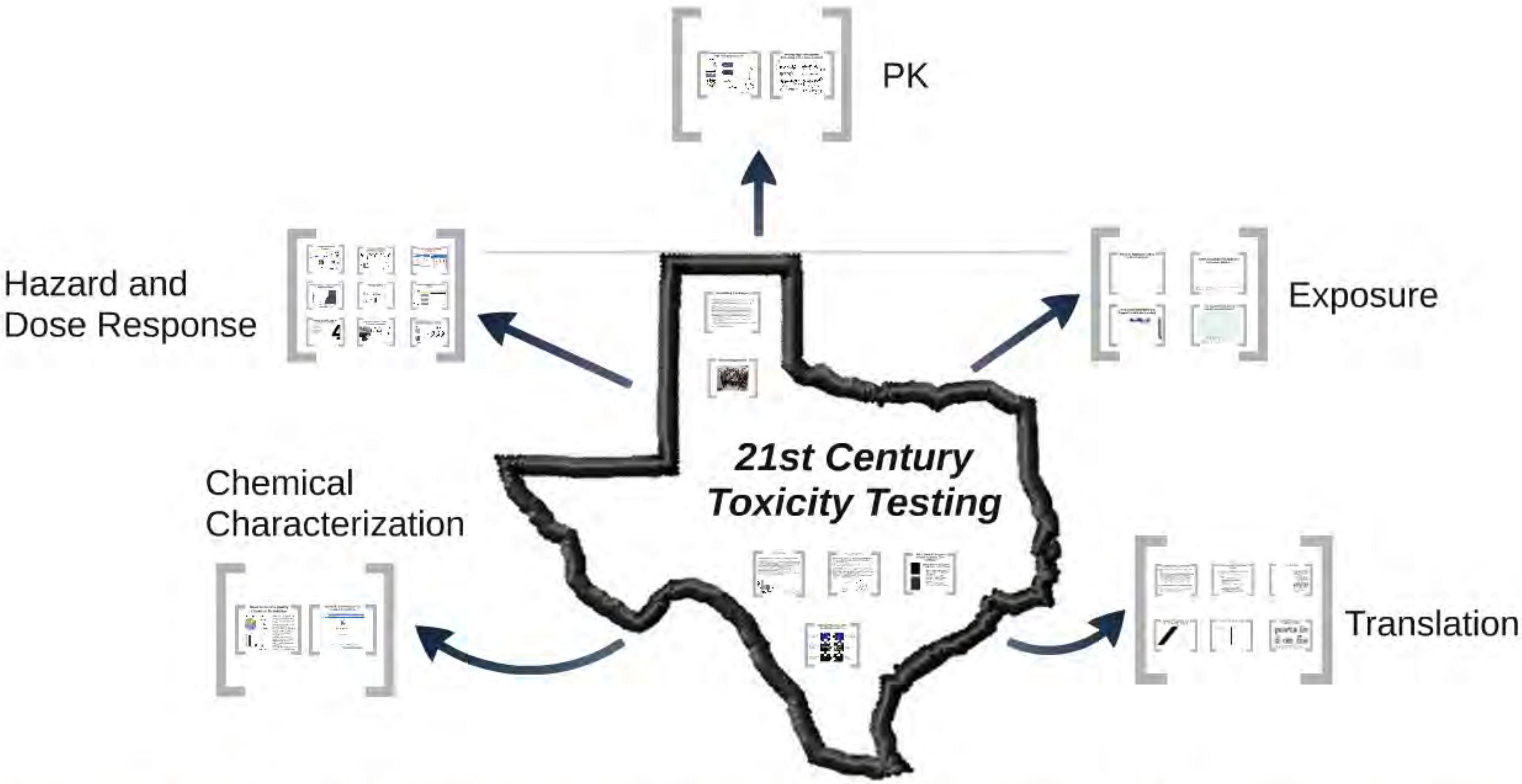
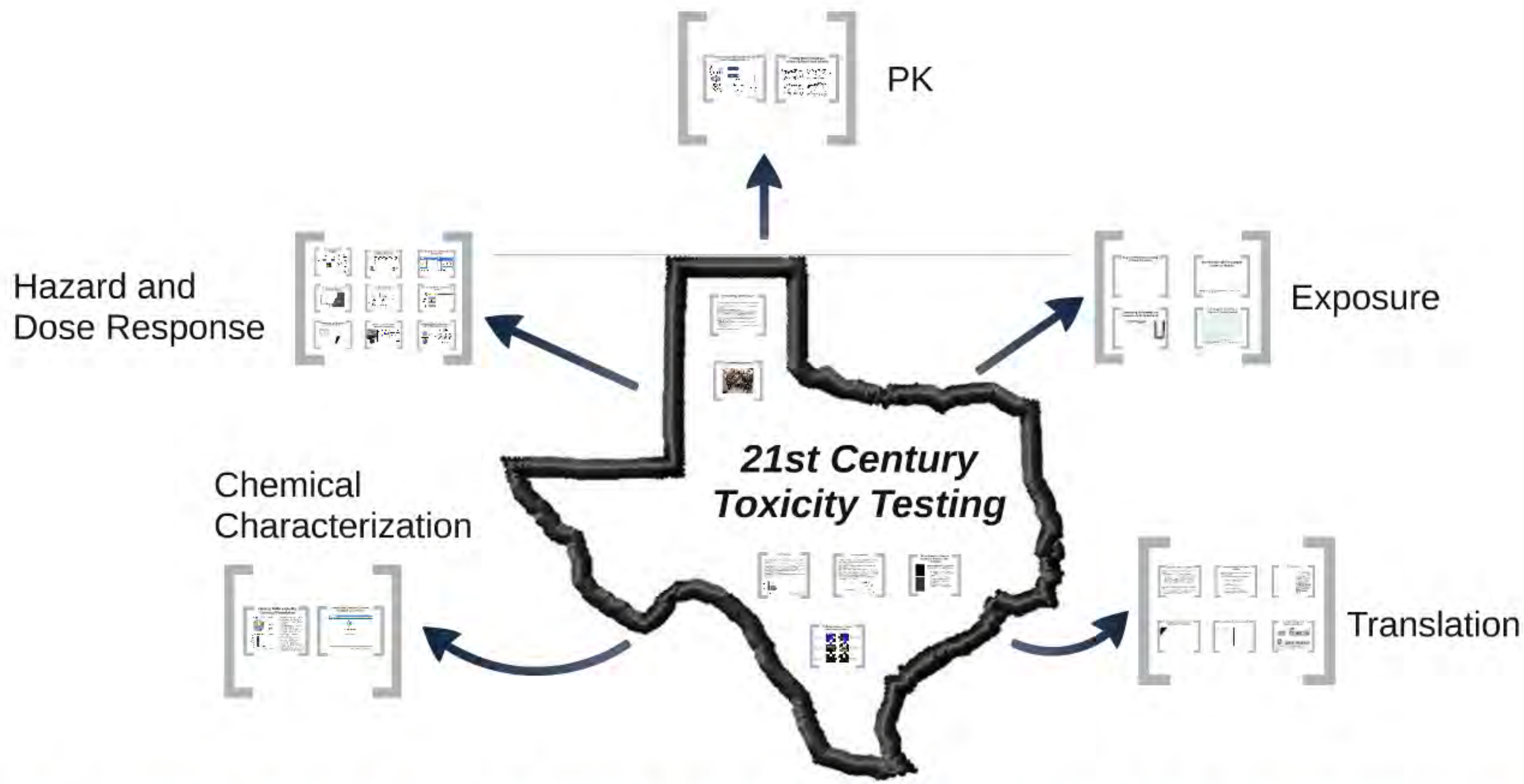


A Texas-Sized Plan for Implementing 21st Century Toxicity Testing

Texas A&M Seminar - April 25, 2016

Russell Thomas
Director, National Center for Computational Toxicology



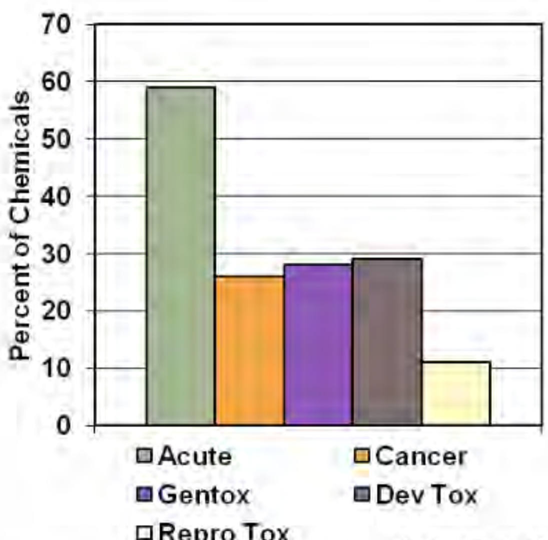
A Texas-Sized Plan for Implementing 21st Century Toxicity Testing

Texas A&M Seminar - April 25, 2016

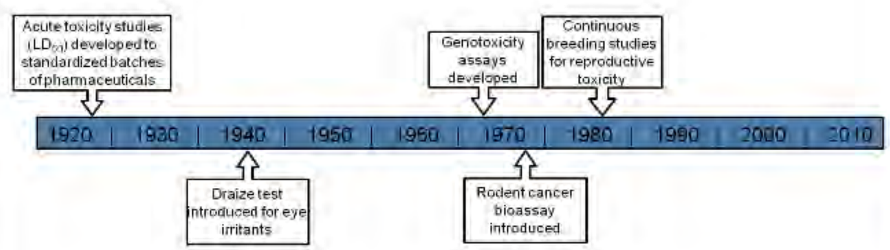
Russell Thomas
Director, National Center for Computational Toxicology

The Challenge

- ~84,000 chemicals are on the US Toxic Substances Control Act inventory
- EPA receives ~1,000 – 2,000 premanufacture notices per year
- A relatively small percentage (< 30%) of chemicals have been more fully tested for potential adverse health effects
- Under current U.S. law, companies are not required to test chemicals for potential adverse health effects prior to manufacture and distribution*
- Traditional methods to test chemicals for toxicity are decades old, costly, and use large numbers of animals



Judson, et al *EHP* (2010)



*Except for pesticides, food additives, drugs, and a few other specific uses.

The Consequences

- The EPA has <90 days to review each premanufacture notice and determine unreasonable risk (usually by chemical structure)
- Only ~500 chemicals and ~600 pesticides have full chemical risk assessments
- At Superfund sites, it is not uncommon to have a large percentage of contaminants with no toxicity information
- Contaminants with no toxicity information are essentially ignored in the overall hazard calculation
- Little is known about the effects or exposure of many commonly used chemicals


EPA

EPA's CompTox Program Initiated to Address These Challenges

Recommendations from 2005 EPA Strategic Plan and 2007 NRC Report

- Broad incorporation of new technologies and computational approaches
- Shift to *in vitro* and high-throughput chemical testing
- Use of human-based test systems
- Focus on pathway-based evaluation of chemical effects

Challenge Requires a Multi-Disciplinary Solution

Computational Chemistry

High-Throughput Hazard Screening

High-Throughput Exposure

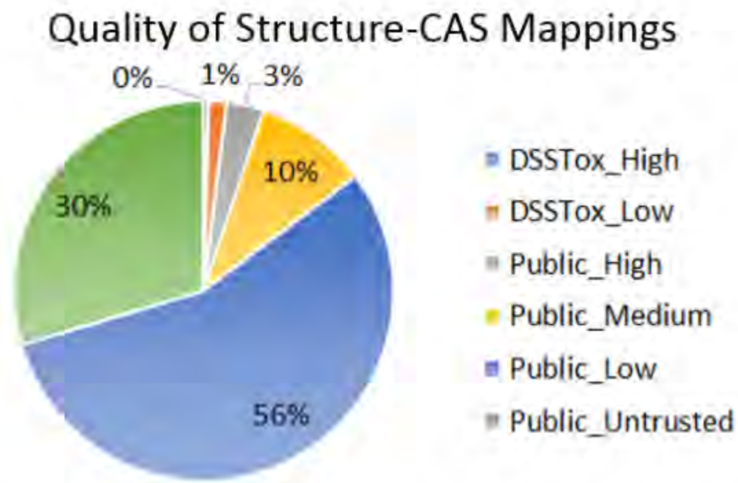
High-Throughput Pharmacokinetics

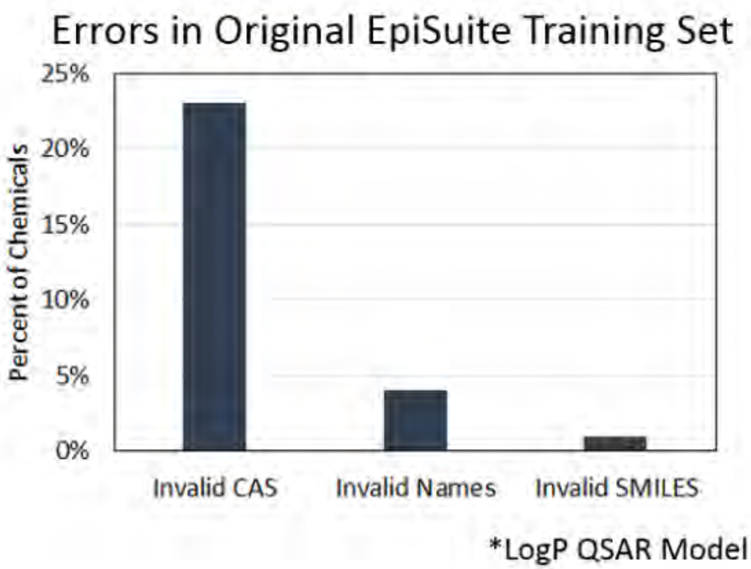
Uncertainty and Variability

Regulatory Translation

Chemical Characterization

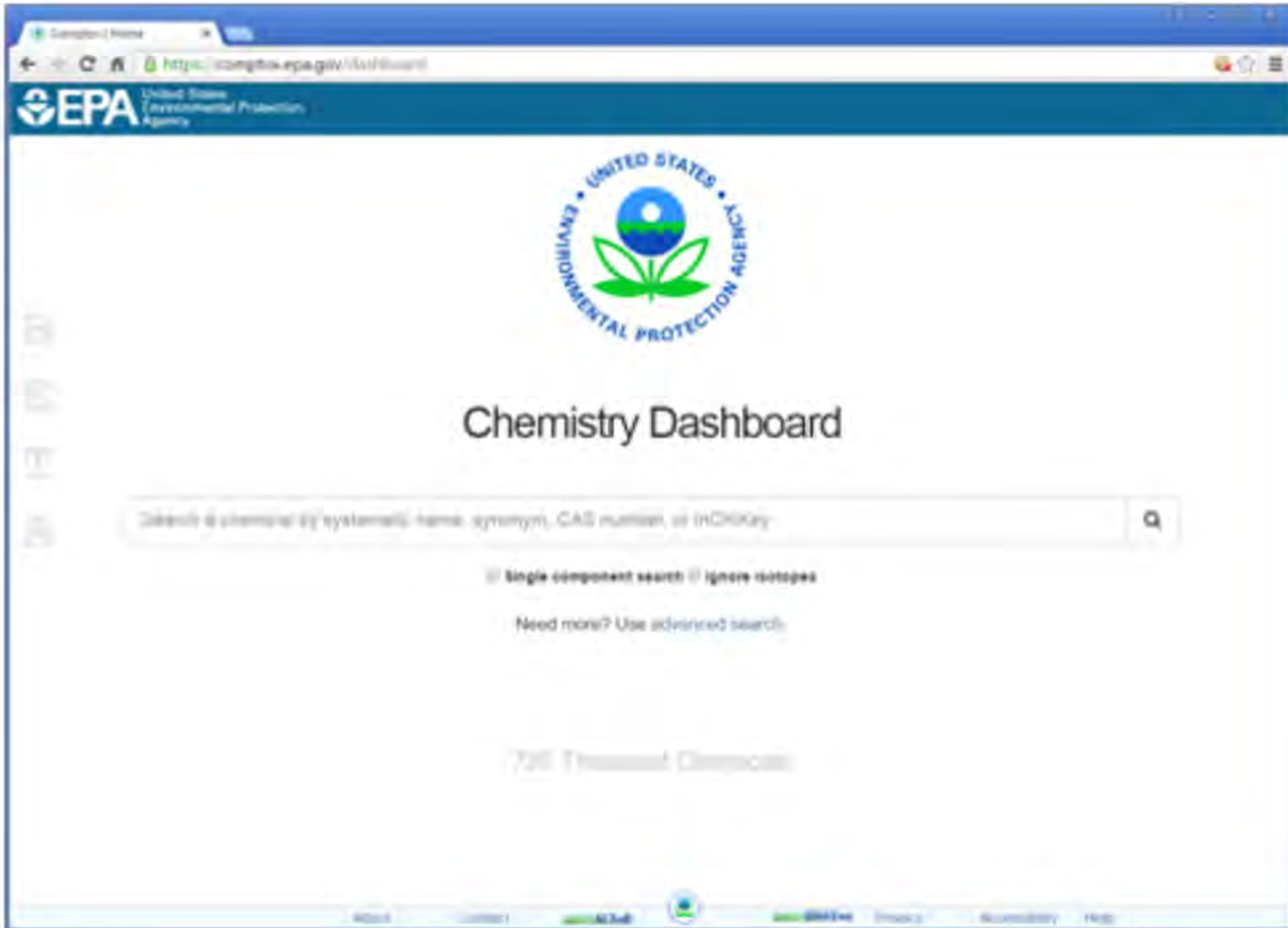
Need to Build a Quality Chemical Foundation





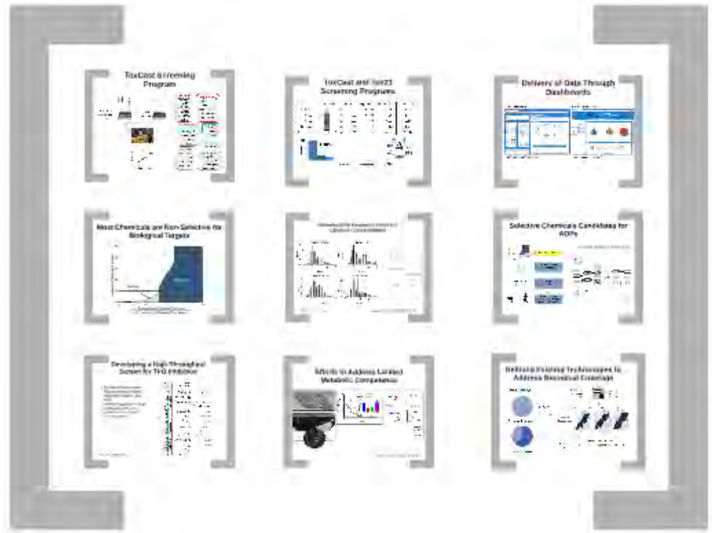
- Significant errors present in public chemistry data and training sets of existing QSAR models
- Creating curated chemical structure database of >700,000 unique substances with QC flags to indicate confidence in structural associations
- Curate training sets for a range of QSAR models for phys-chem properties, environmental fate/transport, and toxicity
- Expand training sets for QSAR models (>450% on average)

Making the Chemistry Content Available to the Public



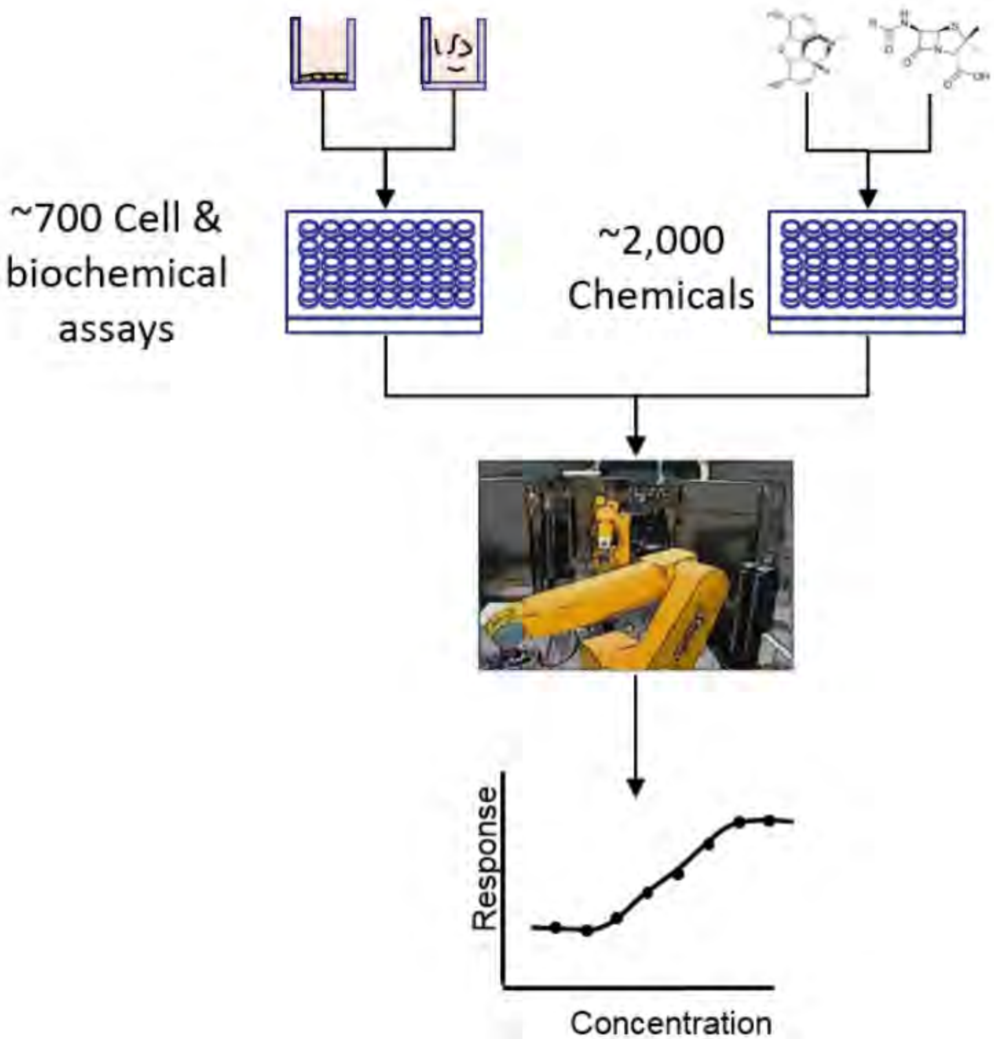
https://comptox.epa.gov

Hazard and Dose Response



Chemical

ToxCast Screening Program



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

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

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

Readout Type
single
multiplexed
multiparametric

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

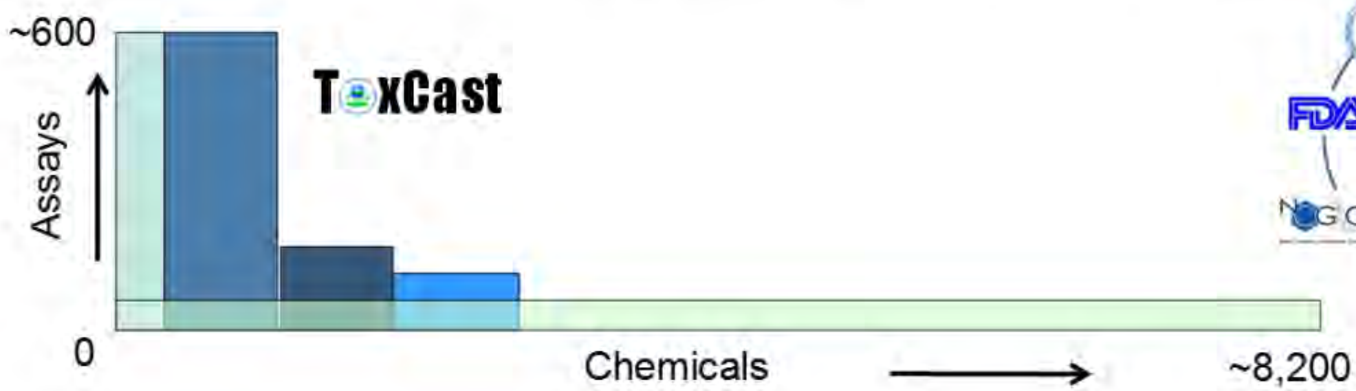
Tissue Source

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

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

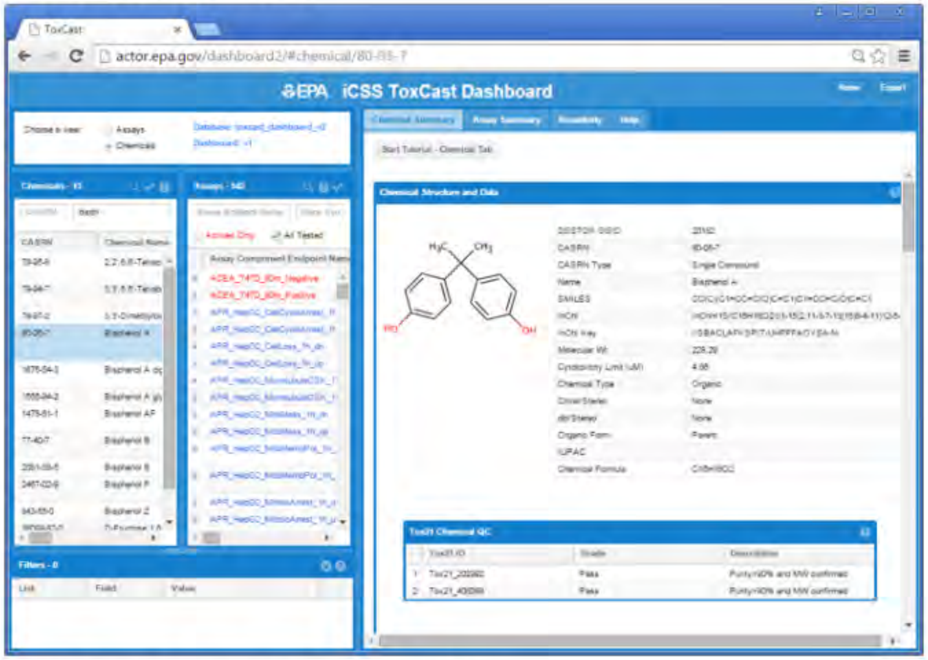
ToxCast and Tox21 Screening Programs

Set	Chemicals	Assays	Endpoints	Completion	Available
ToxCast Phase I	293	~600	~1100	2011	Now
ToxCast Phase II	767	~600	~1100	2013	Now
ToxCast Phase III	1001	~100	~100	Ongoing	2016
E1K (endocrine)	880	~50	~120	2013	Now
Tox21	8,193	~25	~50	Ongoing	Ongoing



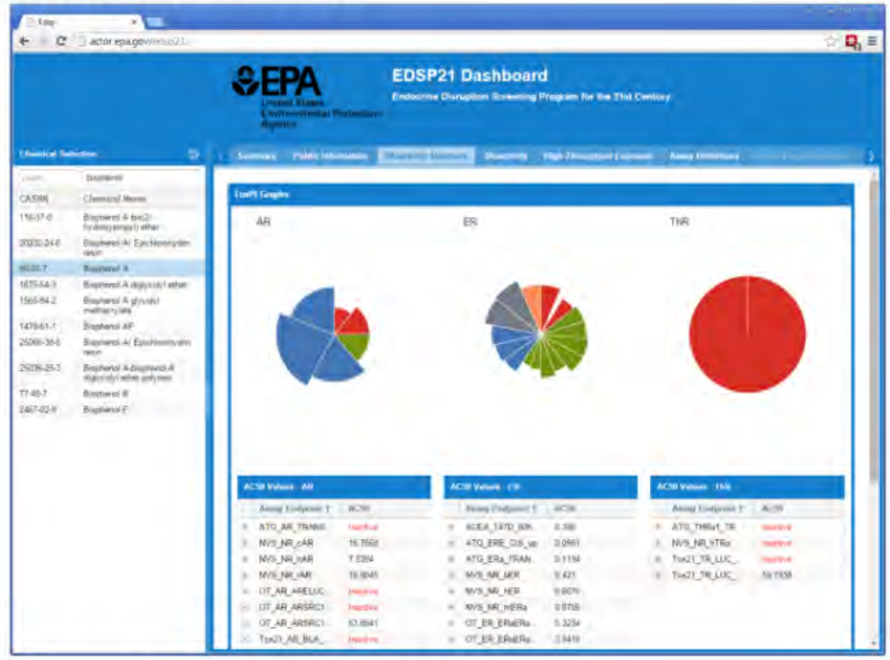
Delivery of Data Through Dashboards

iCSS Dashboard



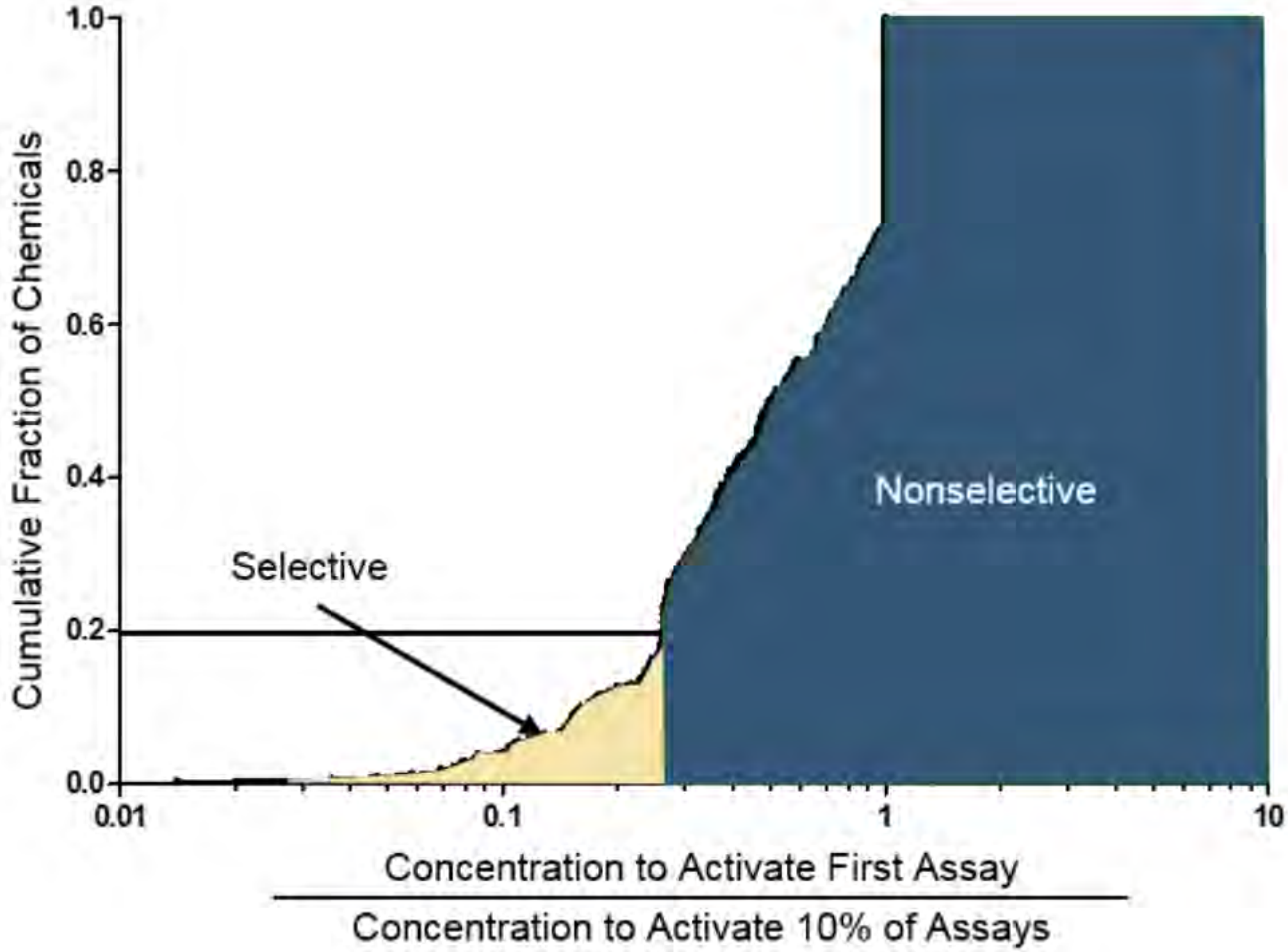
http://actor.epa.gov/dashboard2/

EDSP21 Dashboard

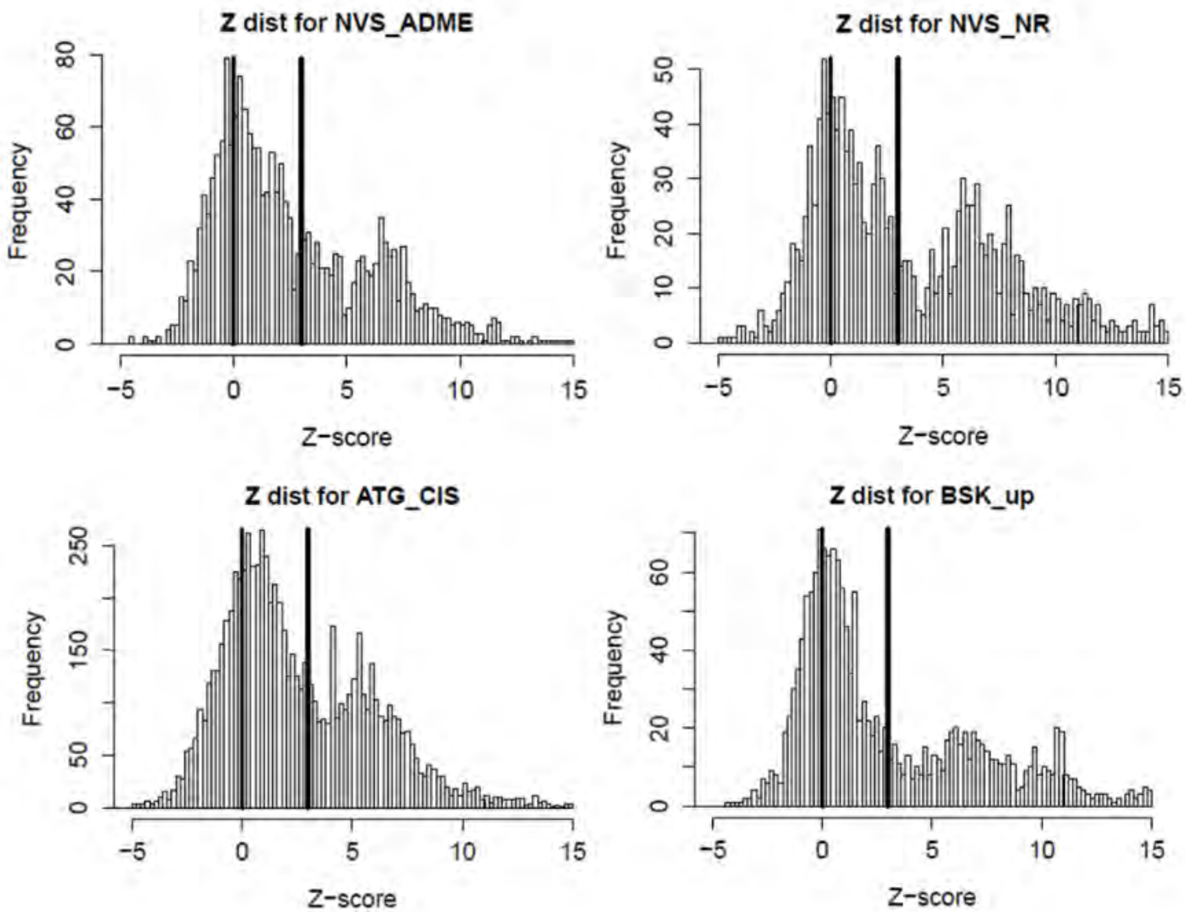


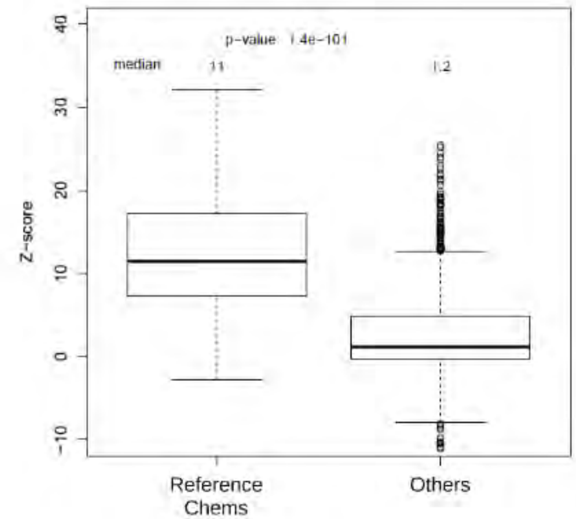
http://actor.epa.gov/edsp21/

Most Chemicals are Non-Selective for Biological Targets



Nonselectivity Frequently Occurs at Cytotoxic Concentrations

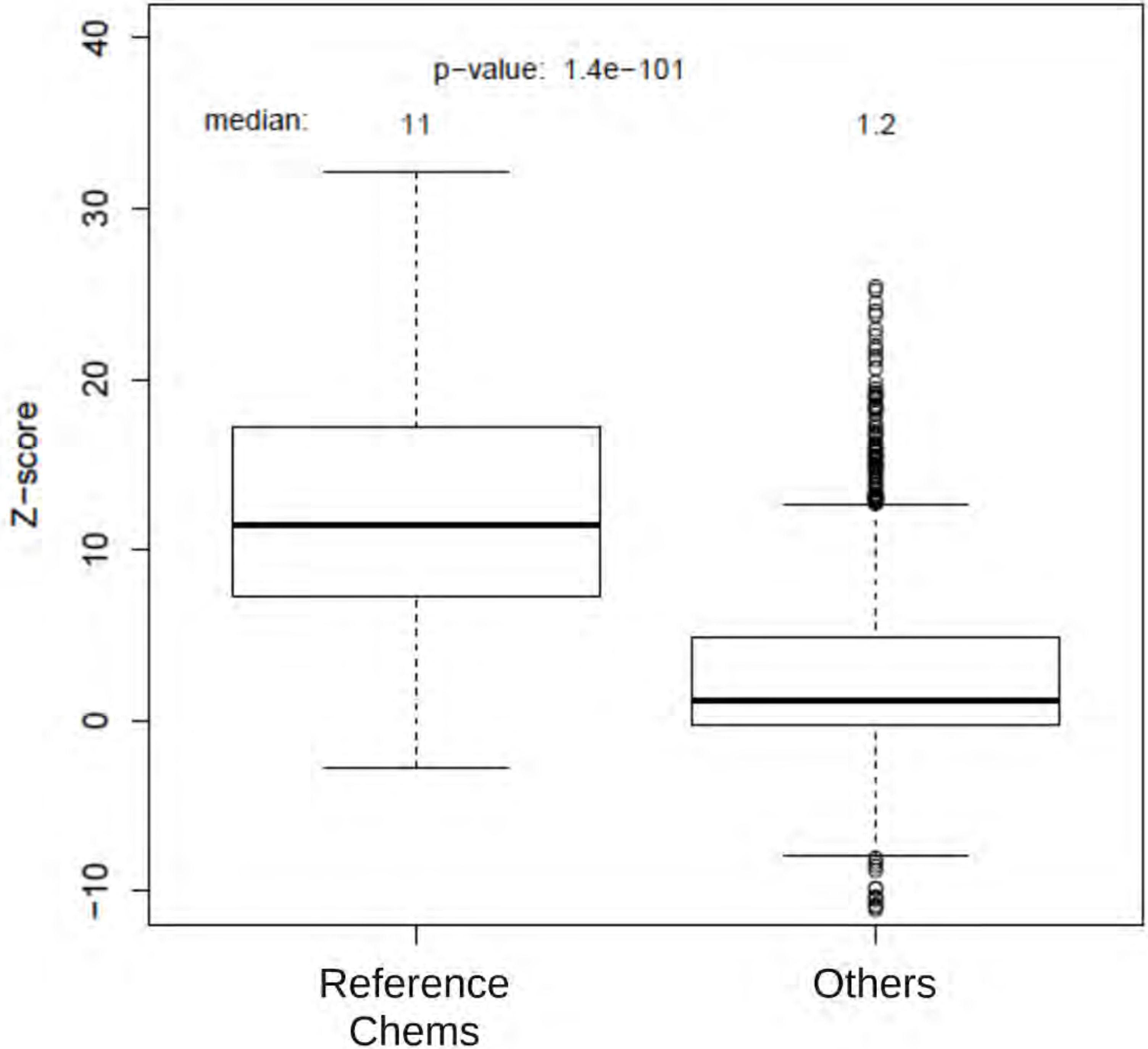




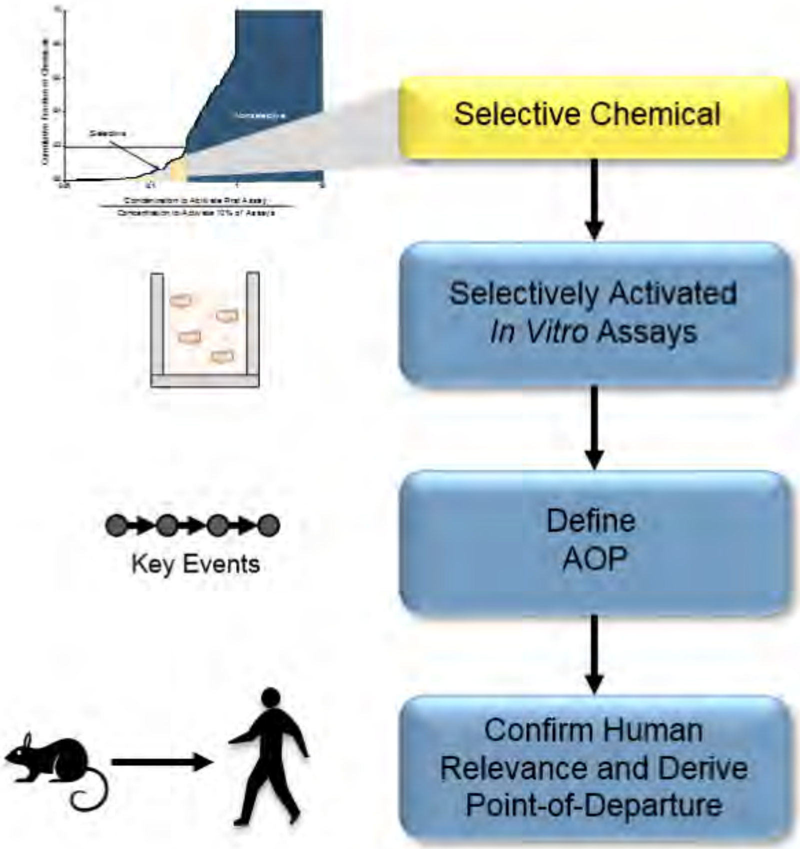
Judson *et al.* Unpublished

*Z-score = #SD from Cytotox Region

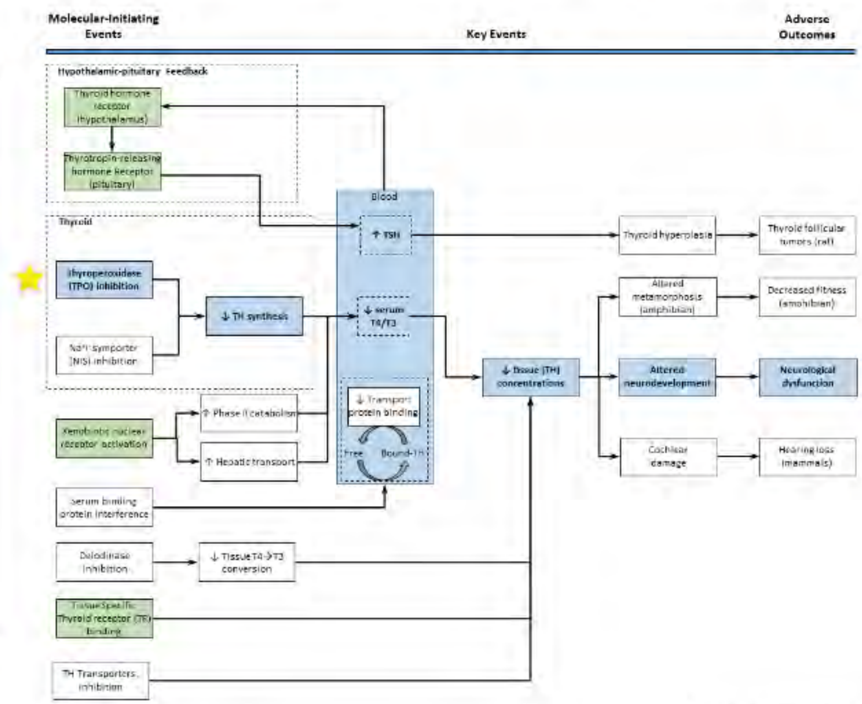

15


p−value: 1.4e−101
median:
11
1.2
Z−score
40
30
20
10
0
−10
Reference Chems
Others

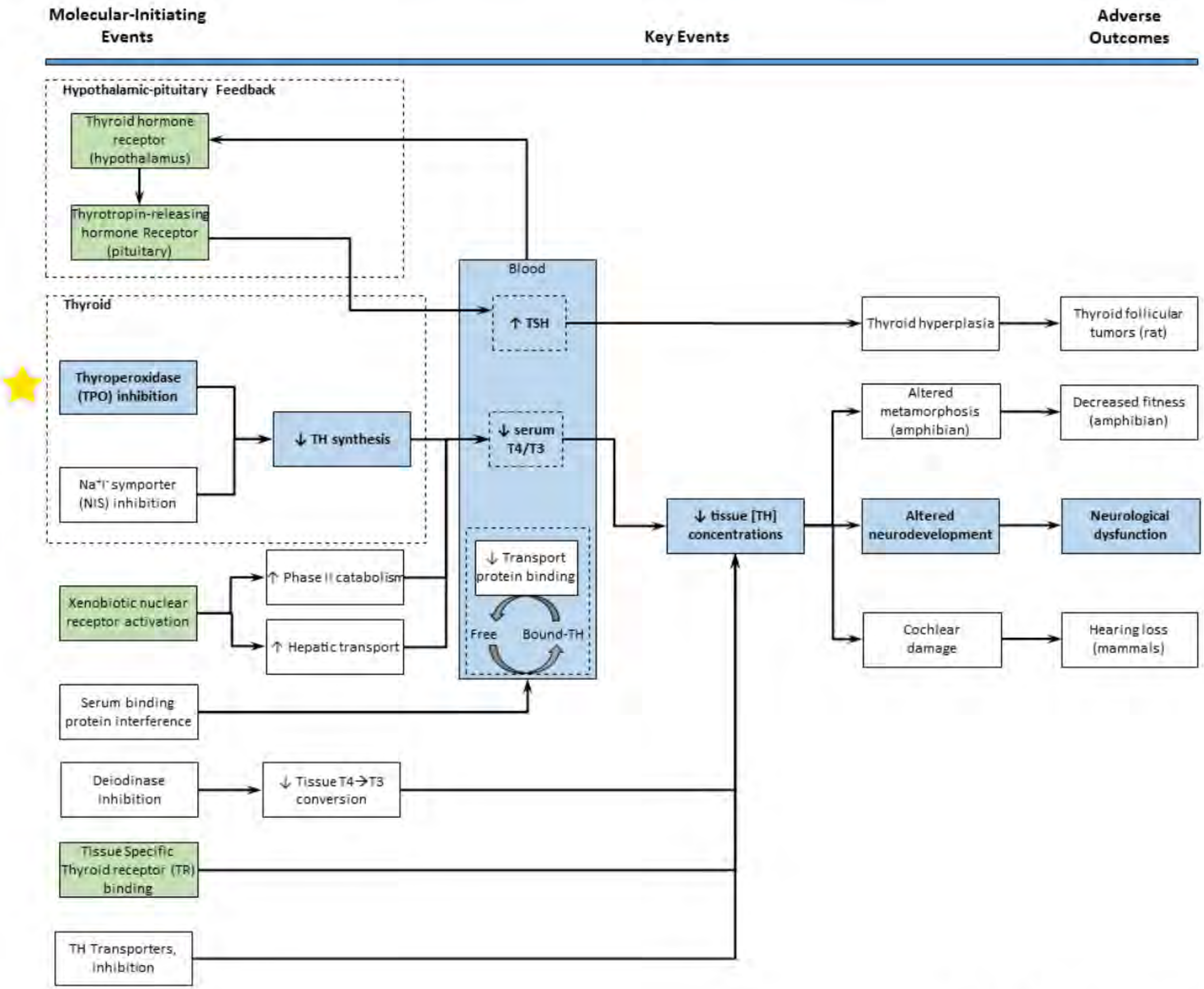
Selective Chemicals Candidates for AOPs



AOP for Chemically-Mediated Thyroid Toxicity



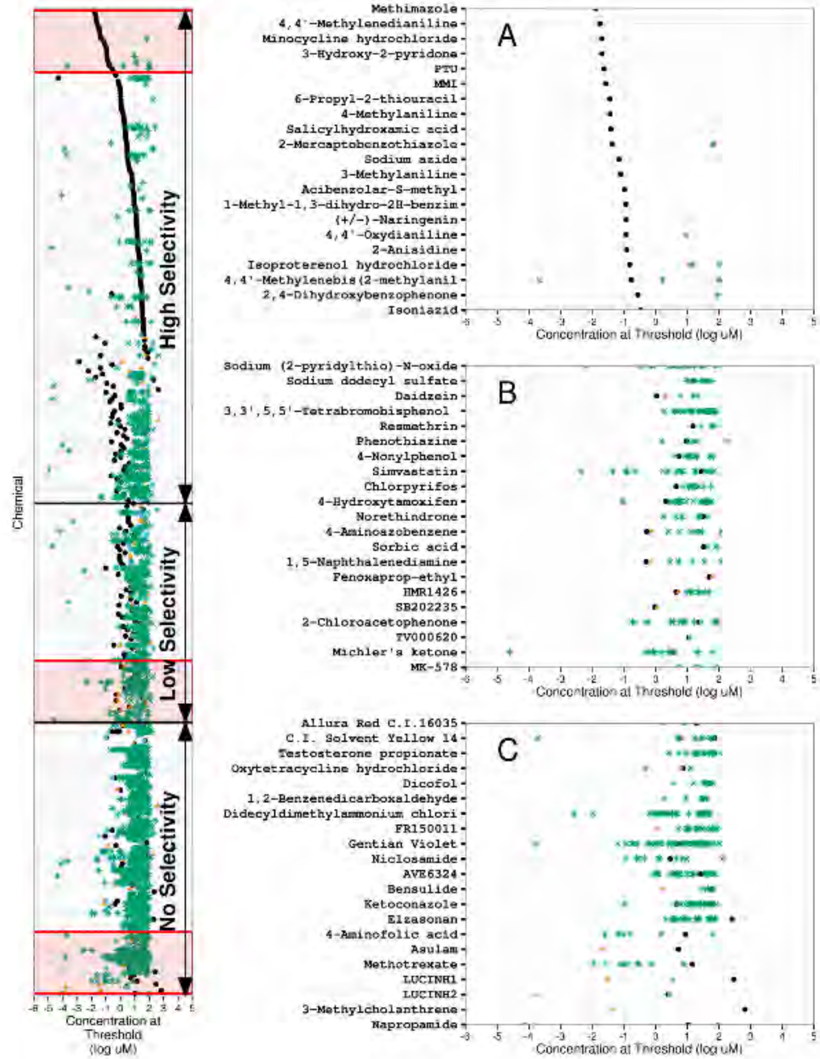
Paul *et al.*, Unpublished



Paul *et al.*, Unpublished

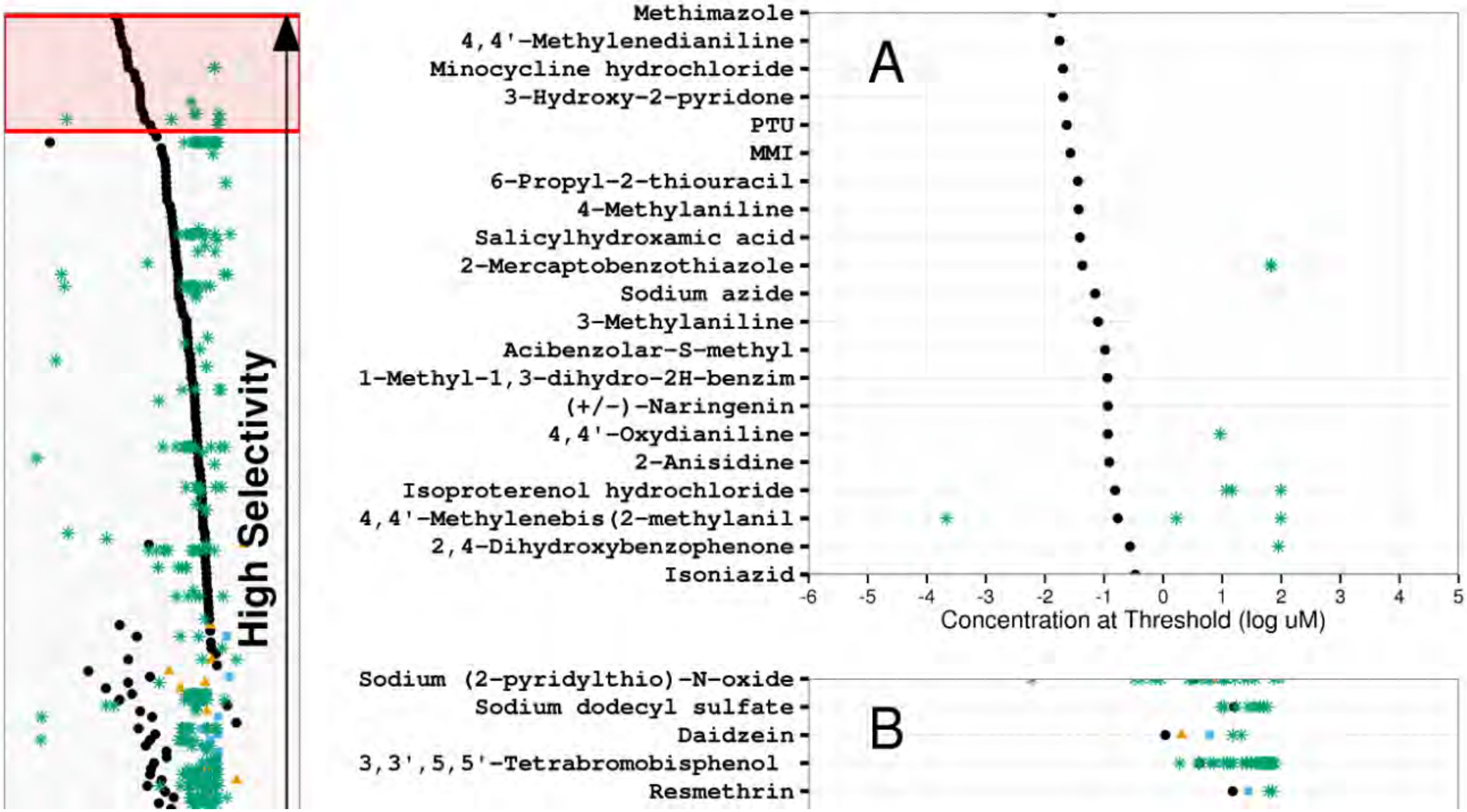
Developing a High-Throughput Screen for TPO Inhibition

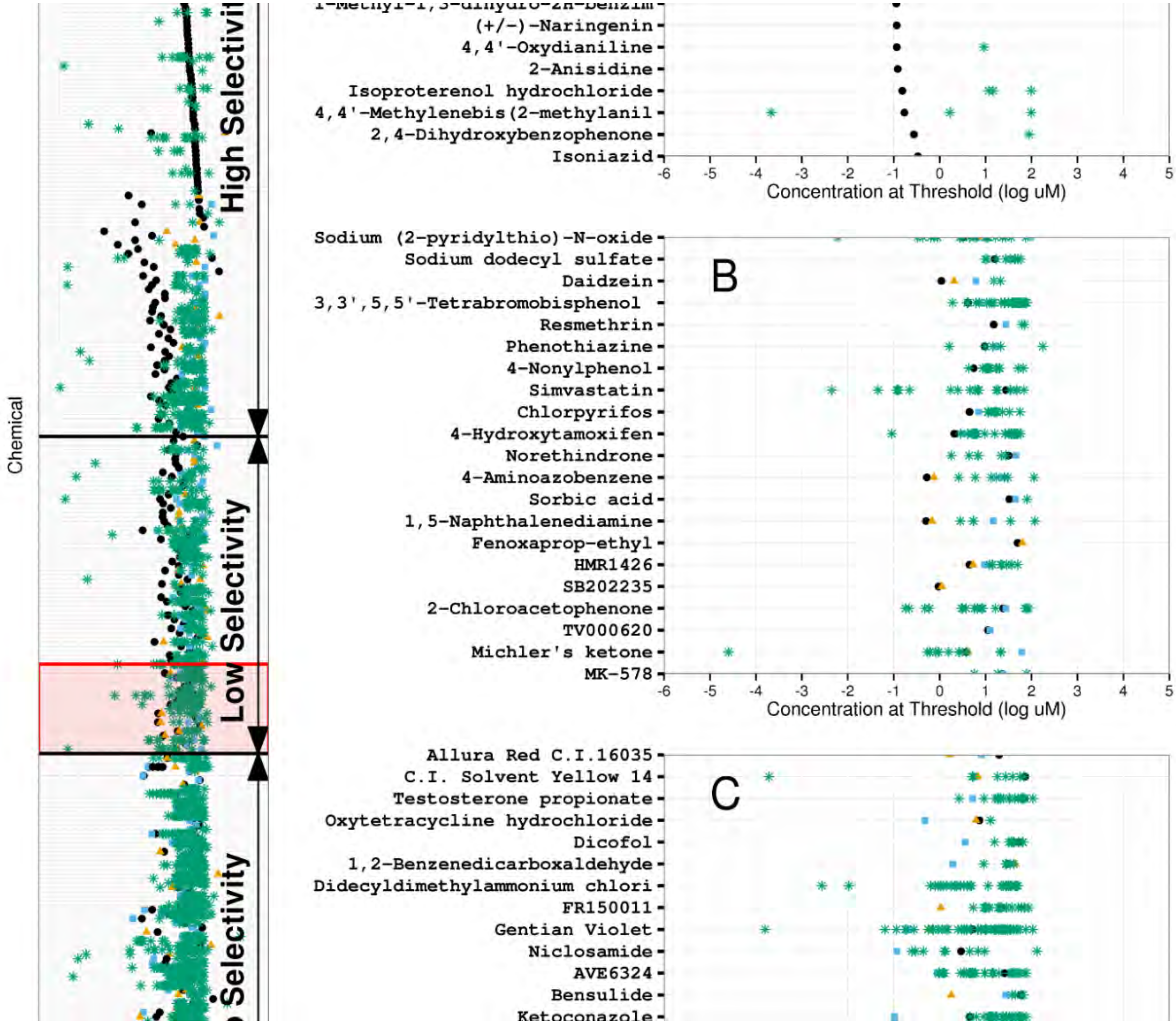
- Rat thyroid microsomes
- Fluorescent peroxidase substrate (Amplex Ultra Red)
- Validated against existing kinetic guaiacol assay
- Luciferase, cytotoxicity counterscreens



Paul *et al.*, Unpublished

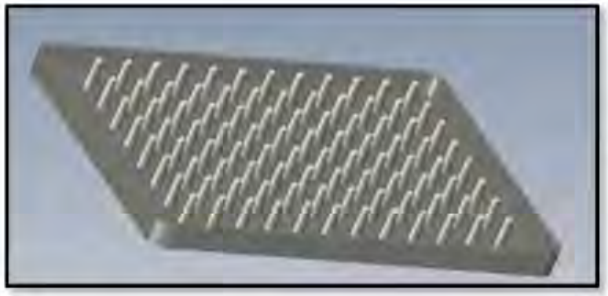
High-Throughput TPO Inhibition

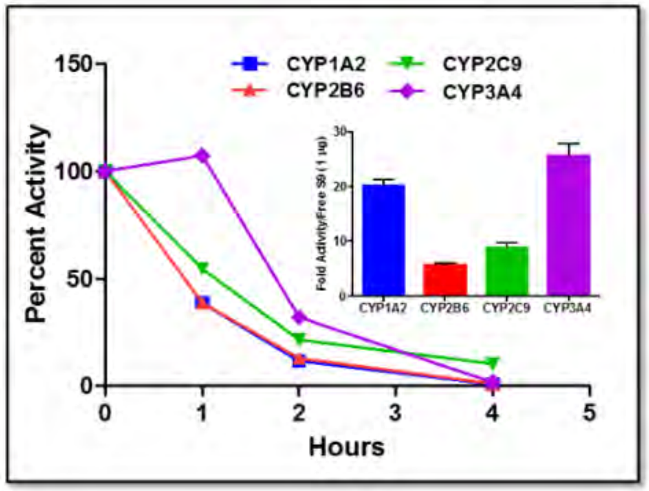


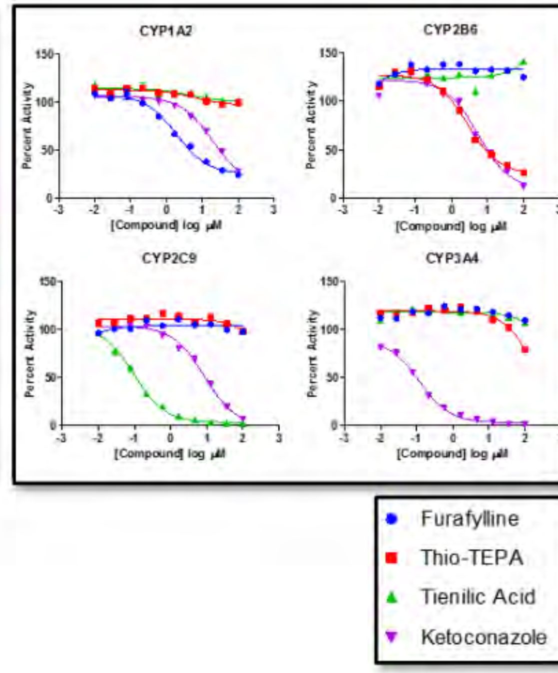


High Selectivity
Low Selectivity
Chemical
(+/-)-Naringenin
4,4'-Oxydianiline
2-Anisidine
Isoproterenol hydrochloride
4,4'-Methylenebis(2-methylanil
2,4-Dihydroxybenzophenone
Isoniazid
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5
Concentration at Threshold (log uM)
B
Sodium (2-pyridylthio)-N-oxide
Sodium dodecyl sulfate
Daidzein
3,3',5,5'-Tetrabromobisphenol
Resmethrin
Phenothiazine
4-Nonylphenol
Simvastatin
Chlorpyrifos
4-Hydroxytamoxifen
Norethindrone
4-Aminoazobenzene
Sorbic acid
1,5-Naphthalenediamine
Fenoxaprop-ethyl
HMR1426
SB202235
2-Chloroacetophenone
TV000620
Michler's ketone
MK-578
-6 -5 -4 -3 -2 -1 0 1 2 3 4 5
Concentration at Threshold (log uM)
C
Allura Red C.I.16035
C.I. Solvent Yellow 14
Testosterone propionate
Oxytetracycline hydrochloride
Dicofol
1,2-Benzenedicarboxaldehyde
Didecyldimethylammonium chlori
FR150011
Gentian Violet
Niclosamide
AVE6324
Bensulide

Efforts to Address Limited Metabolic Competence







DeGroot and Simmons, Unpublished

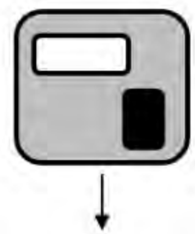
Refining Existing Technologies to Address Biological Coverage

<u>Gene Coverage</u>

<u>Pathway Coverage*</u>

*At least one gene from pathway represented

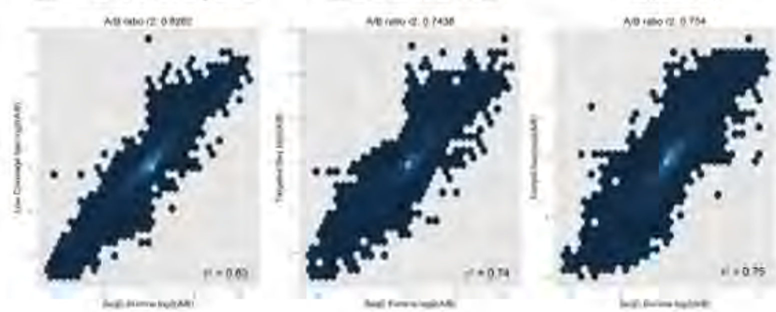
High-Throughput Transcriptomic Platforms



- Low-cost
- Whole genome
- 384-well
- Automatable

Technical Comparison

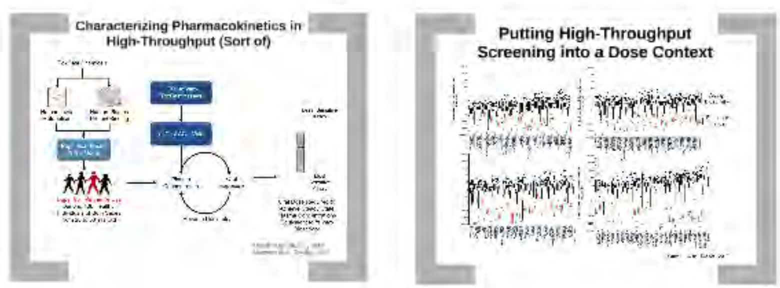
Illumina Low Coverage WT | Illumina RNA Access | TempO-Seq



Functional Comparison

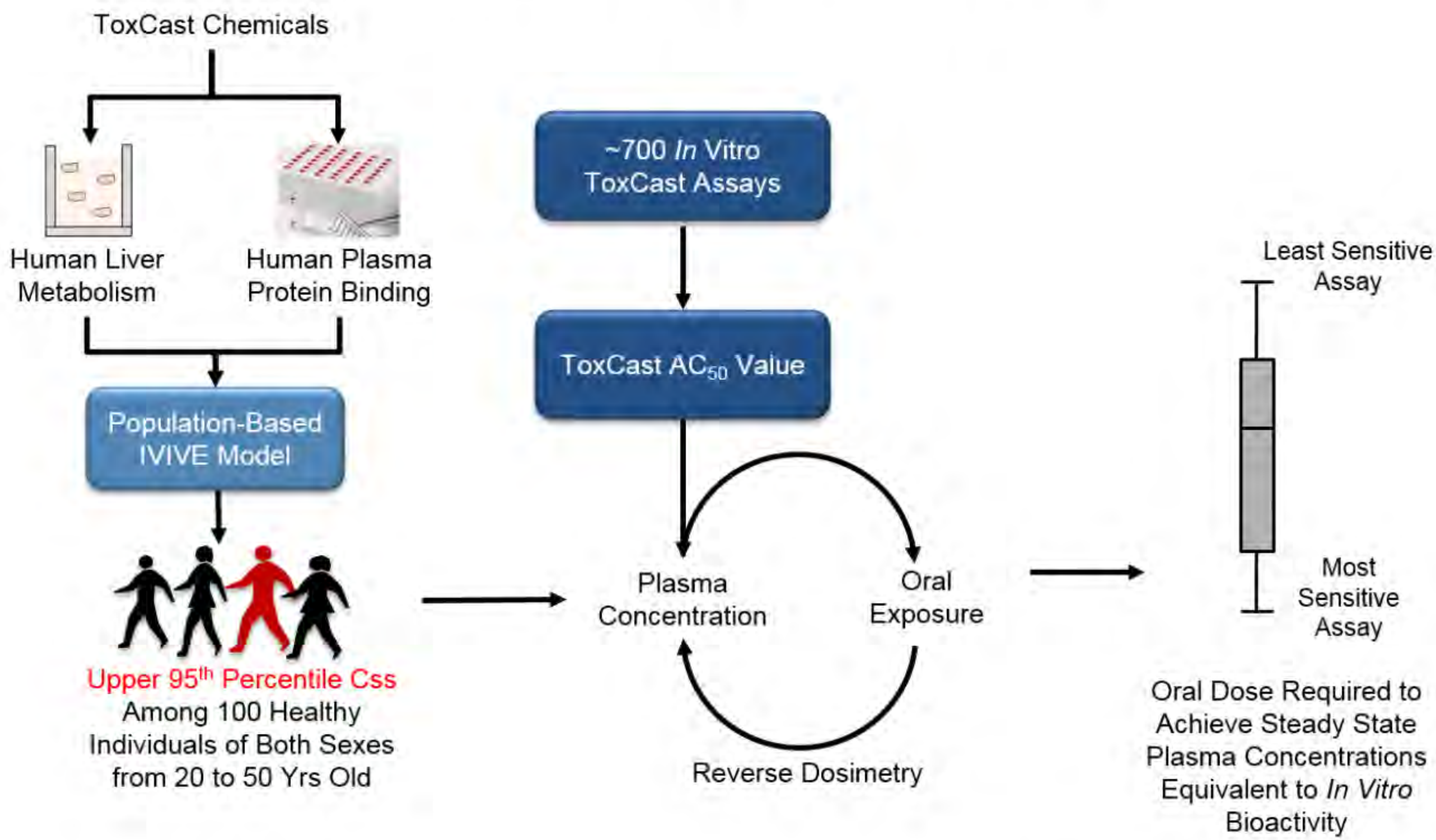
Correct Mechanistic Match

Illumina Low Coverage WT	Illumina RNA Access	TempO-Seq
2/5 (40%)	2/5 (40%)	5/5 (100%)


Characterizing Pharmacokinetics in High-Throughput (Sort of)
Putting High-Throughput Screening into a Dose Context

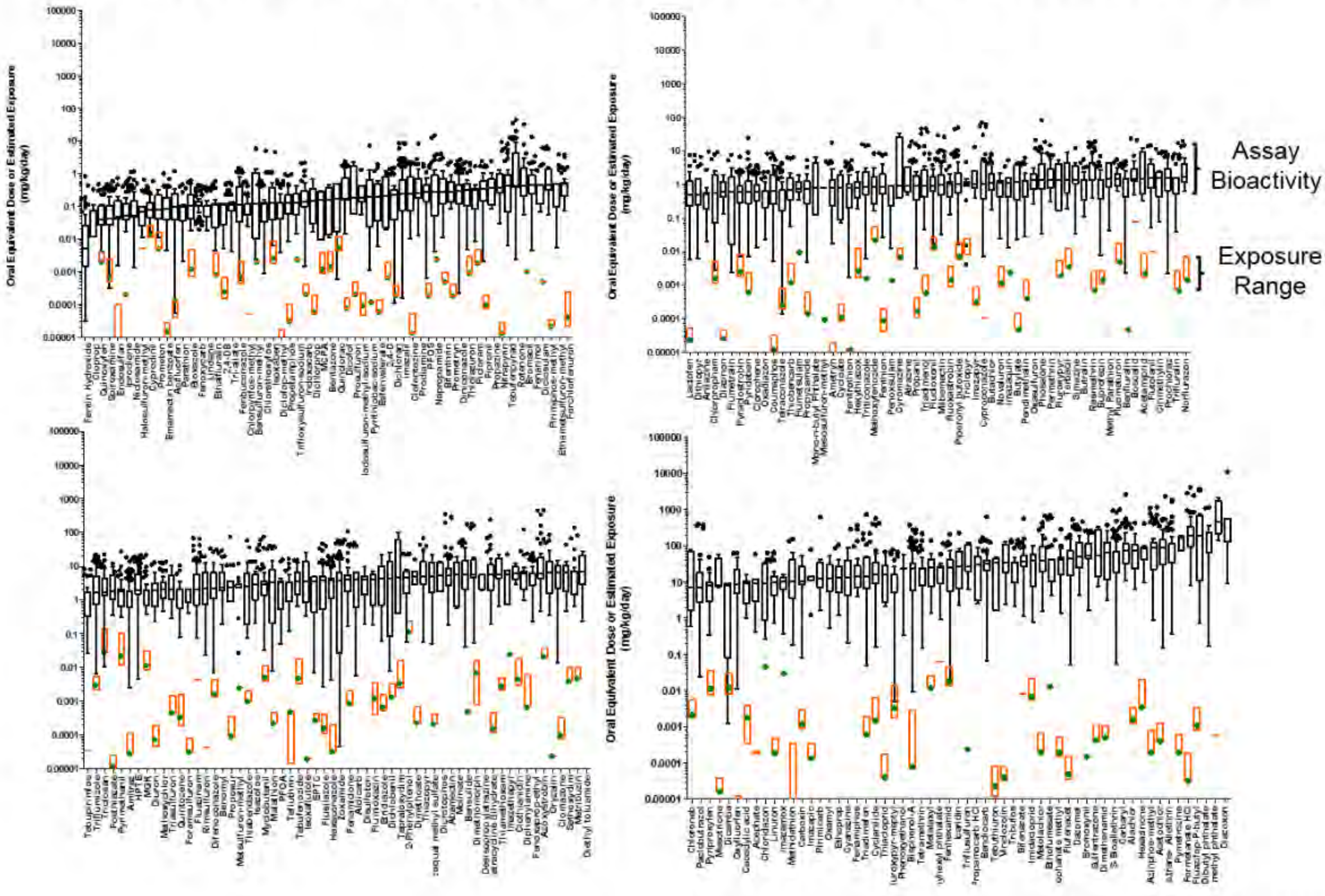
PK

Characterizing Pharmacokinetics in High-Throughput (Sort of)

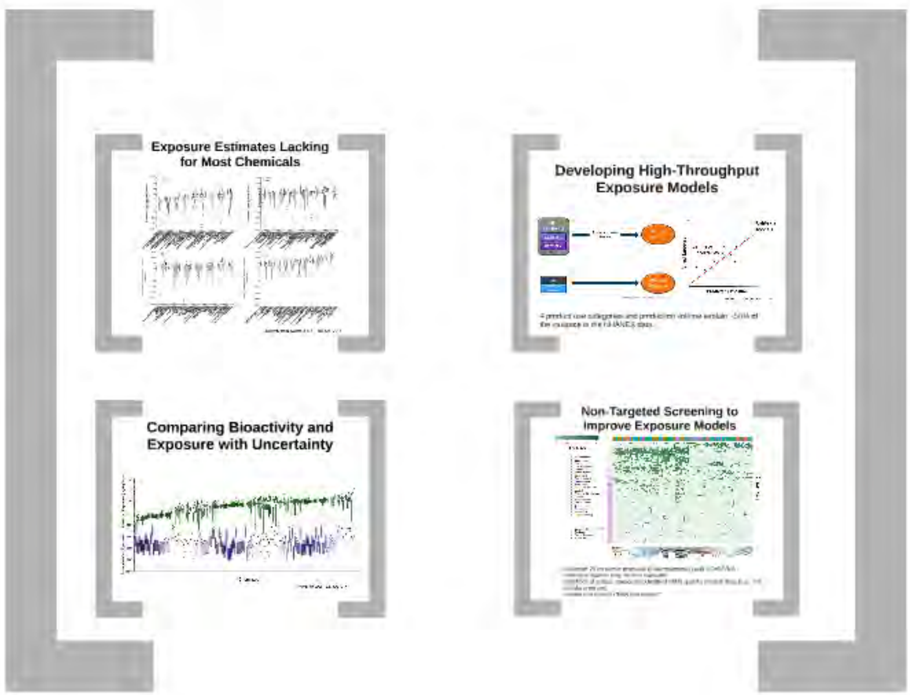


Rotroff et al., Tox Sci., 2010
Wetmore et al., Tox Sci., 2012

Putting High-Throughput Screening into a Dose Context

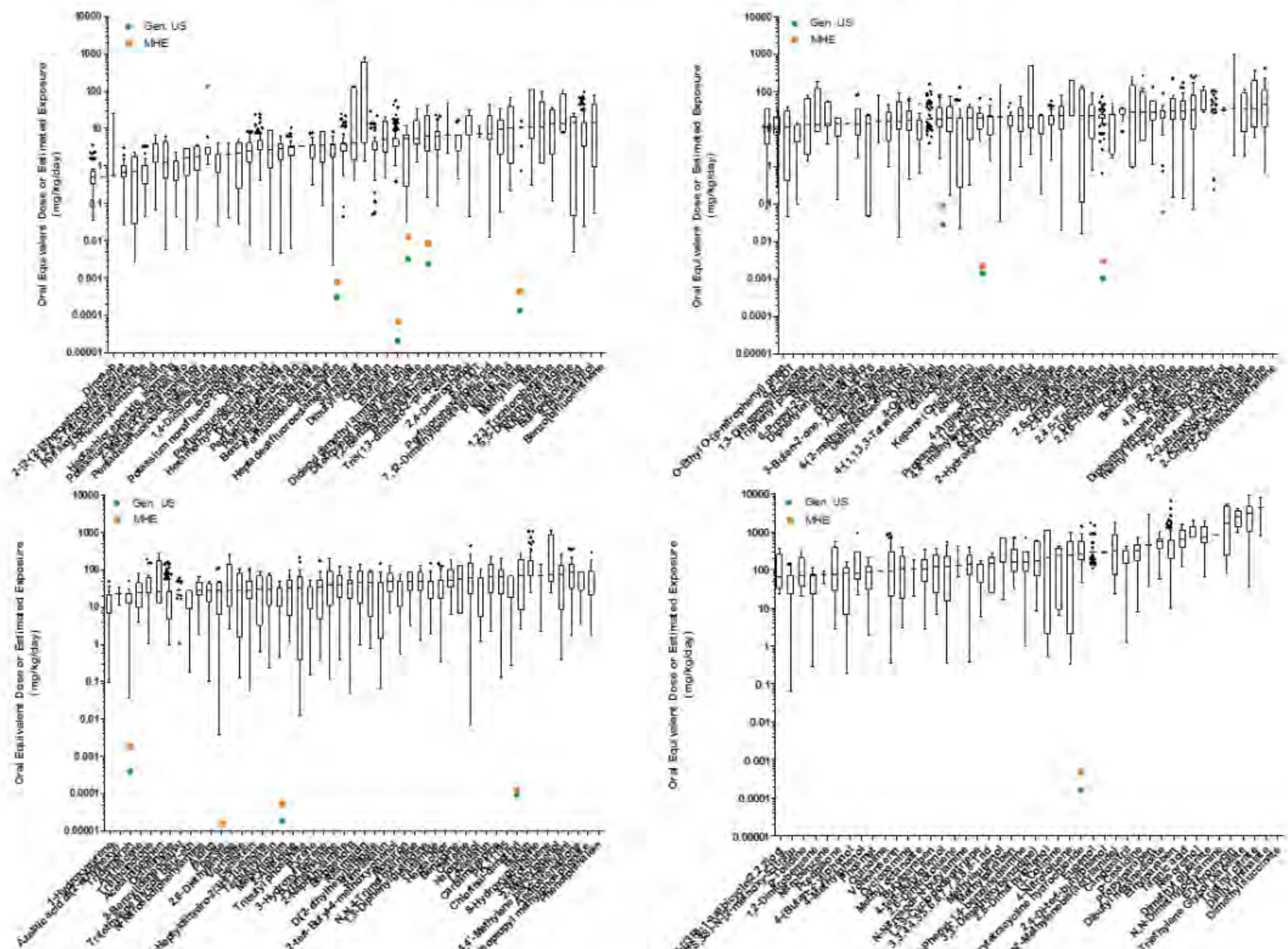


Wetmore *et al.*, *Tox Sci.*, 2012


Exposure Estimates Lacking for Most Chemicals
Developing High-Throughput Exposure Models
Comparing Bioactivity and Exposure with Uncertainty
Non-Targeted Screening to Improve Exposure Models

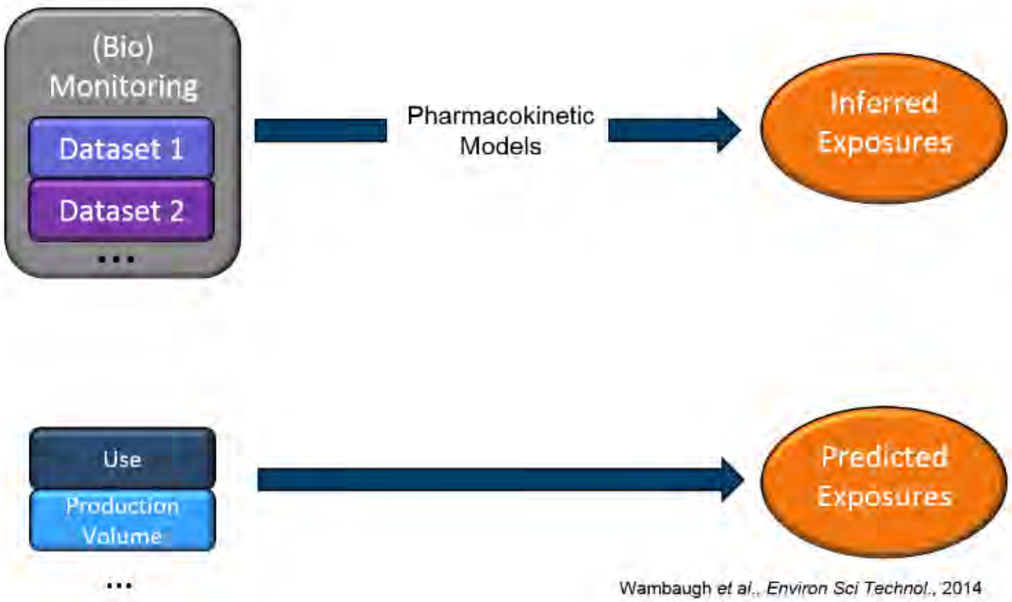
Exposure

Exposure Estimates Lacking for Most Chemicals

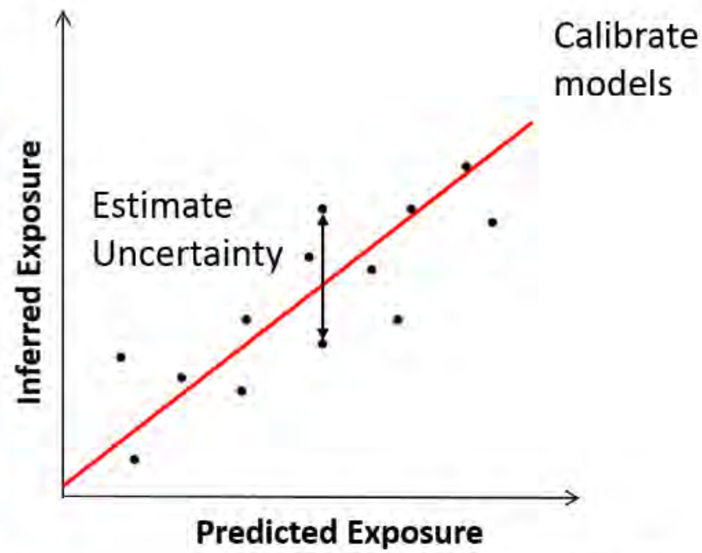


Bioactivity from Wetmore *et al.*, *Tox Sci.*, 2015

Developing High-Throughput Exposure Models



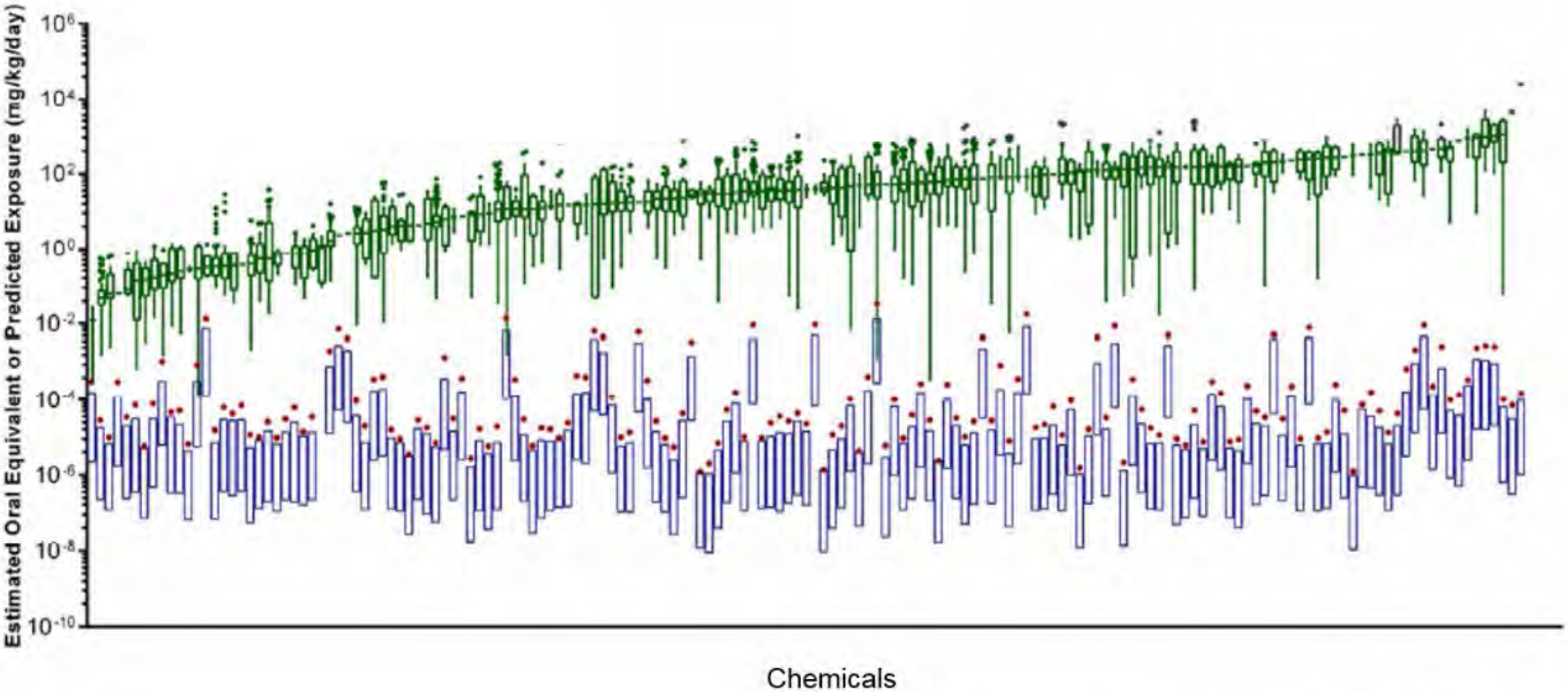
Wambaugh *et al.*, *Environ Sci Technol.*, 2014



Wambaugh *et al.*, *Environ Sci Technol.*, 2014

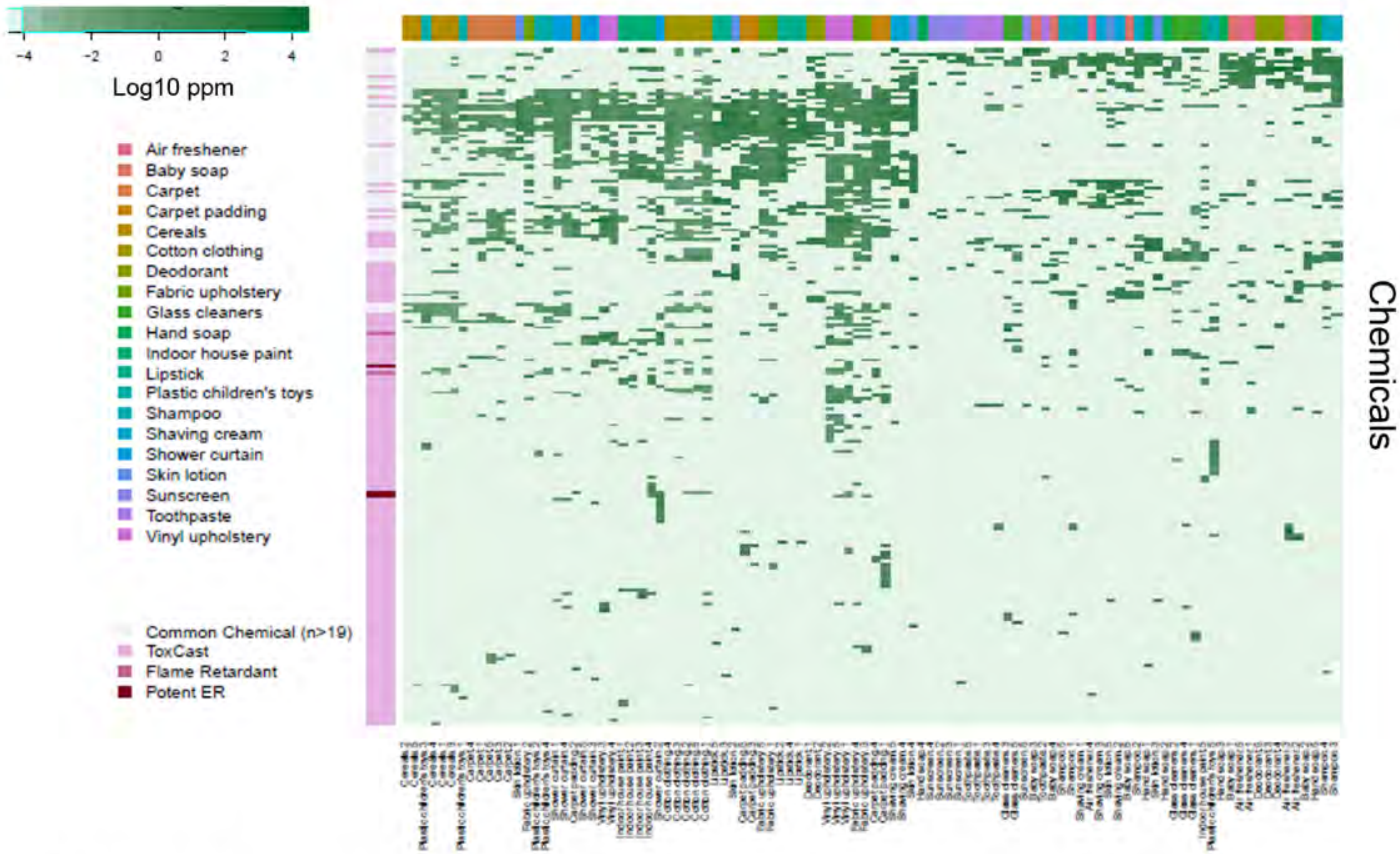
4 product use categories and production volume explain ~50% of the variance in the NHANES data

Comparing Bioactivity and Exposure with Uncertainty

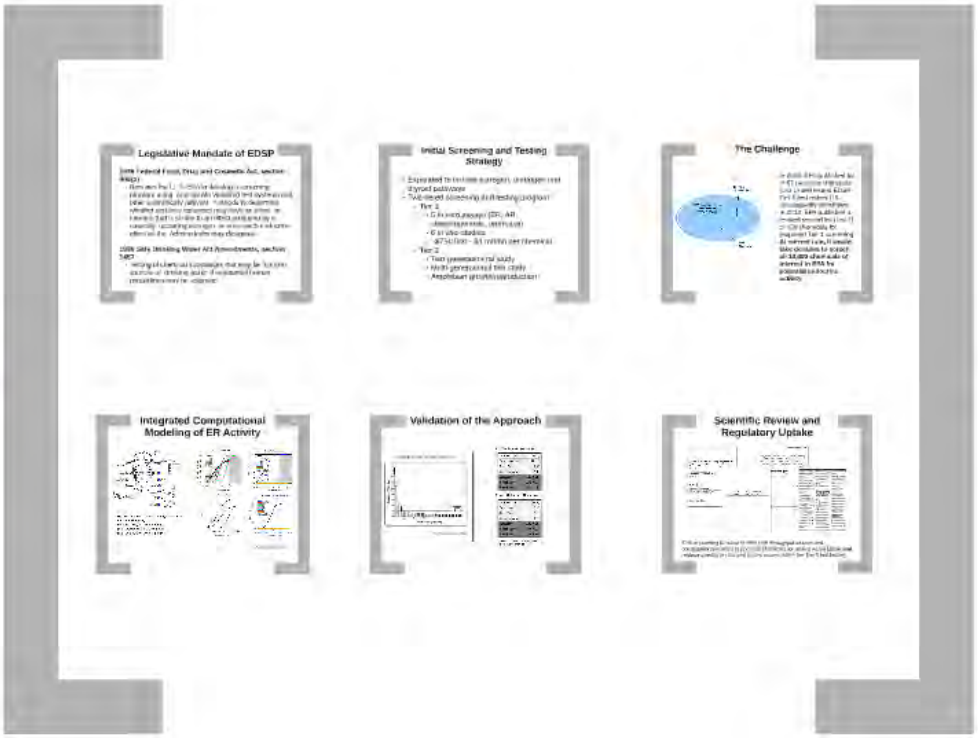


Wetmore *et al., Tox Sci.*, 2015

Non-Targeted Screening to Improve Exposure Models



- Screened 20 consumer produces (5 brands/product) with GCxGC/MS
- Semi-quantitative using internal standards
- Hundreds of unique compounds identified within specific product class (e.g., 306 in baby products).
- Bisphenol A found in “BPA free product”



Translation

Legislative Mandate of EDSP

1996 Federal Food, Drug and Cosmetic Act, section 408(p)

- Requires the U. S. EPA to develop a screening program using appropriate validated test systems and other scientifically relevant methods to determine whether certain substances may have an effect in humans that is similar to an effect produced by a naturally occurring estrogen, or other such endocrine effect as the Administrator may designate.

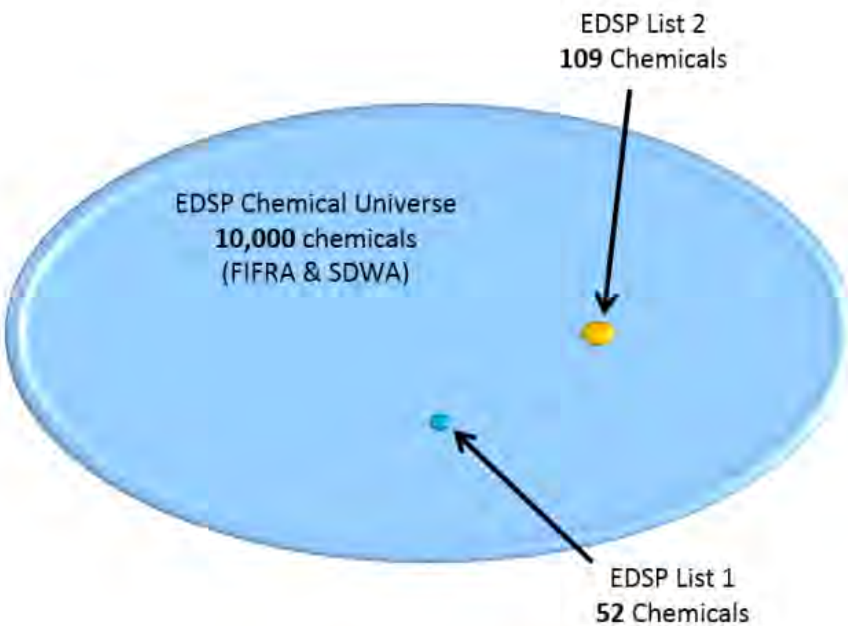
1996 Safe Drinking Water Act Amendments, section 1457

- Testing of chemical substances that may be found in sources of drinking water, if substantial human populations may be exposed.

Initial Screening and Testing Strategy

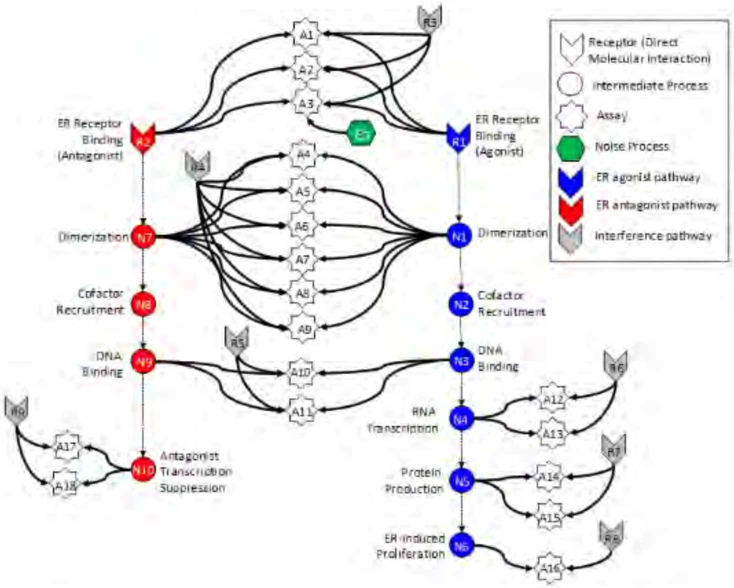
- Expanded to include estrogen, androgen and thyroid pathways
- Two-tiered screening and testing program
  - Tier 1
    - 5 *in vitro* assays (ER, AR, steroidogenesis, aromatse)
    - 6 *in vivo* studies
    - ~$750,000 - $1 million per chemical
  - Tier 2
    - Two-generation rat study
    - Multi-generational fish study
    - Amphibian growth/reproduction

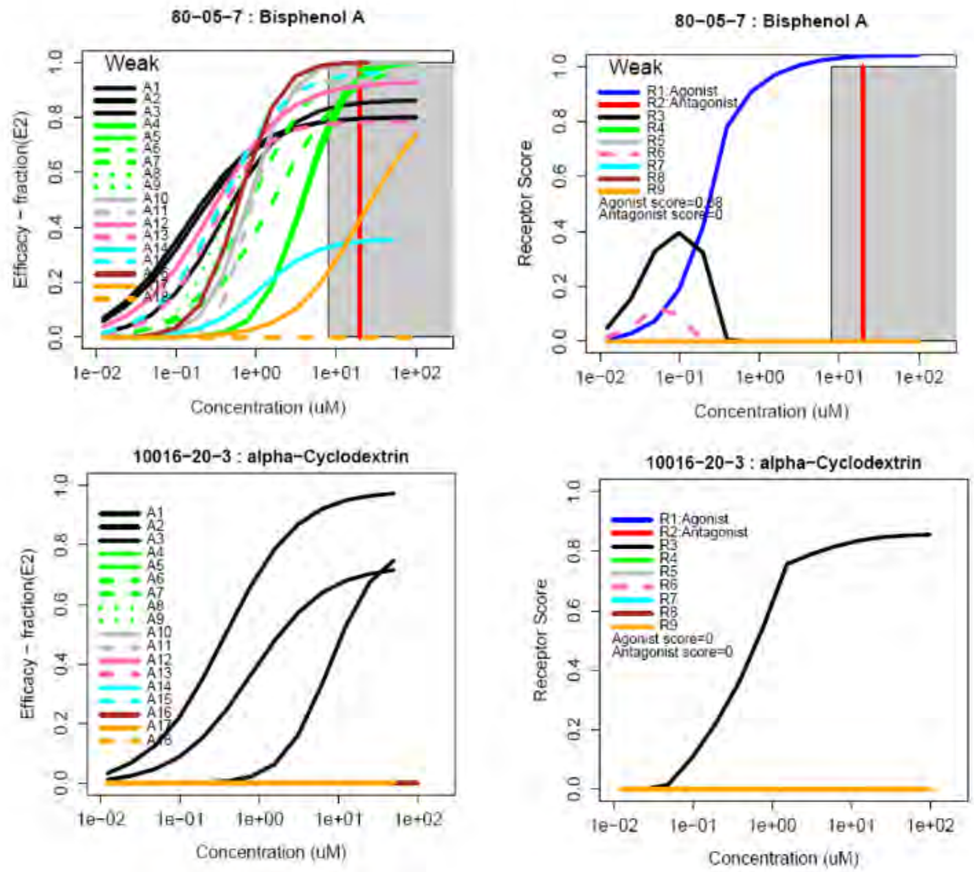
The Challenge



- In 2009, EPA published list of 67 pesticide chemicals (List 1) and issued EDSP Tier 1 test orders (15 subsequently withdrawn)
- In 2013, EPA published a revised second list (List 2) of 109 chemicals for proposed Tier 1 screening
- **At current rate, it would take decades to screen all 10,000 chemicals of interest to EPA for potential endocrine activity**

Integrated Computational Modeling of ER Activity



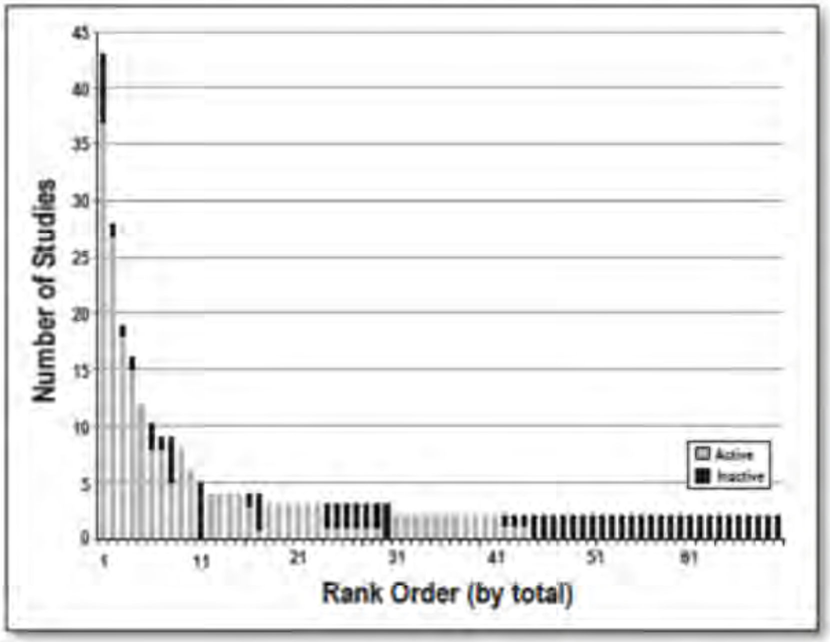


- 18 ToxCast/Tox21 *in vitro* assays measure ER-related activity
- Screened ~2,000 total chemicals
- 38 *in vitro* reference chemicals
- 39 *in vivo* reference chemicals

Judson et al., 2015

Validation of the Approach

Curation of the In Vivo Studies



Kleinstreuer et al.,2016

In Vitro Reference Chemicals*

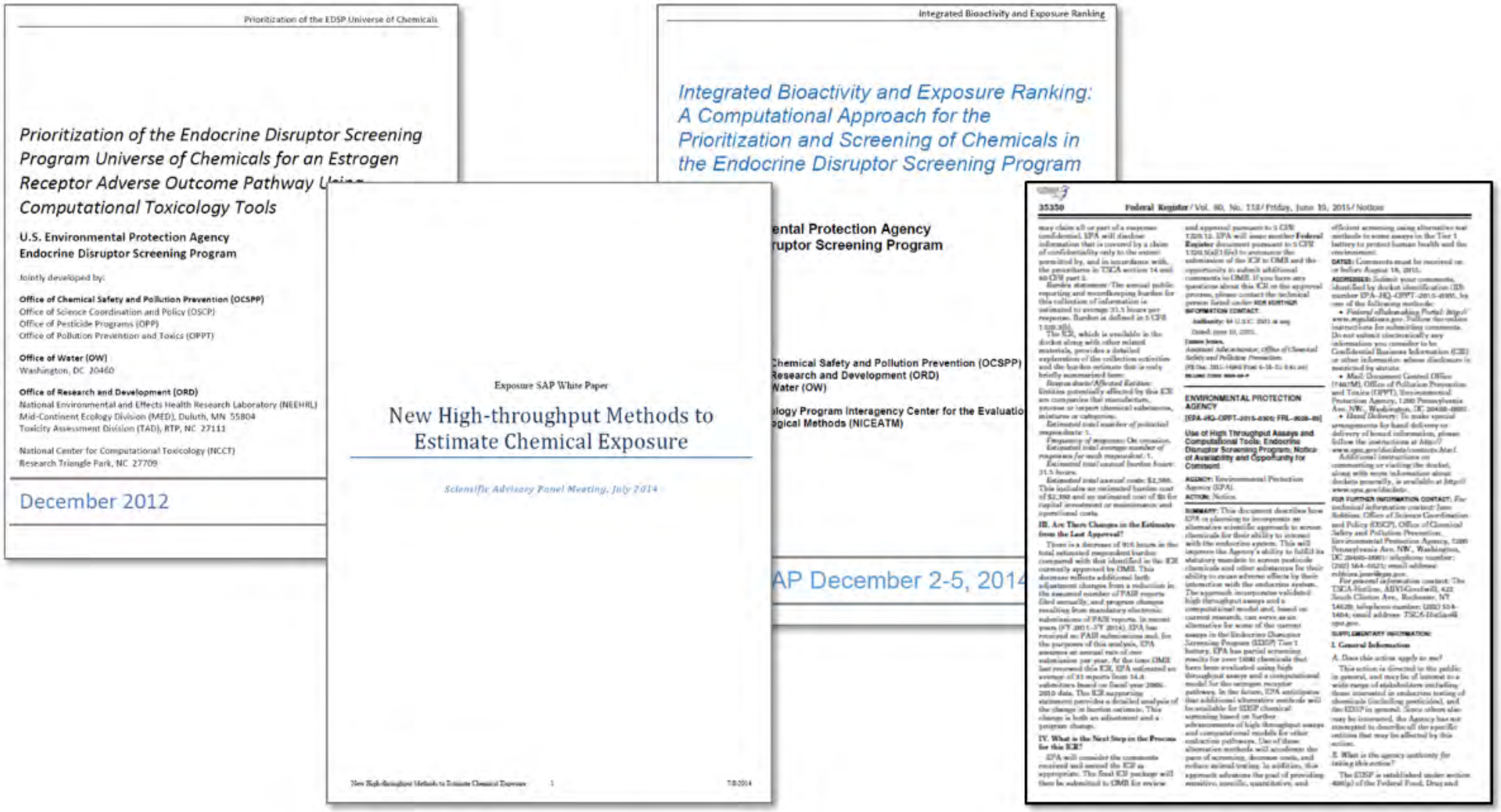
True Positive	26 (25)
True Negative	11 (11)
False Positive	1 (0)
False Negative	2 (2)
Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

In Vivo Reference Chemicals*

True Positive	29 (29)
True Negative	8 (8)
False Positive	5 (1)
False Negative	1 (1)
Accuracy	**0.86 (0.95)**
Sensitivity	**0.97 (0.97)**
Specificity	**0.67 (0.89)**

*Values in parentheses exclude inconclusive chemicals

Scientific Review and Regulatory Uptake



EPA is planning to adopt *in vitro* high throughput assays and computational models to prioritize chemicals for testing within EDSP **and** replace specific *in vitro* and *in vivo* assays within the Tier 1 test battery

Remaining Challenges

- Technical limitations/obstacles associated with each technology (e.g., metabolism, volatiles, etc.)
- Moving from an apical to a molecular paradigm and defining adversity
- Predicting human safety vs. toxicity
- Combining new approaches to have adequate throughput and sufficiently capture higher levels of biological organization
- Systematically integrating multiple data streams from the new approaches in a risk-based, weight of evidence assessment
- Quantifying and incorporating uncertainty and variability
- Dealing with the “V” word
    - Defining a fit-for-purpose framework(s) that is time and resource efficient
    - Role of in vivo rodent studies and understanding their inherent uncertainty
- Legal defensibility of new methods

Acknowledgements