Analysis of Chemical Bioactivity through In Vitro Profiling using ToxCast and Tox21 High-Throughput Screening

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26th-29th July 2015
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Outline

- **The Problem**
- **Addressing the Problem**
  - Chemicals
  - Hazard Predictions for Prioritization
    - Developing data – high-throughput *in vitro*
    - Data interpretation - consensus models and biology
  - Exposure
    - Reverse Toxicokinetics – estimating daily dose
    - High-throughput exposure predictions
- **Putting it all together**
  - Cost efficient and rapid prioritization

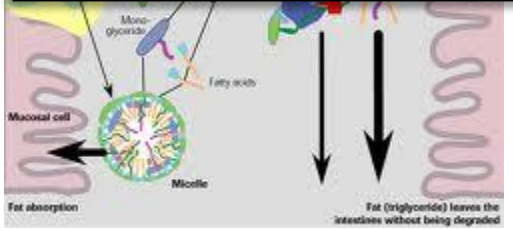
Problem Statement

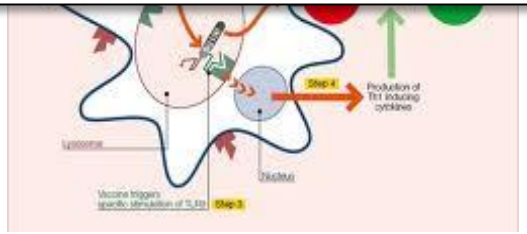
Too many chemicals to test with standard animal-based methods

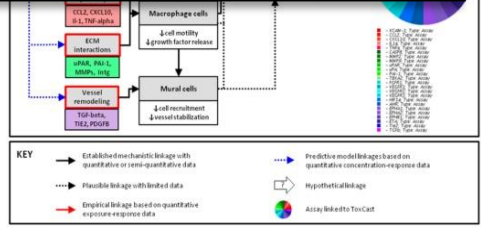
- Cost, time, animal welfare

Need for better mechanistic data

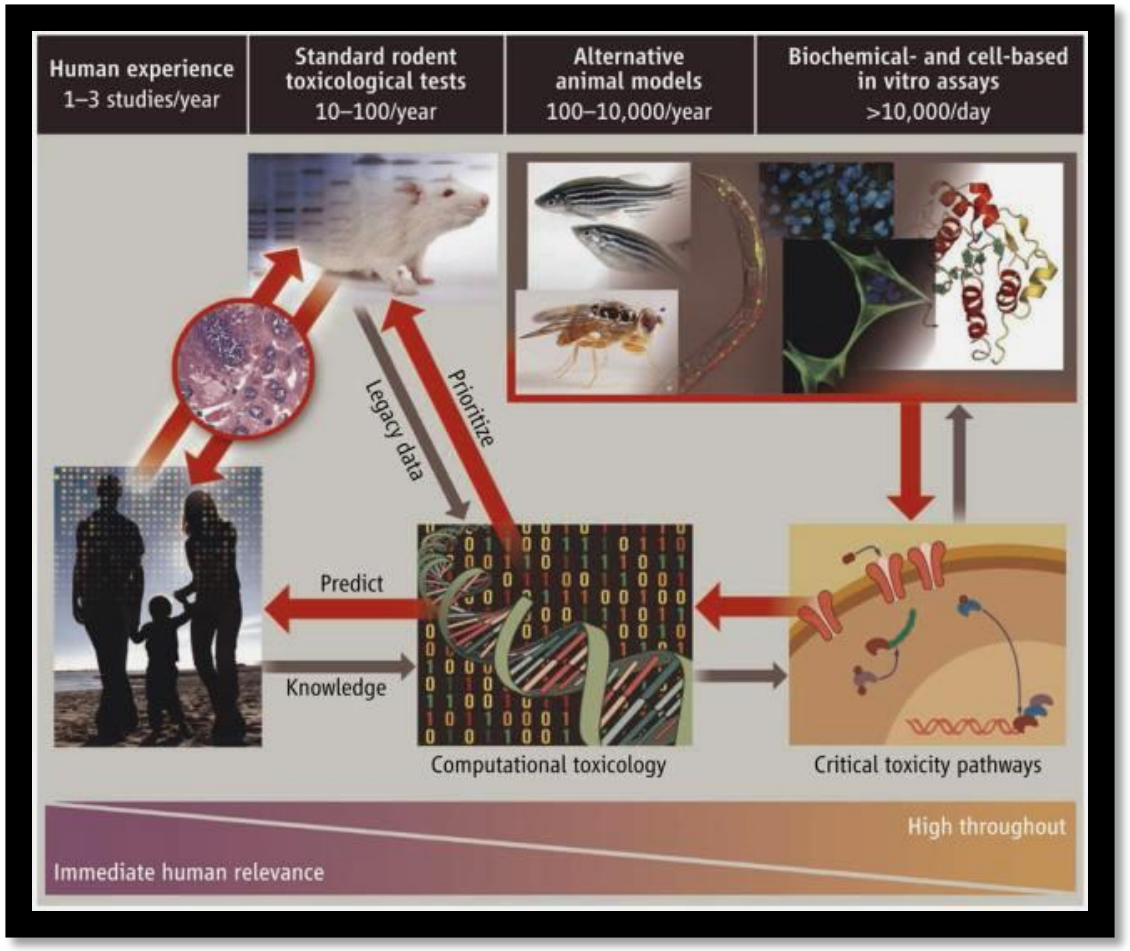
- Determine human relevance
- What is the Mode of Action (MOA) or Adverse Outcome Pathway (AOP)?







Tox21 Vision:
Transforming Toxicity Testing



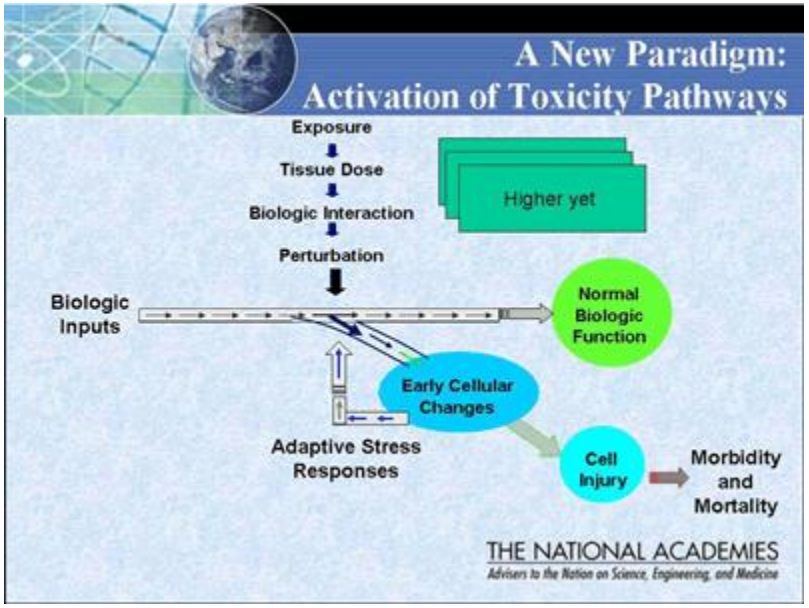
National Center for Advancing Translational Sciences (NCATS)
http://www.ncats.nih.gov/

SOURCE: Collins, Gray and Bucher (2008) Toxicology. Transforming environmental health protection. Science 319: 906

ToxCast / Tox21 Overall Strategy

- Identify targets or pathways linked to toxicity (AOP focus)
- Identify/develop high-throughput assays for these targets or pathways
- Develop predictive systems models
  - *in vitro/in silico→ in vivo*
  - human focus
- Use predictive models (qualitative):
  - Prioritize chemicals for targeted testing
  - Suggest / distinguish possible AOP / MOA for chemicals
- *High-throughput Exposure Predictions*
- *High-throughput Risk Assessments*

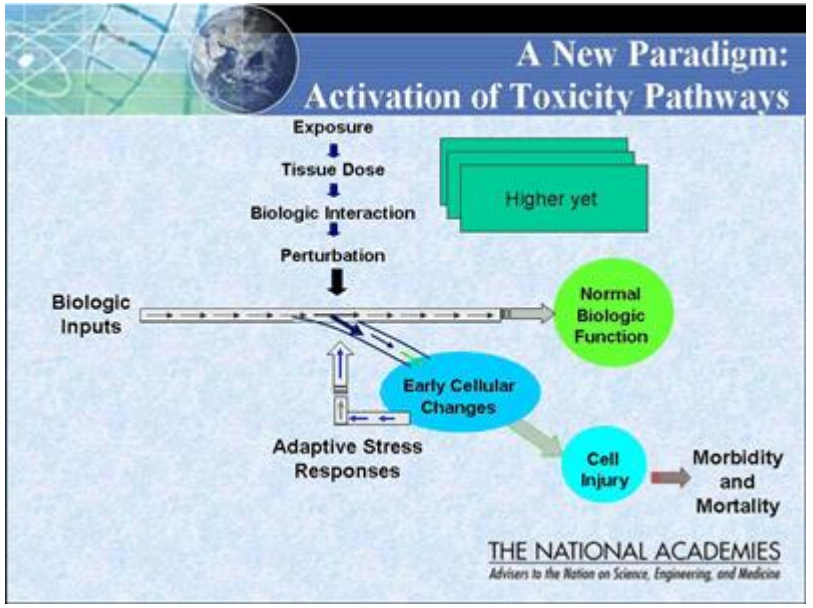
TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY, NRC, 2007.



Toxicity Testing in the 21st Century

"The committee envisions a future in which tests based on human cell systems can serve as better models of human biologic responses than apical studies in different species."

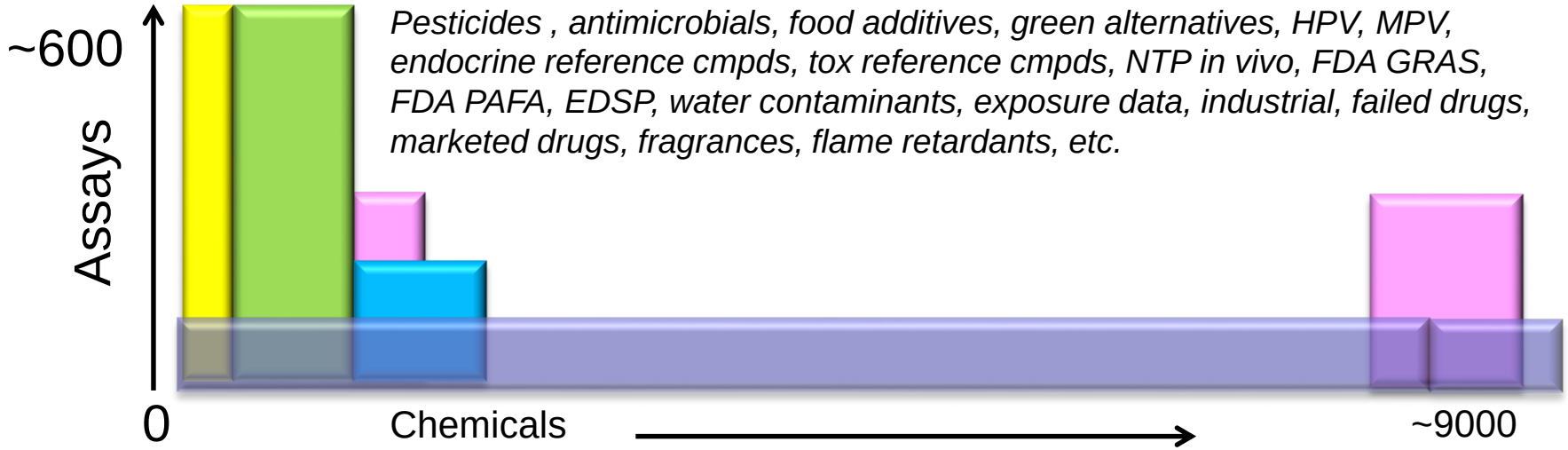
"The committee therefore believes that, given a sufficient research and development effort, human cell systems have the potential to largely supplant testing in animals."



TOXICITY TESTING IN THE 21ST CENTURY: A VISION AND A STRATEGY, NRC, 2007

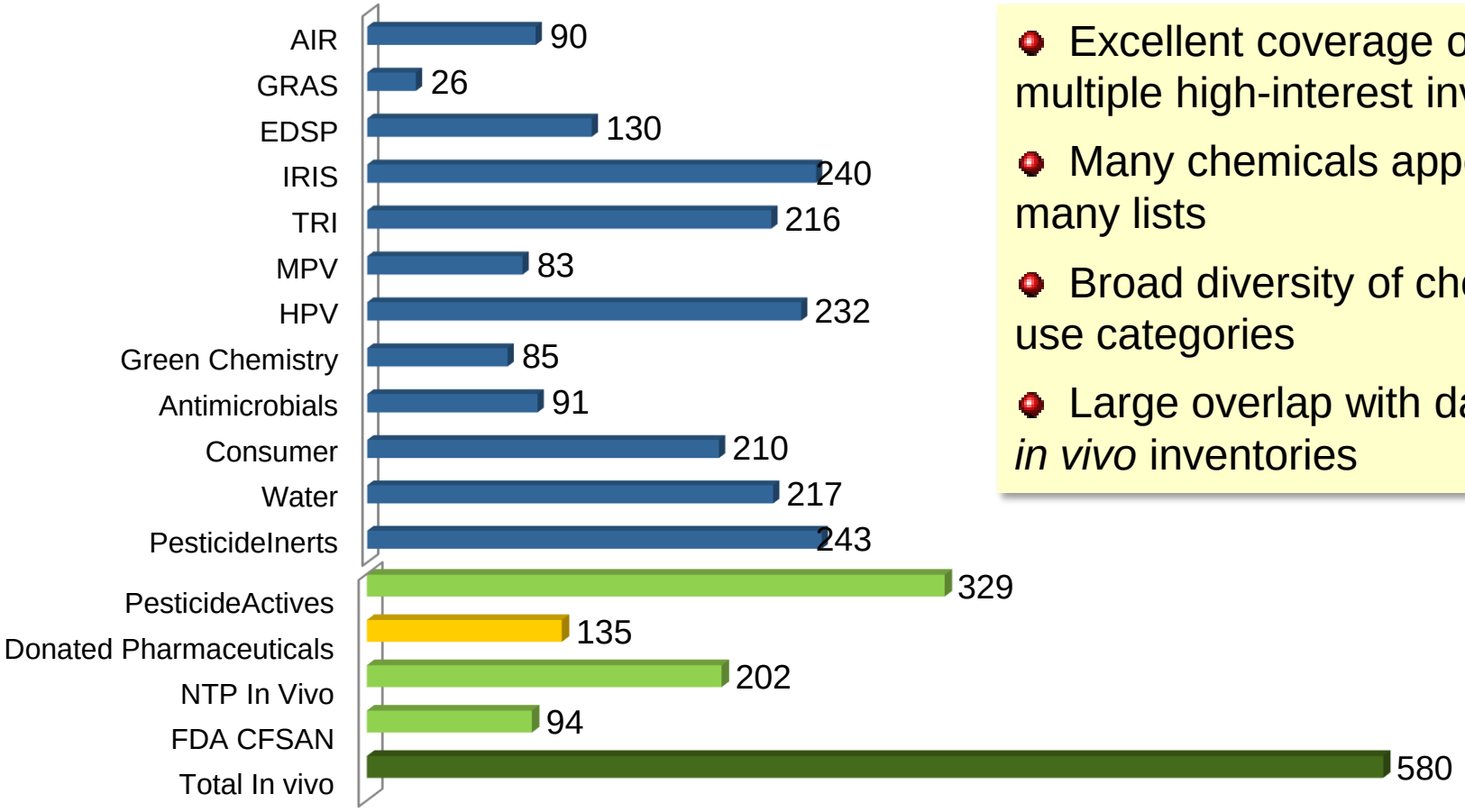
ToxCast & Tox21: Chemicals, Data and Release Timelines

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~700	2011	Now
ToxCast Phase II	767	~600	~700	03/2013	Now
ToxCast E1K	800	~50	~120	03/2013	Now
Tox21	~9000	~80	~150	Ongoing	Ongoing



Pesticides , antimicrobials, food additives, green alternatives, HPV, MPV, endocrine reference cmpds, tox reference cmpds, NTP in vivo, FDA GRAS, FDA PAFA, EDSP, water contaminants, exposure data, industrial, failed drugs, marketed drugs, fragrances, flame retardants, etc.

ToxCast PhI & PhII 1060: # Compounds per Inventory

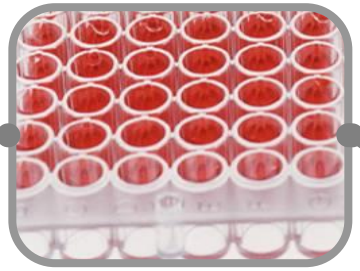


- Excellent coverage of multiple high-interest inventories
- Many chemicals appear on many lists
- Broad diversity of chemical-use categories
- Large overlap with data-rich *in vivo* inventories

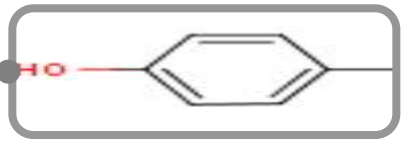
Hazard Predictions for Prioritization: High-Throughput Screening (HTS)

United States Environmental Protection Agency

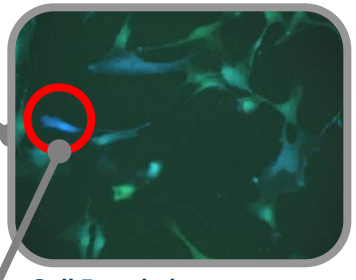
Robots



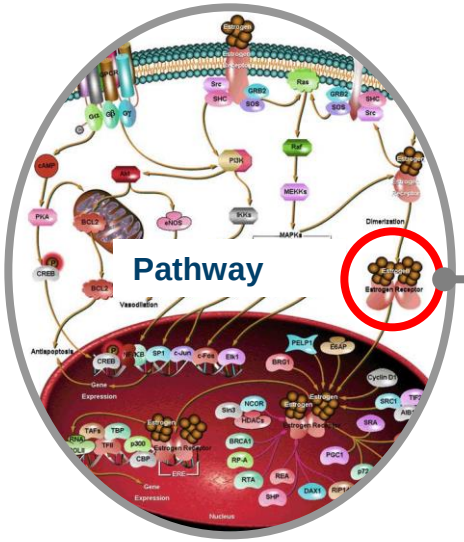
96-, 384-, 1536 Well Plates

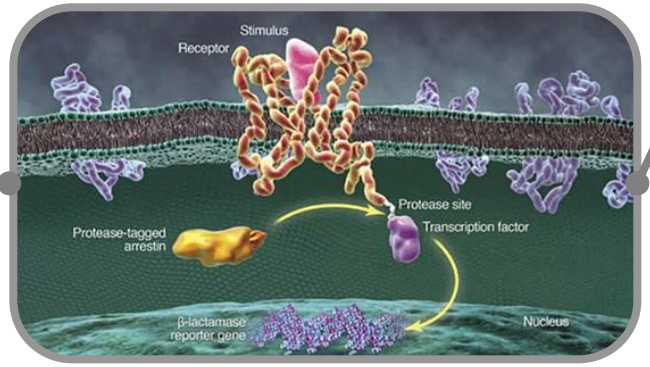


Chemical Exposure

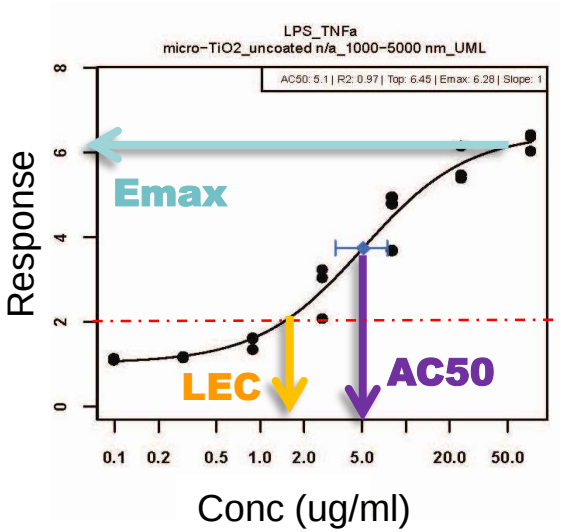


Cell Population

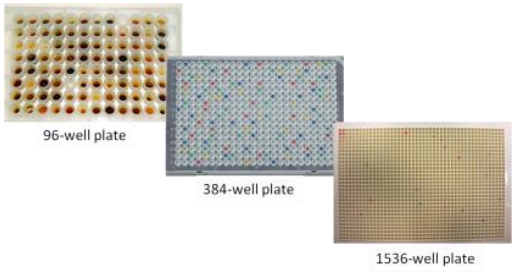




Target Biology (e.g., Estrogen Receptor)



ToxCast Assays (>700 endpoints)



Assay Provider
ACEA
Apredica
Attagene
BioReliance
BioSeek
CeeTox
CellzDirect
Tox21/NCATS
NHEERL MESC
NHEERL Zebrafish
NovaScreen (Perkin Elmer)
Odyssey Thera
Vala Sciences

Biological Response
cell proliferation and death
cell differentiation
Enzymatic activity
mitochondrial depolarization
protein stabilization
oxidative phosphorylation
reporter gene activation
gene expression (qNPA)
receptor binding
receptor activity
steroidogenesis

Target Family
response Element
transporter
cytokines
kinases
nuclear receptor
CYP450 / ADME
cholinesterase
phosphatases
proteases
XME metabolism
GPCRs
ion channels

Assay Design
viability reporter
morphology reporter
conformation reporter
enzyme reporter
membrane potential reporter
binding reporter
inducible reporter

Readout Type
single
multiplexed
multiparametric

Cell Format
cell free
cell lines
primary cells
complex cultures
free embryos

Species
human
rat
mouse
zebrafish
sheep
boar
rabbit
cattle
guinea pig

Tissue Source

Lung	Breast
Liver	Vascular
Skin	Kidney
Cervix	Testis
Uterus	Brain
Intestinal	Spleen
Bladder	Ovary
Pancreas	Prostate
Inflammatory	Bone

Detection Technology
qNPA and ELISA
Fluorescence & Luminescence
Alamar Blue Reduction
Arrayscan / Microscopy
Reporter gene activation
Spectrophotometry
Radioactivity
HPLC and HPEC
TR-FRET

List of assays and related information at: **http://www.epa.gov/ncct/**

ToxCast Results: 1051 Chemicals x 791 Assay Readouts

ACEA: red
Attagene: orange
Apredica: black
BioSeek: green
Novascreen: gray
Tox21: violet
OT: blue

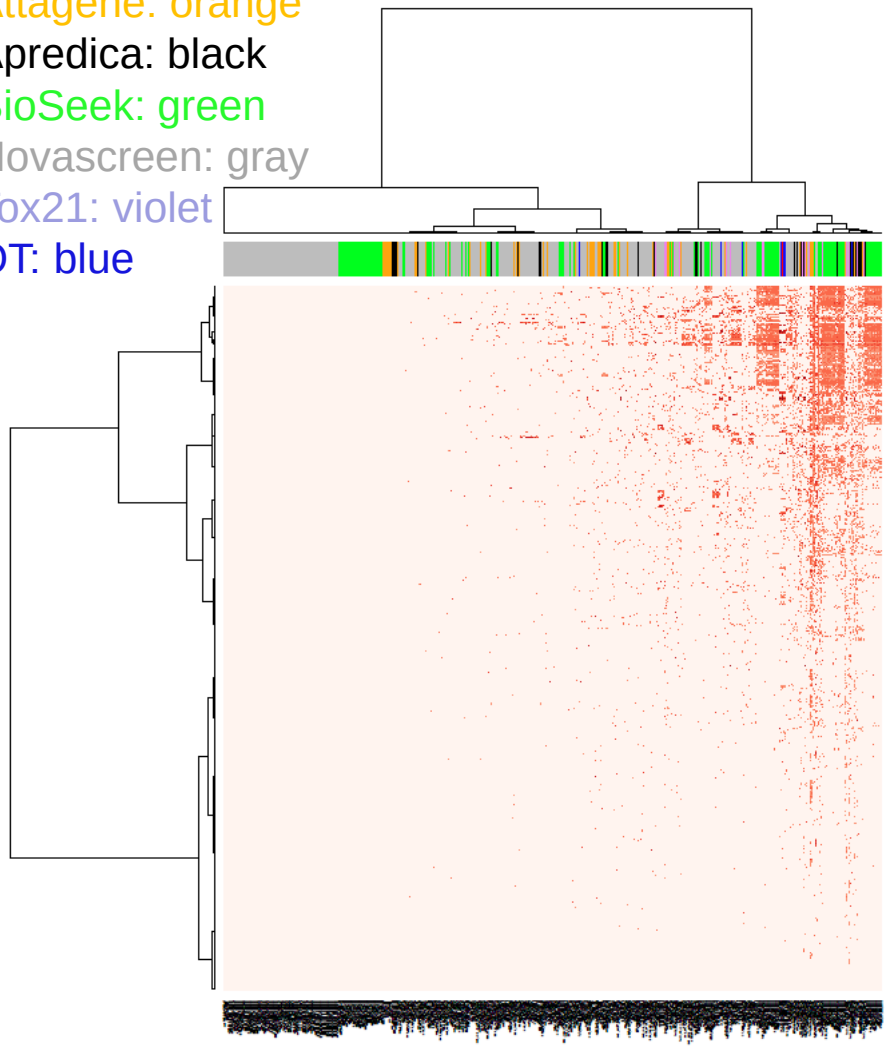


Table 2 Top 20 most promiscuous chemicals[a]

Chemical Name	Total	AC50s <=10µM	AC50s <=1µM
Phenylmercuric acetate	90	47	20
Mancozeb	88	41	13
Gentian violet	86	51	5
Sodium dodecylbenzenesulfonate	82	19	0
Tributyltin methacrylate	79	48	12
Tributyltin chloride	77	45	9
Mercuric chloride	73	45	14
Perfluorooctane sulfonic acid	72	13	2
{4-[3-(aminomethyl)phenyl]piperidin-1-yl}{5-[(2-fluorophenyl)ethynyl]furan-2-yl}methanone (pharma)	71	25	4
Dodecylbenzene sulfonate triethanolamine (1:1)	66	7	1
SSR241586 (pharma)	66	30	8
Emamectin benzoate	65	14	2
{4-[5-(aminomethyl)-2-fluorophenyl]piperidin-1-yl}(4-bromo-3-methyl-5-propoxythiophen-2-yl)methanone hydrochloride (pharma)	64	19	2
(1R)-1-[(ethoxycarbonyl)oxy]ethyl 1-{[5-(5-chlorothiophen-2-yl)-1,2-oxazol-3-yl]methyl}-2-{[1-(propan-2-yl)piperidin-4-yl]carbamoyl}-1H-indole-5-carboxylate hydrochloride(pharma)	63	29	2
Maneb	62	31	16
SSR150106 (pharma)	62	41	13
Didecyl dimethyl ammonium chloride	62	30	2
Zamifenacin (pharma)	60	27	11
SSR125047 (pharma)	59	16	3
Metiram	56	16	4

Sipes *et al.*, Chem Res Toxicol. 26:878-95, 2013

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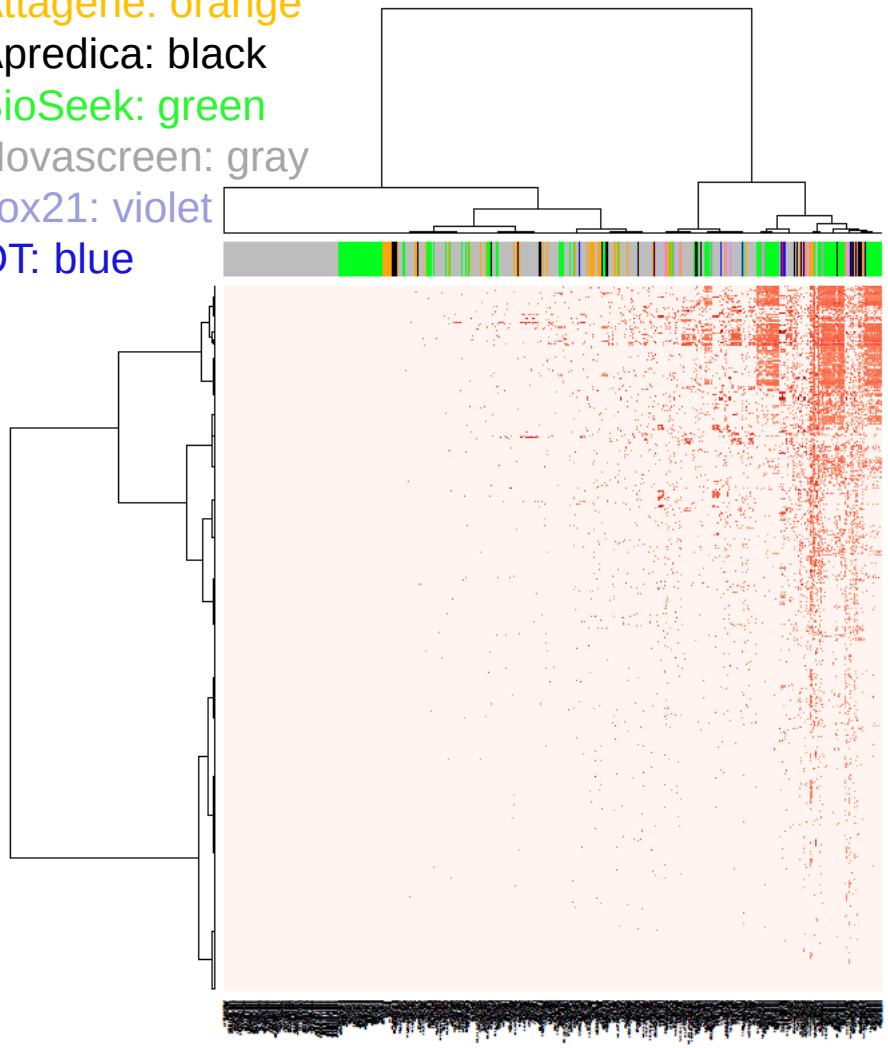
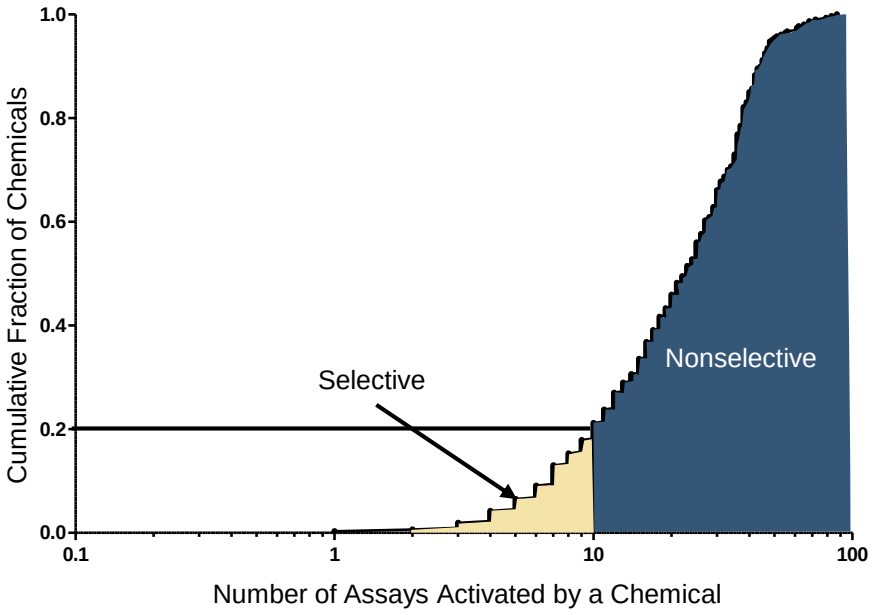


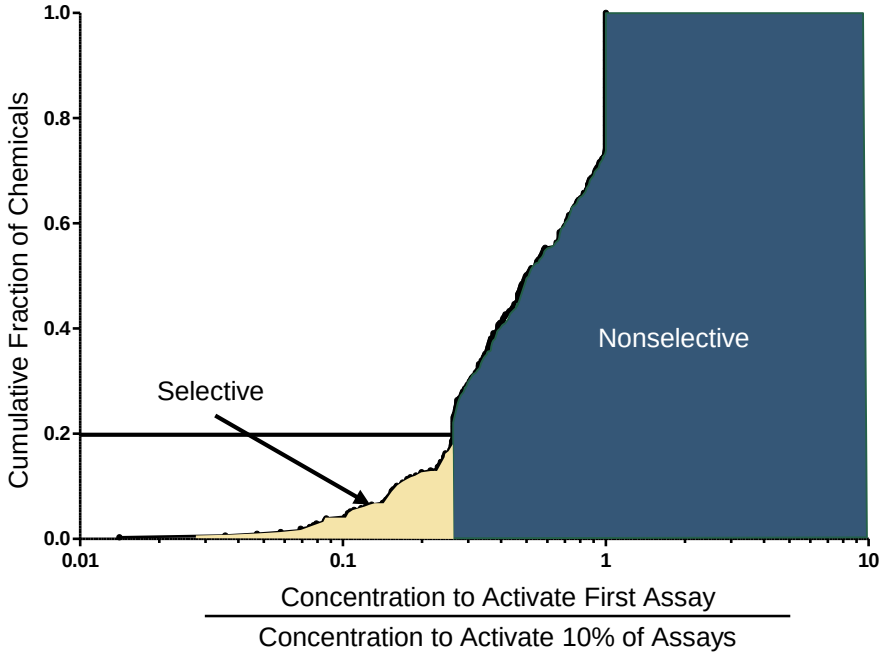
Table 3 Top 20 most promiscuous assays[a]

Assay target	Assay category	Total	AC50s <=10μM	AC50s <=1μM
hCYP2C19	CYP	264	144	53
hCYP2C9	CYP	152	81	19
rPBR	Transporter	147	62	18
hPXR	Nuclear receptor (subfamily 1)	140	73	35
hNET	Transporter	136	48	13
hPBR	Transporter	117	36	5
hDAT	Transporter	117	45	7
hCYP1A2	CYP	108	60	16
gDAT	Transporter	98	26	4
h5HT7	GPCR (aminergic)	96	35	13
hGR	Nuclear receptor (subfamily 3)	96	35	6
hOpiate_mu	GPCR (other)	92	27	5
hDRD1	GPCR (aminergic)	89	36	9
rNaCh_site2	Ion channel	87	37	13
hCYP2B6	CYP	81	43	16
gSIGMA_NonSelective	Other	80	31	13
gOpiateK	GPCR (other)	75	18	4
rMAOAC	Other enzyme	73	15	6
hAR	Nuclear receptor (subfamily 3)	73	33	8
hBACE	Protease	73	28	3

Sipes *et al.*, Chem Res Toxicol. 26:878-95, 2013

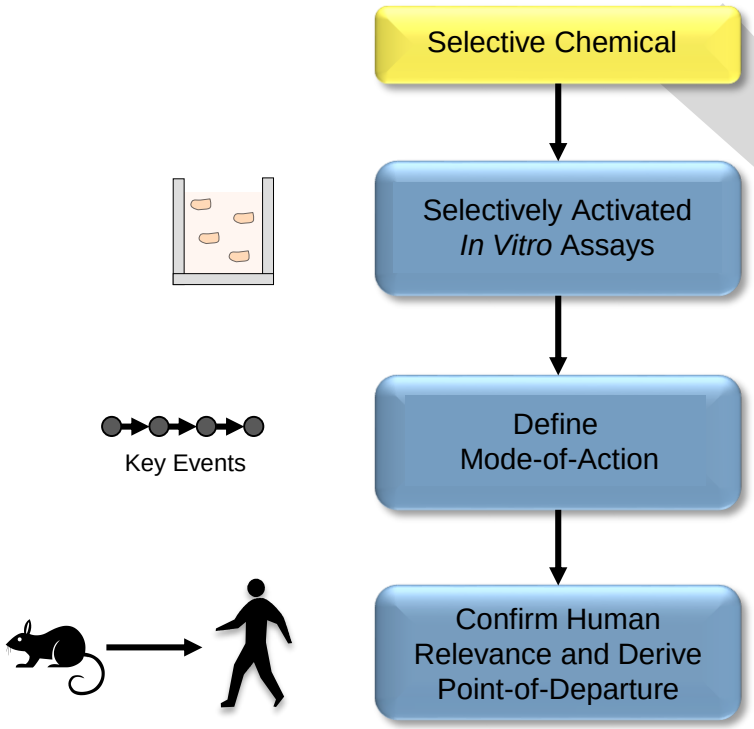
What Did High-Throughput Screening Tell Us?

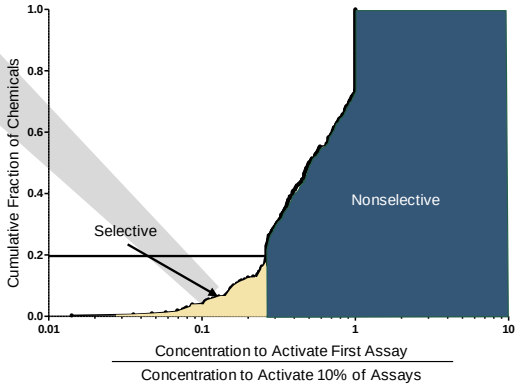




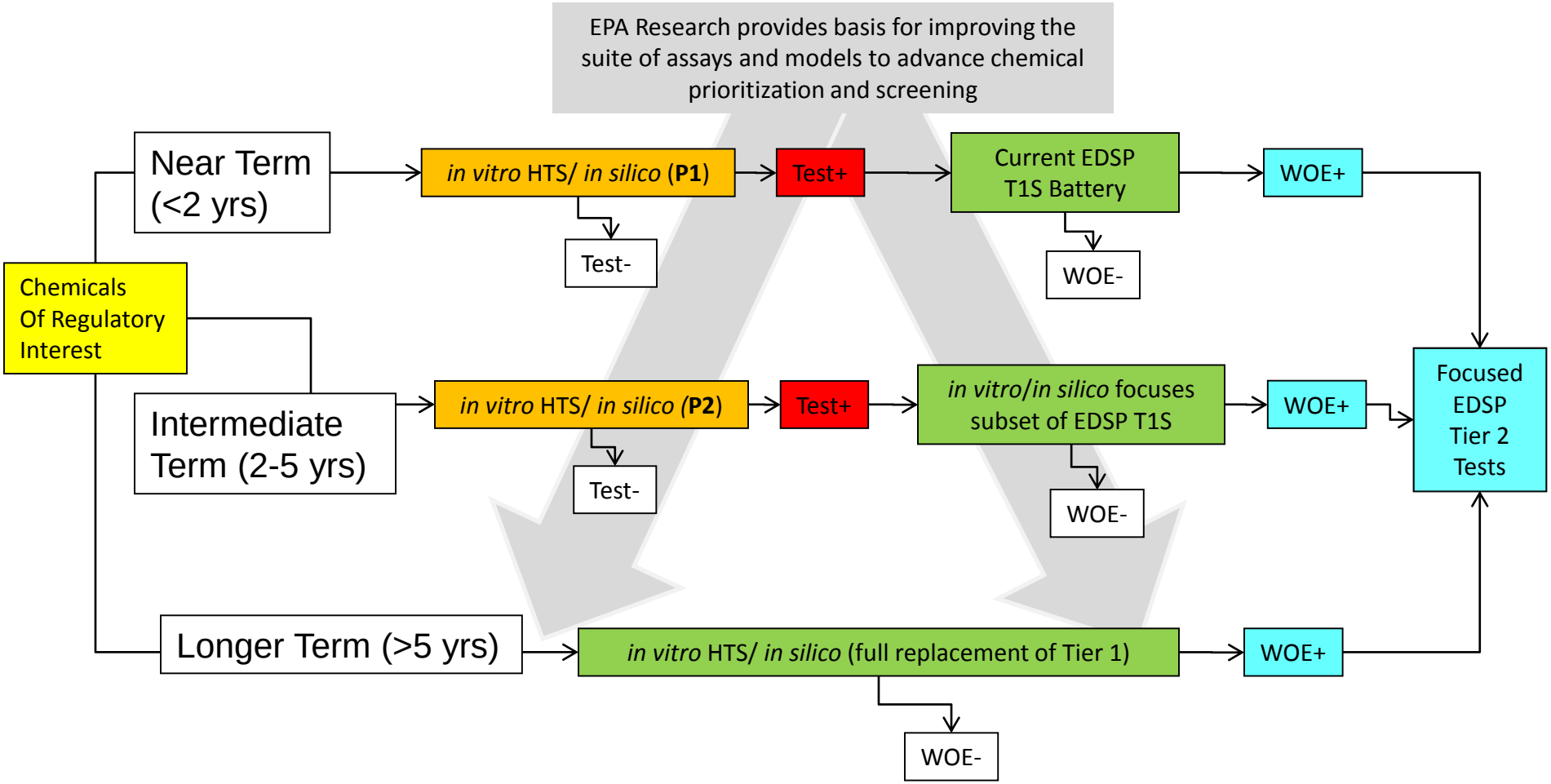
Thomas *et al., Tox Sci.,* 2013

In Vitro Assay Selectivity as a Starting Point for Chemical Mechanisms Of Action/Adverse Outcome Pathways





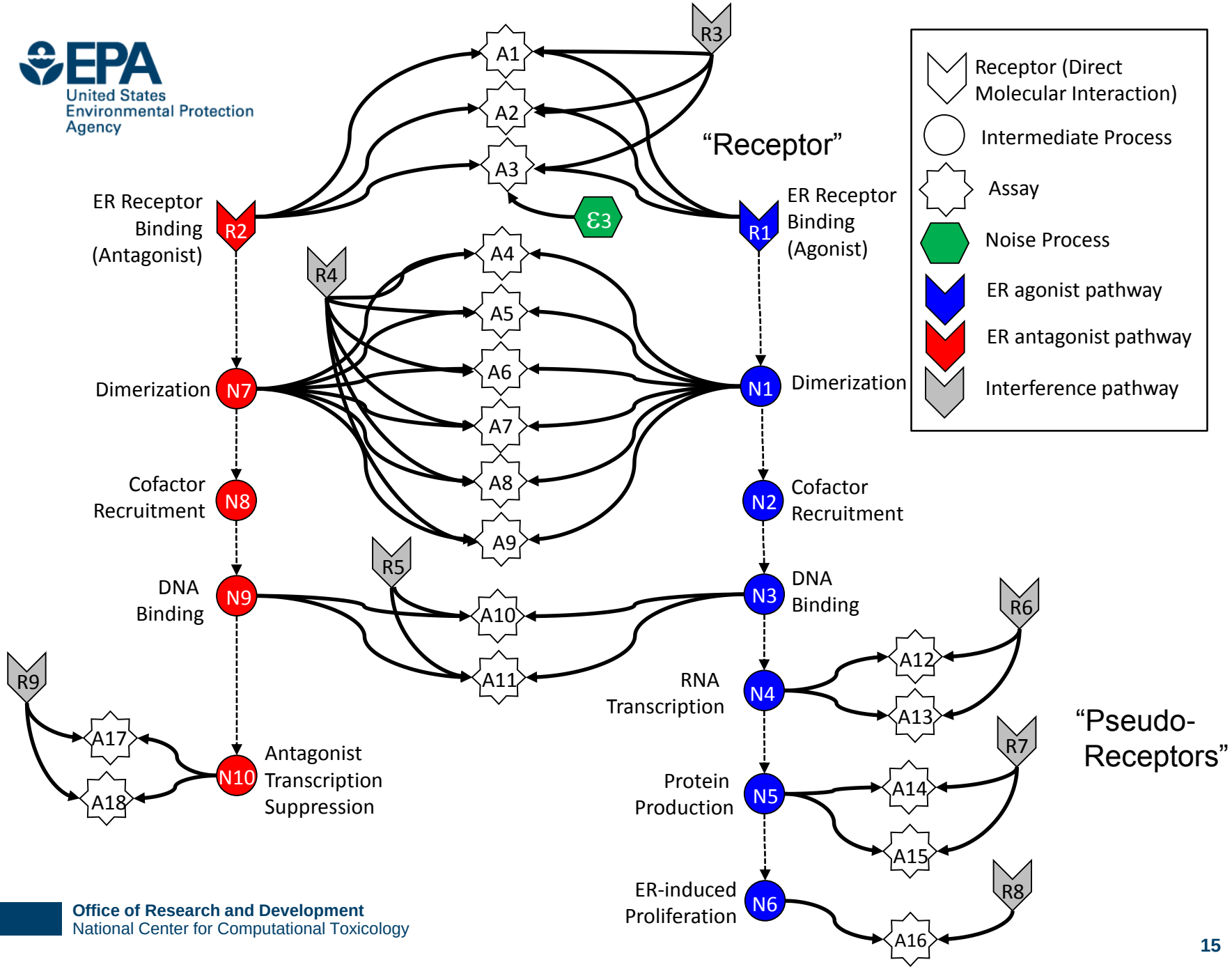
ToxCast and the Endocrine Disruptor Screening Program



Chemical Prioritization
Includes registration review timeline, physico-chemical properties, exposure estimates, *in vitro* assays and computer models (QSAR, expert systems, systems biology models).

Screening Decisions
Near Term = Incorporates HTS/*in silico* prioritization methods for post EDSP List 2
Intermediate = Run subset of T1S assays indicated by HTS and *in silico* predictions
Long Term = Full replacement of EDSP T1S Battery

http://www.epa.gov/endo/pubs/edsp21_work_plan_summary%20_overview_final.pdf


EPA
United States
Environmental Protection
Agency
"Receptor"
ER Receptor
Binding
(Antagonist)
ER Receptor
Binding
(Agonist)
Dimerization
Dimerization
Cofactor
Recruitment
Cofactor
Recruitment
DNA
Binding
DNA
Binding
RNA
Transcription
Protein
Production
ER-induced
Proliferation
Antagonist
Transcription
Suppression
"Pseudo-
Receptors"
Receptor (Direct
Molecular Interaction)
Intermediate Process
Assay
Noise Process
ER agonist pathway
ER antagonist pathway
Interference pathway
R1
R2
R3
R4
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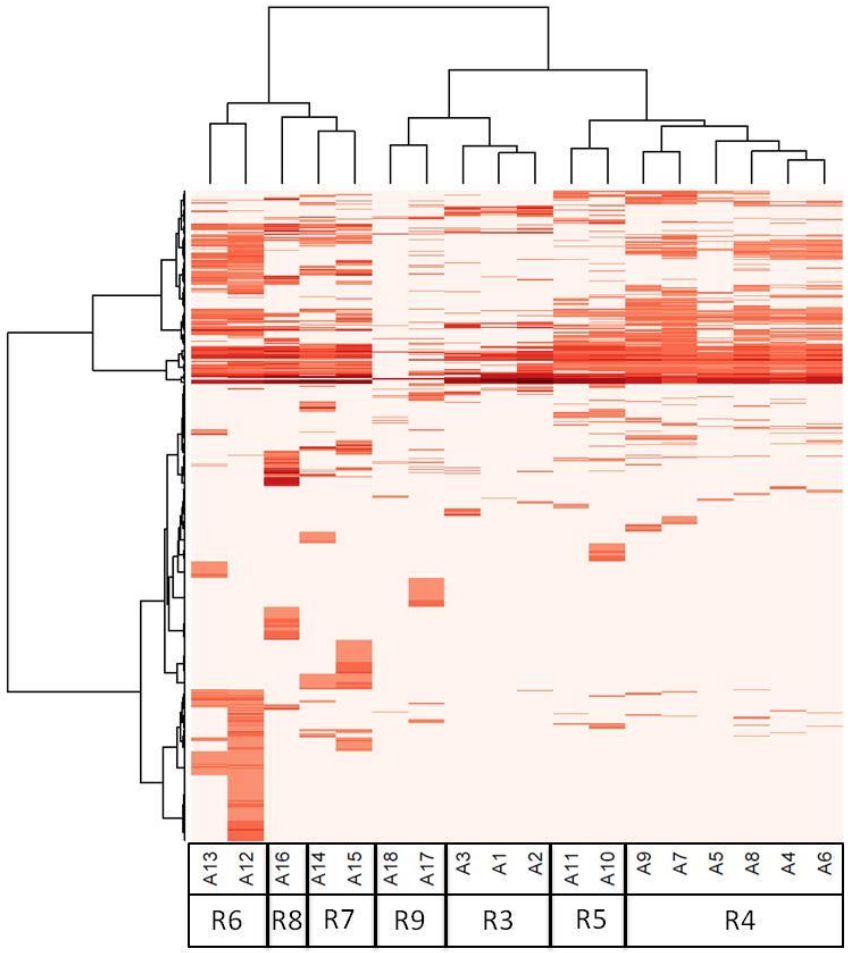
Major theme – all assays have false positives and negative

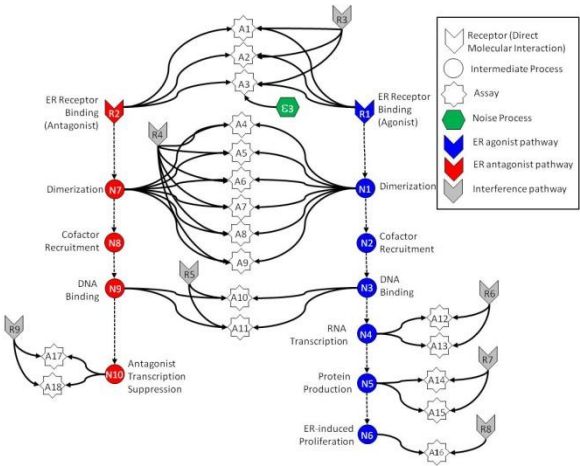
Assays cluster by technology, suggesting technology-specific non-ER activity



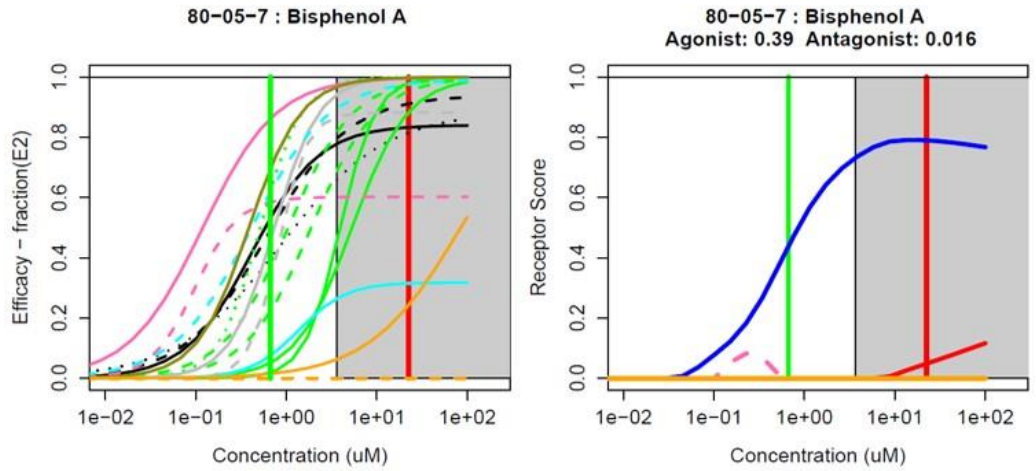
Much of this “noise” is reproducible, i.e. it is “assay interference”

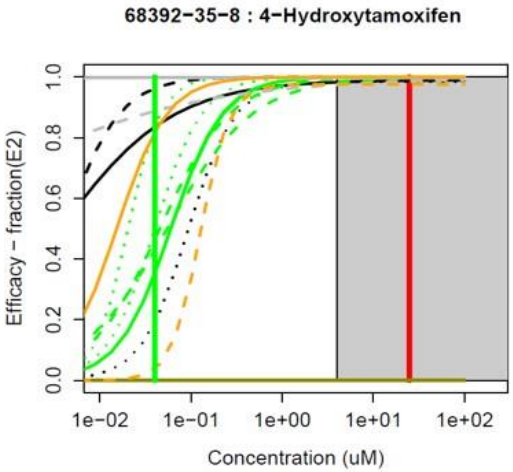
Result of interaction of chemical with complex biology in the assay

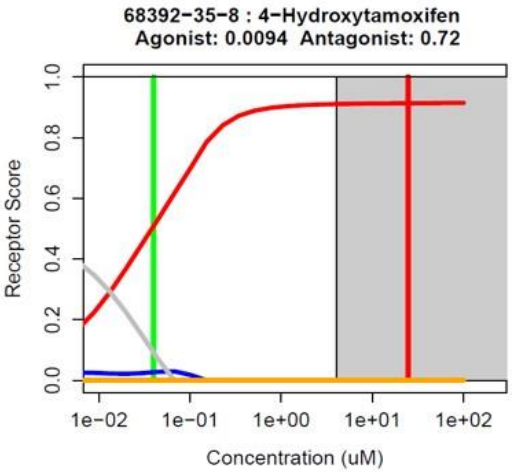
Our chemical library is only partially “drug-like”
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics

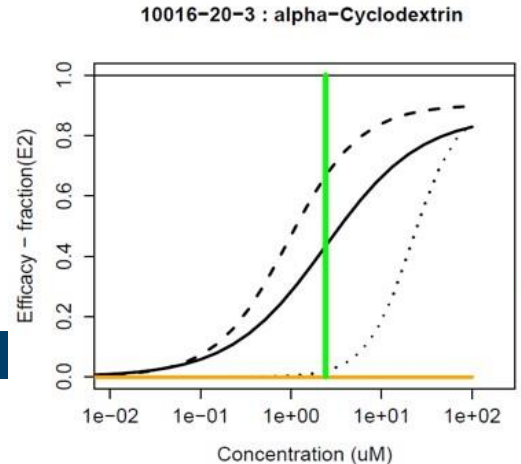


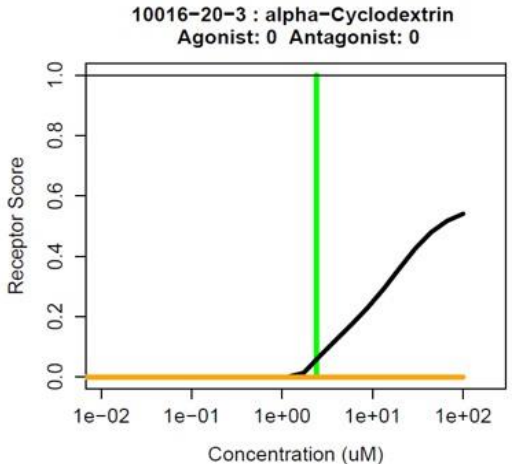
Example Agonist, Antagonist, Interference Chemicals

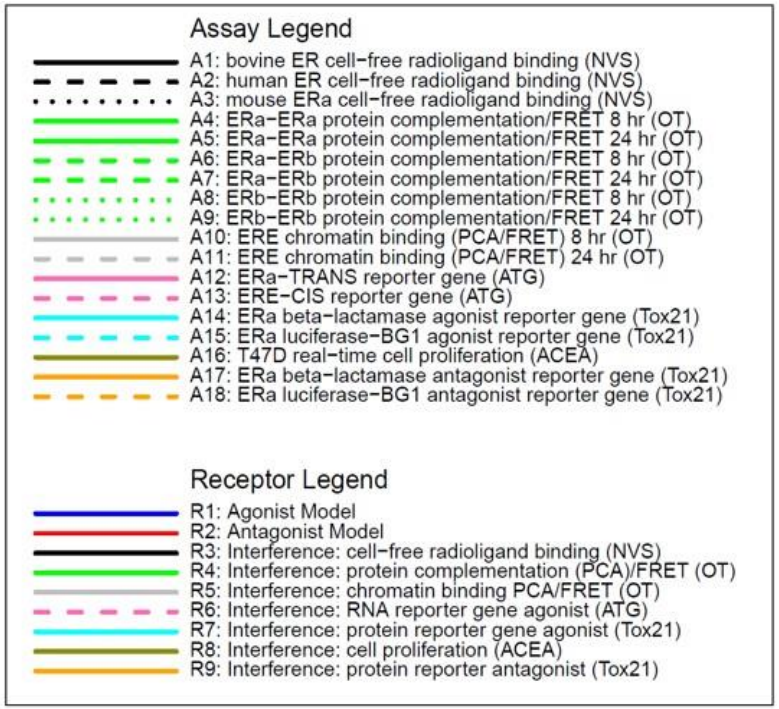


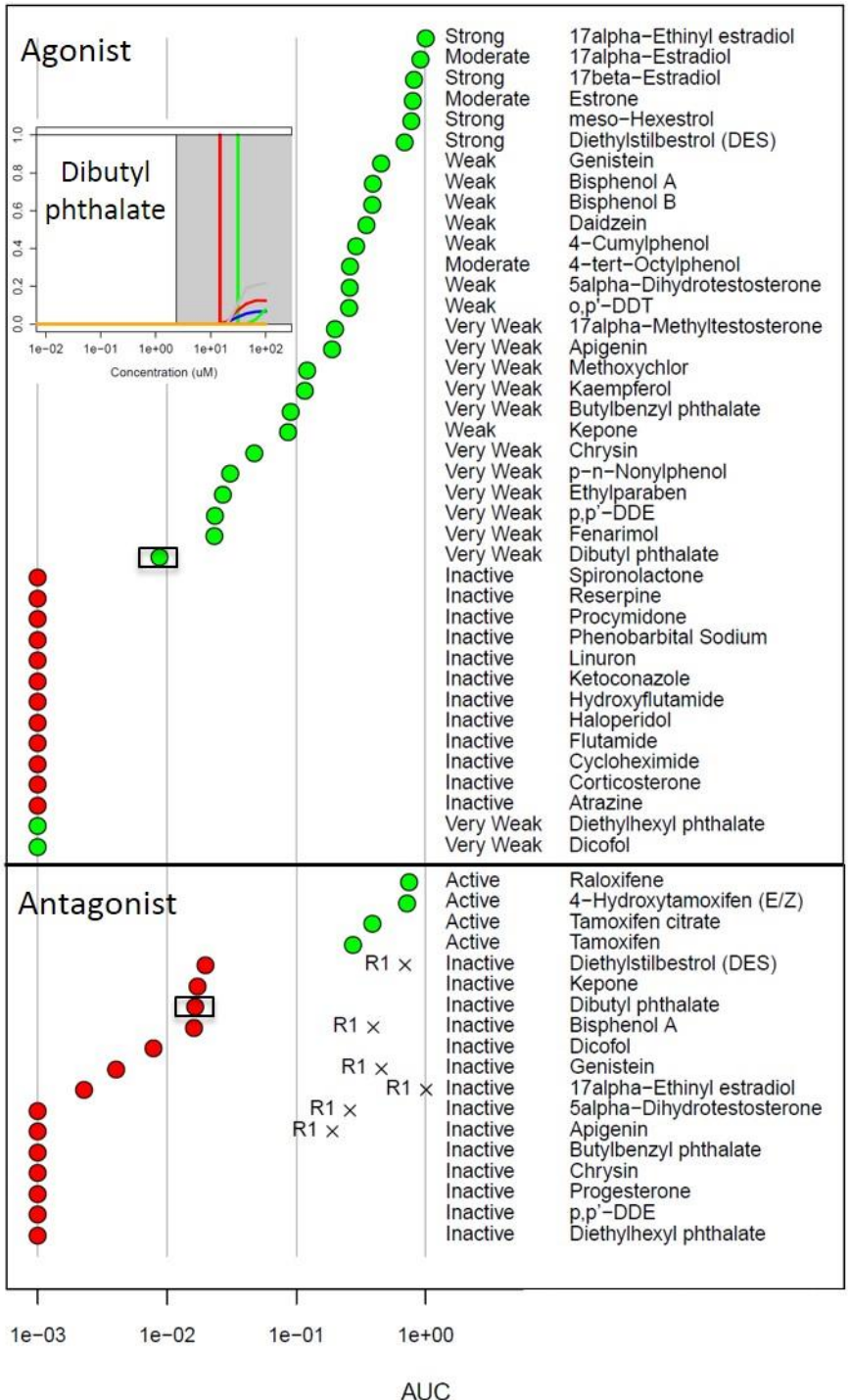




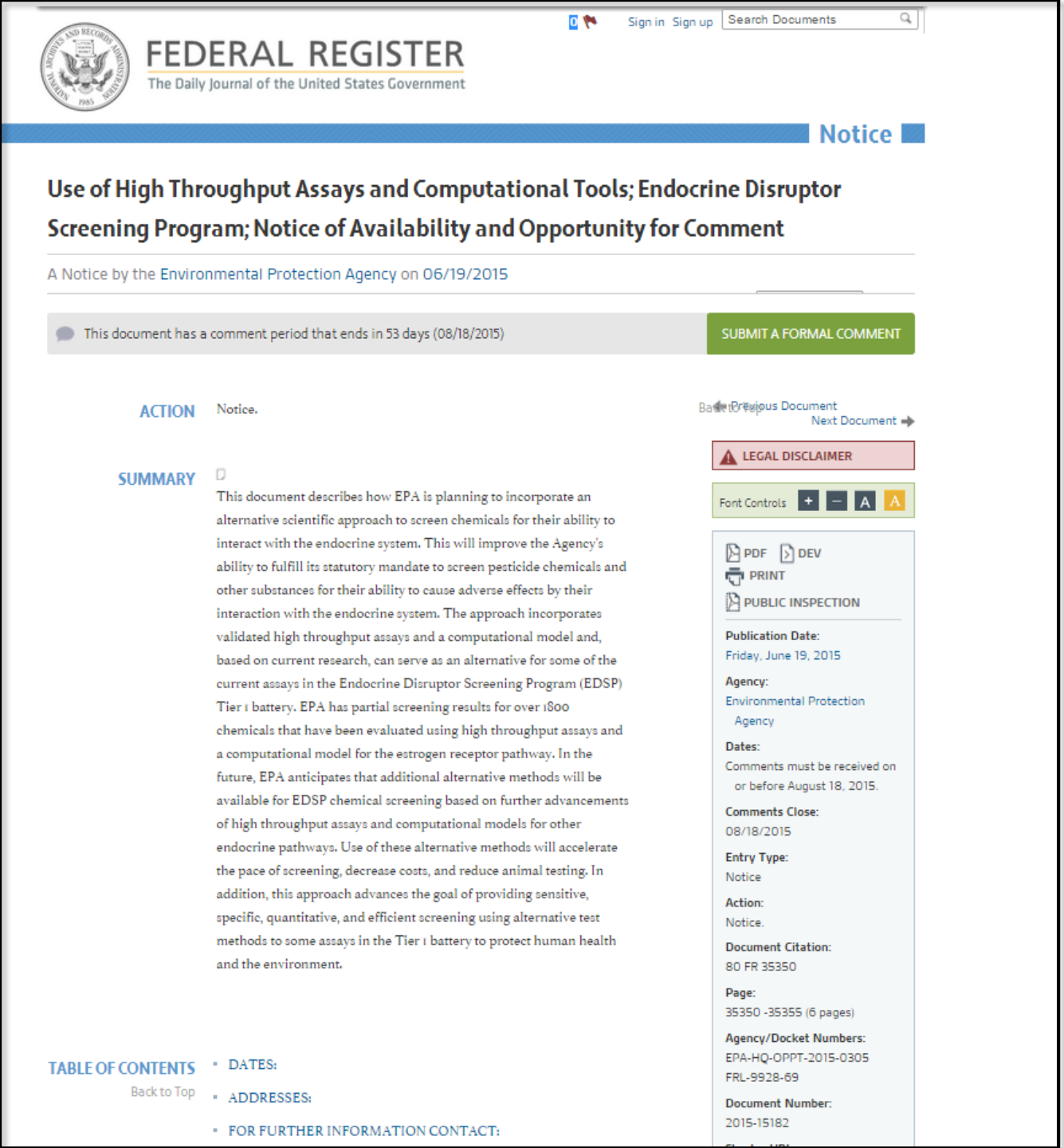








Reference Chemical Classification



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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

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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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Friday, June 19, 2015

Agency:
Environmental Protection Agency

Dates:
Comments must be received on or before August 18, 2015.

Comments Close:
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"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."

Tox21 AhR Agonist qHTS Assay

- Ligand-dependent transcription factor activated by structurally diverse natural and synthetic ligands
- Critical roles in biological processes (development, inflammation)
- Mediates adaptive and toxic response to chemicals
  - HAHs - halogenated aromatic hydrocarbons
  - PAHs – polycyclic aromatic hydrocarbons
- Third-generation CALUX AhR-responsive reporter gene bioassay
  - Human HepG2 cells (HG2L7.5c1)
- Tox21 8.5K Chemical library
  - Environmental, pesticide, industrial, food use, drugs
  - 1536 well-plate format with Tox21 robot
  - 15 concentrations screened in triplicate

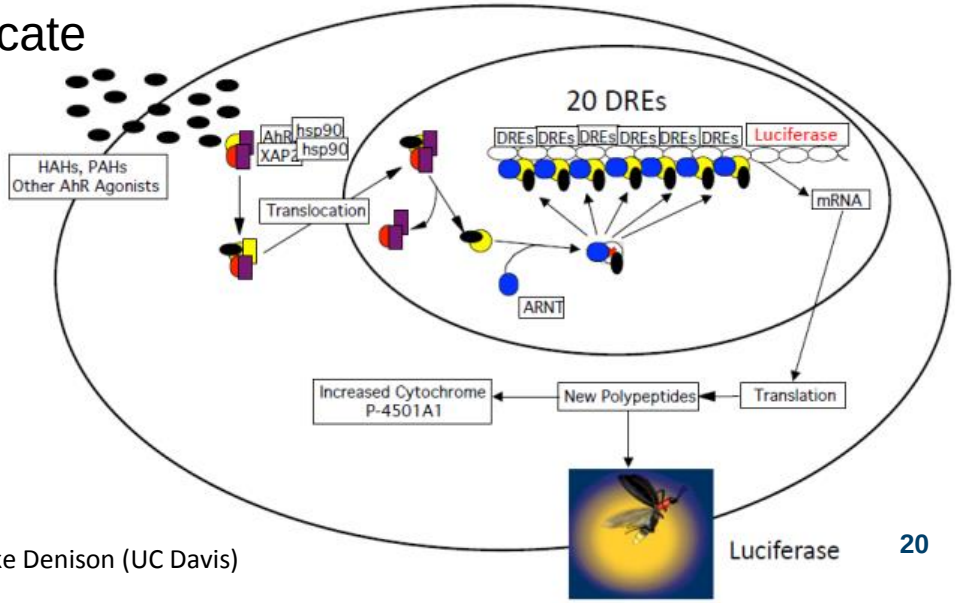


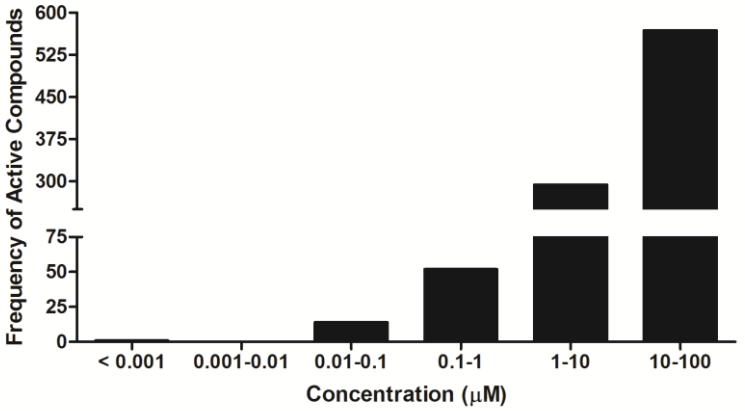
Figure courtesy of Mike Denison (UC Davis)

Tox21 AhR Assay Results

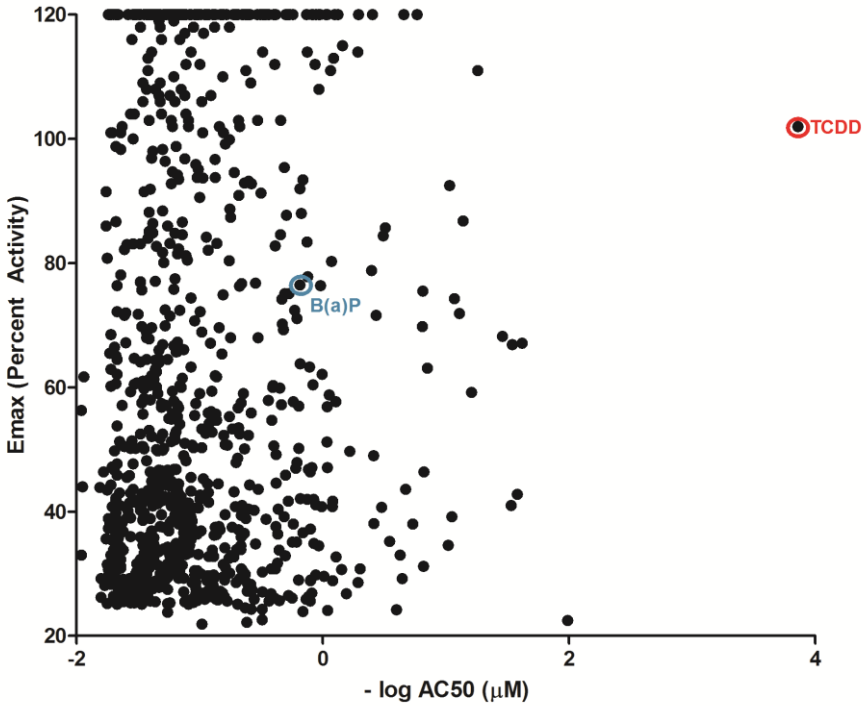
(A)

qHTS Results Summary	
Number of HITS	768
Percentage of HITS	9.2
Concordance (Percentage)	94.3

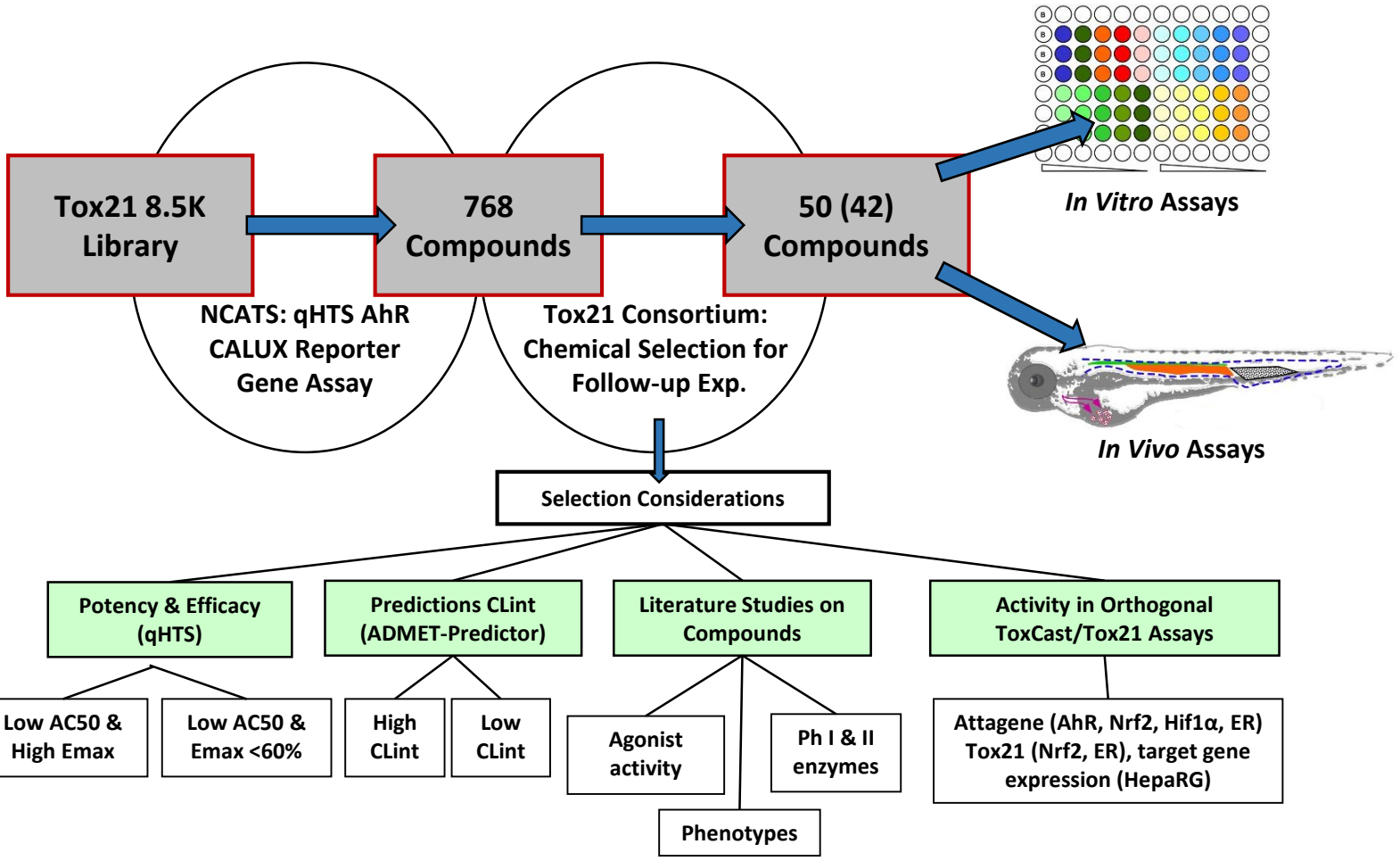
(B)



(C)



Determining Toxicity (dioxin-like effects): Follow-up Assay Strategy



Dioxin-like vs Non-dioxin-like Effects

HepaRG gene expression assay

Chemical ID	CYP1A1	CYP1A2	UGT1A1	IGFBP-1
DMSO	-	-	-	-
20069	+	+	+	+
21135	+	+	+	+
20529	+	+	+	-

Zebrafish larvae development assay

Advantages of Tiered Screening Approach

C.I. Solvent Yellow

IARC Category 3 carcinogen

Trace levels found in food products

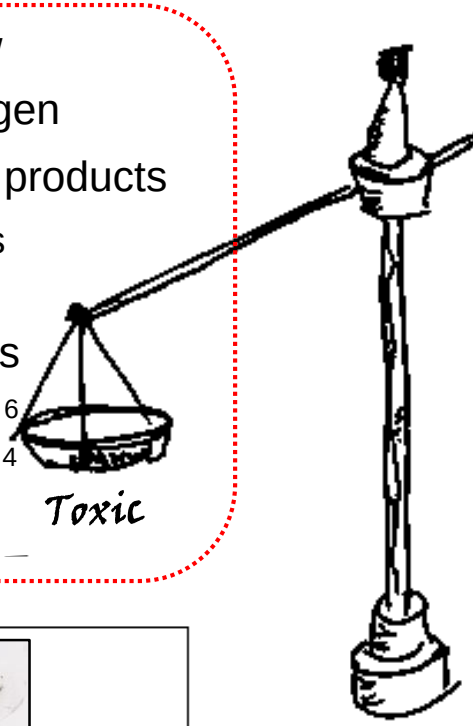
Tumeric, curry, chili powders

- 4.8 to 12.1 mg/g

Impurities in color additives

- 0.008 µg/mL - FD&C Yellow no. 6
- 0.011 ug/mL – D&C Orange no. 4

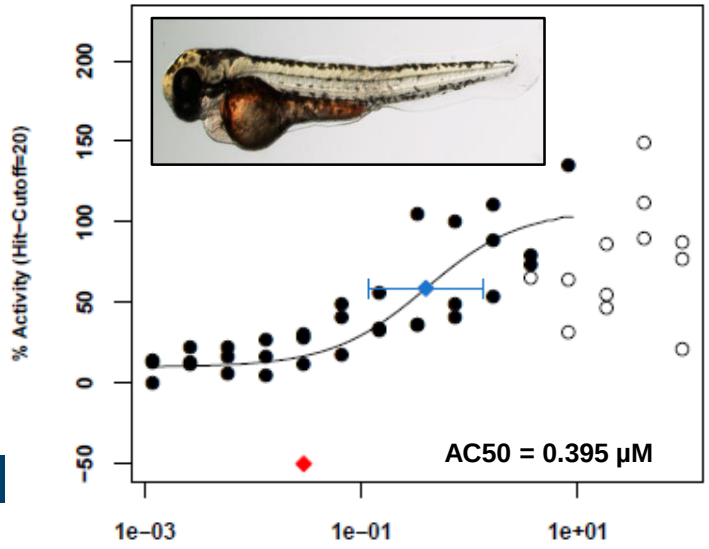
(Fonovich 2013)

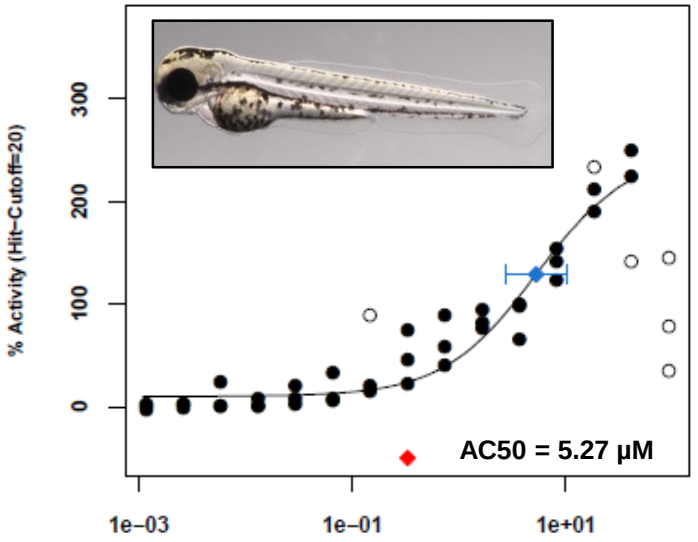


1,4-Dihydroxy-2-naphthoic acid

Probiotic bacterial metabolite isolated from swiss cheese

Inhibits colitis (Fukumoto et al. 2014)





United States Environmental Protection Agency

Exposure

Reverse Toxicokinetics

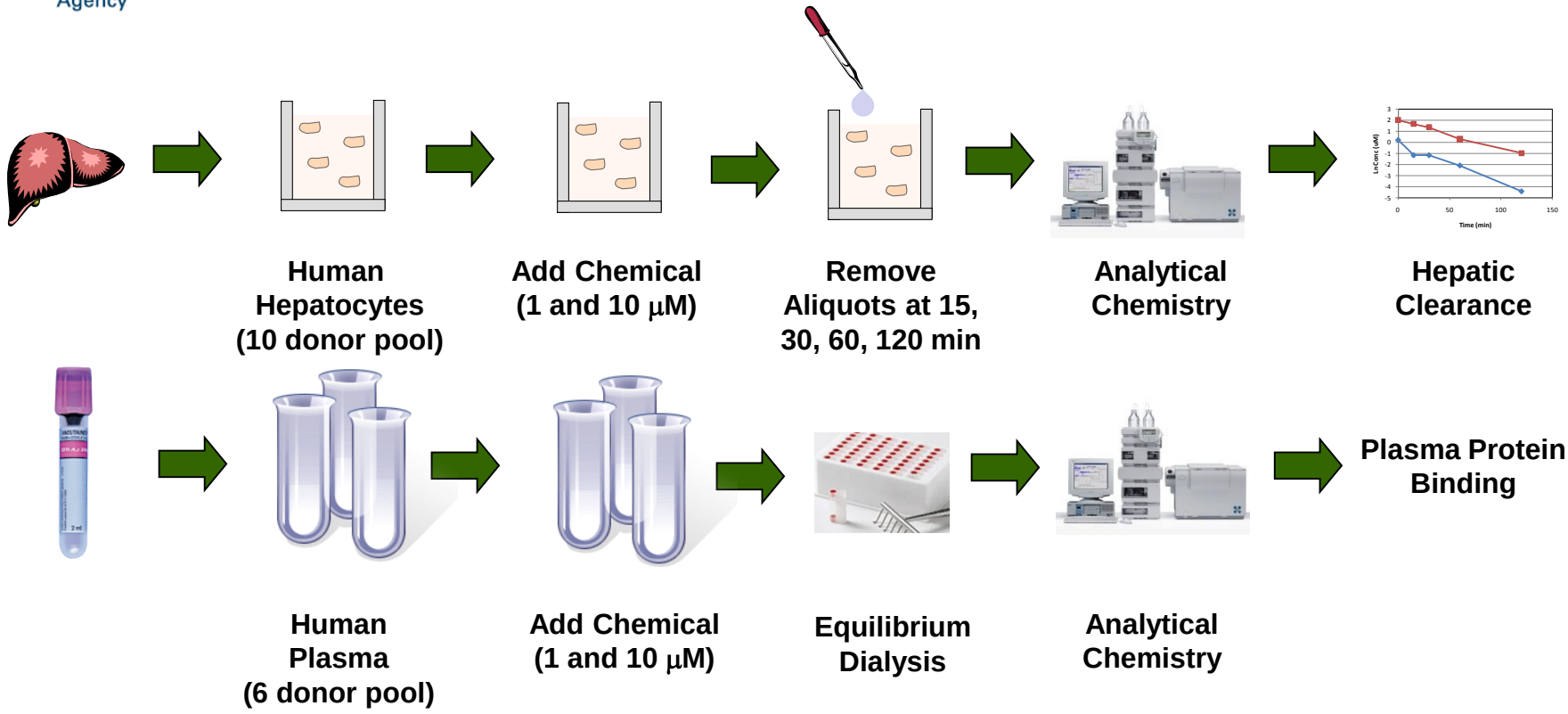
Reverse Toxicokinetics (*In Vitro* Dosimetry)

- **Problem**: How to estimate daily exposure dose from *in vitro* media concentration

- Use Reverse Toxicokinetics (RTK)
  - very simple 2 parameter PK models
  - in vitro measurements of disappearance of parent compound and serum binding values

- Provides scaling from concentration in which there is *in vitro* biological activity to in vivo activity dose (mg/kg/day)

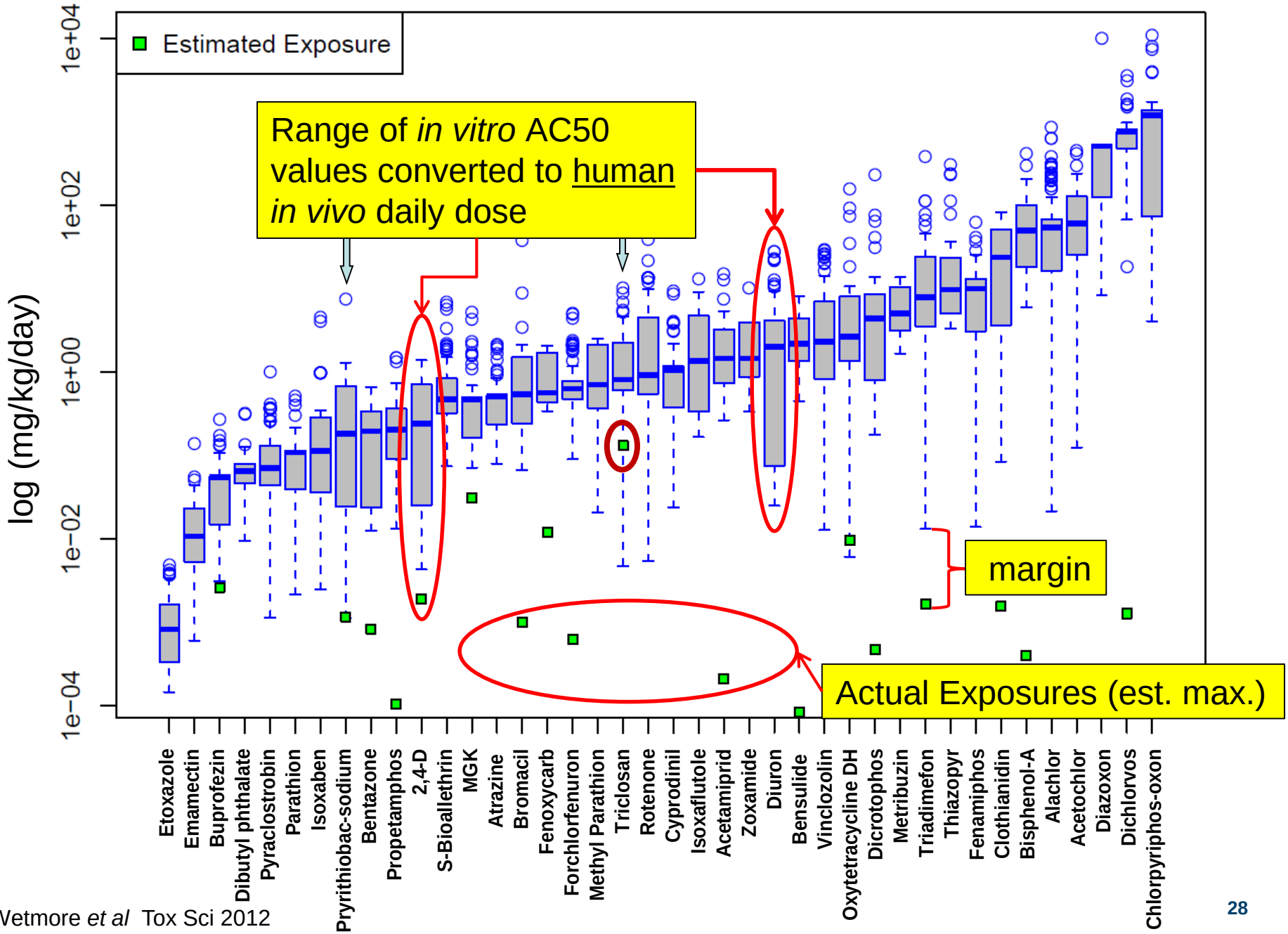
Adding Pharmacokinetics Reverse ToxicoKinetics (rTK)



- Combine experimental data w/ PK Model to estimate dose / concentration scaling
- RatCast: Same experiment, but with rat hepatocytes and plasma

(Rotroff *et al*, ToxSci 2010, Wetmore *et al*, ToxSci 2012)

Combining *in vitro* activity and dosimetry



Wetmore *et al* Tox Sci 2012

United States Environmental Protection Agency

Exposure

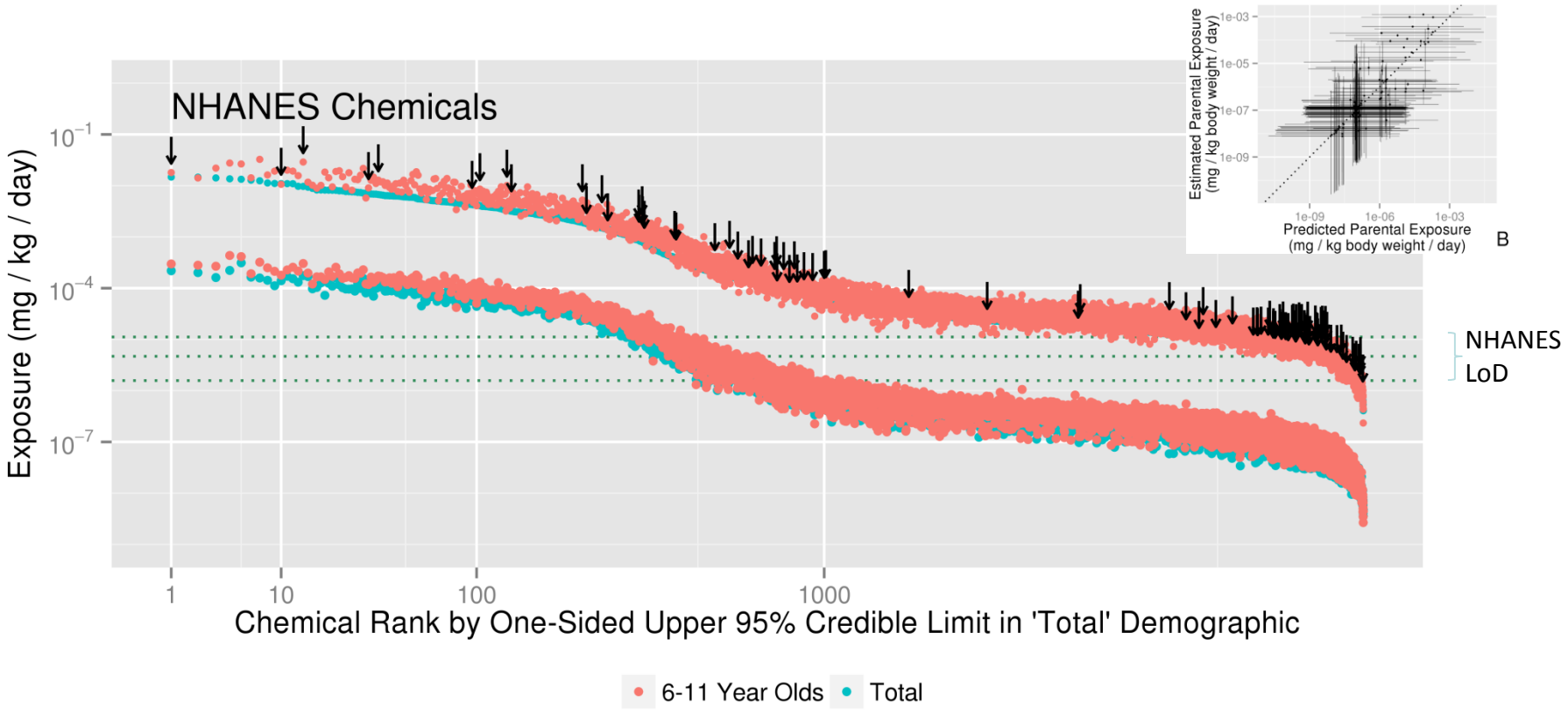
High-throughput exposure predictions

ExpoCast
High-Throughput Exposure Predictions

- **Exposure science lags behind**
  - Most models require extensive information on production, use, fate and transport and rely on empirical data *(no measurement = no exposure?)*

- **ExpoCast**
  - Exposure predictions based on pChem, production values, fate and transport, and product use categories (e.g., industrial, pesticide use, consumer personal care)
  - Industrial vs consumer use
  - Yields exposure estimates and Baysian confidence

Exposure Predictions for 7968 Chemicals & Comparison to NHANES



- NHANES – US National Study – measures exposures in human serum and urine
- Chemicals currently monitored by NHANES are distributed throughput the predictions

Environ. Sci. Technol., **2014**, *48:*12760–12767

Putting it All Together HT Prioritization

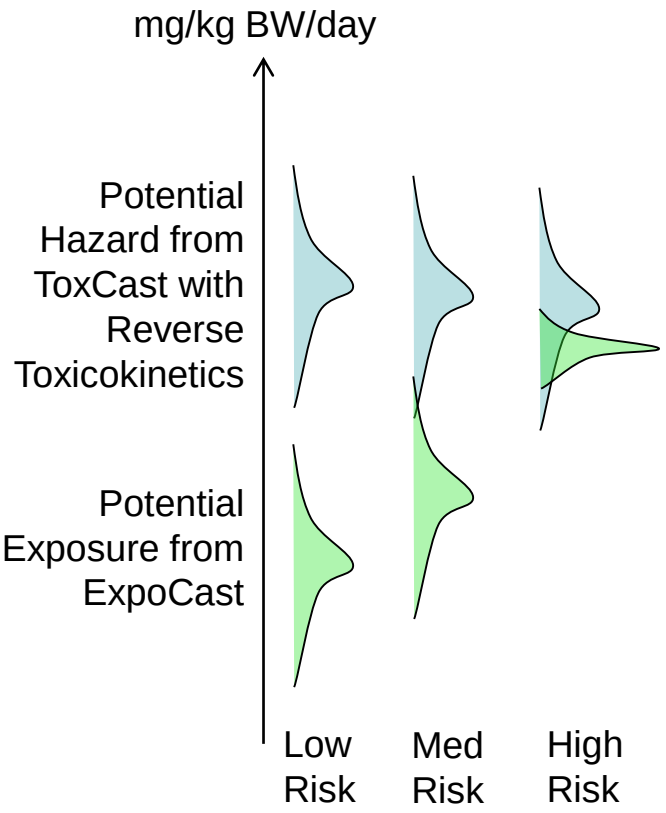
Risk is the product of hazard and exposure

There are thousands of chemicals in commerce, most without enough data for risk evaluation

High throughput *in vitro* methods beginning to bear fruit on potential hazard for many of these chemicals

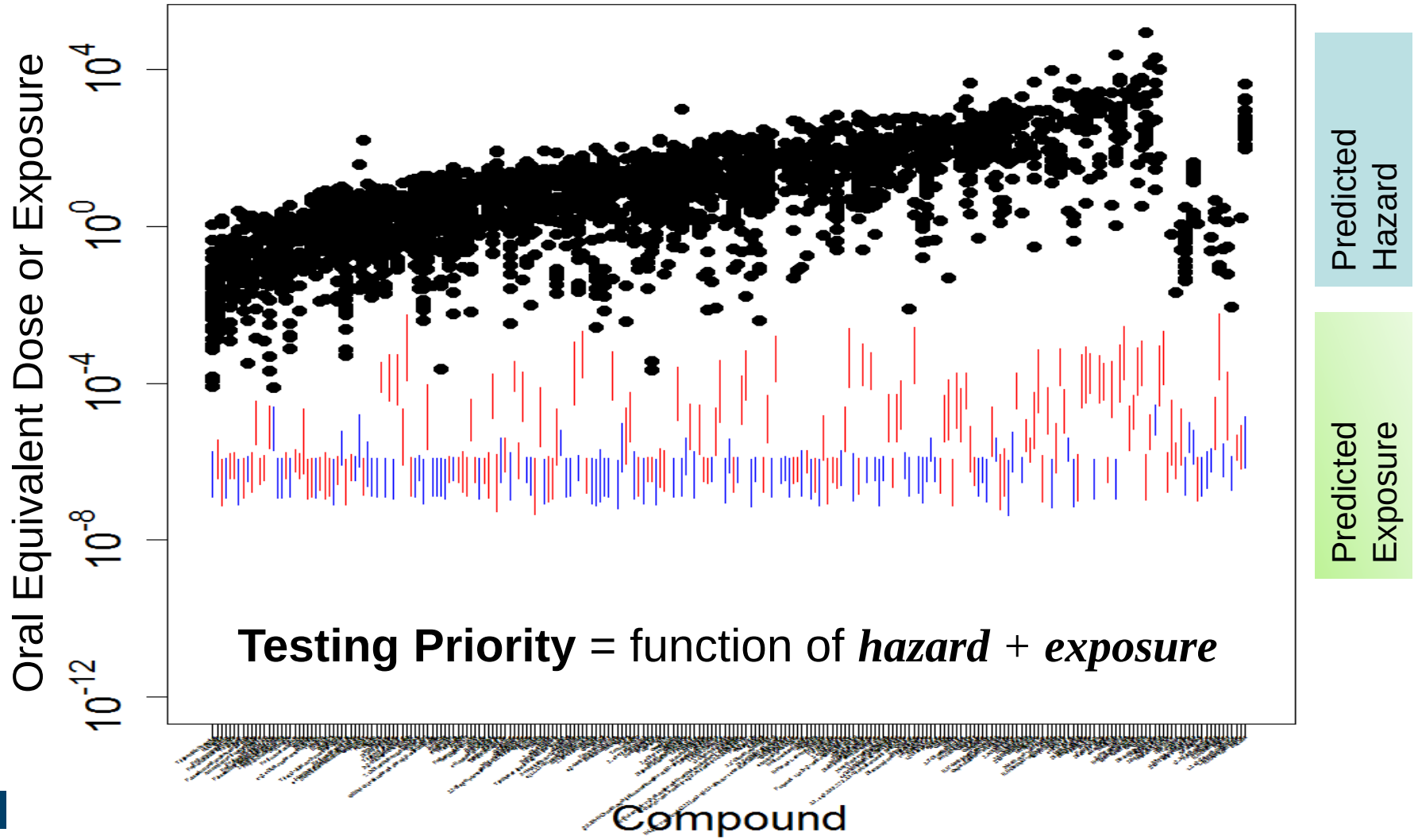
Methods exist for approximately converting these *in vitro* results to daily doses needed to produce similar levels in a human (IVIVE)

What can we say about exposure with the limited data we have?



Judson *et al.*, (2011)
Chemical Research in Toxicology

Combining 2nd Generation ExpoCast Exposure Predictions with Predicted Hazard



Public Data Access using iCSS ToxCast Dashboard


NCCT/ORD/USEPA
NCATS/NIH
– Menghang Xia
– Ruili Huang
North Carolina State University
– Seth Kullman
– Anthony Planchart
University of California at Davis
– Mike Dennison
– Anatoly Soshilov
NCCT 2014