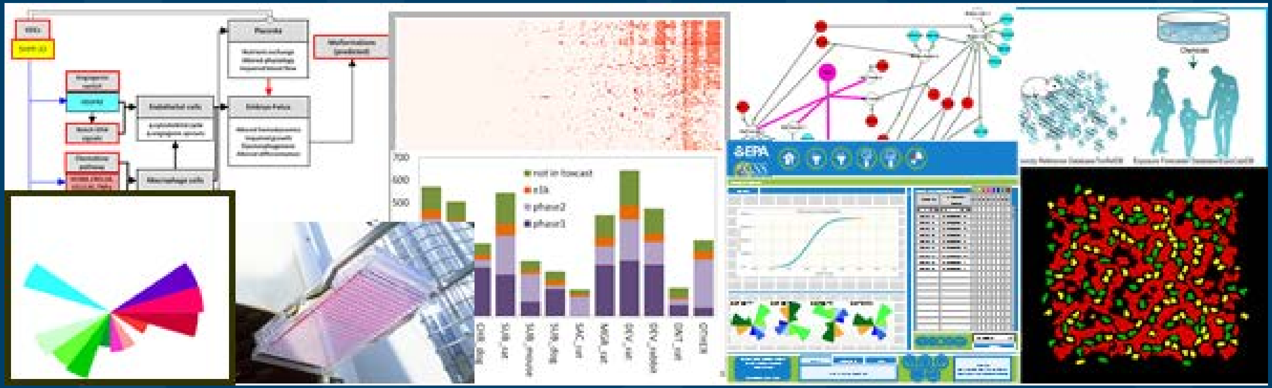
Accelerating the Development and Use of High-Throughput Screening Data for Application to Regulatory Risk Assessments



SETAC Focused Topic Meeting on High Throughput Screening and Environmental Risk Assessment

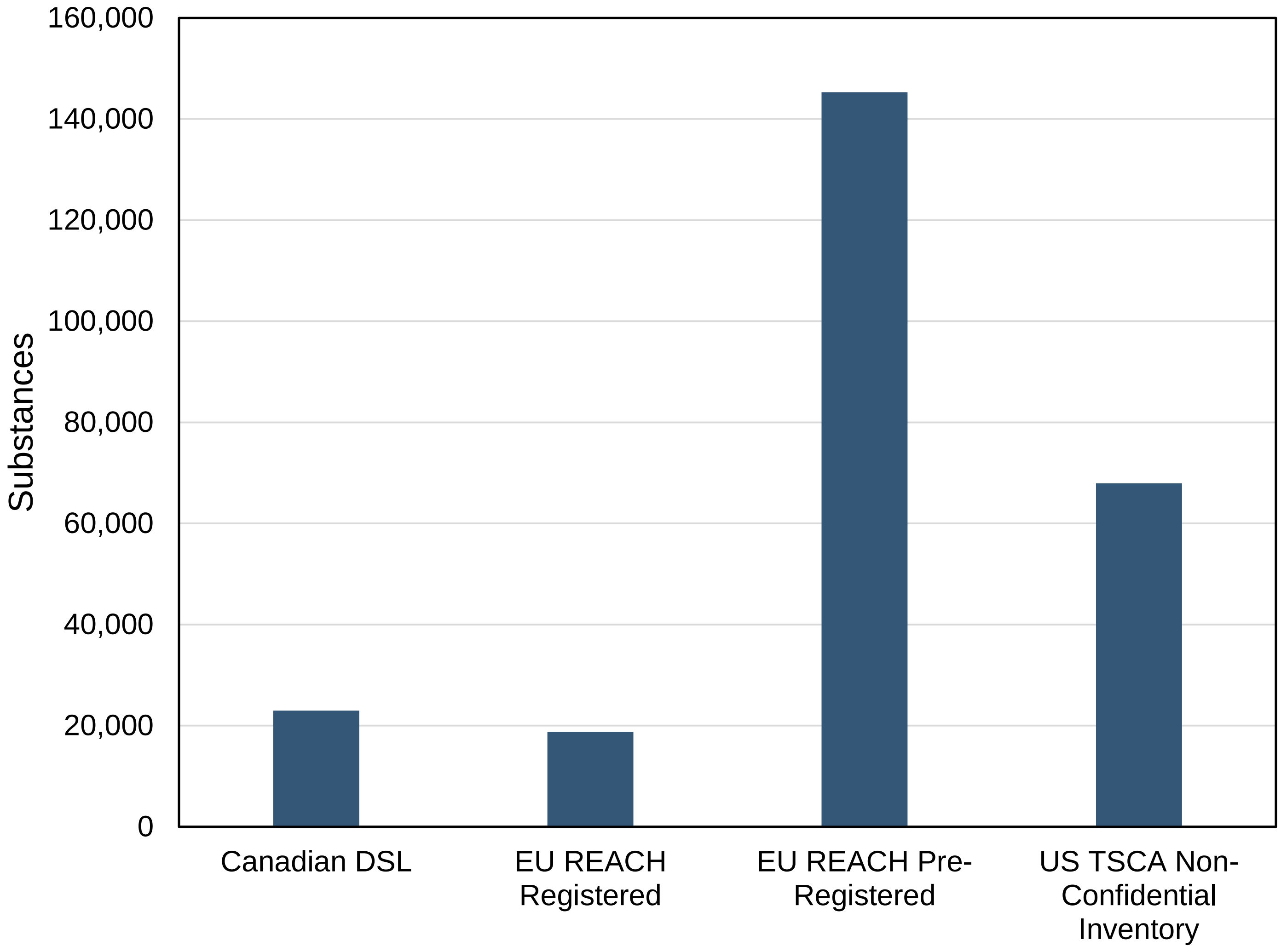
April 16, 2018

Rusty Thomas
Director
National Center for Computational Toxicology

What Do Grandmothers, Regulators, and Toxicologists Have in Common?

http://talesfromthecircus.com/hissing-grandmother-roaming-hands/

Large Numbers of Chemicals in Commerce



Lack of Toxicity Data

United States Environmental Protection Agency

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

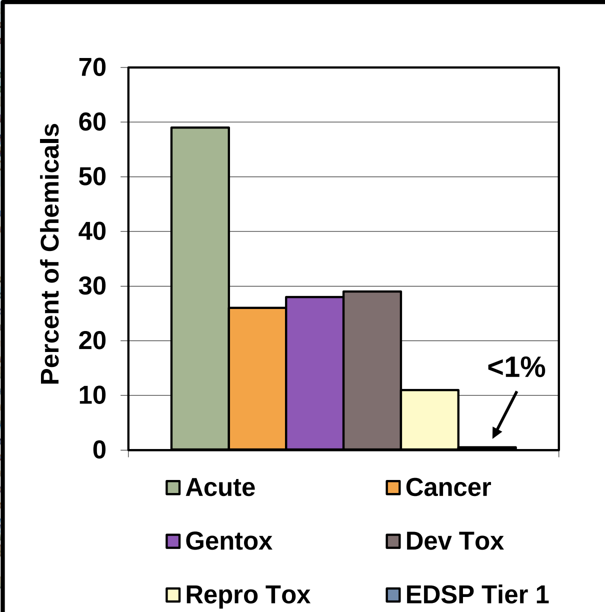
National Research Council

NATIONAL ACADEMY PRESS
Washington, D.C. 1984

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

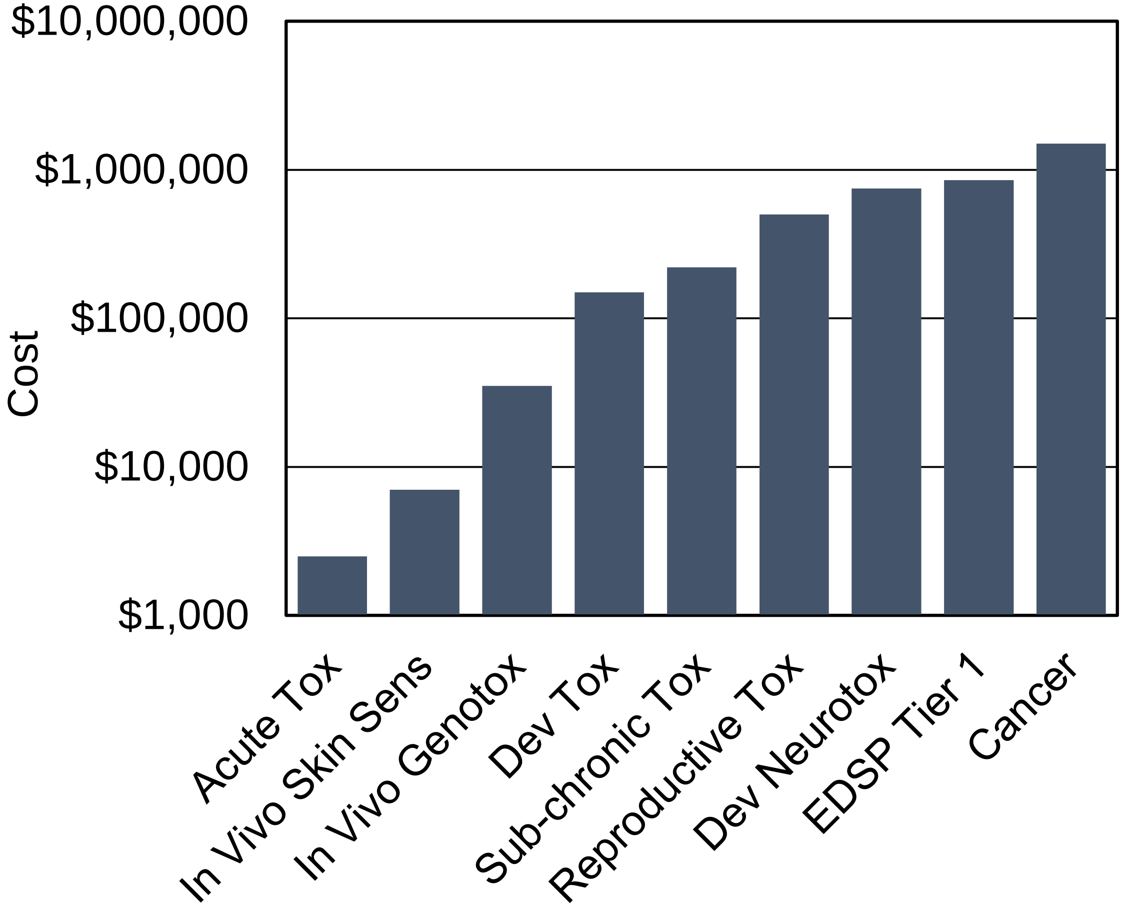
The Toxicity Data Landscape for Environmental Chemicals

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]



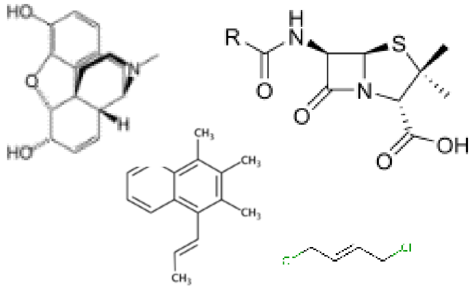
Modified from Judson *et al.*, EHP 2009

Costs of Traditional Toxicity Testing



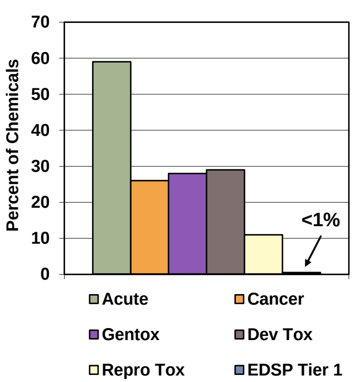
How Can High-Throughput Approaches Address These Challenges?

Number of Chemicals /Combinations



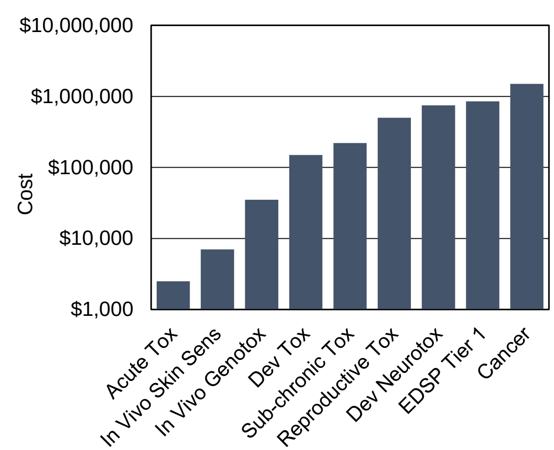
Comprehensive Tox Evaluation

Limited Data

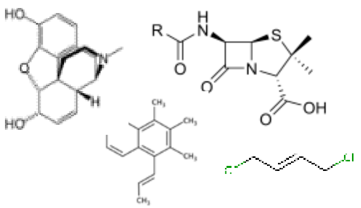


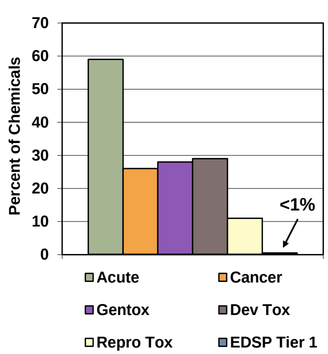
Modified from Judson *et al.*, EHP 2009

Economics



Key Steps in Satisfying Grandma, Toxicologists, and Regulators



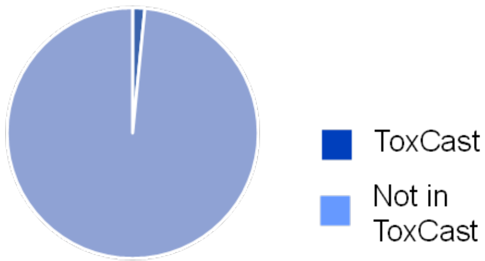


Modified from Judson *et al.*, EHP 2009

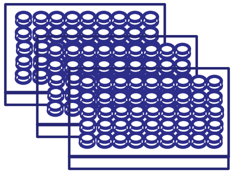
- Systematically addressing limitations in alternative test systems
- Continue putting results in a dose/exposure context
- Characterize of uncertainty
- Emphasize development of computational models to integrate experimental data
- Deliver of data and models through decision support tools
- Translation of results through regulatory focused case studies

Some Existing Limitations in High Throughput and *In Vitro* Test Systems

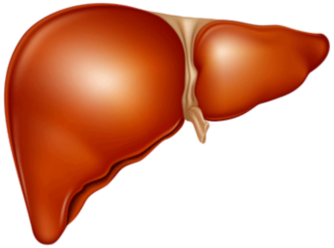
Biological Coverage (Gene Basis)



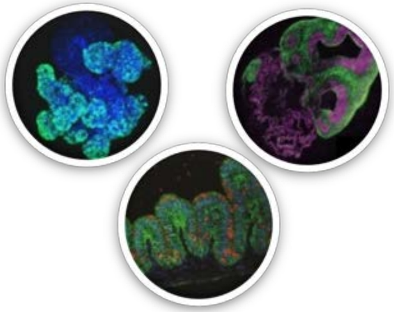
Chemical Coverage and Specific Chemical Types (e.g., VOCs)



Metabolic Competence

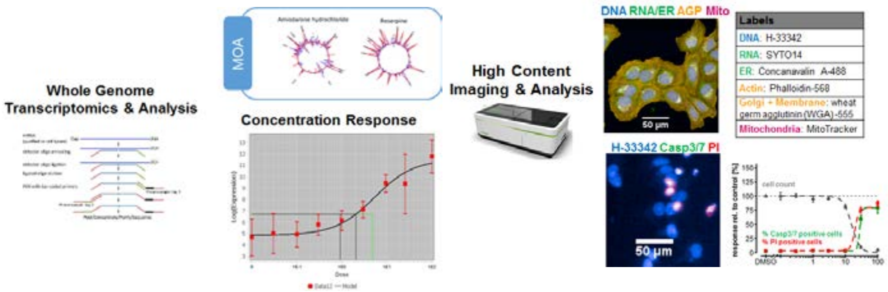


Organ and Tissue Responses

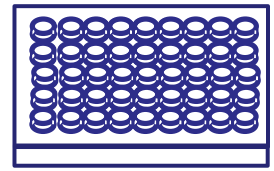


Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptional and Phenotypic Profiling



VOC *In Vitro* Exposure System and Water Library

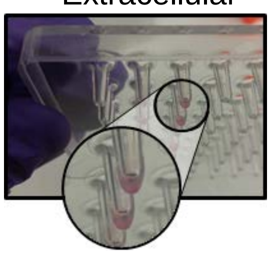


Initial test library of ~70 chemicals

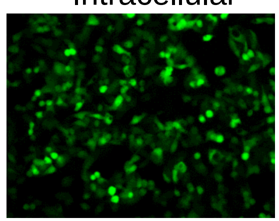
M. Higuchi (EPA-NHEERL)

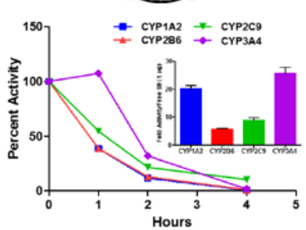
Assay Retrofit for Metabolism

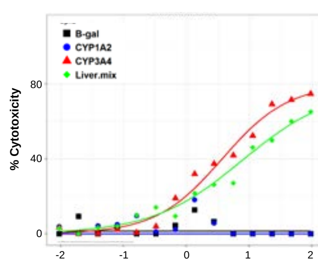
Extracellular



Intracellular







Collaboration with Unilever

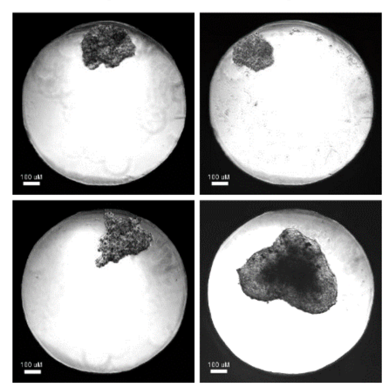
Organotypic Model Development and Virtual Tissue Modeling

3D Organoid Culture

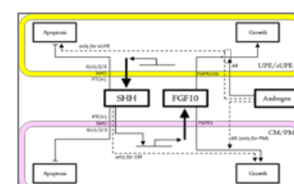
Day 0 Day 10

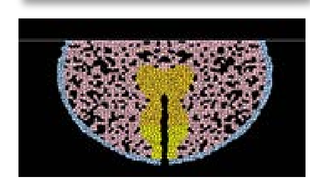
CS-FBS

FBS



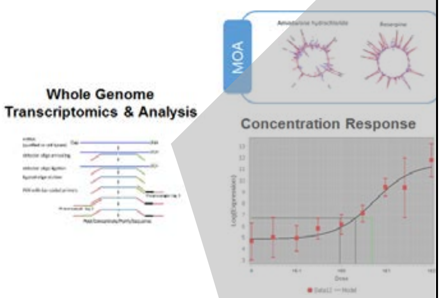
Computational Modeling





Systematically Addressing Limitations in Alternative Test Systems

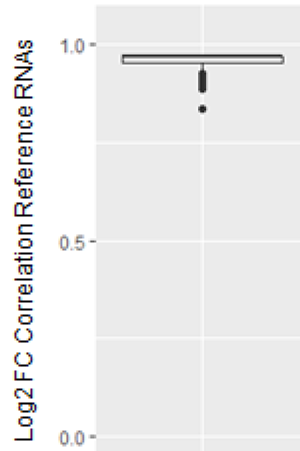
High-Throughput Tra[nscriptional and] Phenotypic I[...]

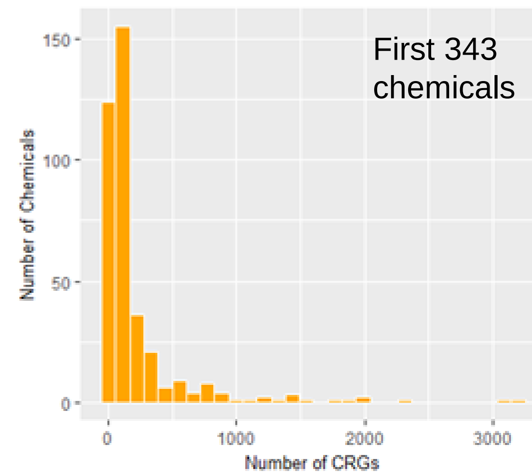


VOC *In Vitro* Exposure System and Water Library

- Whole transcriptome
- Low cost
- 384-well cell lysate
- Performance controls each plate

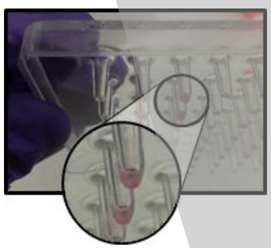
- 2,000 chemical screen
- First cell type (MCF7)
- 8 point conc response
- N = 3 reps

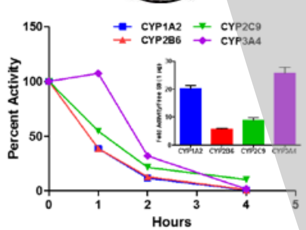


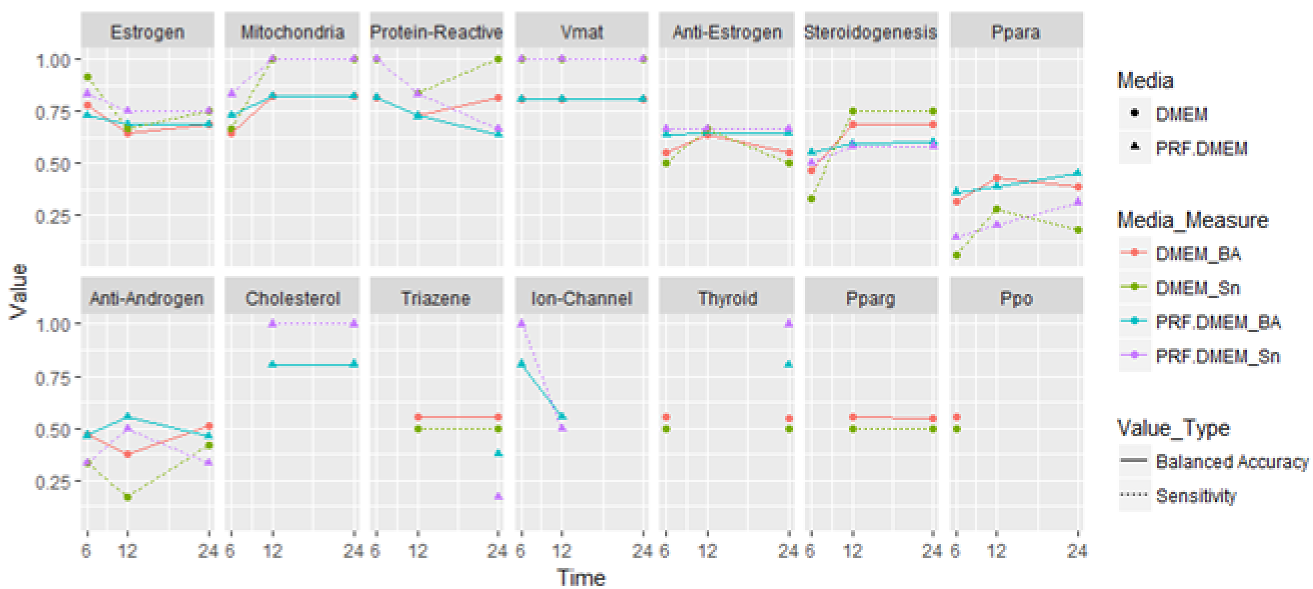


Assay Retrofit

Extracellular



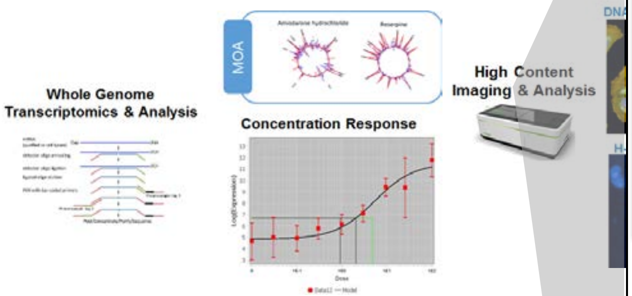




Collaboration with Unilever

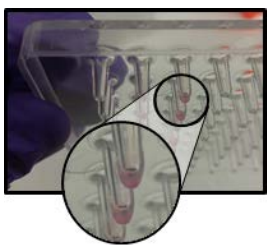
Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptiona… Phenotypic Profiling

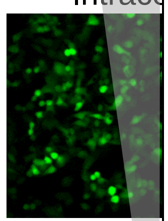


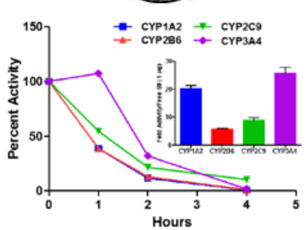
Assay Retrofit for Metabo…

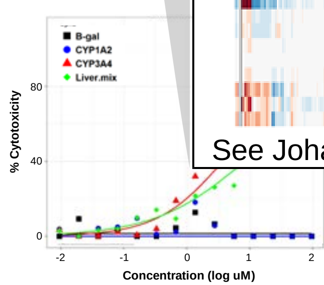
Extracellular



Intrace…







Collaboration with Unilever

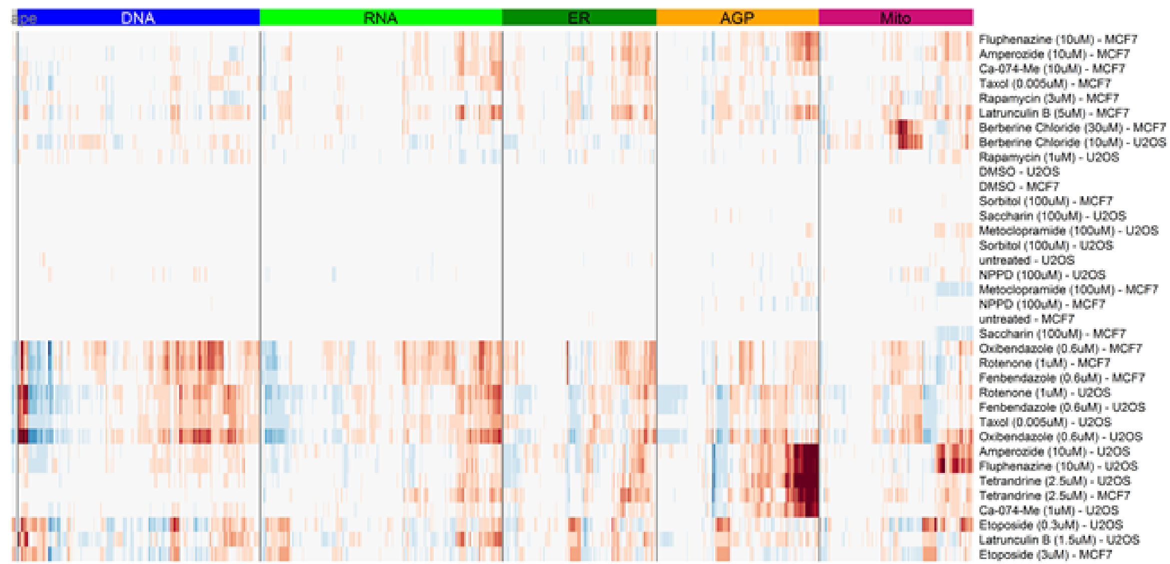
Experimental Design
2 cell types: U-2 OS / MCF7
384-well plates
16 chemicals
7 concentrations (3 $\log_{10}$ units)
3 replicates / plate
3 independent experiments

Image Analysis of 1669 Endpoints

compartments:
- nuclei
- ring
- cytoplasm
- membrane
- cell

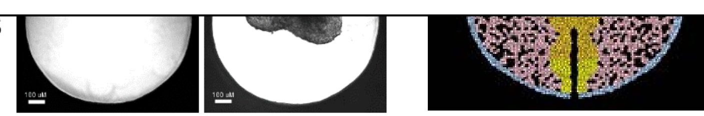
channels (organelles):
- DNA
- RNA
- ER
- AGP (actin skeleton / Golgi/ plasma membrane)
- mitochondria

parameters:
- intensity
- texture
- morphology:
  - symmetry
  - compactness
  - axial
  - radial
  - profile



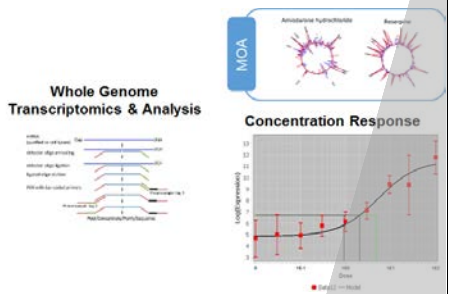
See Johanna Nyffeler Poster

FBS

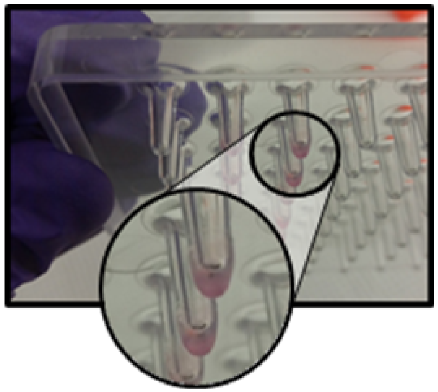


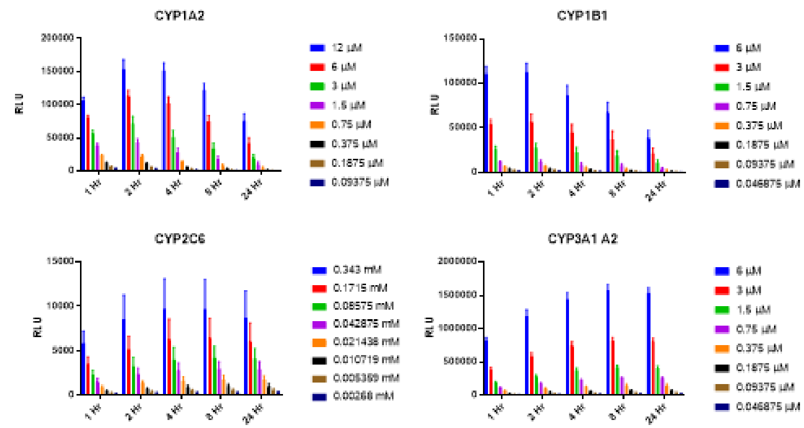
Systematically Addressing Limitations in Alternative Test Systems

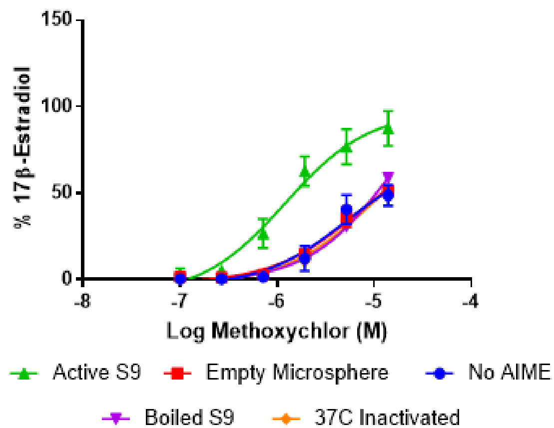
High-Throughput Tra... Phenotypic P...



Assay Retrofit ...
Extracellular

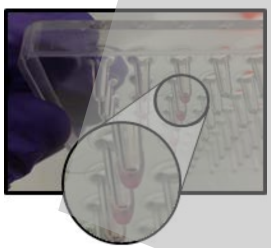


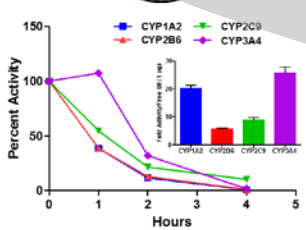


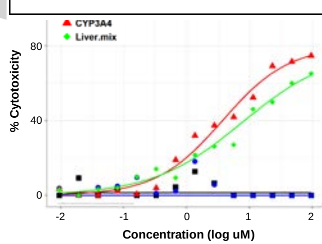


96-Well	EC50 (µM)	EC50 Potency Shift
Active S9	0.71	
Heat Inactivated S9	6.8	9.6
No AIME	4.98	7.0
384-Well	**EC50 (µM)**	**EC50 Potency Shift**
Active S9	1.2	
Heat Inactivated S9	15.9	13.2
No AIME	5.1	4.2

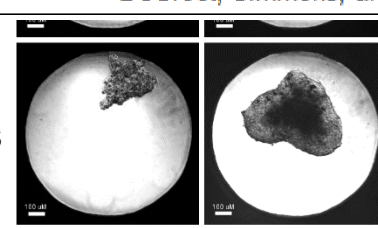
DeGroot, Simmons, and Deisenroth, Unpublished

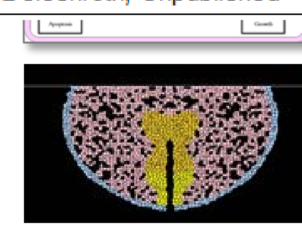






Collaboration with Unilever



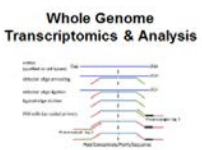


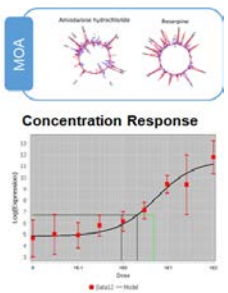
FBS

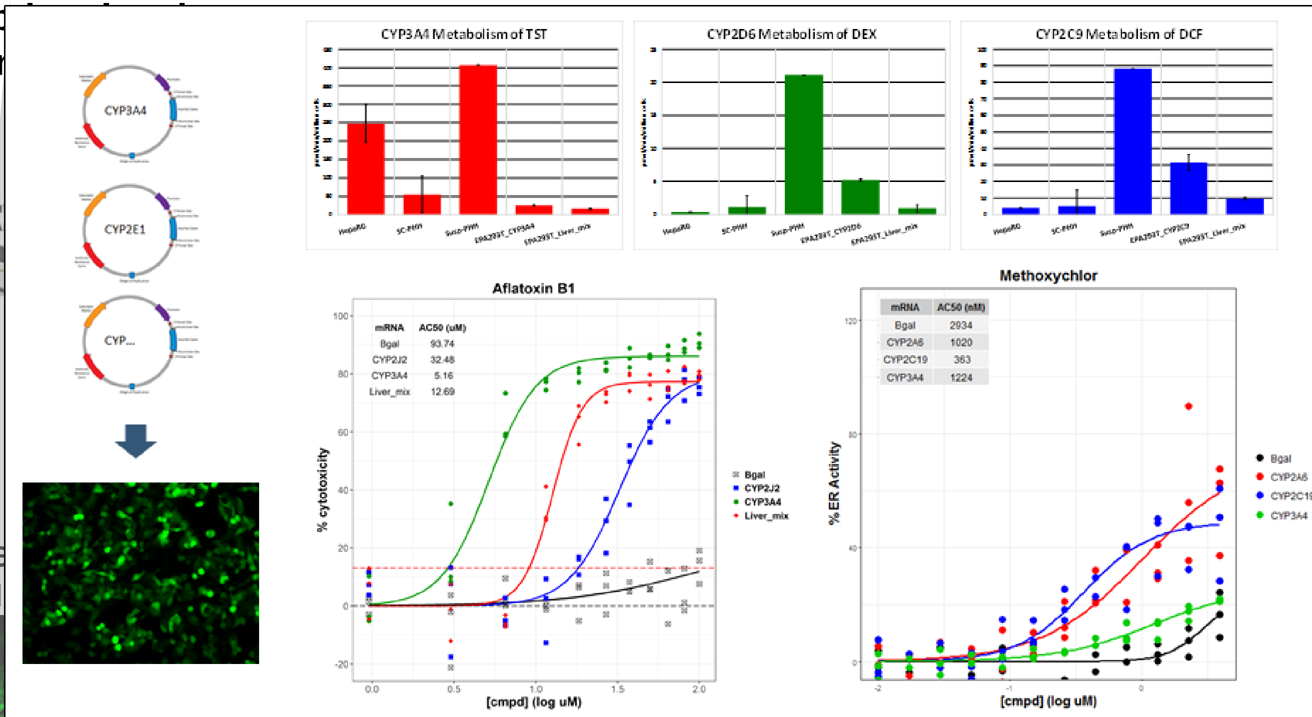
United States Environmental Protection Agency

Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcrip… Phenotypic Profilin…



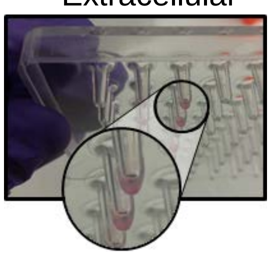


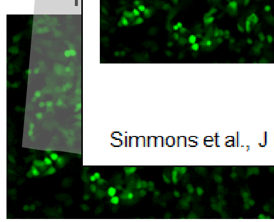


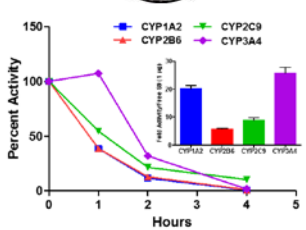
Simmons et al., J Pharmacol Tox Methods, 2018

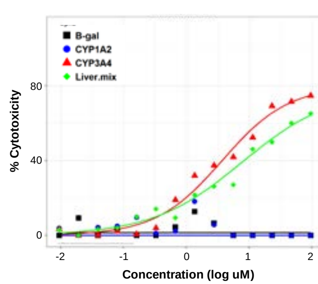
Assay Retrofit for Me…

Extracellular





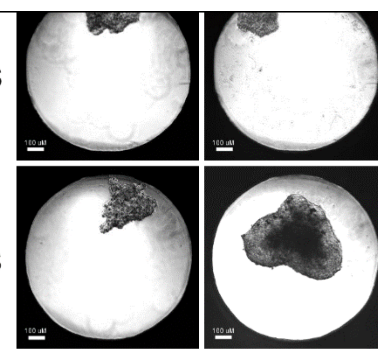


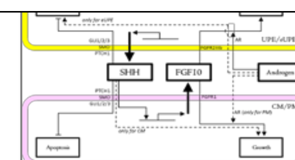


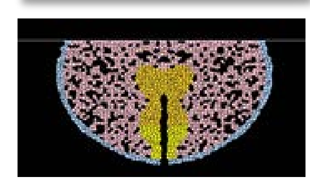
Collaboration with Unilever

CS-FBS

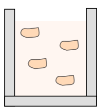
FBS







Putting Alternative Test Results in a Dose and Exposure Context

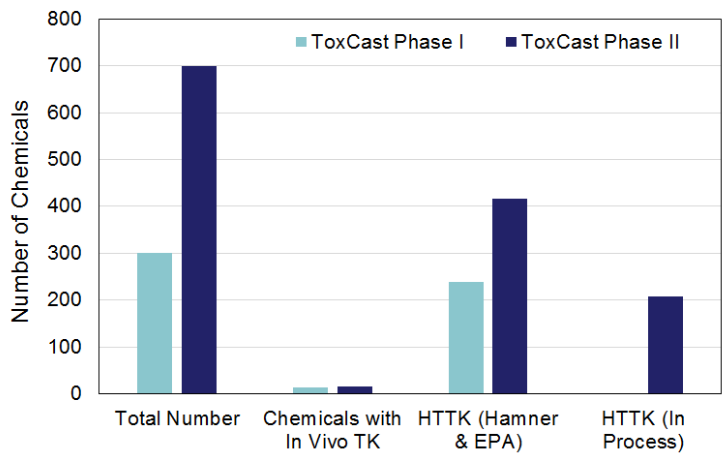


Liver Metabolism

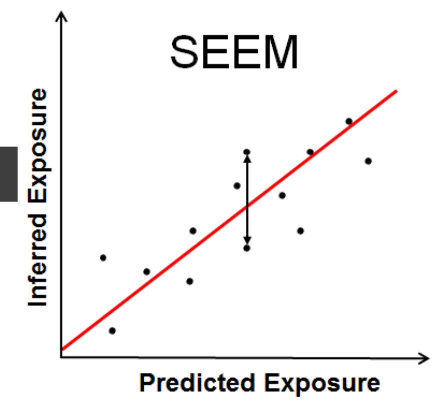
Plasma Protein Binding

Population-Based IVIVE Model

Oral Dose Required to Achieve Steady State Concentrations Equivalent to *In Vitro* Bioactivity



Chemical Use and Production Volume



Biomonitoring Data

Rotroff *et al.*, *Tox Sci.*, 2010
Wetmore *et al.*, *Tox Sci.*, 2012
Wetmore *et al.*, *Tox Sci.*, 2015

Wambaugh *et al.*, 2014

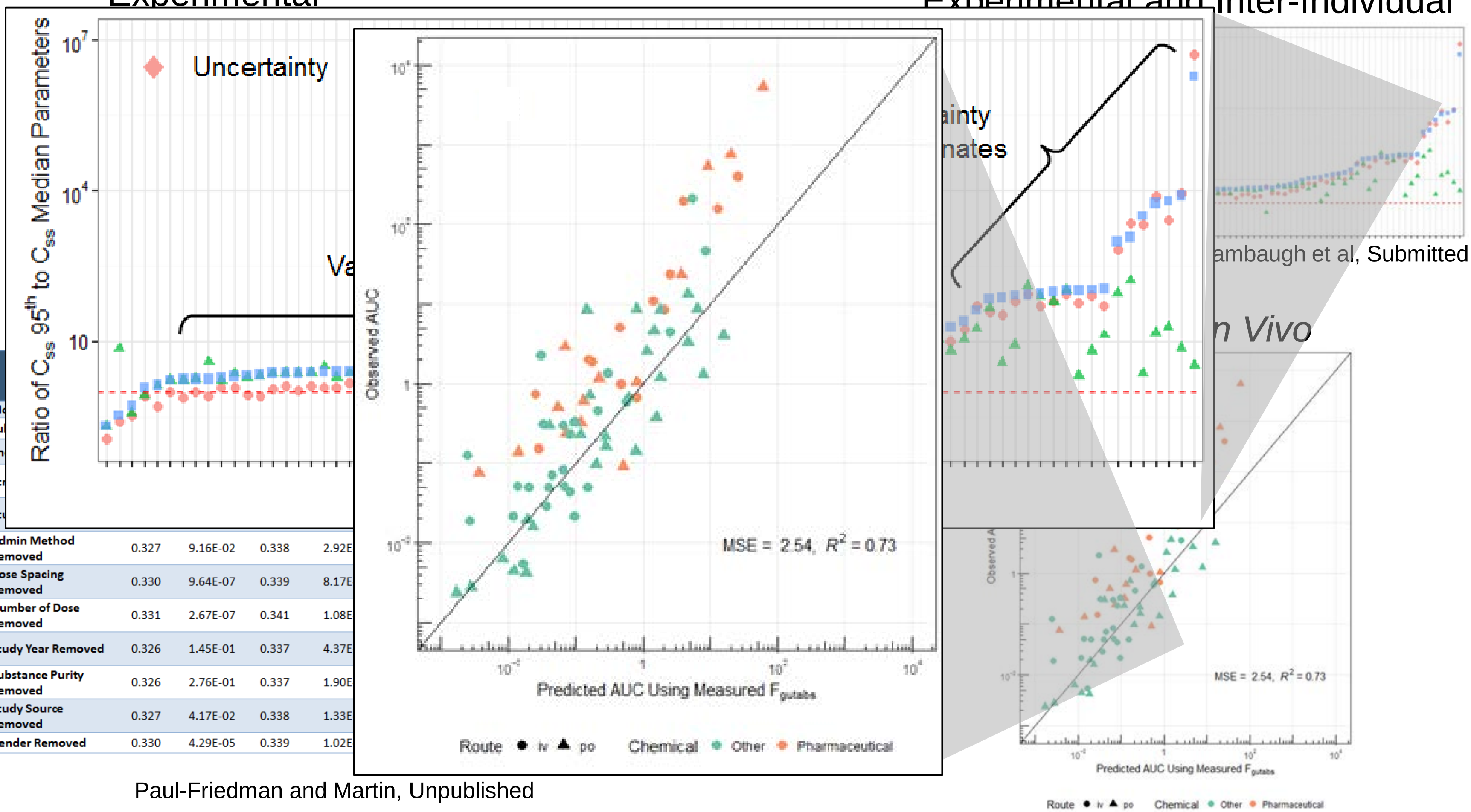
Quantifying Uncertainty and Variability

Pharmacodynamic

Pharmacokinetic

Experimental

Experimental and Inter-Individual



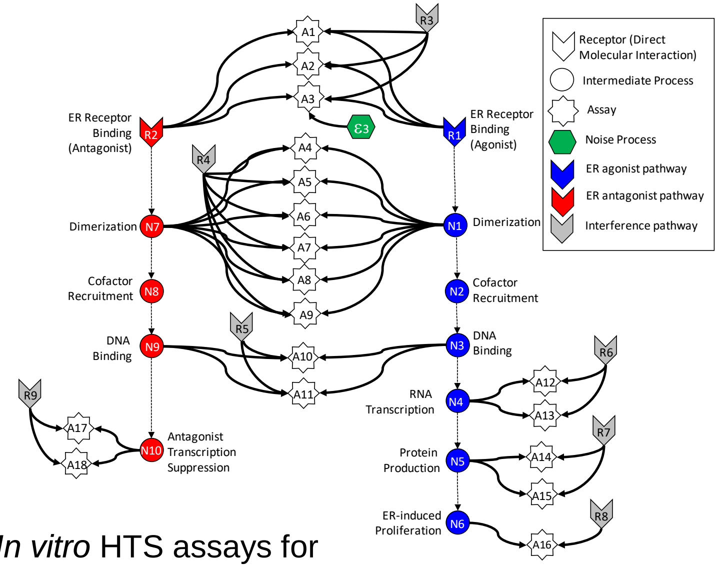
Mo				
Fu				
Ch				
Str				
Stu				
Admin Method Removed	0.327	9.16E-02	0.338	2.92E
Dose Spacing Removed	0.330	9.64E-07	0.339	8.17E
Number of Dose Removed	0.331	2.67E-07	0.341	1.08E
Study Year Removed	0.326	1.45E-01	0.337	4.37E
Substance Purity Removed	0.326	2.76E-01	0.337	1.90E
Study Source Removed	0.327	4.17E-02	0.338	1.33E
Gender Removed	0.330	4.29E-05	0.339	1.02E

Paul-Friedman and Martin, Unpublished

Wambaugh et al, Tox Sci, 2018

Computational Modeling to Integrate Experimental Data

Computational Modeling of Estrogen Receptor Pathway



18 *In vitro* HTS assays for ER bioactivity

In Vitro Reference Chemicals*

Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

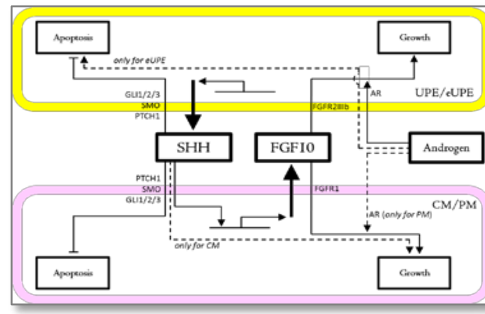
In Vivo Reference Chemicals*

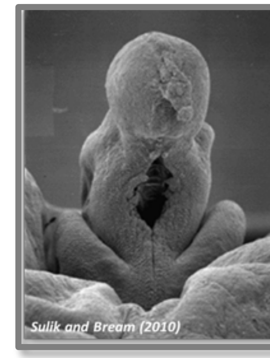
Accuracy	**0.86 (0.95)**
Sensitivity	**0.97 (0.97)**
Specificity	**0.67 (0.89)**

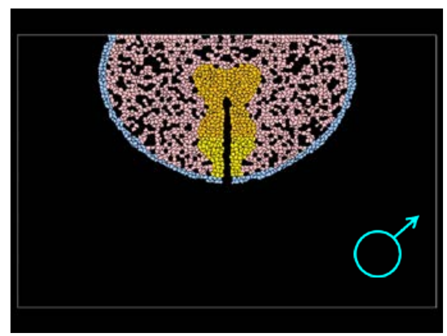
*Values in parentheses exclude inconclusive chemicals

Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T.* 2015

Computational Modeling of Genital Tubercle Development







GD13.5 – 17.5

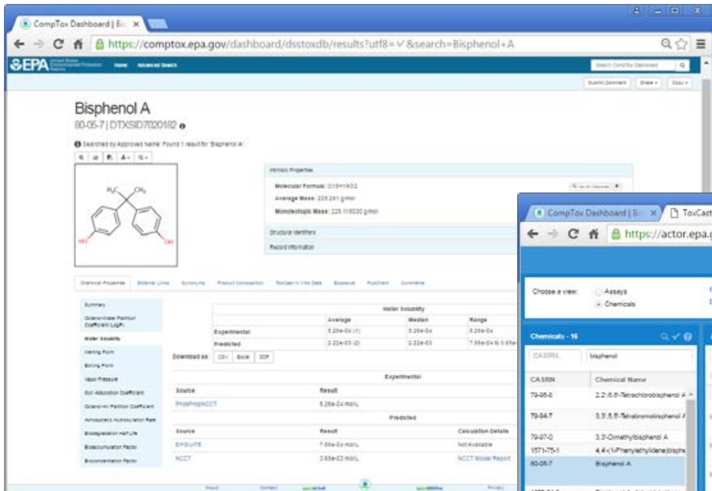
Androgenization *(n = 10 sims)*	***Phenotype (MCS 4000)*** Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

Leung et al., *Repro Toxicol*, 2016

United States Environmental Protection Agency

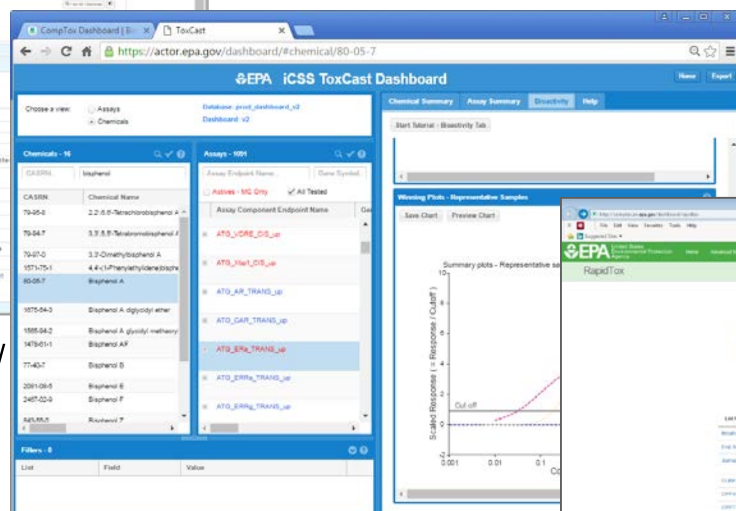
Deliver Data and Models Through Decision Support Tools

Comptox Chemistry Dashboard



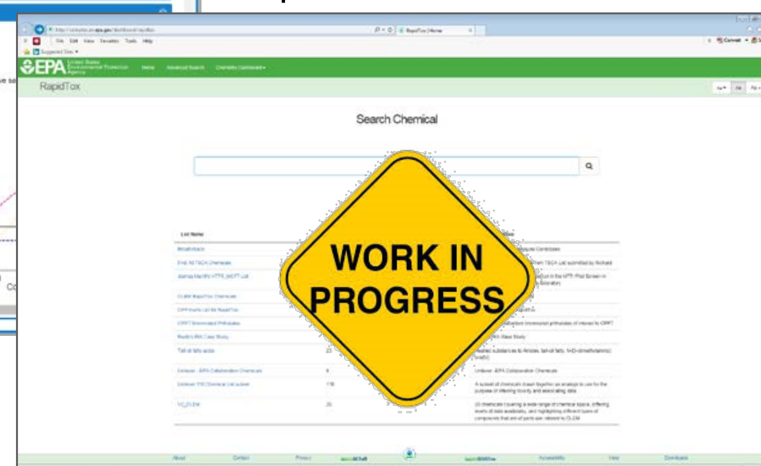
https://comptox.epa.gov/dashboard/

ToxCast Dashboard

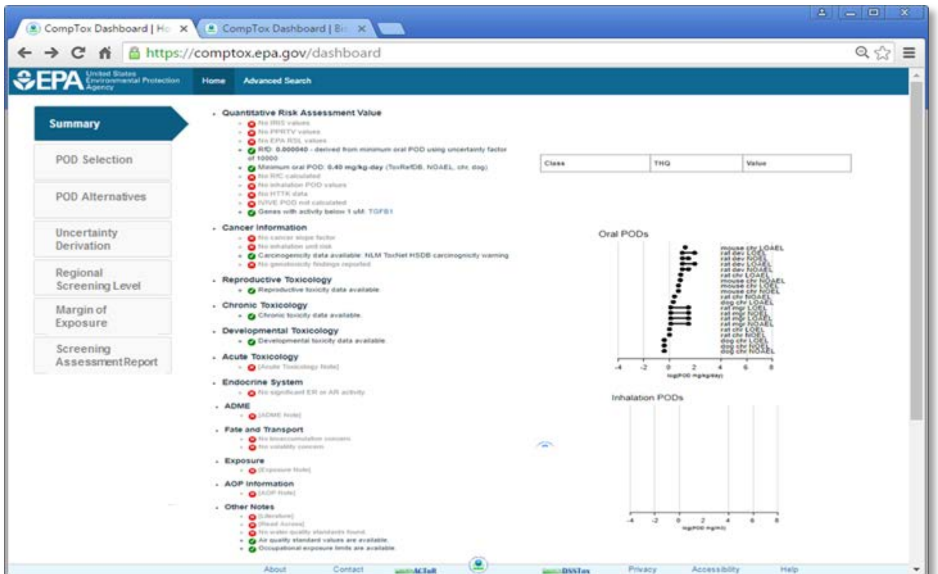


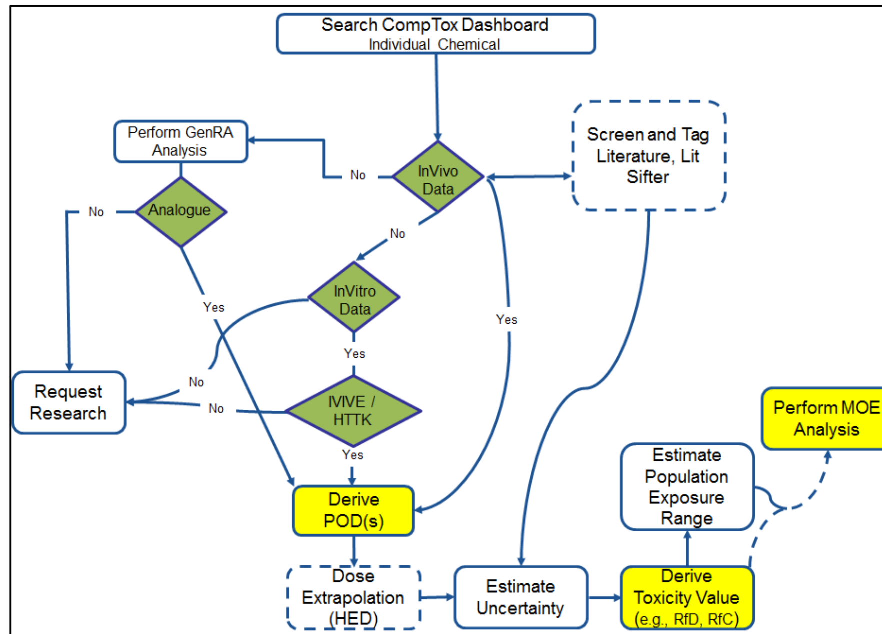
https://actor.epa.gov/dashboard/

RapidTox Dashboard



One Tool, Multiple Workflows to Address Diverse Partner Needs

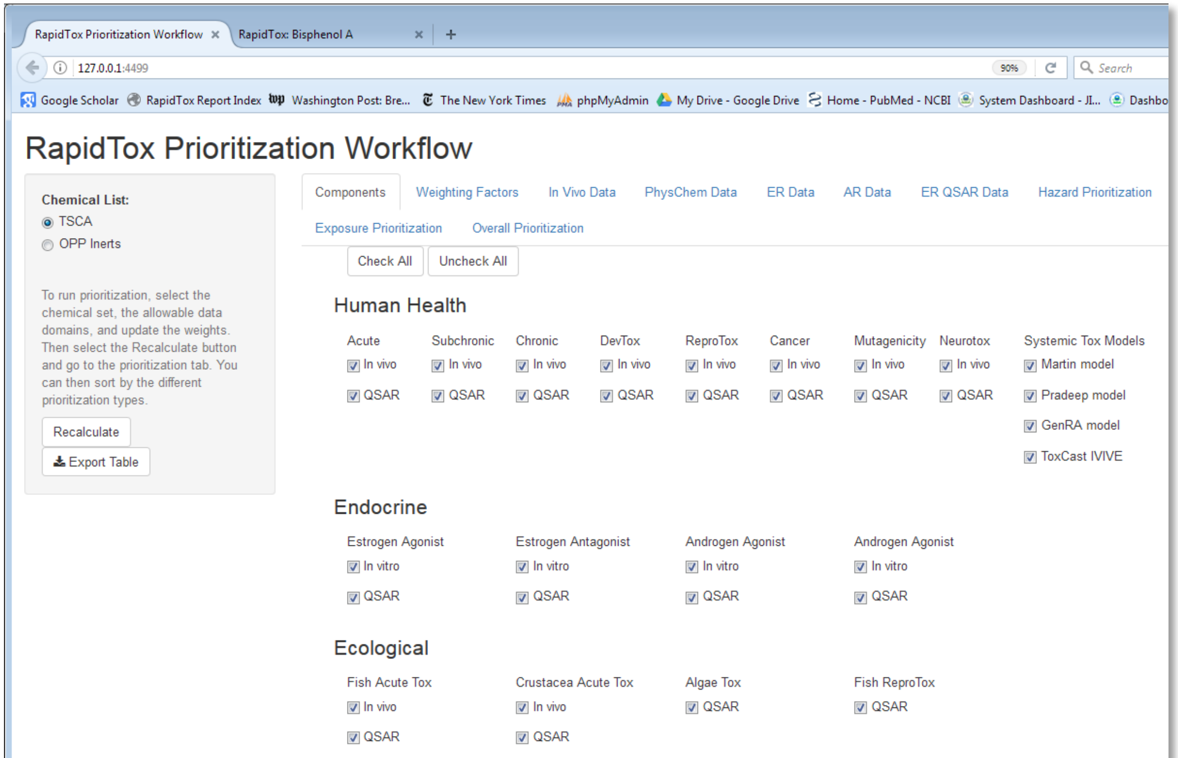


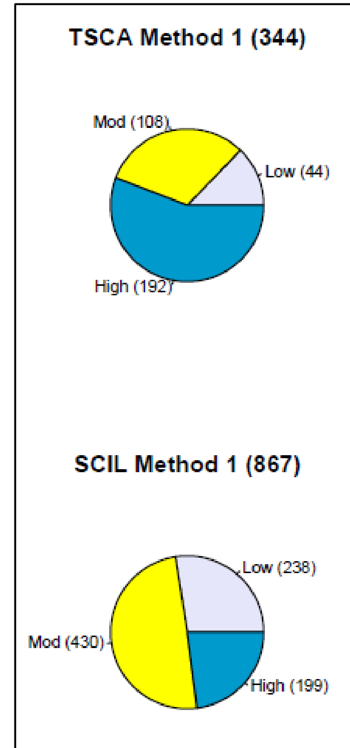


- Semi-automated decision support tool
- Flexible integration of information related to chemical properties, fate and transport, hazard, and exposure
- Enable expert users to review the assumptions made and refine the results
- Presents alternative together with traditional toxicology data

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

One Tool, Multiple Workflows to Address Diverse Partner Needs





EPA-HQ-OPPT-2017-0586

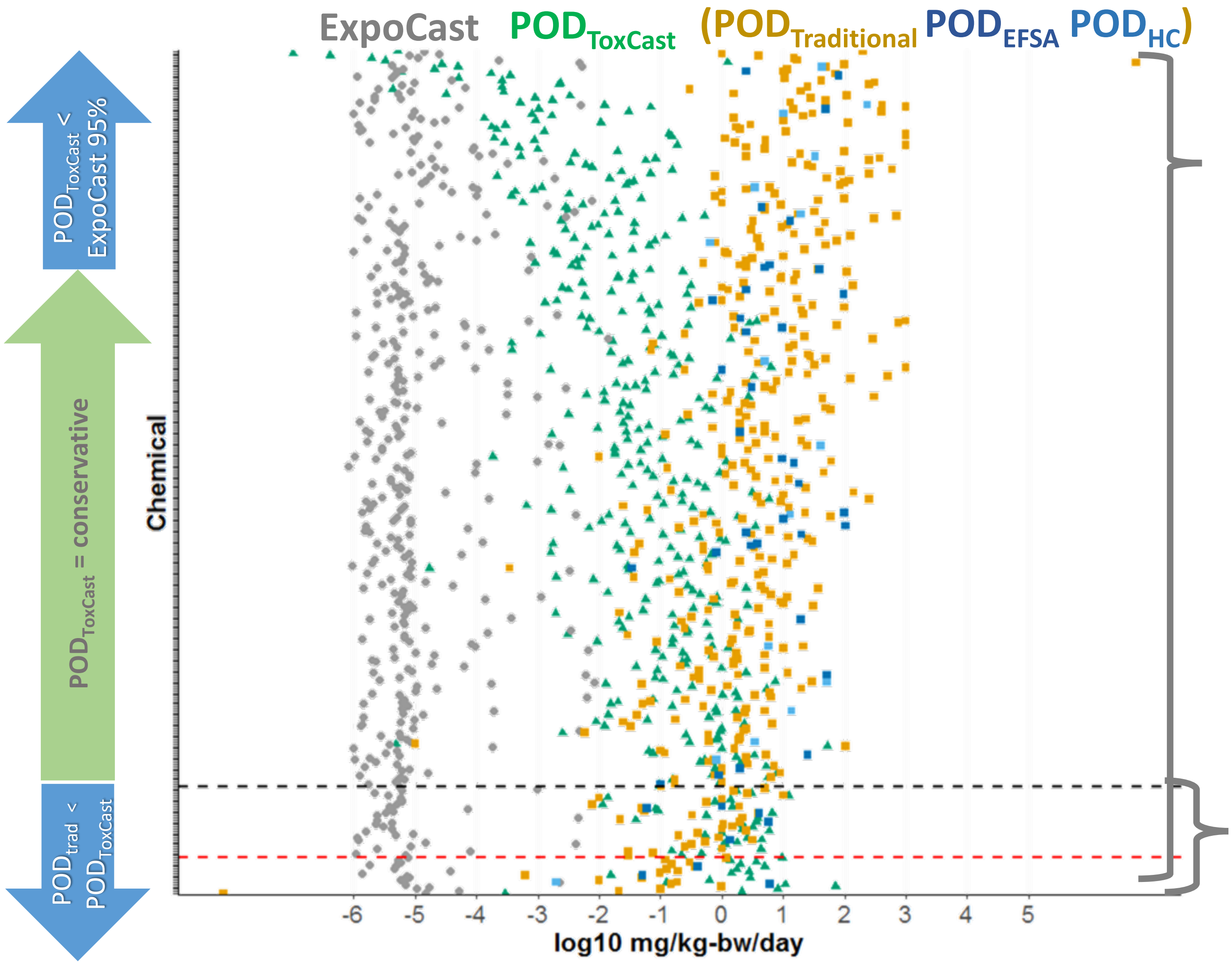
- Flexible exploration of multiple prioritization scenarios
- Selection and weighting of different data streams
- Outputs ordinal and binned prioritization
- Proposed to selected candidates for TSCA prioritization

R.Judson, C.Grulke, EPA Team

Translation of Results Through Regulatory Focused Case Studies

- 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 = 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?

Take Home Messages…

Grandma Approved

https://www.freepik.com/free-photo/senior-woman-with-a-thumbs-up_1014676.htm

- 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 with 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

Acknowledgements and Questions

Tox21 Colleagues:
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- FDA
- NCATS

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- NHEERL
- NCEA

Collaborative Partners:
- Unilever
- A*STAR
- ECHA
- EFSA
- Health Canada
- JRC

EPA's National Center for Computational Toxicology