EPA United States Environmental Protection Agency

# Using Computational Toxicology to Enable Risk-Based Chemical Safety Decision Making

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CSS Communities of Practice

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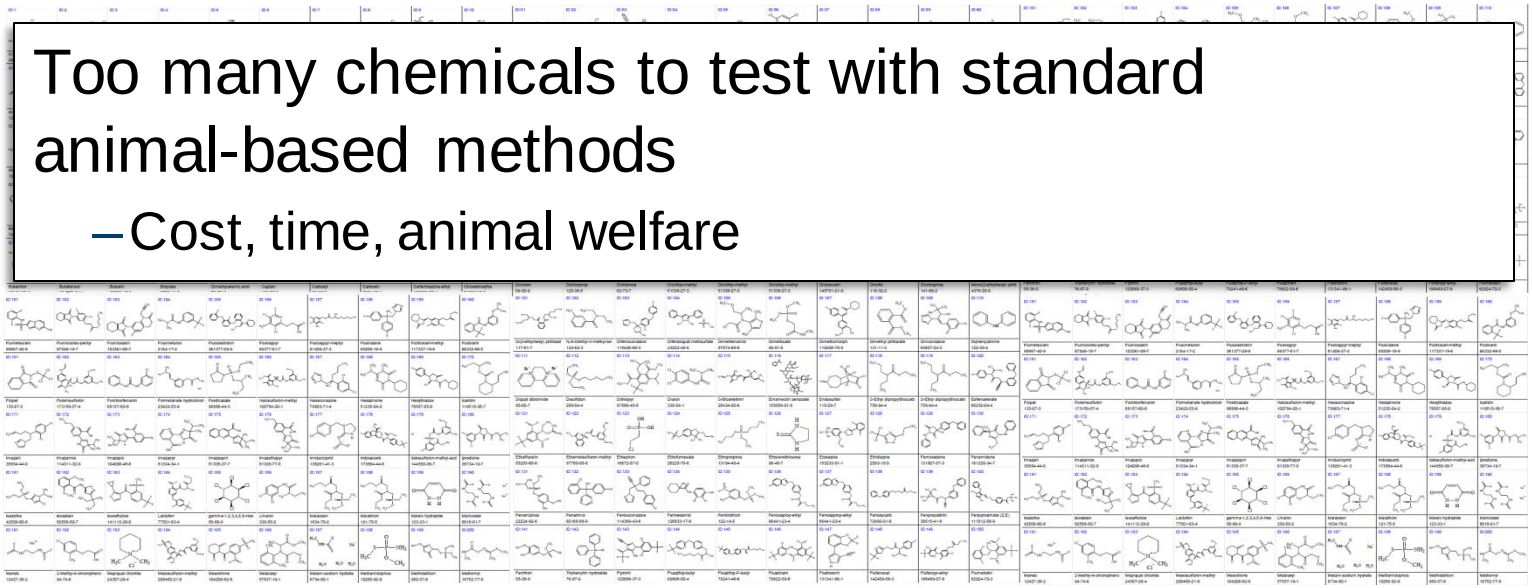
# Problem Statement

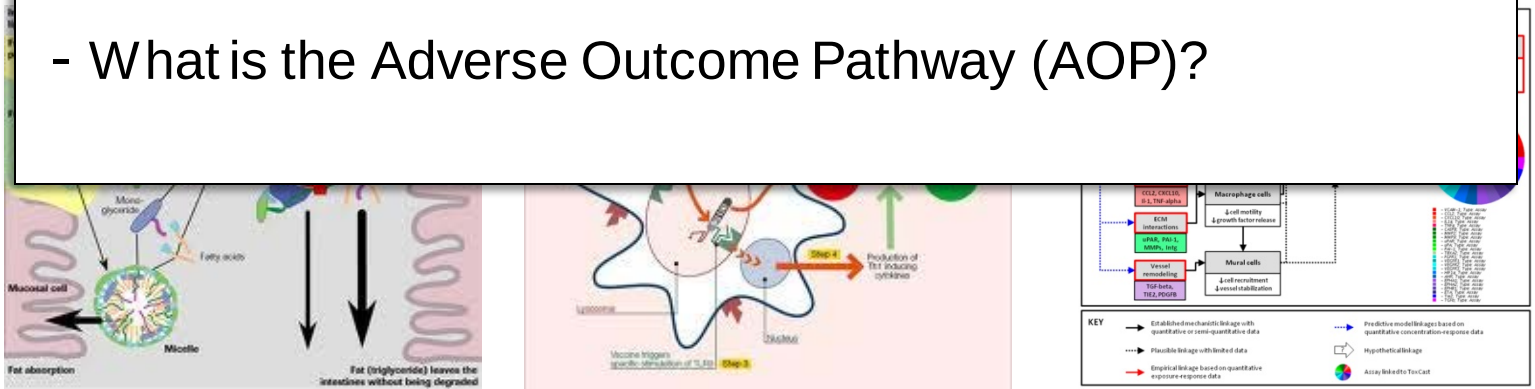
Too many chemicals to test with standard animal-based methods

- Cost, time, animal welfare



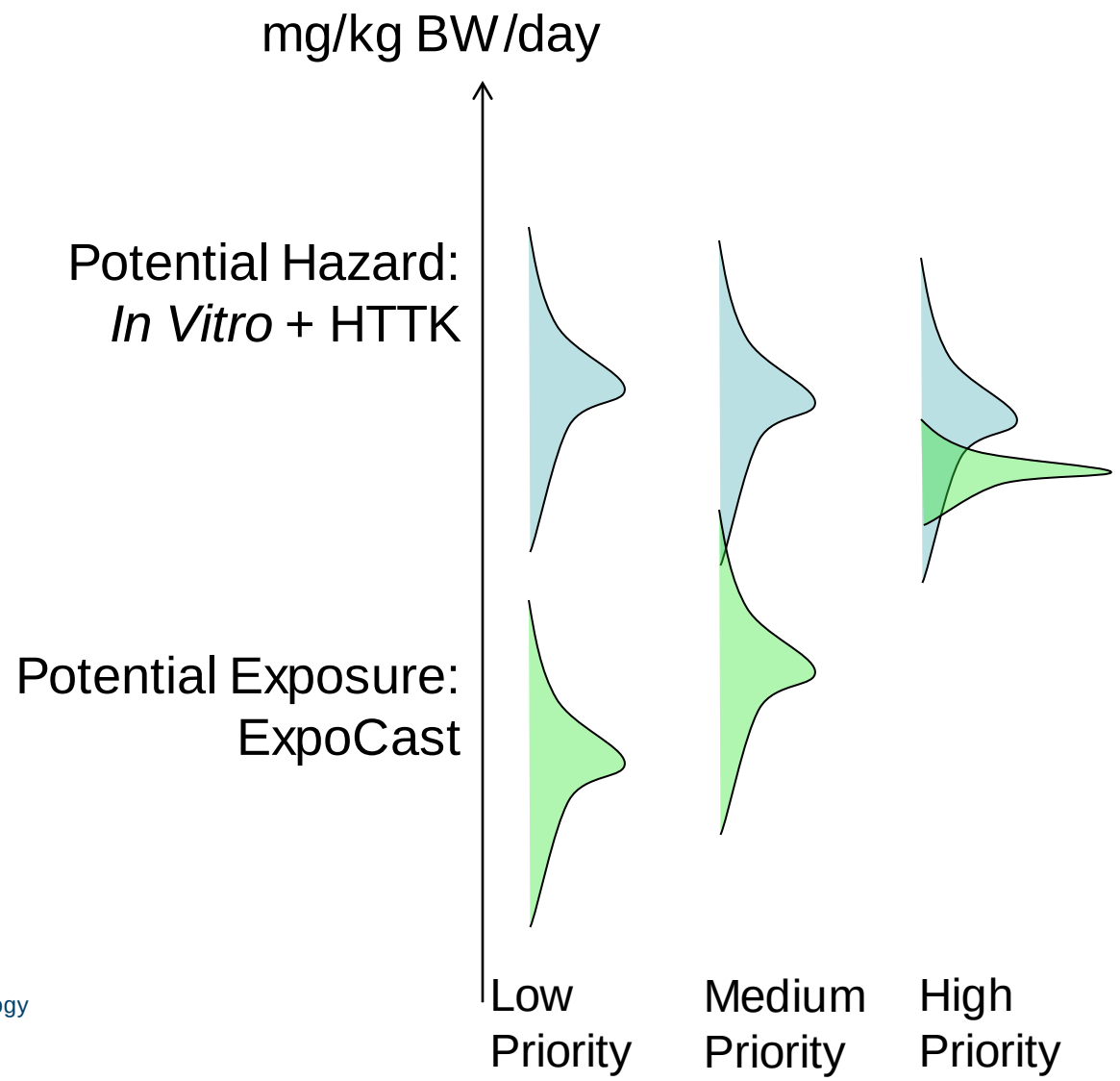
Need for better mechanistic data

- Determine human relevance
- What is the Adverse Outcome Pathway (AOP)?



# Risk-based Prioritization
# Hazard + Exposure

Semi-quantitative
*In Vitro* to *In Vivo*
Approach

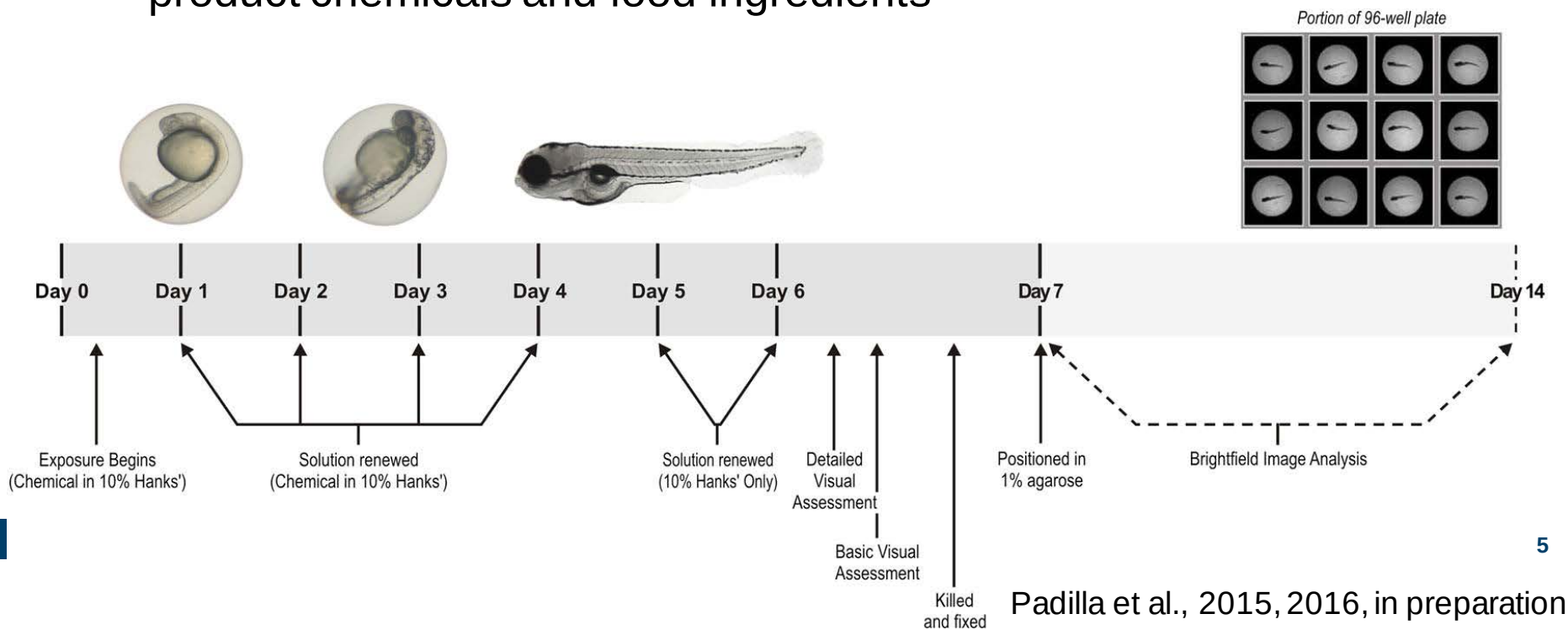


# Computational Toxicology

- Identify biological pathways of toxicity (AOPs)
- Develop high-throughput *in vitro* assays
  - Test "Human Exposure Universe" chemicals in the assays
- Develop models that link *in vitro* to *in vivo* hazard
  - Use pharmacokinetic models to predict activating doses
- Develop exposure models
- Add uncertainty estimates
- Create high-throughput risk assessments

# Zebrafish and Developmental Toxicology

- Goal: Use zebrafish as an *in vivo* model of vertebrate developmental toxicity
- Build *in vitro* to *in vivo* models using ~700 human assays
- ~1000 Chemicals
  - pharmaceuticals, pesticides, industrial chemicals, personal care product chemicals and food ingredients

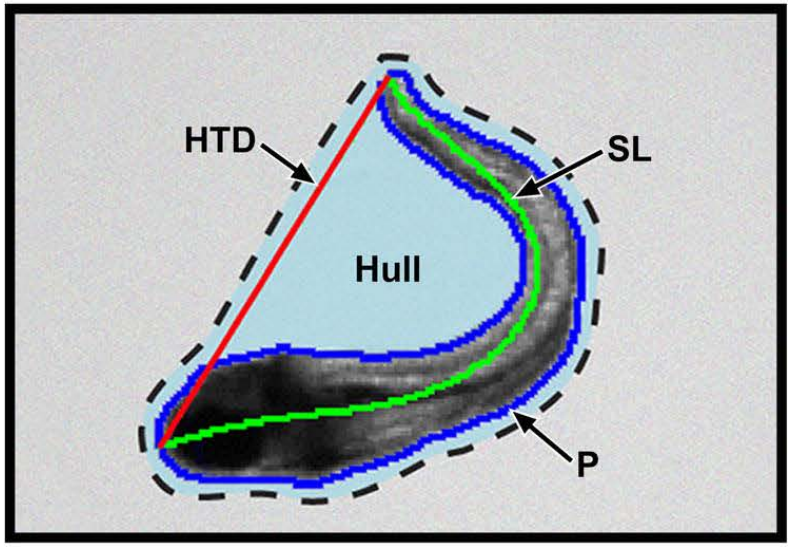





Padilla et al., 2015, 2016, in preparation

# Zebrafish Imaging and scoring

A

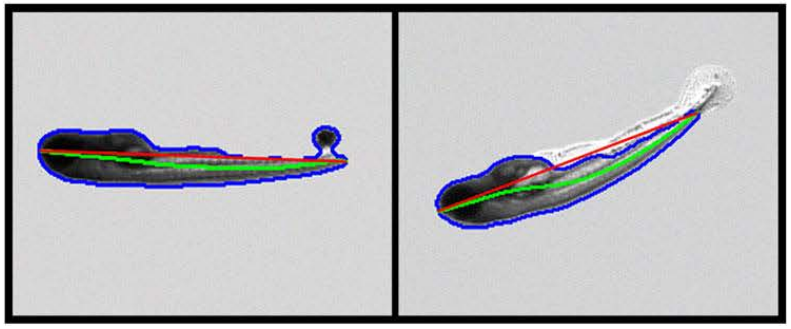



B

| Parameter | Description |
|---|---|
| Area | Area within the mask drawn around the fish, calculated as pixel count or micrometers |
| Perimeter–area (P) | A ratio of the outer perimeter of the fish to the area |
| SL | A line drawn approximately down the middle of the fish from the tip of the larvae's head to the tip of its tail |
| Width | The maximum distance perpendicular to the Spine Length |
| Length–width ratio | A ratio of SL to width |
| HTD | A direct line drawn from the tip of the larvae's head to the tip of the tail |
| Straightness | A ratio of HTD to SL |
| Convexity | A ratio of the fish area to the area of the hull |

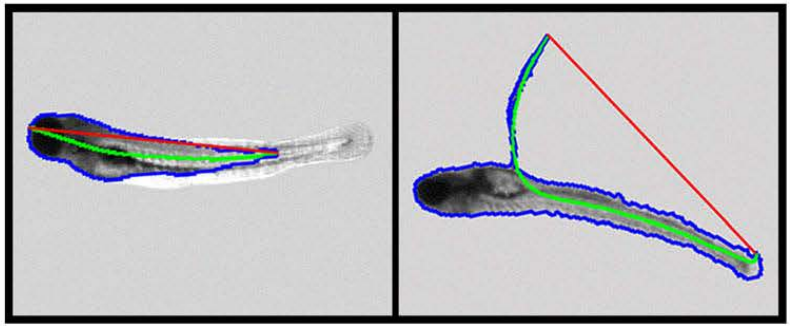
C

Acceptable



D

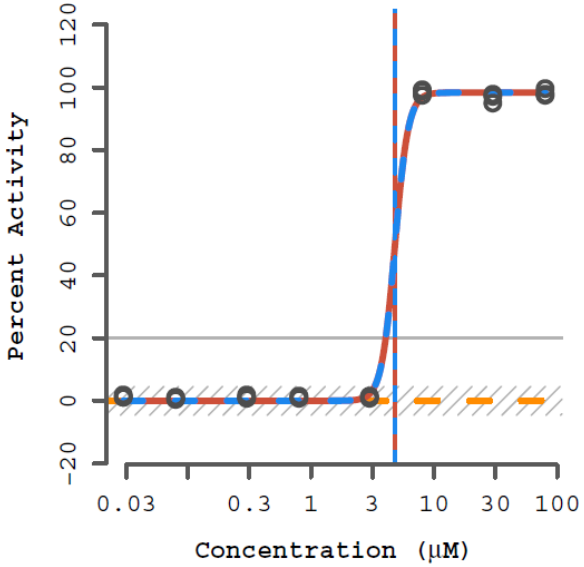
Unacceptable



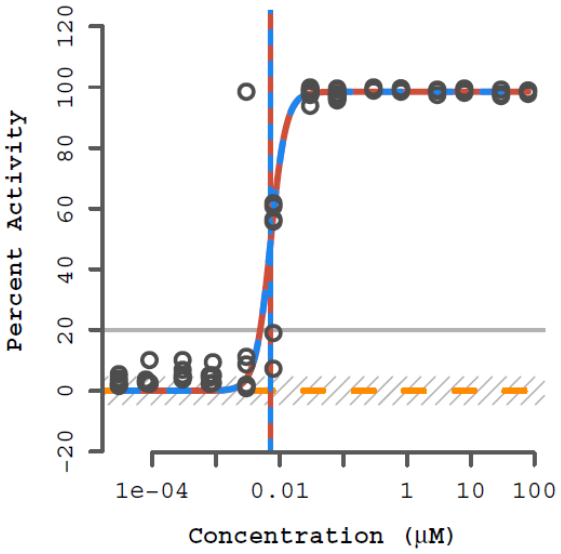
Deal et al. J Applied Tox. 2016

# Example chemicals

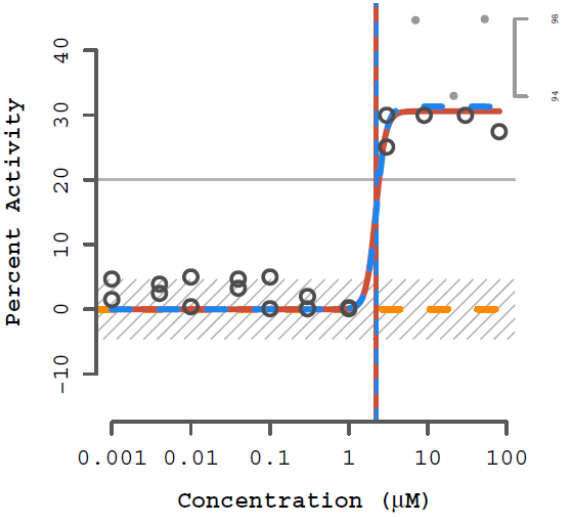
DES




Lovastatin

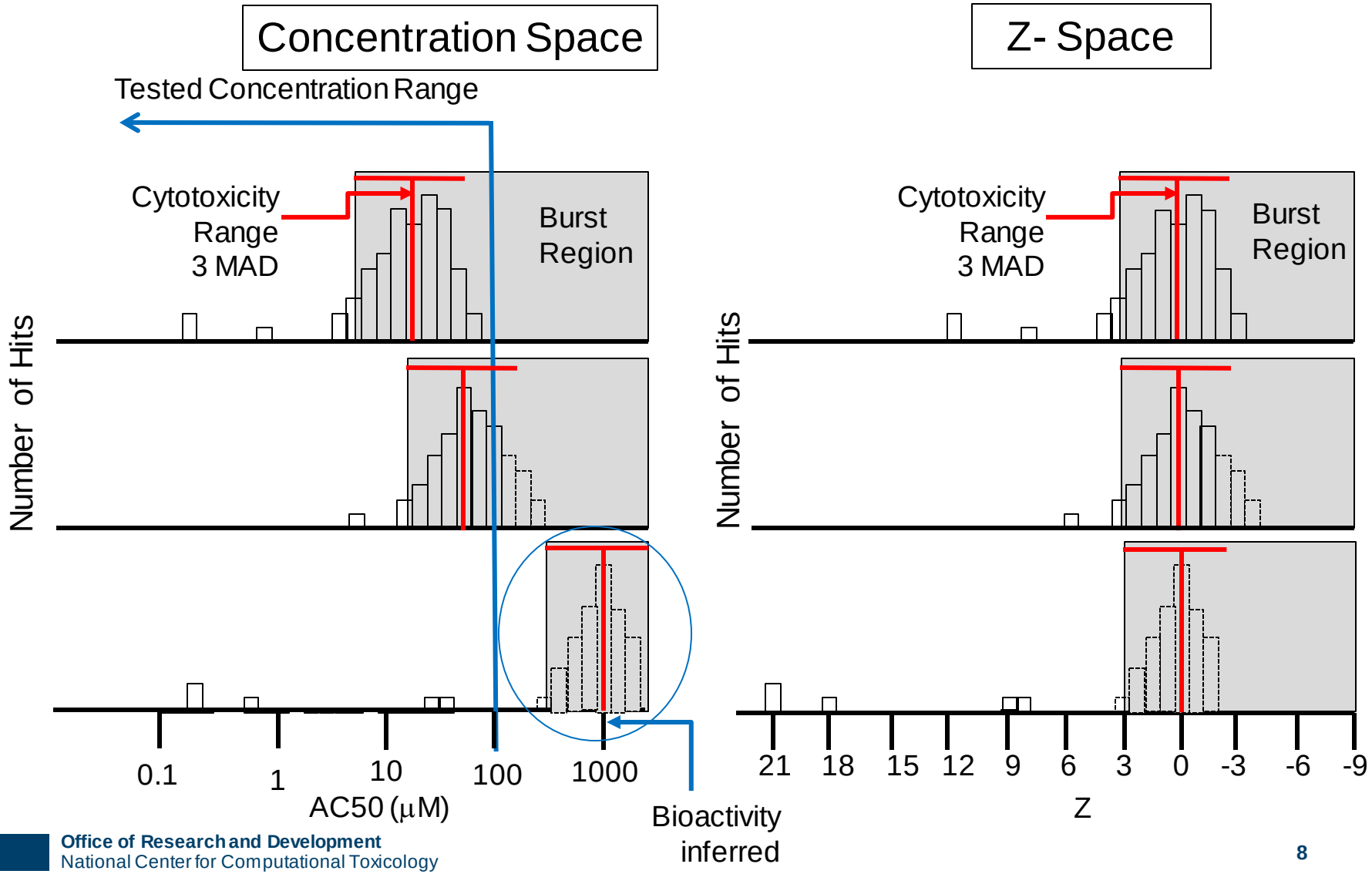



Permethrin




100% = death
<100% = malformations

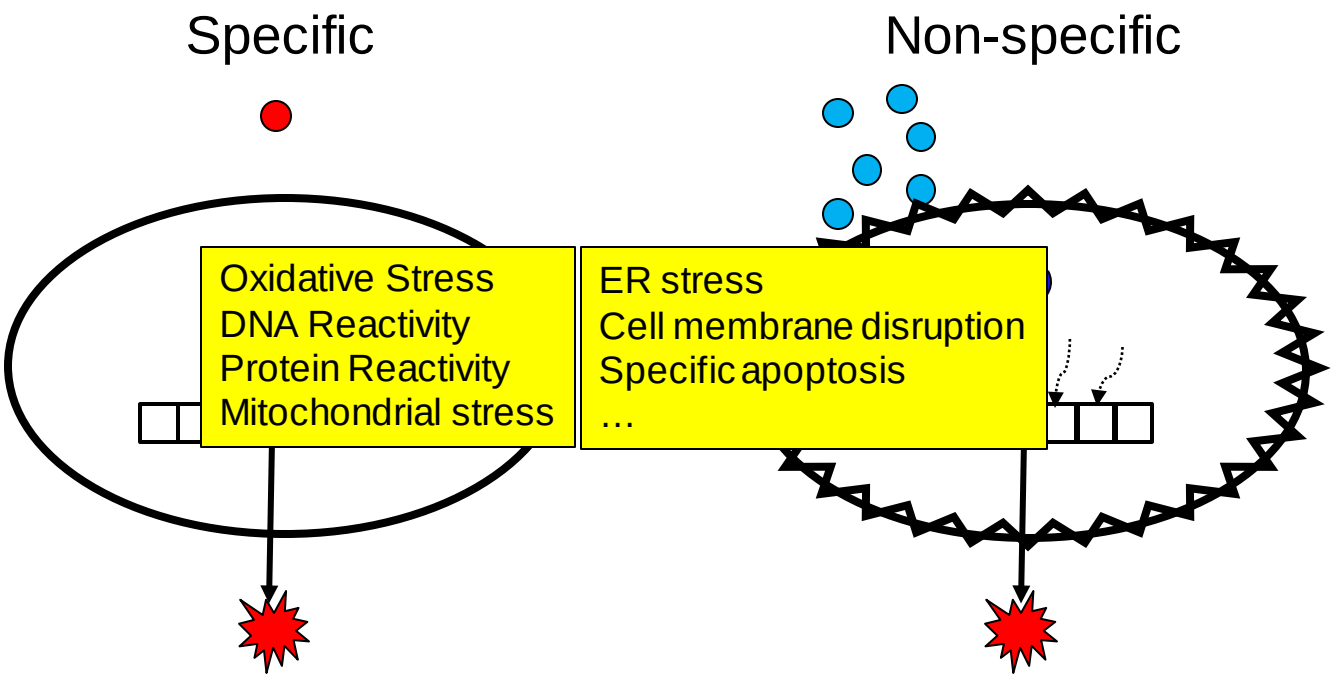
# Most chemicals display a "burst" of potentially non-selective bioactivity near cell-stress / cytotoxity conc.



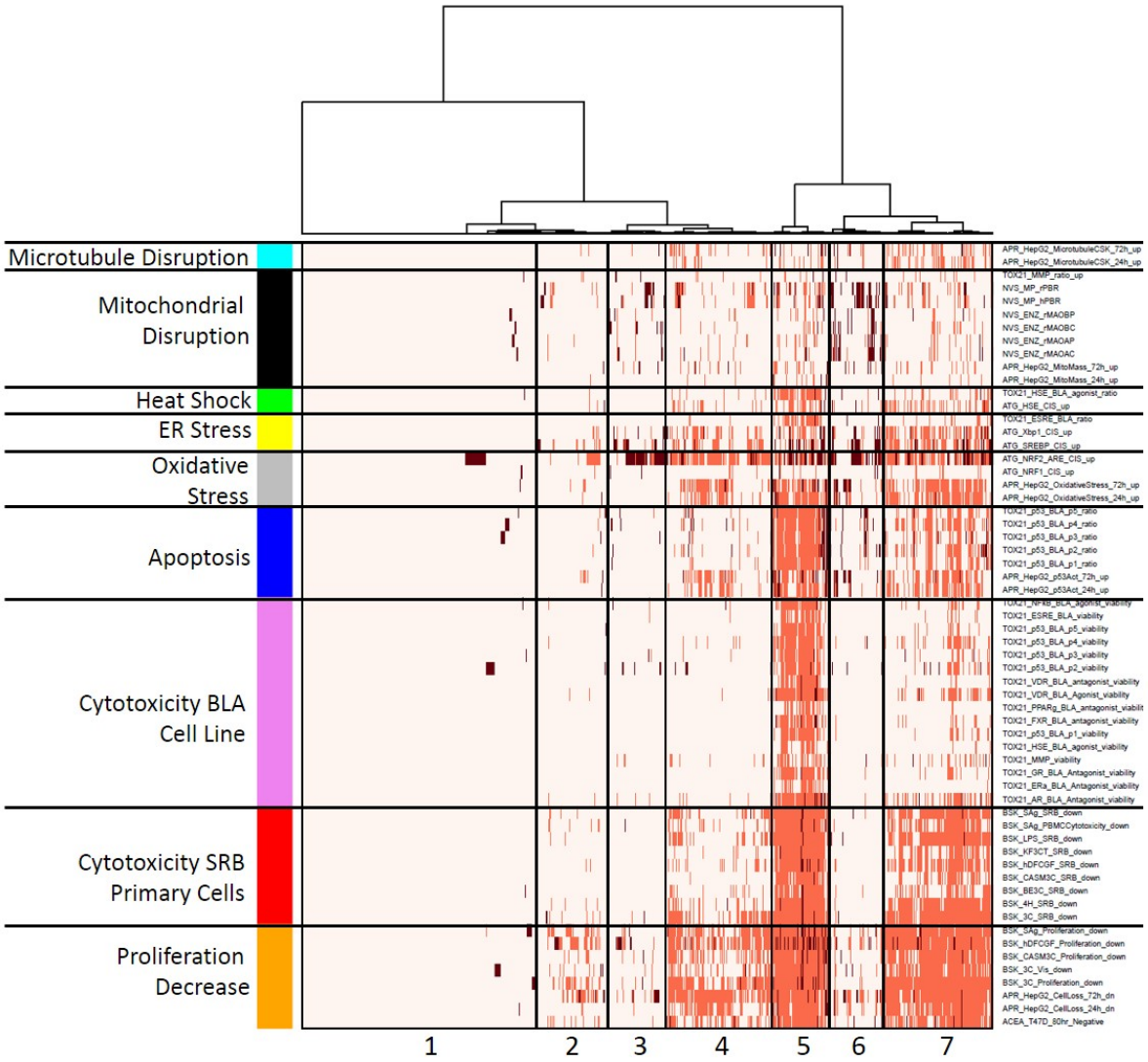



Judson et al. Tox.Sci. (2016)

# Schematic explanation of the burst



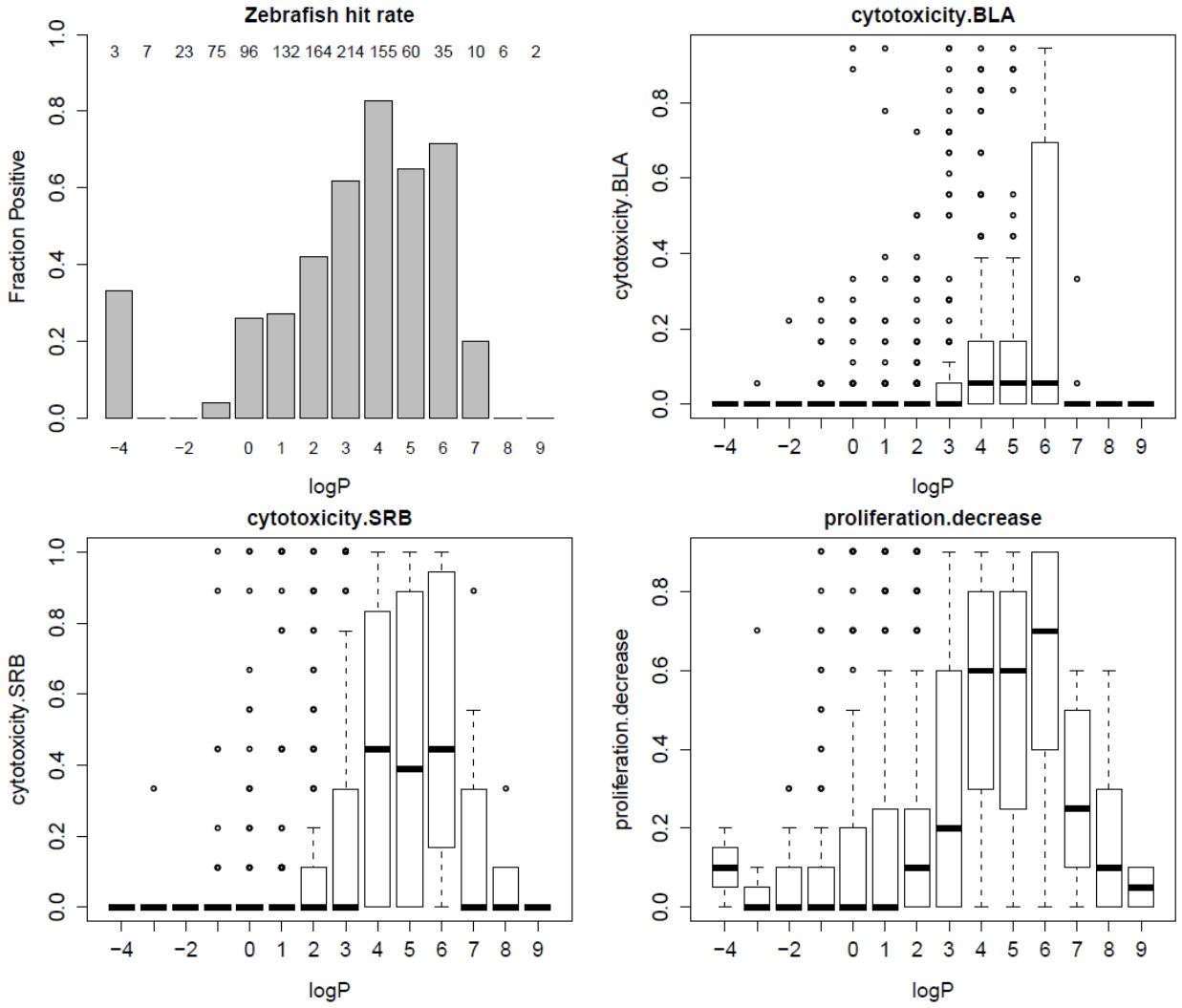
# Heatmap of stress and cytotoxicity assays in 1000 chemicals



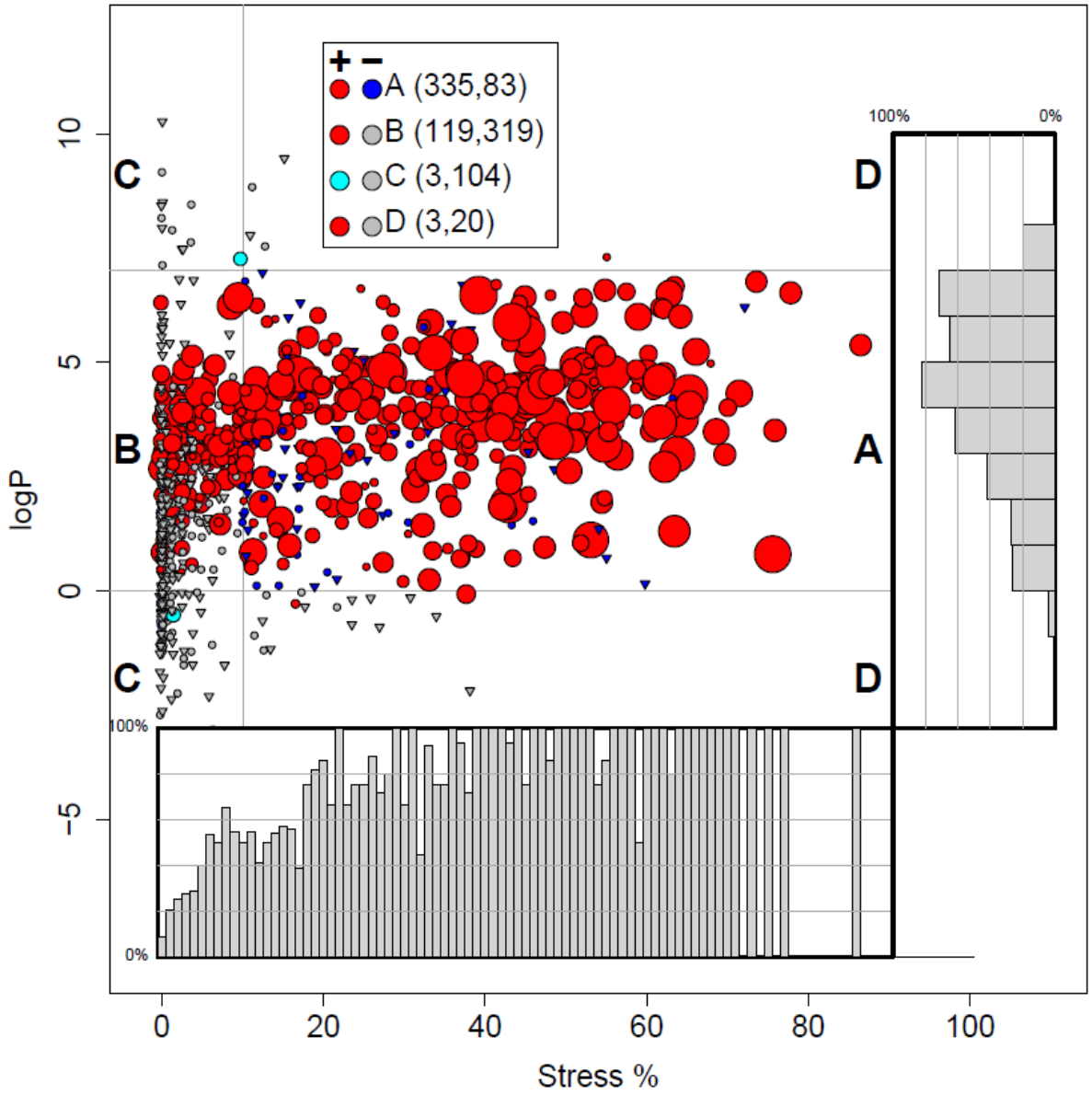

Judson et al. ToxSci (2016)

# Observation about logP

## Human *in vitro* cell stress behaves ~ zebrafish toxicity



# Stress, logP explains ~80% of ZF activity




ZF positive in conc-response

ZF negative in conc-response

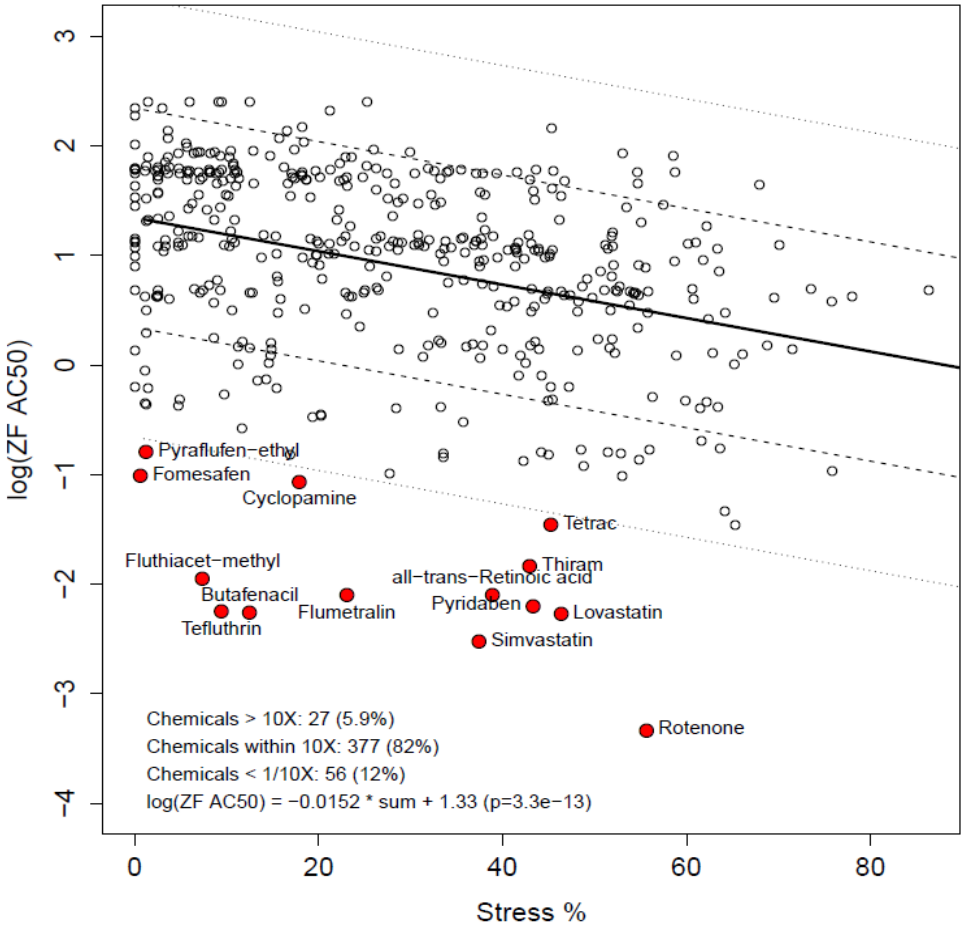
ZF negative in single conc

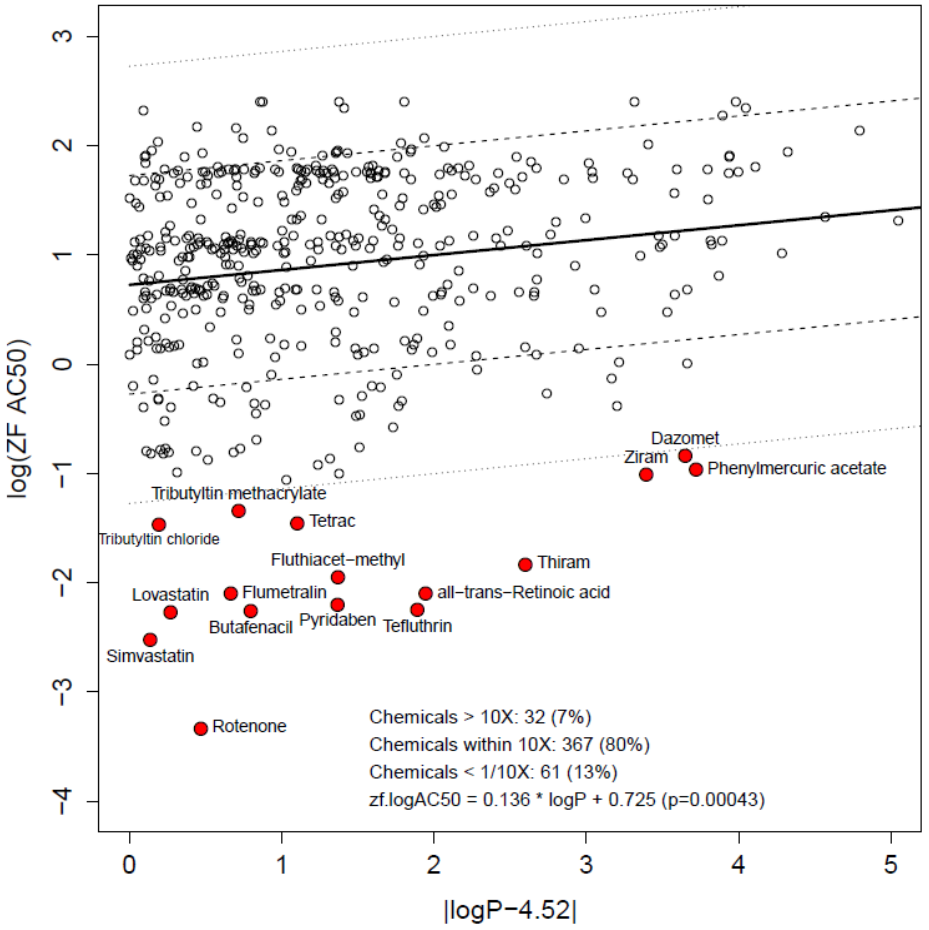
- 83 negatives in region A
  - Blue triangles
  - “false positives”?
- 50 “failed” single screen test?



Judson et al. In preparation

# “Excess Toxicity” points to specific target activity



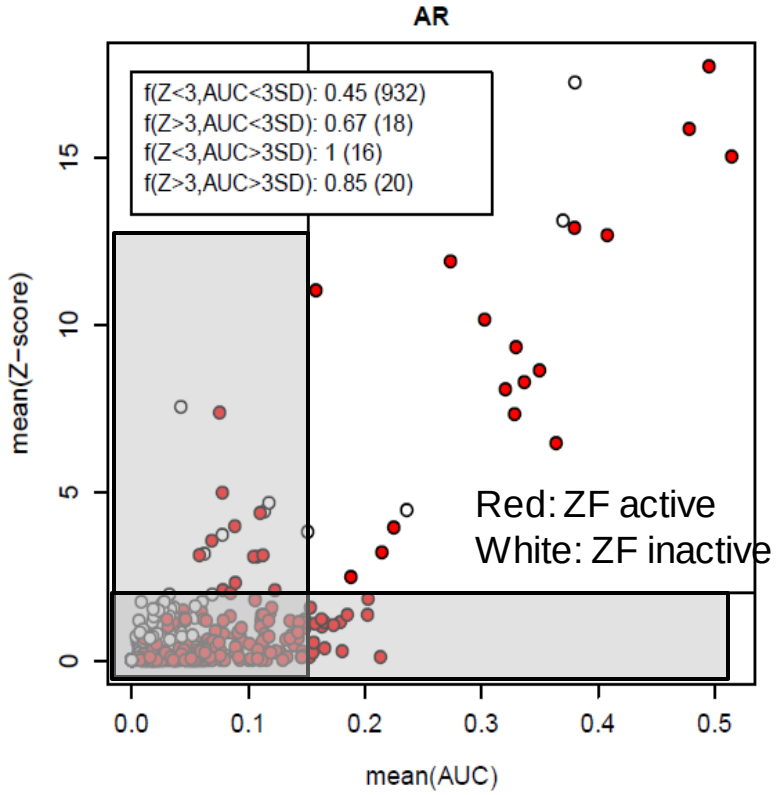
# Chemicals with excess toxicity tend to fall in a few target MOA classes

- ACHE
- Ion channel blockers
- HMGCR
- Mitochondrial disruptors
- PPO inhibitors (disrupts plant cell membranes)
- Chemicals reacting with protein SH groups
- Thyroid hormone receptor blockers

- Some of these classes are over-represented in overall hit predictivity and in excess potency for hits

# Look for specific targets by controlling for stress-related assay confounding

- Are potent actives against specific targets more likely than chance to be ZF-active?




Filter on Z-score (AC50 relative to cytotoxicity)

Filter on AUC (potency x efficacy)
Measure of reproducibility across multiple assays

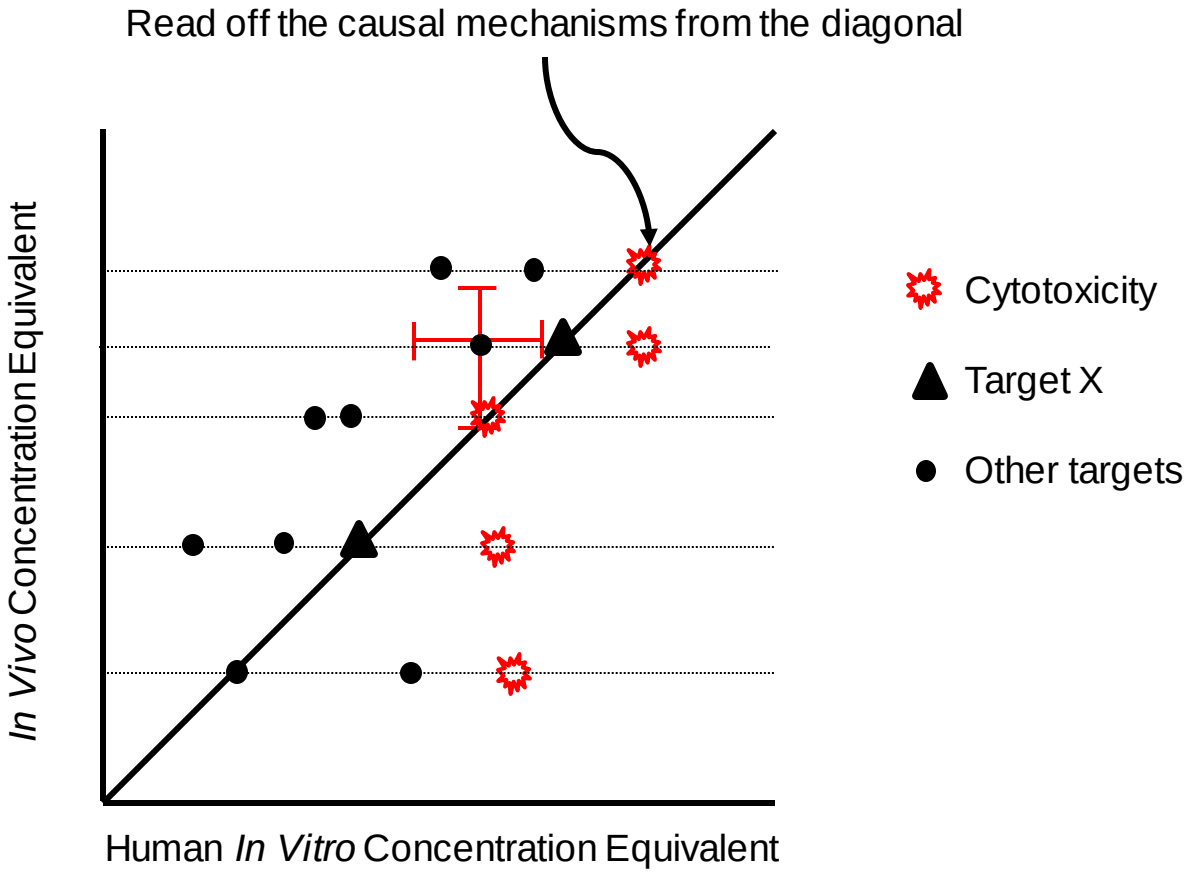
| class | Gene group | annotation | assays | TP | FP | FN | TN | Sens | Spec | BA | OR | PPV | p-value |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| endocrine | AR | Androgen receptor | 11 | 17 | 3 | 443 | 523 | 0.04 | 0.99 | 0.52 | 6.7 | 0.85 | 0.0005 |
| endocrine | CYP19A1 | Aromatase | 2 | | | | | | | 0.52 | 14.4 | 0.92 | 9E-07 |
| endocrine | ESR | Estrogen receptor | 1 | | | | | | | 0.53 | 5.8 | 0.83 | 2E-05 |
| endocrine | NR3C1 | Glucocorticoid receptor | 4 | 14 | 4 | 446 | 522 | 0.03 | 0.99 | 0.51 | 4.1 | 0.78 | 0.0084 |
| endocrine | PGR | Progesterone receptor | 2 | 15 | 3 | 445 | 523 | 0.03 | 0.99 | 0.51 | 5.9 | 0.83 | 0.0016 |
| ER stress | SREBF1 | | 1 | 36 | 10 | 424 | 516 | 0.08 | 0.98 | 0.53 | 4.4 | 0.78 | 1E-05 |
| ER stress | XBP1 | | 1 | 10 | 1 | 450 | 525 | 0.02 | 1.00 | 0.51 | 11.7 | 0.91 | 0.0039 |
| GPCR | LTD4 | | 1 | 11 | 1 | 449 | 525 | 0.02 | 1.00 | 0.51 | 12.9 | 0.92 | 0.002 |
| growth factor | EGR1 | | 1 | 19 | 1 | 441 | 525 | 0.04 | 1.00 | 0.52 | 22.6 | 0.95 | 8E-06 |
| hypoxia | HIF1A | | 1 | 24 | 3 | 436 | 523 | 0.05 | 0.99 | 0.52 | 9.6 | 0.89 | 5E-06 |
| inflammation | CEBPB | | 1 | 30 | 6 | 430 | 520 | 0.07 | 0.99 | 0.53 | 6.0 | 0.83 | 5E-06 |
| inflammation | CREB3 | | 1 | 23 | 1 | 437 | 525 | 0.05 | 1.00 | 0.52 | 27.6 | 0.96 | 5E-07 |
| inflammation | PTGER2 | | 1 | | | | | | | | 5.0 | 0.81 | 3E-05 |
| inflammation | TNF | | 1 | | | | | | | | 2.8 | 0.70 | 0.0026 |
| ion channel | KCNH2 | | 1 | | | | | | | | 7.6 | 0.87 | 0.0026 |
| oncogene | JUN | | 1 | 18 | 6 | 442 | 520 | 0.04 | 0.99 | 0.51 | 3.5 | 0.75 | 0.0062 |
| oxidative stress | NFE2L2 | NRF2, ROS Sensor | 2 | 34 | 5 | 426 | 521 | 0.07 | 0.99 | 0.53 | 8.3 | 0.87 | 1E-07 |
| transcription factor | POU2F1 | | 1 | 17 | 4 | 443 | 522 | 0.04 | 0.99 | 0.51 | 5.0 | 0.81 | 0.0016 |
| transcription factor | SMAD1 | | 1 | 21 | 5 | 439 | 521 | 0.05 | 0.99 | 0.52 | 5.0 | 0.81 | 0.0005 |
| transcription factor | SOX1 | | 1 | 16 | 5 | 444 | 521 | 0.03 | 0.99 | 0.51 | 3.8 | 0.76 | 0.0072 |
| transcription factor | SP1 | | 1 | 18 | 2 | 442 | 524 | 0.04 | 1.00 | 0.52 | 10.7 | 0.90 | 6E-05 |
| transporter | DAT | | 1 | 18 | 6 | 442 | 520 | 0.04 | 0.99 | 0.51 | 3.5 | 0.75 | 0.0062 |
| xenobiotic metabolism | CYP1A | cytochrome P450 | 4 | 18 | 3 | 442 | 523 | 0.04 | 0.99 | 0.52 | 7.1 | 0.86 | 0.0003 |
| xenobiotic metabolism | CYP2A | cytochrome P450 | 3 | 25 | 5 | 435 | 521 | 0.05 | 0.99 | 0.52 | 6.0 | 0.83 | 5E-05 |
| xenobiotic metabolism | CYP2B | cytochrome P450 | 2 | 25 | 2 | 435 | 524 | 0.05 | 1.00 | 0.53 | 15.1 | 0.93 | 4E-07 |
| xenobiotic metabolism | CYP2C | cytochrome P450 | 8 | | | | | | | | 1E+06 | 1.00 | 8E-09 |
| xenobiotic metabolism | CYP2D | cytochrome P450 | 3 | | | | | | | | 5.9 | 0.83 | 0.0016 |
| xenobiotic metabolism | CYP2J | cytochrome P450 | 1 | 21 | 1 | 439 | 525 | 0.05 | 1.00 | 0.52 | 25.1 | 0.95 | 2E-06 |
| xenobiotic metabolism | CYP3A | cytochrome P450 | 4 | 19 | 1 | 441 | 525 | 0.04 | 1.00 | 0.52 | 22.6 | 0.95 | 8E-06 |
| xenobiotic metabolism | NR1I2 | PXR | 3 | 30 | 9 | 430 | 517 | 0.07 | 0.98 | 0.52 | 4.0 | 0.77 | 0.0001 |

Endocrine pathways

Largely stress activity:
more potent than cytotoxicity

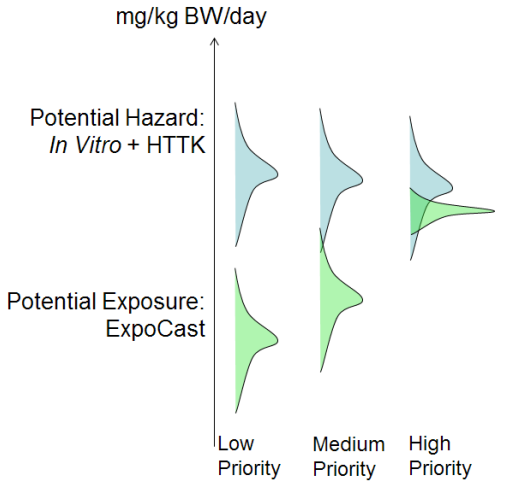
Largely due to conazoles

# The ideal *in vitro* to *in vivo* model
# Zebrafish, rat, mouse, human, …




- Failure so far – concentration equivalents require better understanding of relative kinetics, bioavailability
- Also concentration uncertainty on both axes is ~1 log unit (95% CI)

# Modeling with Uncertainty




- Our first goal is **prediction**
  - What is the highest safe dose of a chemical?
  - What types of harm would a chemical cause above that dose?
- Predictions are based on **models**
  - Computational, statistical, "mental", *in vitro*, *in vivo*
- All models are based on **data**
- Data is always subject to noise, variability
- Therefore, all predictions are subject to **uncertainty**

- Our second goal is estimating **prediction uncertainty**

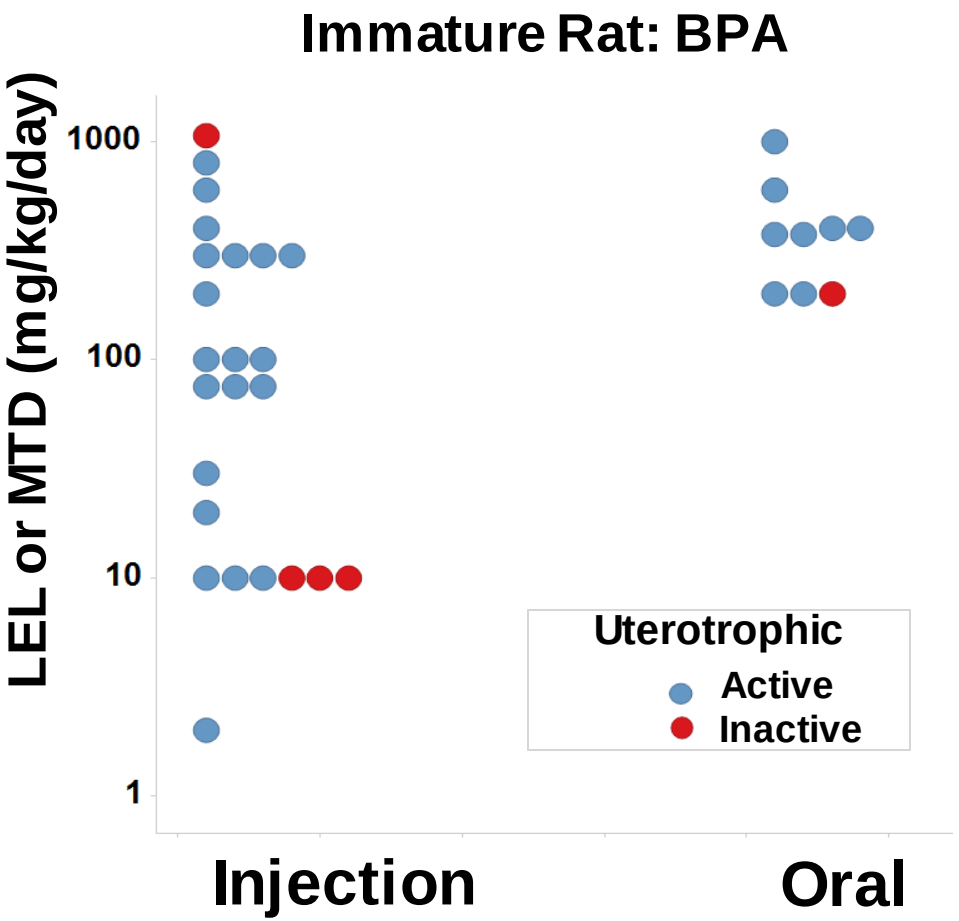


Watt, Kapraun et al. In preparation

# *In vivo* guideline study uncertainty

## 26% of chemicals tested multiple times in the uterotrophic assay gave discrepant results

United States Environmental Protection Agency




Kleinstreuer et al. EHP 2015

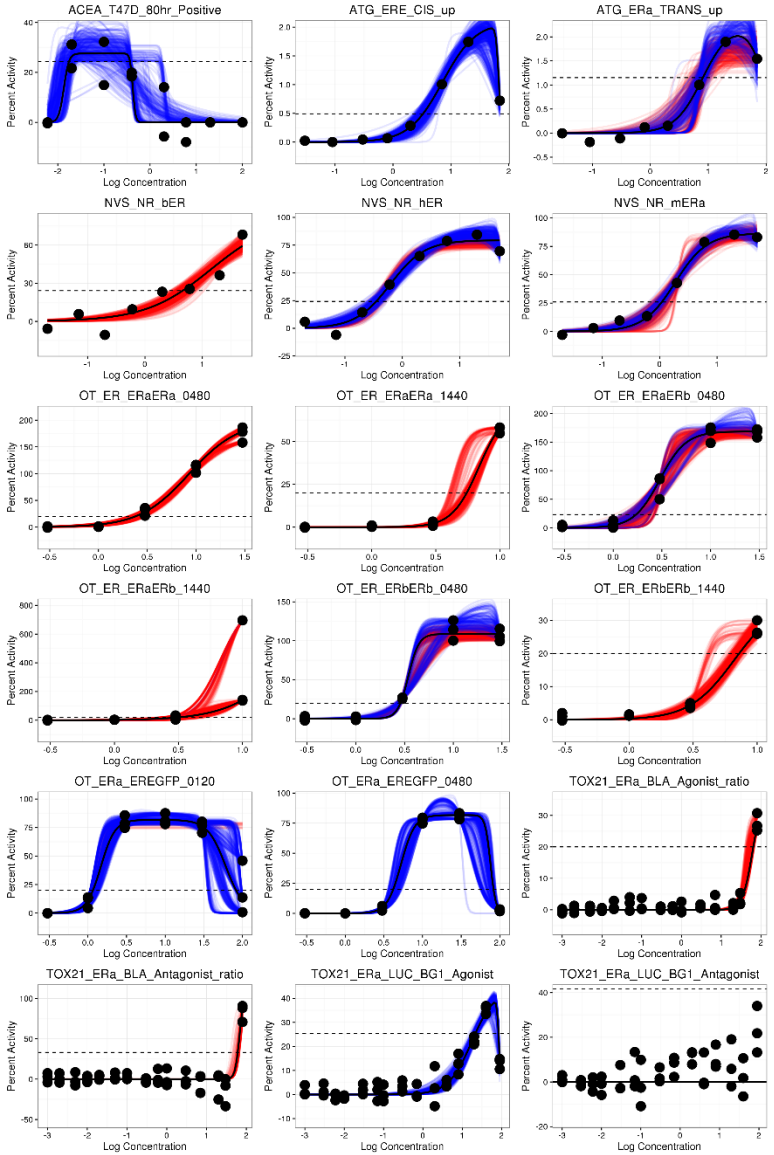
### Anemia Reproducibility

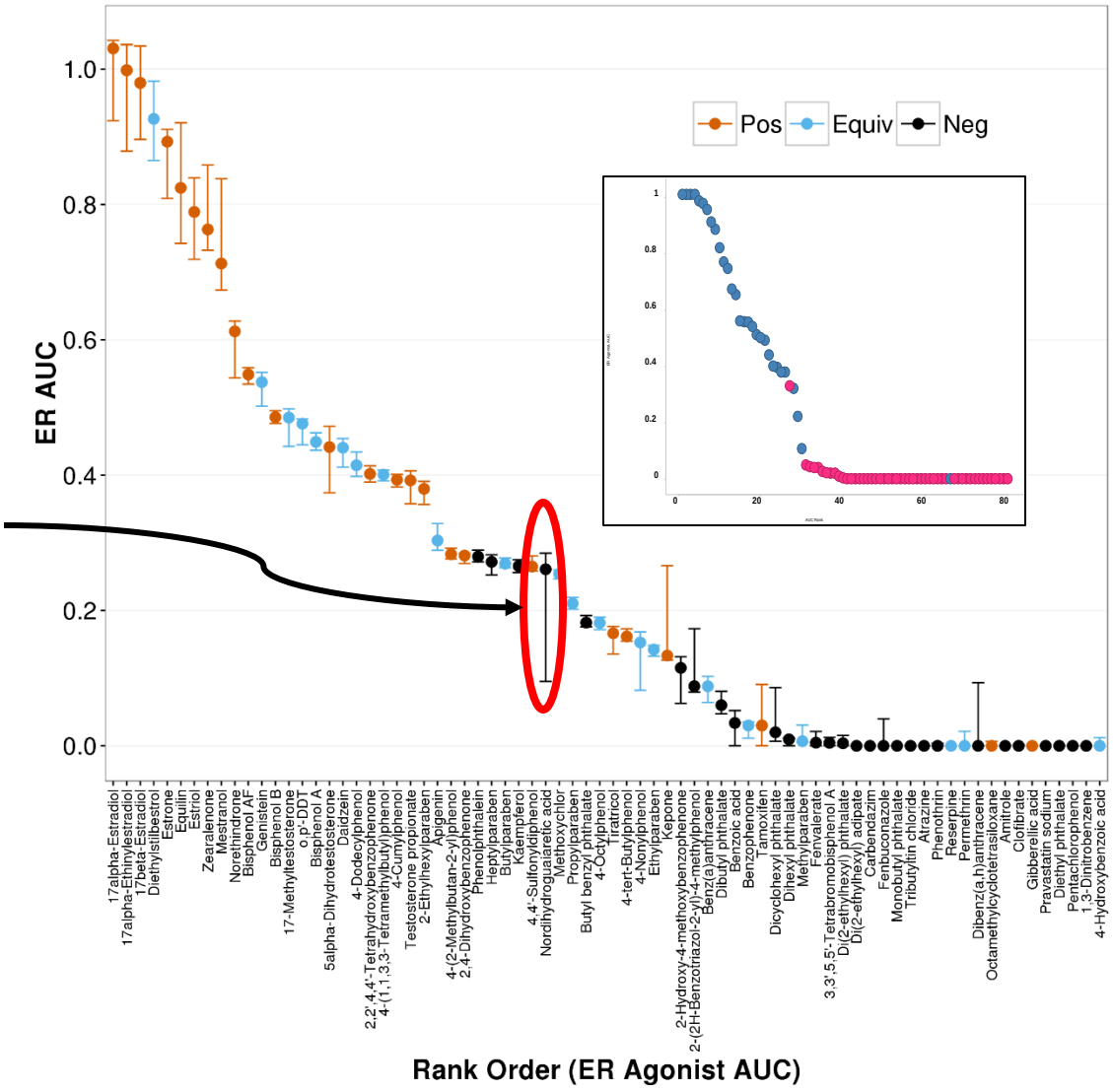
| species / study 1 | species / study 2 | Reproduce | Does Not Reproduce | Fraction Reproduce |
|---|---|---|---|---|
| rat SUB | rat CHR | 18 | 2 | 0.90 |
| rat CHR | dog CHR | 13 | 2 | 0.87 |
| rat CHR | rat SUB | 18 | 4 | 0.82 |
| rat SUB | rat SUB | 16 | 4 | 0.80 |
| rat SUB | dog CHR | 11 | 4 | 0.73 |
| mouse CHR | rat CHR | 11 | 4 | 0.73 |
| mouse CHR | rat SUB | 13 | 7 | 0.65 |
| dog CHR | rat SUB | 11 | 6 | 0.65 |
| dog CHR | rat CHR | 13 | 8 | 0.62 |
| rat CHR | mouse CHR | 11 | 11 | 0.50 |
| mouse CHR | dog CHR | 6 | 6 | 0.50 |
| rat SUB | mouse CHR | 13 | 14 | 0.48 |
| dog CHR | mouse CHR | 6 | 8 | 0.43 |
| mouse CHR | mouse CHR | 2 | 3 | 0.40 |

Judson et al. In Preparation

# *In Vitro* Assay Data is also subject to uncertainty

## See Eric Watt poster



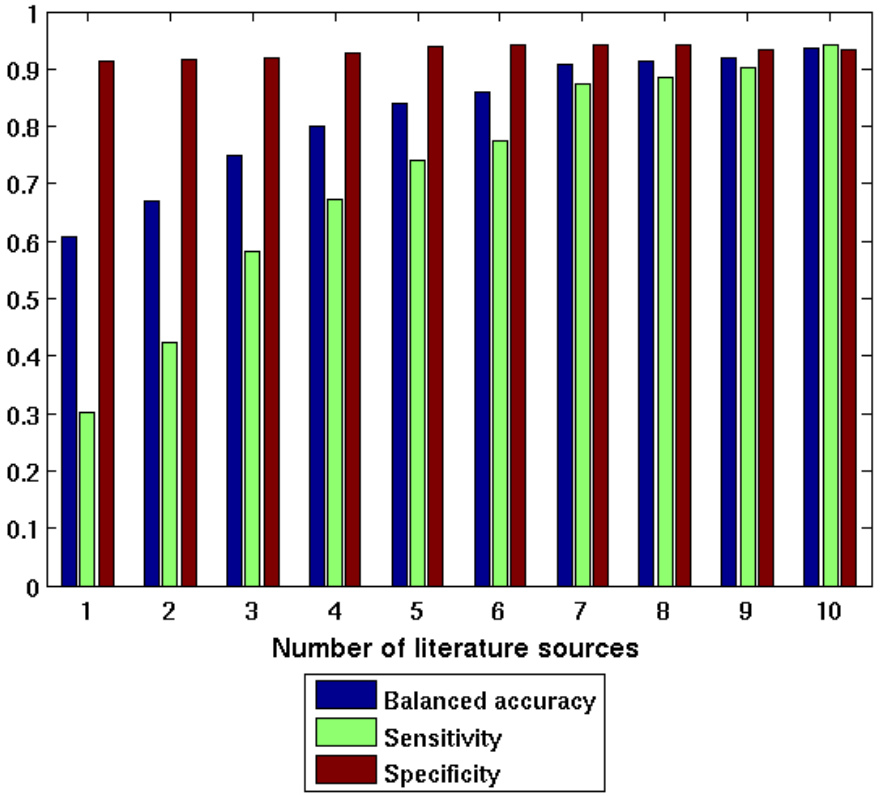



Watt et al. (in prep)

# Uncertainty in data has big impact on model performance

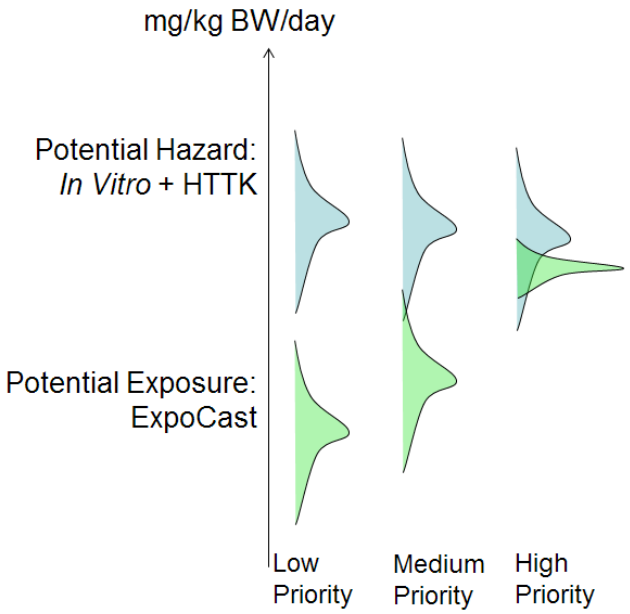
As greater consistency is required from literature sources, QSAR consensus model performance improves

- Source: CERAPP project, Mansouri et al. EHP 2015
- Community development of estrogen receptor models tested against thousands of experimental data points

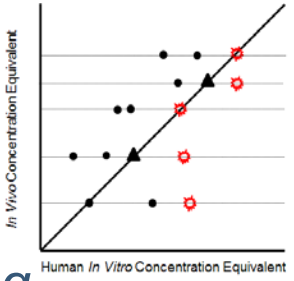


# Given all the uncertainty, is modeling futile?

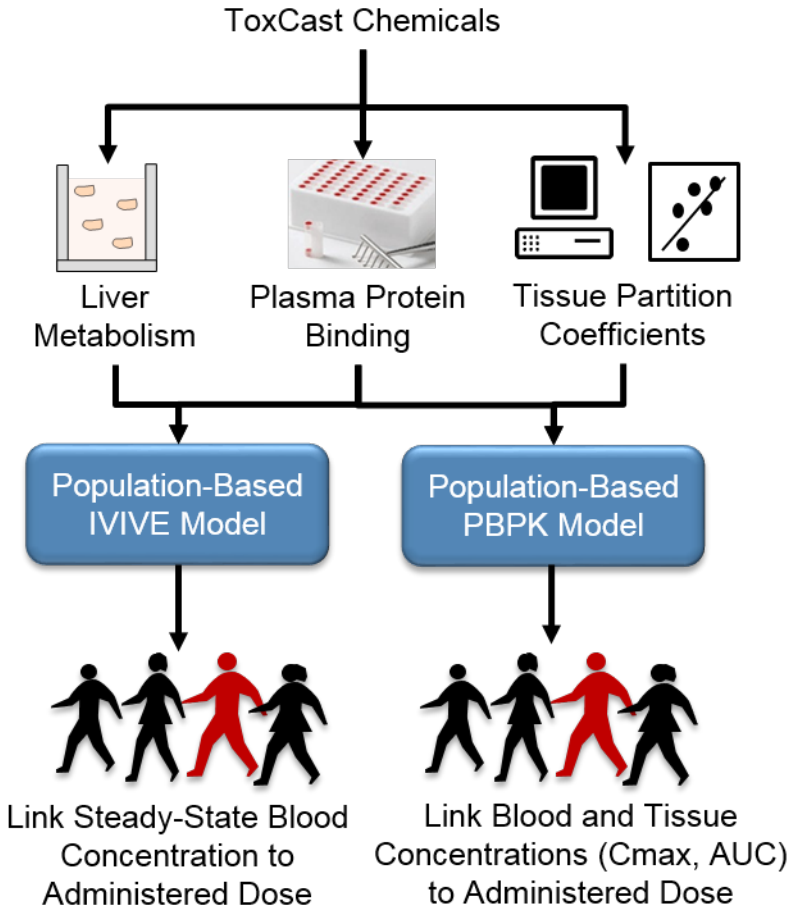
- Not in risk assessment
  - What's important is the difference between hazard and exposure
- Hazard Model:
  - In vitro IC50 (μM) with uncertainty
  - Use toxico / pharmacokinetic model to convert to mg/kg/day (with added uncertainty)
- Exposure model
  - Based on NHANES, other biomonitoring data
  - Add uncertainty
- Compare ranges for margin of exposure

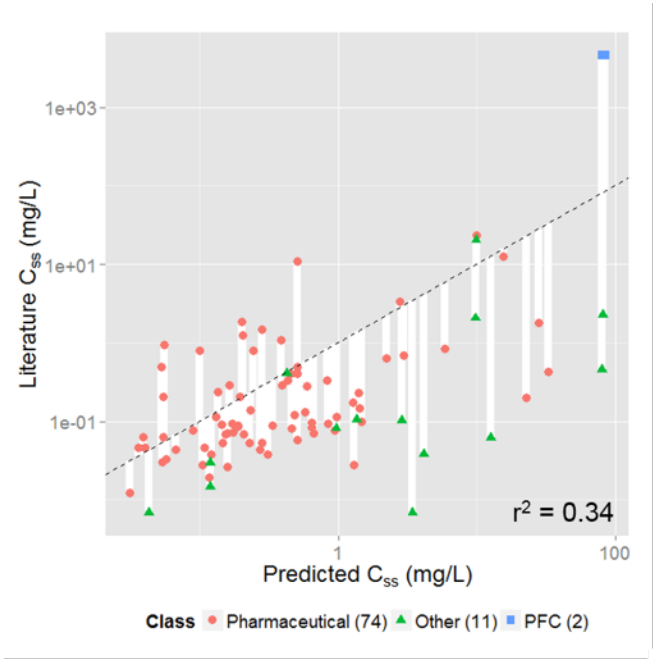


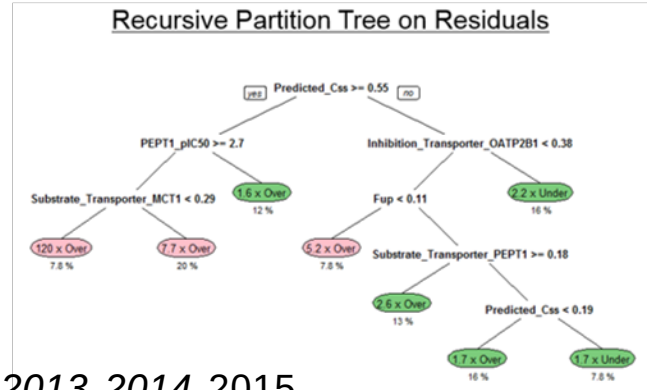
# Toxicokinetics Modeling




## *Incorporating Dosimetry and Uncertainty into In Vitro Screening*



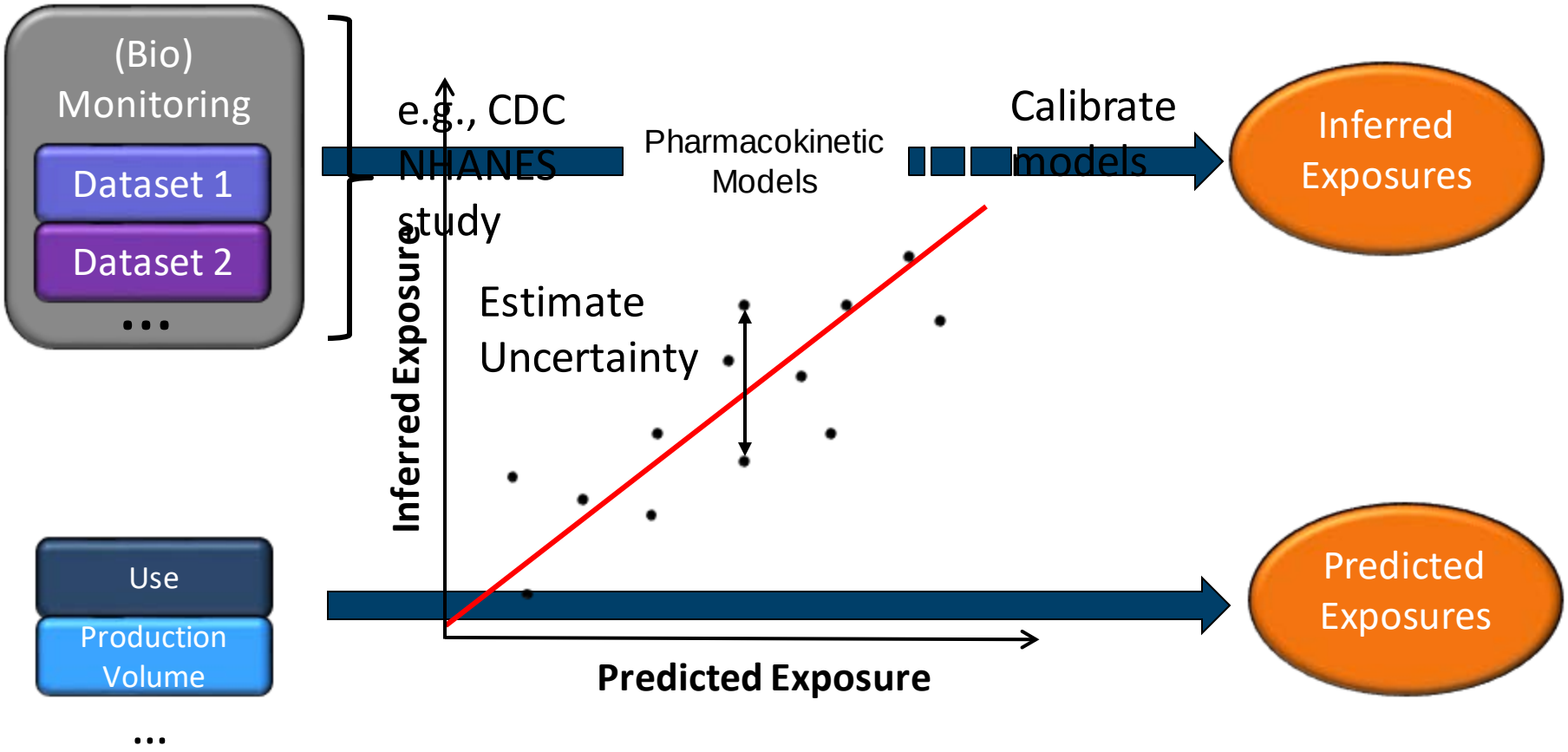






Wetmore, Rotroff, Wambaugh *et al., 2013, 2014,* 2015

# Population and Exposure Modeling

*Estimating Exposure and Associated Uncertainty with Limited Data*



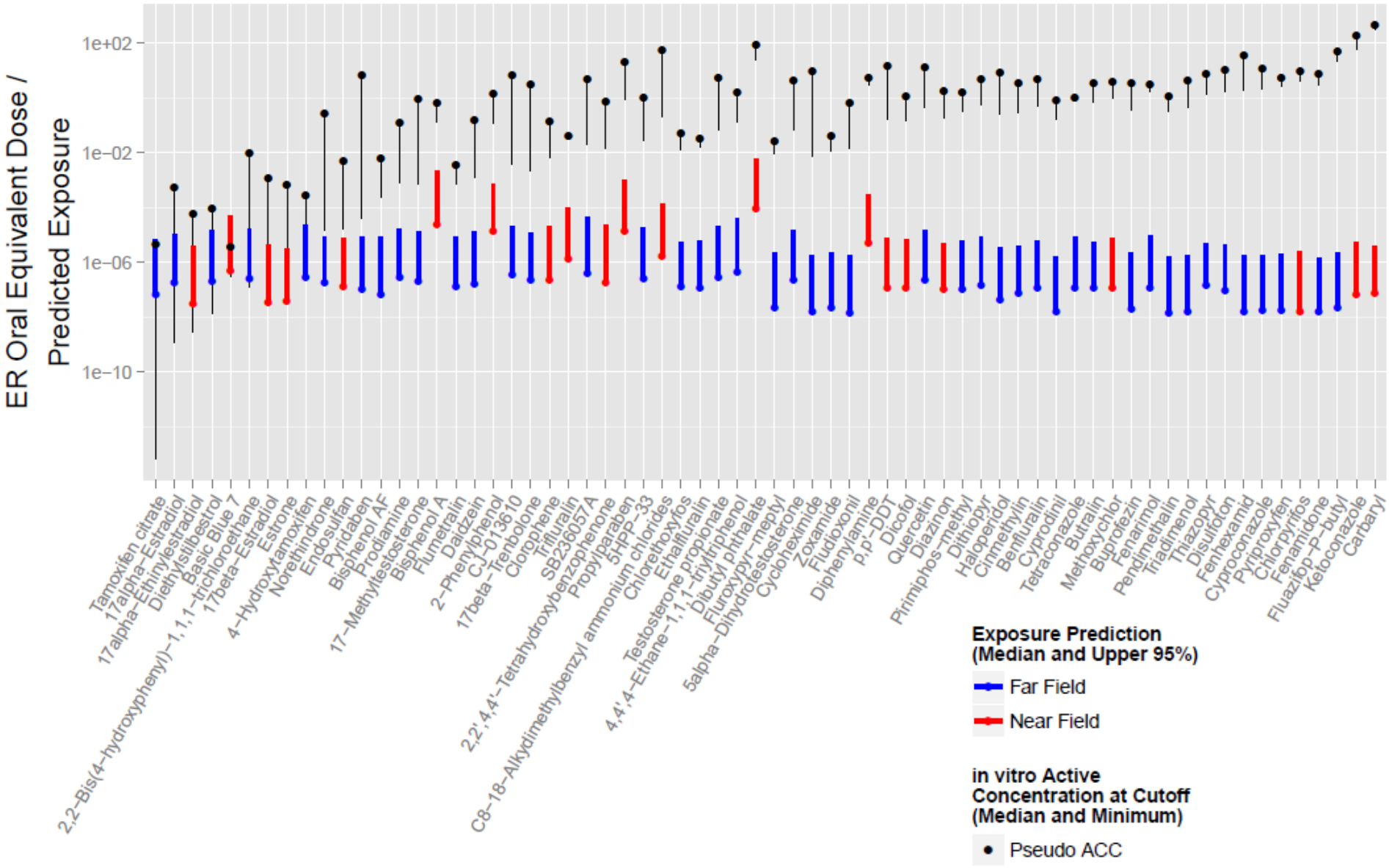





Wambaugh *et al.*, 2014

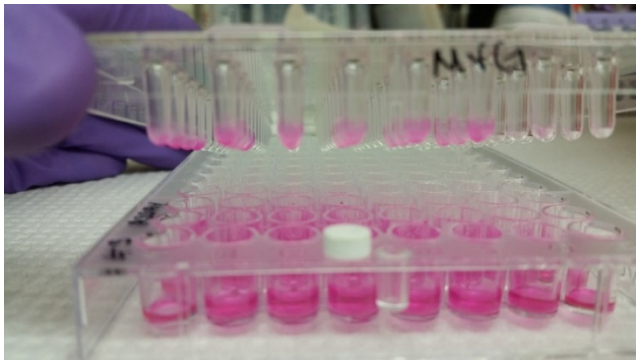
EPA United States Environmental Protection Agency

# High-throughput Risk Assessment for ER
# 290 chemicals with ER bioactivity

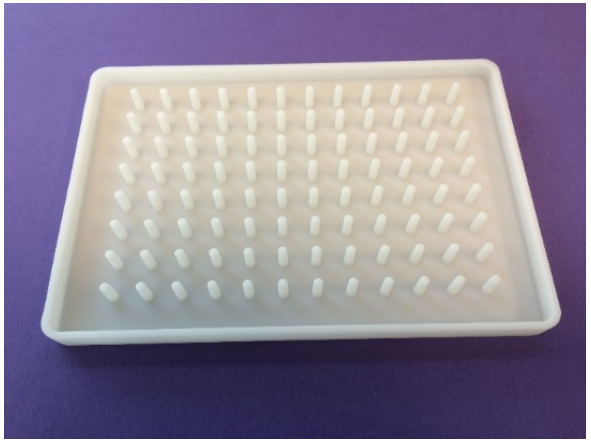


# Retrofitting Assays for Metabolic Competence – Extracellular Approach

Alginate Immobilization of Metabolic Enzymes (AIME)

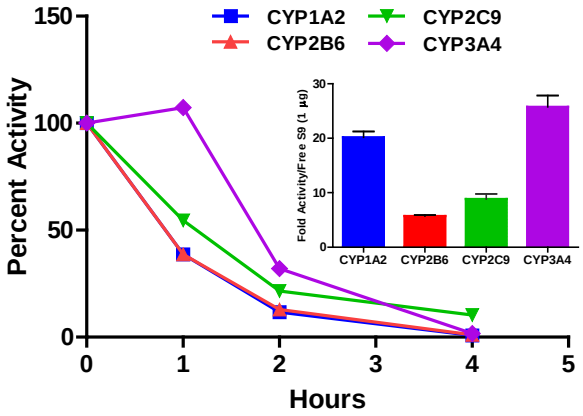


Prototype Lids

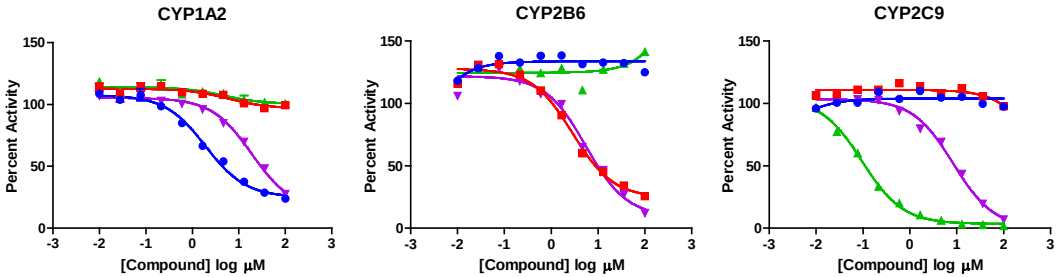


DeGroot et al. 2016 SOT poster #3757

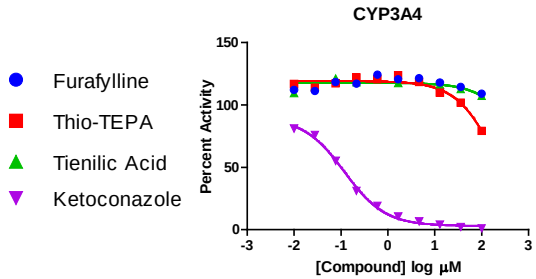
Amount of XME Activity in Microspheres



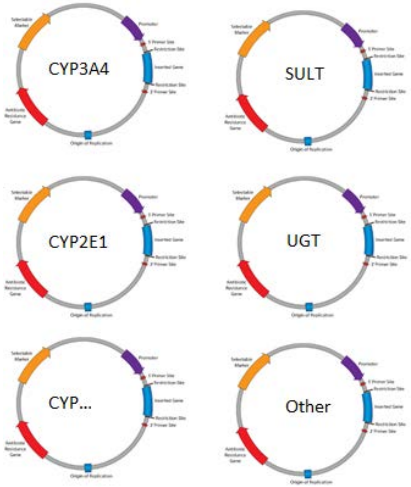

Small Molecule Inhibition of XME Activity



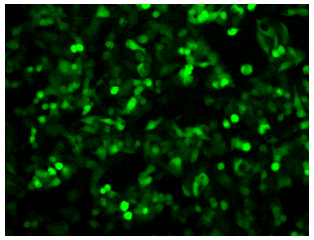

| Compound | Mol. Wt. (g/mol) | Targeted P450 | IC50 Free S9 (µM) | IC50 AIME (µM) |
|---|---|---|---|---|
| Furafylline | 260.25 | 1A2 | 2.39 | 1.92 |
| Thio-TEPA | 189.22 | 2B6 | 7.46 | 2.86 |
| Tienilic Acid | 331.17 | 2C9 | .053 | .096 |
| Ketoconazole | 531.43 | 3A4 | .086 | 0.12 |



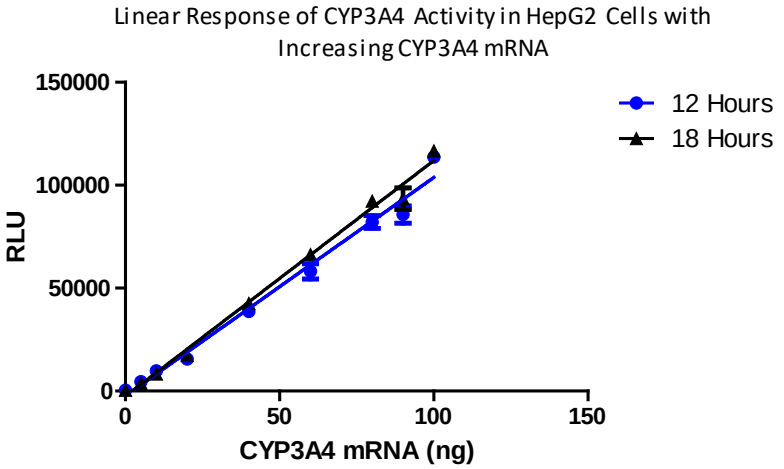
# Retrofitting Assays for Metabolic Competence – mRNA Intracellular Strategy




Pool in vitro transcribed mRNAs chemically modified with pseudouridine ad 5-methylcytidine to reduce immune stimulation

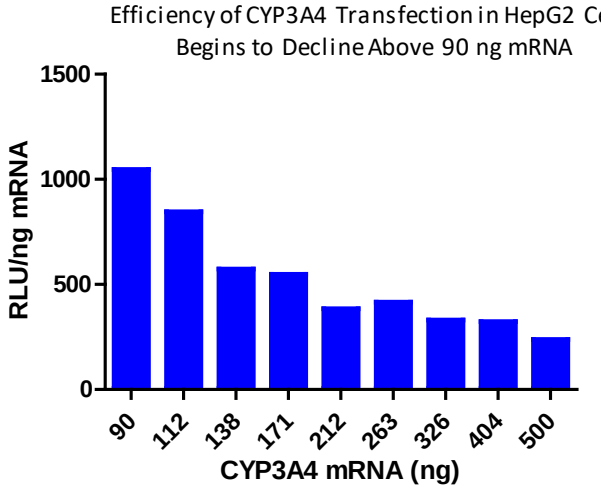


293T cells 21.5 h post transfection with 90 ng of EGFP mRNA using TransIT reagent




Advantage of transfecting with mRNA

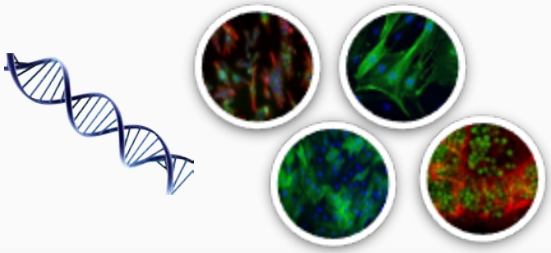
Titrate different CYPs to match different ratios in different tissues



# Developing Approaches for Tiered Testing

United States Environmental Protection Agency

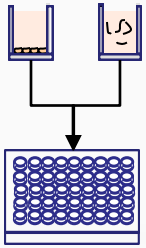
Comprehensive Transcriptomic Screening

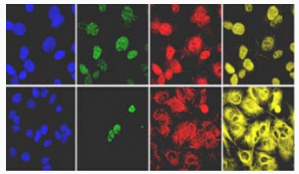


Multiple Human Cell Types

Comprehensive Characterization

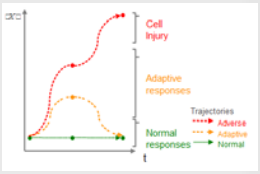
Focused ToxCast/Tox21 Assays

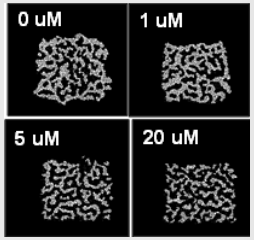




Time Course High Content Assays

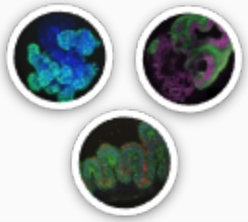
Verification of Affected Processes/ Pathways and Temporal Evaluation




Computational and Statistical Modeling

Organs-on-a-Chip



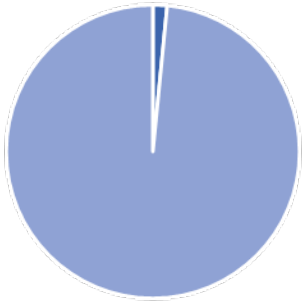
Organotypic and Organoid Models

Interpretation of Affected Process/ Pathways and Population Variability

# Planning for HT Transcriptomics

*New Approaches to Comprehensively Assess Potential Biological Effects*

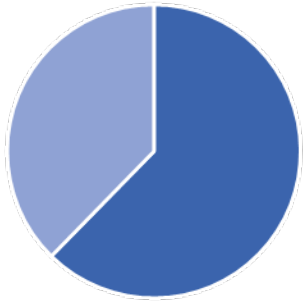
**Gene Coverage**



ToxCast

Not in ToxCast

**Pathway Coverage***

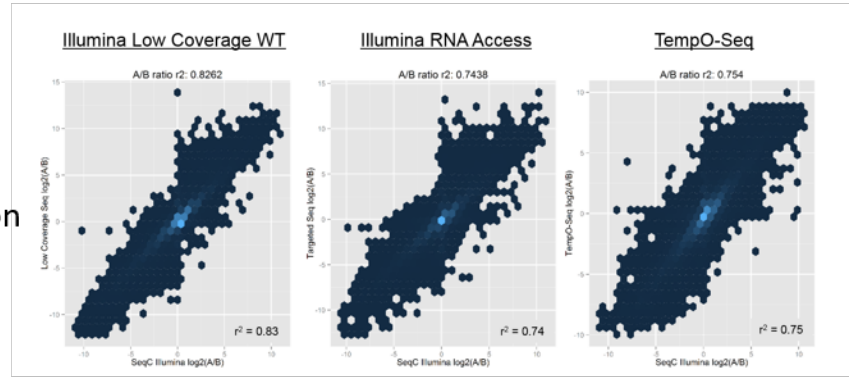


*At least one gene from pathway represented

High-Throughput Transcriptomic Platforms

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

Technical Comparison

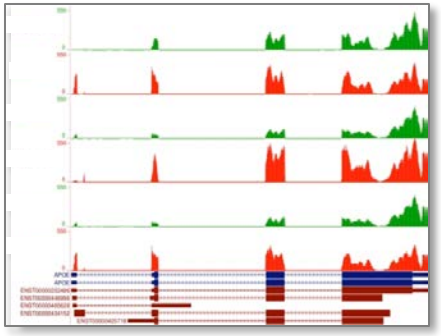


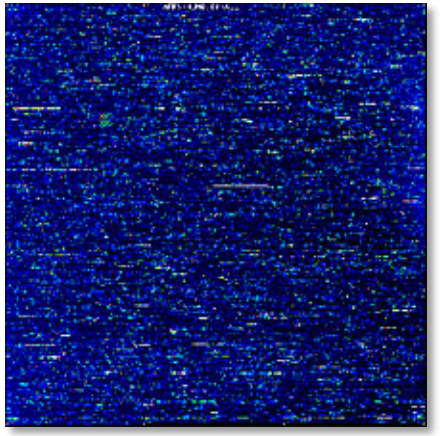

Functional Comparison

| | Correct Mechanistic Match | | |
|---|---|---|---|
| | 2/5 (40%) | 2/5 (40%) | 5/5 (100%) |

Karmaus and Martin, Unpublished

# Requirements and Potential Platforms for HT Transcriptomics





## Requirements

- Measure or infer transcriptional changes across the whole genome (or very close to it) (e.g. not subsets of 1000, 1500, 2500 genes)
- Compatible with 96- and 384-well plate formats (maybe 1536?) and laboratory automation
- Work directly with cell lysates (no separate RNA purification)
- Compatible with multiple cell types and culture conditions
- Low levels of technical variance and robust correlation with orthogonal measures of gene expression changes
- Low cost ($30 - $45 per sample or less)

## Potential Platforms

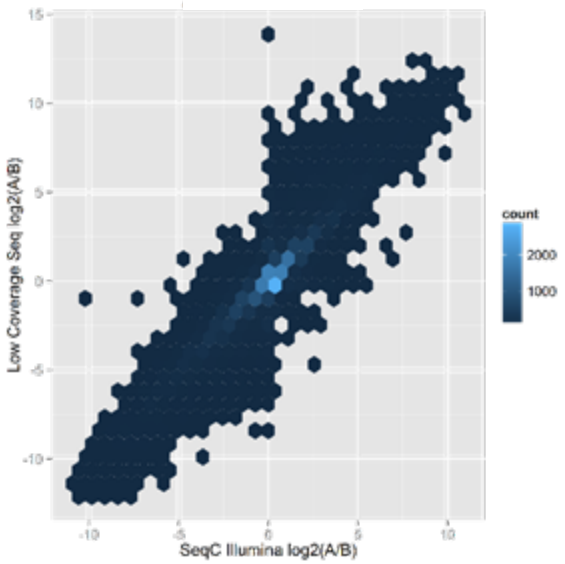
- Low coverage whole transcriptome RNA-seq (3 – 5 million mapped reads)
- Targeted RNA-seq (e.g., TempO-seq, TruSeq, SureSelect)
- Microarrays (e.g., Genechip HT)
- Bead-based (e.g., L1000)

# Technical Performance of the Three Sequencing Platforms

United States Environmental Protection Agency

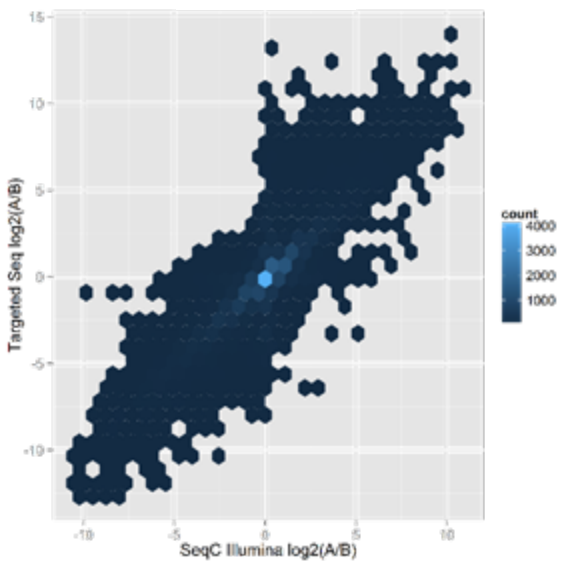
## Low Coverage

$r^2$ 0.83



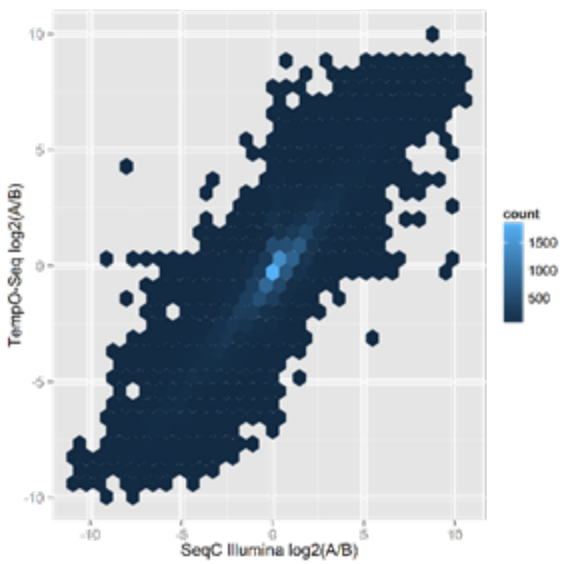

## TruSeq

$r^2$ 0.74




## TempO-Seq

$r^2$ 0.75




Data from MAQC II Samples

# HT Transcriptomics Next Steps

- Perform pilot study (Summer) to validate workflow and refine experimental design
- Initiate large scale screen (Fall/Winter)
    - Cell type: MCF7
    - Compounds: 1,000 (ToxCast Phase I/II)
    - Time Point: Single
    - Concentration Response: 8 (?)
- Perform secondary pilot study looking at cell type selection/ pooling strategies (Fall/Winter)
- Integrate HT transcriptomic platform with metabolic retrofit solution to allow screening +/- metabolism (FY17)
- **Explore partnerships to build community database of common chemical set across multiple cell types/lines**

# Other Ongoing Efforts

- **Curated chemical structure database of >1 million unique substances**
- Capability to retrofit high-throughput *in vitro* assays for metabolic competence
- **Software infrastructure to manage, use and share big data in toxicology**
- Methods to quantify uncertainty in all quantities
- **Read-across approaches that quantitatively include uncertainty**
- Pharmacokinetic models for hundreds of chemicals while understanding which chemical classes are well predicted and which ones have greater uncertainty
- High-throughput exposure models for thousands of chemicals with estimates of uncertainty
- **Non-targeted analytical measurements of chemical constituents in hundreds of consumer products**
- **Framework for streamlined validation of high-throughput *in vitro* assays**

EPA United States Environmental Protection Agency

# 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 validation
  - Defining a fit-for-purpose framework(s) that is time and resource efficient
  - Performance-based technology standards vs. traditional validation
  - Role of *in vivo* rodent studies and understanding their inherent uncertainty
- Legal defensibility of new methods and assessment products

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