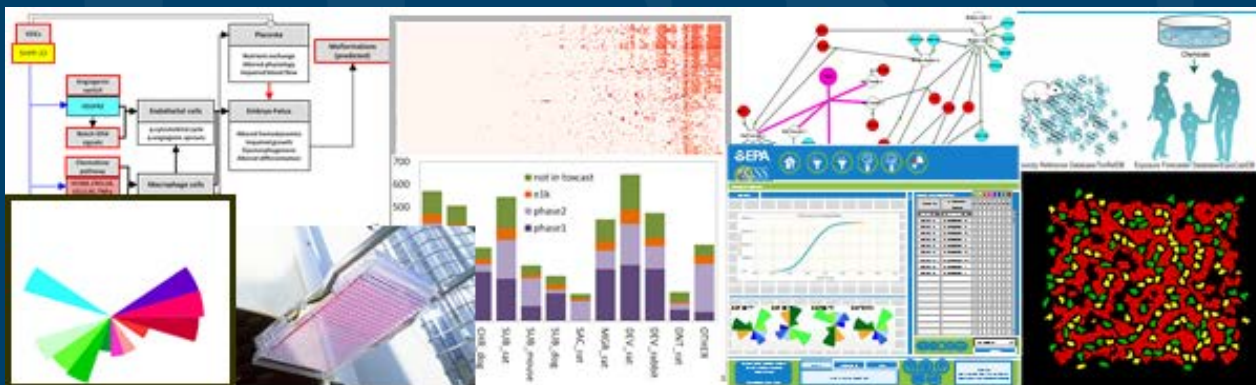


Accelerating the Development and Use of High-Throughput Screening Data for Application to Regulatory Risk Assessments

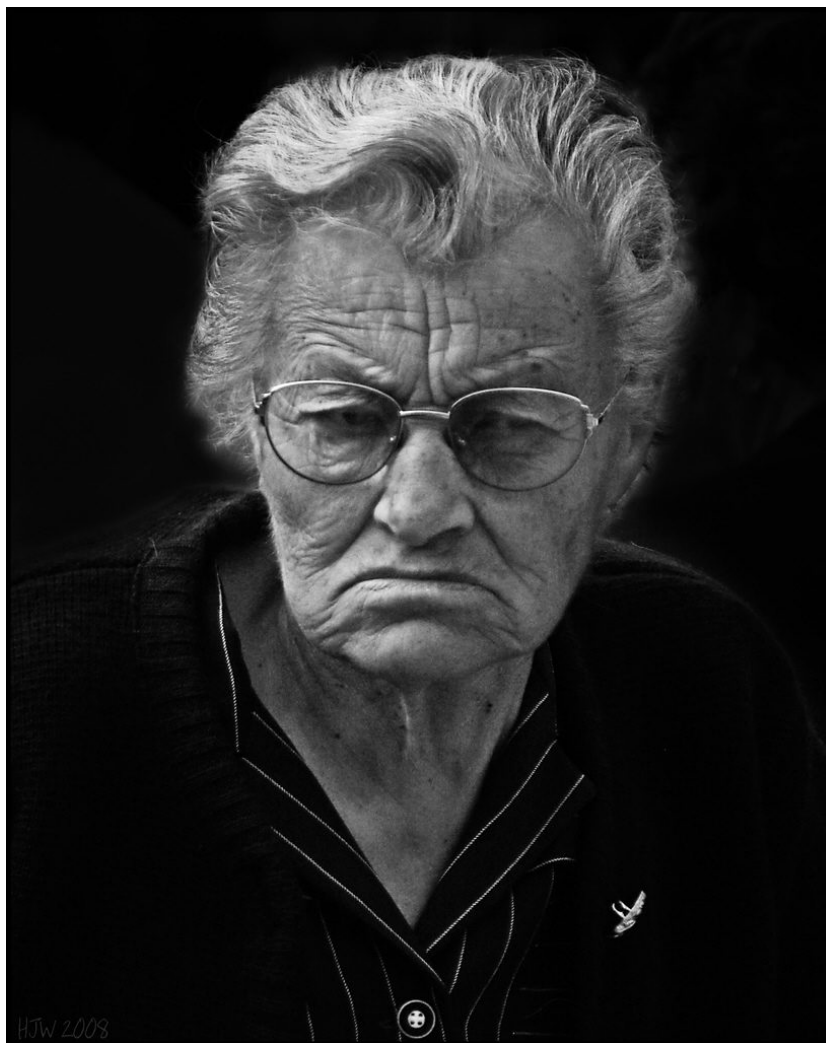


SETAC Focused Topic Meeting on High Throughput Screening and Environmental Risk Assessment

April 16, 2018

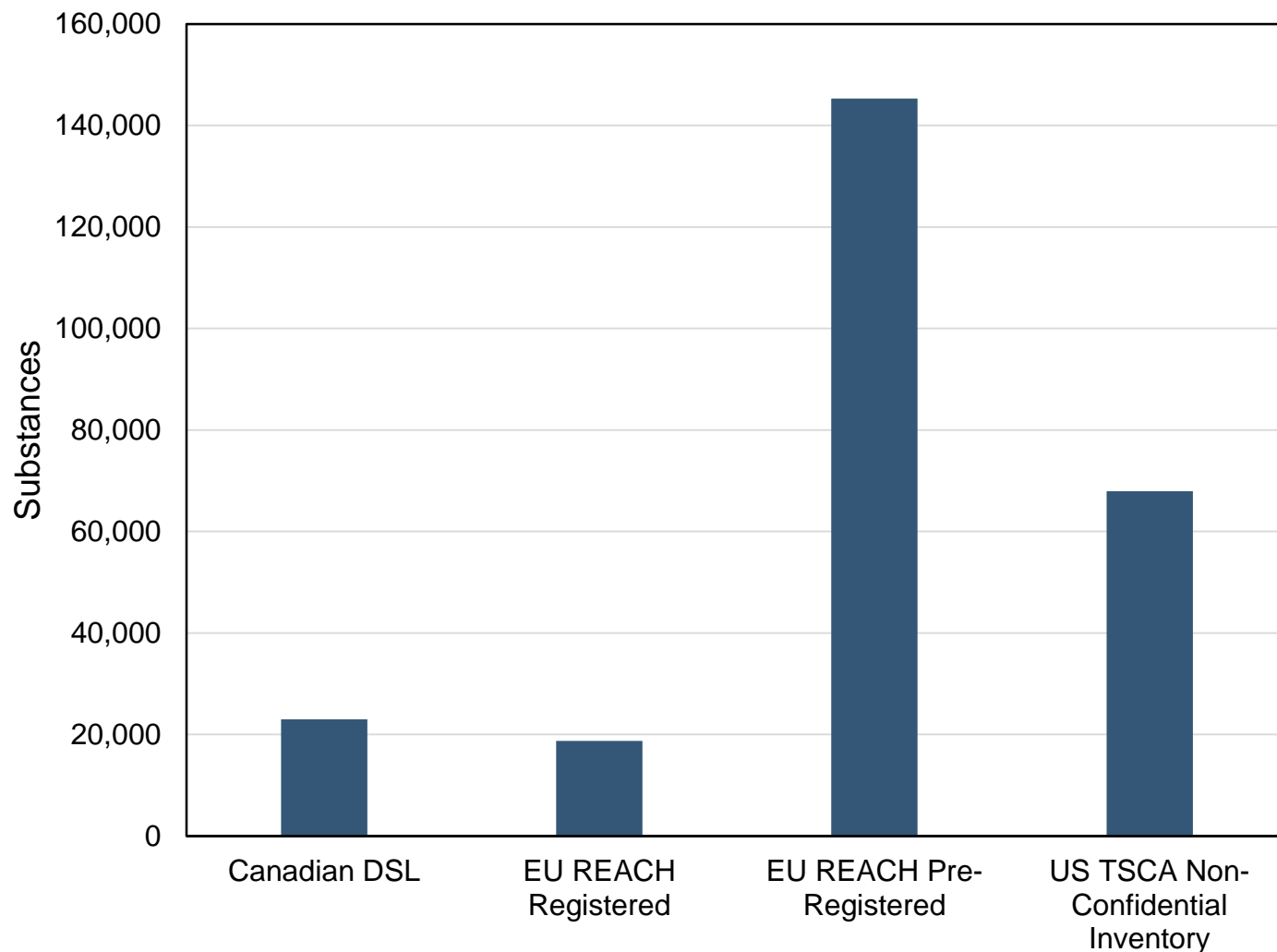
Rusty Thomas
Director
National Center for Computational Toxicology

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

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,² Todd Holderman,² Philip Sayre,² Shirlee Tan,⁴ Thomas Carpenter,⁵ and Edwin Smith⁶

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

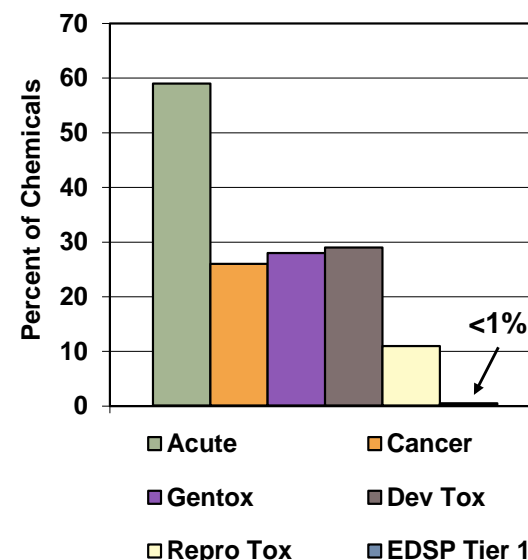
DATA EXTRACTION: ACToR contains chemical structure information; physical-chemical properties; *in vitro* assay data; tabular *in vivo* data; summary toxicology calls (e.g., a statement that a chemical is considered to be a human carcinogen); and links to online toxicology summaries. Here, we use data 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 ToxCast and medium-production-volume chemical water contaminants.

CONCLUSIONS: Approximately two-thirds of the chemicals in the U.S. EPA ToxCast evaluation database such as the U.S. Risk Information System, and the National Key Words: ACToR, carcinogenicity, reproductive, toxicity. *Environ Health Perspect* available via <http://ehpnet1.niehs.nih.gov/docs/2006/112-10/112-10-judson/abstract.html>

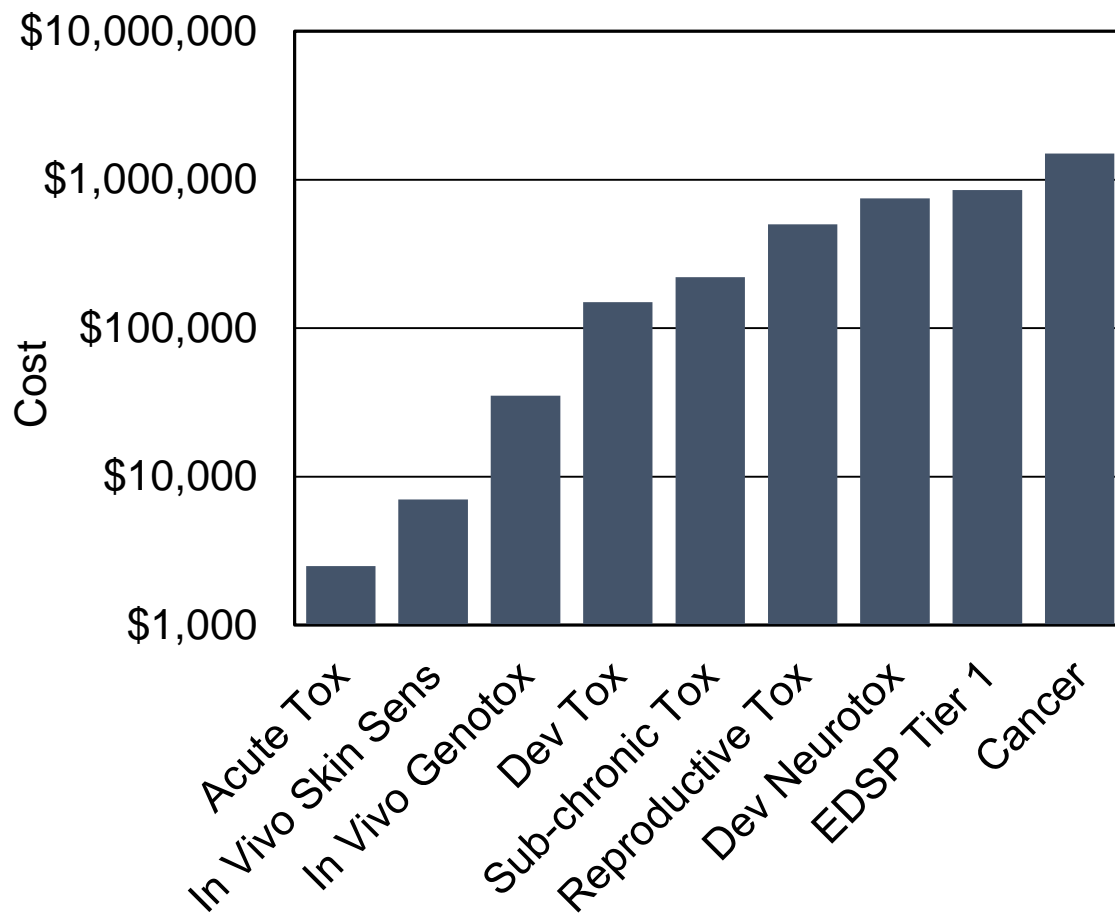
The U.S. Environmental Protection Agency (EPA) has a significant interest in making more efficient and informative determination approaches in part of the large number of chemicals under its jurisdiction. Ultimately, it would be ideal to characterize the toxicology of all chemicals in use in the United States. However, the size of this chemical inventory (in excess of 75,000 chemicals, with estimated number in the Toxic Substances Control Act (TSCA 1976) inventory EPA 2004b) makes this goal too using current approaches to toxicity testing that rely on extensive animal testing, cost millions of dollars, and 2-3 years per chemical. The International Science Institute/Health and Environmental Sciences Institute (IHSI/HESI) released several reports describing focused, tier-based approach for testing of agricultural chemicals, which ultimately lead to the use of fewer (Barton et al. 2006; Carmichael et al. 2006) 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 ecological toxicity to support regulatory action. This "data gap" is well documented (Allanous



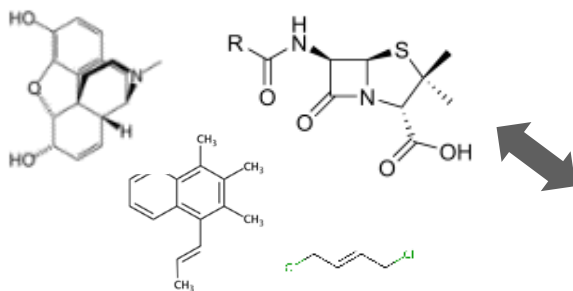
Modified from Judson *et al.*, EHP 2009

Costs of Traditional Toxicity Testing



How Can High-Throughput Approaches Address These Challenges?

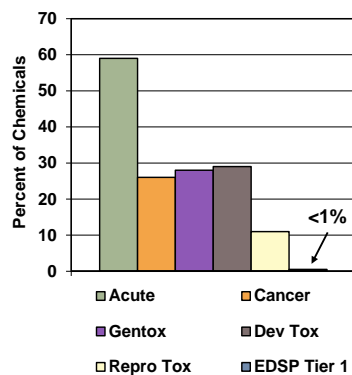
Number of Chemicals
/Combinations



Comprehensive Tox
Evaluation

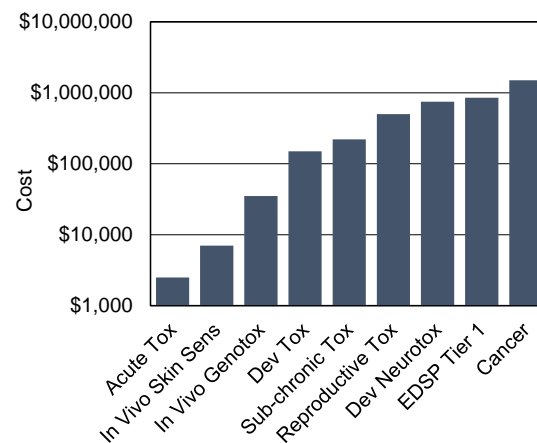
DevTox
ImmuTox
MGR
RepeatDoseTox
AcuteTox
Genotox
Zyrcare
SkinSens

Limited Data

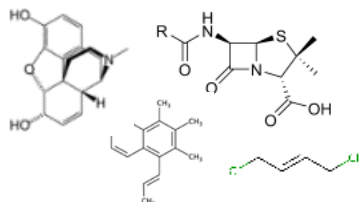


Modified from Judson *et al.*, EHP 2009

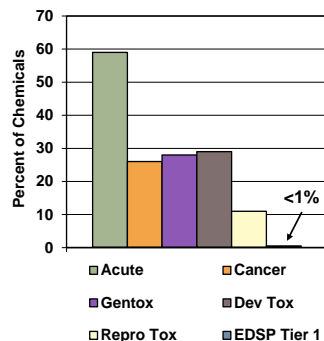
Economics



Key Steps in Satisfying Grandma, Toxicologists, and Regulators



DevTox
Skinsens
ImmunoTox
MGR
RepeatDoseTox
AcuteTox
Genotox
Zyrcarc

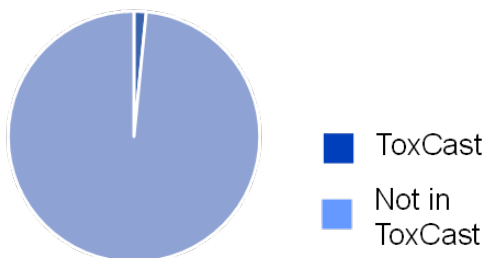


Modified from Judson *et al.*, EHP 2009

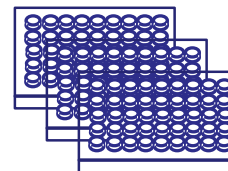
- Systematically addressing limitations in alternative test systems
- Continue putting results in a dose/exposure context
- Characterize of uncertainty
- Emphasize development of computational models to integrate experimental data
- Deliver of data and models through decision support tools
- Translation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

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

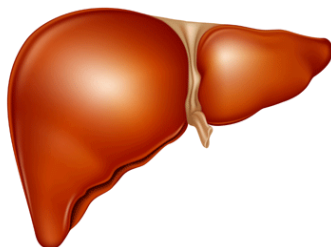
**Biological Coverage
(Gene Basis)**



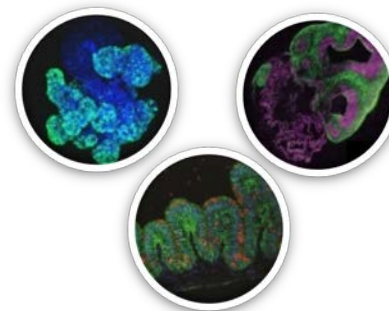
**Chemical Coverage and
Specific Chemical Types
(e.g., VOCs)**



**Metabolic
Competence**

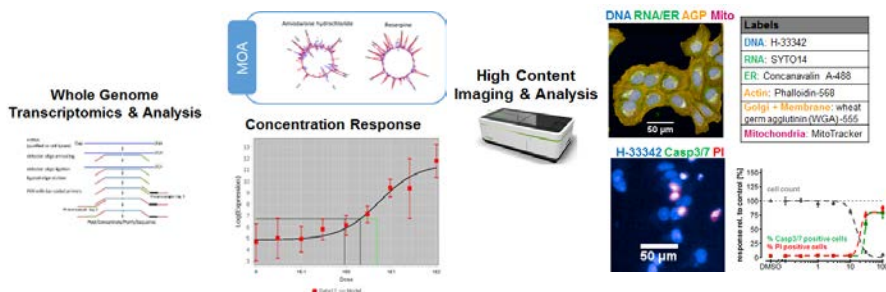


**Organ and Tissue
Responses**

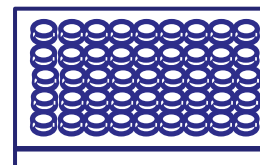


Systematically Addressing Limitations in Alternative Test Systems

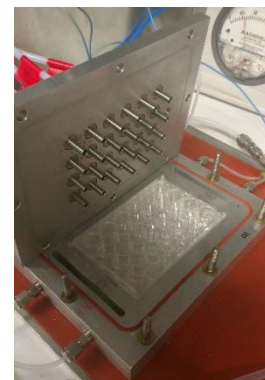
High-Throughput Transcriptional and Phenotypic Profiling



VOC *In Vitro* Exposure System and Water Library



Initial test library of
~70 chemicals

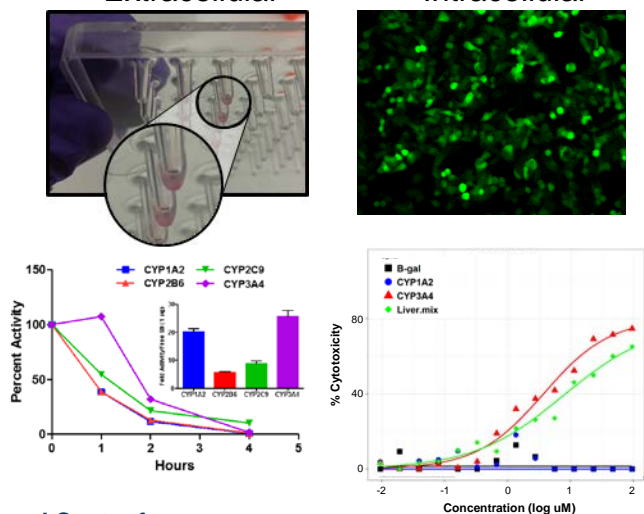


M. Higuchi (EPA-NHEERL)

Assay Retrofit for Metabolism

Extracellular

Intracellular



Organotypic Model Development and Virtual Tissue Modeling

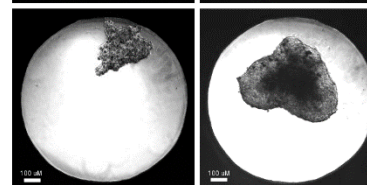
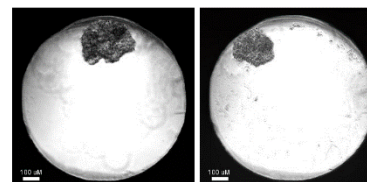
3D Organoid Culture

Day 0

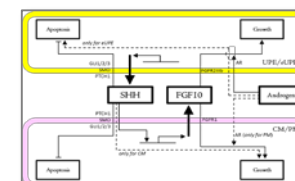
Day 10

Computational Modeling

CS-FBS



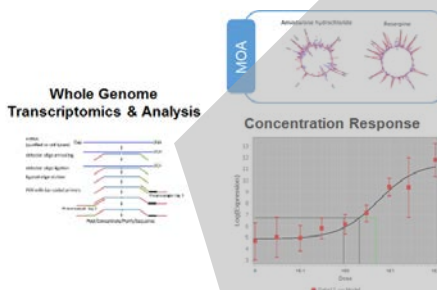
FBS



Systematically Addressing Limitations in Alternative Test Systems

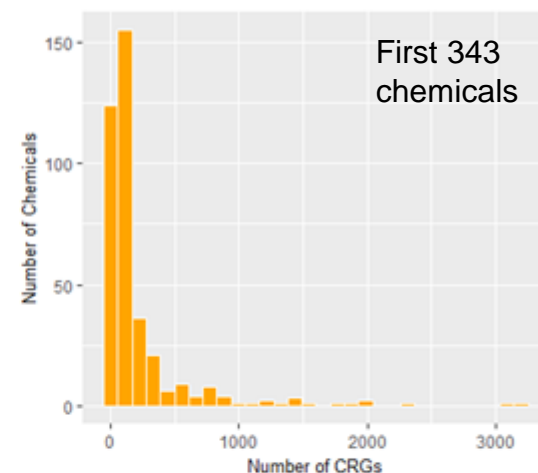
High-Throughput Transcriptional and Phenotypic

Phenotypic

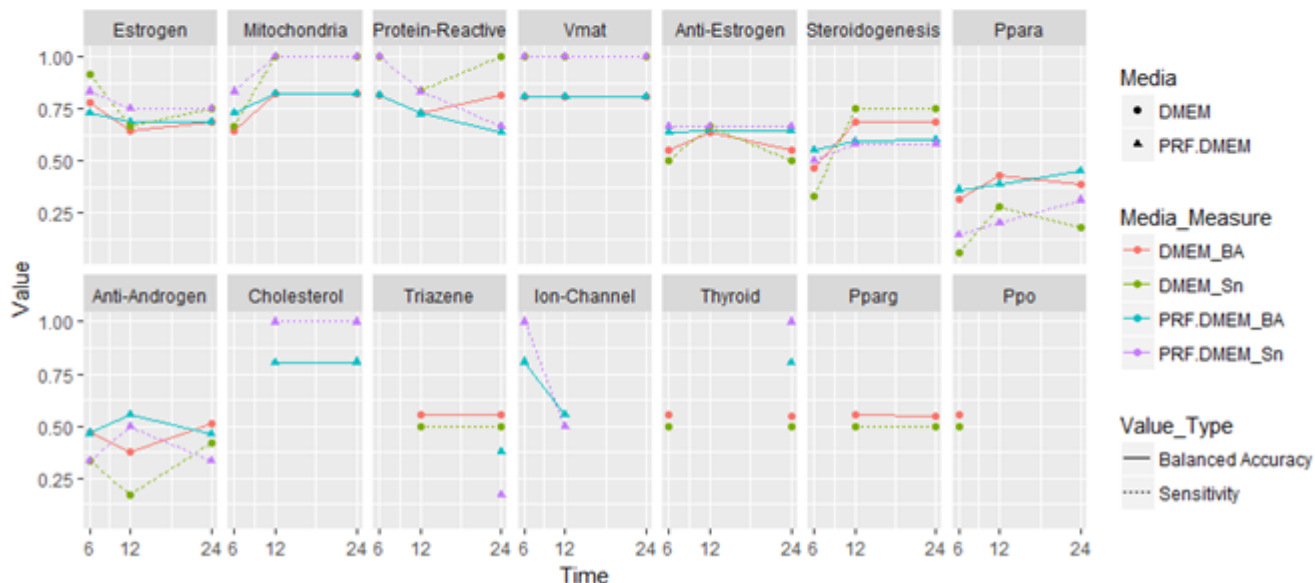
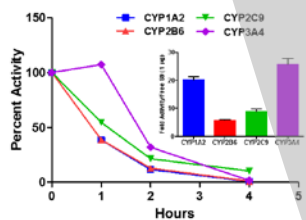


- Whole transcriptome
- Low cost
- 384-well cell lysate
- Performance controls each plate

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

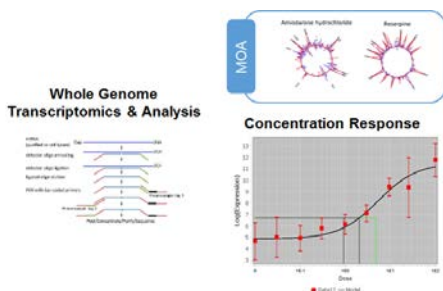


Assay Retrofit Extracellular



Systematically Addressing Limitations in Alternative Test Systems

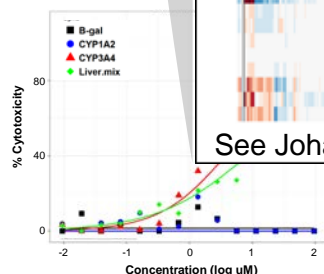
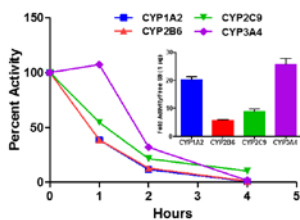
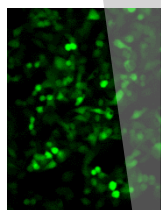
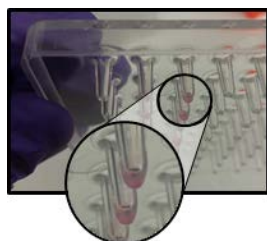
High-Throughput Transcriptional Phenotypic Profiling



High Content Imaging & Analysis



Assay Retrofit for Metabolic Extracellular Intracellular



Experimental Design

- 2 cell types: U-2 OS / MCF7
- 384-well plates
- 16 chemicals
- 7 concentrations (3 log₁₀ units)
- 3 replicates / plate
- 3 independent experiments

compartments:

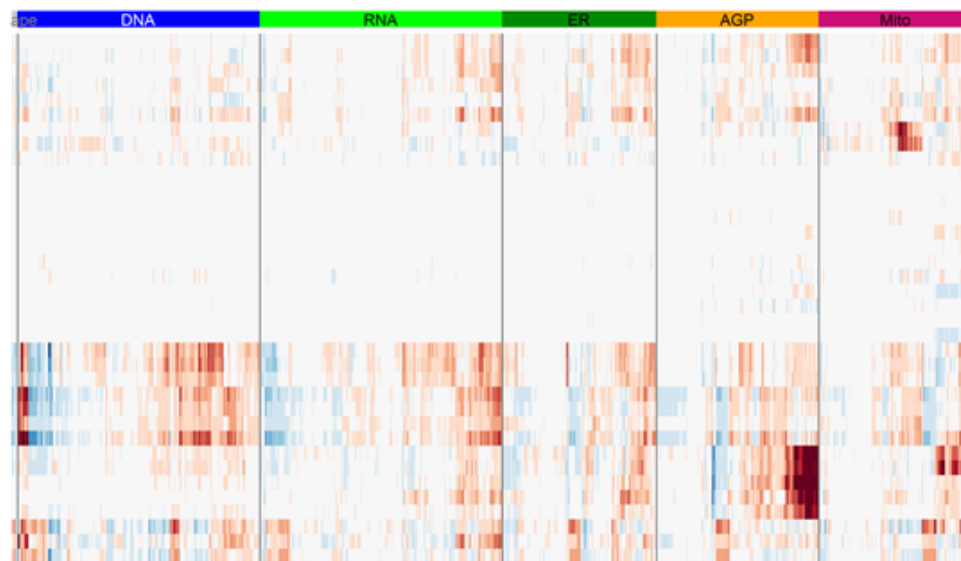
- nuclei
- ring
- cytoplasm
- membrane
- cell

channels (organelles):

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

parameters:

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



Fluphenazine (10uM) - MCF7
Amperozide (10uM) - MCF7
Ca-074-Me (10uM) - MCF7
Taxol (0.005uM) - MCF7
Rapamycin (3uM) - MCF7
Latrunculin B (5uM) - MCF7
Berberine Chloride (30uM) - MCF7
Berberine Chloride (10uM) - U2OS
Rapamycin (1uM) - U2OS
DMSO - U2OS
DMSO - MCF7
Sorbitol (100uM) - MCF7
Saccharin (100uM) - U2OS
Metoclopramide (100uM) - U2OS
Sorbitol (100uM) - U2OS
untreated - U2OS
NPPD (100uM) - U2OS
Metoclopramide (100uM) - MCF7
NPPD (100uM) - MCF7
untreated - MCF7
Saccharin (100uM) - MCF7
Oxibendazole (0.6uM) - MCF7
Rotenone (1uM) - MCF7
Fenbendazole (0.6uM) - MCF7
Rotenone (1uM) - U2OS
Fenbendazole (0.6uM) - U2OS
Taxol (0.005uM) - U2OS
Oxibendazole (0.6uM) - U2OS
Amperozide (10uM) - U2OS
Fluphenazine (10uM) - U2OS
Tetrandrine (2.5uM) - U2OS
Tetrandrine (2.5uM) - MCF7
Ca-074-Me (1uM) - U2OS
Etoposide (0.3uM) - U2OS
Latrunculin B (1.5uM) - U2OS
Etoposide (3uM) - MCF7

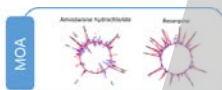
See Johanna Nyffeler Poster

FBS

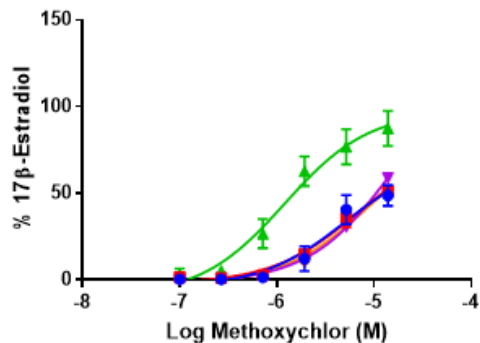
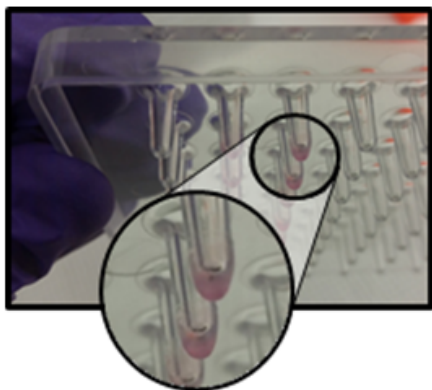
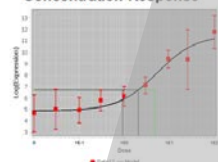


Systematically Addressing Limitations in Alternative Test Systems

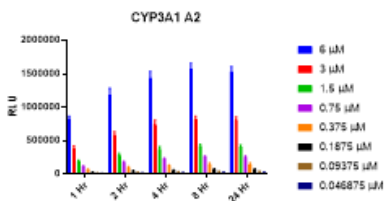
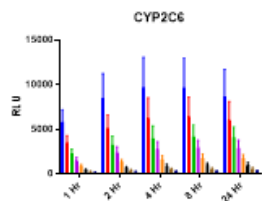
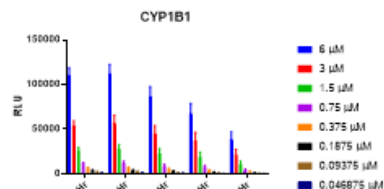
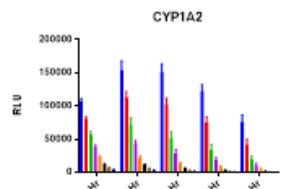
High-Throughput Trauma Phenotypic P



Concentration Response

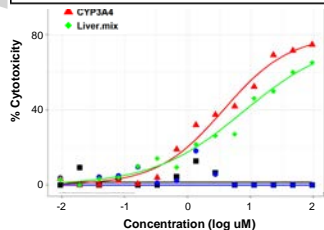
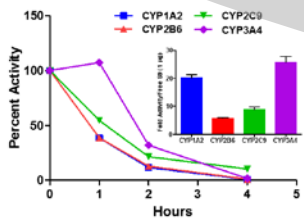


Active S9 Empty Microsphere No AIME
Boiled S9 37C Inactivated

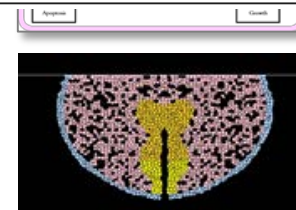
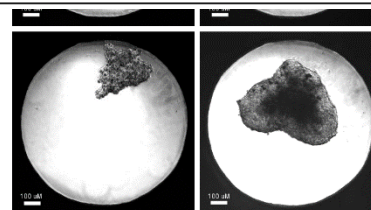


96-Well	EC50 (μM)	EC50 Potency Shift
Active S9	0.71	
Heat Inactivated S9	6.8	9.6
No AIME	4.98	7.0
384-Well	EC50 (μM)	EC50 Potency Shift
Active S9	1.2	
Heat Inactivated S9	15.9	13.2
No AIME	5.1	4.2

DeGroot, Simmons, and Deisenroth, Unpublished

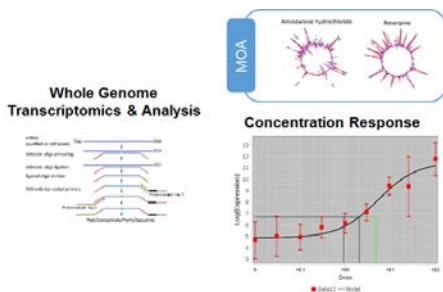


FBS



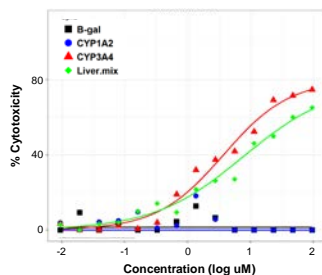
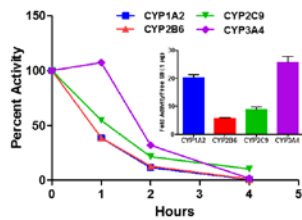
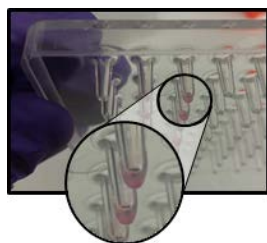
Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptomic Phenotypic Profiling

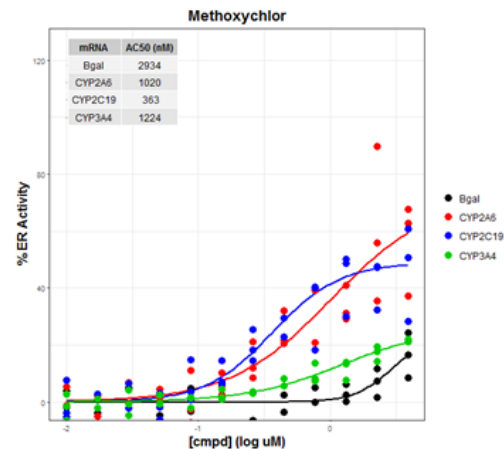
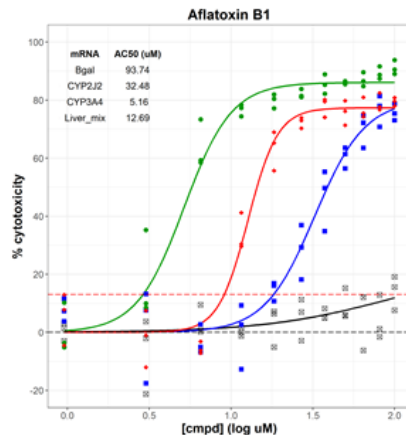
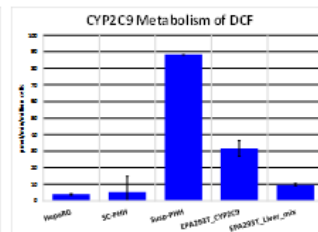
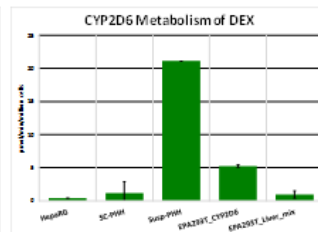
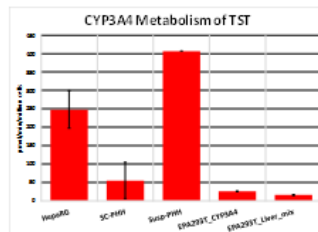


High Content Imaging & Analysis

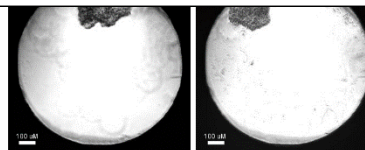
Assay Retrofit for Metabolic Extracellular



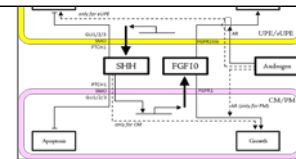
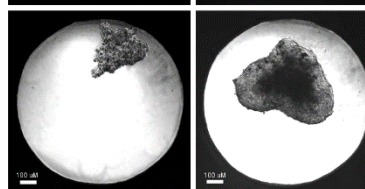
Simmons et al., J Pharmacol Tox Methods, 2018



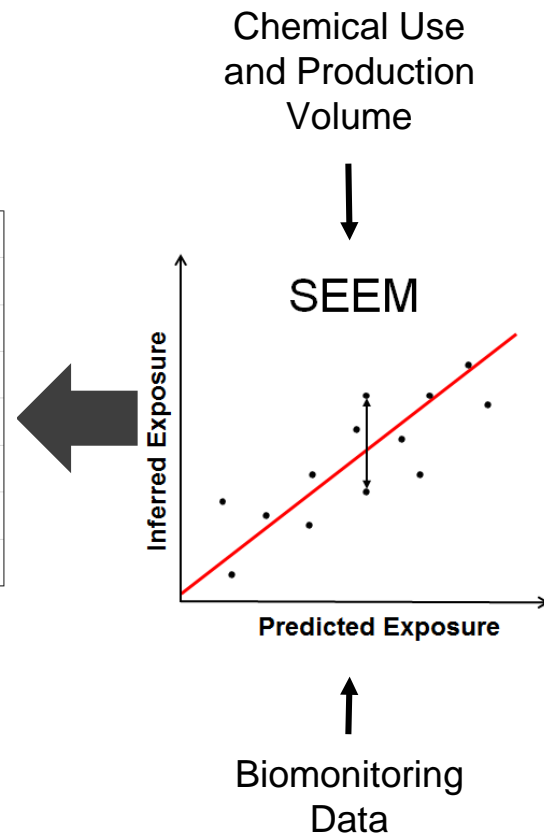
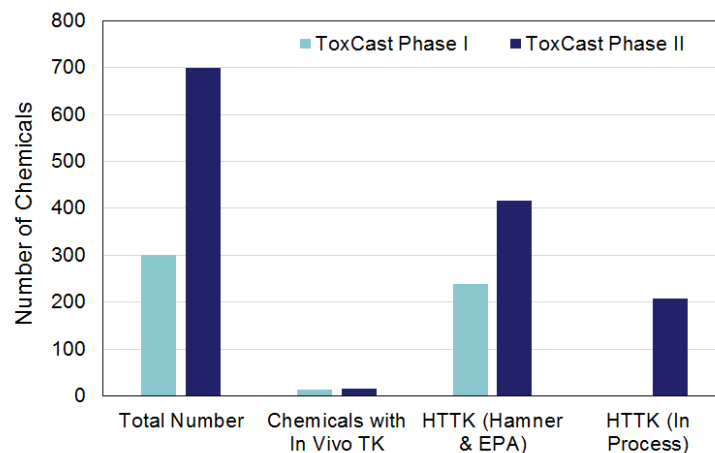
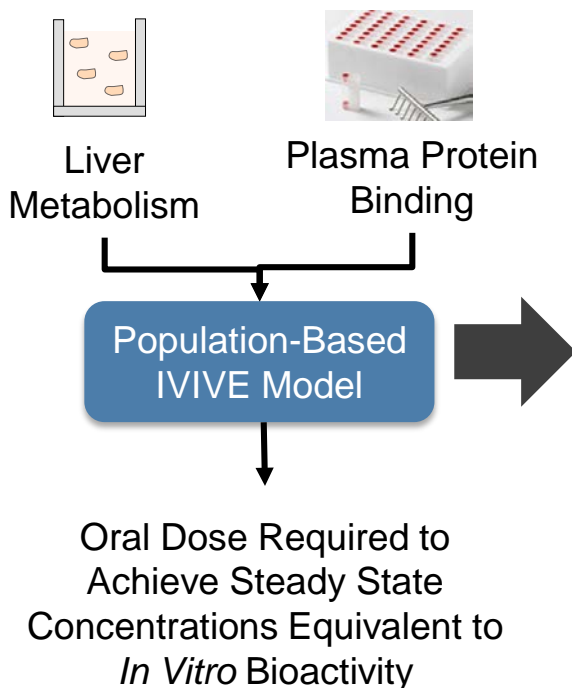
CS-FBS



FBS



Putting Alternative Test Results in a Dose and Exposure Context



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

Wambaugh *et al.*, 2014

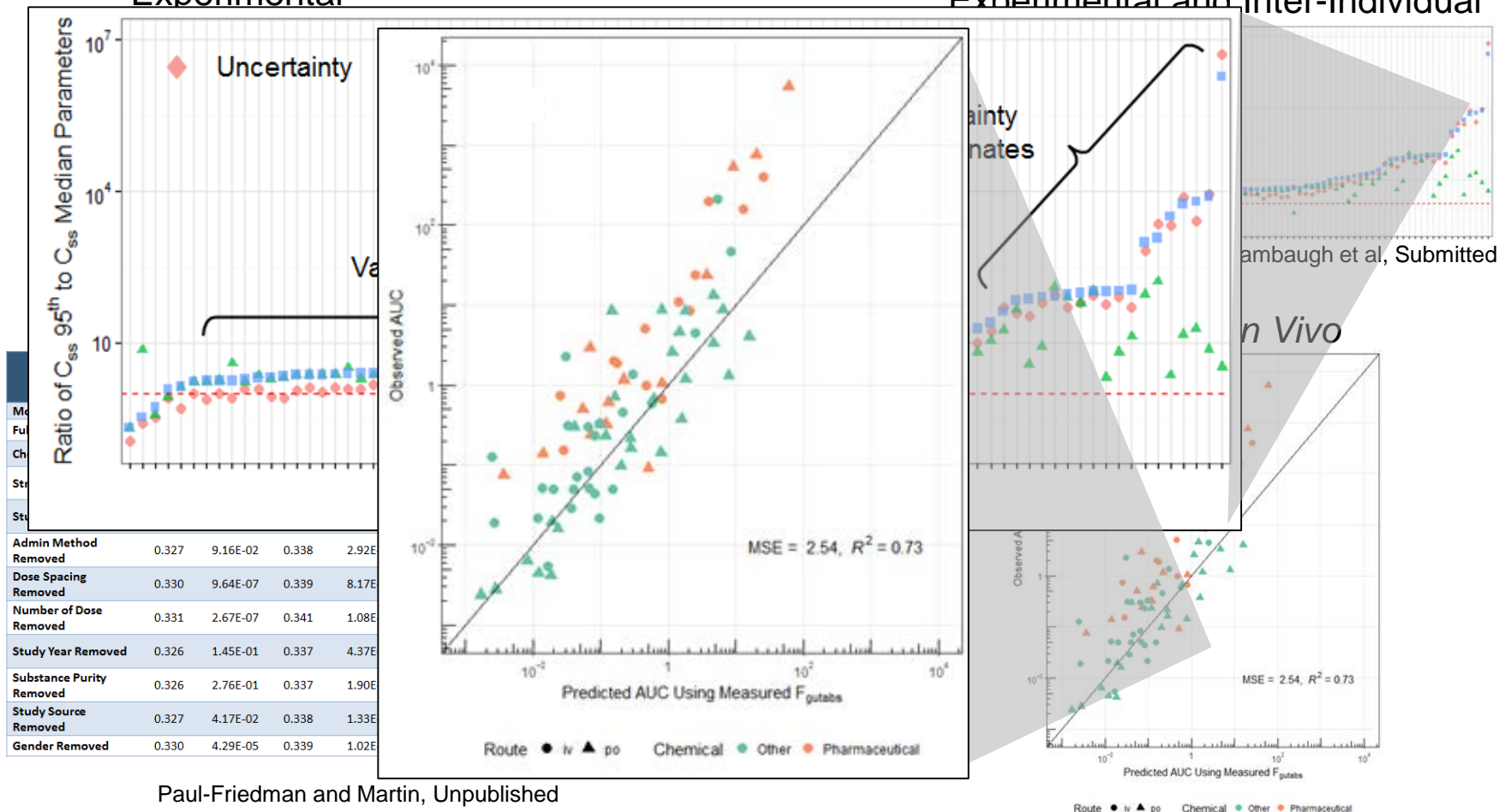
Quantifying Uncertainty and Variability

Pharmacodynamic

Pharmacokinetic

Experimental

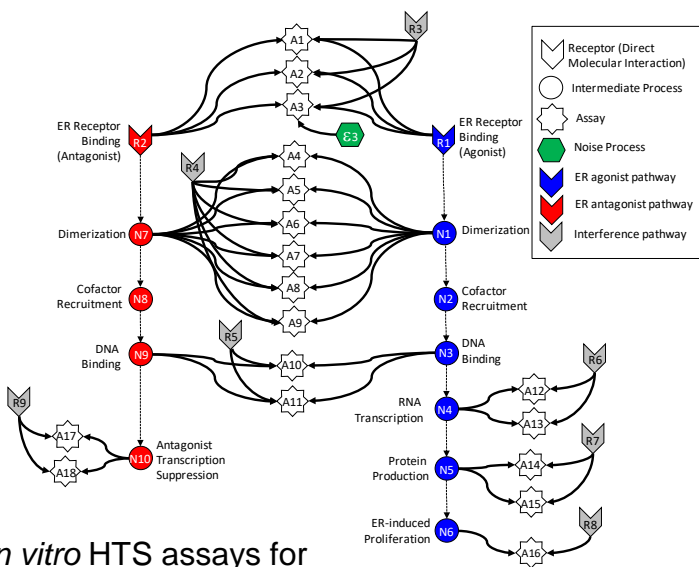
Experimental and Inter-Individual



Paul-Friedman and Martin, Unpublished

Computational Modeling to Integrate Experimental Data

Computational Modeling of Estrogen Receptor Pathway



18 *In vitro* HTS assays for ER bioactivity

In Vitro Reference Chemicals*

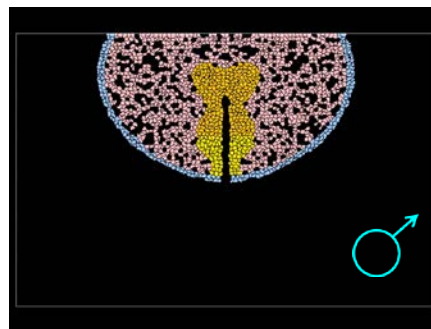
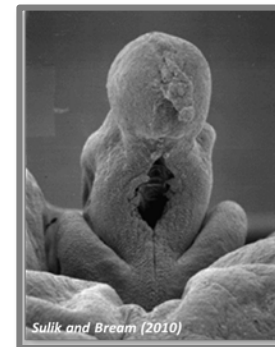
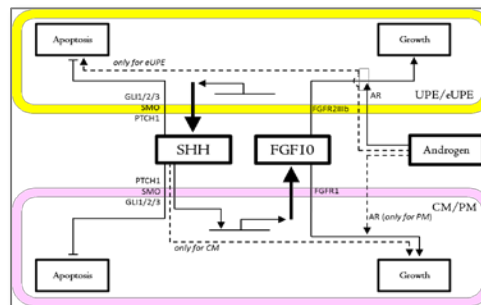
Accuracy	0.93 (0.95)
Sensitivity	0.93 (0.93)
Specificity	0.92 (1.0)

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

Computational Modeling of Genital Tubercle Development



GD13.5 – 17.5

Androgenization
(n = 10 sims)

Phenotype (MCS 4000)

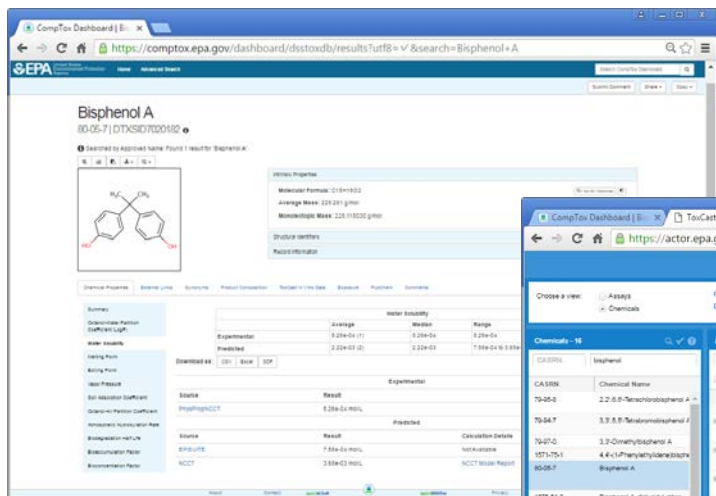
	Septation	Fusion	Conden.	Closure Index
100%	6/10	8/10	10/10	0.80
67%	2/10	5/10	10/10	0.57
33%	0/10	4/10	0/10	0.13
0%	0/10	2/10	0/10	0.07

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

Leung *et al.*, *Repro Toxicol.* 2016

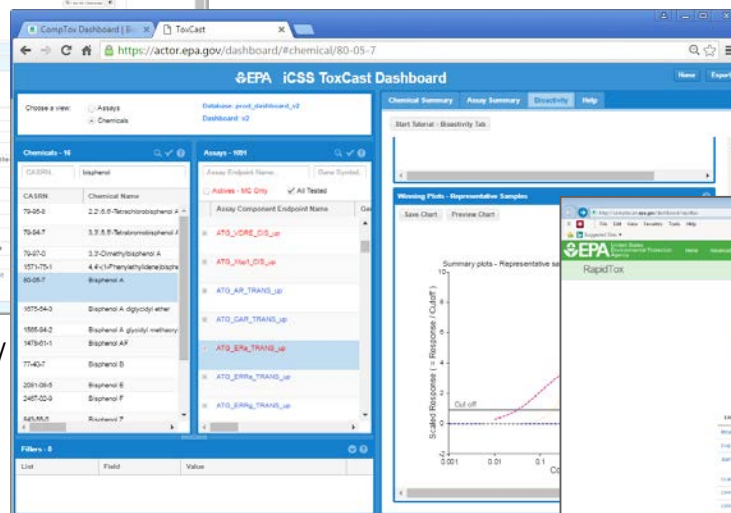
Deliver Data and Models Through Decision Support Tools

Comptox Chemistry Dashboard



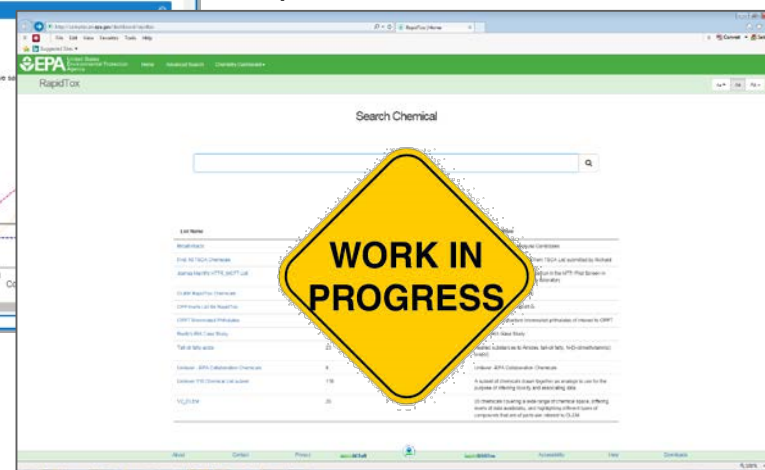
<https://comptox.epa.gov/dashboard/>

ToxCast Dashboard

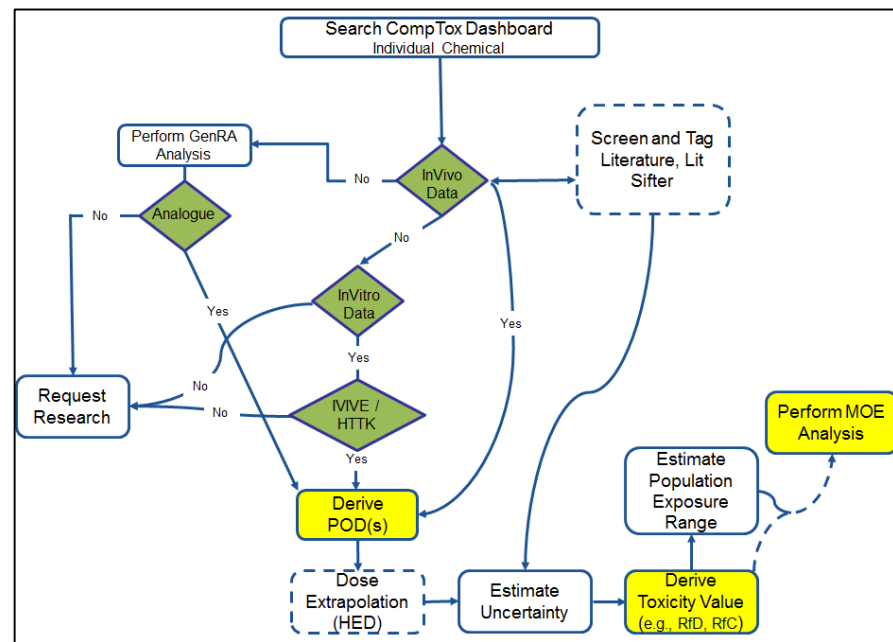
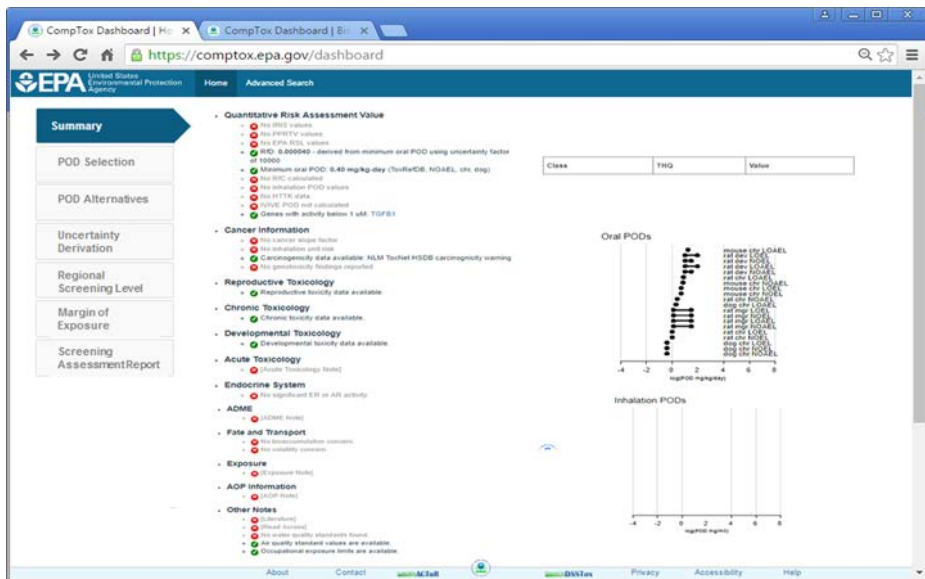


<https://actor.epa.gov/dashboard/>

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

One Tool, Multiple Workflows to Address Diverse Partner Needs

RapidTox Prioritization Workflow

Chemical List:
☒ TSCA
☐ OPP Inerts

To run prioritization, select the chemical set, the allowable data domains, and update the weights. Then select the Recalculate button and go to the prioritization tab. You can then sort by the different prioritization types.

Recalculate
Export Table

Components Weighting Factors In Vivo Data PhysChem Data ER Data AR Data ER QSAR Data Hazard Prioritization

Exposure Prioritization Overall Prioritization

Check All Uncheck All

Human Health

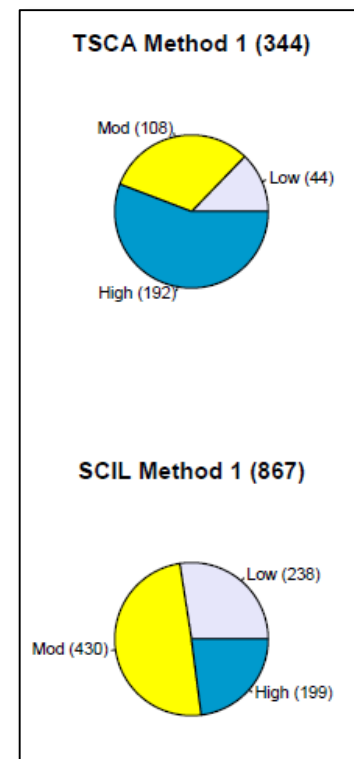
Acute	Subchronic	Chronic	DevTox	ReproTox	Cancer	Mutagenicity	Neurotox	Systemic Tox Models
<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> Martin model
<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> Pradeep model
								<input checked="" type="checkbox"/> GenRA model
								<input checked="" type="checkbox"/> ToxCast IVIVE

Endocrine

Estrogen Agonist	Estrogen Antagonist	Androgen Agonist	Androgen Antagonist
<input checked="" type="checkbox"/> In vitro	<input checked="" type="checkbox"/> In vitro	<input checked="" type="checkbox"/> In vitro	<input checked="" type="checkbox"/> In vitro
<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR

Ecological

Fish Acute Tox	Crustacea Acute Tox	Algae Tox	Fish ReproTox
<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> In vivo	<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR
<input checked="" type="checkbox"/> QSAR	<input checked="" type="checkbox"/> QSAR		



EPA-HQ-OPPT-2017-0586

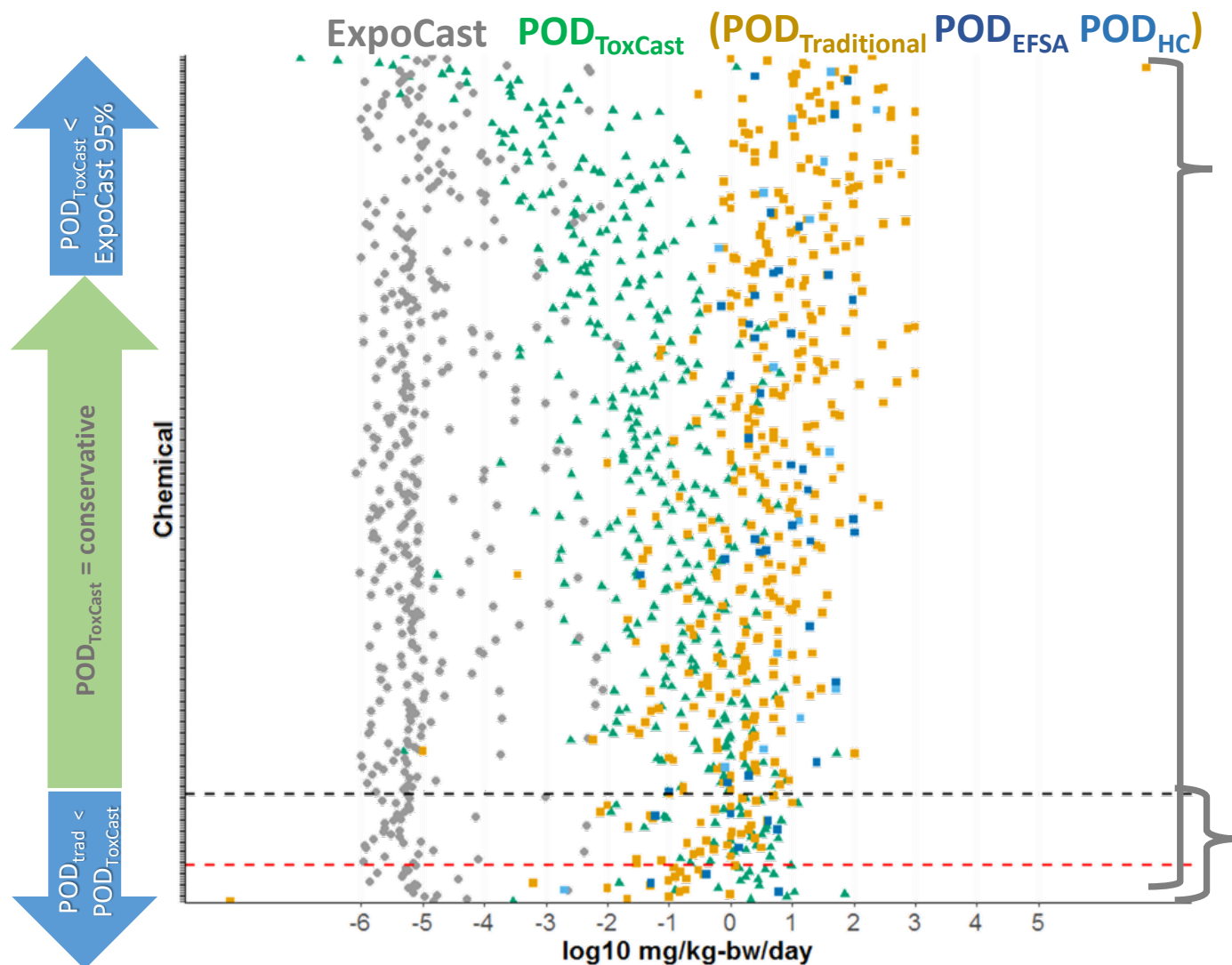
- Flexible exploration of multiple prioritization scenarios
- Selection and weighting of different data streams
- Outputs ordinal and binned prioritization
- Proposed to selected candidates for TSCA prioritization

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 HTKK
~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 will 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

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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

