Use of the ToxCast and Tox21 Screening Strategies in Support of Chemical Prioritization for Risk Assessment

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IEBMC

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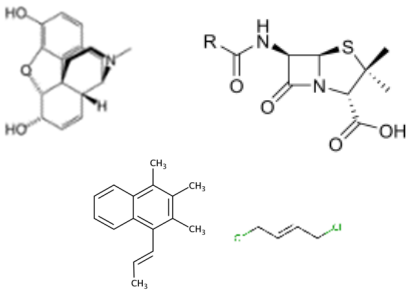
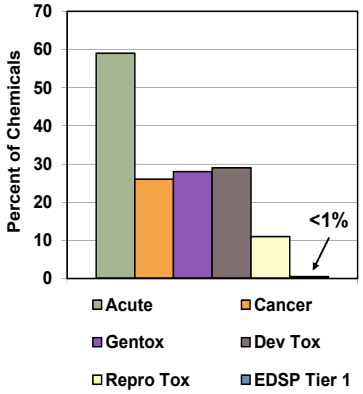
U.S. Environmental Protection Agency

Regulatory Agencies Make a Broad Range of Decisions on Chemicals…

Number of Chemicals /Combinations



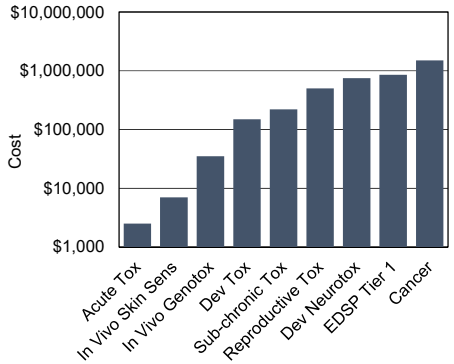
Lack of Data



Modified from Judson *et al.*, EHP 2010

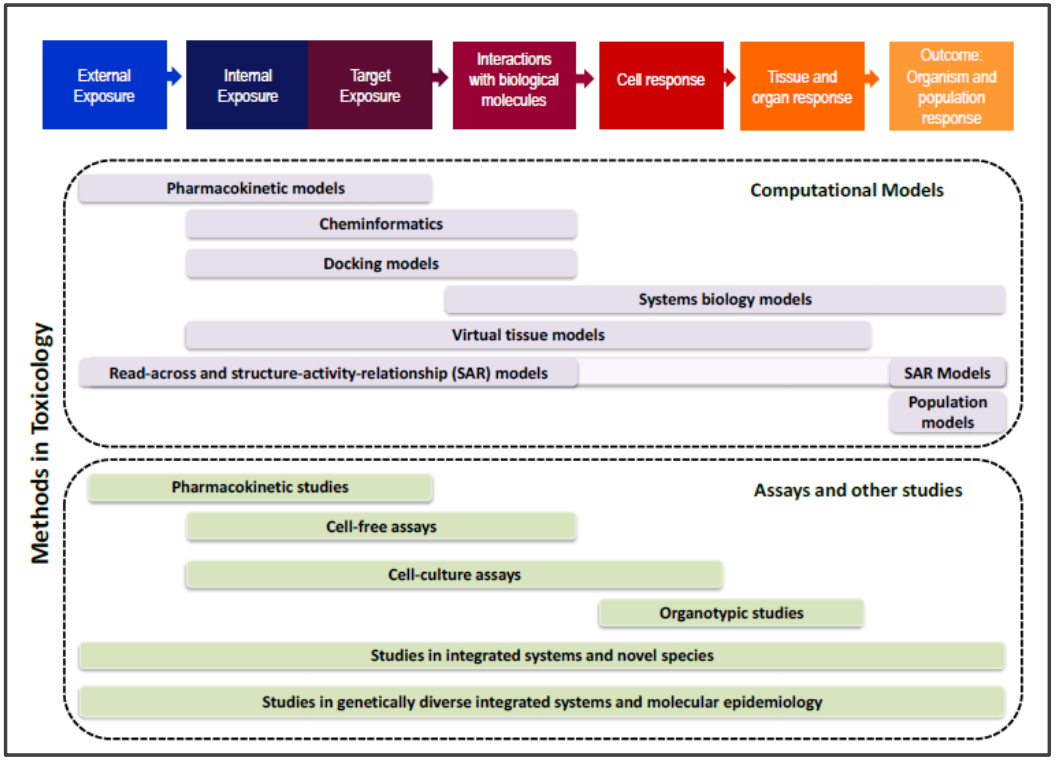
Ethics/Relevance Concerns

Economics

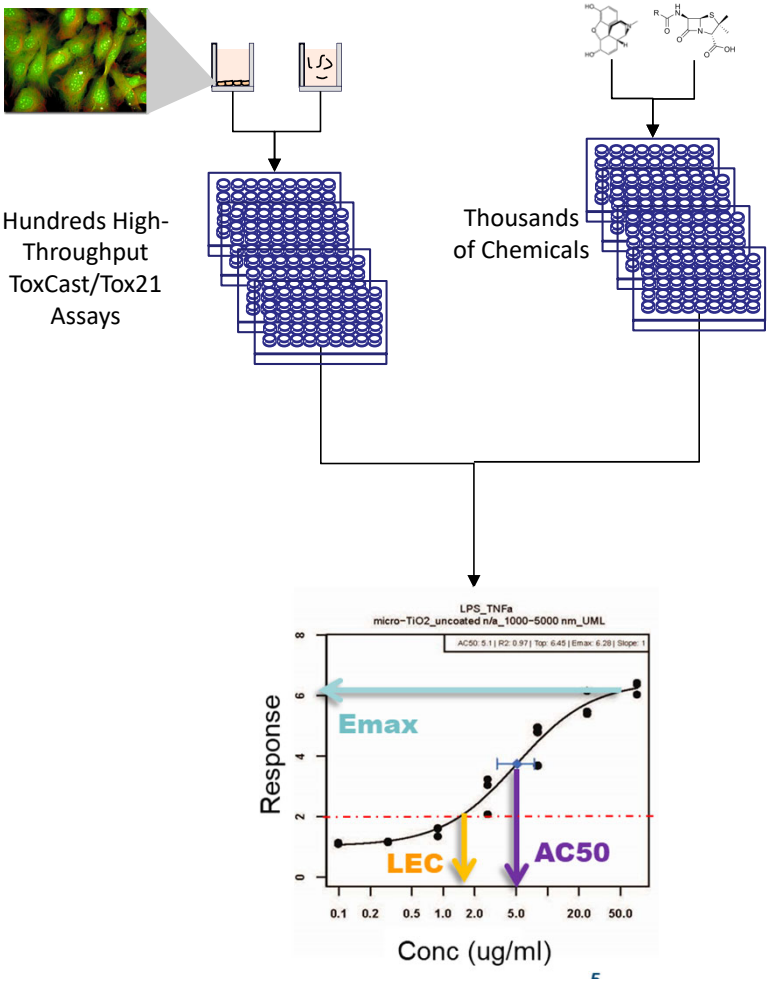


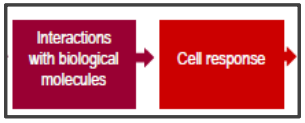
- Number of chemicals and combinations of chemicals is extremely large (>20,000 substances on active TSCA inventory)
- Due to historical regulatory requirements, most chemicals lack traditional toxicity testing data
- Traditional toxicology testing is expensive and time consuming
- Traditional animal-based testing has issues related to ethics and relevance

Toxicology Moving to Embrace 21st Century Methods



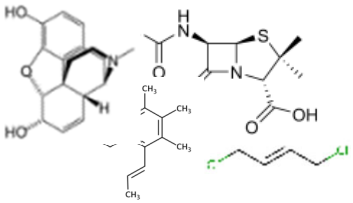
High-Throughput Assays Used to Screen Chemicals for Potential Toxicity

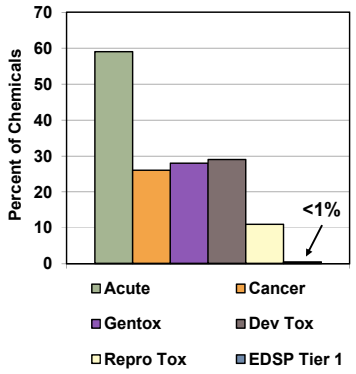




- Understanding of what cellular processes/pathways may be perturbed by a chemical
- Understanding of what amount of a chemical causes these perturbations

Key Steps in Satisfying Toxicologists and Regulators



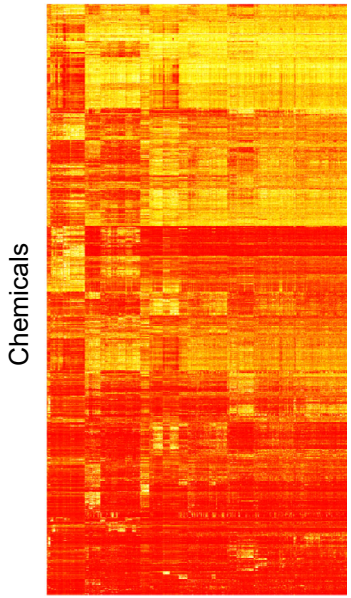


Modified from Judson *et al.*, EHP 2009

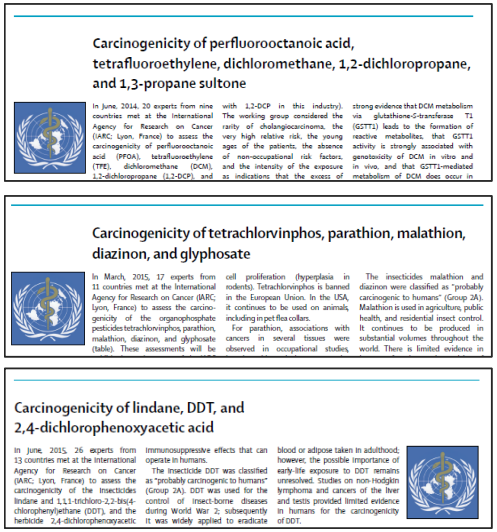
- Transparency and validation
- Systematically addressing limitations in alternative test systems
- Put results in a dose/exposure context
- Characterize uncertainty
- Emphasize development of computational models to integrate experimental data
- Deliver of data and models through decision support tools

Broad Success Derived from High-Throughput Screening Approaches

Group Chemicals by Similar Bioactivity and Predictive Modeling



Provide Mechanistic Support for Hazard ID



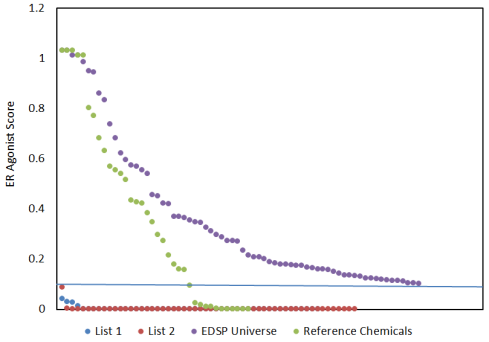
Carcinogenicity of perfluorooctanoic acid, tetrafluoroethylene, dichloromethane, 1,2-dichloropropane, and 1,3-propane sultone

Carcinogenicity of tetrachlorvinphos, parathion, malathion, diazinon, and glyphosate

Carcinogenicity of lindane, DDT, and 2,4-dichlorophenoxyacetic acid

IARC Monographs

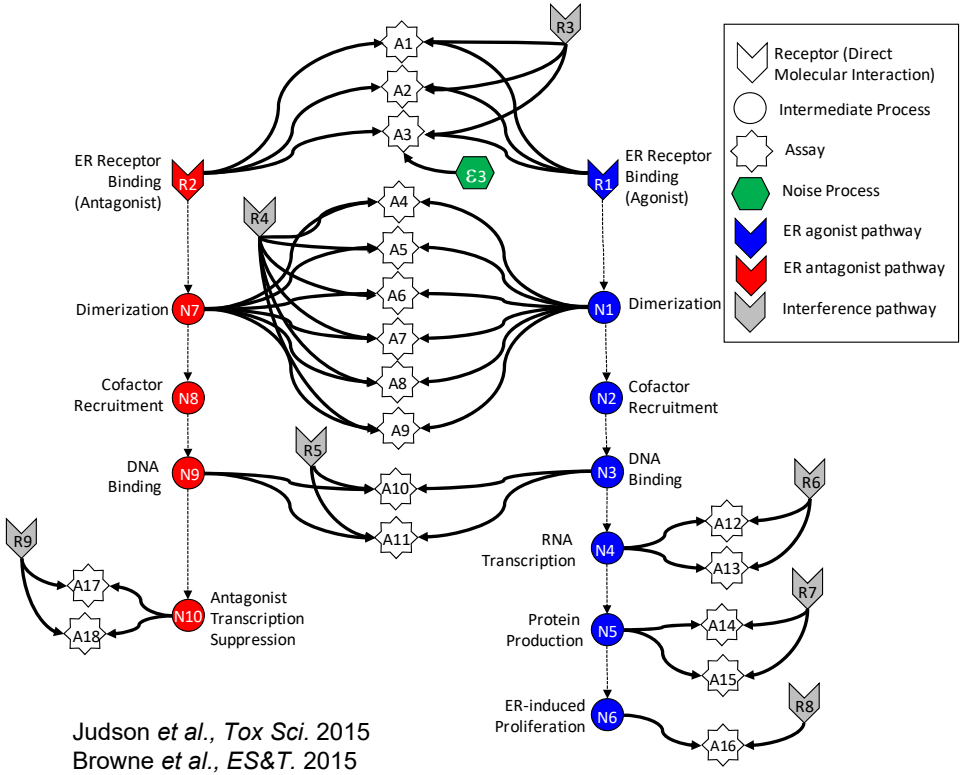
Prioritization of Chemicals for Further Testing



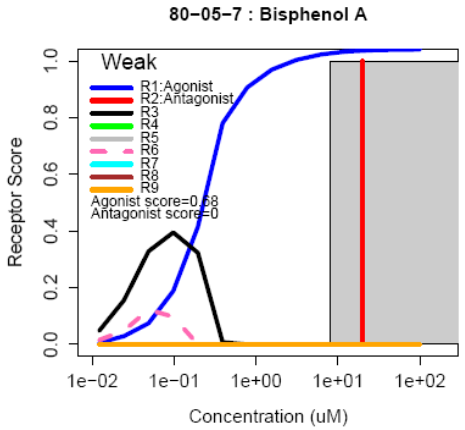
FIFRA SAP, Dec 2014

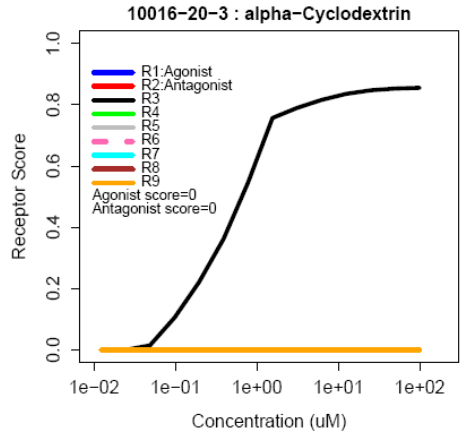
Targeted Pathways (AOP Approach)

18 *In Vitro* Assays Measure ER-Related Activity

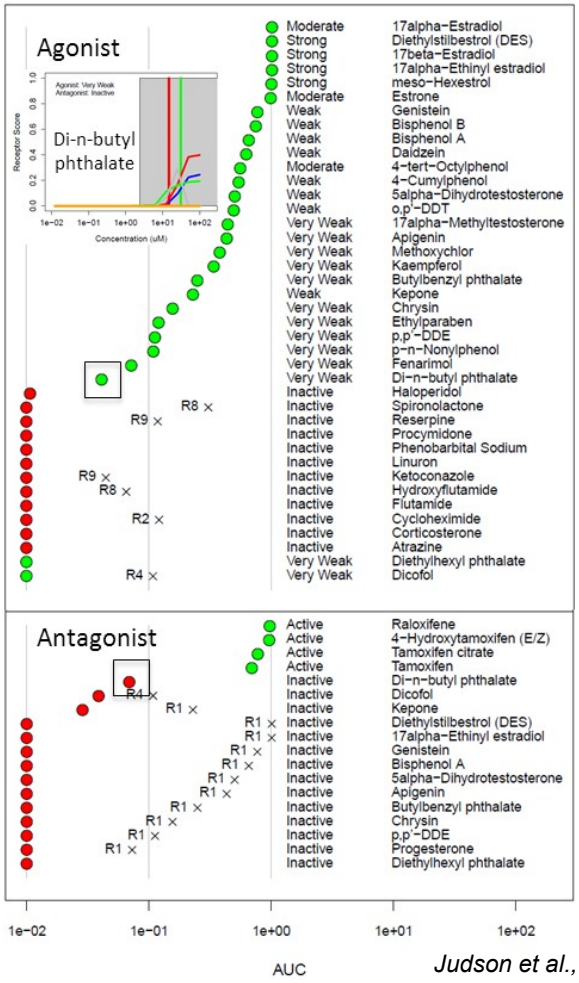


Judson *et al., Tox Sci.* 2015
Browne *et al., ES&T.* 2015
Kleinstreuer *et al., EHP* 2016

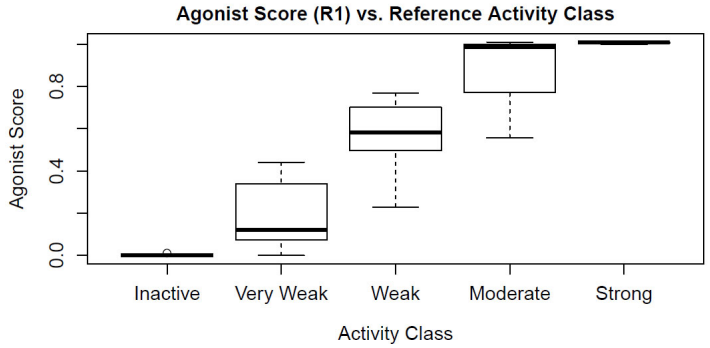


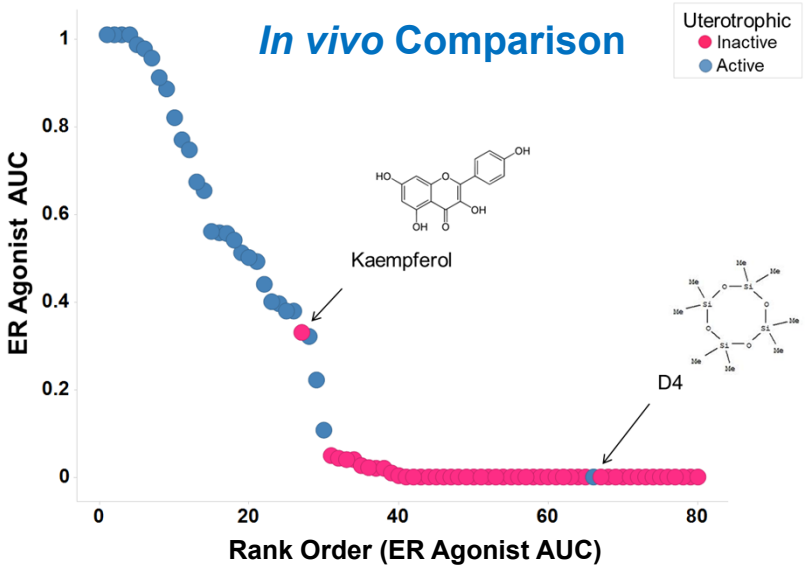


ER Model Performance



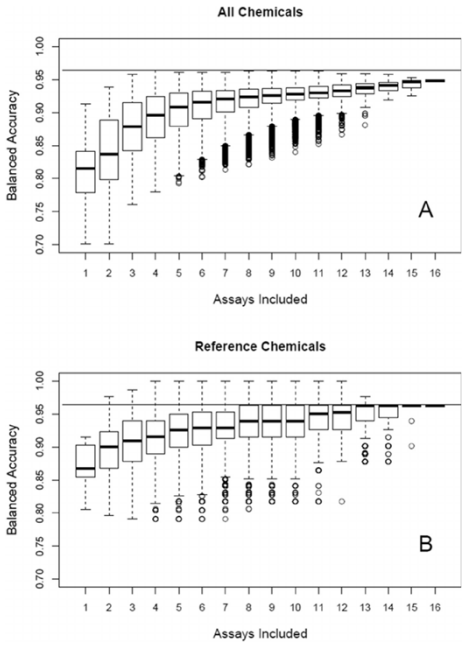
Judson et al., Tox Sci 2015

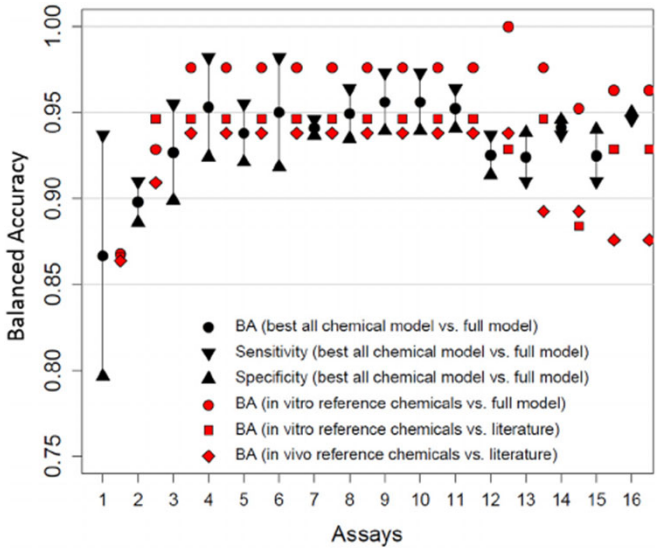




Browne et al., Environ. Sci. Technol., **2015**

ER Minimal Model

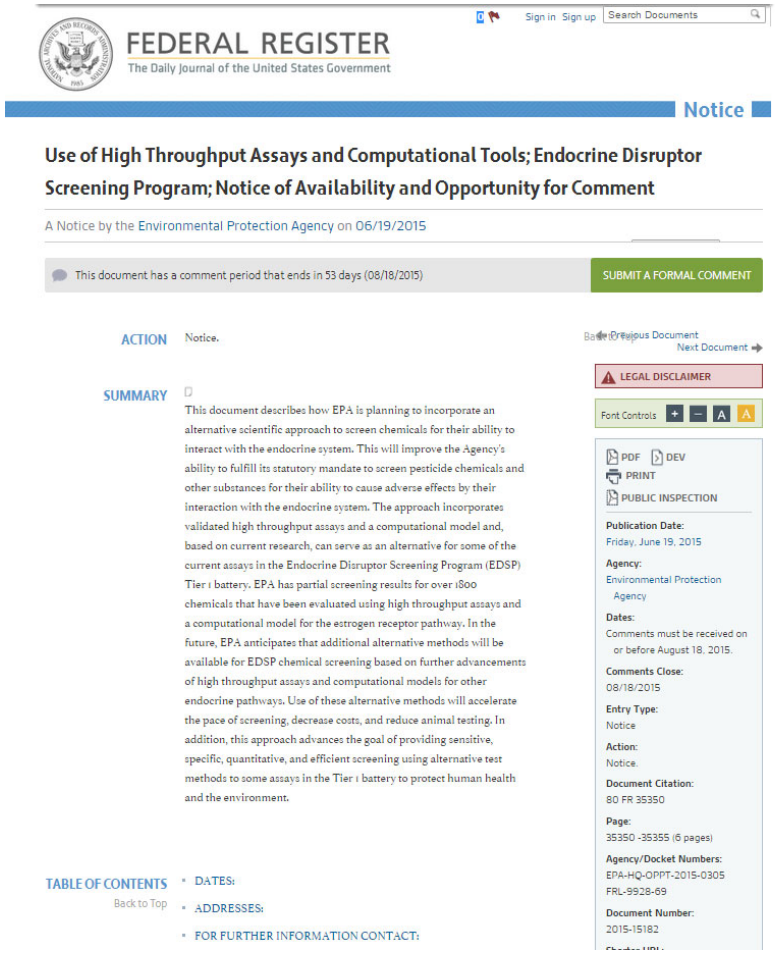




Combinations of four assays provide good balanced accuracy

R.S. Judson et al. / Regulatory Toxicology and Pharmacology 91 (2017) 39e49

Regulatory Applications: EDSP



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The Daily Journal of the United States Government

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Notice

Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment

A Notice by the Environmental Protection Agency on 06/19/2015

This document has a comment period that ends in 53 days (08/18/2015) SUBMIT A FORMAL COMMENT

ACTION Notice.

SUMMARY

This document describes how EPA is planning to incorporate an alternative scientific approach to screen chemicals for their ability to interact with the endocrine system. This will improve the Agency's ability to fulfill its statutory mandate to screen pesticide chemicals and other substances for their ability to cause adverse effects by their interaction with the endocrine system. The approach incorporates validated high throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery. EPA has partial screening results for over 1800 chemicals that have been evaluated using high throughput assays and a computational model for the estrogen receptor pathway. In the future, EPA anticipates that additional alternative methods will be available for EDSP chemical screening based on further advancements of high throughput assays and computational models for other endocrine pathways. Use of these alternative methods will accelerate the pace of screening, decrease costs, and reduce animal testing. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.

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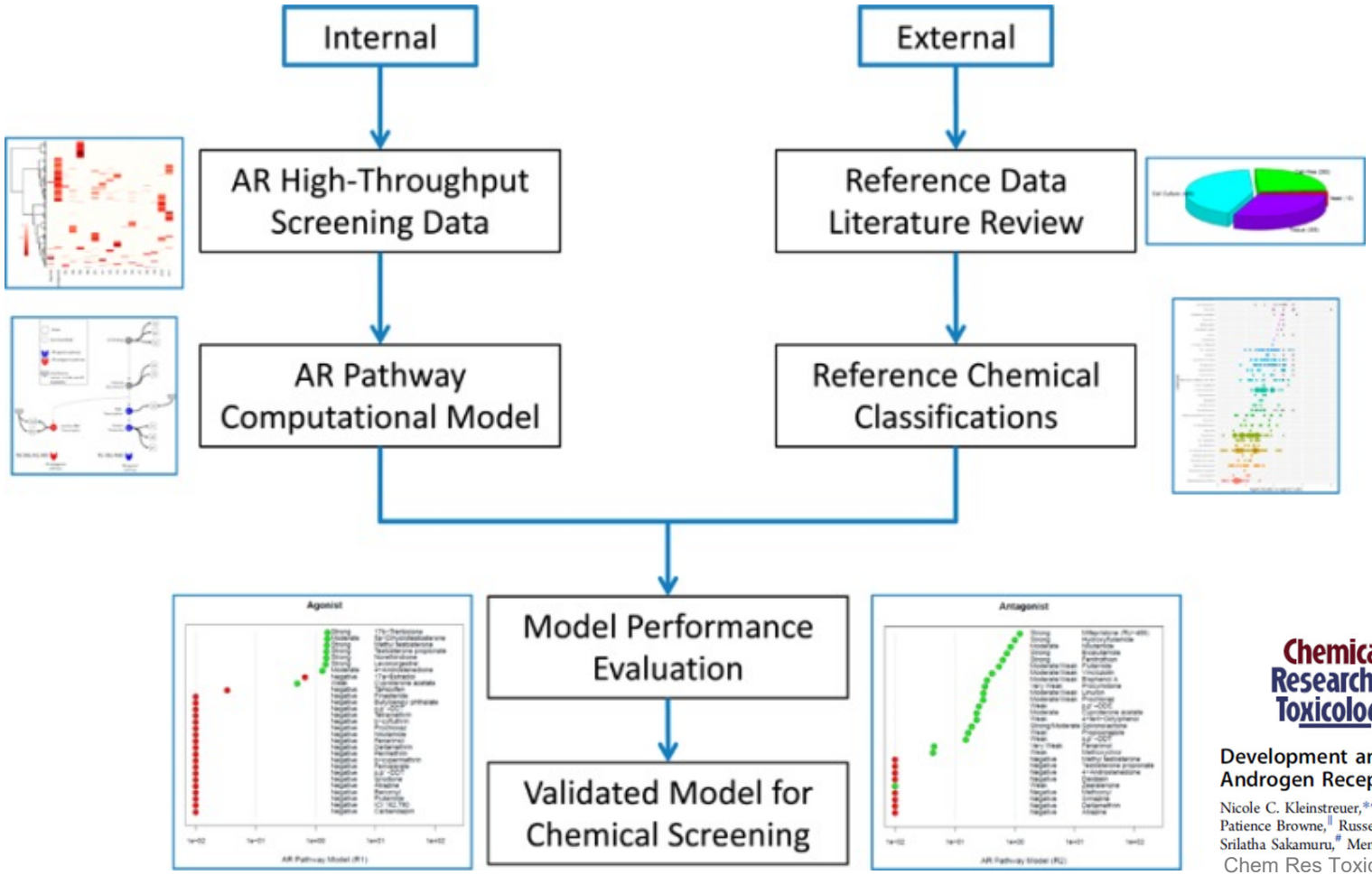
Document Number: 2015-15182

"The approach incorporates validated high-throughput assays and a computational model and, based on current research, can serve as an alternative for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery."

Androgen Receptor Model



Chemical Research in Toxicology

Development and Validation of a Computational Model for Androgen Receptor Activity

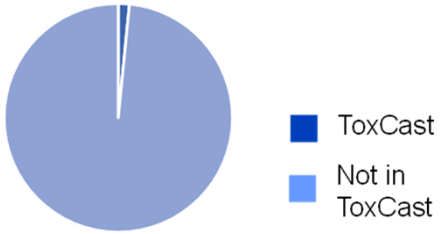
Nicole C. Kleinstreuer,[*,†] Patricia Ceger,[‡] Eric D. Watt,[§] Matthew Martin,[§] Keith Houck,[§] Patience Browne,[∥] Russell S. Thomas,[§] Warren M. Casey,[†] David J. Dix,[⊥] David Allen,[‡] Srilatha Sakamuru,[#] Menghang Xia,[#] Ruili Huang,[#] and Richard Judson[§]

Chem Res Toxicol. 30:946-964, 2017.

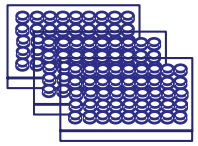
Some Existing Limitations in High-Throughput and *In Vitro* Test Systems

Human Focus

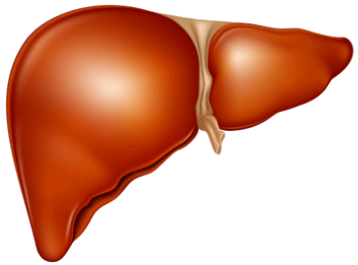
Biological Coverage (Gene Basis)



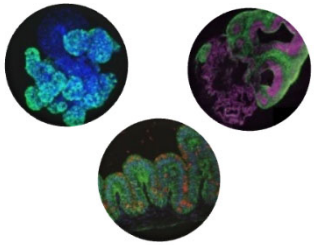
Chemical Coverage and Specific Chemical Types (e.g., VOCs)



Metabolic Competence

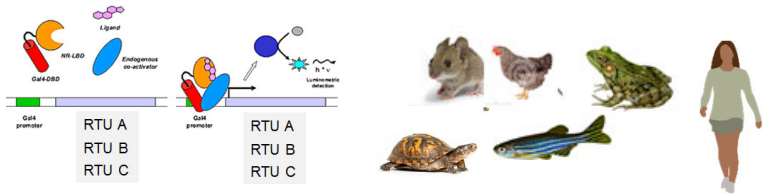


Organ and Tissue Responses

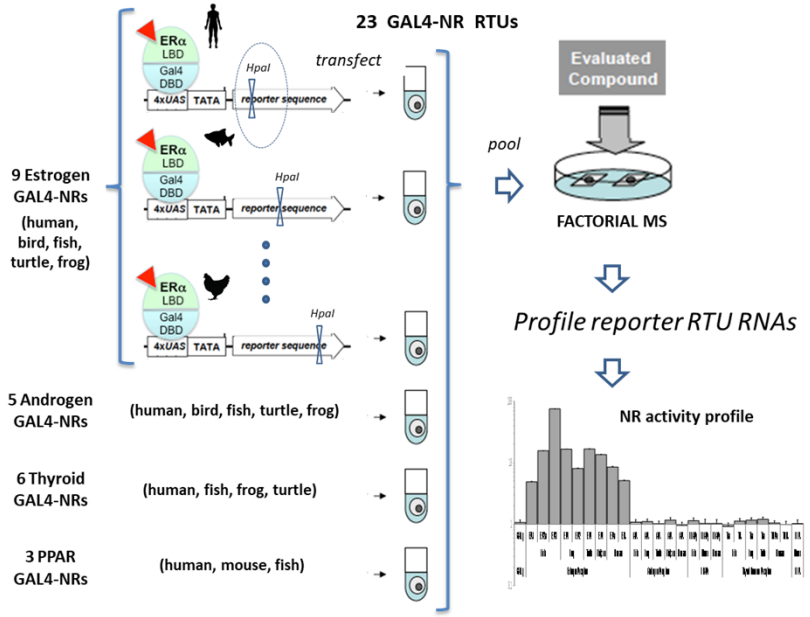


Assessing Cross-Species Differences in Response

Multispecies Attagene *Trans* Reporter Assay



Highly multiplexed reporter gene assay

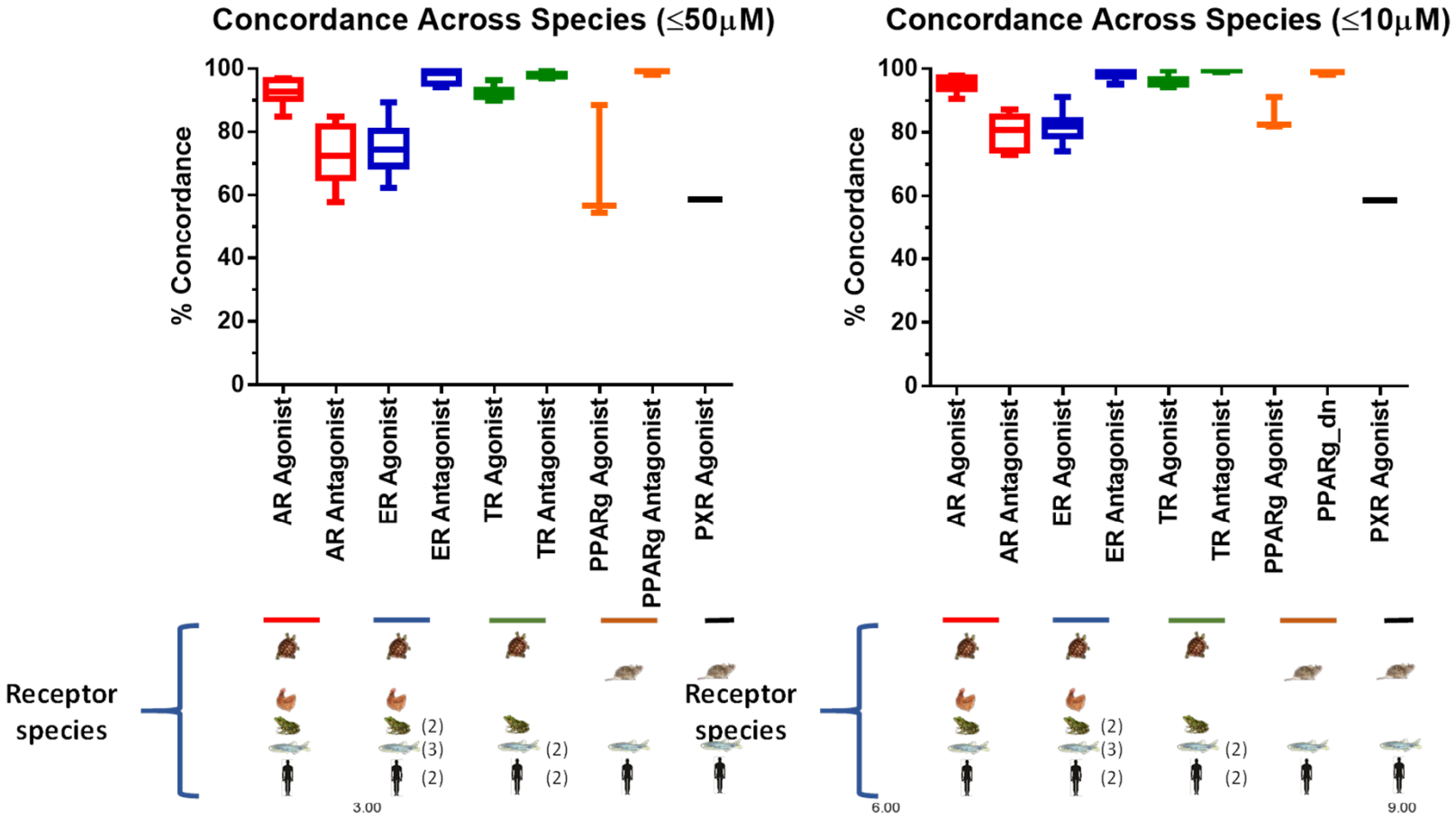


Houck et al., unpublished

NR family	NR	Class	Species	Sequence ID
Estrogen	ER1	Fish	*Danio rerio*	BC162466
	ER2a		*Danio rerio*	BC044349
	ER2b		*Danio rerio*	BC086848
	ER1	Amphibian	*Xenopus laevis*	NM_001089617
	ER2		*Xenopus laevis*	NM_001130954
	ER1	Reptilian	*Chrysemys picta*	NM_001282246
	ER1	Avian	*Gallus gallus*	NM_205183
	ERa	Mammalian	*Homo Sapiens*	NM_000125
	ERb		*Homo Sapiens*	NM_001437
Androgen	AR	Fish	*Danio rerio*	NM_001083123
	AR	Amphibian	*Xenopus laevis*	NM_001090884
	AR	Reptilian	*Chrysemys picta*	XM_005279527
	AR	Avian	*Gallus gallus*	NM_001040090
	AR	Mammalian	*Homo Sapiens*	NM_000044
Thyroid	TRa	Fish	*Danio rerio*	BC096778
	TRb		*Danio rerio*	BC163114
	TRa	Amphibian	*Xenopus laevis*	NM_001088126
	TRa	Reptilian	*Chrysemys picta*	XM_005294120
	THRa	Mammalian	*Homo Sapiens*	NM_199334
	THRb		*Homo Sapiens*	NM_000461
PPAR	PPARg	Fish	*Danio rerio*	NM_131467
	PPARg	Mammalian	*Mus musculus*	NM_001127330
	PPARg		*Homo Sapiens*	BC006811

- Host cell: human HepG2
- Agonist mode for all receptors
- Antagonist for ER and AR

Cross-Species Differences in Nuclear Receptor Responses



- 180 Chemicals tested in concentration-response
- Chemicals selected for NR activity

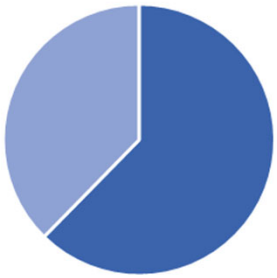
Beginning to Address Concerns for Increased Biological Coverage

Gene Coverage

ToxCast

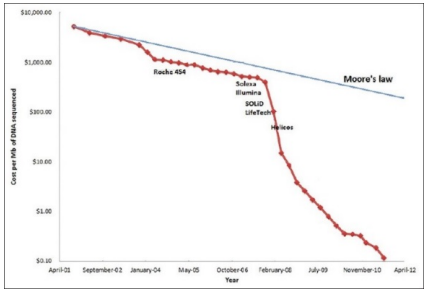
Not in ToxCast

Pathway Coverage*

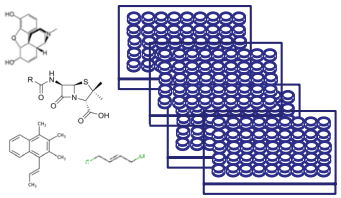


*At least one gene from pathway represented

High-throughput Genomics (HTTr)

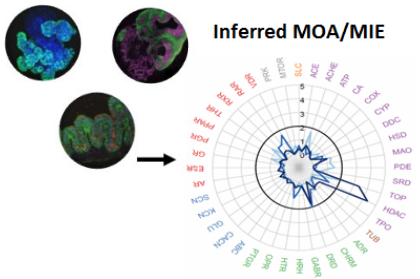


Thousands of chemicals



X

Multiple Cell Types



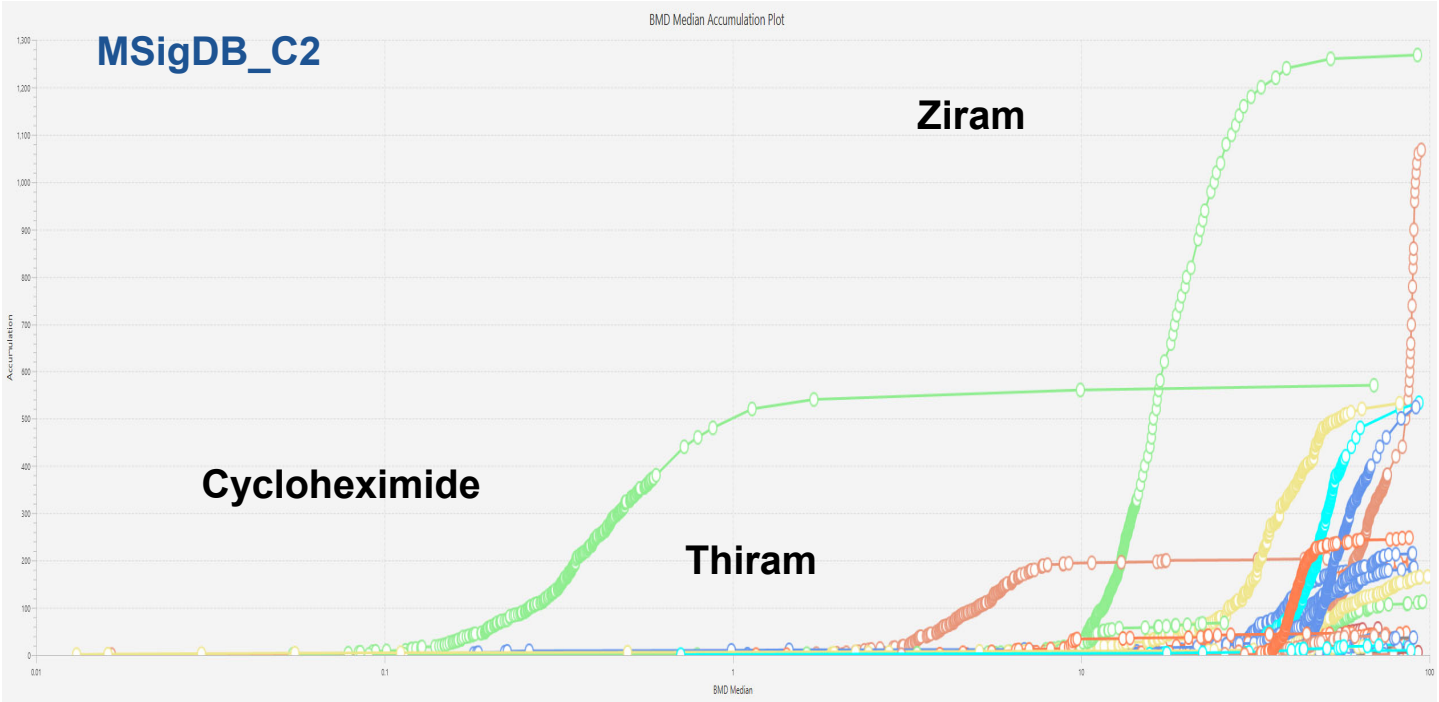
Requirements:

- Low cost
- Whole genome
- 384 well
- Automatable

Pathway Potencies by Benchmark Dose Analysis

Pilot Study

- Reference chemicals for a variety of MoA's
- MCF7 human breast cancer cell line

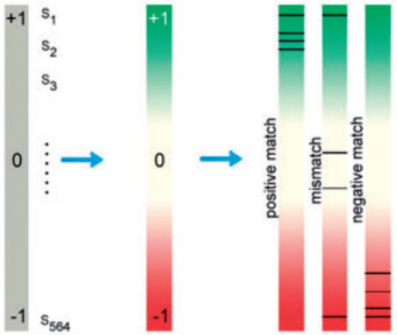


- Broad range of pathway level potency estimates and number of pathways affected across chemicals.

Josh Harrill, NCCT, unpublished

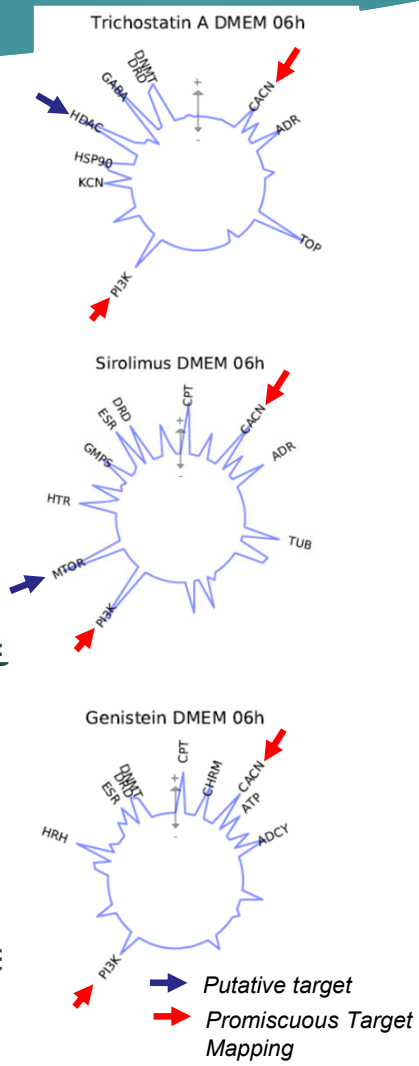
Predicting MoA by Connectivity Mapping

Connectivty mapping



Lamb *et al* (2006)
Musa *el al* (2017)

- Connectivity Mapping
  - Use DEGs / CRGs to define “signatures” for each chemical or treatment
  - Search signature database annotated with MoA
  - Infer MoA using pair-wise similarity between signature



- Differential gene expression observed with reference chemicals
- Putative targets identified using Connectivity Mapping
- Large degree of promiscuity of predicted targets observed
- Currently evaluating additional methods for MIE prediction

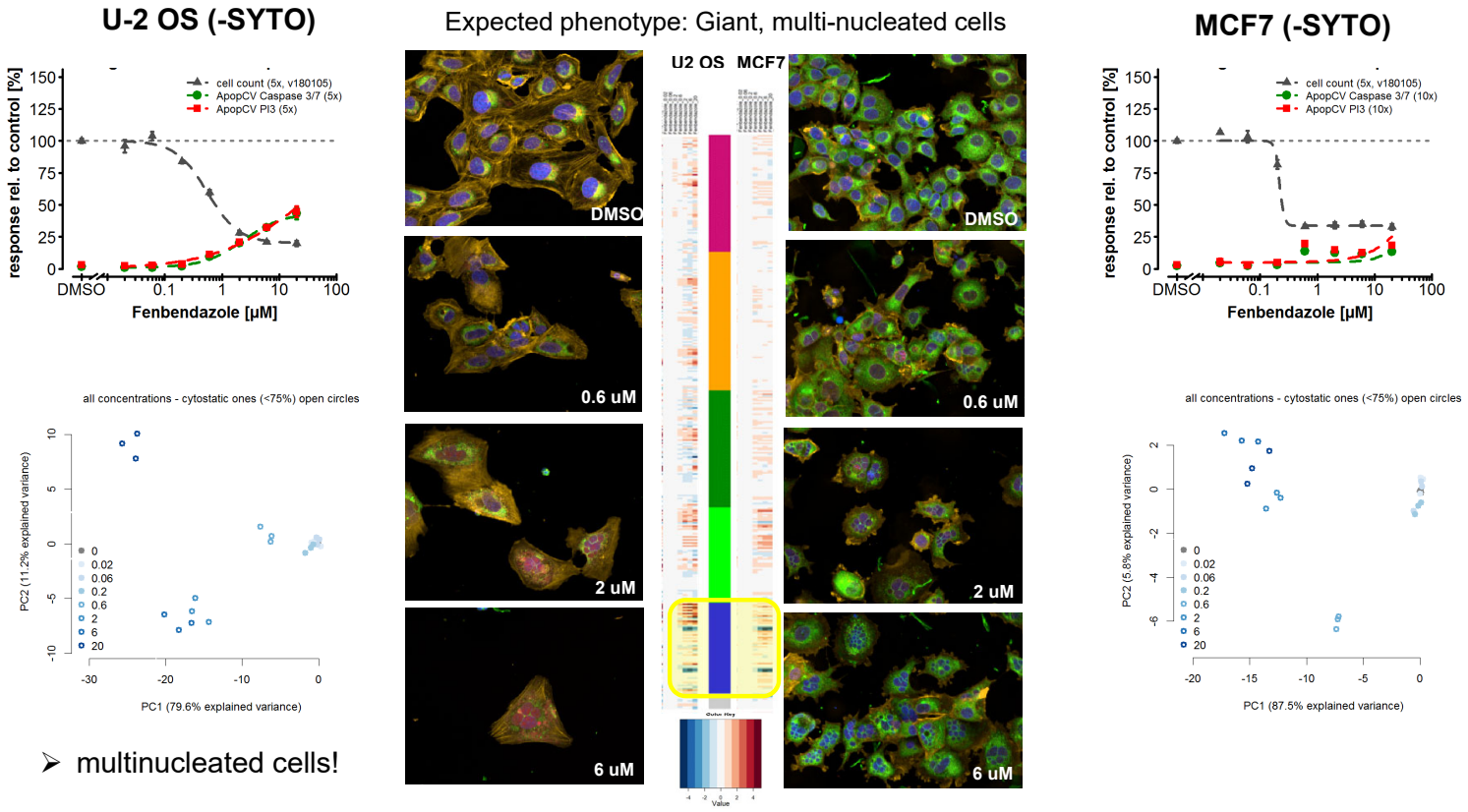
Imran Shah, NCCT, unpublished

Cell Painting Phenotypic Screen Background

- Cell Painting (Bray et al., 2016, *Nature Protocols*): A cell morphology-based phenotypic profiling assay multiplexing six fluorescent "non-antibody" labels, imaged in five channels, to evaluate multiple cellular compartments and organelles.
- Key Features:
  - Non-targeted screening (i.e. target agnostic)
  - Tractable across different adherent cell lines
  - High content 100s – 1000s of features measured at the cell level
  - Concentration-response analysis
  - Fingerprinting and clustering

Marker	Cellular Component	Labeling Chemistry	Labeling Phase	Opera Phenix	
				Excitation	Emission
Hoechst 33342	Nucleus	Bisbenzamide probe that binds to dsDNA	Fixed	405	480
Concanavalin A – AlexaFluor 488	Endoplasmic reticulum	Lectin that selectively binds to α-mannopyranosyl and α-glucopyranosyl residues enriched in rough endoplasmic reticulum		435	550
SYTO 14 nucleic acid stain	Nucleoli	Cyanine probe that binds to ssRNA		435	550
Wheat germ agglutinin (WGA) – AlexaFluor 555	Golgi Apparatus and Plasma Membrane	Lectin that selectively binds to sialic acid and N-acetylglucosaminyl residues enriched in the trans-Golgi network and plasma membrane		570	630
Phalloidin – AlexaFluor 568	F-actin (cytoskeleton)	Phallotoxin (bicyclic heptapeptide) that binds filamentous actin			
MitoTracker Deep Red	Mitochondria	Accumulates in active mitochondria	Live	650	760

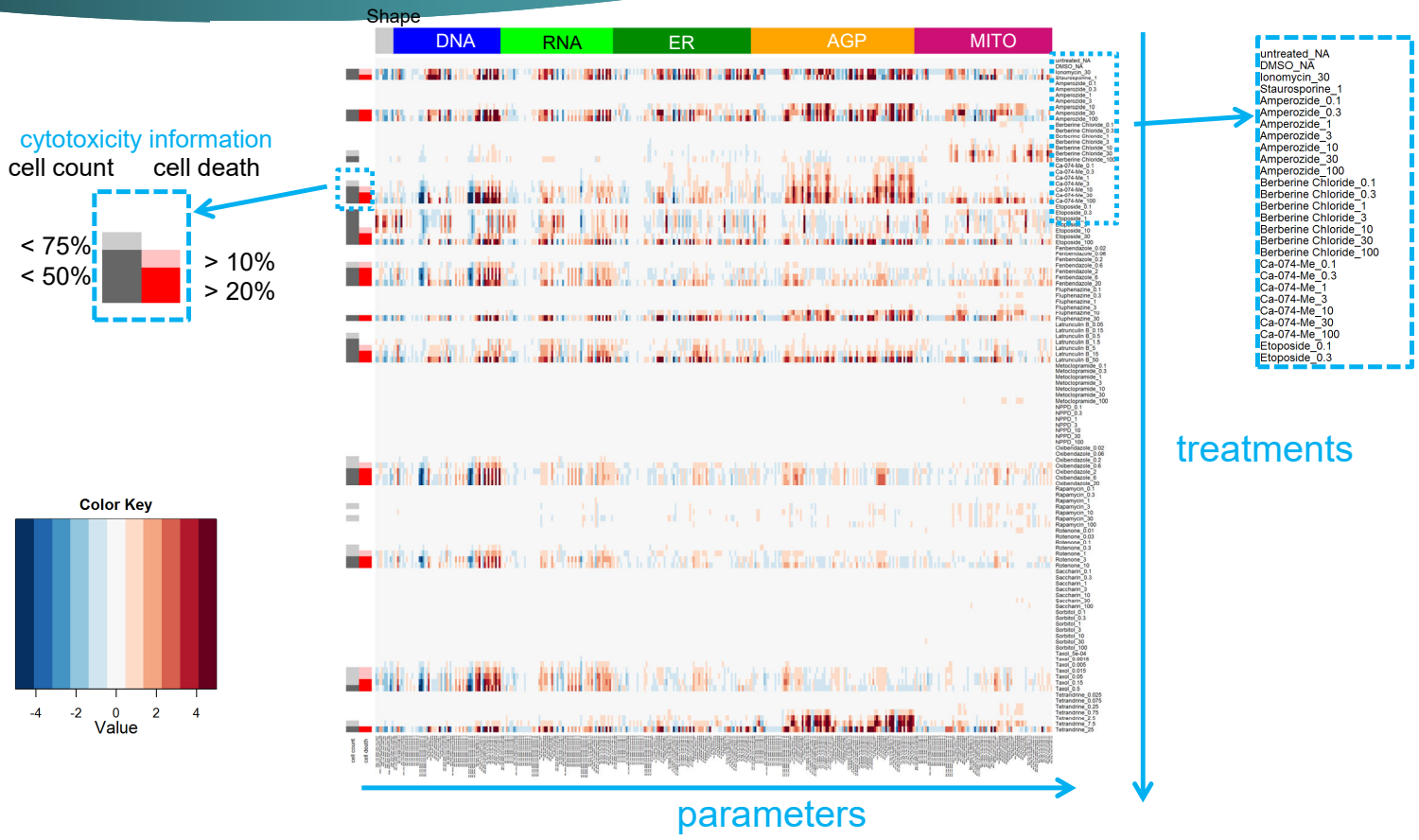
Reference Compound Effects



➢ multinucleated cells!

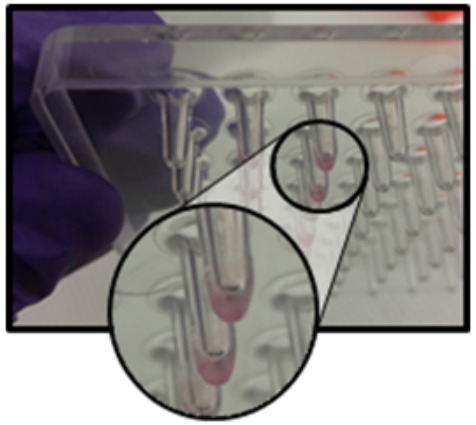
Josh Harrill, NCCT, unpublished

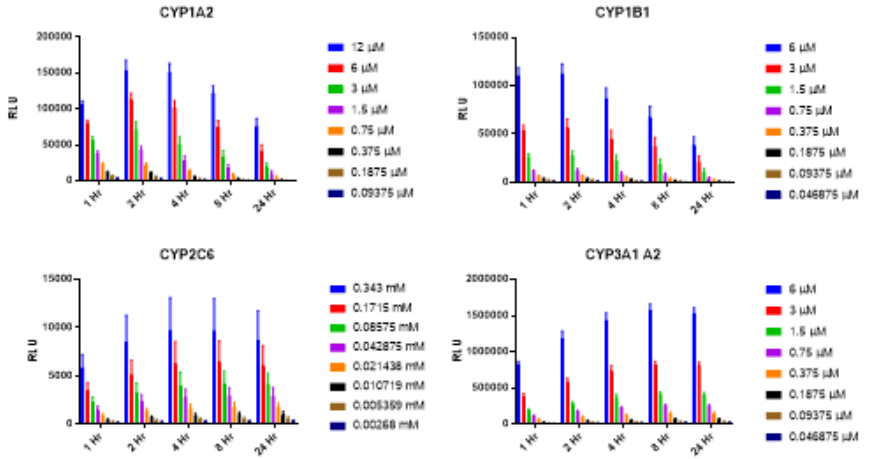
Preliminary Results

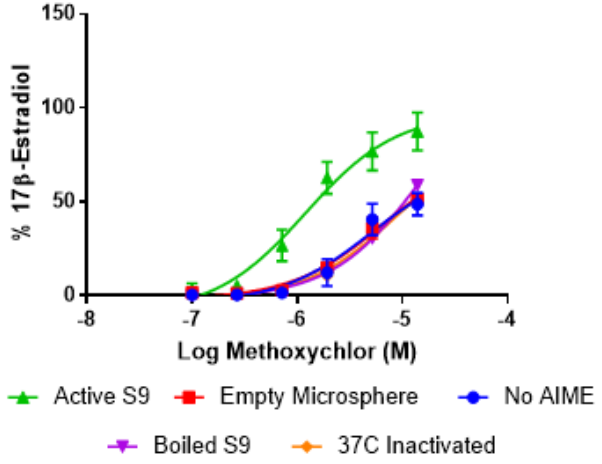


Josh Harrill, NCCT, unpublished

Assays Retrofit for Xenobiotic Metabolism: Extracellular



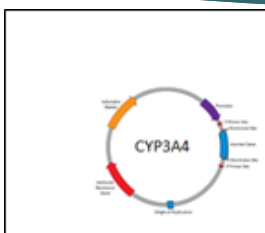


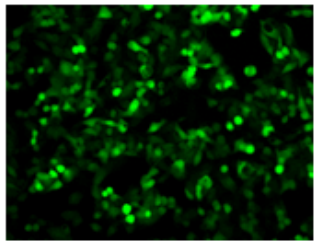


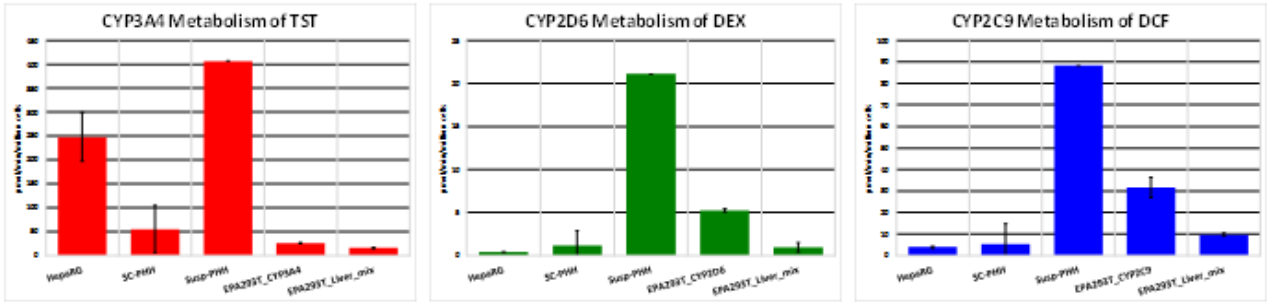
96-Well	EC50 (µM)	EC50 Potency Shift
Active S9	0.71	
Heat Inactivated S9	6.8	9.6
No AIME	4.98	7.0
384-Well	**EC50 (µM)**	**EC50 Potency Shift**
Active S9	1.2	
Heat Inactivated S9	15.9	13.2
No AIME	5.1	4.2

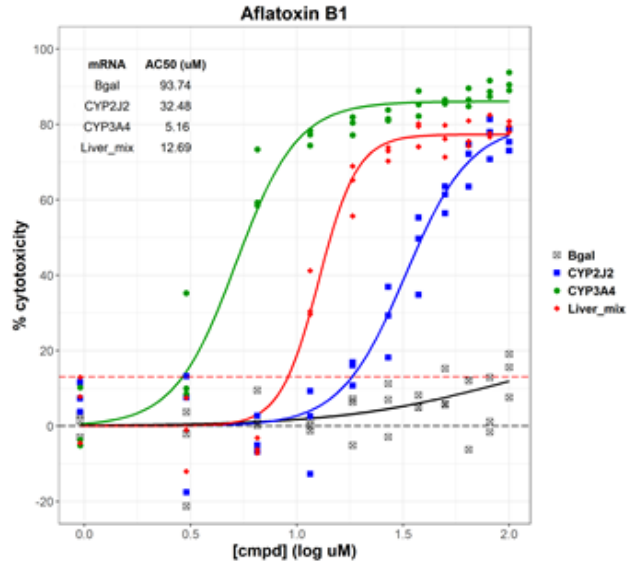
DeGroot, Simmons, and Deisenroth, Unpublished

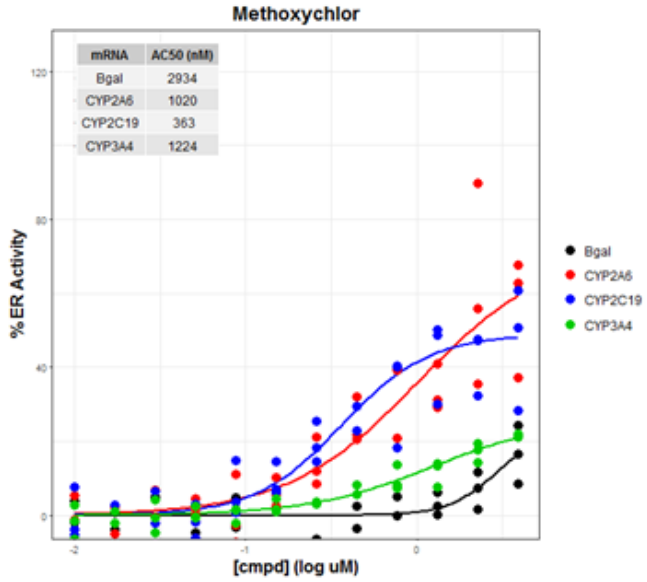
Assays Retrofit for Xenobiotic Metabolism: Intracellular





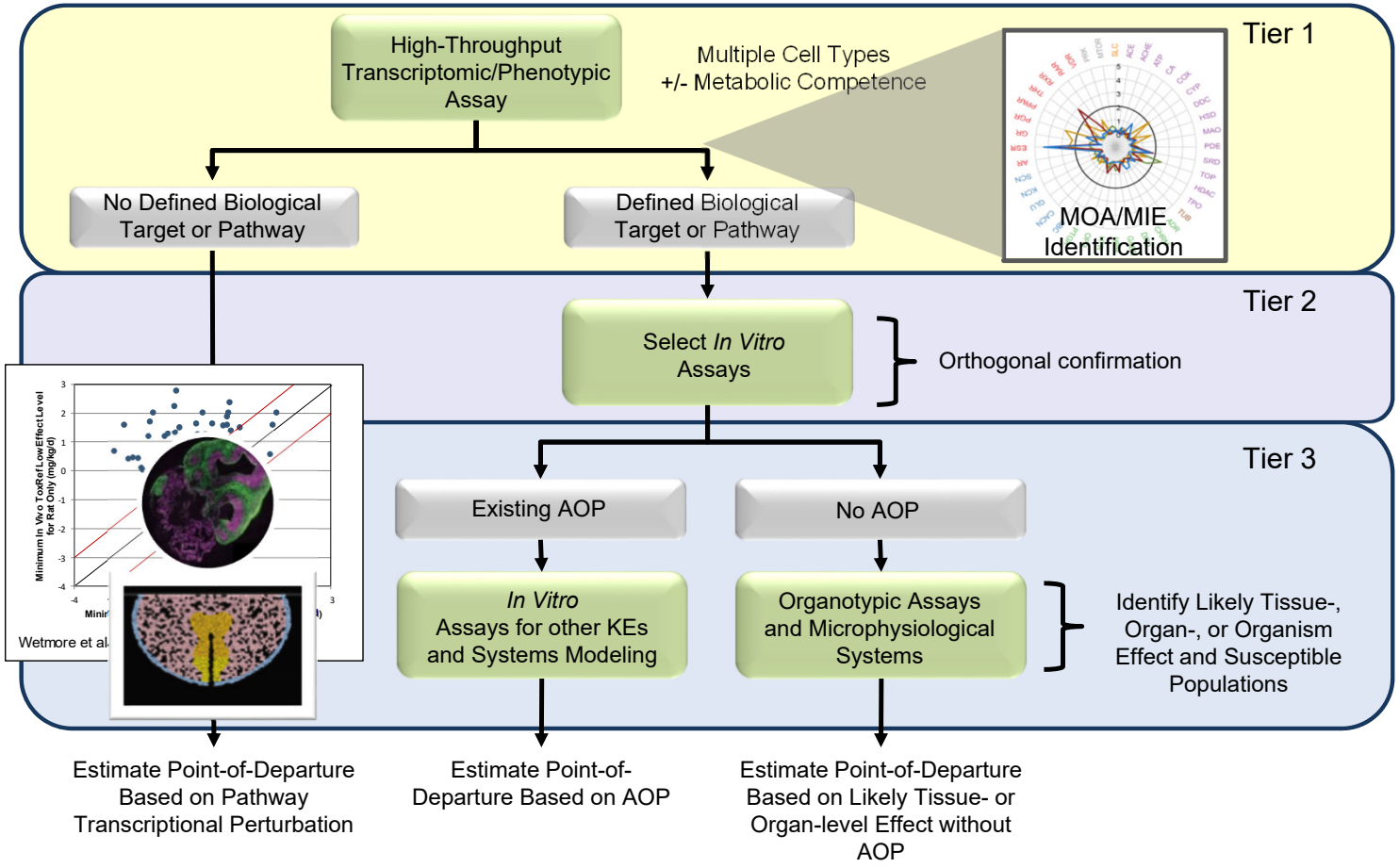






Simmons et al., J Pharmacol Tox Methods, 2018

Framework for Integrating Hazard Components…



Moving Towards Risk: Adding a High-Throughput Toxicokinetic Component

EPA ToxCast Phase I and II Chemicals

Human Liver Metabolism

Human Plasma Protein Binding

Population-Based IVIVE Model

Upper 95th Percentile Css Among 100 Healthy Individuals of Both Sexes from 20 to 50 Yrs Old

- Currently evaluated ~700 ToxCast Phase I and II chemicals
- Models available through "'httk" R package (https://cran.r-project.org/web/packages/httk/)

In Vitro Potency Value

Plasma Concentration

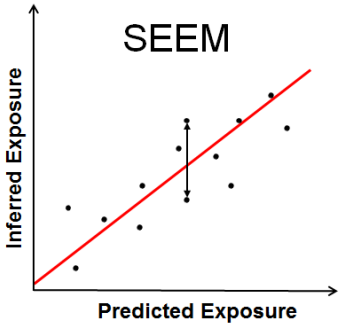
Oral Exposure

Reverse Dosimetry

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

Chemical Use and Production Volume

SEEM

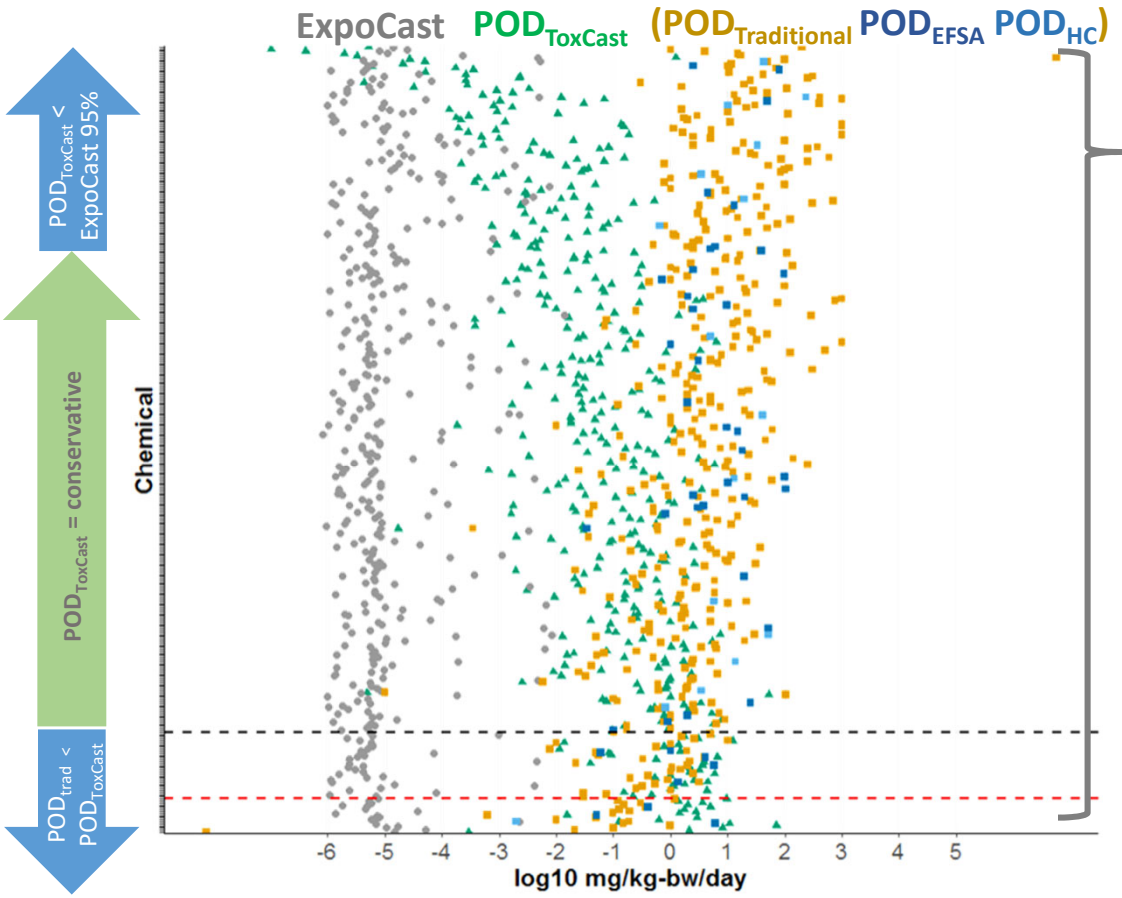


Biomonitoring Data

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

Wambaugh *et al.,* 2014

Bioactivity Provides a Conservative Estimate of a NOAEL/LOAEL



Total =

380 chemicals

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

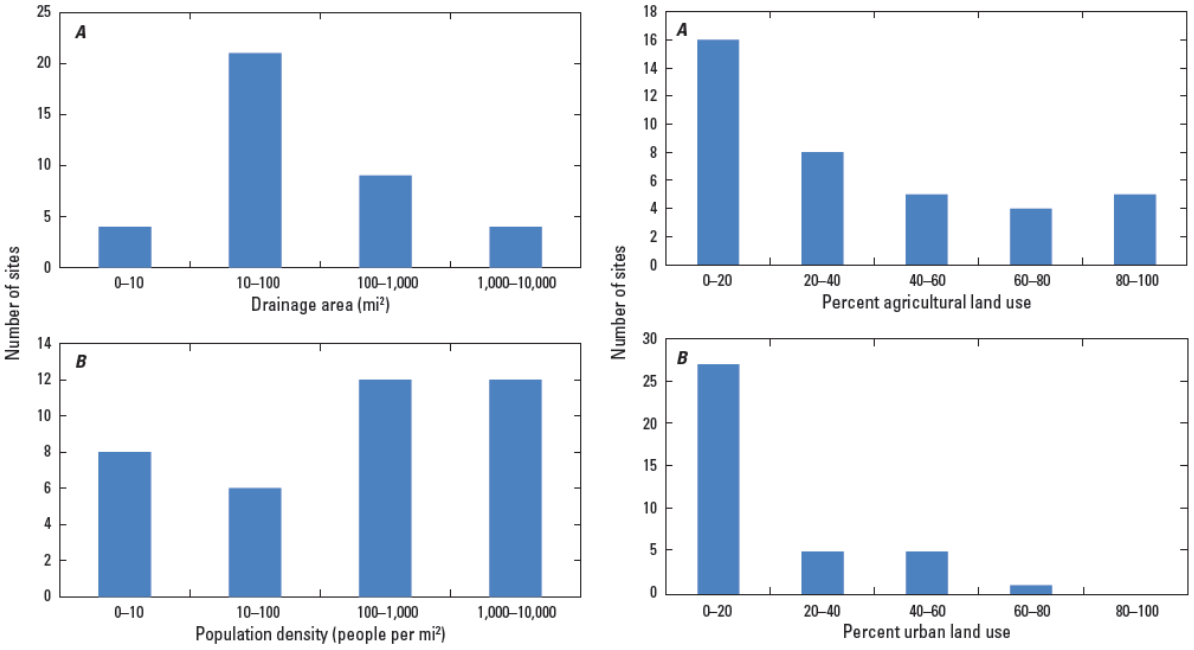
For ~91.3% of the chemicals, $POD_{ToxCast}$ was conservative.

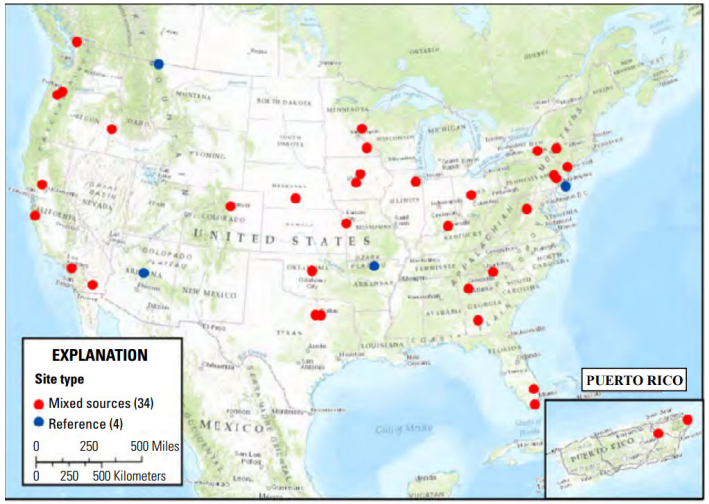
(~130-fold with human HTTK

~40-fold with rat HTTK)

Environmental Monitoring Application: Nationwide Streams Surveillance

- 38 total sites (4 reference sites) across US and PR
- Water samples collected 2012-2014
- Locations varied by watershed drainage





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Brett Blackwell/ORD/EPA

Bioassay Analysis Workflow

Ambient Water Sample

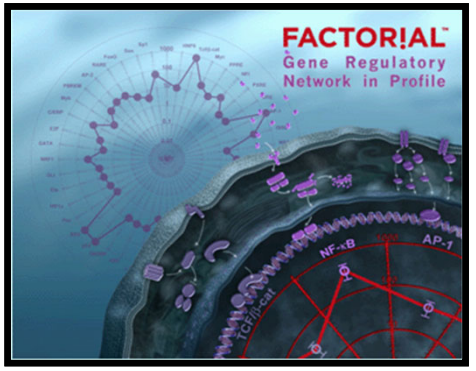
Filtered

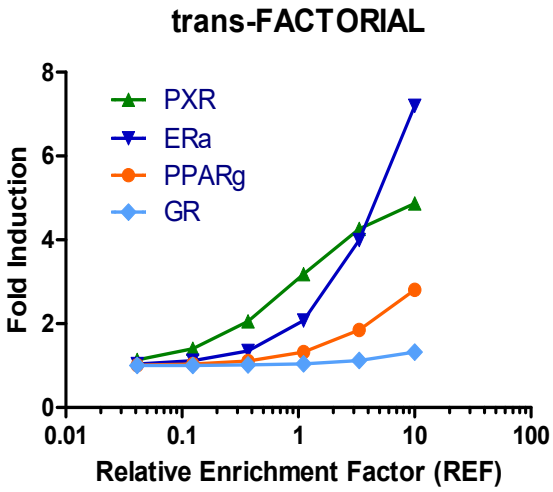
Extracted 200mg HLB

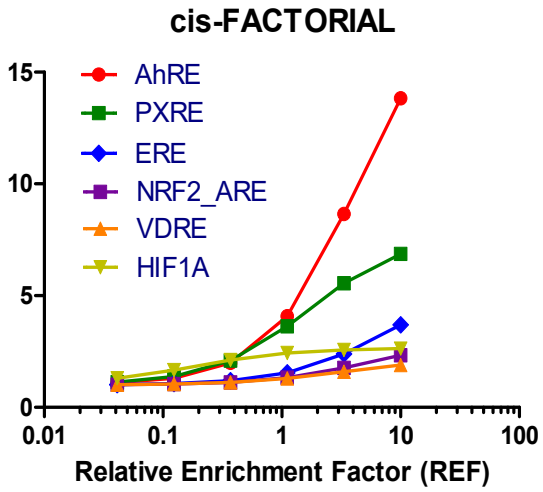
"Unknown" Chemical Mixture

DMSO

1000x conc.







Extract Analysis

- 6-point curve; 3-fold dilution
- 24h exposure

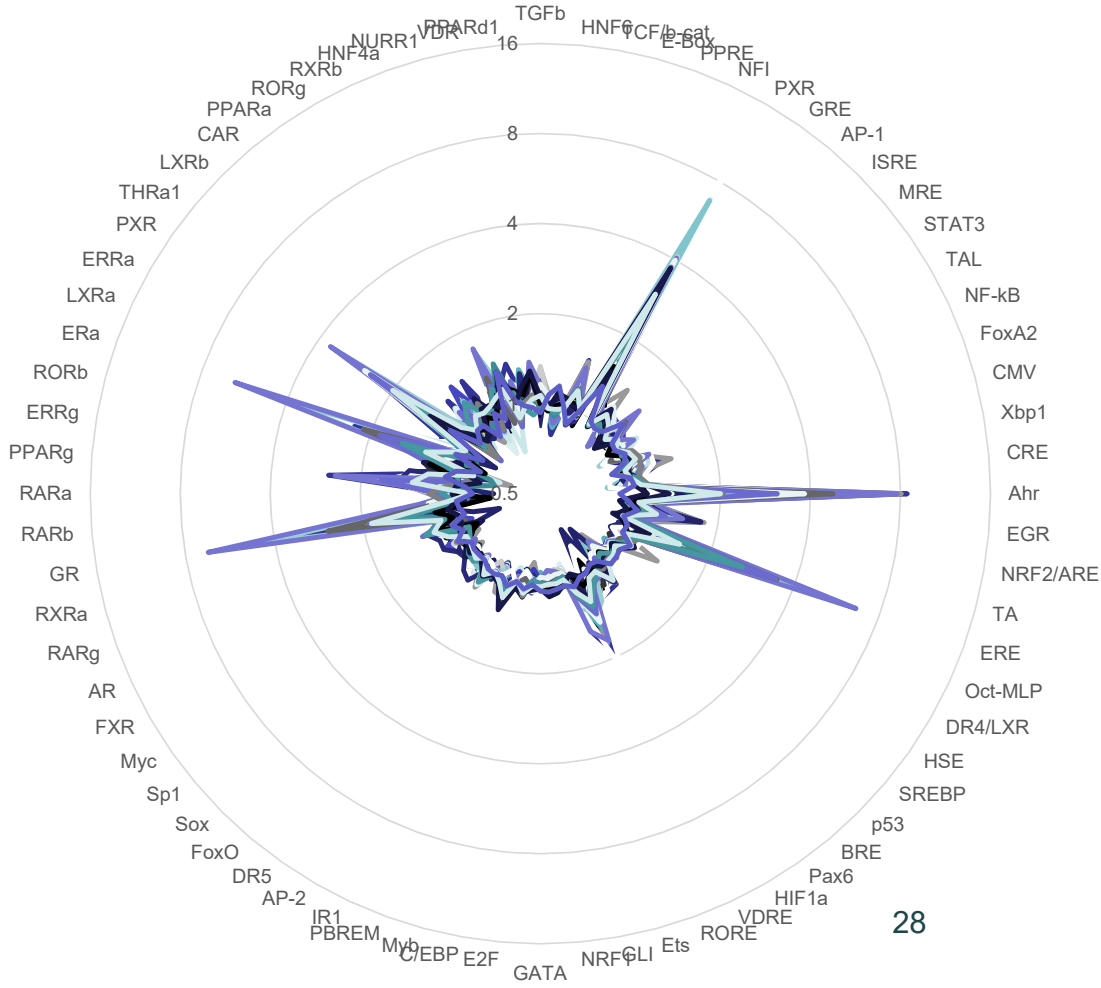
- Area Under Curve (AUC)
  - Response relative to extract blank

Bioassay Results

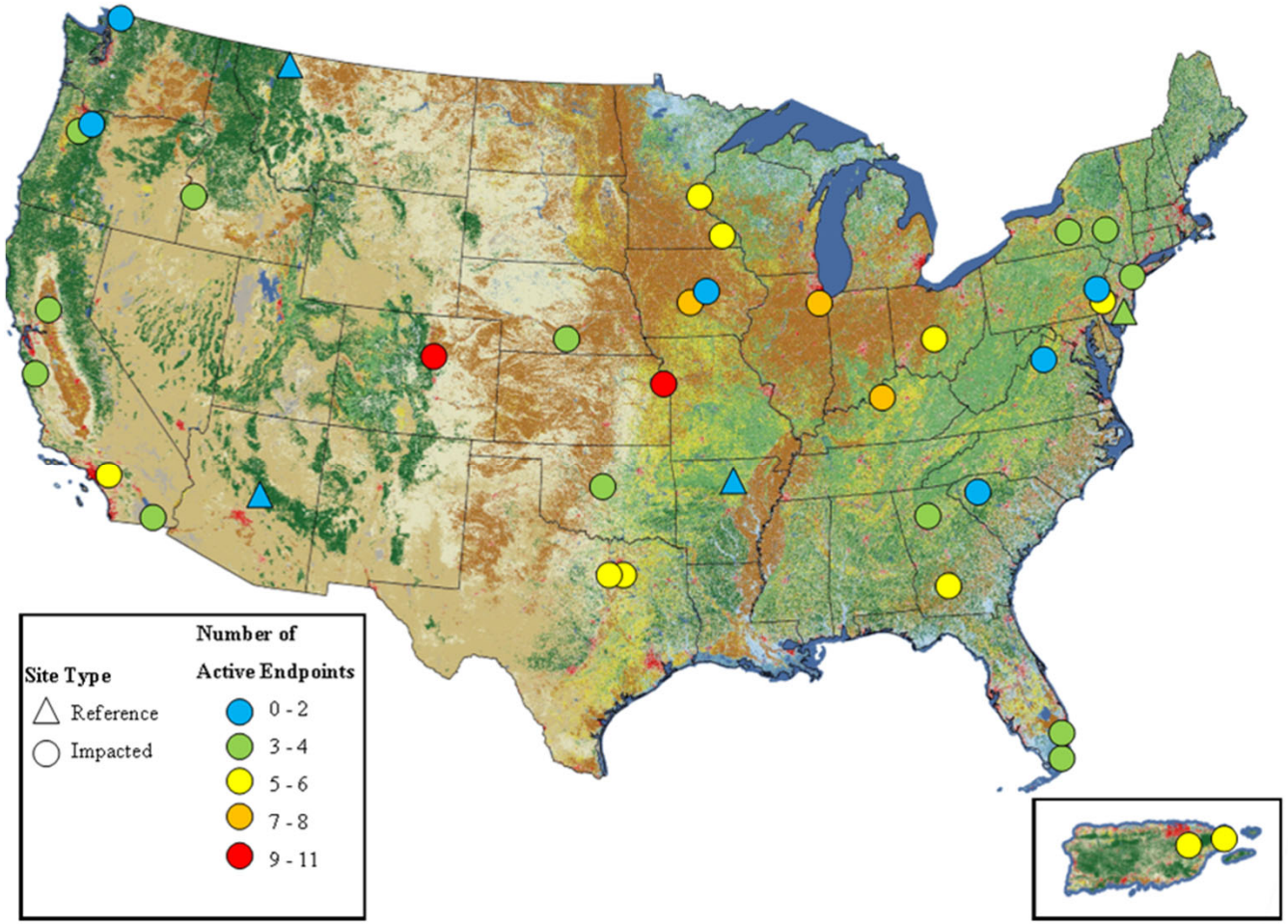
- 26/70 endpoints AUC >1.25-fold (borderline active)
- 11/70 endpoints AUC >1.5-fold (active)

<u>Active Endpoints</u>

- PXRE, PXR, AhRE – 30-36 sites
- ERE – 17 sites
- ERα, PPARγ – 10 sites
- GR, VDRE, NRF2 – 6-8 sites
- RORE, RXRβ – 2 sites

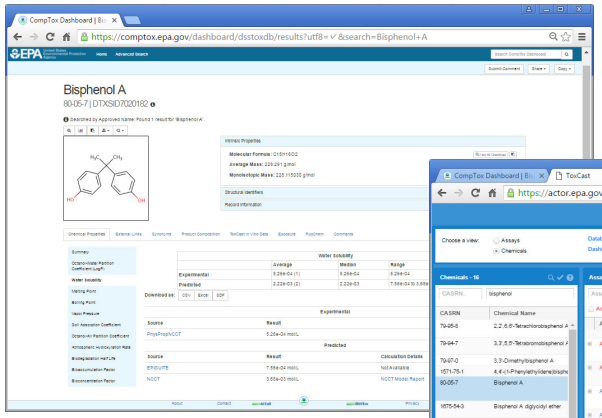


Bioassay Results



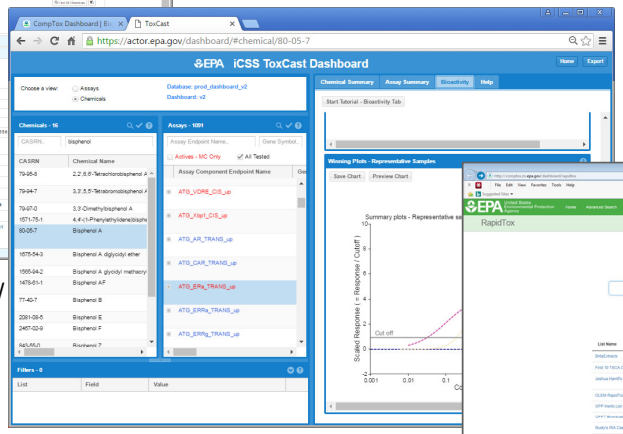
Deliver Data and Models Through Decision Support Tools

Comptox Chemistry Dashboard



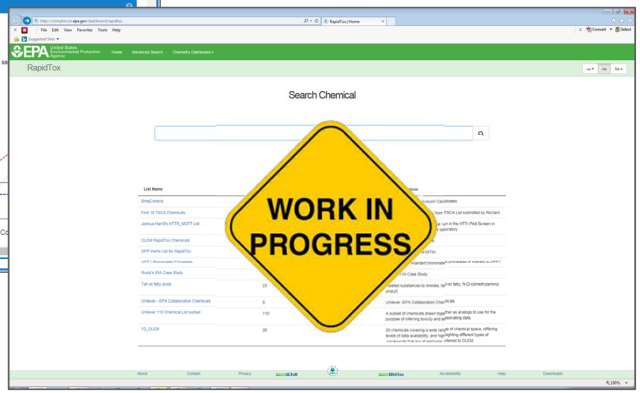
https://comptox.epa.gov/dashboard/

ToxCast Dashboard



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

RapidTox Dashboard



Concluding Remarks

- Multiple opportunities exist for using high-throughput and computational approaches to address challenges in toxicology and risk assessment
- In vitro/alternative approaches valuable for chemical prioritization, especially where we understand the toxicity pathways
- May also be useful in conservative point-of-departure approaches for unknown/non-selective effects
- Combining with exposure predictions allows prioritization for risk
- Using high-throughput approaches will require systematically addressing key technical and data analysis challenges
- Enabling application of high-throughput data to chemical safety decisions with require delivery and integration using a broad range of IT tools

Thank You for Your Attention!

EPA's National Center for Computational Toxicology