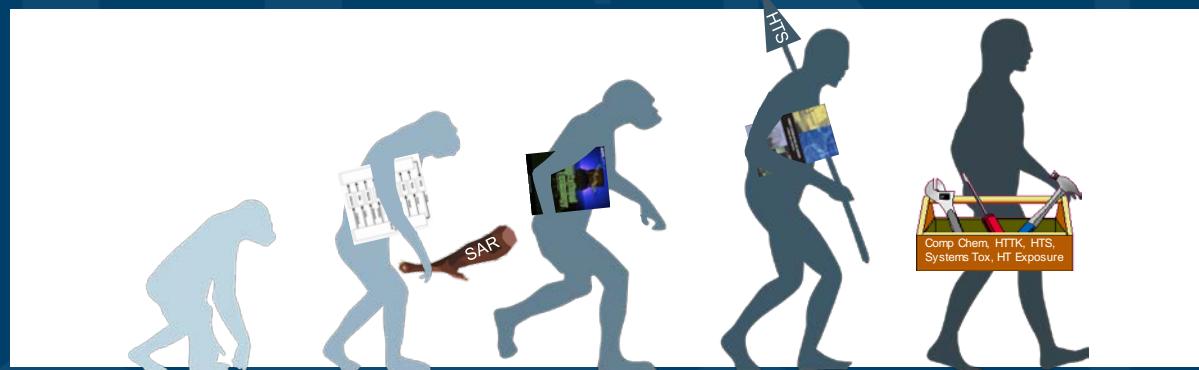


Evolution of Computational Toxicology

From Primitive Beginnings to Sophisticated Application

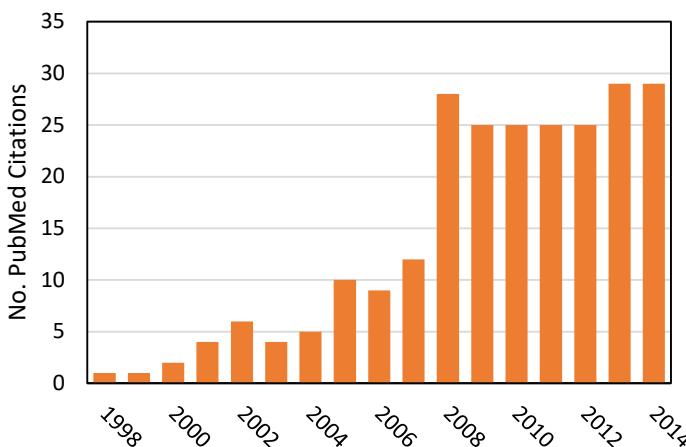
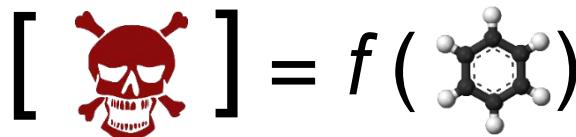


Health Canada Seminar

November 15, 2016

Rusty Thomas
Director
National Center for Computational Toxicology

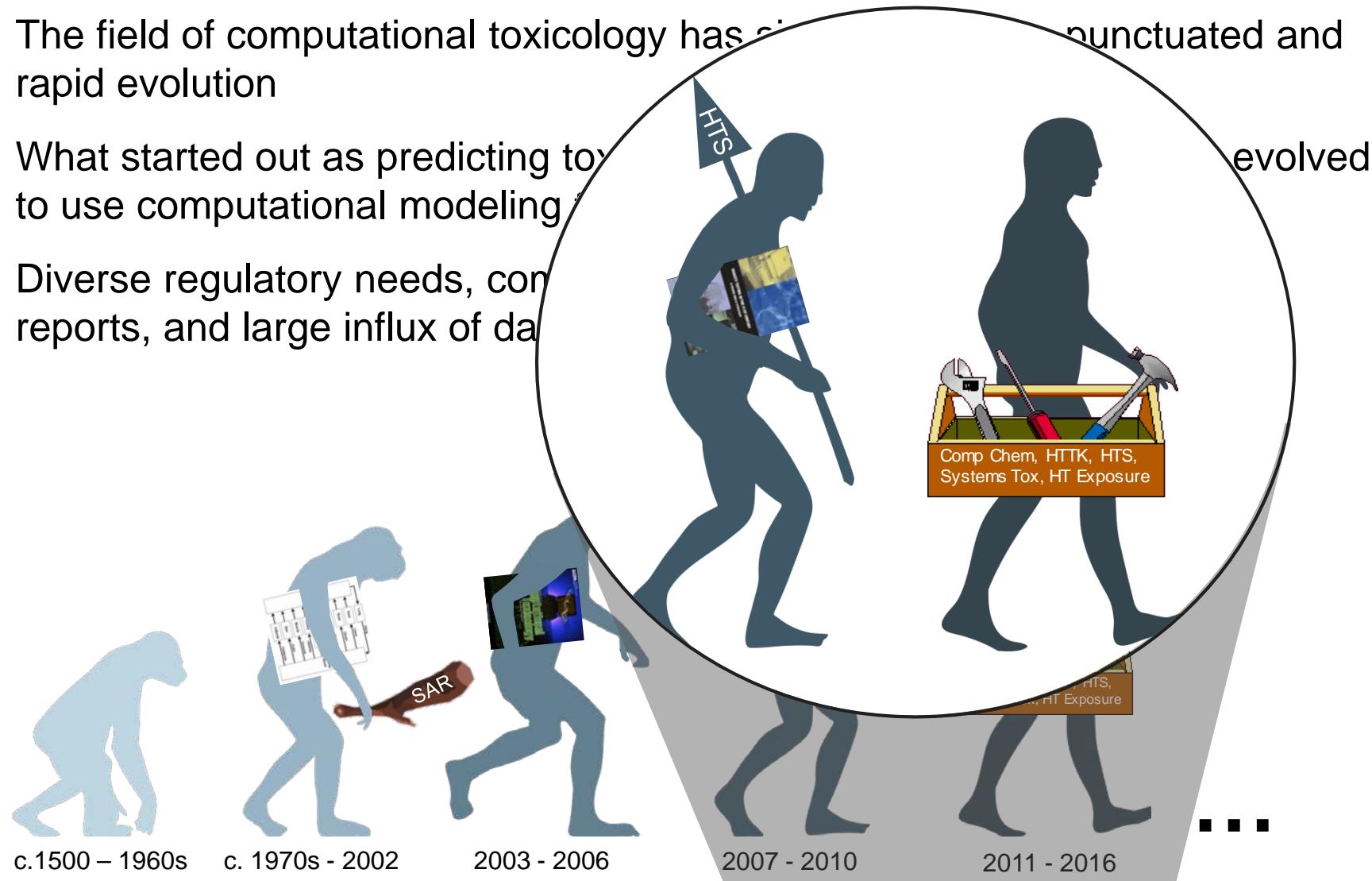
Early Beginnings of Computational Toxicology...



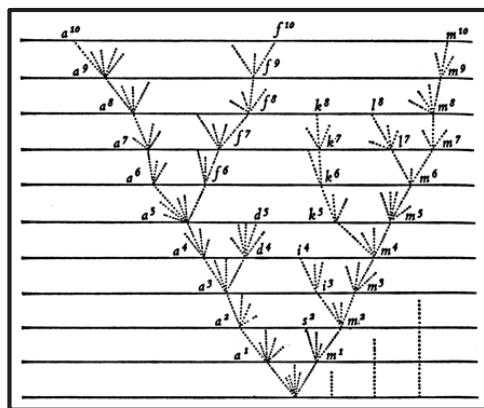
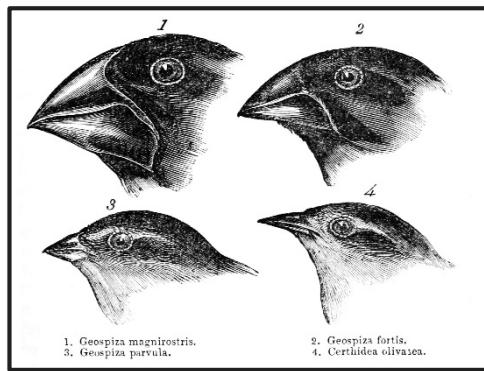
- Application of computational modeling to toxicological endpoints began with development of QSARs in 1970s.
- In early 1970s, first physiologically-based pharmacokinetic (PBPK) models were developed
- In 1980s, significant growth in computer modeling in QSAR and PBPK modeling
- In 1990s, development of physiologically-based pharmacodynamic (PBPD) models for AChE inhibition and cell death/proliferation/mutation
- In the late 1990s, use of the term 'computational toxicology' appeared in the literature

Rapid Evolution of Computational Toxicology

- The field of computational toxicology has seen punctuated and rapid evolution
- What started out as predicting toxicity has evolved to use computational modeling
- Diverse regulatory needs, complex reports, and large influx of data

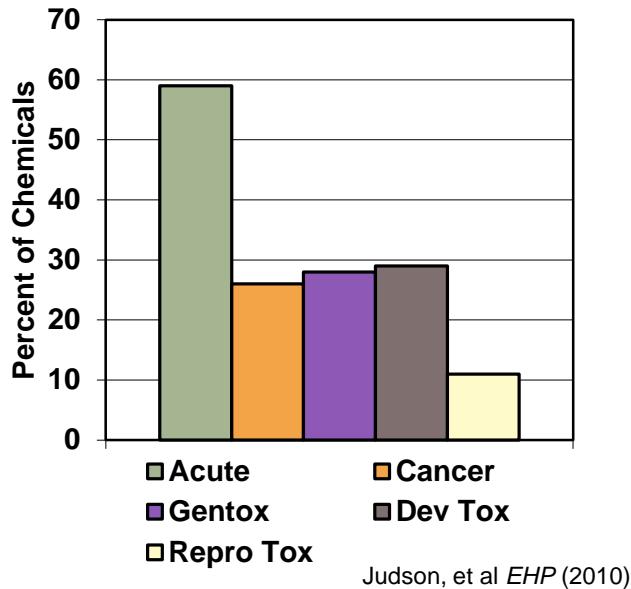


What Traits Have Been Under Selection?

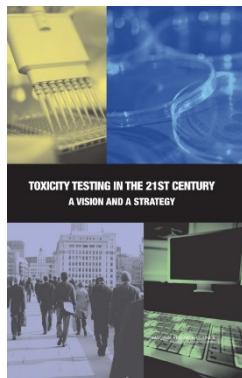


- Increased throughput and biological coverage
- Interrogation of effects at the molecular and pathway level
- Increasingly relevant test systems
- Putting results in a dose/exposure context
- Characterization of uncertainty
- Computational modeling to integrate experimental data
- Delivery of data and models through decision support tools
- Transparency and performance-based validation

Throughput Spurred by Regulatory Demand and Expert Guidance

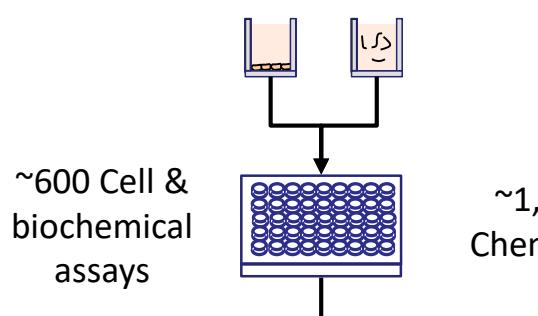


- In 1976, the U.S. Toxic Substances Control Act put burden on EPA to demonstrate “unreasonable risk” for industrial chemicals
- Increased recognition of lack of safety data for many environmental/industrial chemicals
 - 1984 U.S. NRC report
- Guidance from internal strategy reports and expert committees highlighted need for increased throughput
 - 2005 EPA CompTox Report
 - 2007 U.S. NRC report

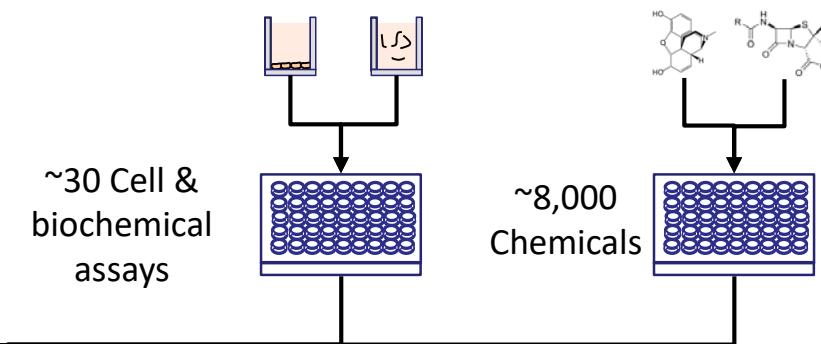


Increased Throughput Required Shift to Molecular/Pathway Approaches

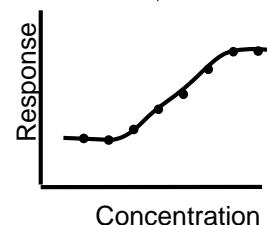
ToxCast



Tox21

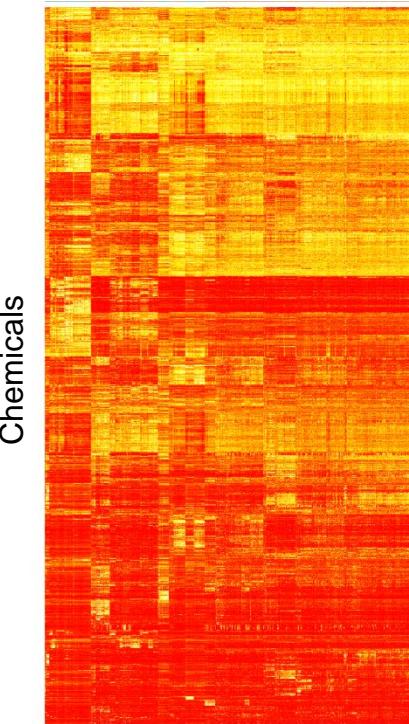


Set	Chemicals	Assays	Completion
ToxCast Phase I	293	~600	2011
ToxCast Phase II	767	~600	2013
ToxCast Phase III	1001	~100	Ongoing
E1K (endocrine)	880	~50	2013



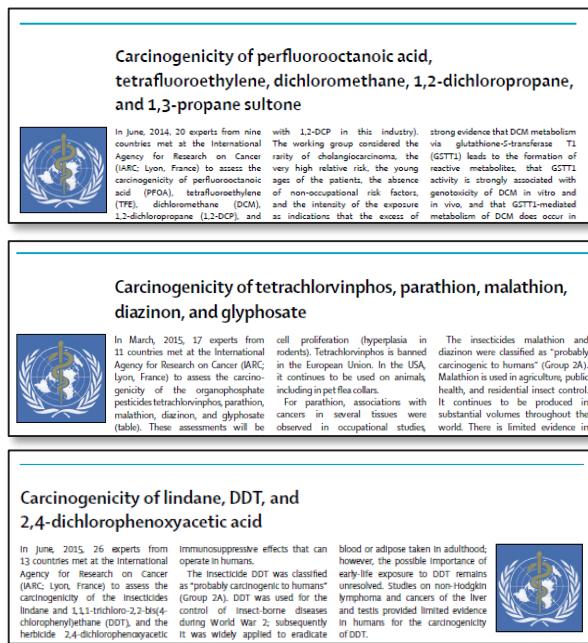
Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling



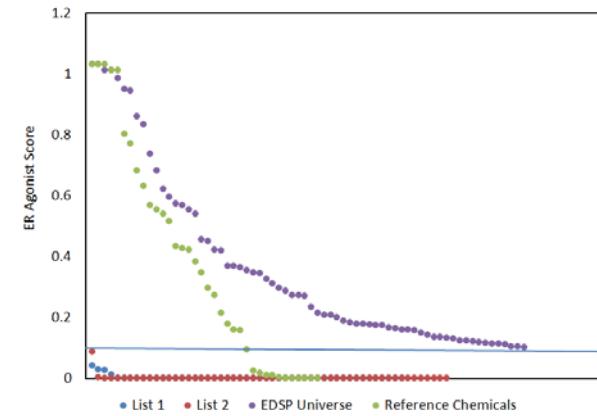
Assays/Pathways

Provide Mechanistic Support for Hazard ID



IARC Monographs 110, 112, 113

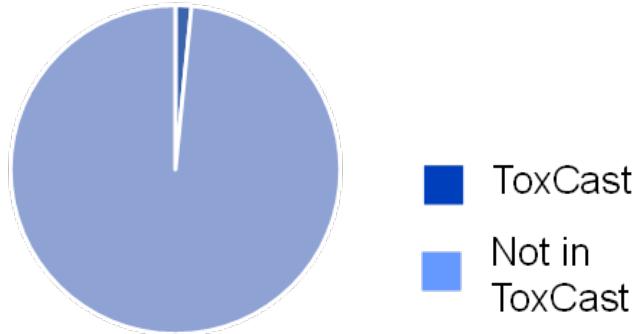
Prioritization of Chemicals for Further Testing



FIFRA SAP, Dec 2014

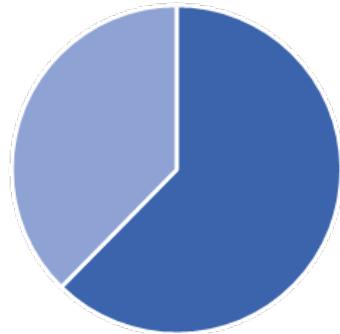
Continuing Pressure Towards Increased Biological Coverage

Gene Coverage

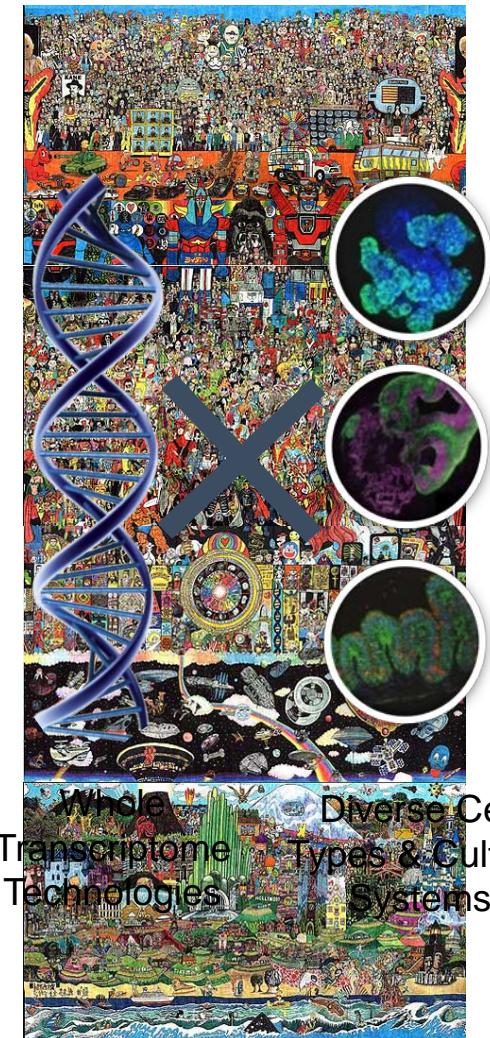


- ToxCast
- Not in ToxCast

Pathway Coverage*



*At least one gene from pathway represented



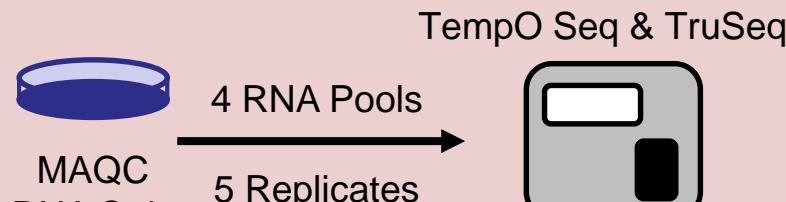
Picture of Everything - Howard Hallis

Searching for a Platform

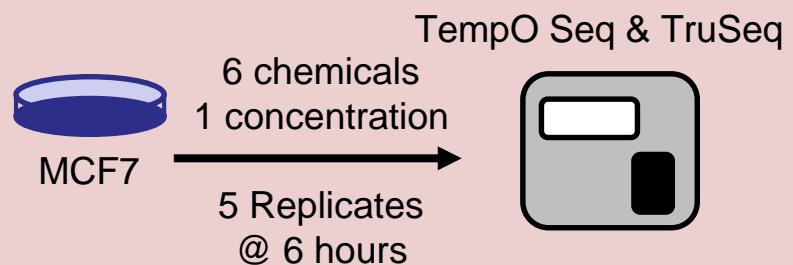
Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Targeted RNA-seq

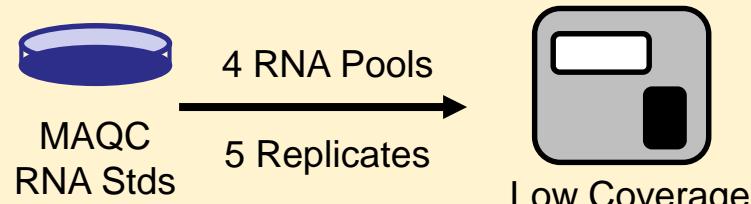


Technical Comparison

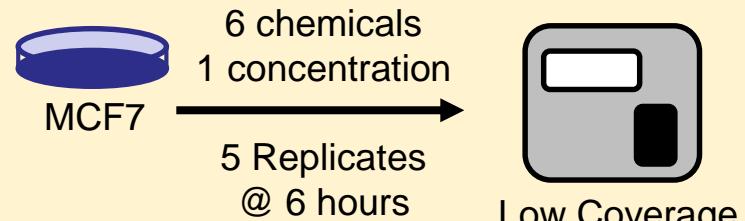


Functional Comparison

Low Coverage RNA-seq



Technical Comparison

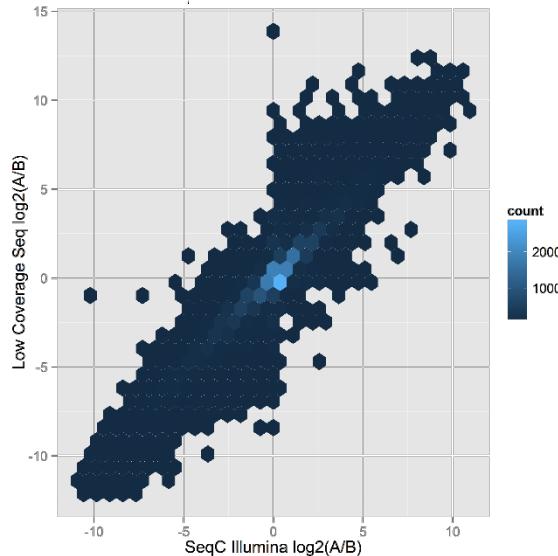


Functional Comparison

Technical Performance of the Three Sequencing Platforms

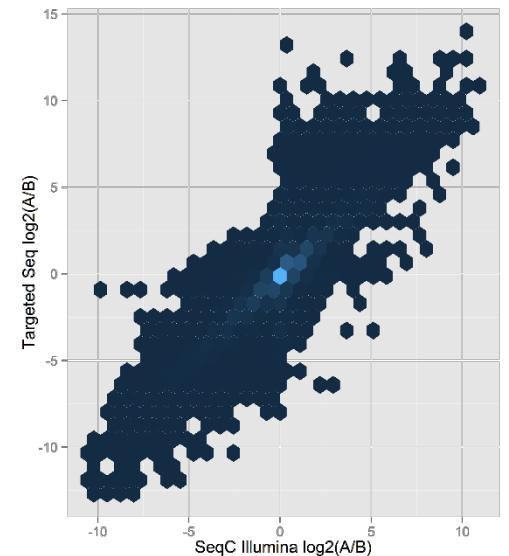
Low Coverage

r^2 0.83



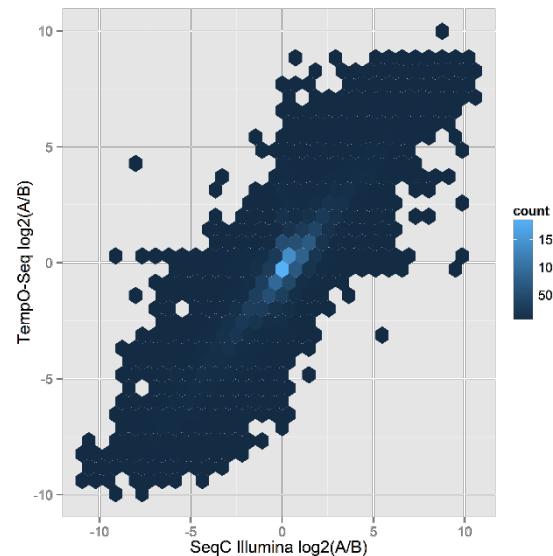
TruSeq

r^2 0.74



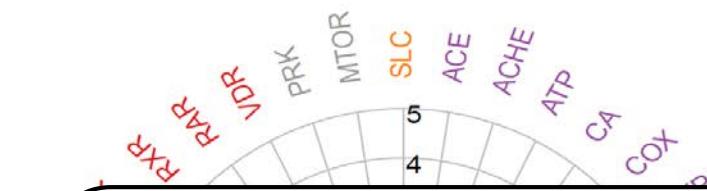
TempO-Seq

r^2 0.75

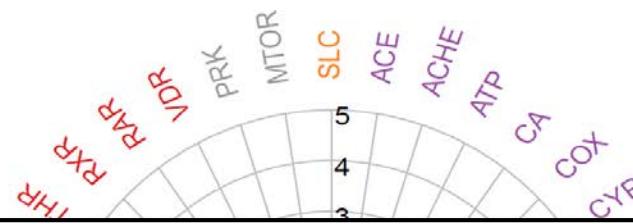


Functional Performance of the Three Sequencing Platforms

Genistein

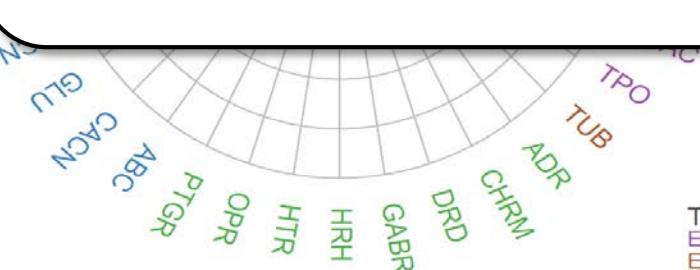


Trichostatin

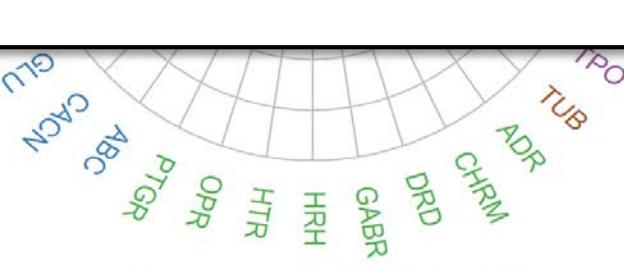


**COMING
SOON!**

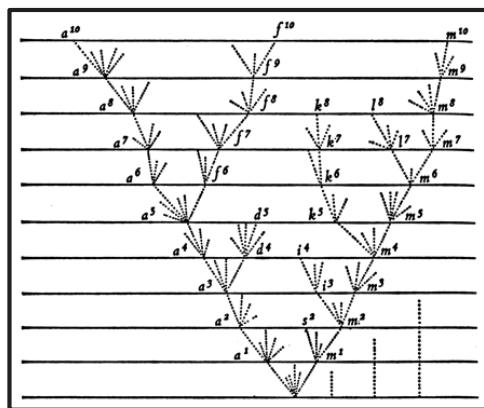
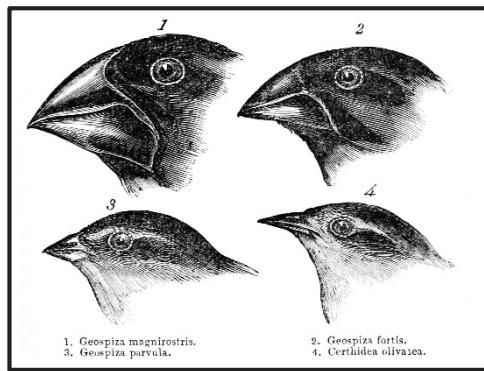
- Large scale screen of 1,000 chemicals (ToxCast I/II) in single cell type
- Additional screens across multiple cell types/lines
- Additional reference chemicals and genetic perturbations (RNAi/CRISPR/cDNA)



Target Family
Enzymes
Exosome
G Protein-coupled receptors
Ion channels
Nuclear receptors
Protein kinases
Transporters



What Traits Have Been Under Selection?

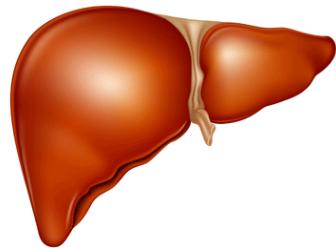


- Increased throughput and biological coverage
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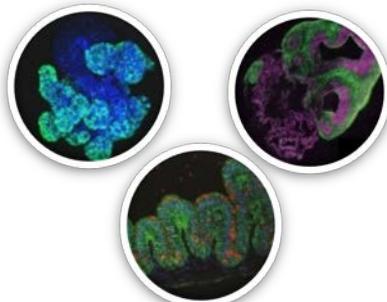
Increasing Relevance of Test Systems Necessary at Multiple Levels



Relevant Species Responses



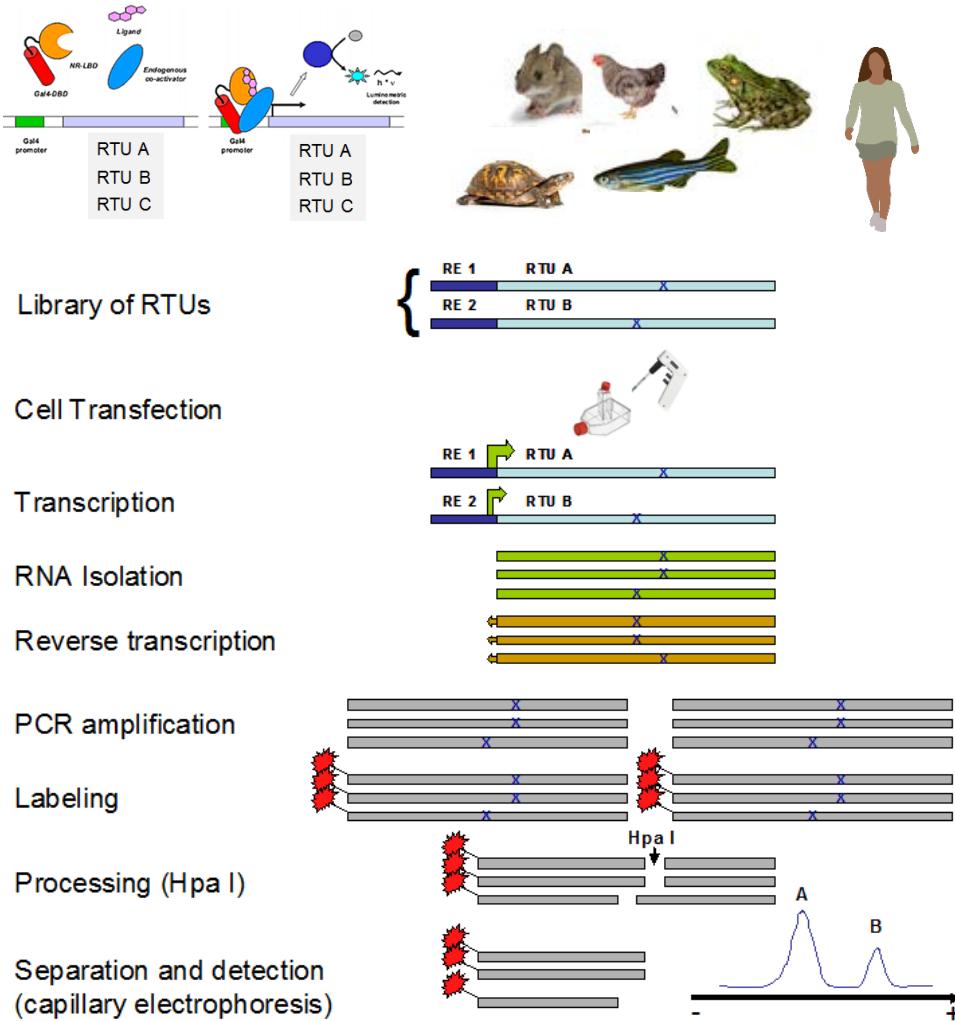
Relevant Metabolism



Relevant Tissue and Organ Responses

Assessing Cross-Species Differences in Response

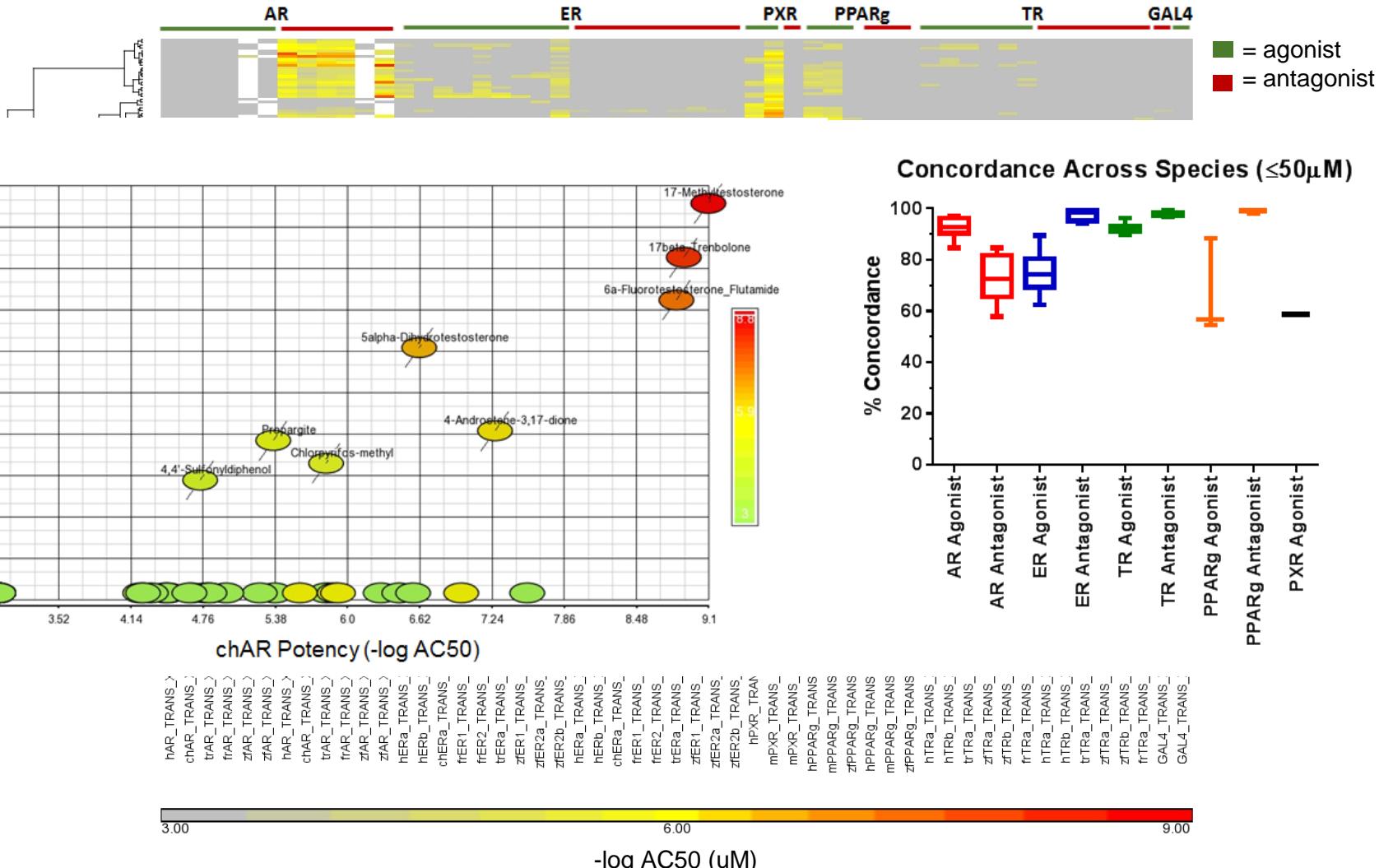
Multispecies Attagene Trans Reporter Assay



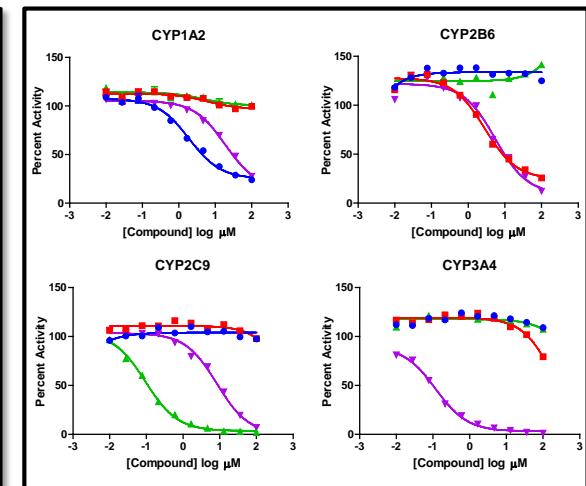
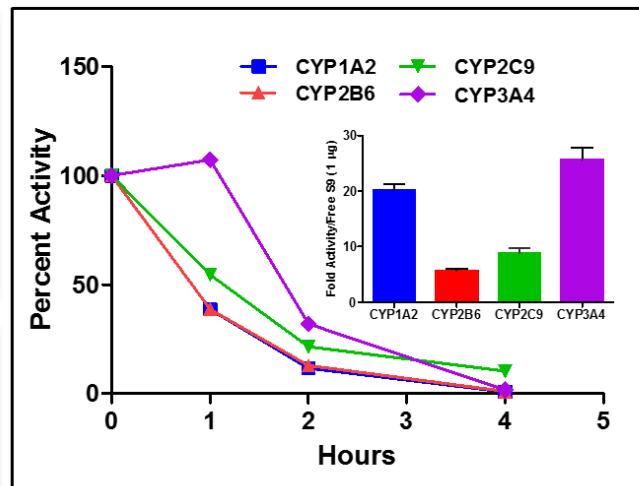
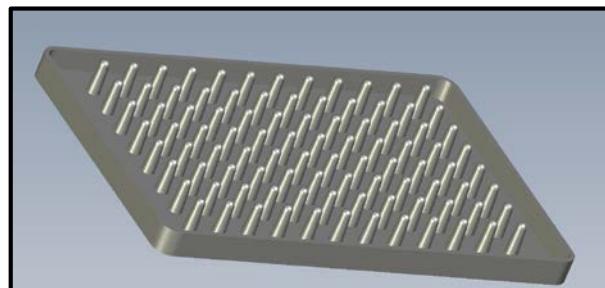
Receptor Family	Receptor Name	Species
Estrogen Receptor	ER _a	Human
Estrogen Receptor	ER _b	Human
Estrogen Receptor	ER1	Zebrafish
Estrogen Receptor	ER2a	Zebrafish
Estrogen Receptor	ER2b	Zebrafish
Estrogen Receptor	ER _a	Chicken
Estrogen Receptor	ER1	Frog
Estrogen Receptor	ER2	Frog
Estrogen Receptor	ER _a	Turtle
Estrogen Receptor	AR	Human
Estrogen Receptor	AR	Chicken
Estrogen Receptor	AR	Turtle
Estrogen Receptor	AR	Frog
Estrogen Receptor	AR	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPAR γ	Mouse
Peroxisome Proliferator Activated Receptor γ	PPAR γ	Zebrafish
Peroxisome Proliferator Activated Receptor γ	PPAR γ	Human
Pregnane X Receptor	PXR	Mouse
Thyroid Receptor	TR _a	Turtle
Thyroid Receptor	TR _b	Zebrafish
Thyroid Receptor	TR _b	Zebrafish
Thyroid Receptor	TR _a	Frog
Thyroid Receptor	TR _a	Human
Thyroid Receptor	TR _b	Human
Controls	M-06	NA
Controls	GAL4	NA
Controls	M-19	NA
Controls	m-32	NA
Controls	m-61	NA

- Host cell: human HepG2
- Stimulation with EC20 of 6a-fluorotestosterone for detection of androgen receptor antagonists
- 100 chemicals with ER, AR, PPAR activity tested in concentration-response
- Data calculated as fold-change over control (6a-fluorotestosterone/DMSO)

Cross-Species Differences in Nuclear Receptor Responses

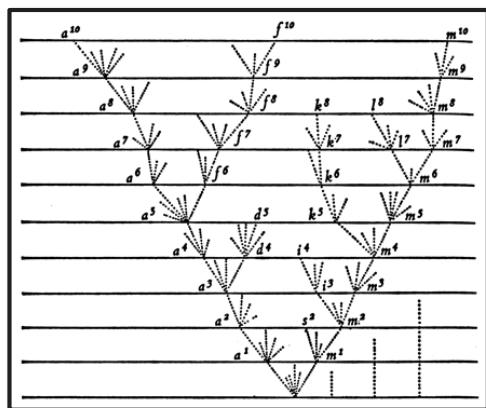
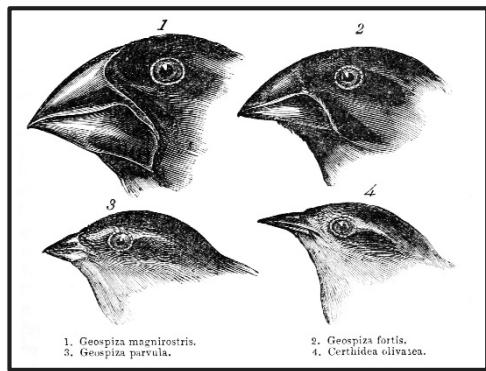


Adding Relevant Metabolic Activity



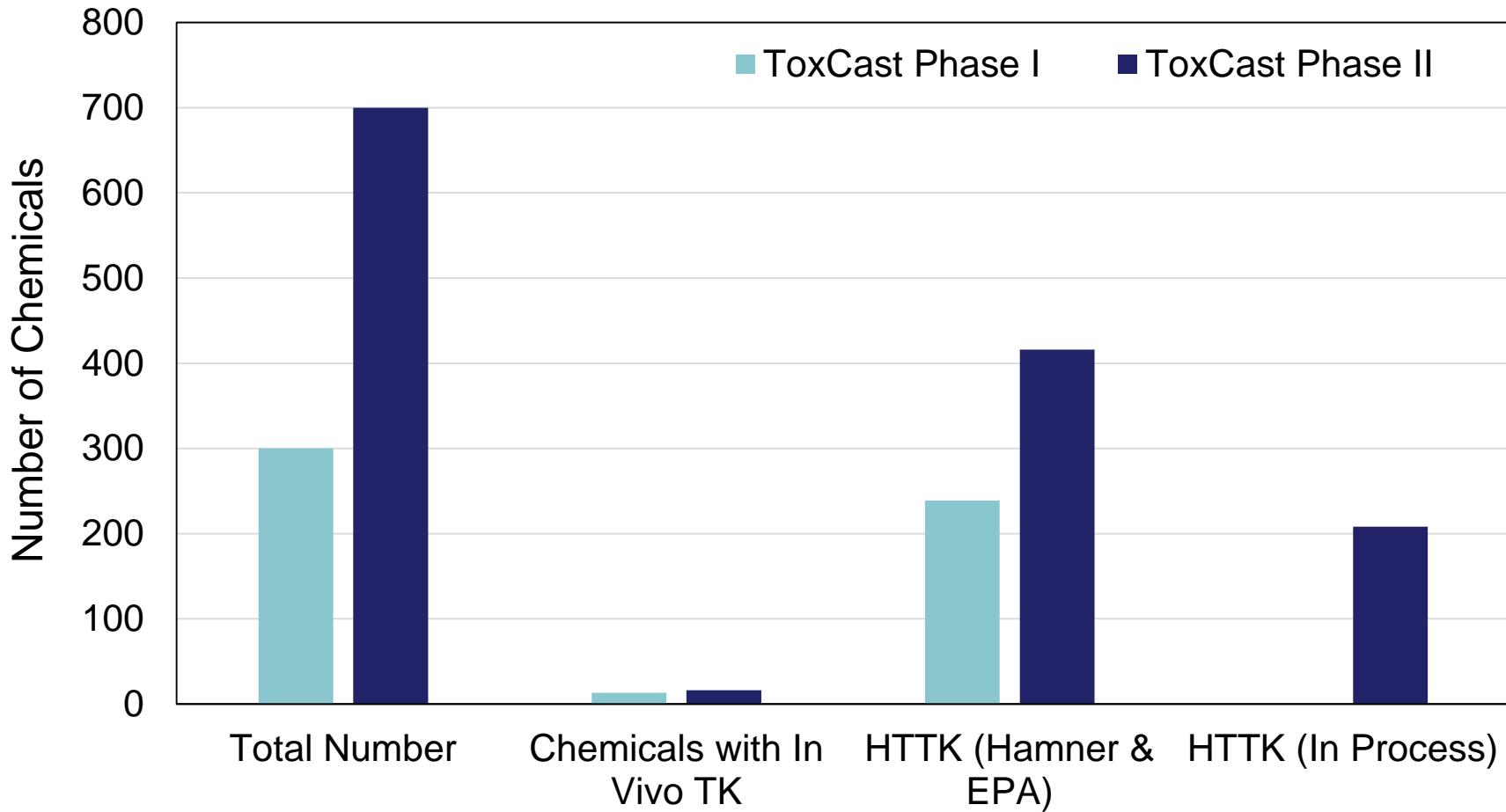
- Furafylline
- Thio-TEPA
- Tienilic Acid
- Ketoconazole

What Traits Have Been Under Selection?

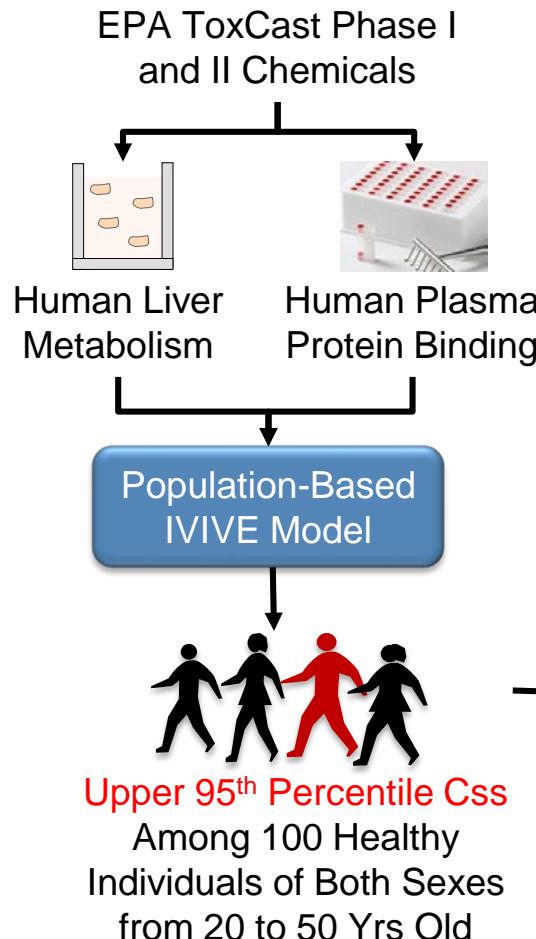


- Increased throughput and biological coverage
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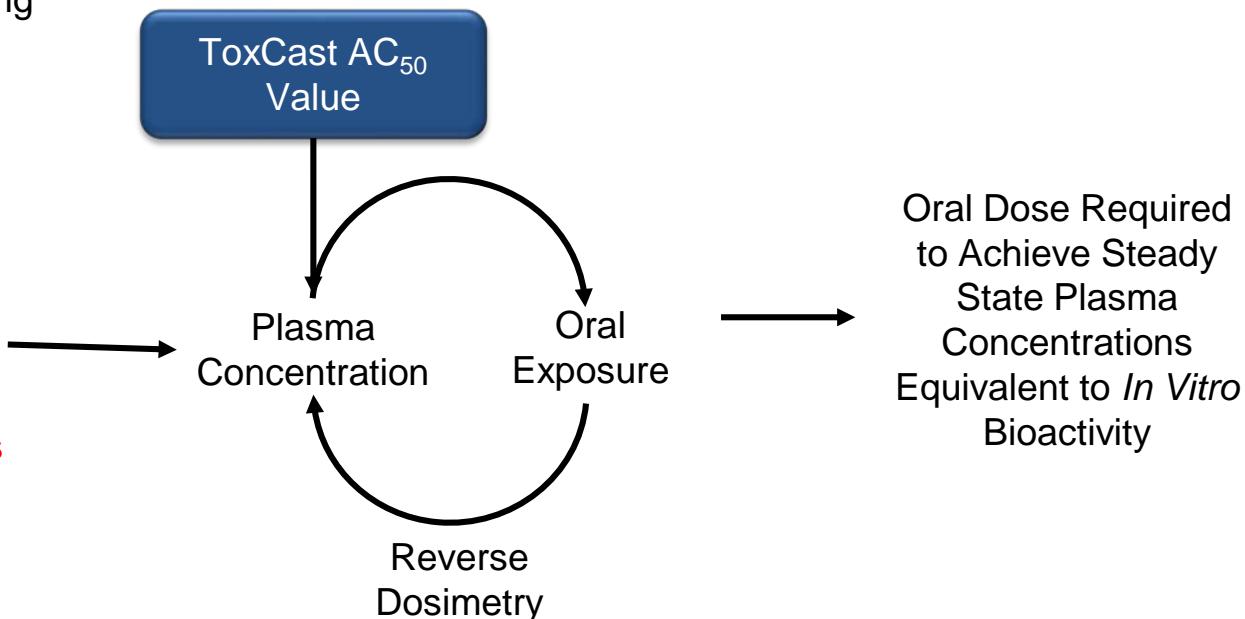
The Need for High(er) Throughput TK Approaches



Incorporating a High-Throughput Toxicokinetic Approach

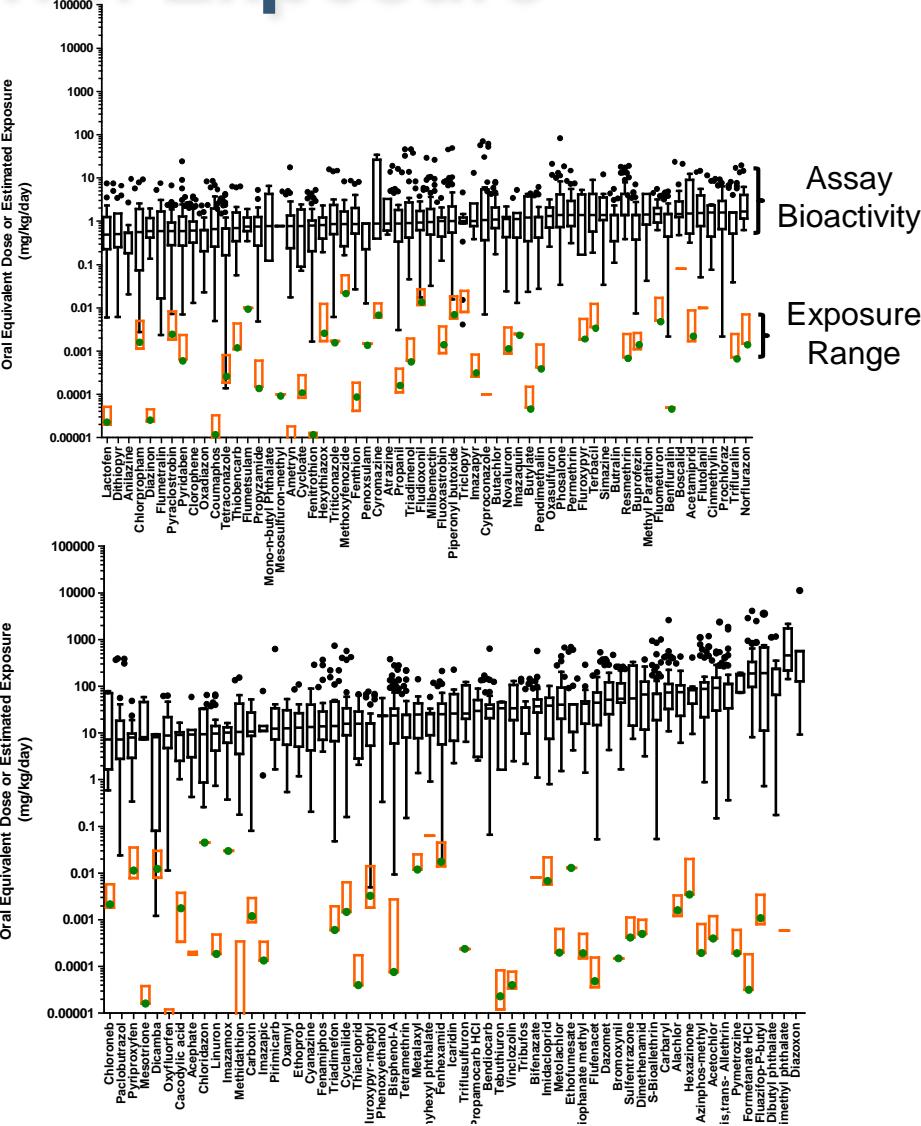
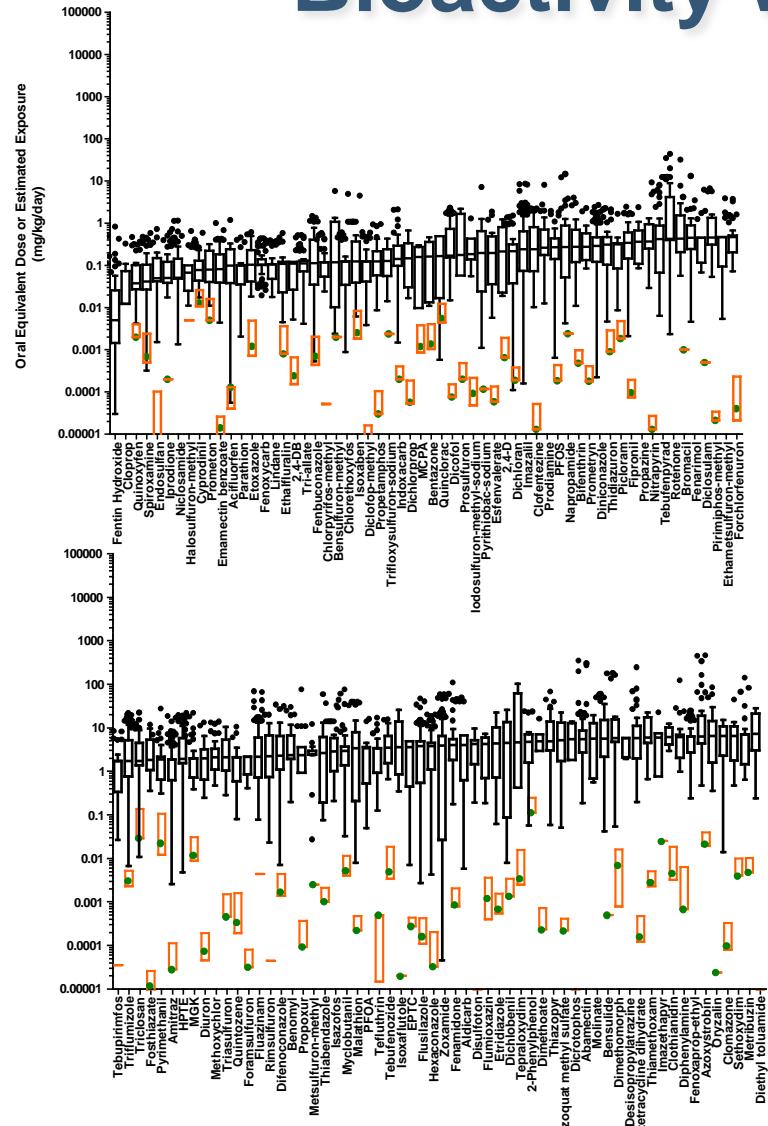


- Models available through “httk” R package (<https://cran.r-project.org/web/packages/httk/>)

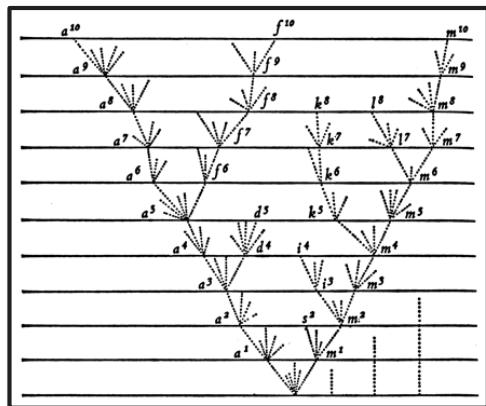
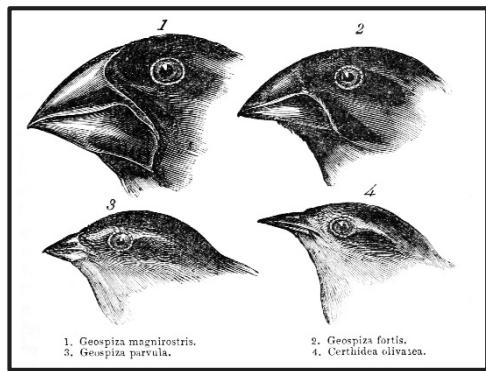


Rotroff et al., *Tox Sci.*, 2010
Wetmore et al., *Tox Sci.*, 2012

Comparing Dosimetry Adjusted Bioactivity with Exposure



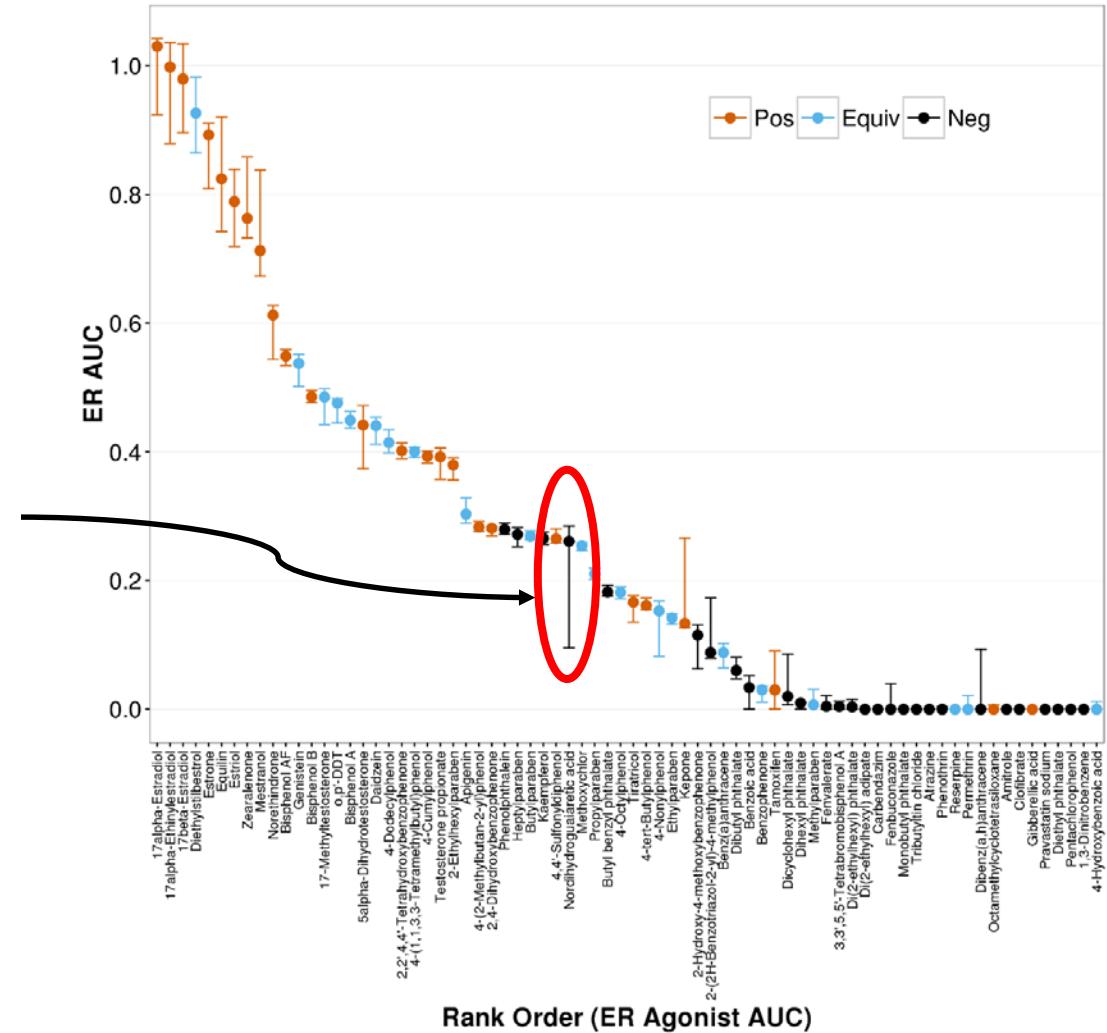
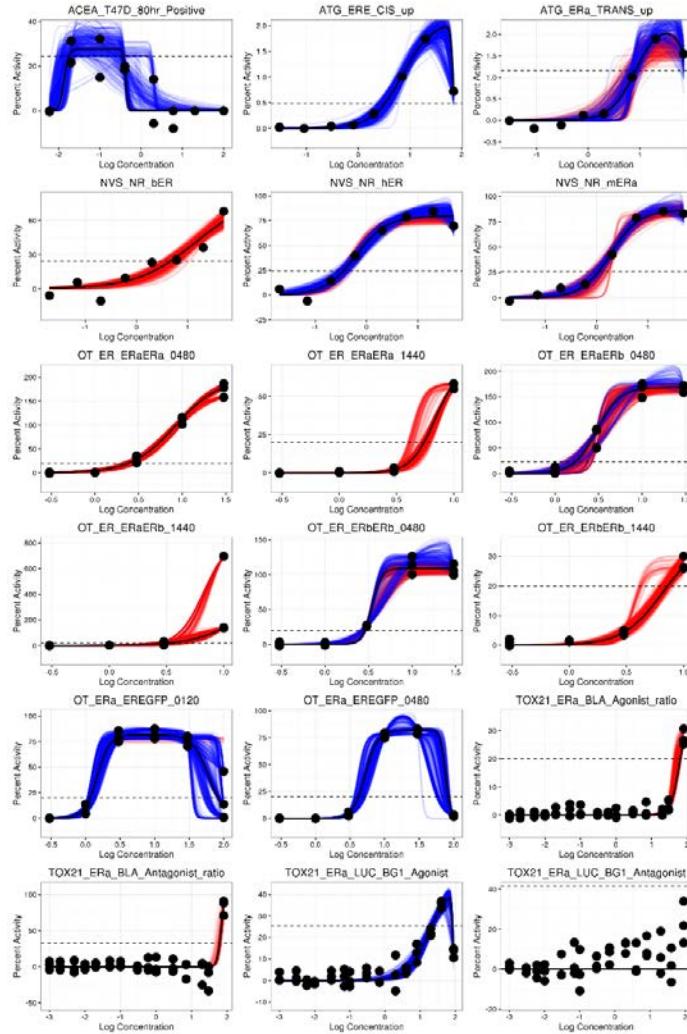
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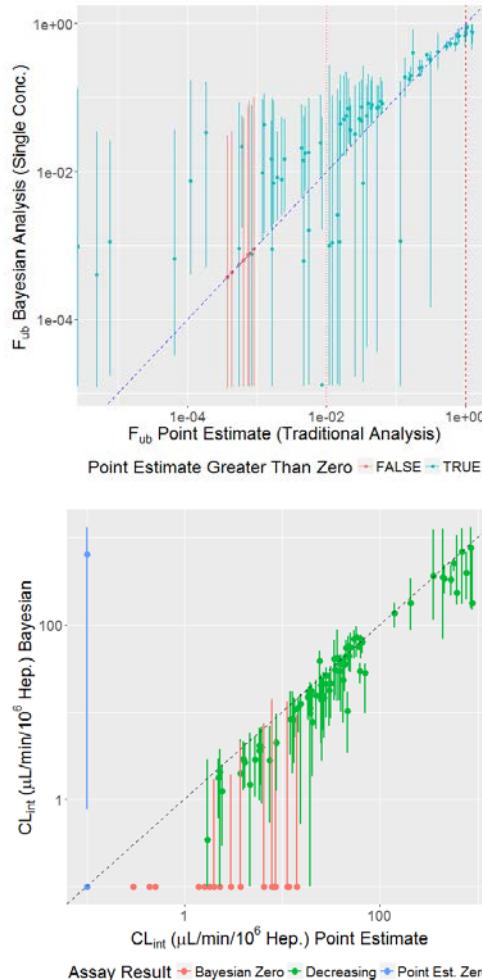
Incorporating Uncertainty in the Pharmacodynamic Modeling

Bootstrapped Modeling of CR Curves

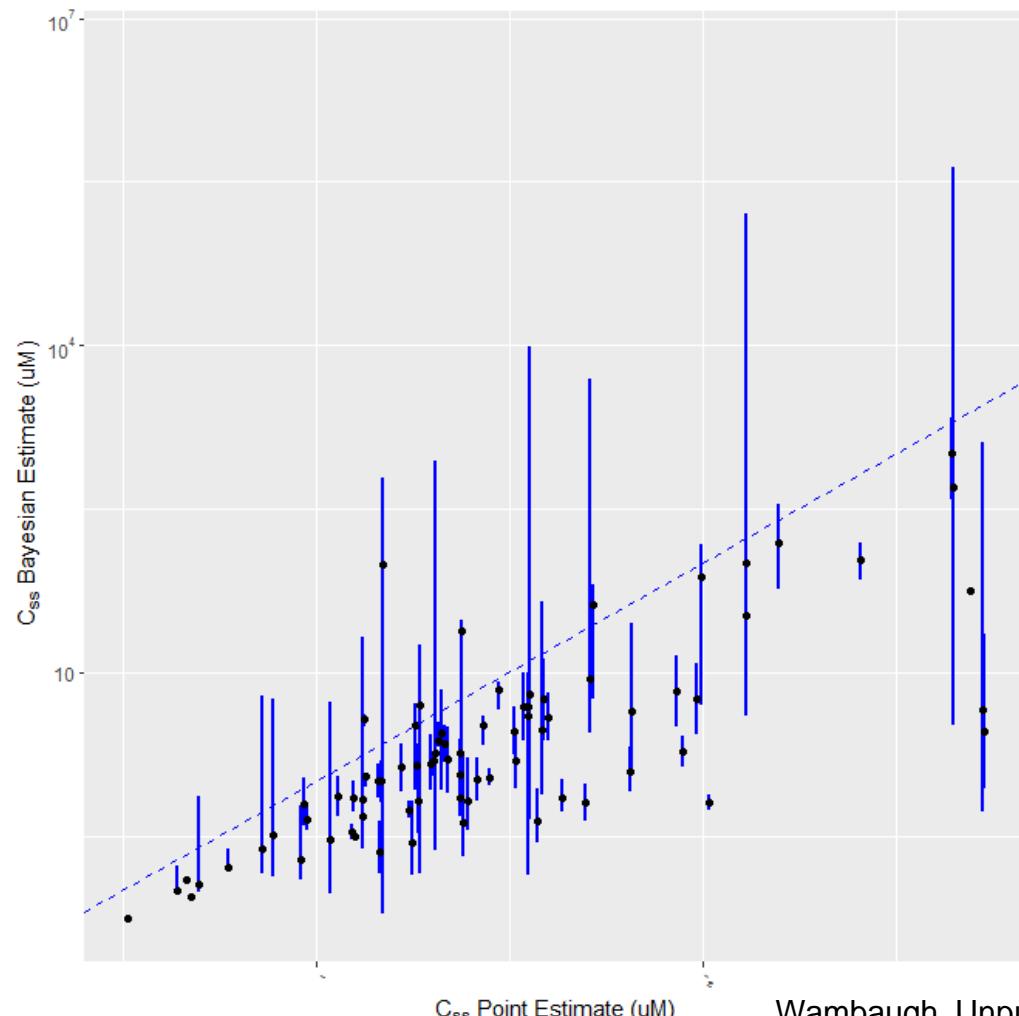


Incorporating Uncertainty Into the Pharmacokinetic Modeling

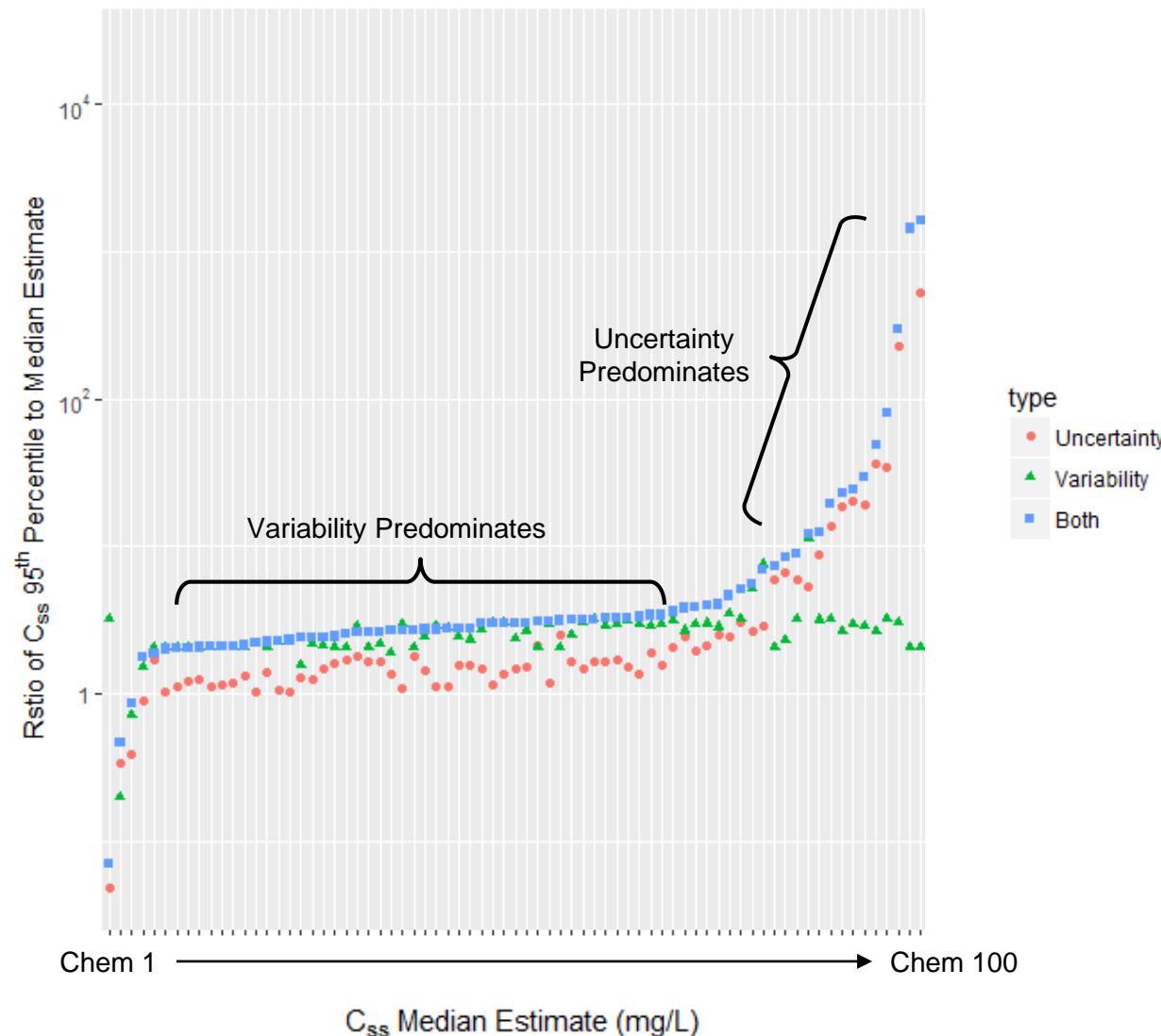
Bayesian Modeling of Plasma Protein Binding and Intrinsic Clearance



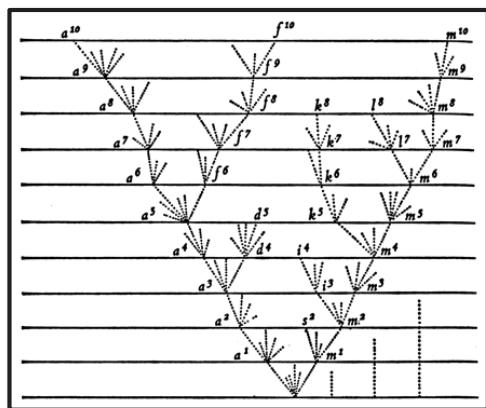
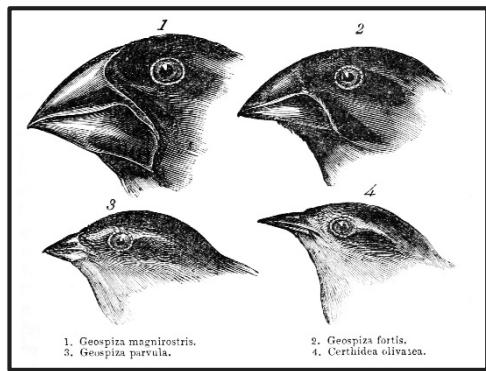
Propagation of Experimental Uncertainty to Steady State Plasma Concentrations



Impact of Incorporating Uncertainty and Variability is Chemical Specific



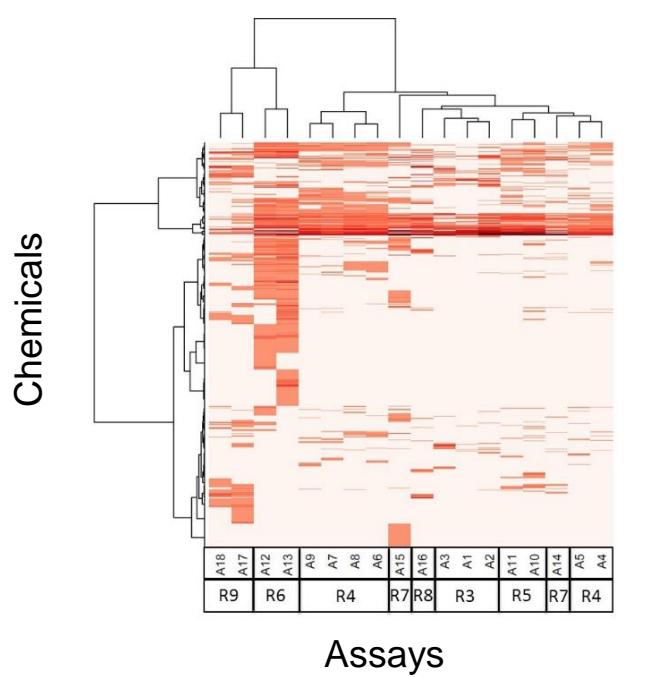
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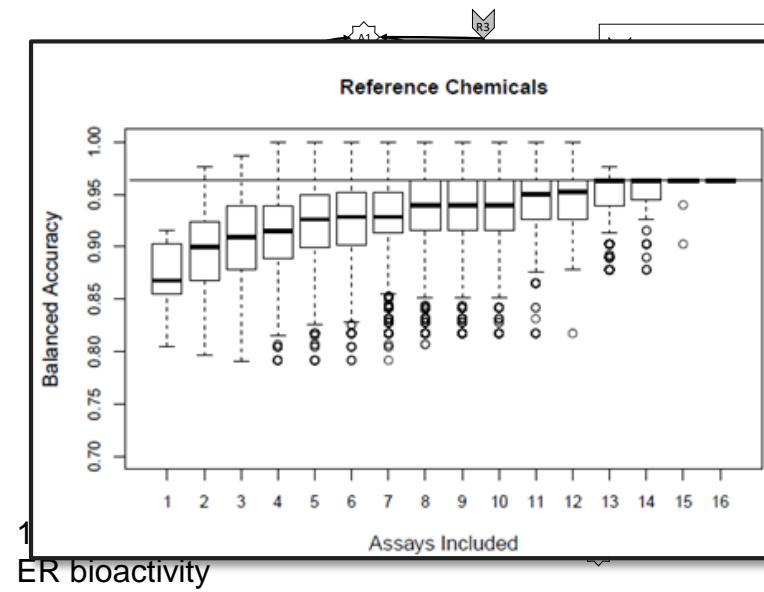
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Relatively Simple Models Used to Reduce Assay Interference

Assays cluster by technology,
suggesting technology-specific non-ER bioactivity



Computational Modeling of Estrogen Receptor Pathway



Judson, Unpublished

In Vitro Reference Chemicals* *In Vivo* Reference Chemicals*

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

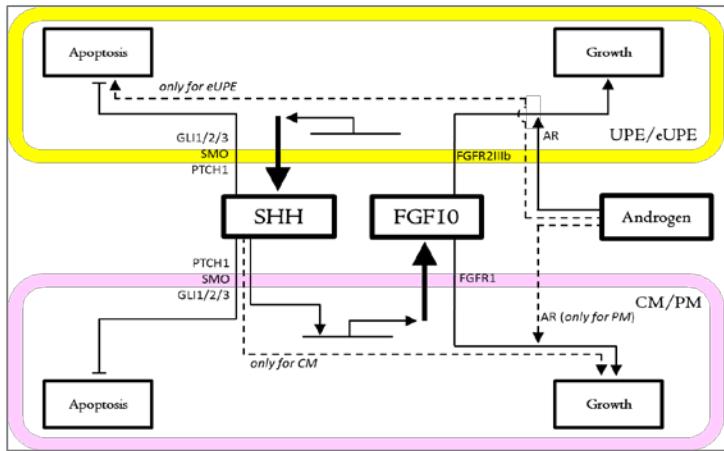
Accuracy	0.86 (0.95)
Sensitivity	0.97 (0.97)
Specificity	0.67 (0.89)

*Values in parentheses exclude inconclusive chemicals

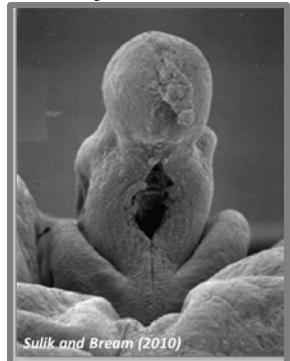
Judson *et al.*, *Tox Sci.* 2015
Browne *et al.*, *ES&T*. 2015

Complex Systems Models to Predict Phenotypic Responses to Chemicals

Signaling Network Underlying Virtual Genital Tubercl Model (Mouse)



Embryonic GT

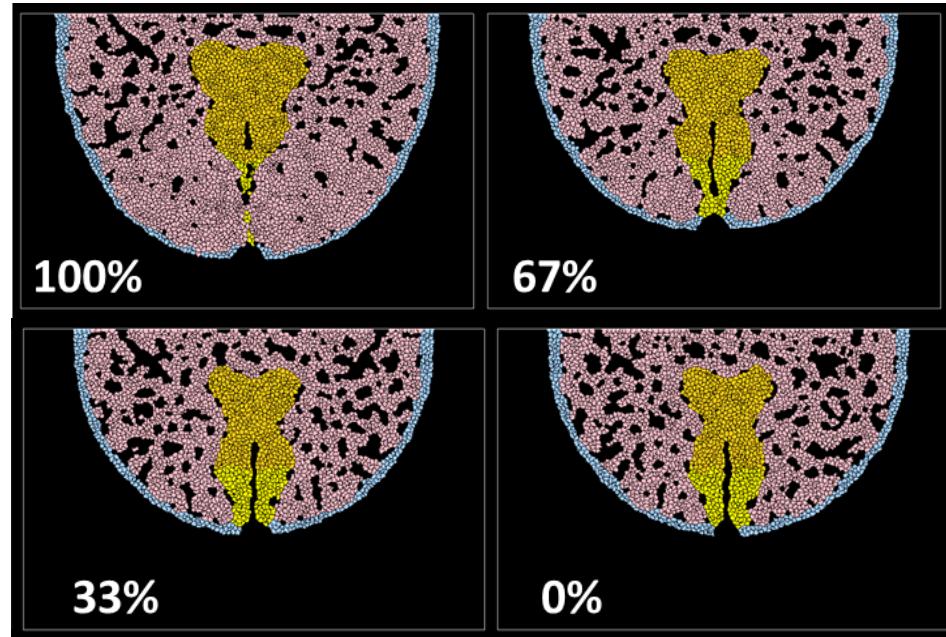


Abstracted GT



Leung et al., *Repro Toxicol*, 2016

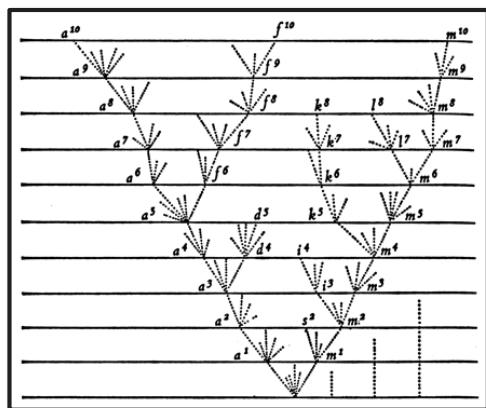
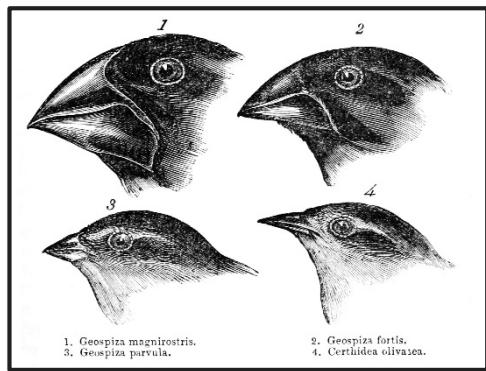
Simulation of Genital Tubercl Closure



GD13.5 – 17.5

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

What Traits Have Been Under Selection?



- Increased throughput and biological coverage
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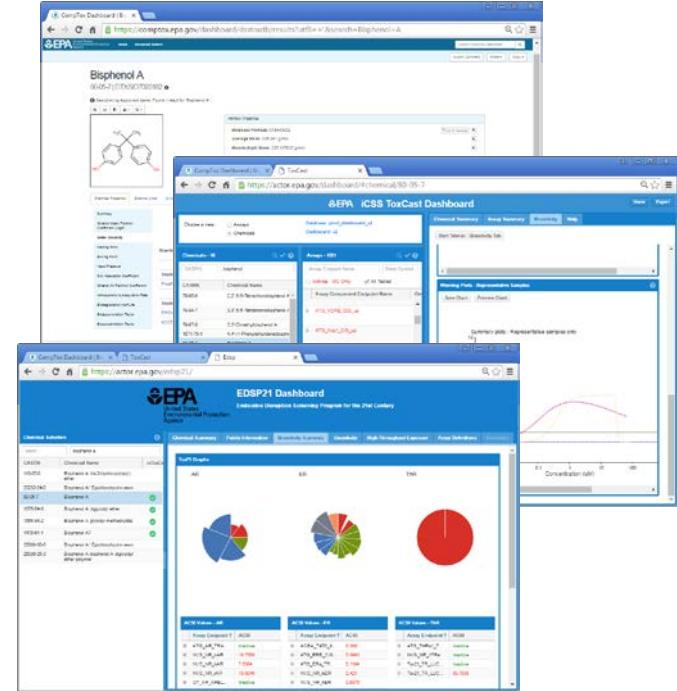
Significant Pressure to Deliver Data and Models in Useful Format



Initial Data Delivered as Flat Files

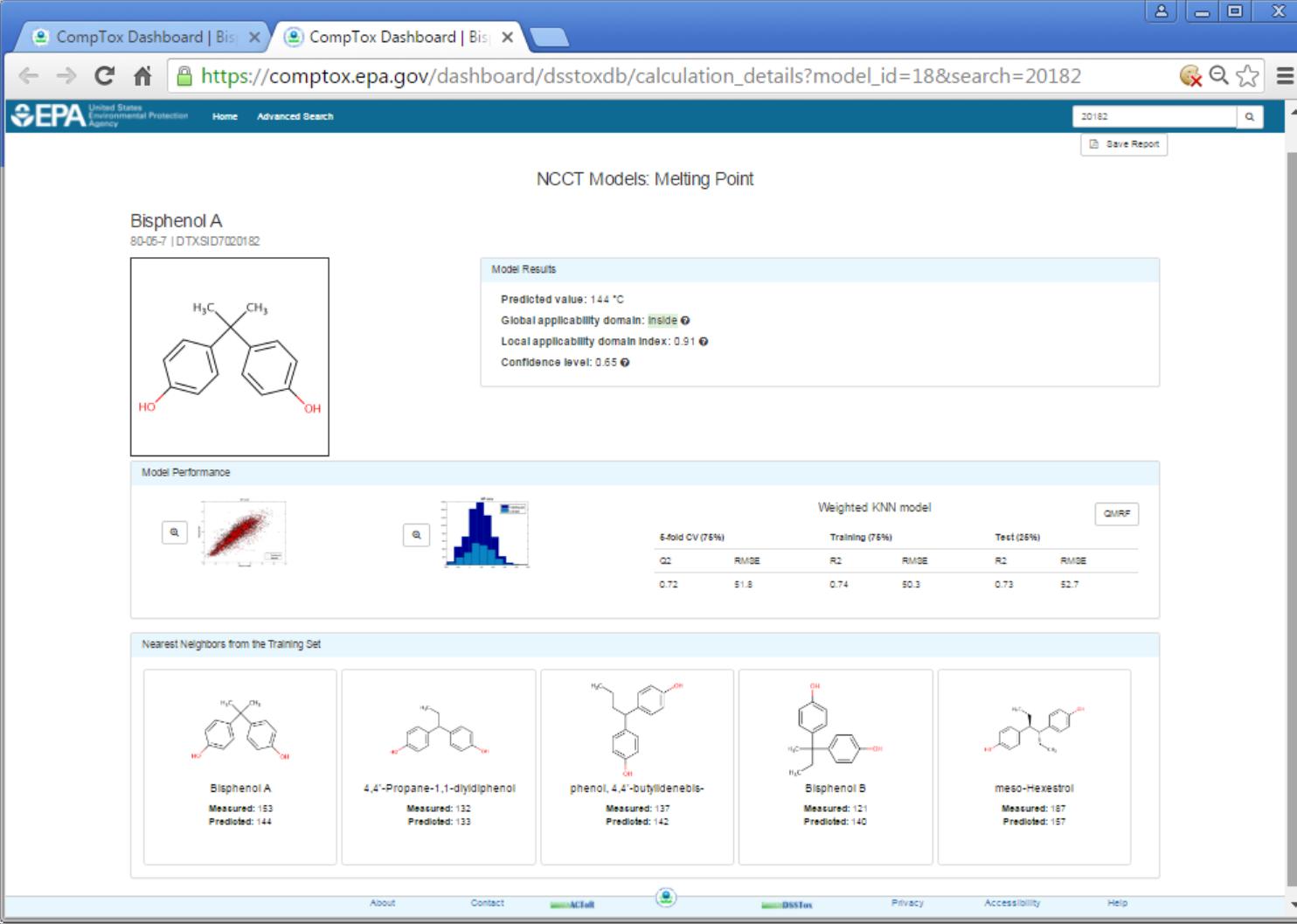


Progress to Dashboard with Limited Search, Visualization, and Export Functionality



Currently Providing Cross-Functional and Decision Support Dashboards

New Chemistry Dashboard Delivers Structural and Property Data



The screenshot displays the CompTox Dashboard interface for Bisphenol A (80-05-7 | DTXSID7020182). The main content area is titled "NCCT Models: Melting Point".

Bisphenol A
80-05-7 | DTXSID7020182

Model Results

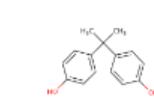
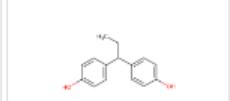
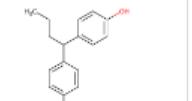
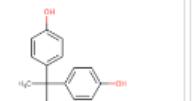
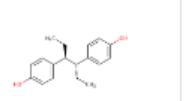
- Predicted value: 144 °C
- Global applicability domain: inside
- Local applicability domain index: 0.91
- Confidence level: 0.65

Model Performance

Weighted KNN model

6-fold CV (75%)		Training (75%)		Test (25%)	
R ²	RMSE	R ²	RMSE	R ²	RMSE
0.72	51.8	0.74	50.3	0.73	52.7

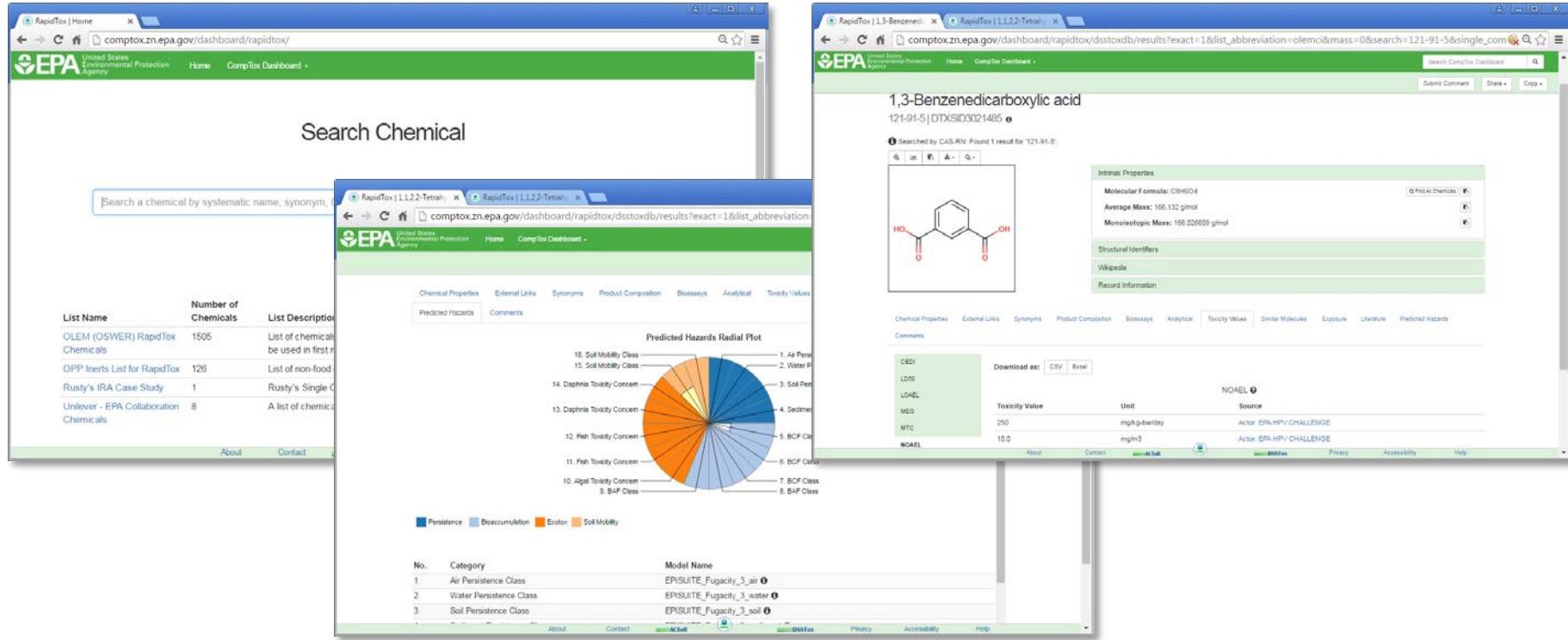
Nearest Neighbors from the Training Set

 Bisphenol A Measured: 153 Predicted: 144	 4,4'-Propane-1,1-diyldiphenol Measured: 132 Predicted: 133	 phenol, 4,4'-butylenedibutylidenebis- Measured: 137 Predicted: 142	 Bisphenol B Measured: 121 Predicted: 140	 meso-Hexestrol Measured: 187 Predicted: 157
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RapidTox Decision Support Dashboard in Development



The dashboard features a search bar at the top left. Below it is a table listing various chemical lists:

List Name	Number of Chemicals	List Description
OLEM (OSWER) RapidTox Chemicals	1505	List of chemicals that will be used in first risk assessments.
OPP Inerts List for RapidTox	126	List of non-food inerts.
Rusty's IRA Case Study	1	Rusty's Single Chemicals
Unilever - EPA Collaboration Chemicals	8	A list of chemicals from Unilever.

The central part of the dashboard displays a "Predicted Hazards Radial Plot" for 1,3-Benzenedicarboxylic acid (121-91-5). The plot shows the relationship between persistence, bioaccumulation, ecotoxicity, and soil mobility. The plot is divided into 18 segments, with the following segments labeled:

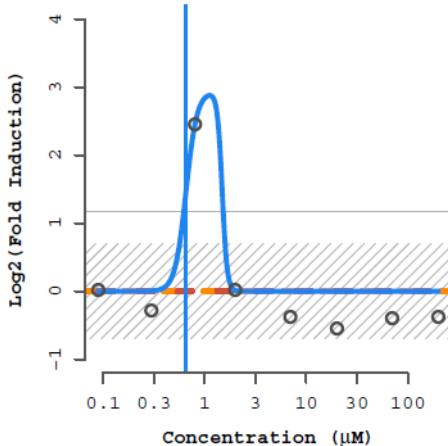
- 1. Air Persistence
- 2. Water persistence
- 3. Soil Persistence
- 4. Sediment
- 5. BCF Class
- 6. BCF Curve
- 7. BCF Class
- 8. BAF Class
- 9. BAF Class
- 10. Algal Toxicity Concern
- 11. Fish Toxicity Concern
- 12. Daphnia Toxicity Concern
- 13. Daphnia Toxicity Concern
- 14. Daphnia Toxicity Concern
- 15. Soil Mobility Class
- 16. Soil Mobility Class
- 17. Soil Mobility Class

Below the plot is a table of model names corresponding to the persistence categories:

No.	Category	Model Name
1	Air Persistence Class	EPISUITE_Fugacity_3_air
2	Water Persistence Class	EPISUITE_Fugacity_3_water
3	Soil Persistence Class	EPISUITE_Fugacity_3_soil

- Semi-automated decision support tool with dashboard interface for high-throughput risk assessments
- Combining diverse data streams into quantitative toxicity values with associated uncertainty

Regulatory Applications Require More Focus on Quality and Transparency



ASSAY: AEID117 (ATG ERA_TRANS)

NAME: Thioglycolic acid
CHID: 26141 CASRN: 68-11-1
SPID(S): TX007664
L4ID: 420385

HILL MODEL (in red):
 tp ga gw
 val: 3.1e-11 -2.15 0.416
 sd: NaN NaN NaN

GAIN-LOSS MODEL (in blue):
 tp ga gw la lw
 val: 2.93 -0.184 8 0.173 18
 sd: 3.56 0.334 9.48 5.82 814

CNST	HILL	GNLSS
AIC: 20.14	26.14	17.79
PROB: 0.23	0.01	0.76
RMSE: 0.92	0.92	0.32

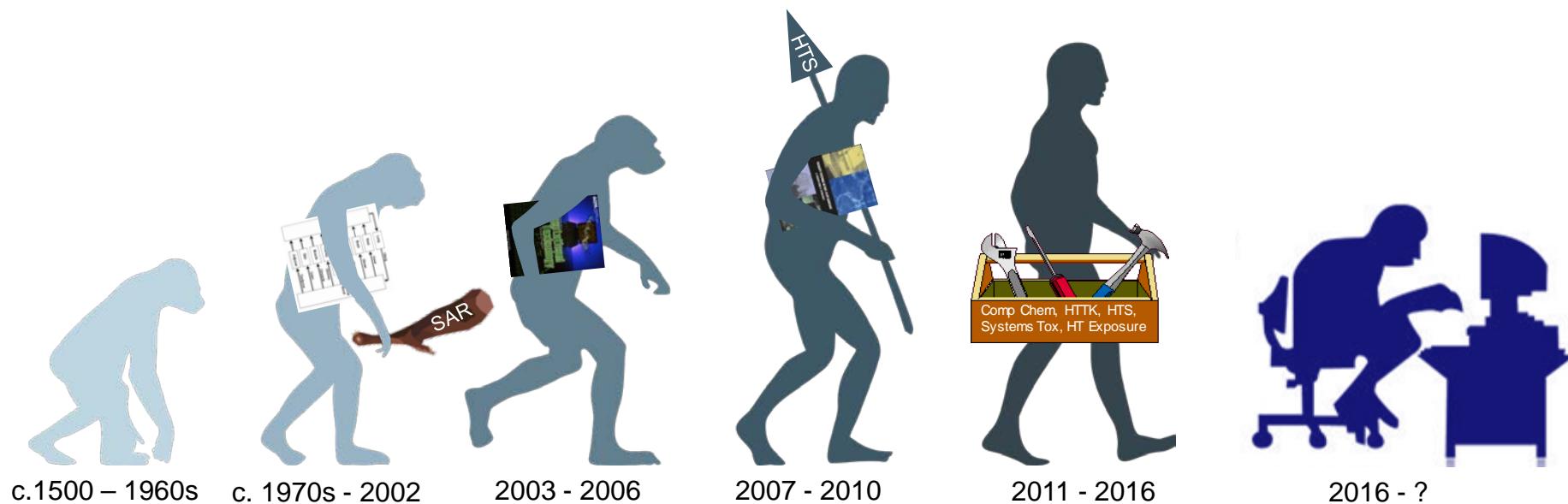
MAX_MEAN: 2.45 MAX_MED: 2.45 BMAD: 0.233

COFF: 1.17 HIT-CALL: 1 FITC: 50 ACTP: 0.77

FLAGS:
Only one conc above baseline, active
Borderline active

- Public release of Tox21 and ToxCast data on PubChem and EPA web site (raw and processed data)
- Transparent ToxCast data analysis pipeline
 - Data quality flags to indicate concerns with chemical purity and identity, noisy data, and systematic assay errors
 - Publicly available as an R package
- Tox21 and ToxCast chemical libraries have undergone analytical QC and results publicly available
- Public posting of ToxCast procedures
 - Chemical Procurement and QC
 - Data Analysis
 - Assay Characteristics and Performance
- External audit on ToxCast data and data analysis pipeline

Next Phase... Evolution Towards a Truly Predictive Science



Acknowledgements and Questions

Tox21 Colleagues:

NTP Crew

FDA Collaborators

NCATS Collaborators

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NERL

NHEERL

NCEA



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