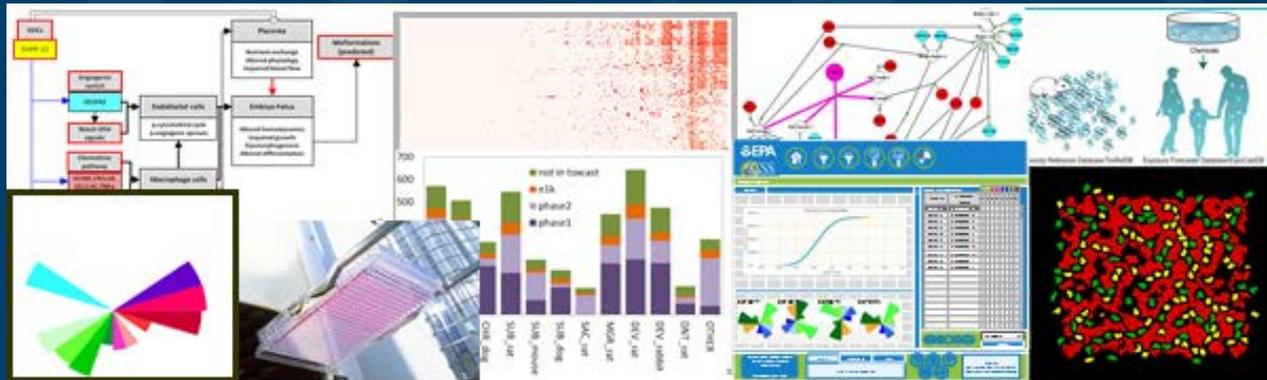


Accelerating the Development and Use of High-Throughput Screening Data for Application to Regulatory Risk Assessments



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

April 16, 2018

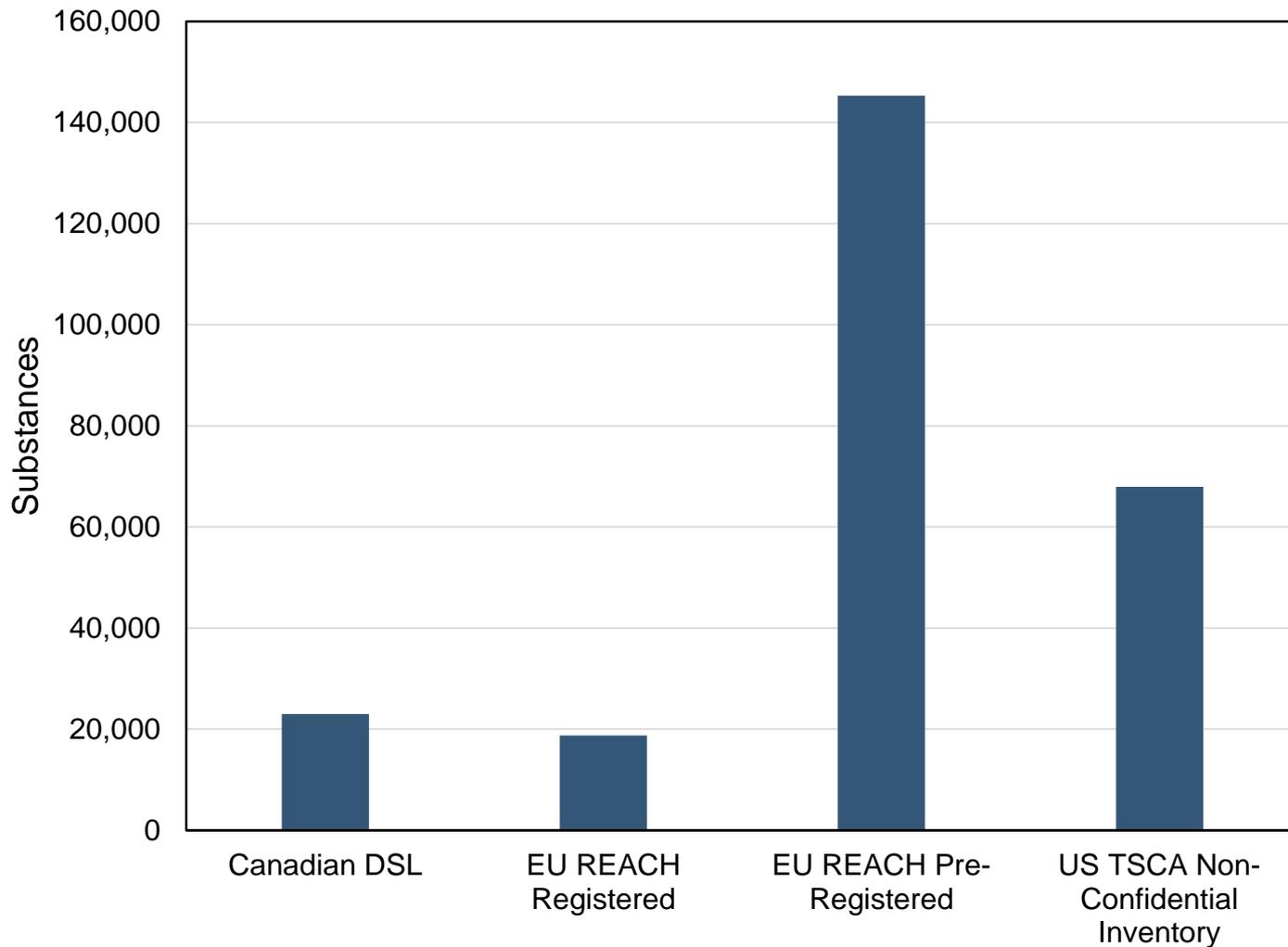
Rusty Thomas
Director
National Center for Computational Toxicology

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,³ 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.

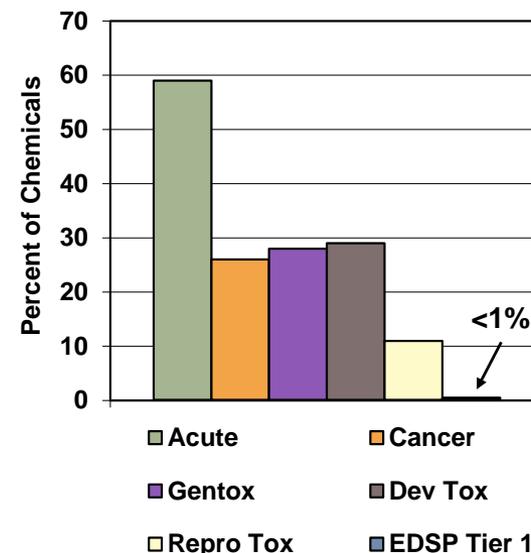
DATA EXTRACTION: ACToR contains chemical structure information; physical-chemical properties; *in vitro* assay data; tabular *in vivo* data; summary toxicology calls (e.g., a statement that a chemical is considered to be a human carcinogen); and links to online toxicology summaries. Here, we use data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

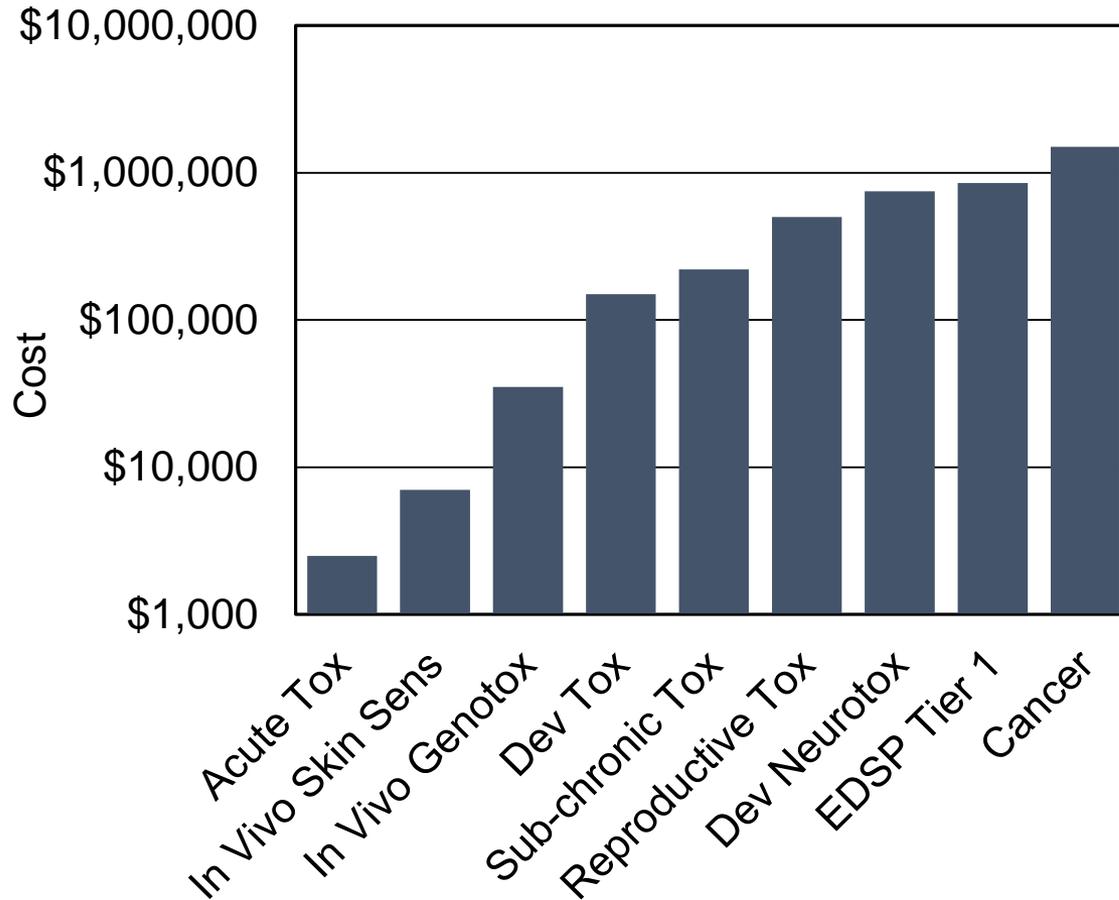
CONCLUSIONS: Approximately two-thirds of the data are available. About one-quarter of the evaluation database such as the U.S. Risk Information System, and the National Health and Environmental Effects Research Laboratory. **KEY WORDS:** ACToR, carcinogenicity, reproductive, toxicity. *Environ Health Perspect* available via <http://dx.doi.org/> [Online].

The U.S. Environmental Protection Agency (EPA) has a significant interest in developing more efficient and informative determination approaches in part of the large number of chemicals in its jurisdiction. Ultimately, it would be beneficial to characterize the toxicologic of all chemicals in use in the United States. However, the size of this chemical [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 determination 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. The National Research Council

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

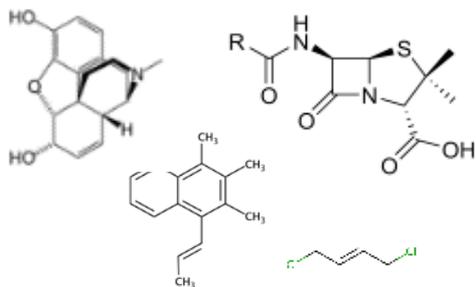


Costs of Traditional Toxicity Testing



How Can High-Throughput Approaches Address These Challenges?

Number of Chemicals
/Combinations

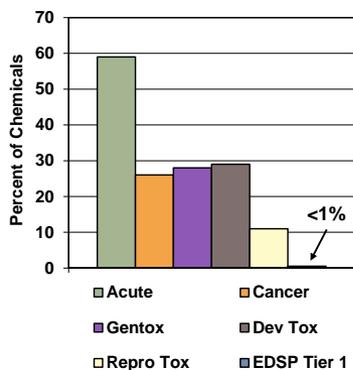


Comprehensive Tox
Evaluation

Skincens
DevTox
ImmunoTox
MGR
RepeatDoseTox
AcuteTox
Genotox
Zyrcare

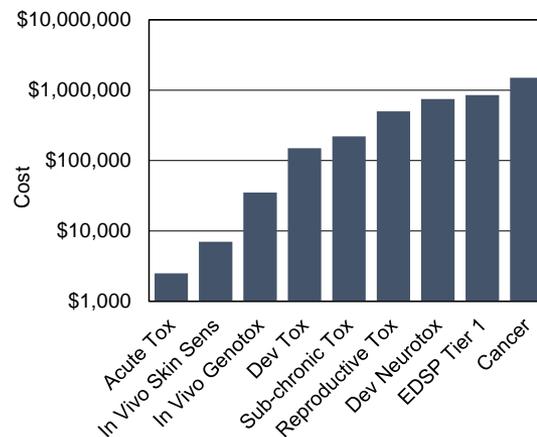


Limited Data

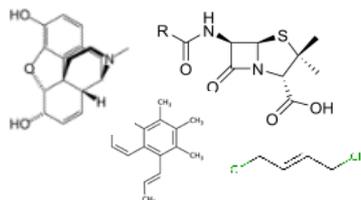


Modified from Judson *et al.*, EHP 2009

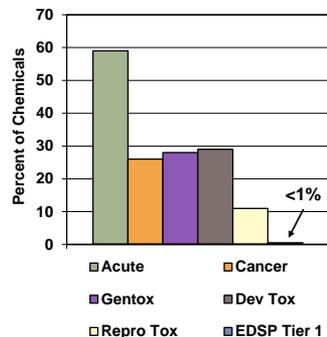
Economics



Key Steps in Satisfying Grandma, Toxicologists, and Regulators



DevTox
 SkinSens
 ImmunoTox
 MGR
 RepeatDoseTox
 AcuteTox
 Genotox
 ZyrCare

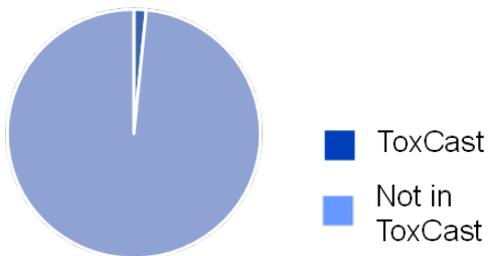


Modified from Judson *et al.*, EHP 2009

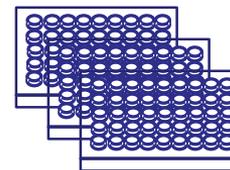
- Systematically addressing limitations in alternative test systems
- Continue putting results in a dose/exposure context
- Characterize of uncertainty
- Emphasize development of computational models to integrate experimental data
- Deliver of data and models through decision support tools
- Translation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

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

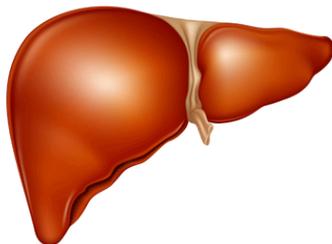
Biological Coverage (Gene Basis)



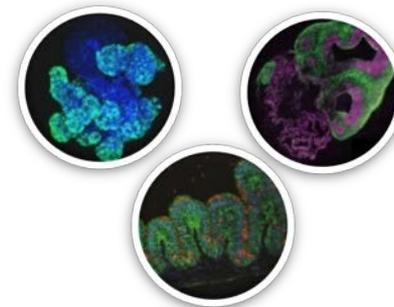
Chemical Coverage and Specific Chemical Types (e.g., VOCs)



Metabolic Competence

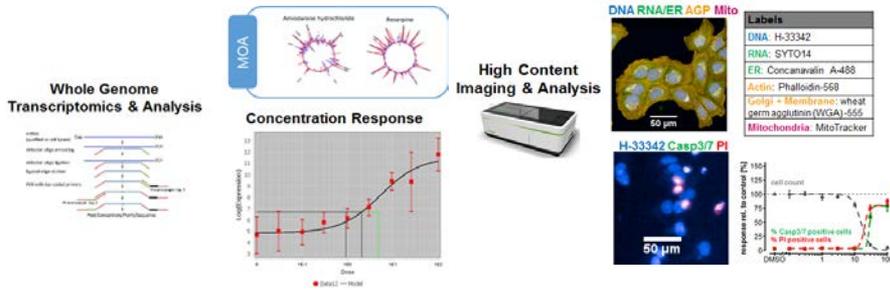


Organ and Tissue Responses

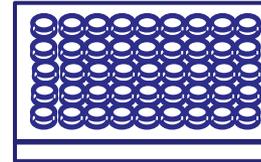


Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptional and Phenotypic Profiling



VOC *In Vitro* Exposure System and Water Library



Initial test library of ~70 chemicals

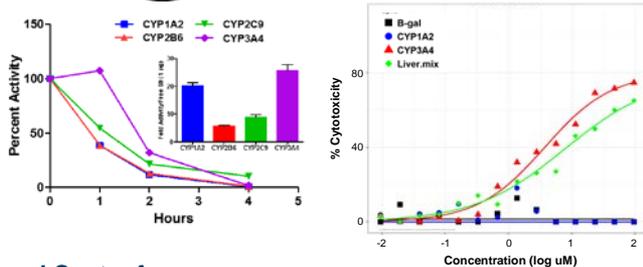
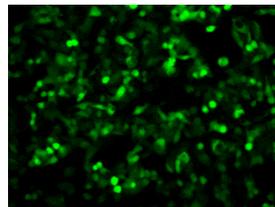
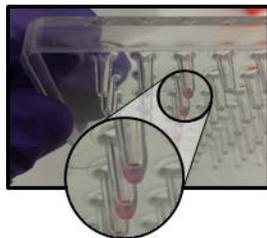


M. Higuchi (EPA-NHEERL)

Assay Retrofit for Metabolism

Extracellular

Intracellular

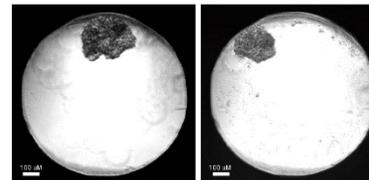


Collaboration with Unilever

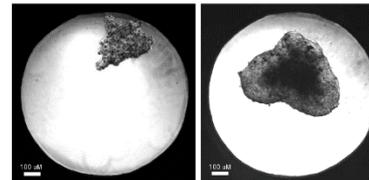
Organotypic Model Development and Virtual Tissue Modeling

3D Organoid Culture
Day 0 Day 10

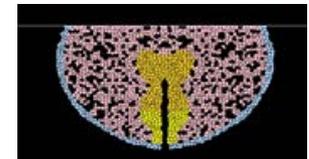
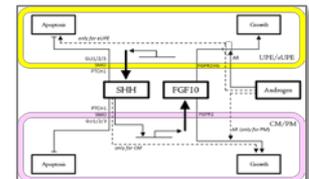
CS-FBS



FBS

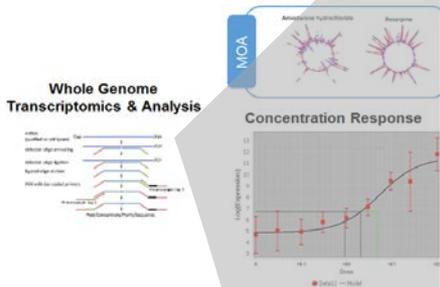


Computational Modeling

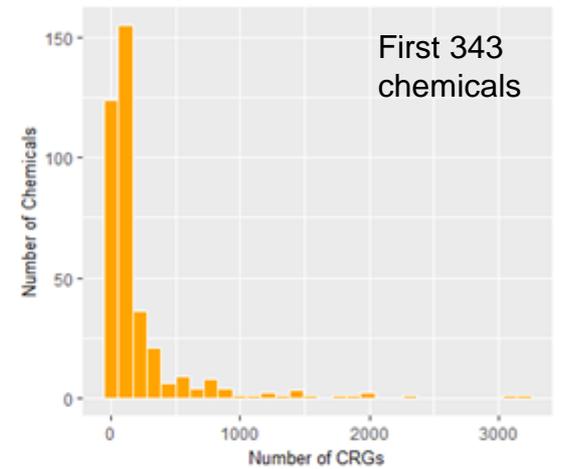
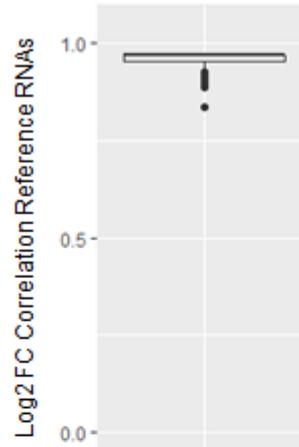


Systematically Addressing Limitations in Alternative Test Systems

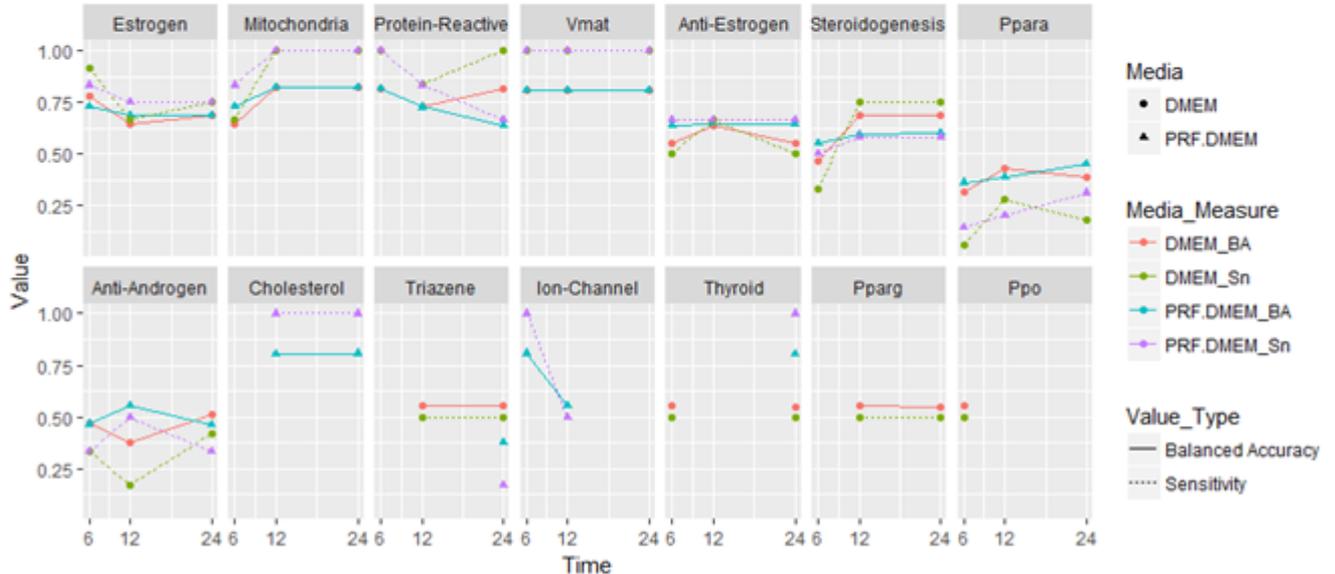
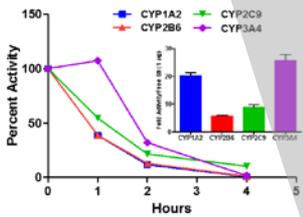
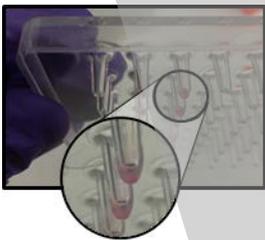
High-Throughput Transcriptional and Phenotypic



- 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

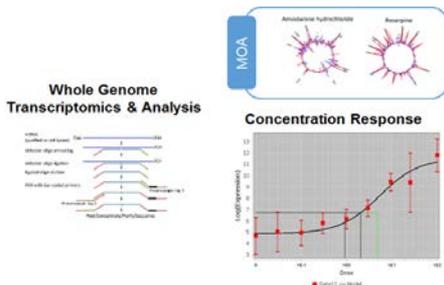


Assay Retrofit Extracellular



Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptional Phenotypic Profiling



High Content Imaging & Analysis



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

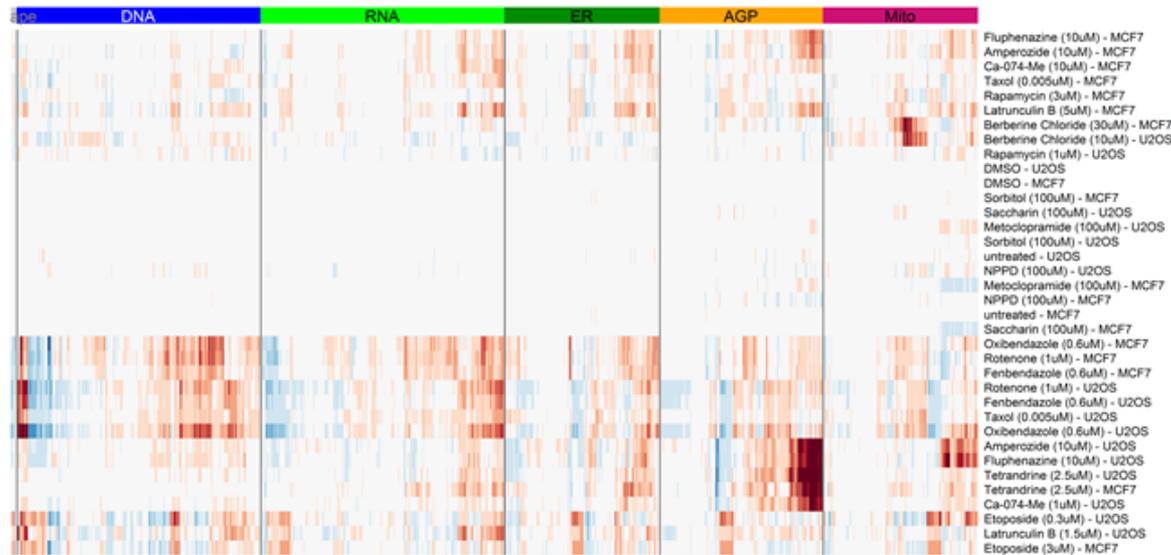
channels (organelles):

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

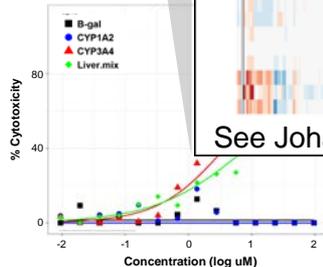
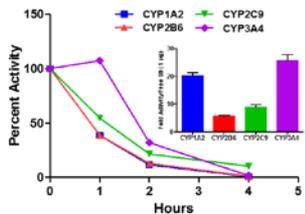
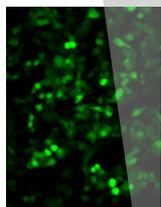
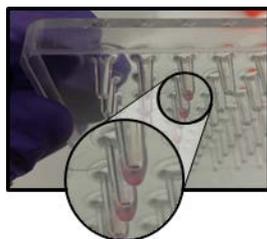
parameters:

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

Image Analysis of 1669 Endpoints



Assay Retrofit for Metabolic Extracellular Intracellular

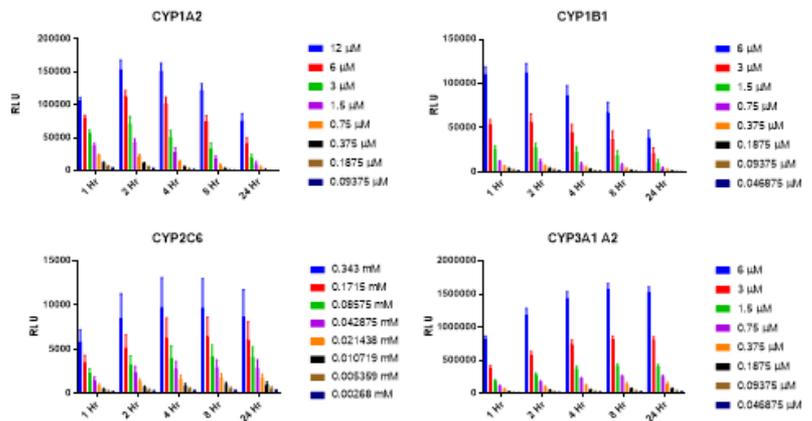
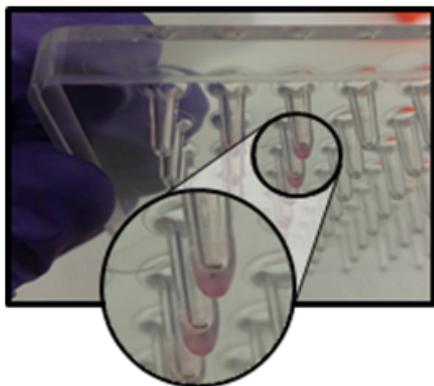
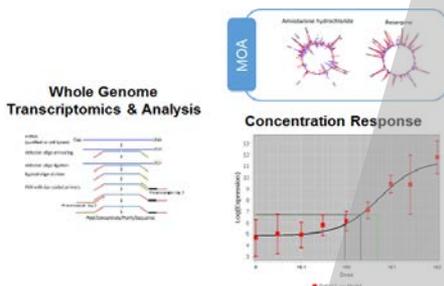


FBS

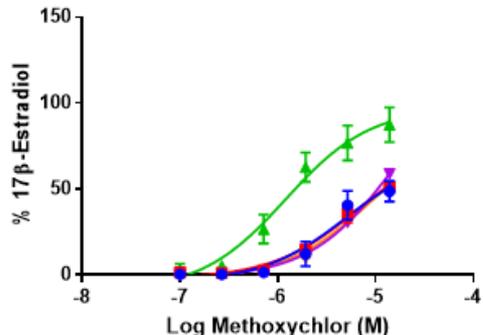
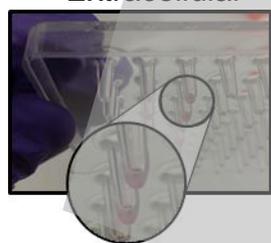


Systematically Addressing Limitations in Alternative Test Systems

High-Throughput Transcriptional Phenotypic Profiling



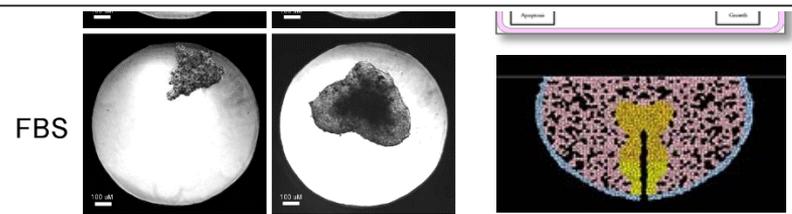
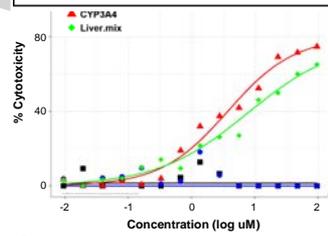
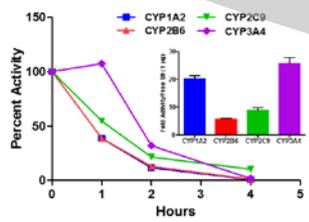
Assay Retrofit Extracellular



▲ Active S9
 ■ Empty Microsphere
 ● No AIME
▼ Boiled S9
 ◆ 37C Inactivated

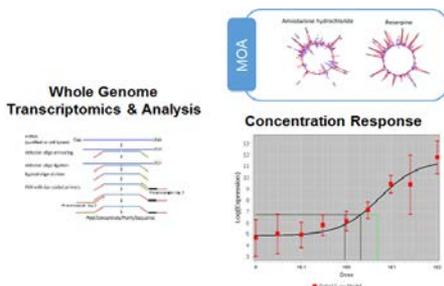
96-Well	EC50 (μM)	EC50 Potency Shift
Active S9	0.71	
Heat Inactivated S9	6.8	9.6
No AIME	4.98	7.0
384-Well	EC50 (μM)	EC50 Potency Shift
Active S9	1.2	
Heat Inactivated S9	15.9	13.2
No AIME	5.1	4.2

DeGroot, Simmons, and Deisenroth, Unpublished

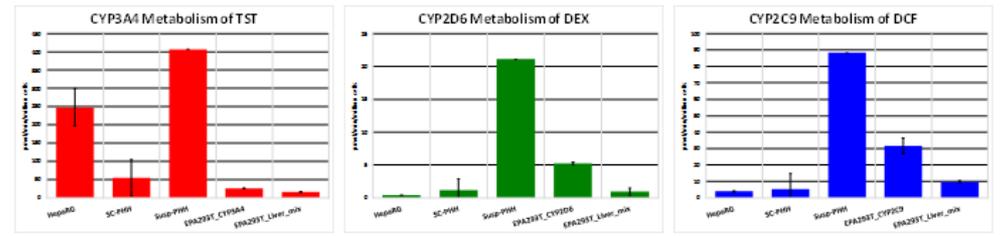
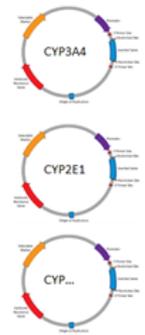


Systematically Addressing Limitations in Alternative Test Systems

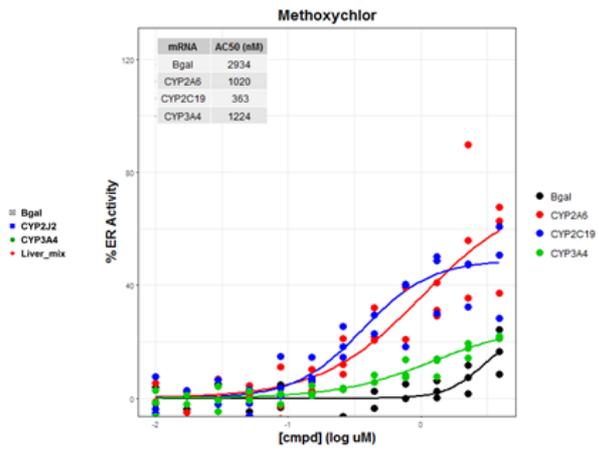
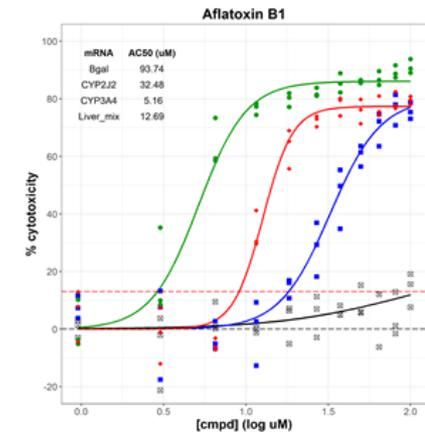
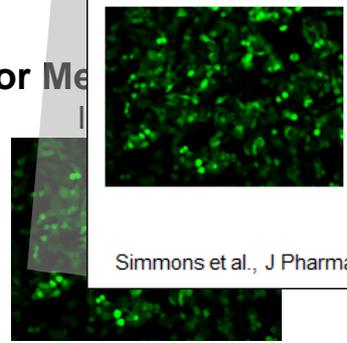
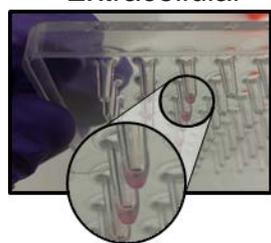
High-Throughput Transcriptomic Phenotypic Profiling



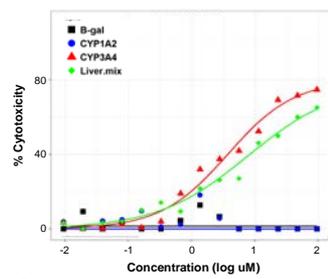
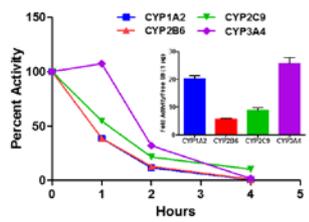
High Content Imaging & Analysis



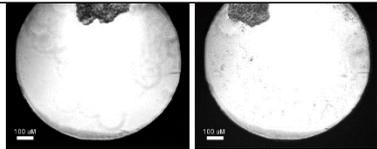
Assay Retrofit for Metabolic Extracellular



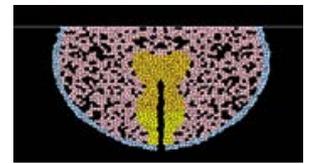
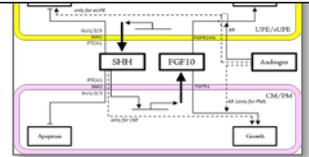
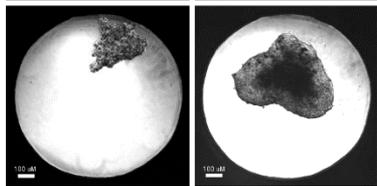
Simmons et al., J Pharmacol Tox Methods, 2018



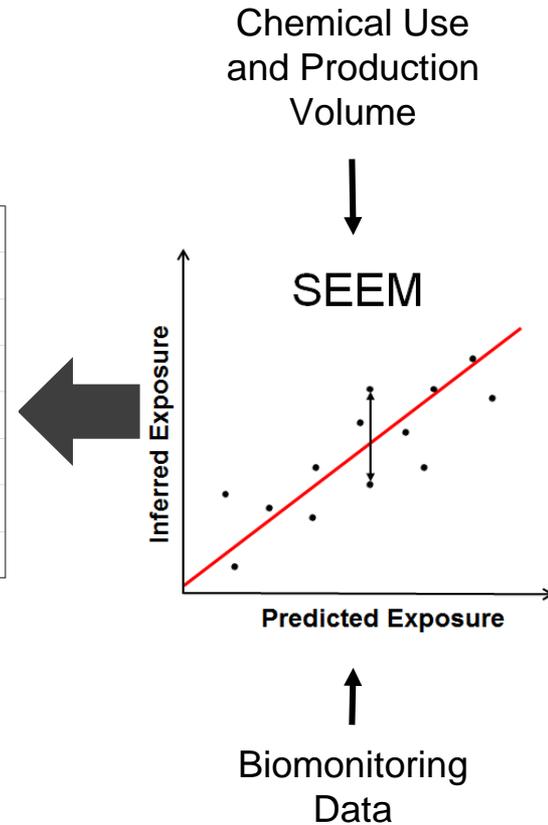
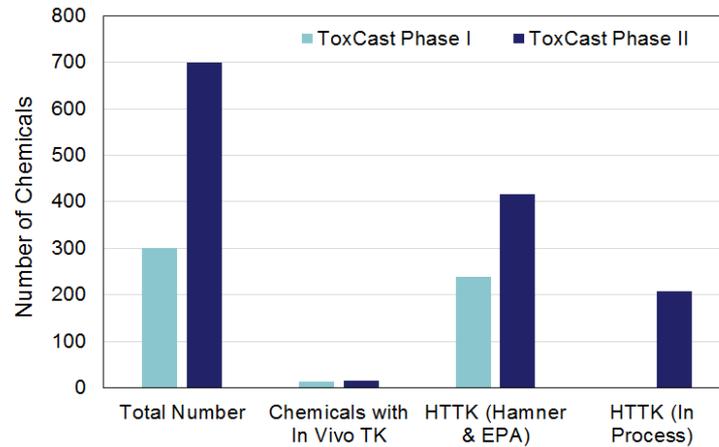
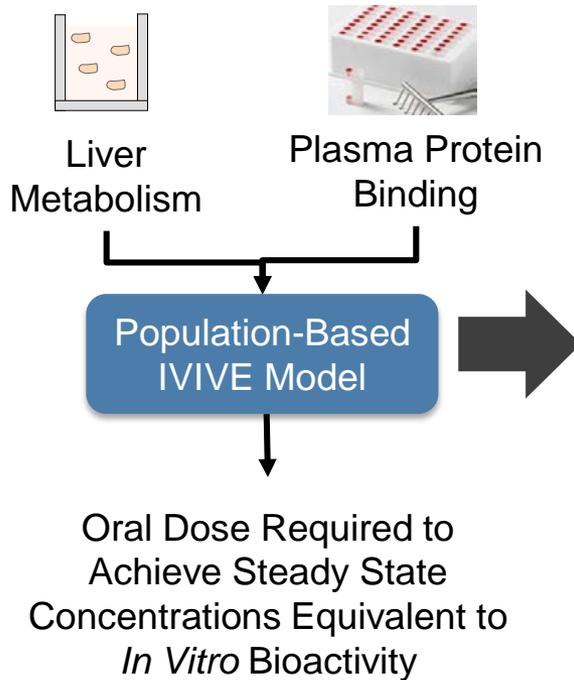
CS-FBS



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

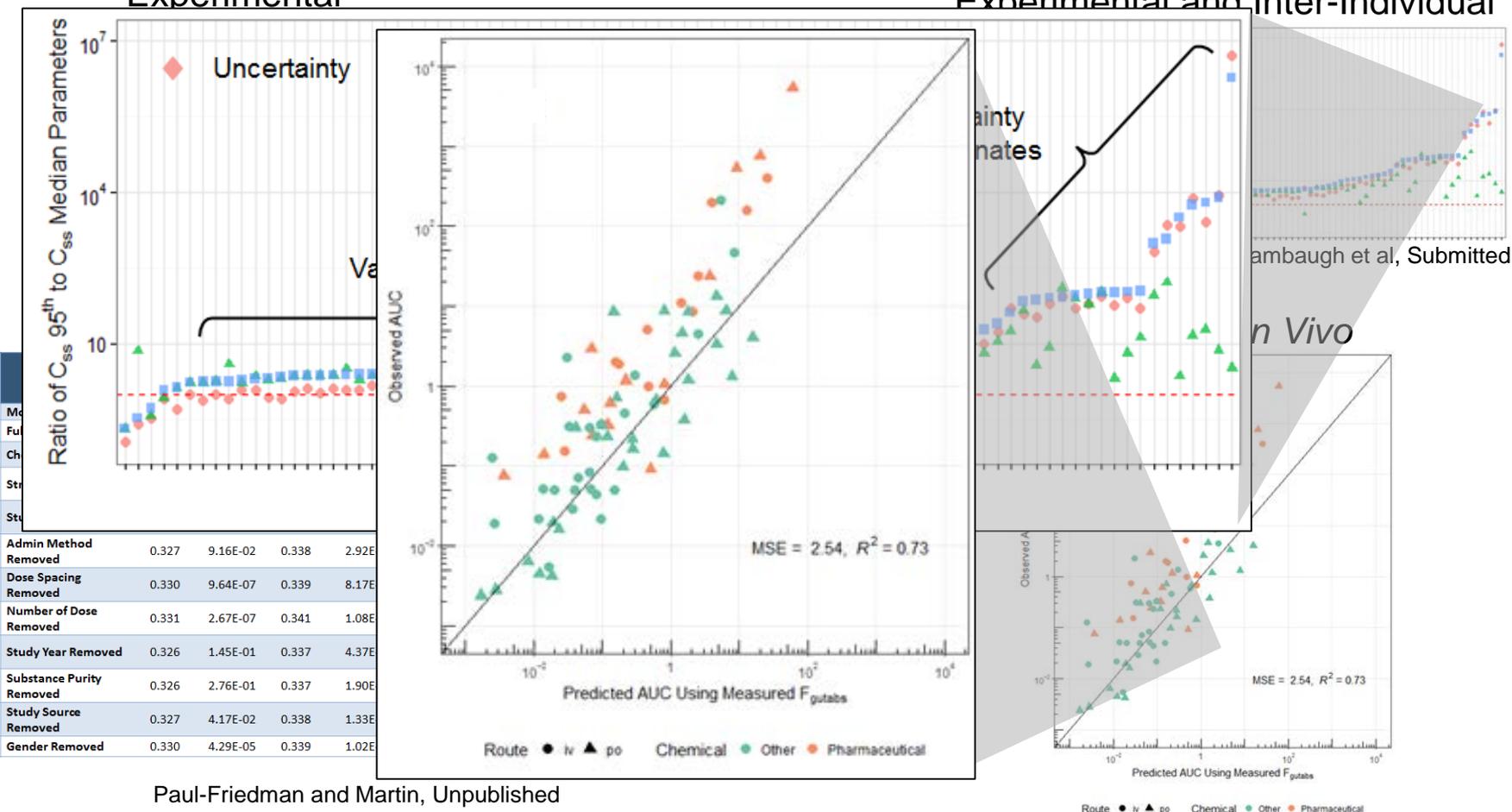
Quantifying Uncertainty and Variability

Pharmacodynamic

Pharmacokinetic

Experimental

Experimental and Inter-Individual

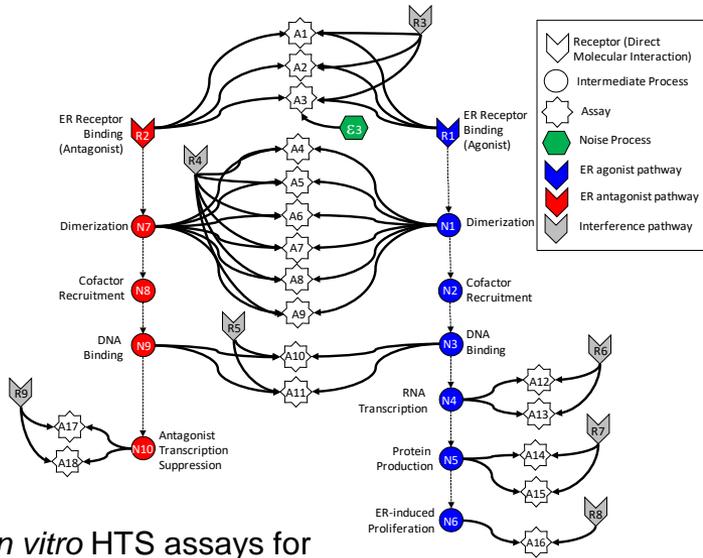


Admin Method Removed	0.327	9.16E-02	0.338	2.92E
Dose Spacing Removed	0.330	9.64E-07	0.339	8.17E
Number of Dose Removed	0.331	2.67E-07	0.341	1.08E
Study Year Removed	0.326	1.45E-01	0.337	4.37E
Substance Purity Removed	0.326	2.76E-01	0.337	1.90E
Study Source Removed	0.327	4.17E-02	0.338	1.33E
Gender Removed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

Paul-Friedman and Martin, Unpublished

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)

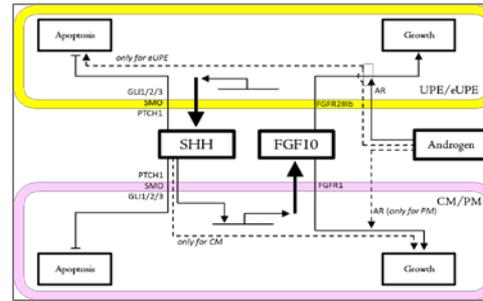
In Vivo Reference Chemicals*

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



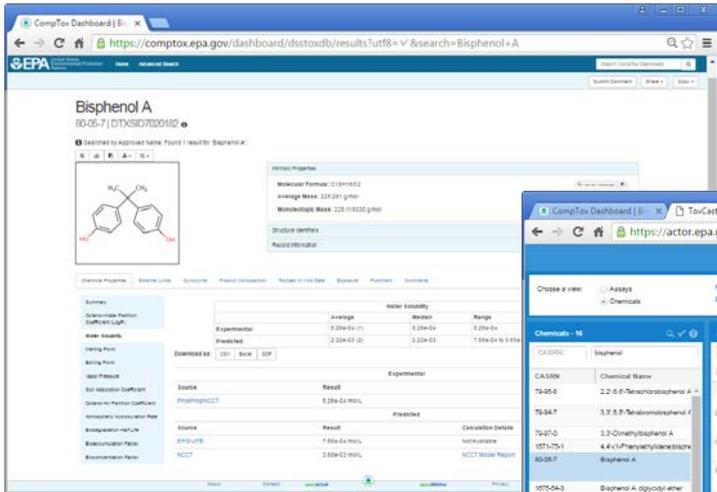
GD13.5 – 17.5

Androgenization (n = 10 sims)	Phenotype (MCS 4000)			Closure Index
	Septation	Fusion	Conden.	
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

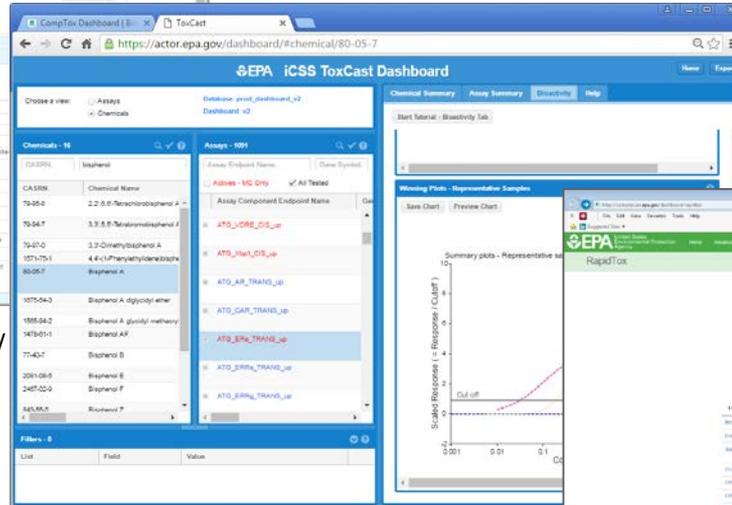
Deliver Data and Models Through Decision Support Tools

Comptox Chemistry Dashboard



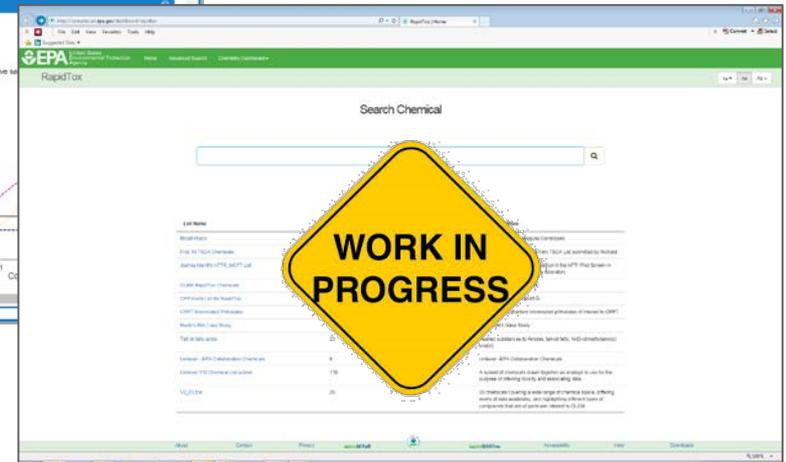
<https://comptox.epa.gov/dashboard/>

ToxCast Dashboard

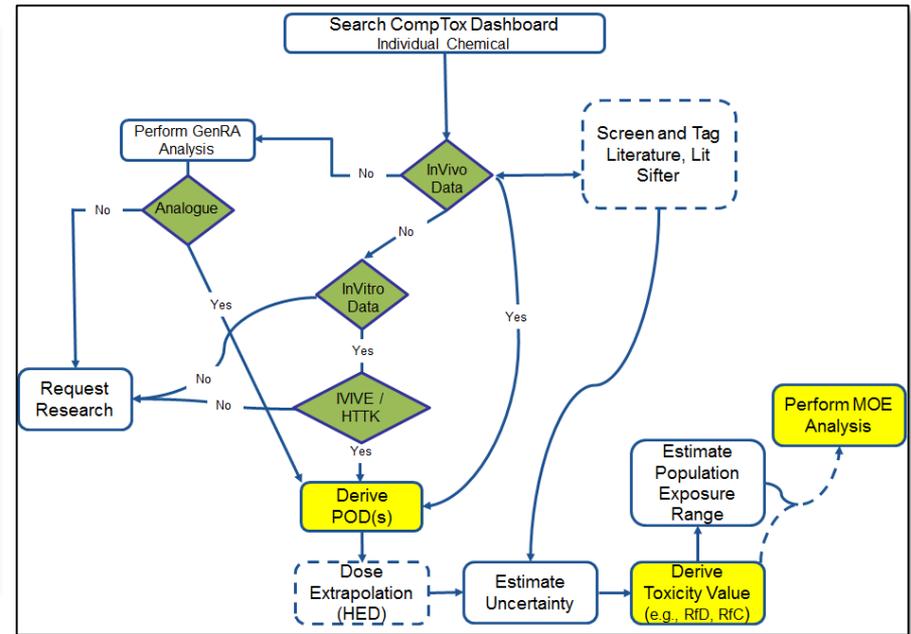
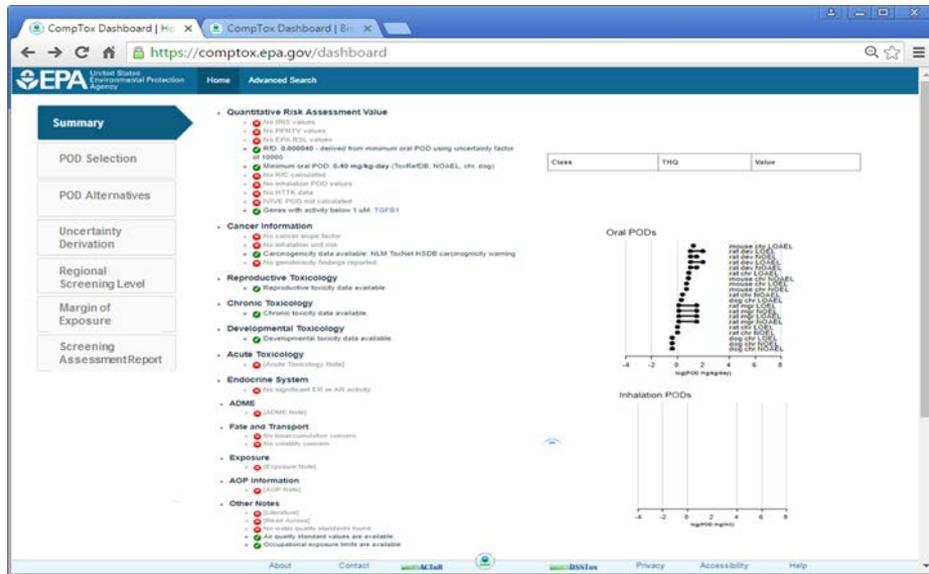


<https://actor.epa.gov/dashboard/>

RapidTox Dashboard

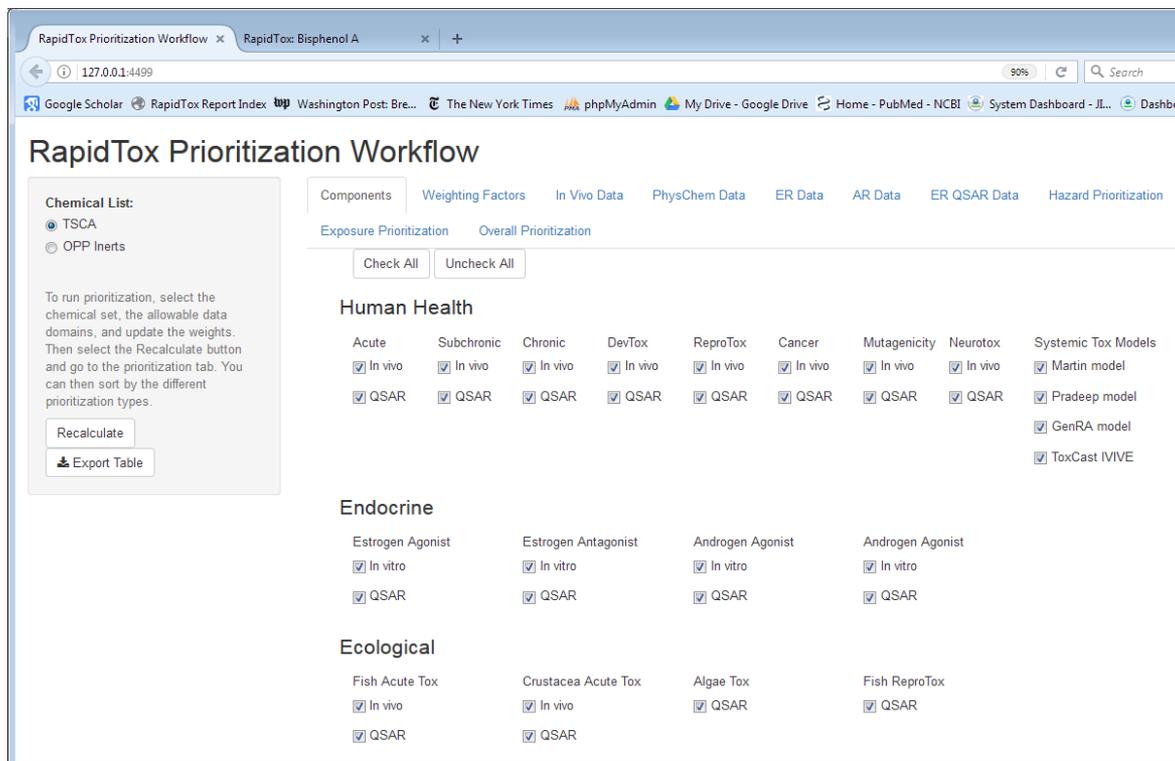


One Tool, Multiple Workflows to Address Diverse Partner Needs

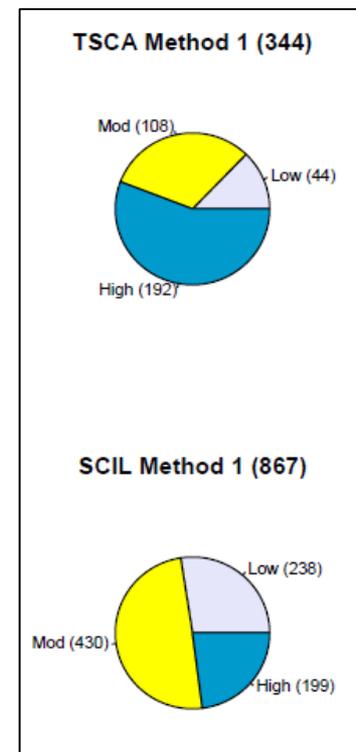


- Semi-automated decision support tool
- Flexible integration of information related to chemical properties, fate and transport, hazard, and exposure
- Enable expert users to review the assumptions made and refine the results
- Presents alternative together with traditional toxicology data

One Tool, Multiple Workflows to Address Diverse Partner Needs



The screenshot shows the 'RapidTox Prioritization Workflow' web application. The browser address bar shows '127.0.0.1:4499'. The page title is 'RapidTox Prioritization Workflow'. On the left, there is a 'Chemical List' section with radio buttons for 'TSCA' (selected) and 'OPP Inerts'. Below this is a 'Recalculate' button and an 'Export Table' button. The main content area has tabs for 'Components', 'Weighting Factors', 'In Vivo Data', 'PhysChem Data', 'ER Data', 'AR Data', 'ER QSAR Data', and 'Hazard Prioritization'. Under 'Components', there are sub-tabs for 'Exposure Prioritization' and 'Overall Prioritization', along with 'Check All' and 'Uncheck All' buttons. The 'Human Health' section includes categories like Acute, Subchronic, Chronic, DevTox, ReproTox, Cancer, Mutagenicity, Neurotox, and Systemic Tox Models, each with checkboxes for 'In vivo' and 'QSAR'. Other sections include 'Endocrine' (Estrogen Agonist/Antagonist, Androgen Agonist) and 'Ecological' (Fish Acute Tox, Crustacea Acute Tox, Algae Tox, Fish ReproTox).



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.

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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, toxic prevention scientist, and director of the national division: Industrial for the protection of

Robert Kavlock is an Administrator for the Administrator for the Office of Research Development (ORD) in the scientific whose leading-edge the understanding of for the agency. The views expressed those of the author represent the views Environmental Protection Administration, Washington, D.C. 20460, United States

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Chemical Research in Toxicology

5 On This Cover: See Pages 400A, 400L, 400I, 400J

Accelerating the Pace of Chemical Risk Assessment

Robert J. Kavlock,¹ Tina Bahadon,¹ Tara S. Barton-Madren,¹ Maureen R. Guinn,¹ Mike Ravensberg,¹ and Russell S. Thomas^{2,3,4}

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 vitro* approaches and *in silico* 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 exposure with the new data and to advance the application of NAMs 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 involves with direct input from and engagement by risk managers and regulatory decision makers, a workshop was convened among science leaders from international regulatory agencies to identify common barriers for using NAMs and to propose next steps to address them. Central to the workshop was 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* bioassay 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. This development and application of NAMs will play an increasing role in filling important data gaps on the toxicity of chemicals, but confidence in NAMs will only come with learning by doing and sharing in the experience.

CONTENTS

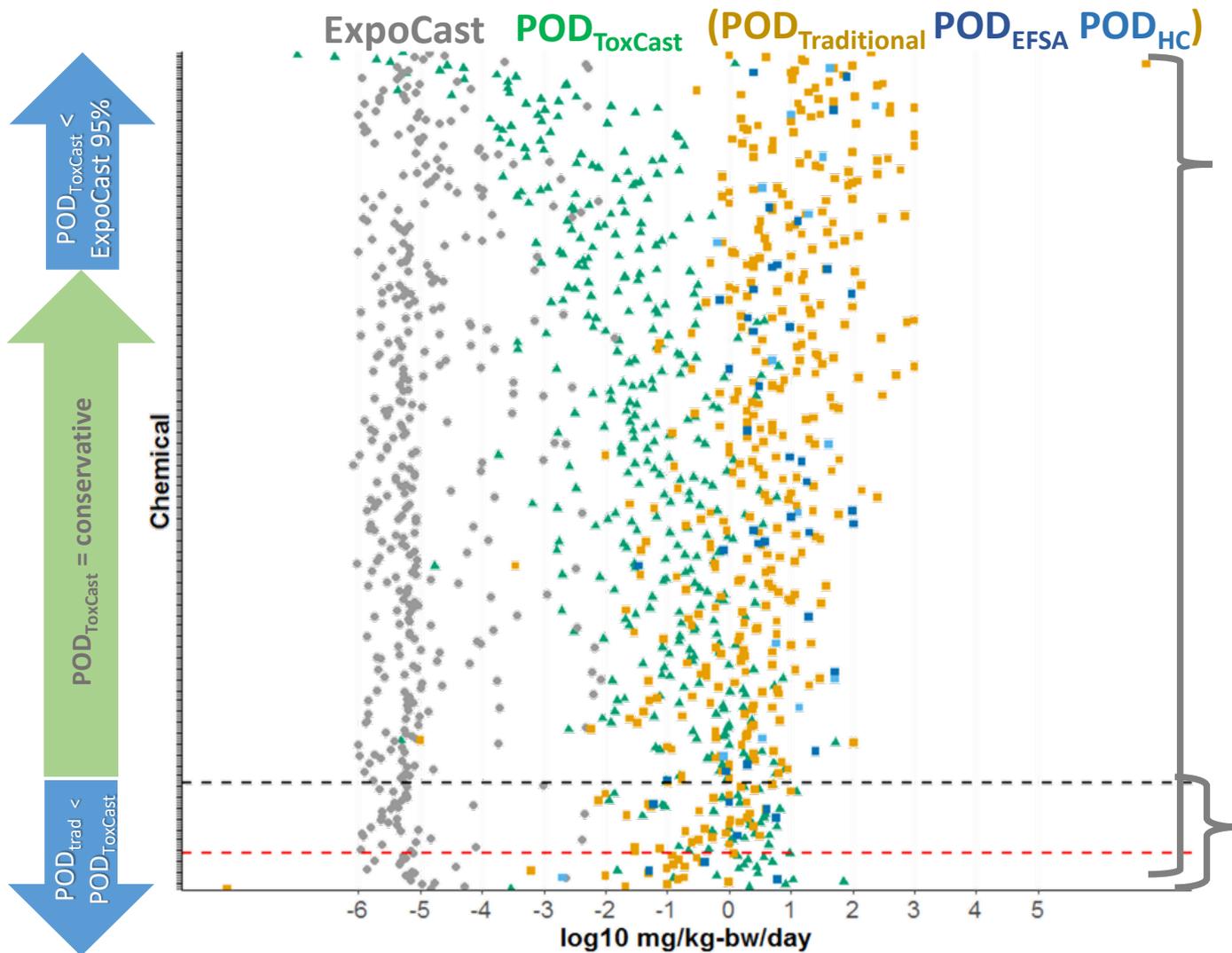
1. Overview
2. Next Steps
3. Conclusion
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5. OCID
6. NRES
7. Biographies
8. References

1. Overview
The modernization of the U.S. Toxic Substances Control Act (TSCA) and the implementation of European Union's Registration, Evaluation, Authorization and Restriction of Chemicals (REACH), the new phase of the Canadian Chemicals Management Plan (CCMP), and many international chemical management policies and laws have increased 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 support traditional toxicology data which have traditionally relied on animal models. The new data are diverse and include data from high-throughput toxicity and toxicokinetics testing, molecular epidemiology, toxicogenomics, exposure science, computational chemistry, and new animal models, among others.

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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 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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Health Canada
JRC

EPA's National Center for Computational Toxicology

