systematically addressing technical limitations of *in vitro* test systems
- Continued integration of high-throughput results into tiered testing
- Characterization of uncertainty and variability
- Delivery of data and models through decision support tools
- Building confidence through regulatory focused case studies

Integrating of High Throughput Results into Tiered Testing Framework



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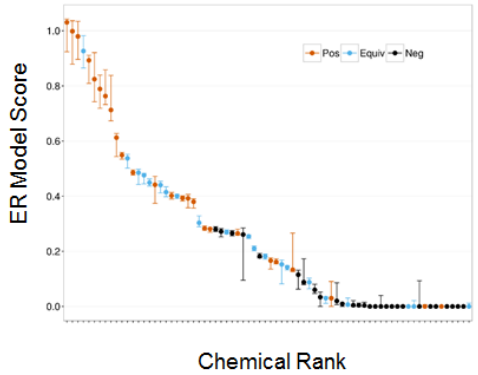
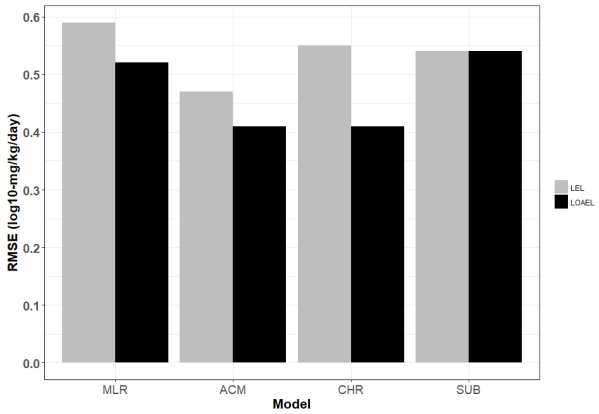
Characterization of Uncertainty and Variability

Pharmacodynamic



Experimental

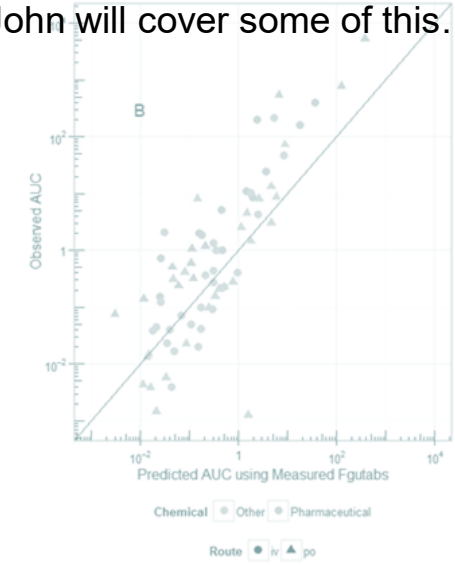


In Vivo

Pharmacokinetic

Experimental and Inter-Individual

John will cover some of this…

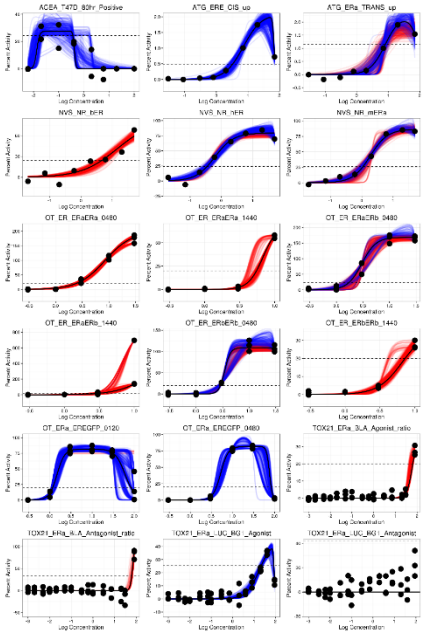


In Vitro-to-*In Vivo*

Characterizing Uncertainty for *In Vitro* Testing Systems and Computational Modeling

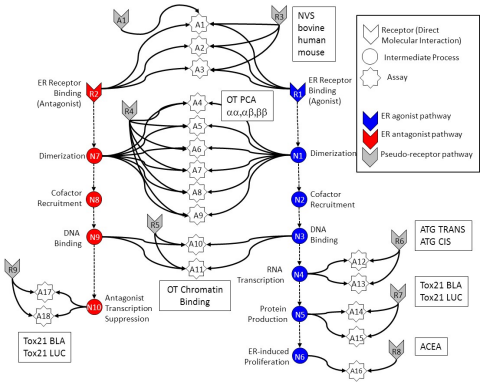
United States Environmental Protection Agency

Bootstrap Uncertainty in *In Vitro* Potency Values



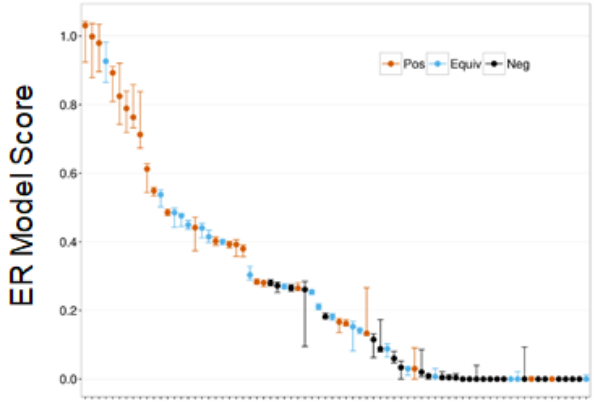
18 ER *In Vitro* Assays

Computational Modeling



ER Pathway Model

Propagation of Uncertainty in Modeling Output



Chemical Rank

Watt and Judson, PLOS One 2018

Characterizing Uncertainty for *In Vivo* Toxicity Studies

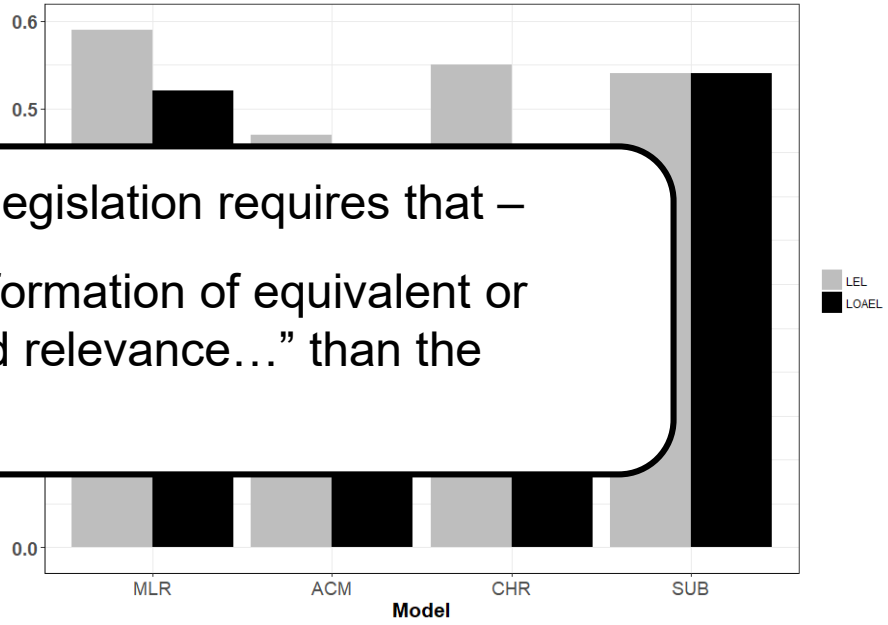
Qualitative Reproducibility in Target Organ Effects in Repeat Dose Toxicity Studies

Organ	Species	Repeated negative	Mixed effects	Repeated positive	% Concordance
Liver	dog	20	26	46	71.7
	mouse				
	rat				
Kidney	dog				
	mouse				
	rat				
Spleen	dog				
	mouse				
	rat				
Testes	dog	65	20	7	78.3
	mouse	110	20	9	85.6
	rat	135	87	23	64.5
Adrenal gland	dog	76	12	4	87.0
	mouse	109	23	7	83.5
	rat	142	83	20	66.1

Section 4(h) in the new TSCA legislation requires that –

NAMs need to provide "information of equivalent or better scientific quality and relevance…" than the traditional animal models

Quantitative Variability in Effect Levels from *In Vivo* Repeat Dose Toxicity Studies



Using an RMSE=0.59, the 95% CI of an LEL/LOAEL is:
1 mg/kg/day ➔ 0.07 – 14 mg/kg/day.
10 mg/kg/day ➔ 0.7 – 143 mg/kg/day.

LyLy Pham and Katie Paul-Friedman, Unpublished

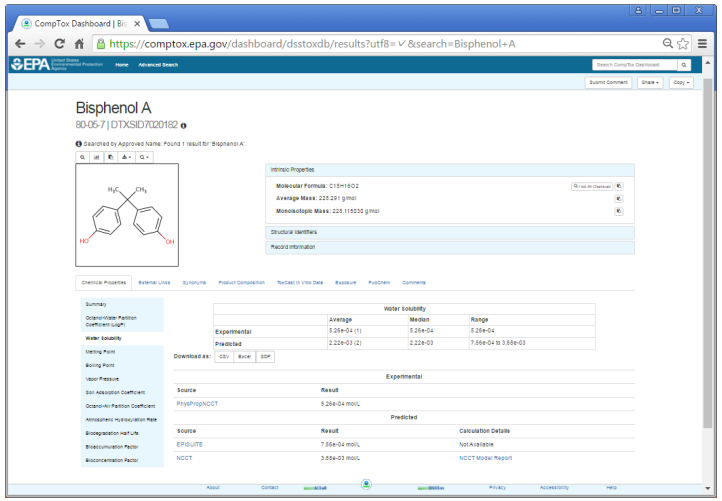
EPA United States Environmental Protection Agency

Research Focus Areas

- Increasing biological coverage in high-throughput *in vitro* test systems
- Systematically addressing technical limitations of *in vitro* test systems
- Continued integration of high-throughput results into tiered testing
- Characterization of uncertainty and variability
- Delivery of data and models through decision support tools
- Building confidence through regulatory focused case studies

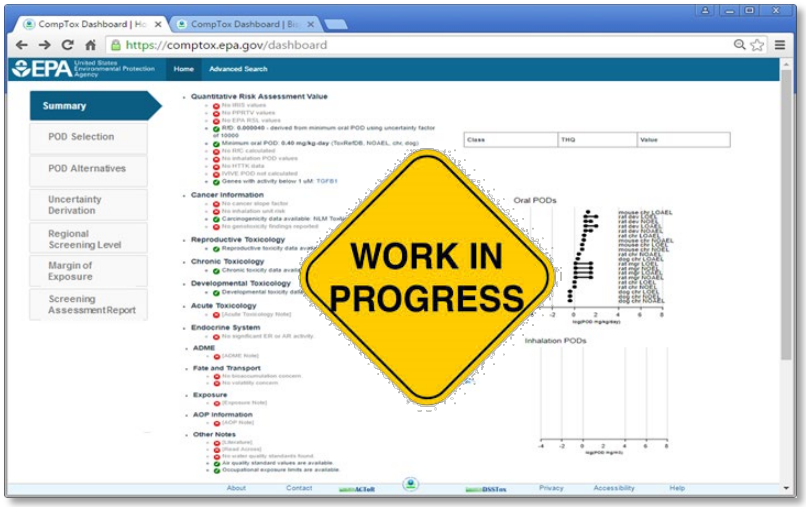
Enable Translation Through Data Visualization and Decision Support Tools

Comptox Chemicals Dashboard

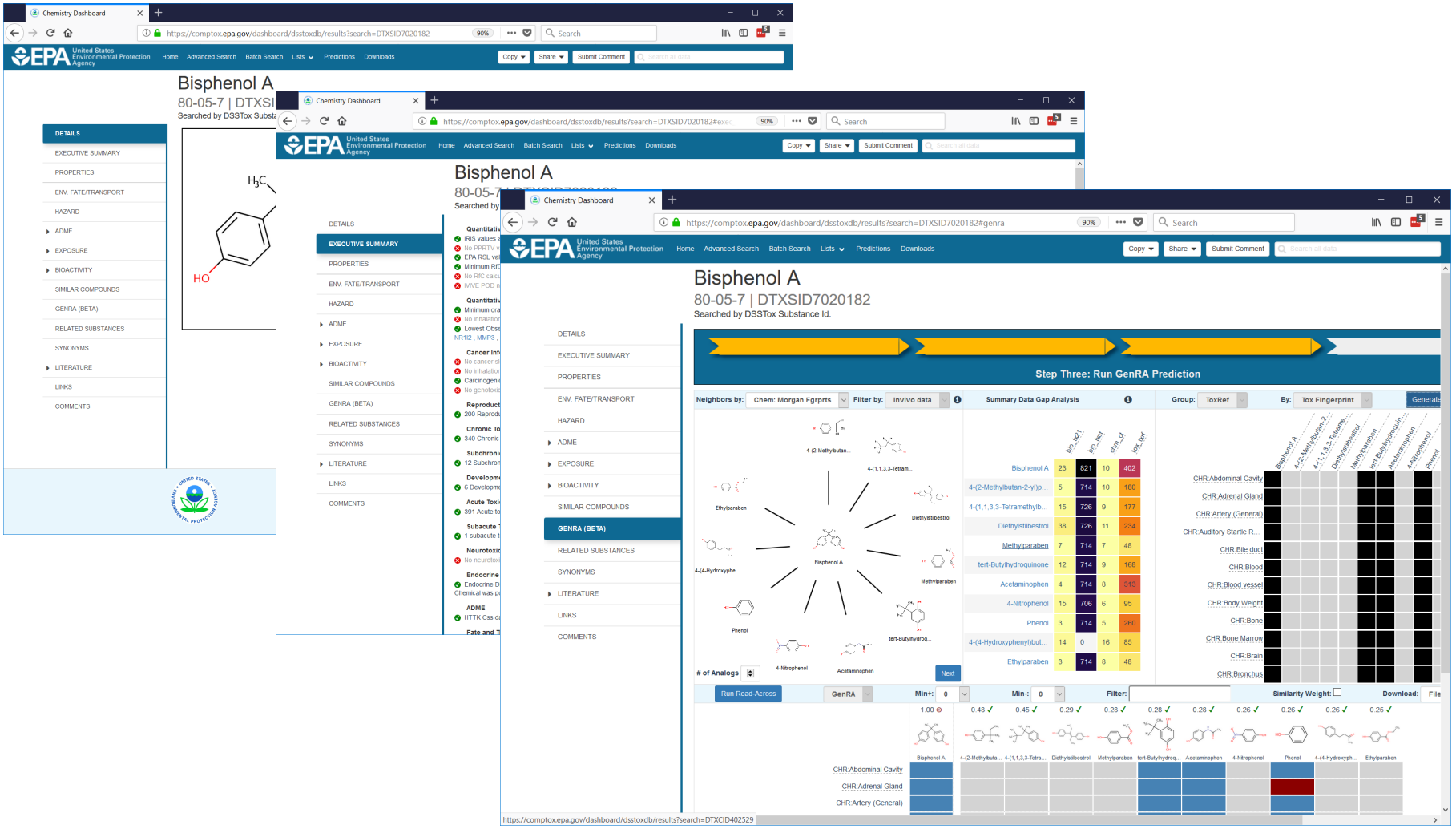


https://comptox.epa.gov/dashboard/

RapidTox Dashboard

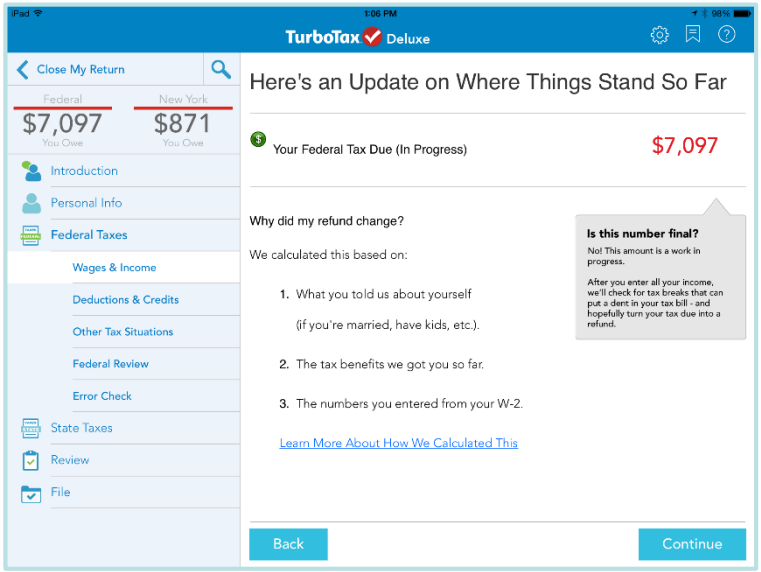


Significantly Enhanced Functionality in New Release of CompTox Chemicals Dashboard



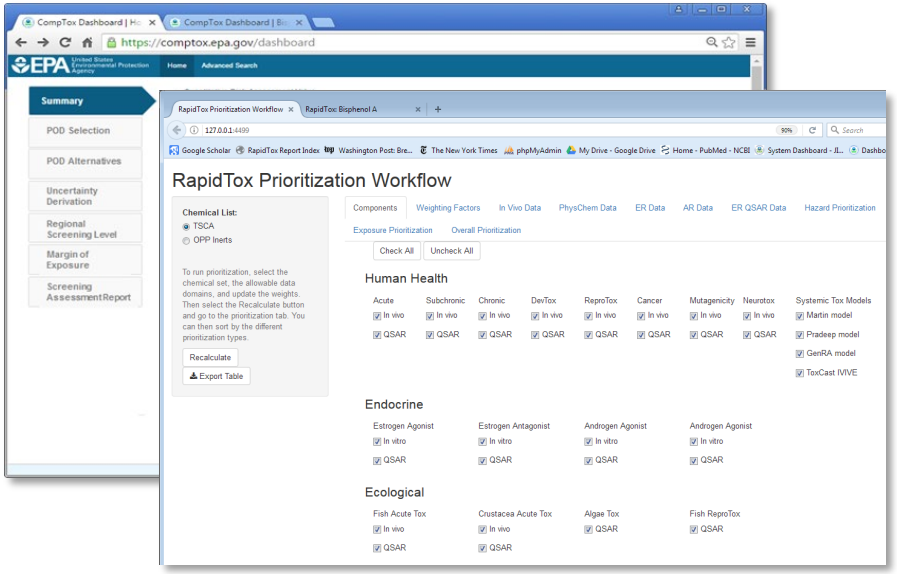
Similar to Financial Tools, RapidTox will Have Multiple Workflows to Address Different Decision Contexts

Workflow to Calculate Your Taxes



https://turbotax.intuit.com/

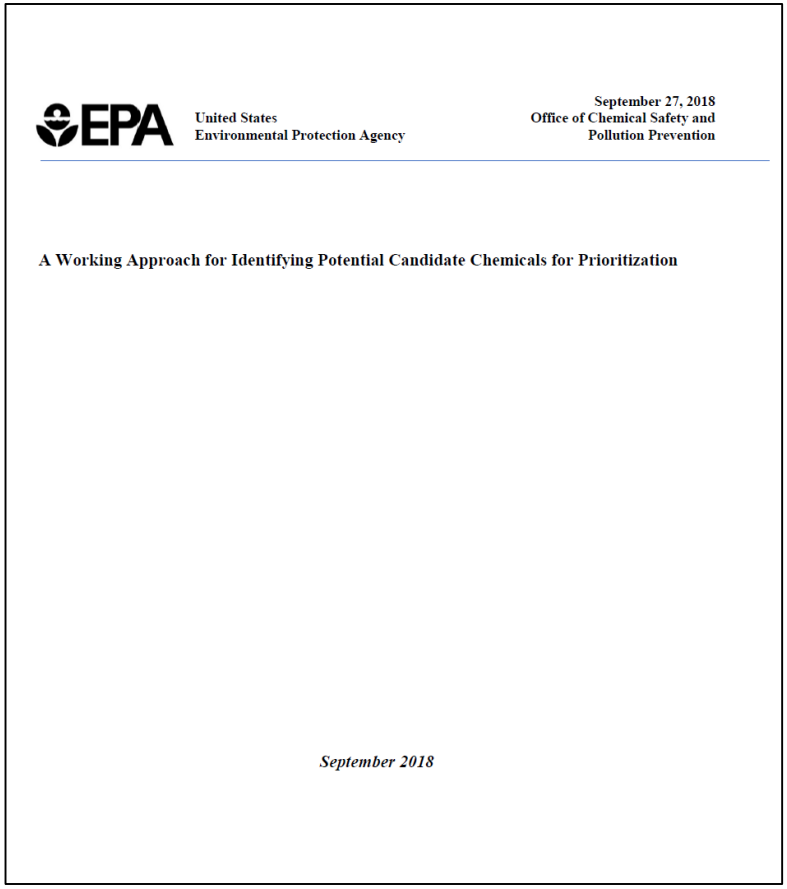
Workflows to Integrate Safety Data for Regulatory Decisions



- Semi-automated decision support workflows
- Flexible integration of information related to chemical properties, fate and transport, hazard, and exposure
- Enable expert users to review the assumptions made and refine the results
- Presents alternative data together with traditional toxicology data

R.Sams, J. Lambert, R.Judson, A.Williams, EPA Team

Beginning to Incorporate RapidTox Workflows into Regulatory Decision Making



United States
Environmental Protection Agency

September 27, 2018
Office of Chemical Safety and
Pollution Prevention

A Working Approach for Identifying Potential Candidate Chemicals for Prioritization

September 2018

https://www.epa.gov/assessing-and-managing-chemicals-under-tsca/prioritizing-existing-chemicals-risk-evaluation#prepriorization

EPA United States Environmental Protection Agency

Research Focus Areas

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Translation of Results Through Regulatory Focused Case Studies

Bloomberg BNA

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Practitioner Insights: Bringing New Methods for Chemical Safety into the Regulatory Toolbox; It is Time to Get Serious

Chemicals

The recently amended toxics law requires the EPA to take significant strides towards using non-animal safety tests for chemicals. EPA's Dr. Robert Kavlock explores this challenge and reports on a recent international workshop the agency convened that lays the groundwork for tests that can reduce reliance on animals, costs and in many cases provide better information.

DR. ROBERT KAVLOCK

Chemical Research in Toxicology

Perspective

pubs.acs.org/crt

Accelerating the Pace of Chemical Risk Assessment

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‡Healthy Environments and Consumer Safety Branch, Health Canada, Ottawa, ON K1A 0K9, Canada
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∥Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, North Carolina 27711, United States

ABSTRACT: Changes in chemical regulations worldwide have increased the demand for new data on chemical safety. New approach methodologies (NAMs) are defined broadly here as including *in silico* approaches and *in chemico* and *in vitro* assays, as well as the inclusion of information from the exposure of chemicals in the context of hazard [European Chemicals Agency, "New Approach Methodologies in Regulatory Science", 2016]. NAMs for toxicity testing, including alternatives to animal testing approaches, have shown promise to provide a large amount of data to fill information gaps in both hazard and exposure. In order to increase experience with the new data and to advance the applications of NAM data to evaluate the safety of data-poor chemicals, demonstration case studies have to be developed to build confidence in their usability. Case studies can be used to explore the domains of applicability of the NAM data and identify areas that would benefit from further research, development, and application. To ensure that this science evolves with direct input from and engagement by risk managers and regulatory decision makers, a workshop was convened among senior leaders from international regulatory agencies to identify common barriers for using NAMs and to propose next steps to address them. Central to the workshop were a series of collaborative case studies designed to explore areas where the benefits of NAM data could be demonstrated. These included use of *in vitro* bioassays data in combination with exposure estimates to derive a quantitative assessment of risk, use of NAMs for updating chemical categorizations, and use of NAMs to increase understanding of exposure and human health toxicity of various chemicals. The case study approach proved effective in building collaborations and engagement with regulatory decision makers and to promote the importance of data and knowledge sharing among international regulatory agencies. The case studies will be continued to explore new ways of describing hazard (i.e., pathway perturbations as a measure of adversity) and new ways of describing risk (i.e., using NAMs to identify protective levels without necessarily being predictive of a specific hazard). Importantly, the case studies also highlighted the need for increased training and communication across the various communities including the risk assessors, regulators, stakeholders (e.g., industry, non-governmental organizations), and the general public. The development and application of NAMs will play an increasing role in filling important data gaps on the safety of chemicals, but confidence in NAMs will only come with learning by doing and sharing in the experience.

Accelerating the Pace of Chemical Risk Assessment APCRA

CONTENTS

1. OVERVIEW

The modernization of the U.S. Toxic Substances Control Act (TSCA), the implementation of European Union's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the next phase of the Canadian Chemicals Management Plan (CMP), and many international chemical management policies and laws have escalated the demand for data on the safety of chemicals. To meet this demand, a variety of new data streams—in hazard, exposure, and dose evaluation—are being considered to augment traditional toxicology data which has mostly relied on animal models. The new data are diverse and include data from high-throughput toxicity and toxicokinetic testing, molecular epidemiology, toxicogenomics, exposure science, computational chemistry, and new animal models, among others.

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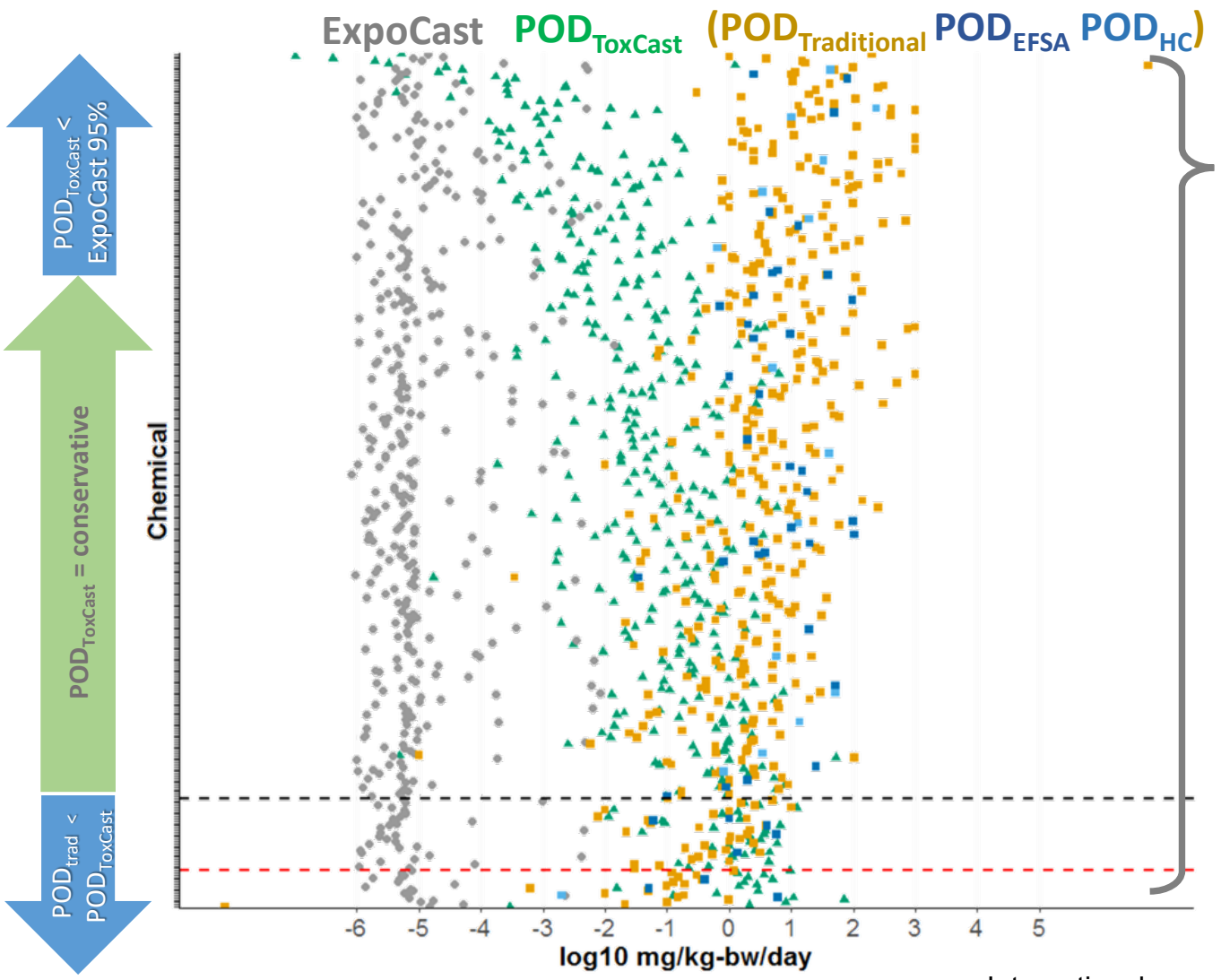
- Multiple international case studies stemming from 2016 inter-governmental workshop
- Example: *In Vitro* Bioactivity as a Conservative Point of Departure
- Participants include EPA, Health Canada, ECHA, EFSA, JRC, and A*STAR
- Goal: Determine whether *in vitro* bioactivity from broad high-throughput screening studies (e.g., ToxCast) can be used as a conservative point-of-departure and when compared with exposure estimates serve to prioritize chemicals for future study or as lower tier risk assessment.

Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL



Total =

448 chemicals

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

For ~92% of the chemicals, POD$_{ToxCast}$ was conservative.

(~100-fold with human HTTK

~50-fold with rat HTTK)

Less conservative than TTC

International case study with EPA, ASTAR, ECHA, Health Canada, and EFSA

EPA United States Environmental Protection Agency

Take Home Messages…

- Applying and refining new technologies for comprehensively evaluating toxicological space at significantly less cost
- Systematically addressing previous technical limitations such as a lack of metabolism, limited chemical space, and organ/tissue effects
- Making progress in integrating new technologies into tiered toxicity testing framework
- Rigorously characterizing uncertainties and variability in both *in vitro* test systems and traditional *in vivo* models
- Enabling application of new technologies to chemical safety decisions through delivery and integration using a broad range of IT tools
- Partnering with regulators on case studies to increase confidence and accelerate application to chemical risk assessment

Acknowledgements and Questions

Tox21 Colleagues:
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- FDA
- NCATS

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- NHEERL
- NCEA

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- A*STAR
- ECHA
- EFSA
- Health Canada
- JRC

EPA's National Center for Computational Toxicology