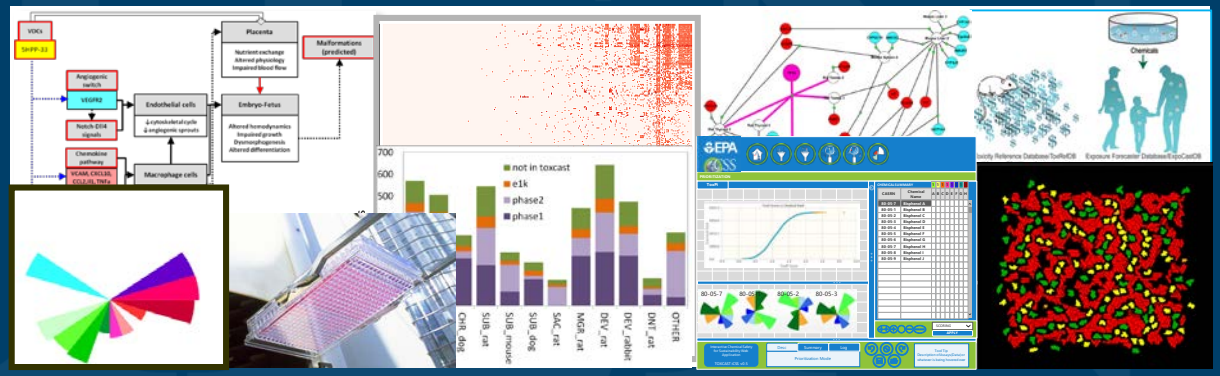
EPA United States Environmental Protection Agency

Translating Innovations in Toxicology into Chemical Safety Decision Making



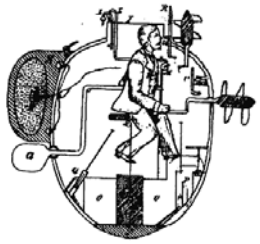
Total Exposure Health Conference

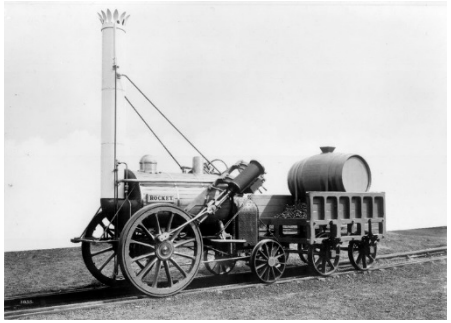
September 6, 2018

Rusty Thomas
Director
National Center for Computational Toxicology

EPA United States Environmental Protection Agency

Version 1.0 is Never Perfect…

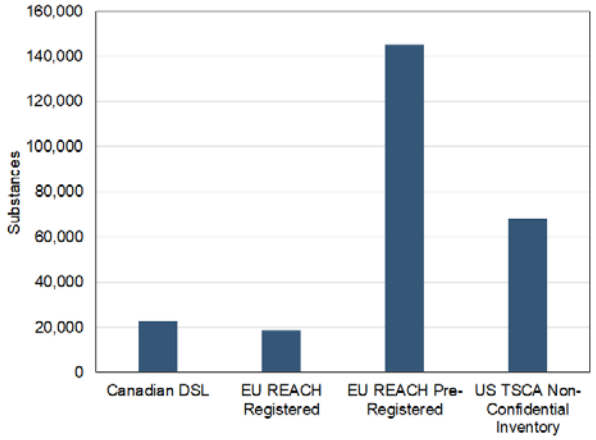




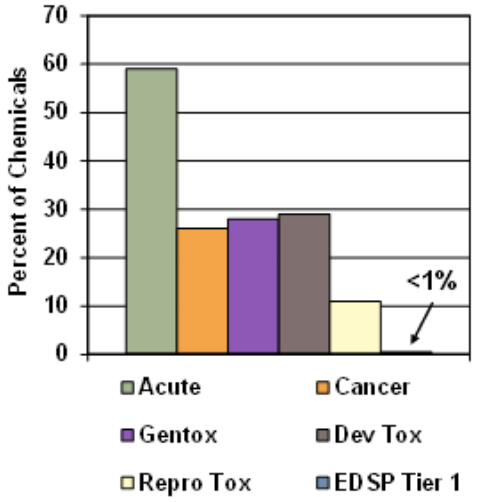
Early Versions of Toxicology Left Challenges for Fully Evaluating Safety

United States Environmental Protection Agency

Number of Substances

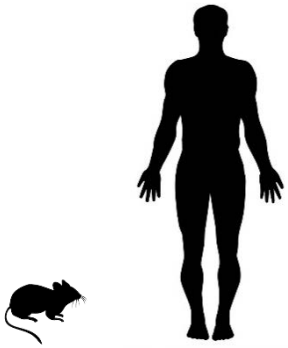


Amount of Data

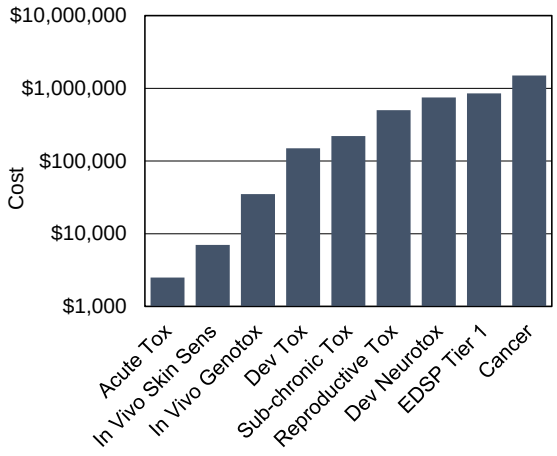


Modified from Judson *et al.*, EHP 2009

Relevance



Economics



Solving the Challenges Will Require Both Innovation and Translational Tools

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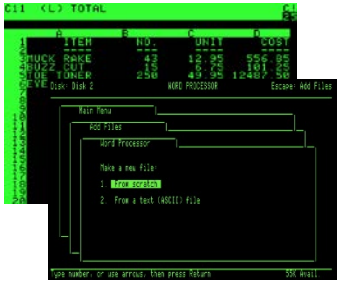

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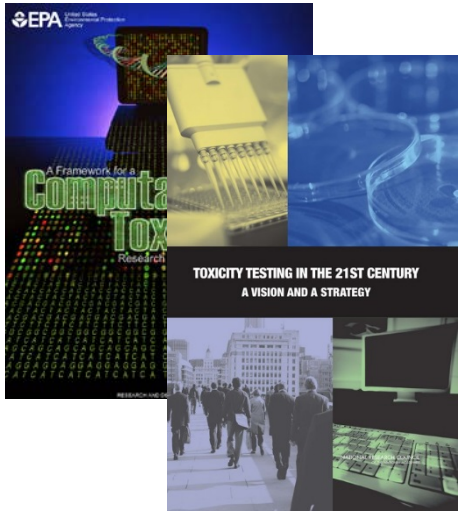

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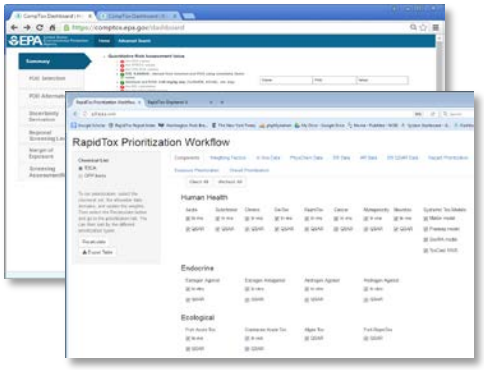
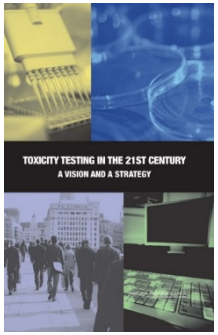
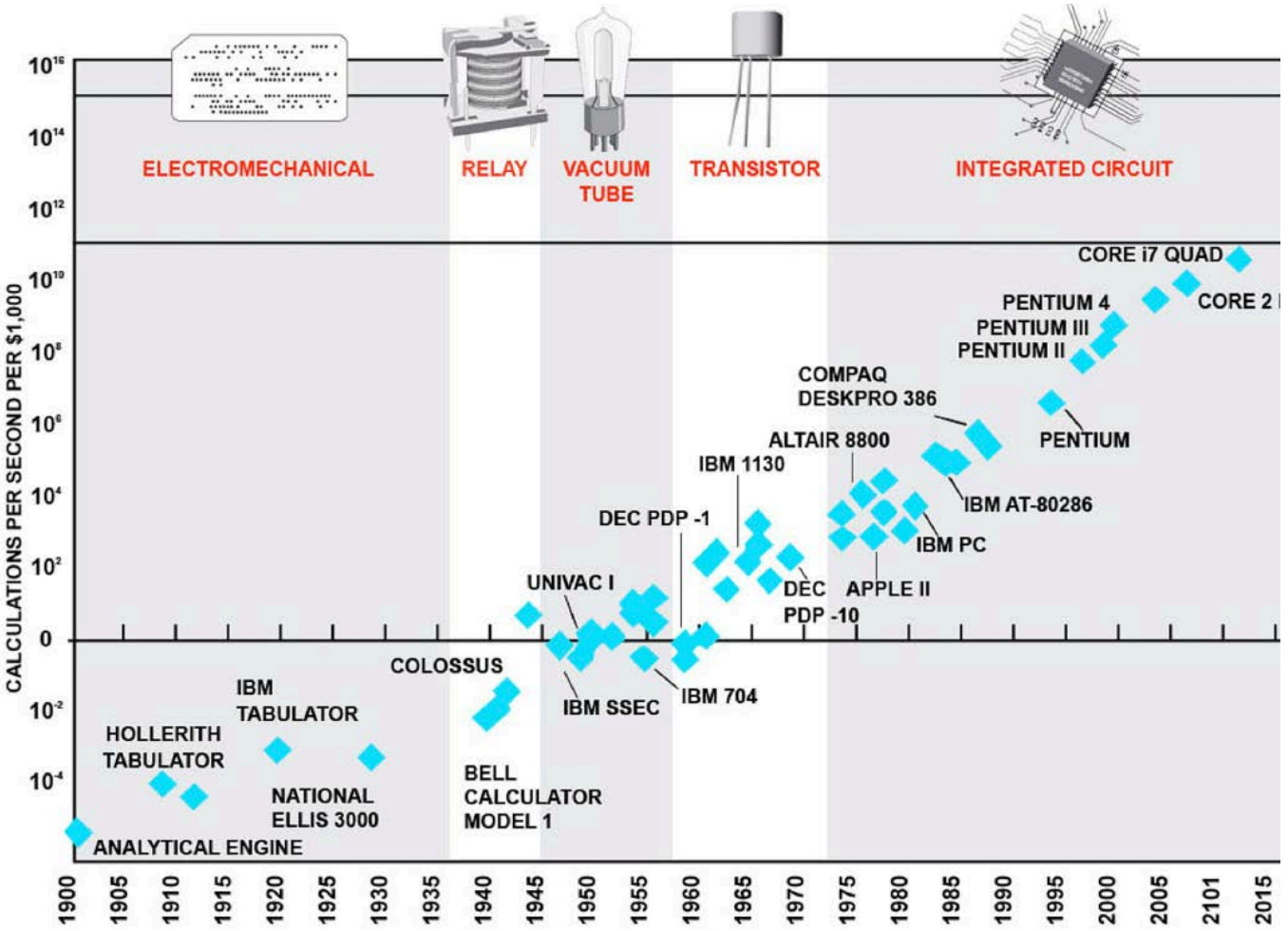


Highlights of Technical Innovations and Translational Tools at EPA



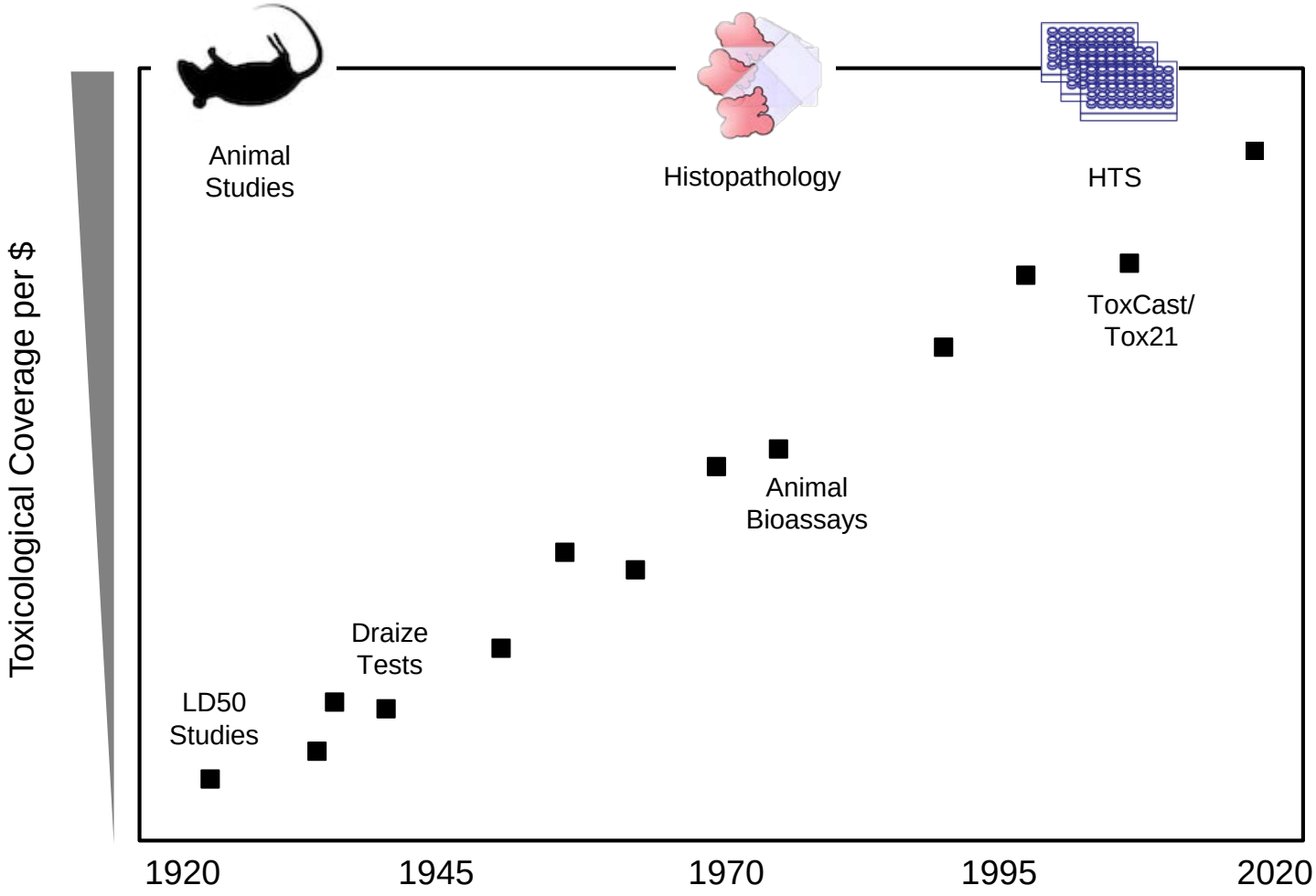
- Move towards increased throughput
- Understanding promiscuity is the norm for environmental/commercial chemicals
- Focus on comprehensive biological coverage
- Increasingly relevant test systems
- Delivery of data and models through decision support tools
- Building confidence through regulatory focused case studies

Moore's Law for Computational "Throughput" and Coverage



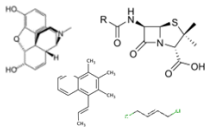
https://sites.google.com/site/hmtcamobilecsp20152016/word-of-the-week/831

Rusty's Law for Toxicological "Throughput" and Coverage



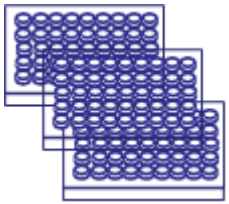
*Graph for illustrative purposes only

Innovations in High-Throughput Screening



Thousands of Chemicals

Concentration Response Screening

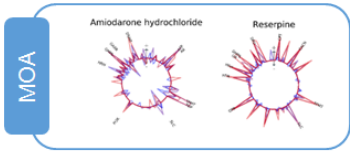


ToxCast Assays

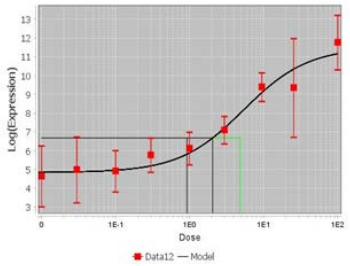
Transcription Factors
Transporter
Cytokines
Kinases
Nuclear Receptors
CYP450 / ADME
Cholinesterase
Phosphatases
Proteases
XME metabolism
GPCRs
Ion channels

~700 Assay Endpoints

Mode-of-Action Identification



Concentration Response Modeling

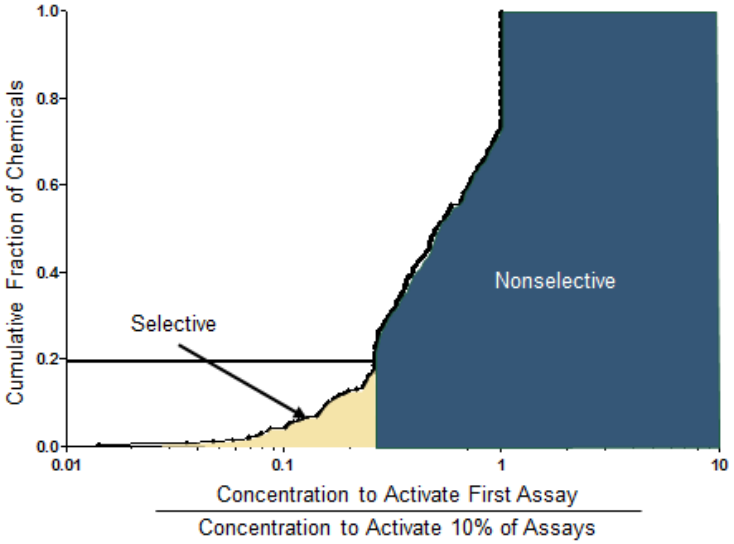


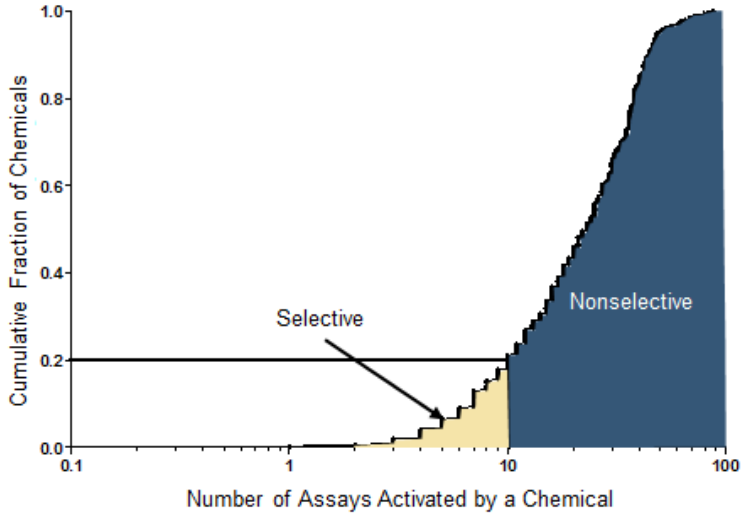
- 96, 384, and 1536-well, laboratory automation compatible
- Relatively expensive (~$20,000 - $30,000 / chemical)
- Coverage of molecular and phenotypic responses
- Multiple assay vendors/labs

Biological Promiscuity is the Norm for Most Chemicals

- Most histological changes do not occur without upstream or downsream changes at the molecular level

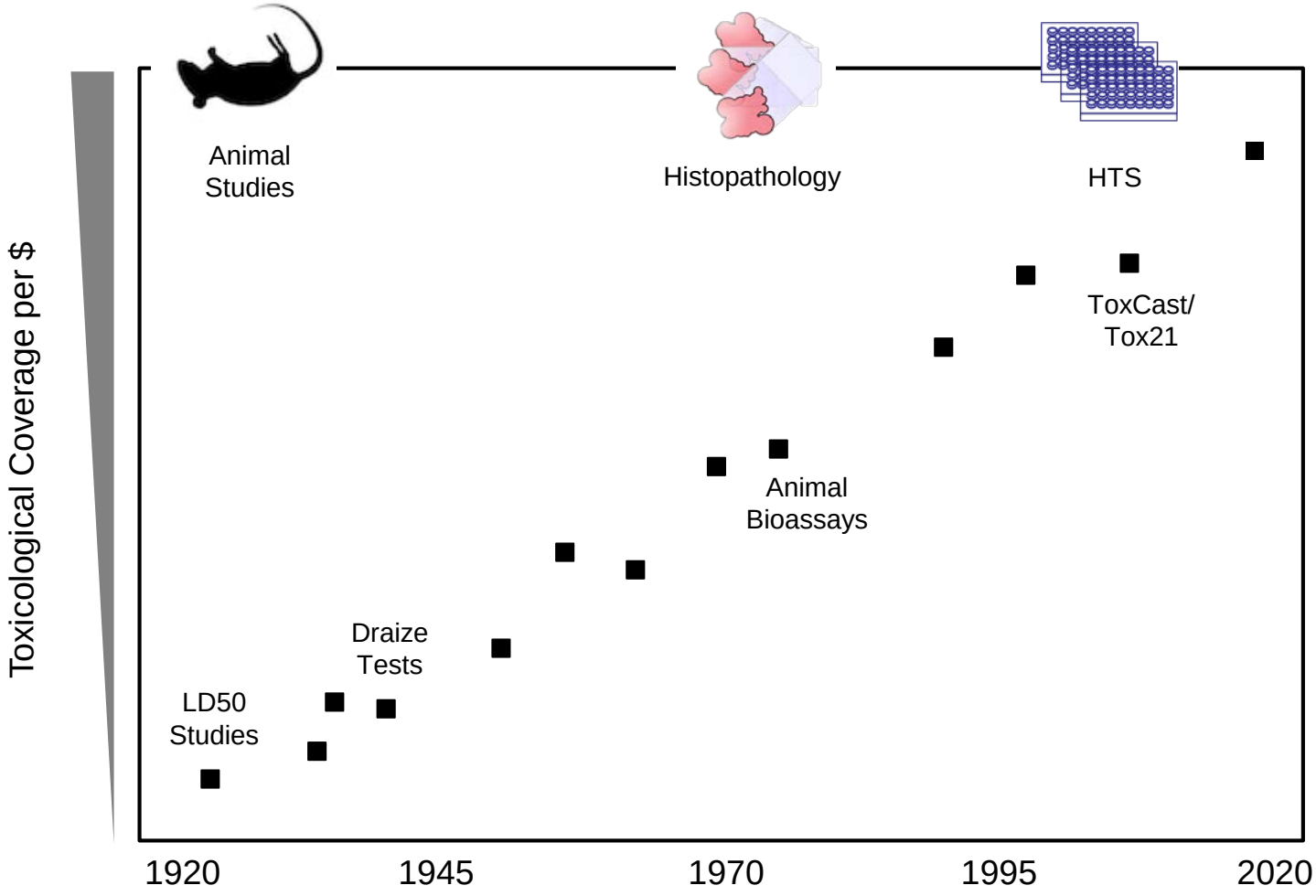
- Most environmental/commercial chemicals are highly non-selective in their interactions with biological systems





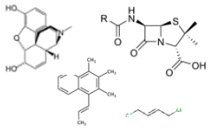
Thomas *et al., Tox Sci.,* 2013

Rusty's Law for Toxicological "Throughput" and Coverage

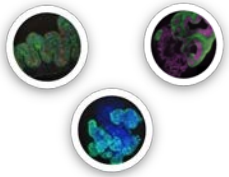


*Graph for illustrative purposes only

Innovations in High-Throughput and High-Content Screening

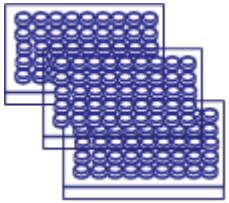


Thousands of Chemicals

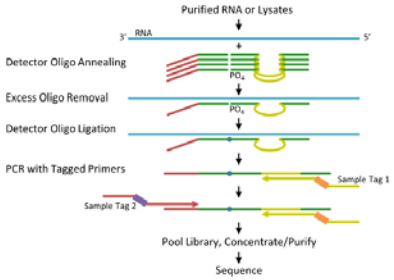


Multiple Cell Types

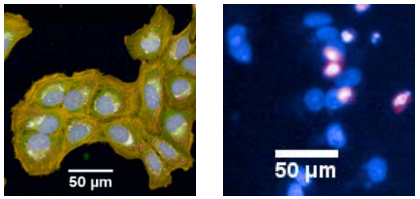
Concentration Response Screening



Whole Genome Transcriptomics

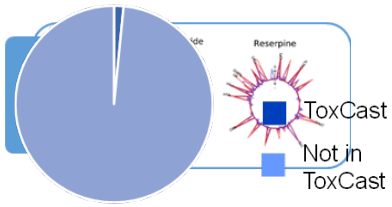


Multi-Parameter Cellular Phenotypic Profiling



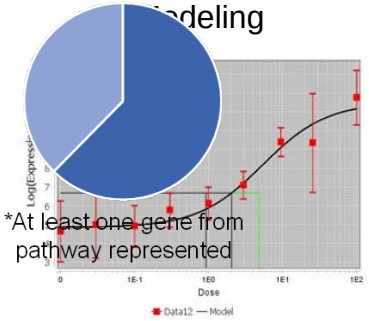
Mode-of-Action Identification

Gene Coverage



Pathway Coverage*

Concentration Response Modeling



*At least one gene from pathway represented

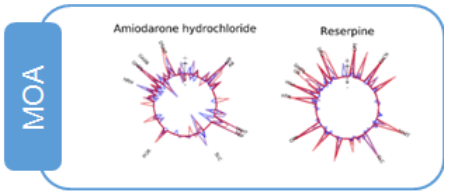
- 384-well, laboratory automation compatible
- Relatively inexpensive ($2.50 - $1,500 per chemical)
- Broad complementary coverage of molecular and phenotypic responses
- Integration of reference materials and controls for performance standards
- Increased portability

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

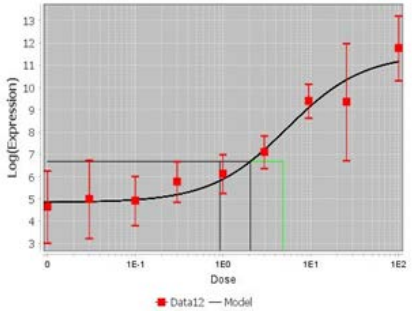
- **Number of samples:** 54,432
- **Number of endpoints:** 1.15×10^9
- **Total amount of data:** ~50 TB

Mode-of-Action Identification



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

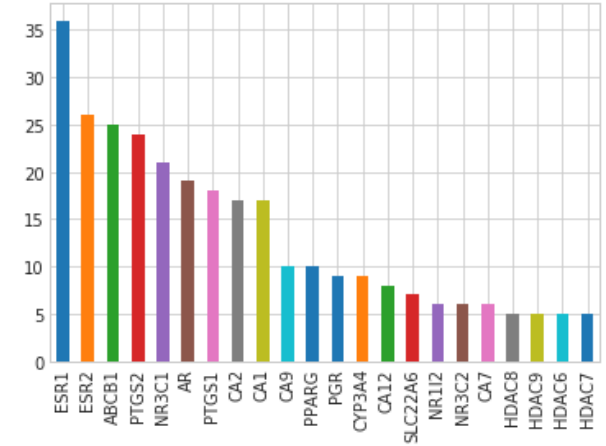
Concentration Response Modeling



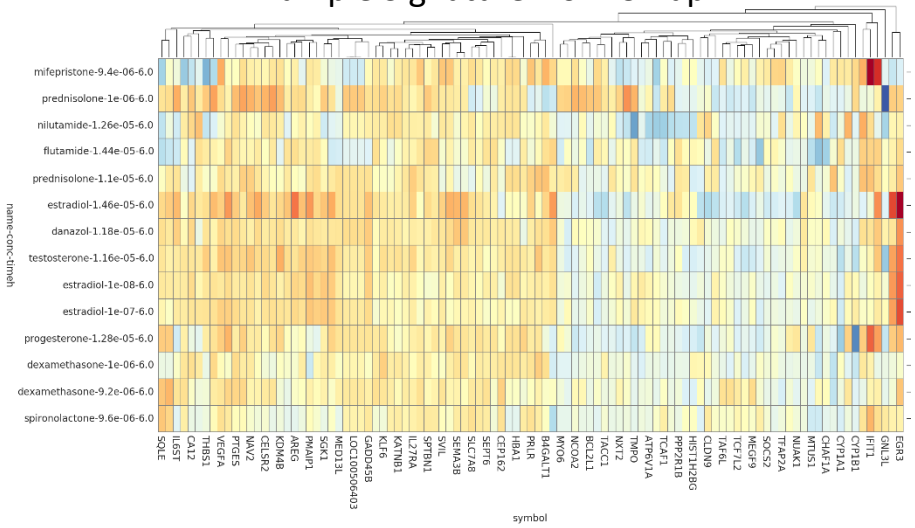
Currently comparing a range of approaches…
BMDExpress, Proast, tcpl, and new NB model

Identifying Potential Biological Targets

Annotated Targets in CMap v2 and RefChem



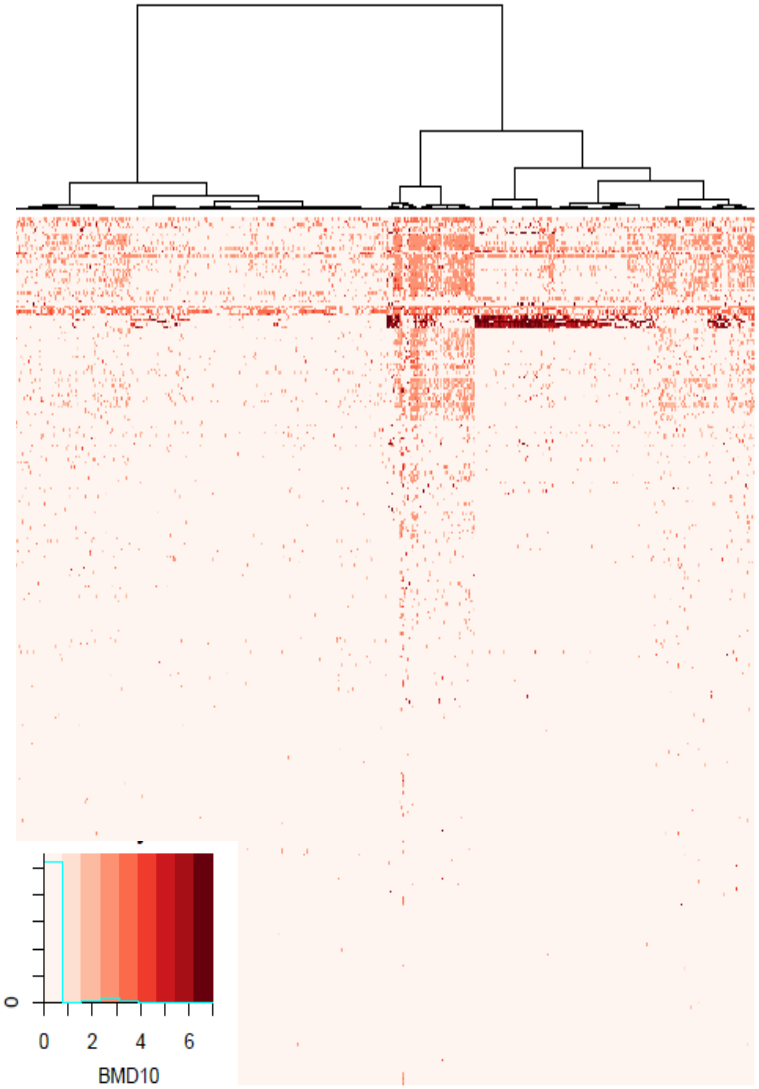
Example Signature from CMap v2

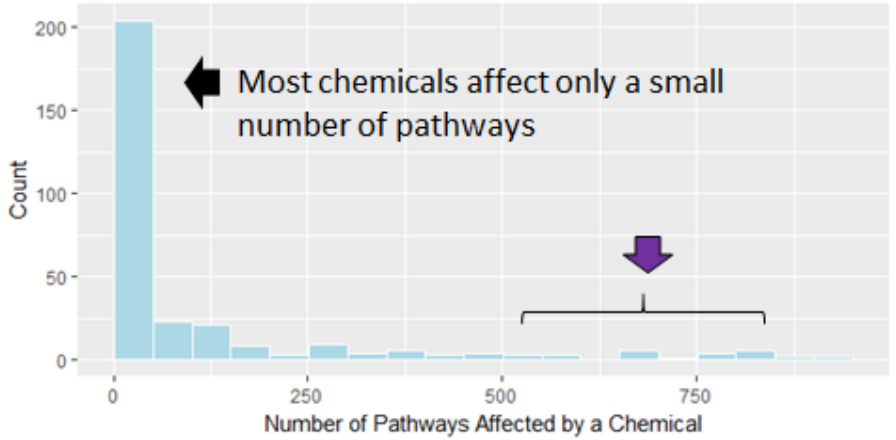


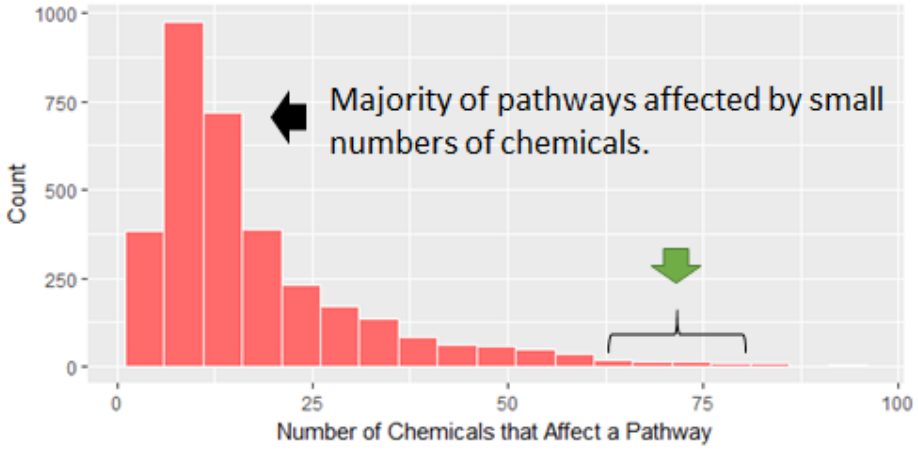
	CMap v2 / Affymetrix	HTTr-Phase I RefChem Hits	
Target	Signature size	Sensitivity	Positives
CYP2C9	131	1	1
ESR1	257	1	11
HDAC1	124	1	2
DHFR	215	1	2
NR1I2	139	1	2
PGR	115	1	1
HMGCR	236	1	1
ABCC2	357	1	1
TYMS	329	1	1
ESR2	281	0.86	7
AR	261	0.78	9
NR3C2	352	0.5	2
ABCB1	117	0.5	2
NR3C1	148	0.5	4
CA1	176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







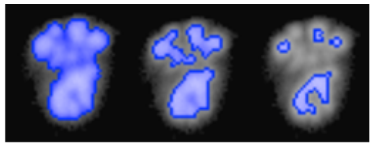
J. Harrill, I. Shah, W. Setzer, Judson Unpublished

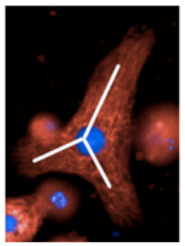
Development of High-Throughput Phenotypic Profiling

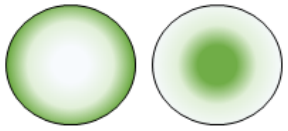
Cell Compartments

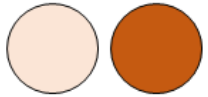
Non-Ab Dyes	NUCLEUS	RING	CYTOPLASM	MEMBRANE	CELL
DNA	S,C,A,R, P,I,T,M	--	--	--	S,C,A,R, P,M
RNA	S,C,A,R, P,I,T	--	--	--	S,C,A,R, P
ER	S,C,A,R, P,I,T	I,T	I,T	I	S,C,A,R, P
AGP	S,C,A,R, P,I,T	I,T	I,T	I,T	S,C,A,R, P
MITO	S,C,A,R, P,I,T	I,T	I,T	I	S,C,A,R, P

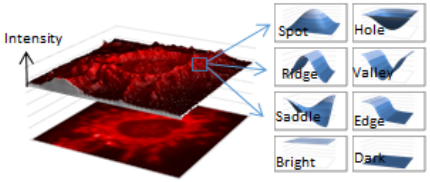

Shape (M)


Threshold Compactness (C)

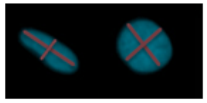

Symmetry (S)


Radial distribution (R)


Intensity (I)



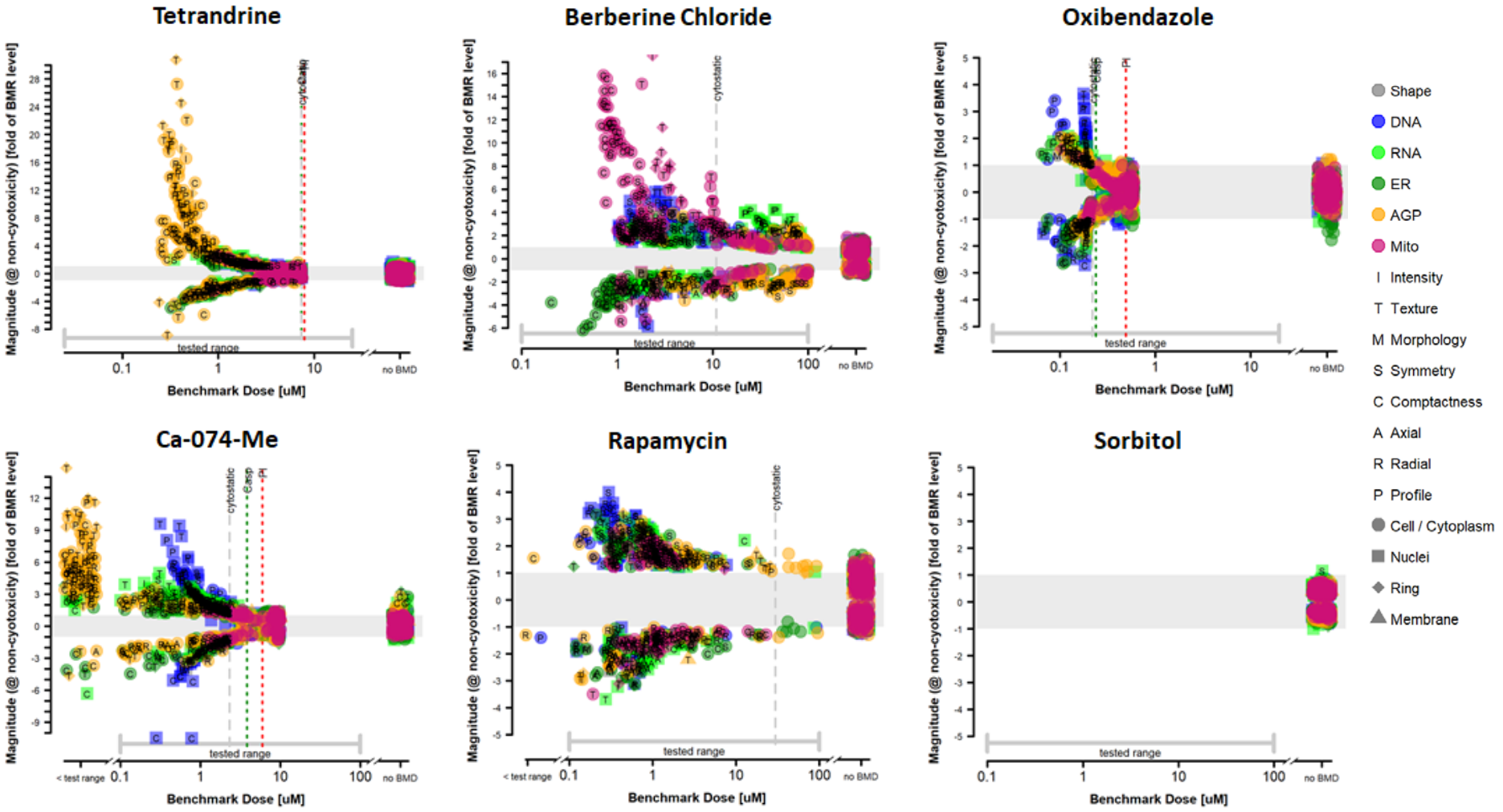
Texture (T)


Axial (A)


Profile (P)

~1,300 total phenotypic endpoints

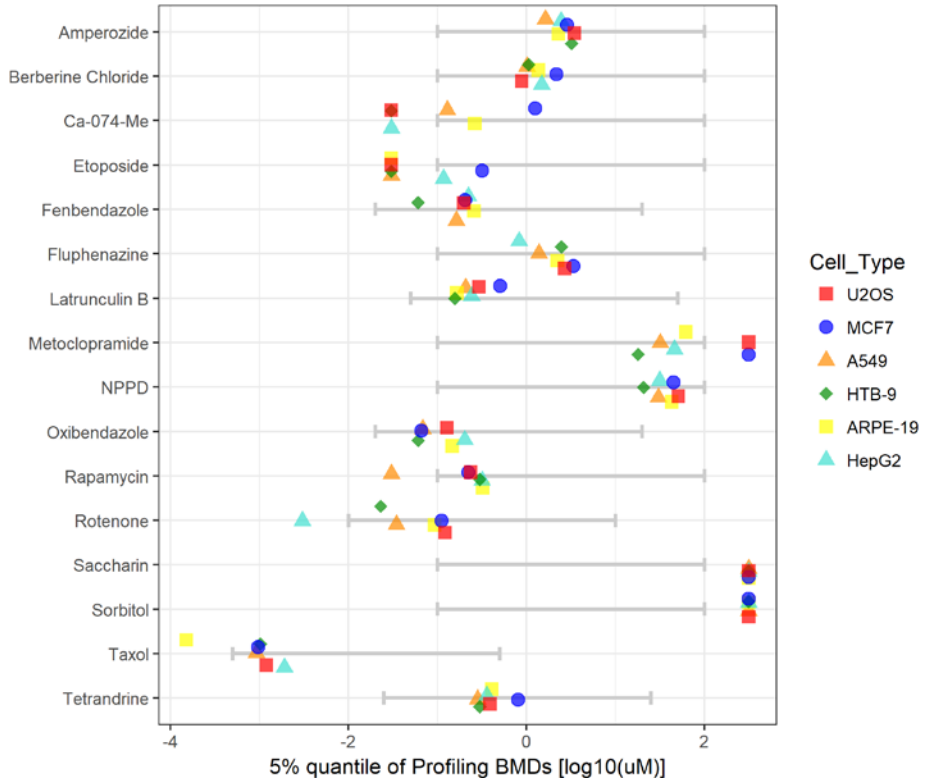
Unique Phenotypic Responses Associated with Different MOAs



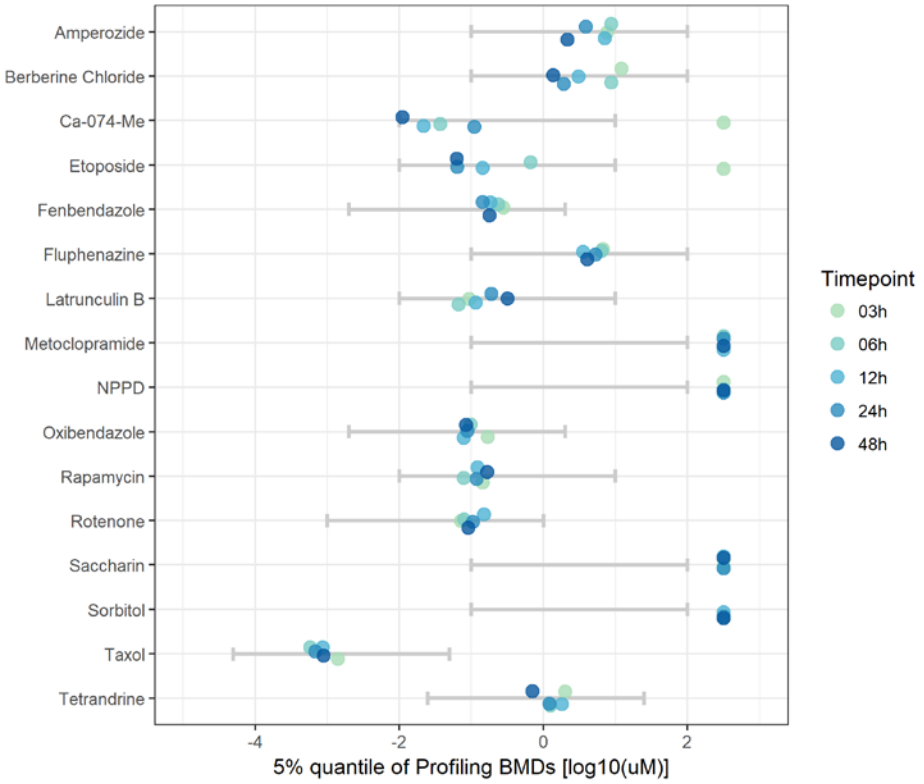
J. Nyffeler, J. Harrill, Unpublished

Variation in Phenotypic Potencies Across Cell Type and Time

Cell Type Differences (48 hr)



Time Point Differences (U2OS cells)



*Data points represent 5th percentile of phenotypic BMDs

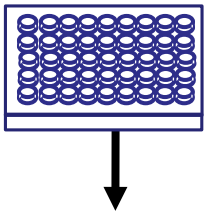
Tested range

J. Nyffeler, J. Harrill, Unpublished

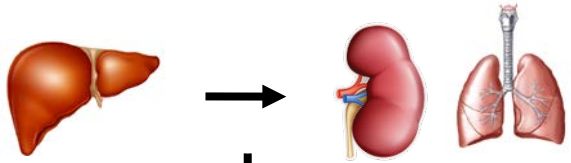
Innovations in Incorporating Xenobiotic Metabolism

"Extracellular" Approach

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

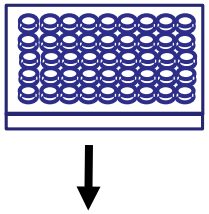


More closely models effects of hepatic metabolism and generation of circulating metabolites

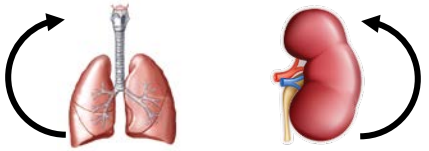


"Intracellular" Approach

Chemical metabolism inside the cell in cell-based assays



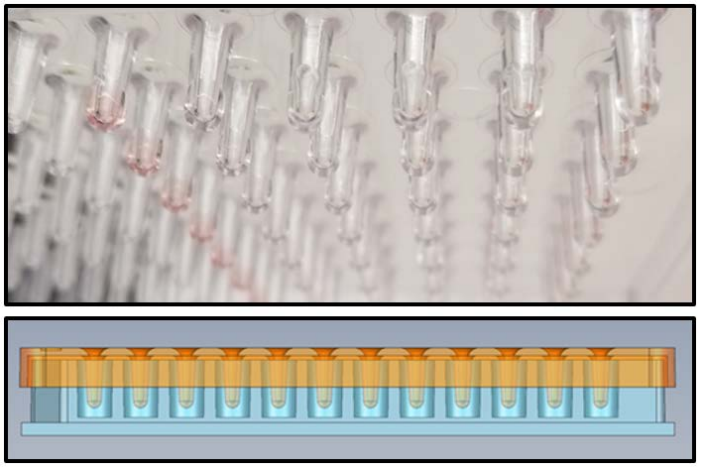
More closely models effects of target tissue metabolism



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

Application of Extracellular Strategy to Identify Estrogenic Metabolites

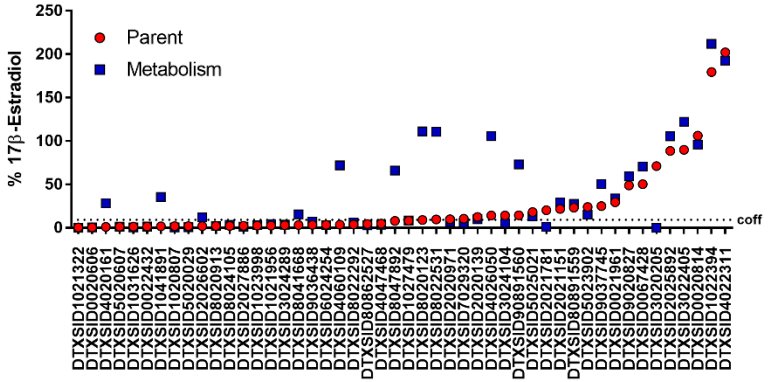
AIME Method: S9 Fraction Immobilization in Alginate Microspheres on 96- or 384-well peg



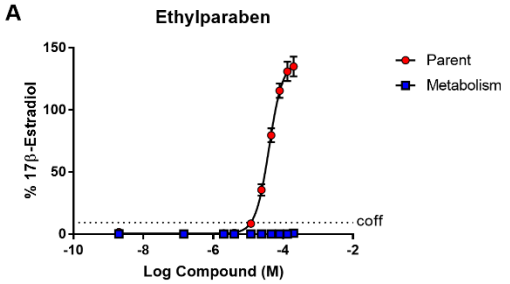
Screening Window of VM7 (formerly BG1) ER Transactivation Assay

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

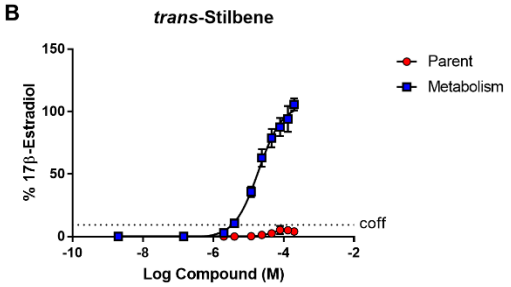
Pilot Screening Results of Pinto et al., 2016 Library



Example Detoxification

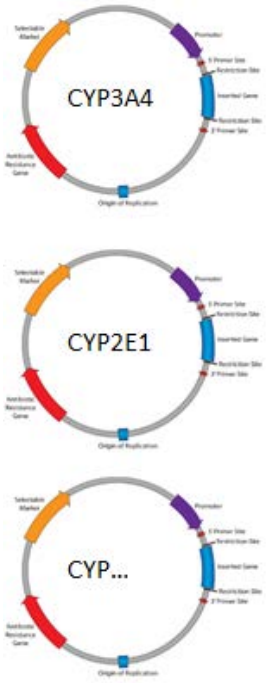


Example Bioactivation

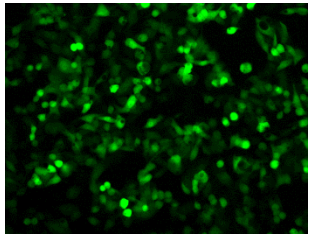


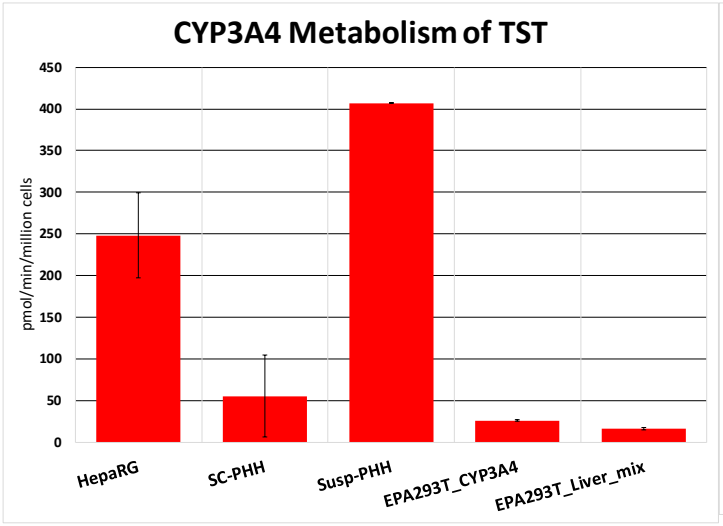
D. DeGroot, C. Deisenroth, Unpublished

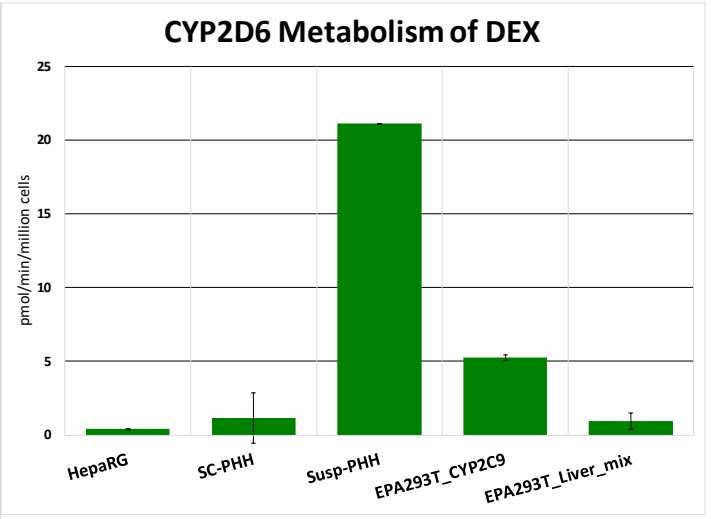
Application of Intracellular Strategy to Identify Cytotoxic Metabolites

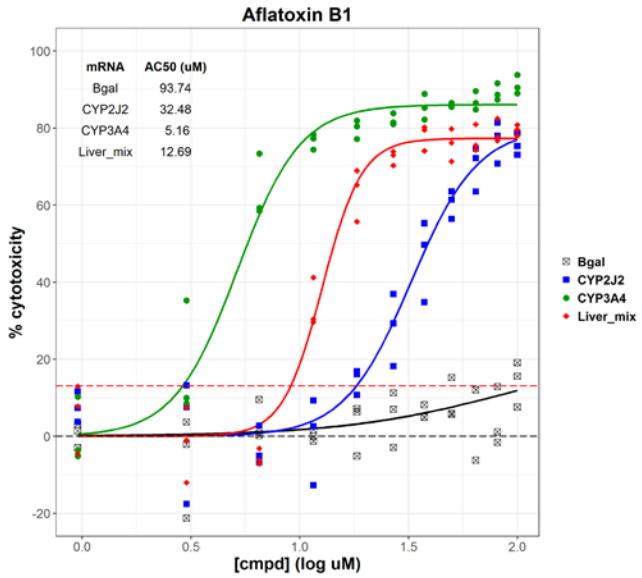


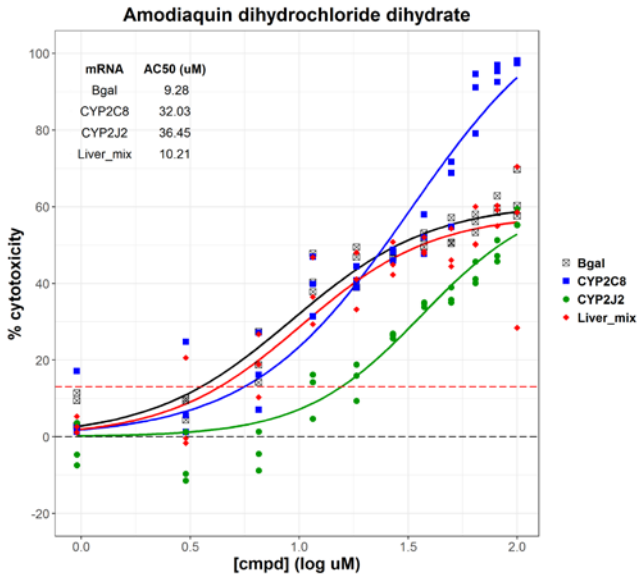
mRNA









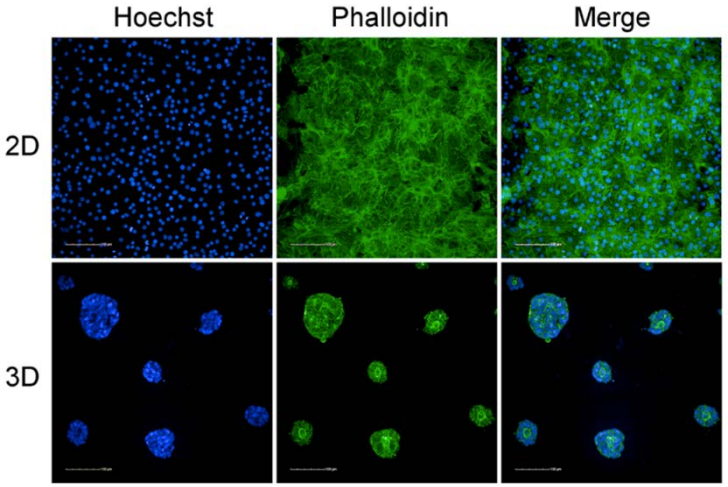


S. Simmons, Unpublished

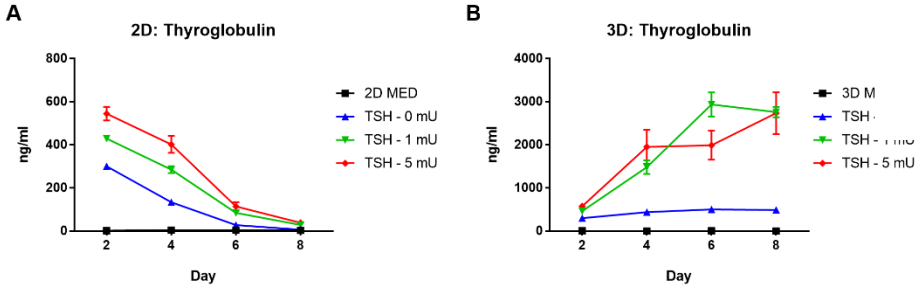
Innovating in Organotypic Culture Models to Predict Tissue Effects

United States Environmental Protection Agency

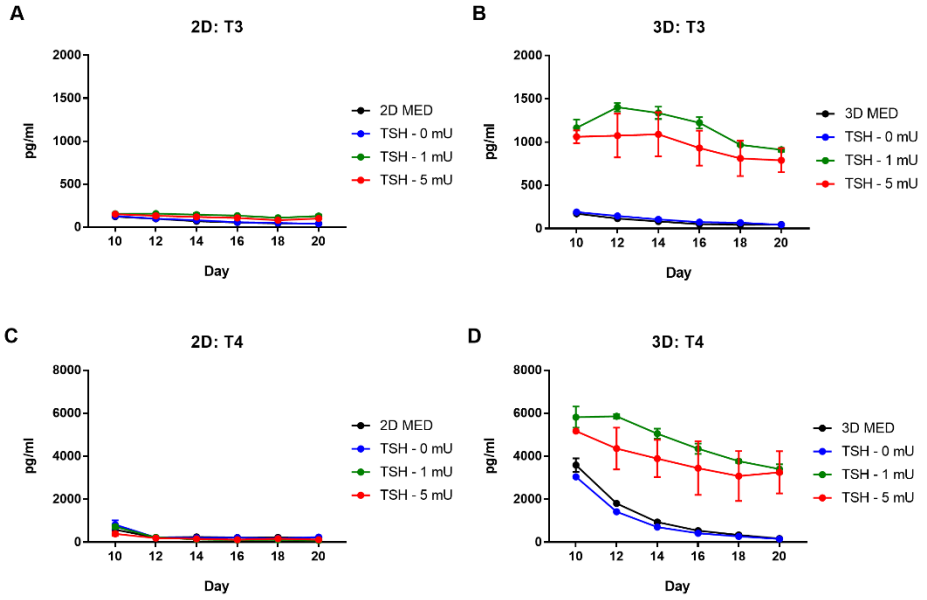
3D Microtissue Model of Primary Human Thyrocytes



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

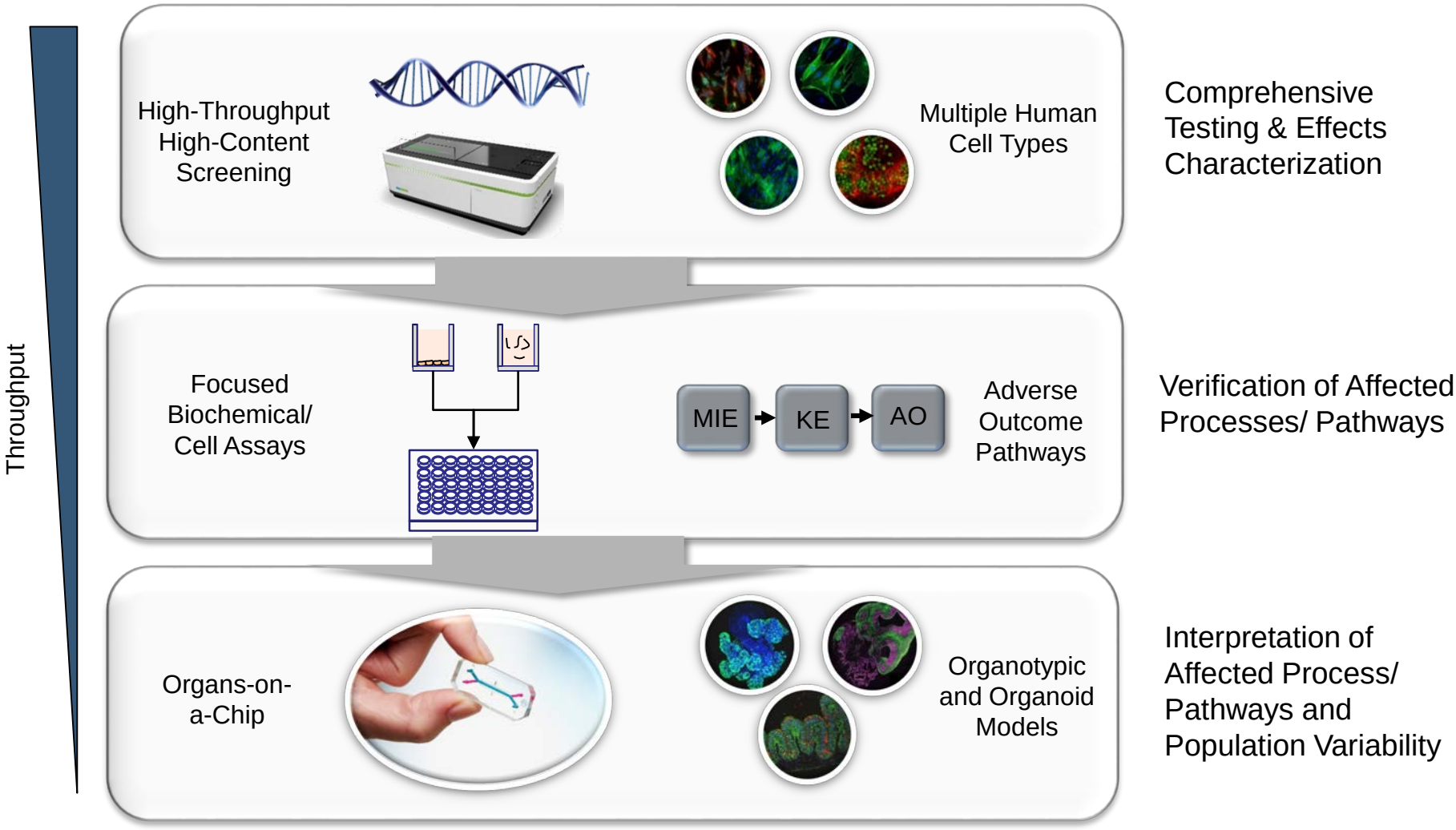


Thyroid hormone is synthesized and secreted over time in a 3D culture model



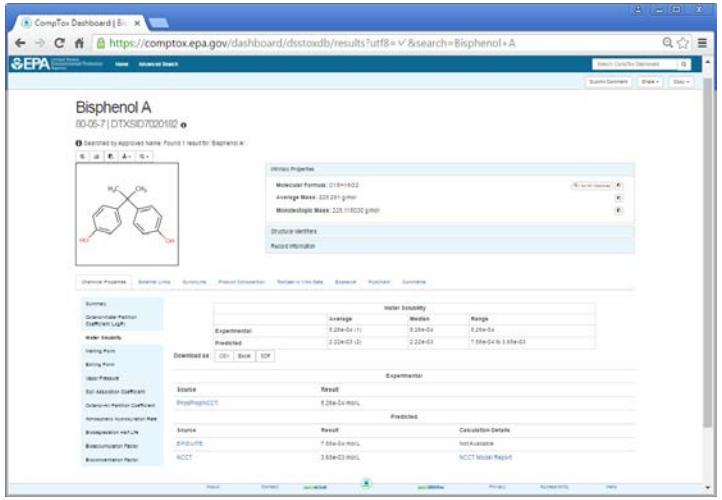
C. Deisenroth, Unpublished

Integrating New Technologies for Efficient Chemical Testing



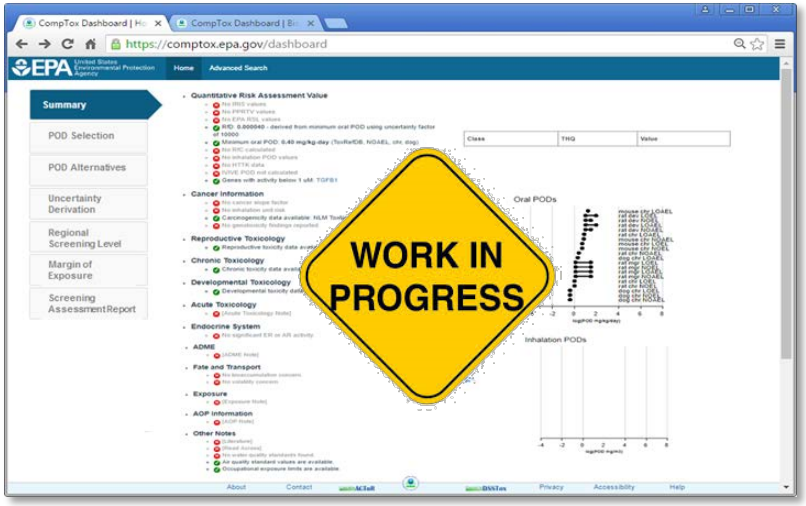
Enable Translation Through Data Visualization and Decision Support Tools

Comptox Chemicals Dashboard



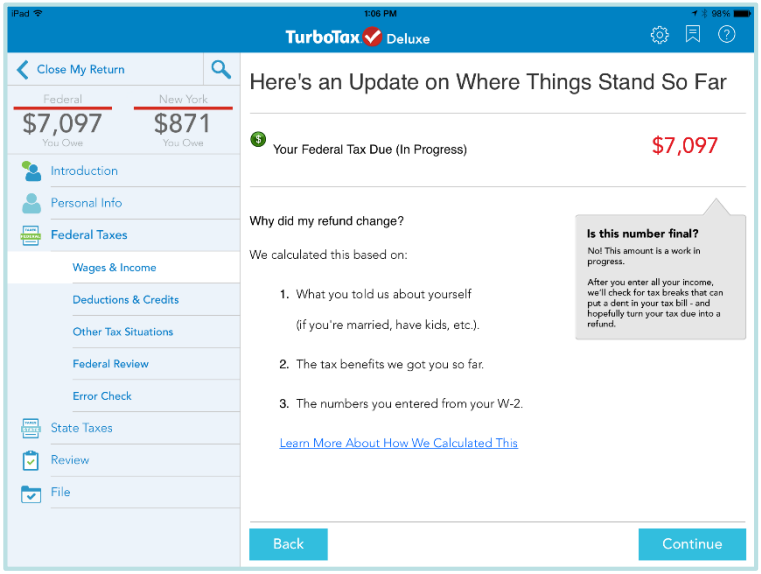
https://comptox.epa.gov/dashboard/

RapidTox Dashboard



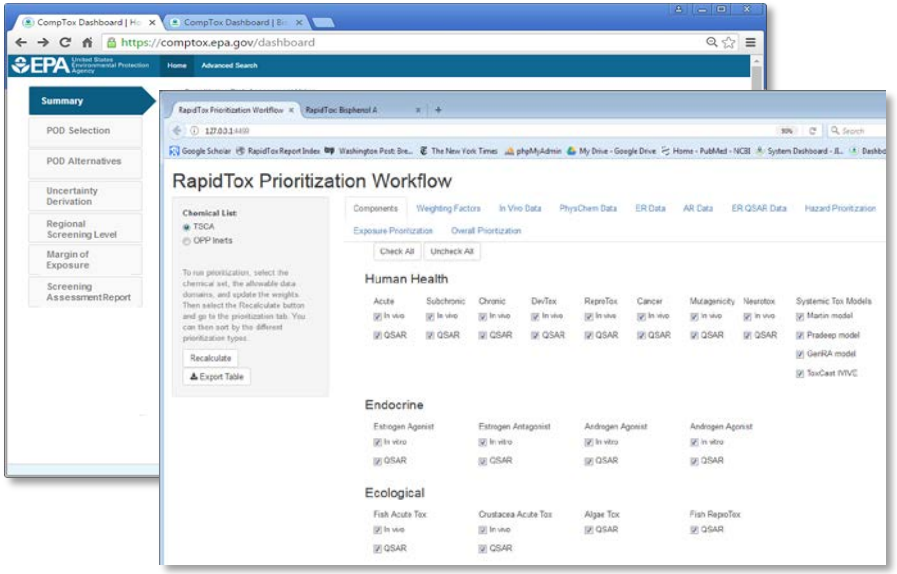
Similar to Financial Tools, RapidTox will Have Multiple Workflows to Address Different Decision Contexts

Workflow to Calculate Your Taxes



https://turbotax.intuit.com/

Workflows to Integrate Safety Data for Regulatory Decisions



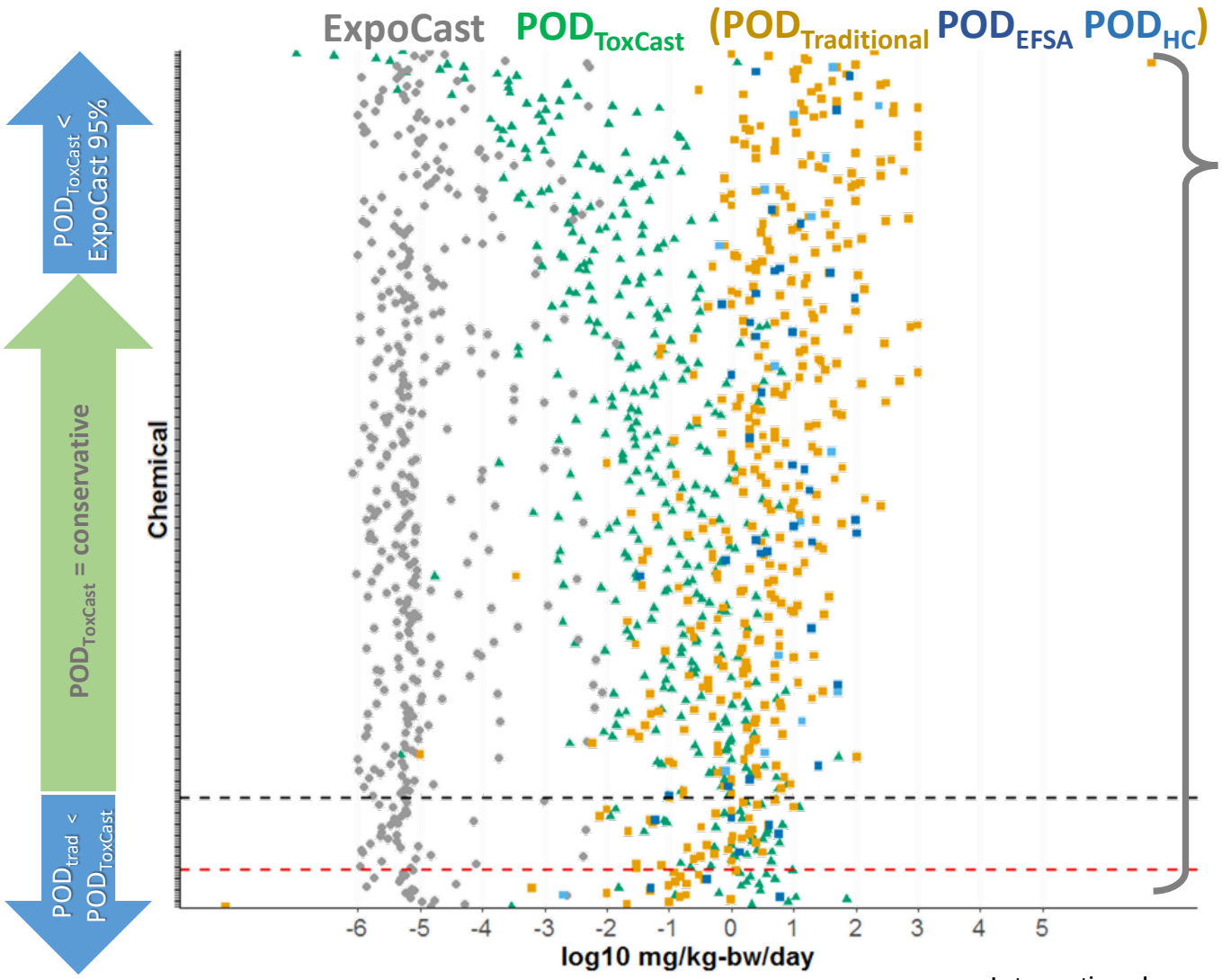
- Semi-automated decision support workflows
- Flexible integration of information related to chemical properties, fate and transport, hazard, and exposure
- Enable expert users to review the assumptions made and refine the results
- Presents alternative data together with traditional toxicology data

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

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 =

448 chemicals

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

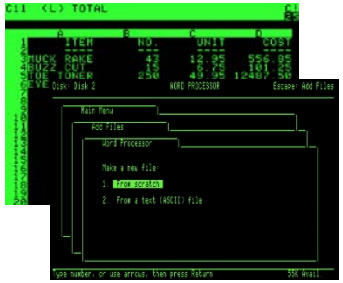
For ~92% of the chemicals, $POD_{ToxCast}$ was conservative.

(~100-fold with human HTTK

~50-fold with rat HTTK)

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

Take Home Messages…



- Advancing toxicology to the new and improved version will require both continued technical innovation as well as translational efforts
- New technologies exist for rapidly and comprehensively covering toxicological space at significantly less cost
- Addressing previous technical limitations such as a lack of metabolism and organ/tissue effects are within reach
- Enabling application of new technologies 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:
- NTP
- FDA
- NCATS

EPA Colleagues:
- NERL
- NHEERL
- NCEA

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

EPA's National Center for Computational Toxicology