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

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Drug Safety Gordon Conference
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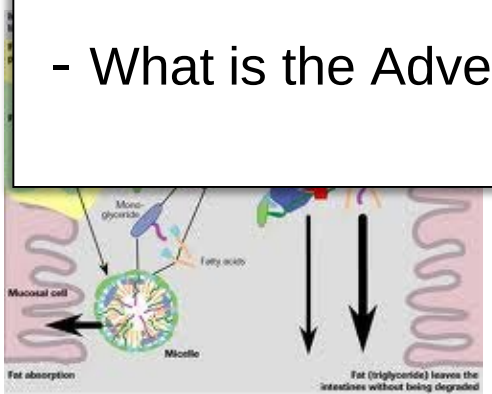
Problem Statement

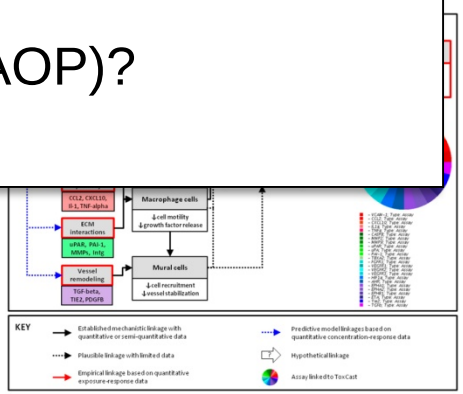
Too many chemicals to test with standard animal-based methods

- Cost, time, animal welfare

Need for better mechanistic data

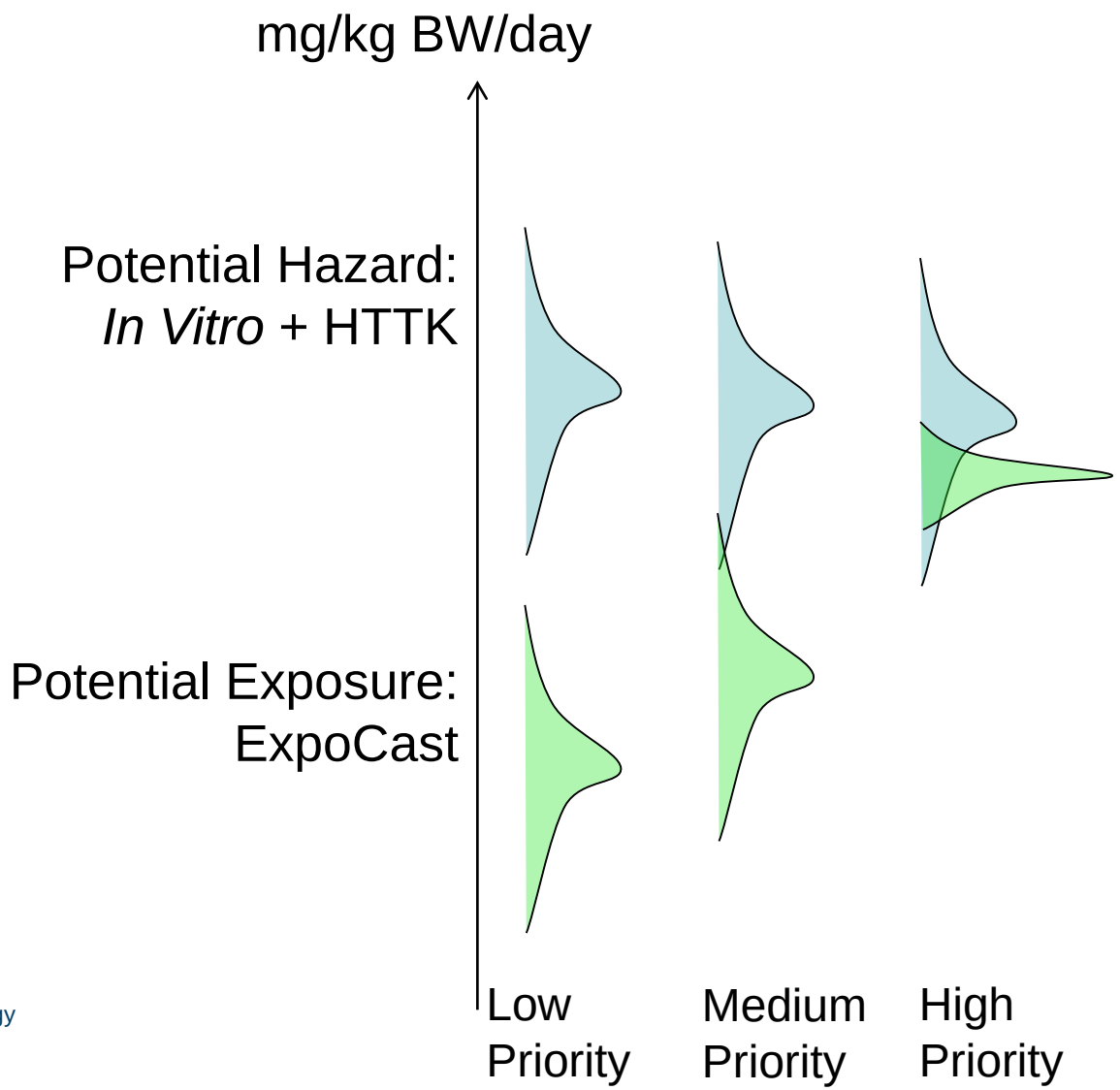
- Determine human relevance
- What is the Adverse Outcome Pathway (AOP)?





Risk-based Prioritization
Hazard + Exposure

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

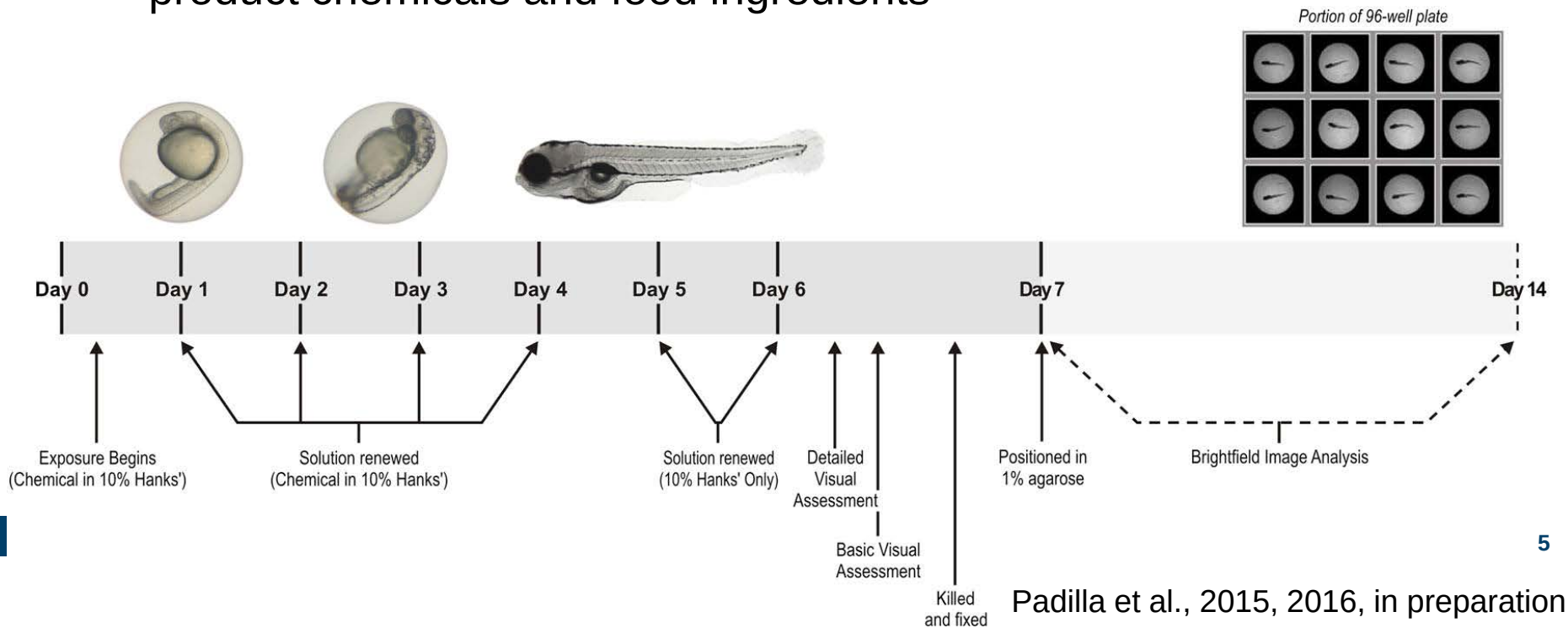


Computational Toxicology

- 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

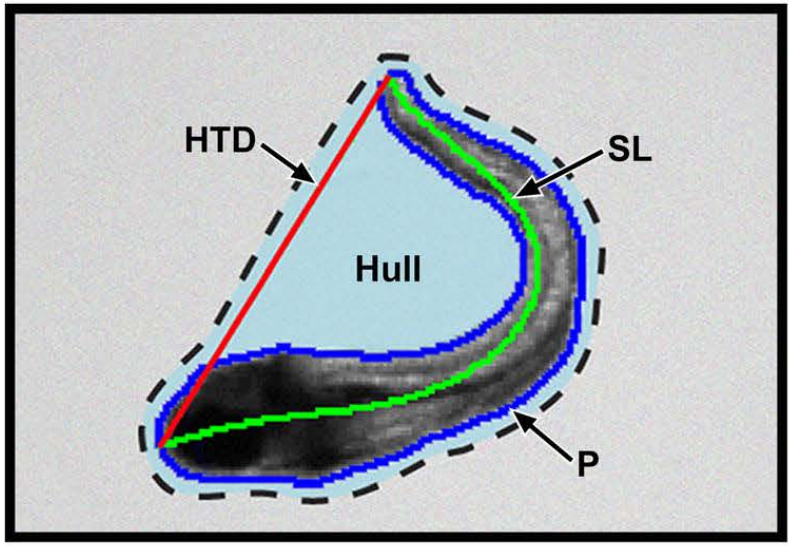
- Goal: Use zebrafish as an *in vivo* model of vertebrate developmental toxicity
- Build *in vitro* to *in vivo* models using ~700 human assays
- ~1000 Chemicals
  - pharmaceuticals, pesticides, industrial chemicals, personal care product chemicals and food ingredients



Padilla et al., 2015, 2016, in preparation

Zebrafish Imaging and scoring

A

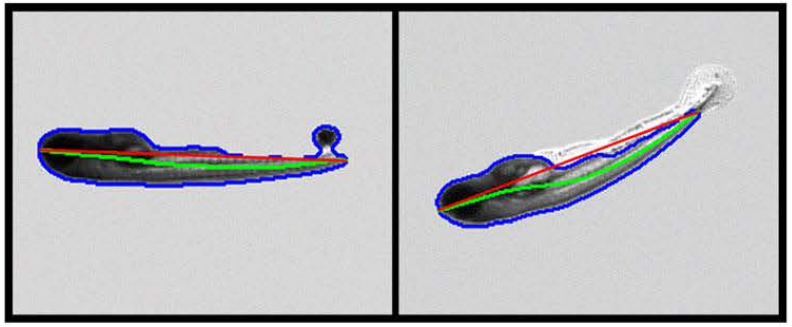


B

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

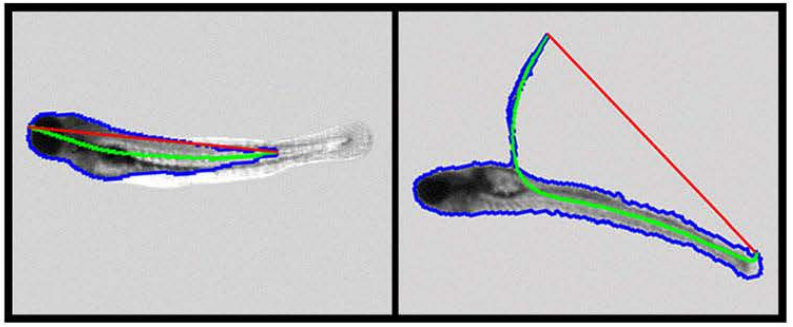
C

Acceptable



D

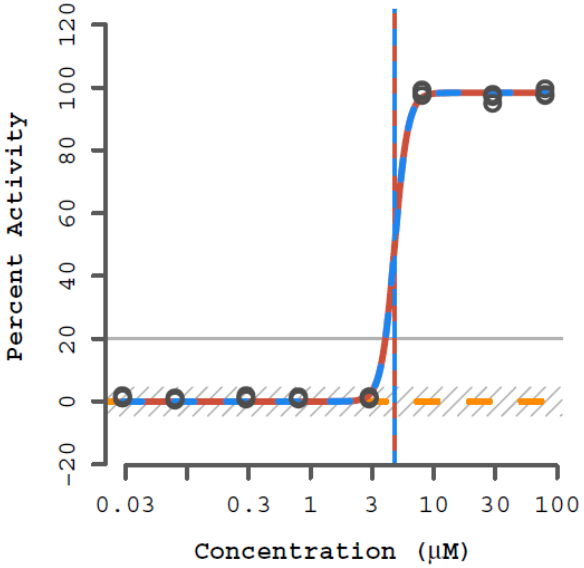
Unacceptable



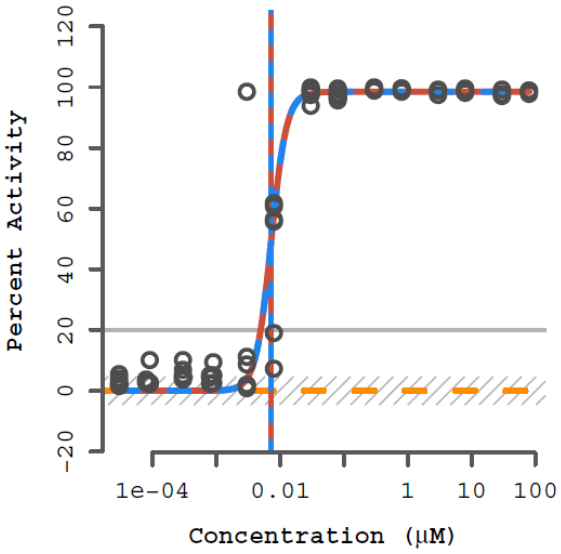
Deal et al. J Applied Tox. 2016

Example chemicals

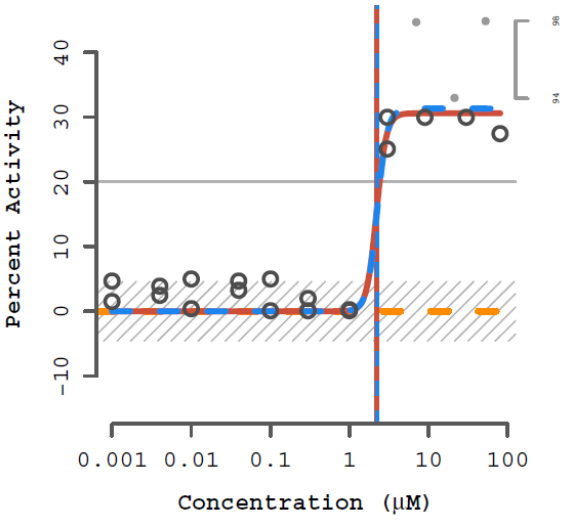
DES



Lovastatin

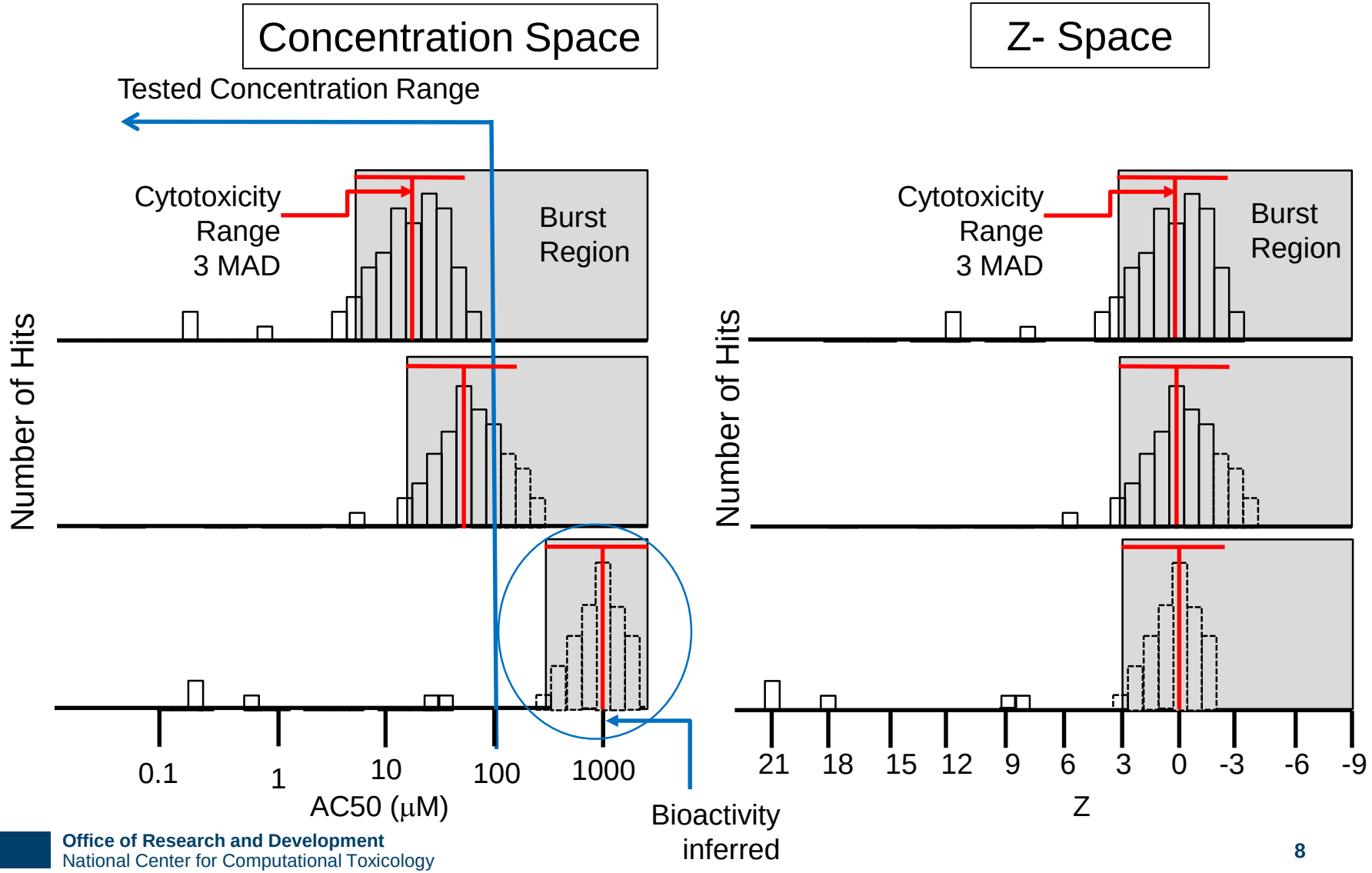


Permethrin



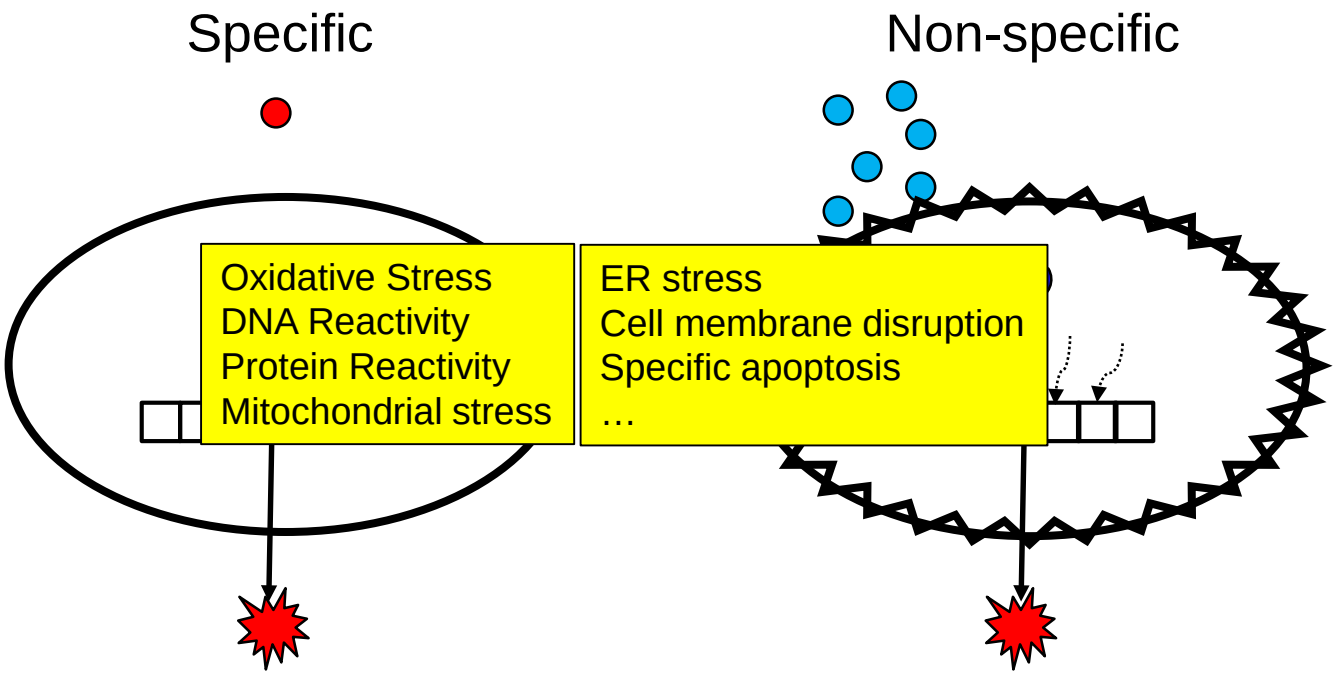
100% = death
<100% = malformations

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

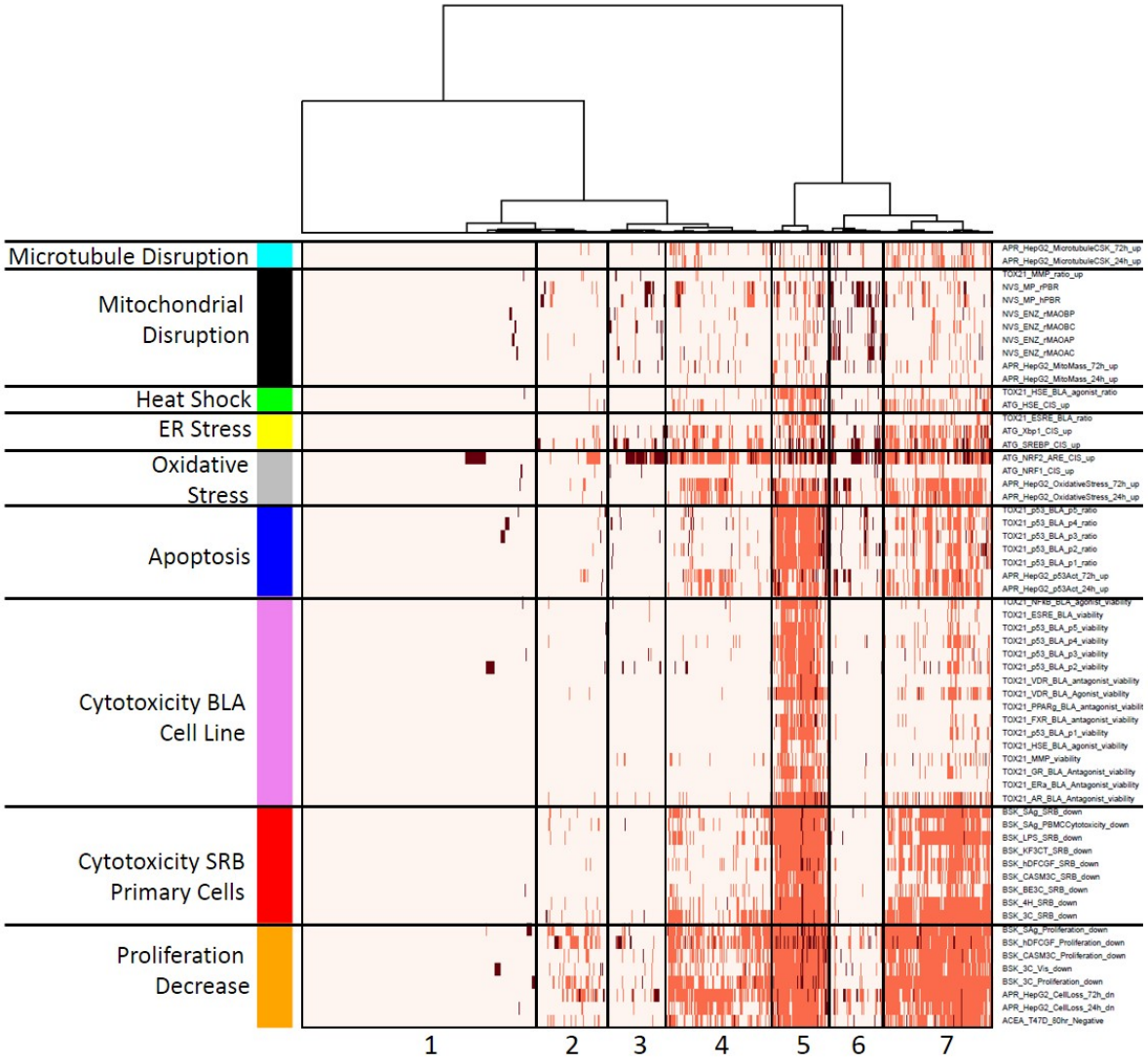


Judson et al. Tox.Sci. (2016)

Schematic explanation of the burst



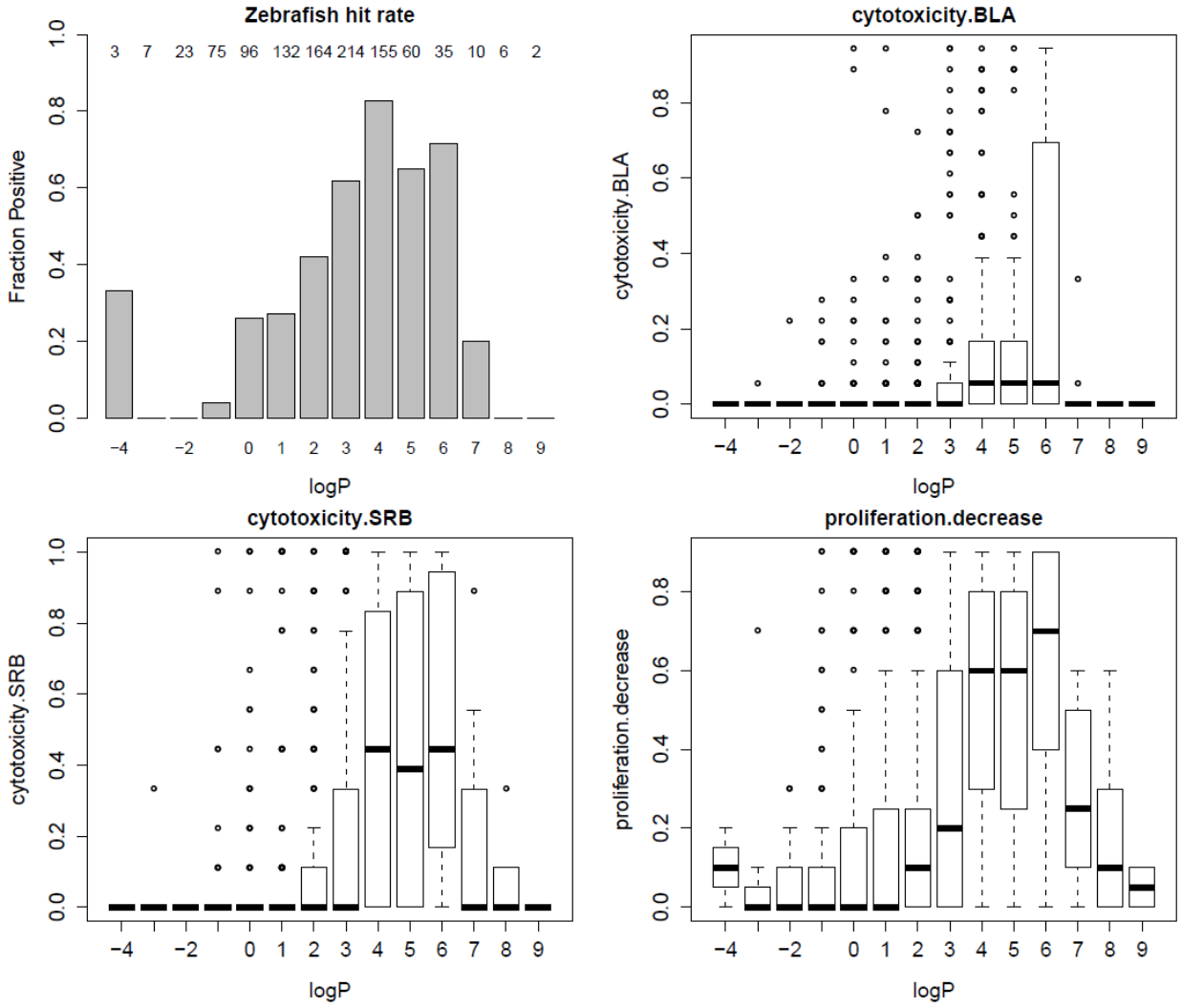
Heatmap of stress and cytotoxicity assays in 1000 chemicals



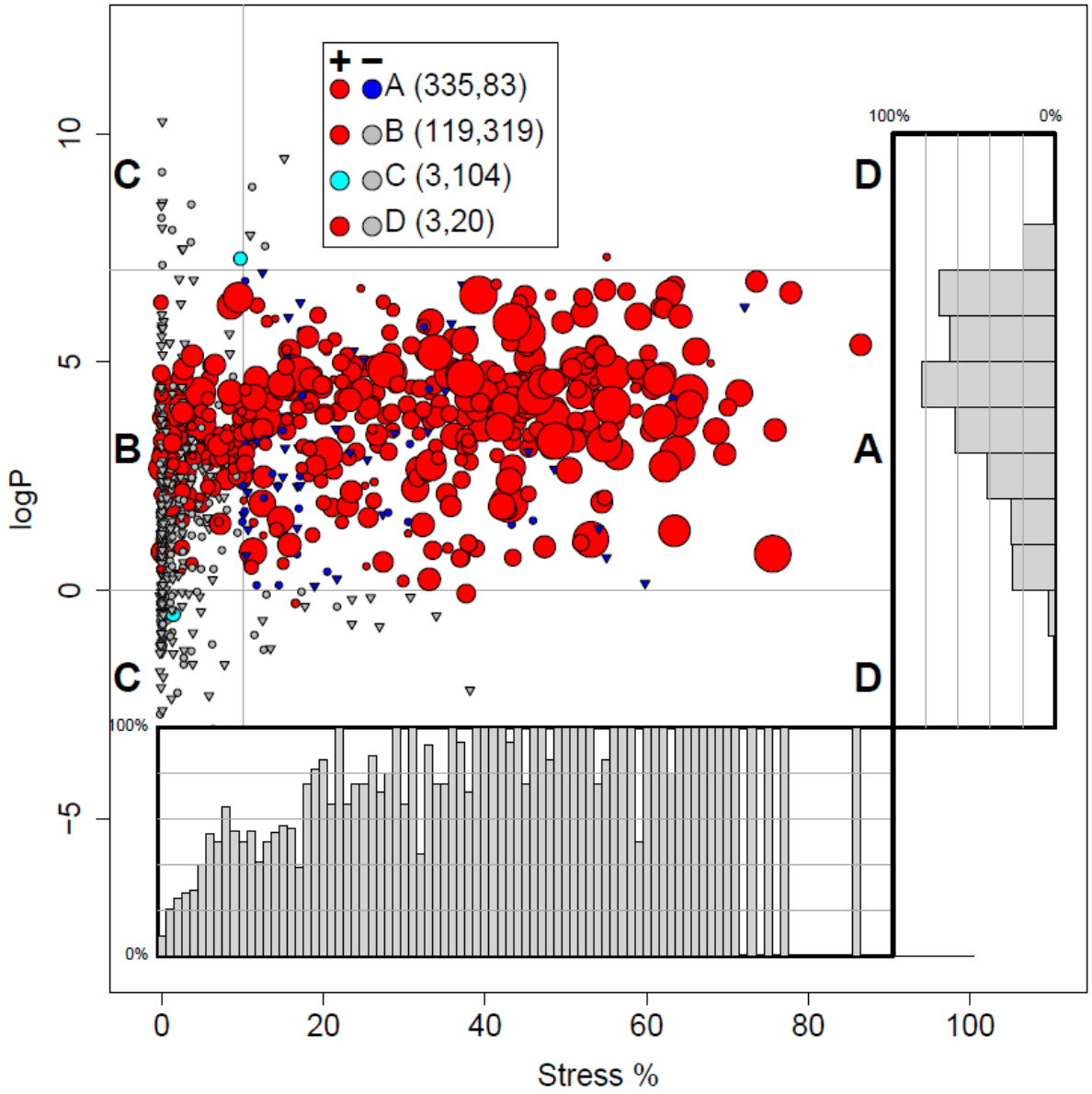
Judson et al. ToxSci (2016)

Observation about logP

Human *in vitro* cell stress behaves ~ zebrafish toxicity



Stress, logP explains ~80% of ZF activity



ZF positive in conc-response

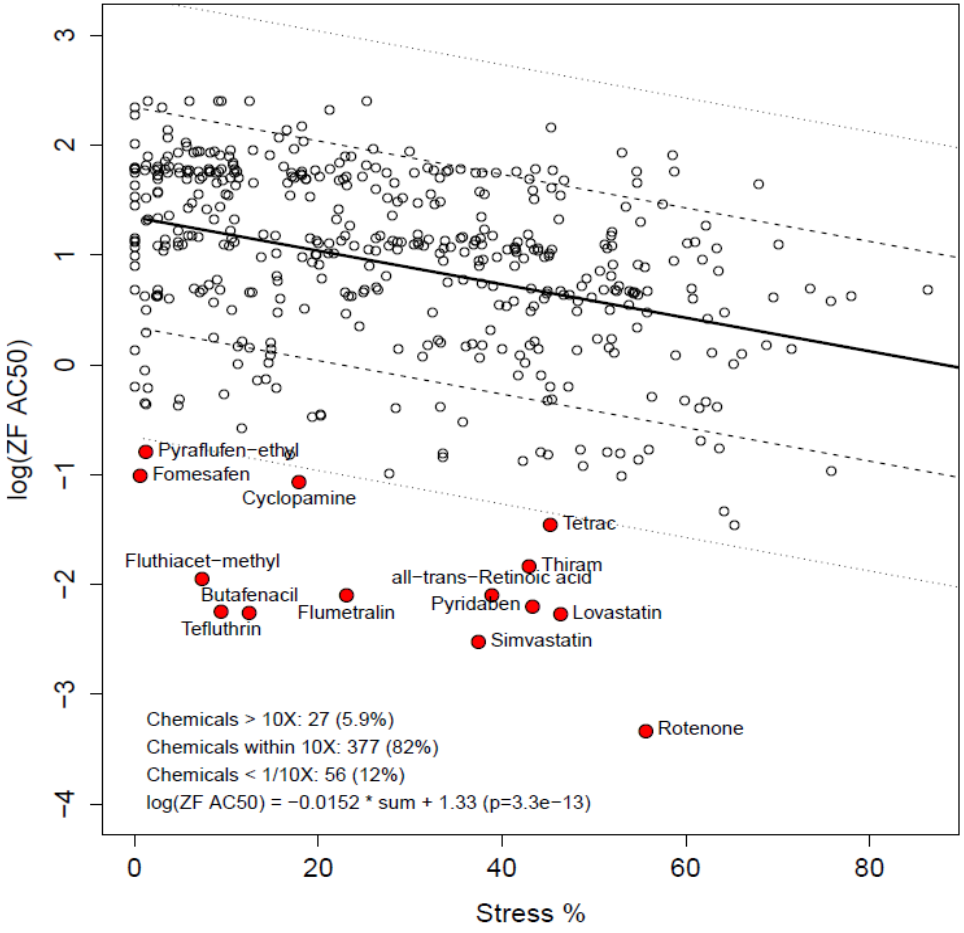
ZF negative in conc-response

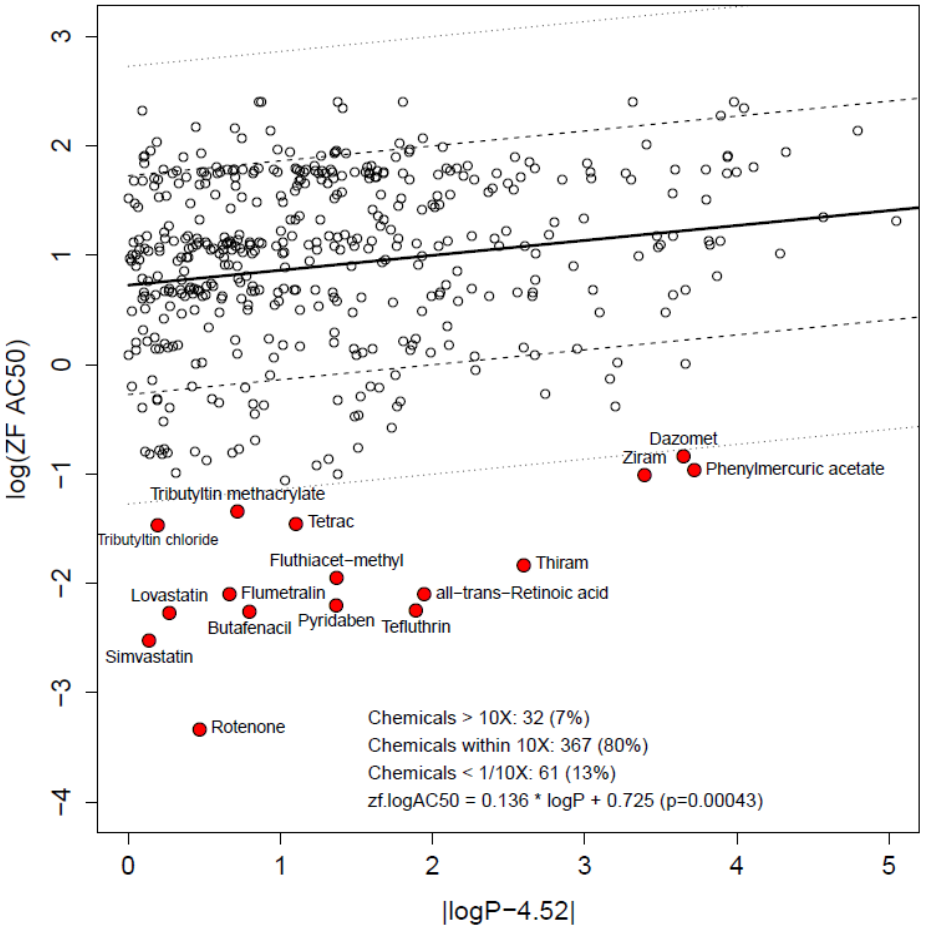
ZF negative in single conc

- 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





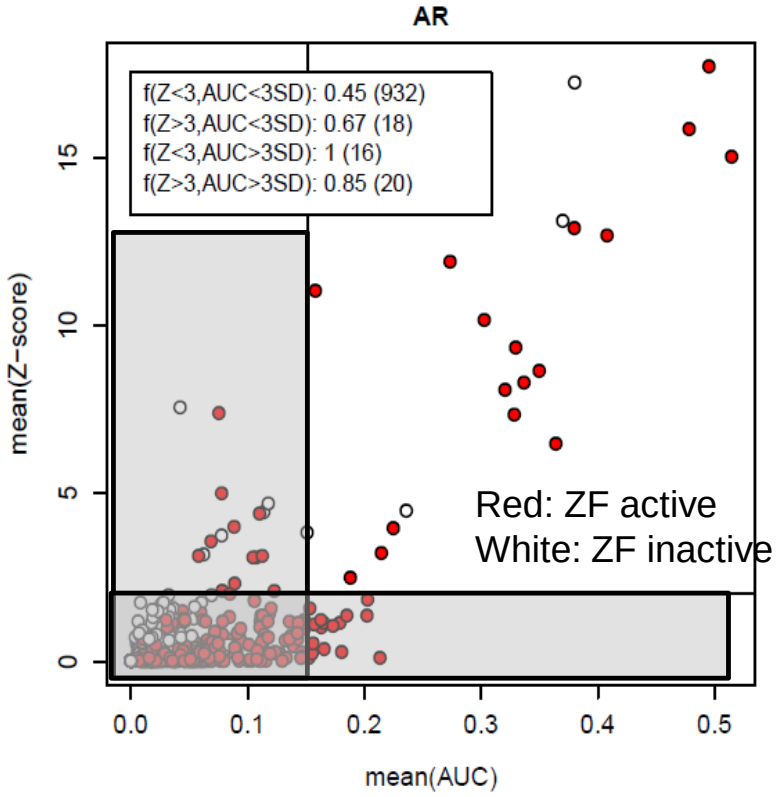
Chemicals with excess toxicity tend to fall in a few target MOA classes

- 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

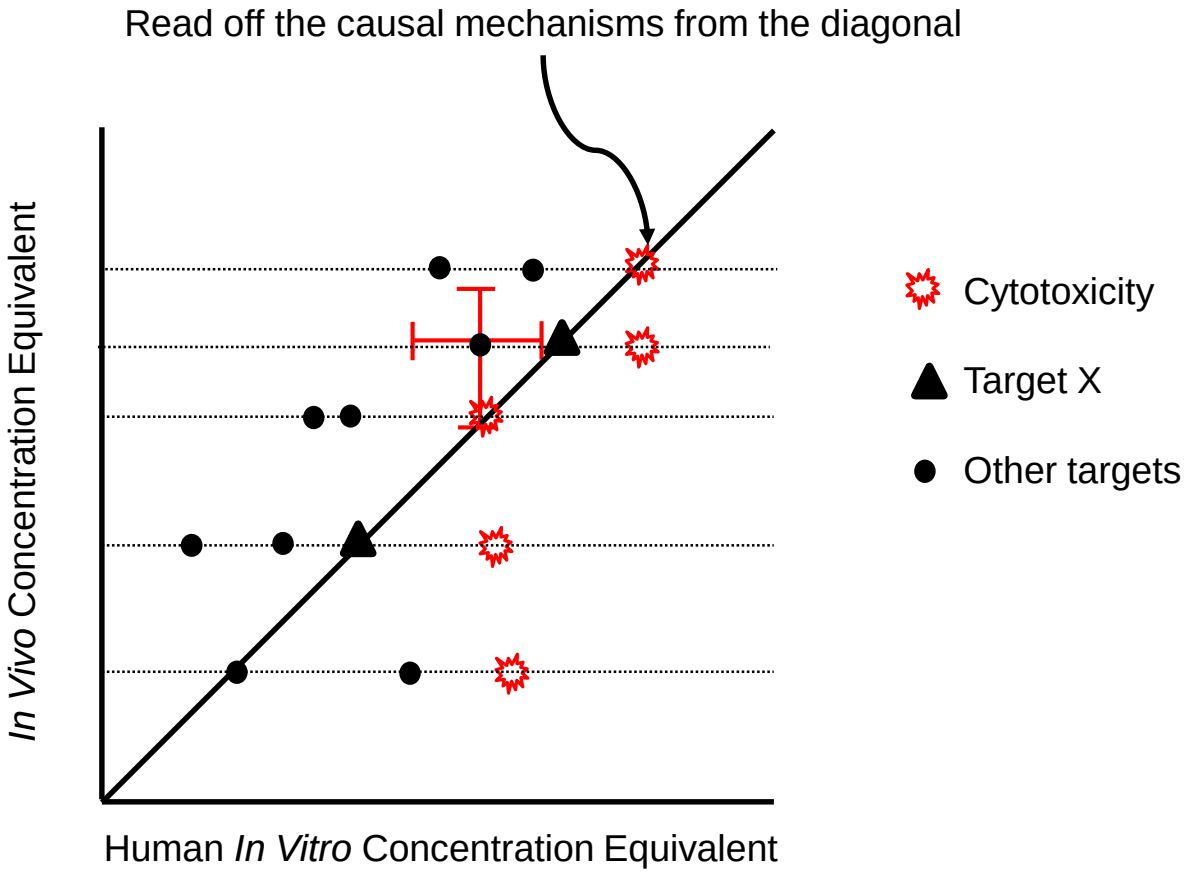
class	Gene group	annotation	assays	TP	FP	FN	TN	Sens	Spec	BA	OR	PPV	p-value
endocrine	AR	Androgen receptor	11	17	3	443	523	0.04	0.99	0.52	6.7	0.85	0.0005
endocrine	CYP19A1	Aromatase	2							0.52	14.4	0.92	9E-07
endocrine	ESR	Estrogen receptor	17							0.53	5.8	0.83	2E-05
endocrine	NR3C1	Glucocorticoid 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

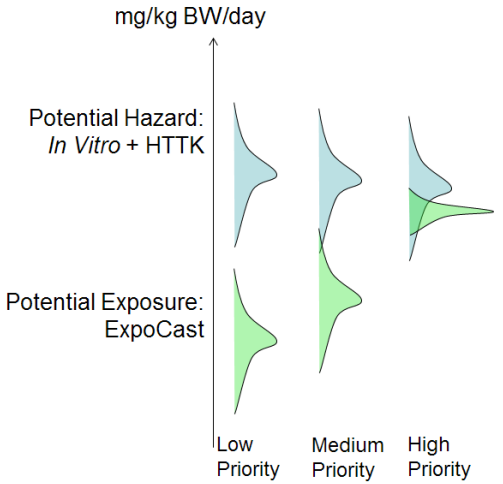
Largely due to conazoles

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



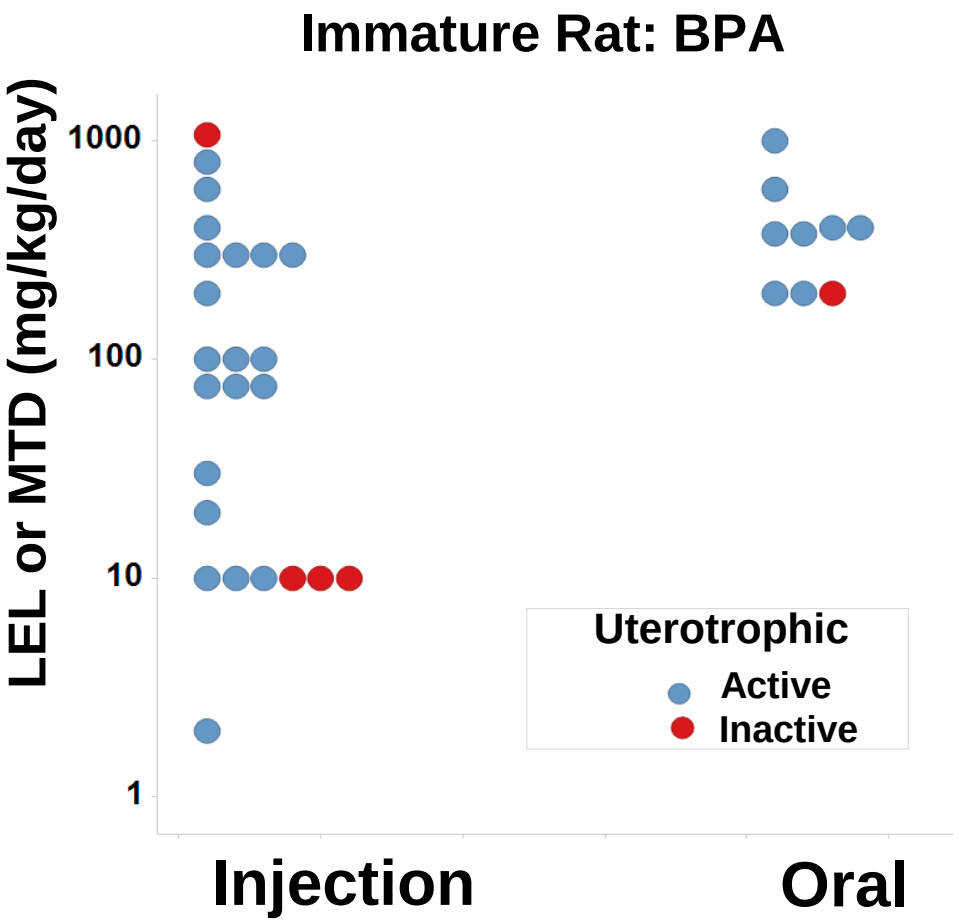
- Our first goal is **prediction**
  - What is the highest safe dose of a chemical?
  - What types of harm would a chemical cause above that dose?
- Predictions are based on **models**
  - Computational, statistical, "mental", *in vitro*, *in vivo*
- All models are based on **data**
- Data is always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



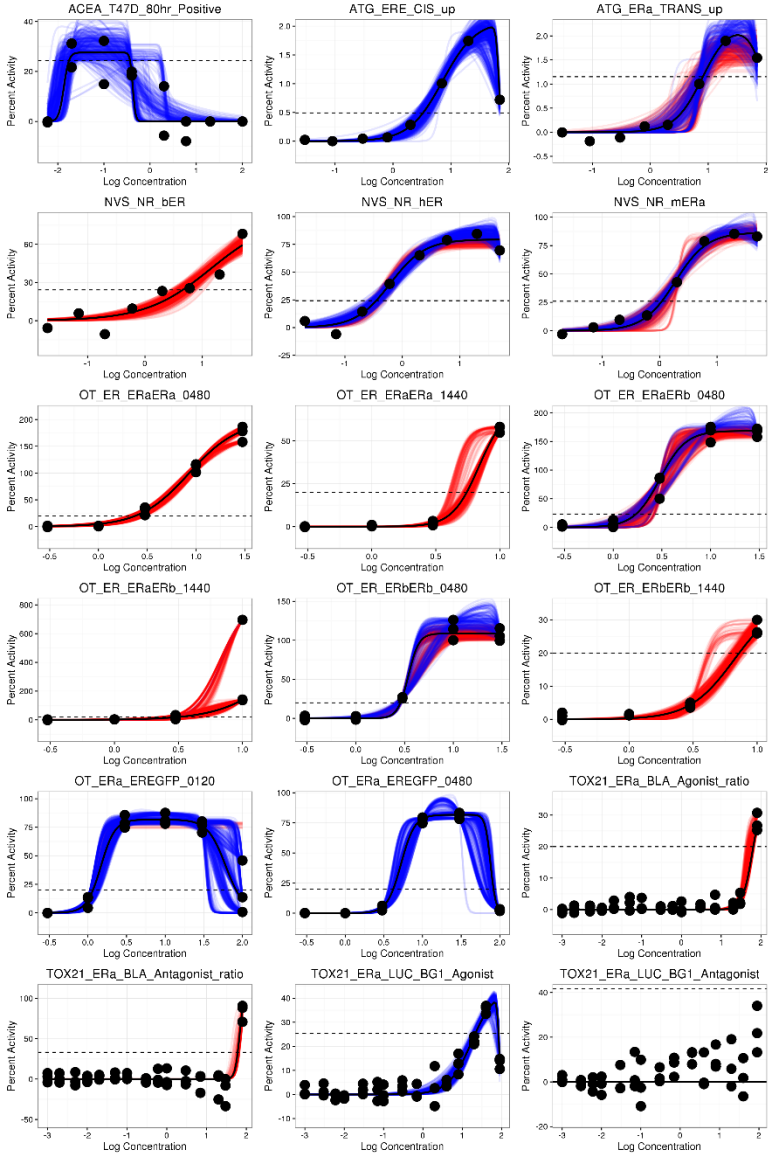
Kleinstreuer et al. EHP 2015

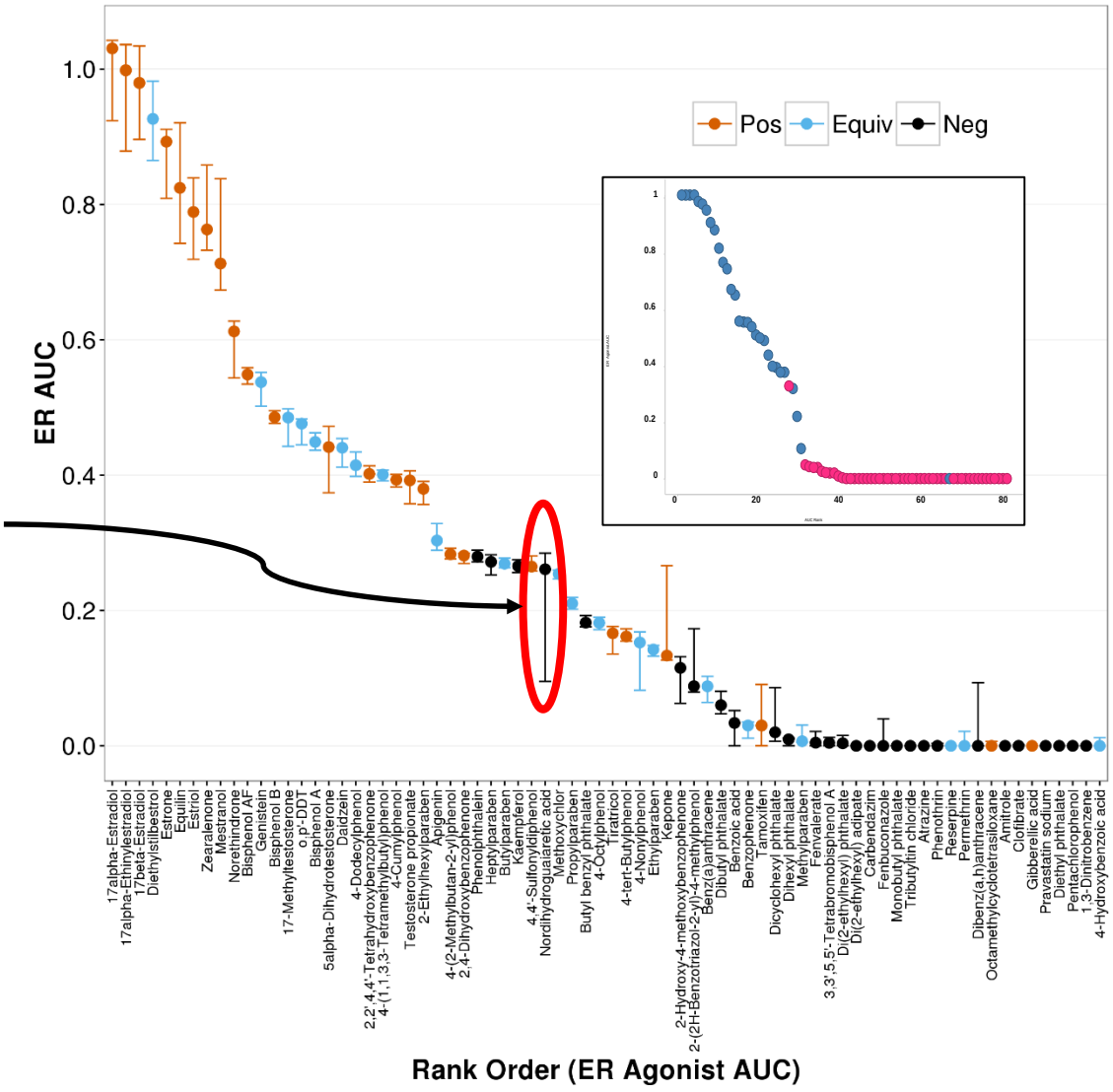
Anemia Reproducibility

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



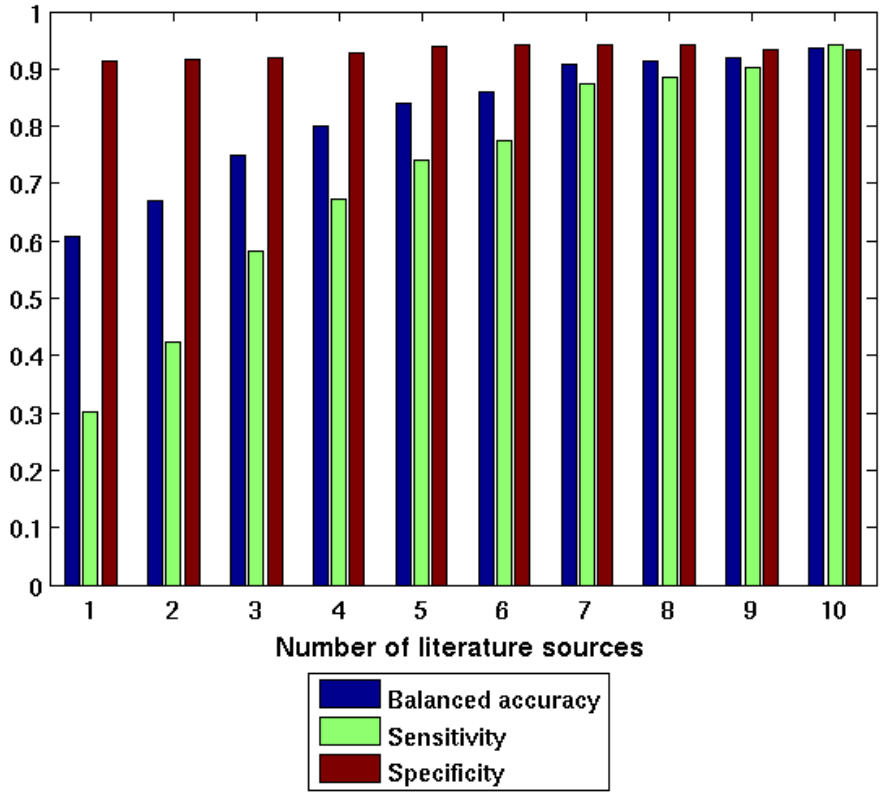


Watt et al. (in prep)

Uncertainty in data has big impact on model performance

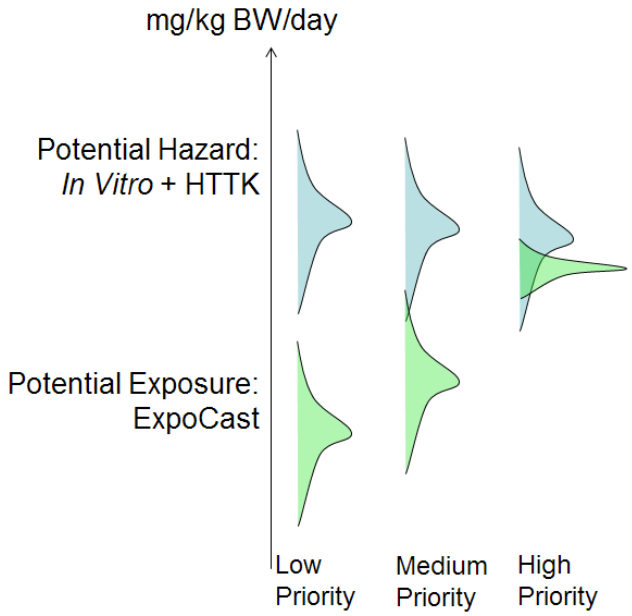
As greater consistency is required from literature sources, QSAR consensus model performance improves

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

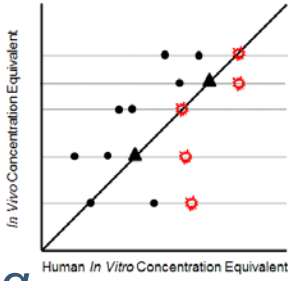


Given all the uncertainty, is modeling futile?

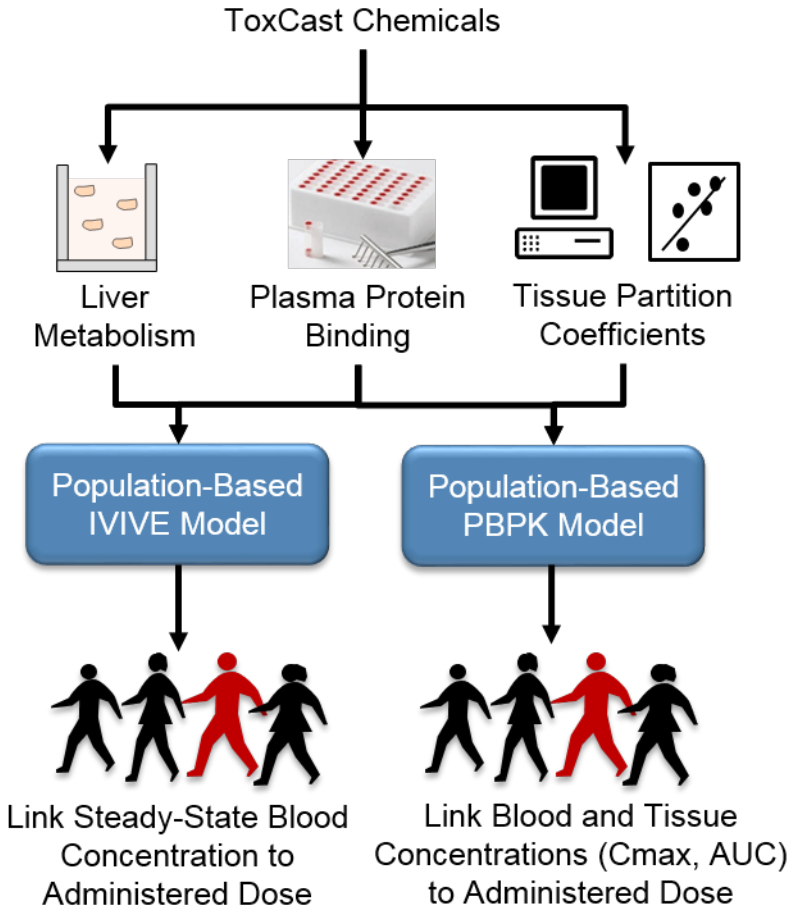
- Not in risk assessment
  - What's important is the difference between hazard and exposure
- Hazard Model:
  - In vitro IC50 (μM) with uncertainty
  - Use toxico / pharmacokinetic model to convert to mg/kg/day (with added uncertainty)
- Exposure model
  - Based on NHANES, other biomonitoring data
 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

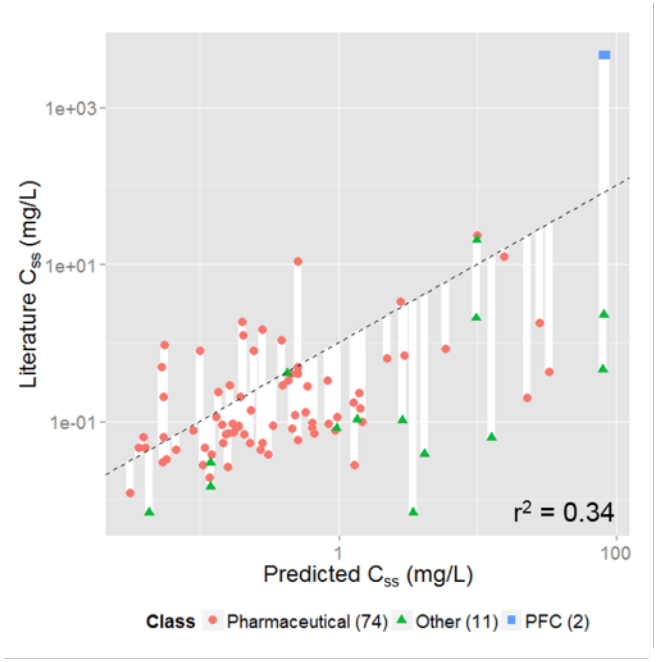


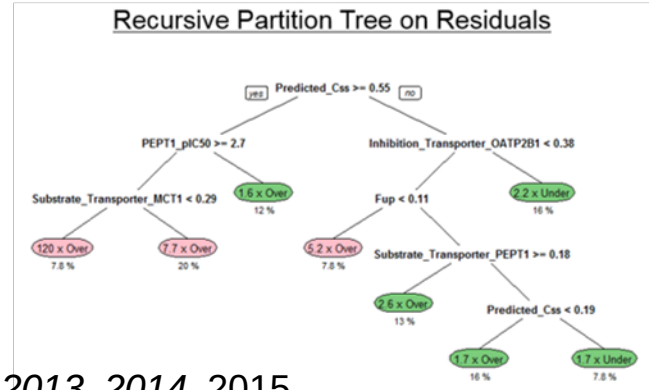
Toxicokinetics Modeling



Incorporating Dosimetry and Uncertainty into In Vitro Screening



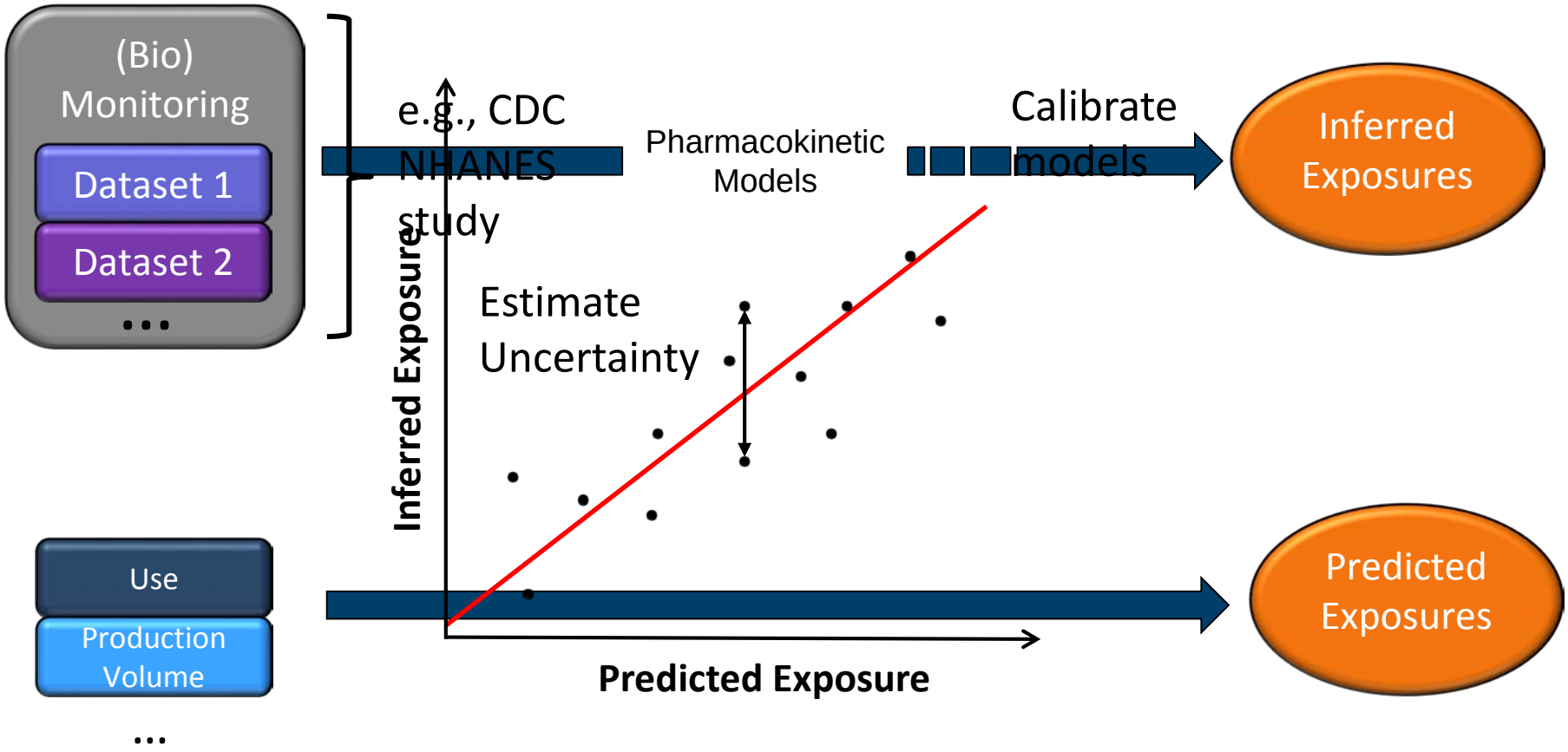




Wetmore, Rotroff, Wambaugh *et al., 2013, 2014,* 2015

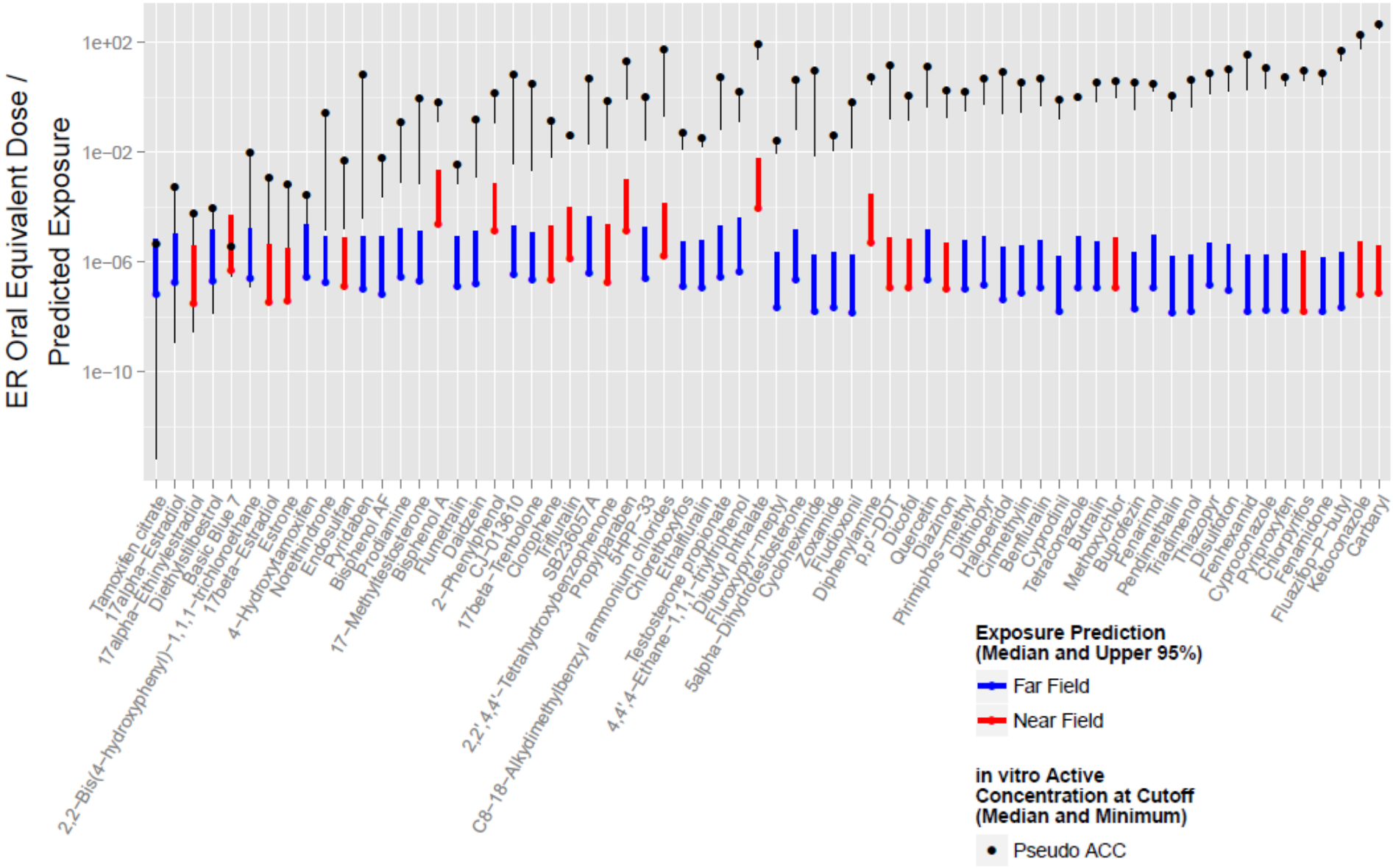
Population and Exposure Modeling

Estimating Exposure and Associated Uncertainty with Limited Data



Wambaugh *et al.*, 2014

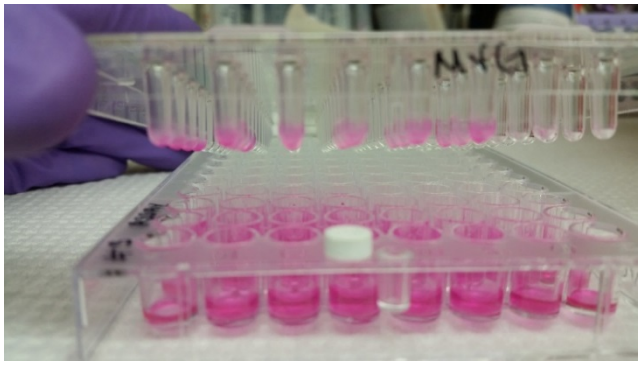
High-throughput Risk Assessment for ER
290 chemicals with ER bioactivity



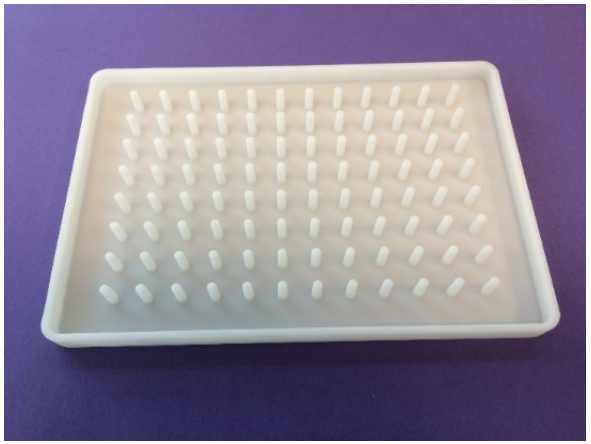
Retrofitting Assays for Metabolic Competence – Extracellular Approach

United States Environmental Protection Agency

Alginate Immobilization of Metabolic Enzymes (AIME)

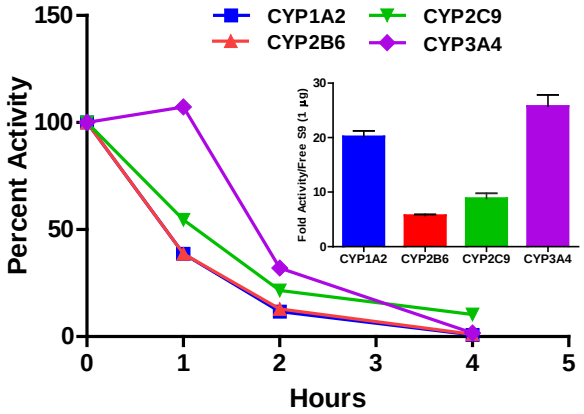


Prototype Lids

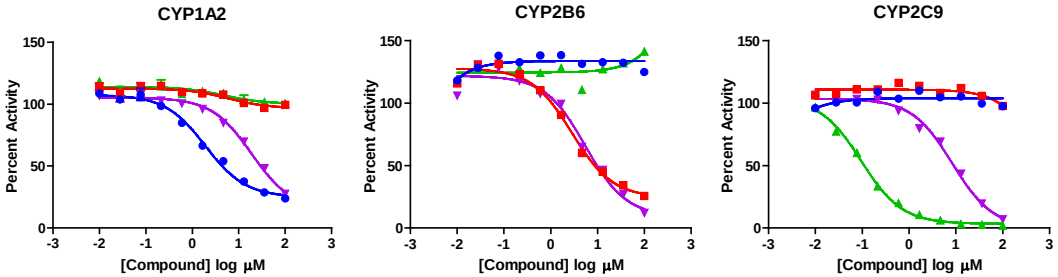


DeGroot et al. 2016 SOT poster #3757

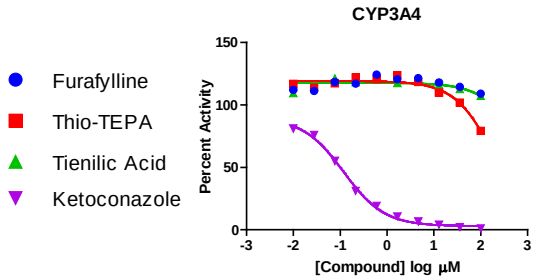
Amount of XME Activity in Microspheres



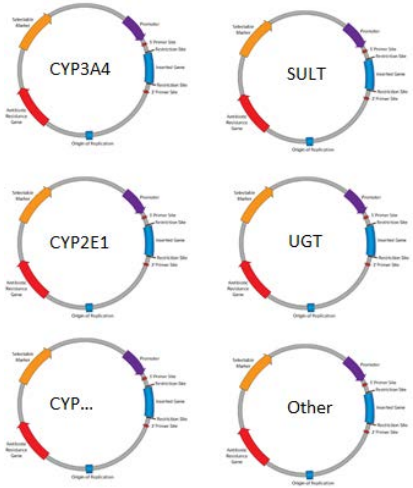
Small Molecule Inhibition of XME Activity



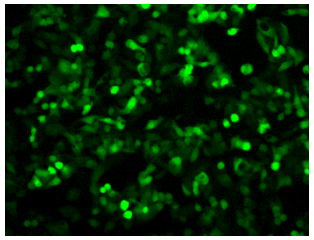
Compound	Mol. Wt. (g/mol)	Targeted P450	IC50 Free S9 (µM)	IC50 AIME (µM)
Furafylline	260.25	1A2	2.39	1.92
Thio-TEPA	189.22	2B6	7.46	2.86
Tienilic Acid	331.17	2C9	.053	.096
Ketoconazole	531.43	3A4	.086	0.12



Retrofitting Assays for Metabolic Competence – mRNA Intracellular Strategy

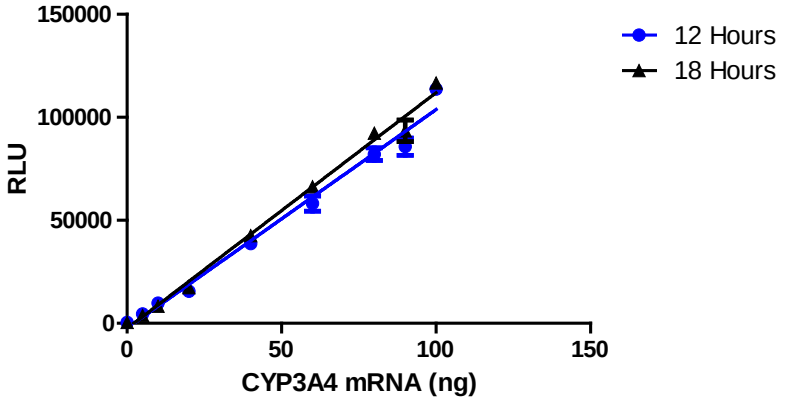


Pool in vitro transcribed mRNAs chemically modified with pseudouridine ad 5-methylcytidine to reduce immune stimulation



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

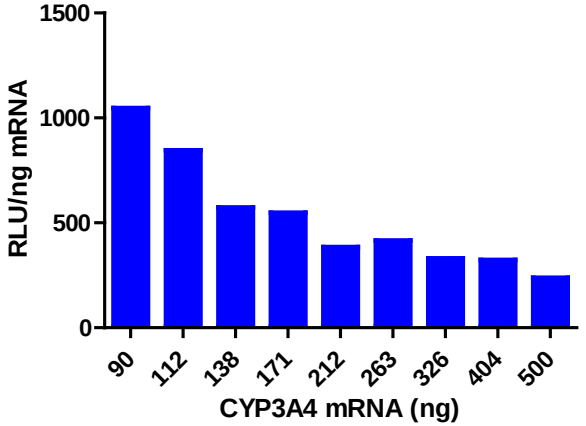
Linear Response of CYP3A4 Activity in HepG2 Cells with Increasing CYP3A4 mRNA



Advantage of transfecting with mRNA

Titrate different CYPs to match different ratios in different tissues

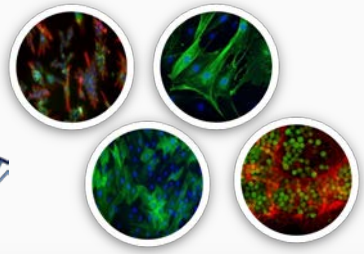
Efficiency of CYP3A4 Transfection in HepG2 Cells Begins to Decline Above 90 ng mRNA



Developing Approaches for Tiered Testing

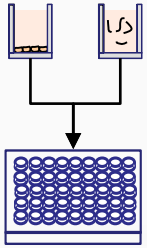
United States Environmental Protection Agency

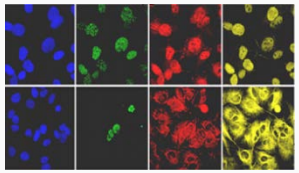
Comprehensive Transcriptomic Screening



Multiple Human Cell Types

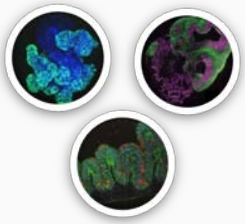
Focused ToxCast/Tox21 Assays



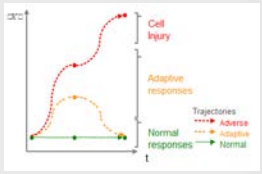


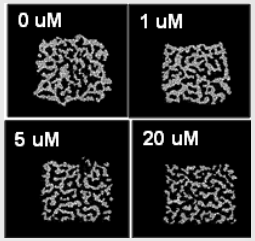
Time Course High Content Assays

Organs-on-a-Chip



Organotypic and Organoid Models





Computational and Statistical Modeling

Comprehensive Characterization

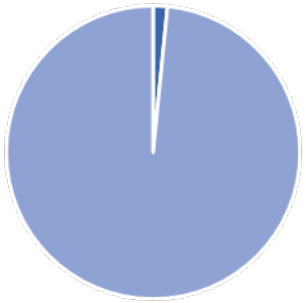
Verification of Affected Processes/ Pathways and Temporal Evaluation

Interpretation of Affected Process/ Pathways and Population Variability

Planning for HT Transcriptomics

New Approaches to Comprehensively Assess Potential Biological Effects

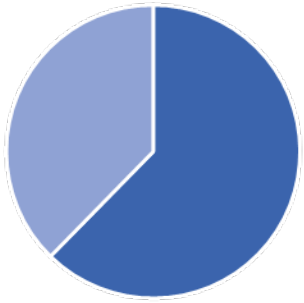
Gene Coverage



ToxCast

Not in ToxCast

Pathway Coverage*

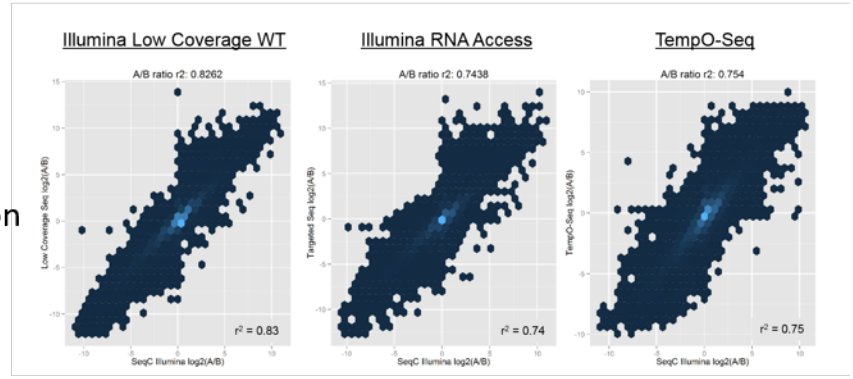


*At least one gene from pathway represented

High-Throughput Transcriptomic Platforms

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

Technical Comparison

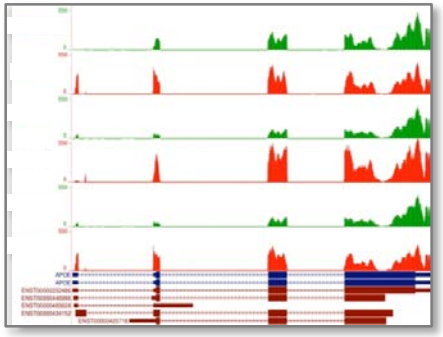


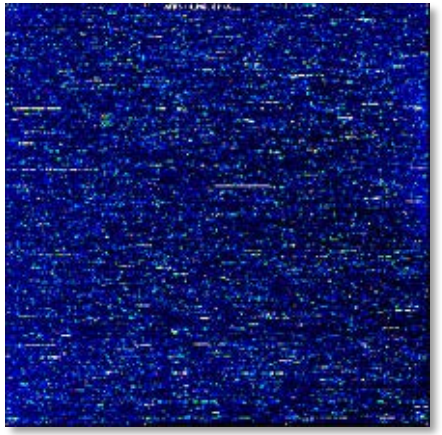
Functional Comparison

	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

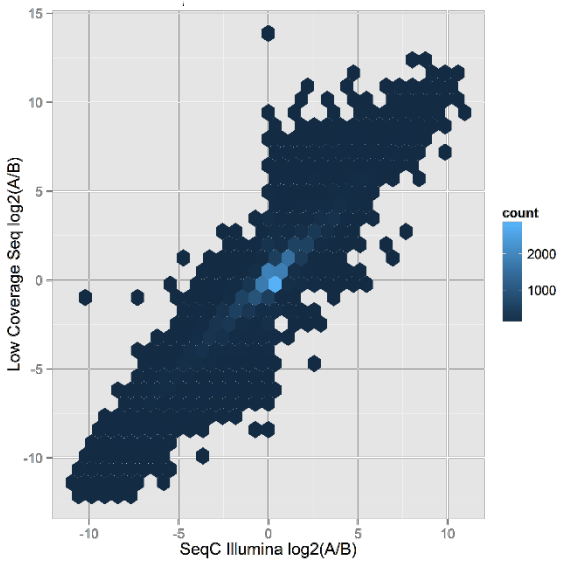
- Low coverage whole transcriptome RNA-seq (3 – 5 million mapped reads)
- Targeted RNA-seq (e.g., TempO-seq, TruSeq, SureSelect)
- Microarrays (e.g., Genechip HT)
- Bead-based (e.g., L1000)

Technical Performance of the Three Sequencing Platforms

United States Environmental Protection Agency

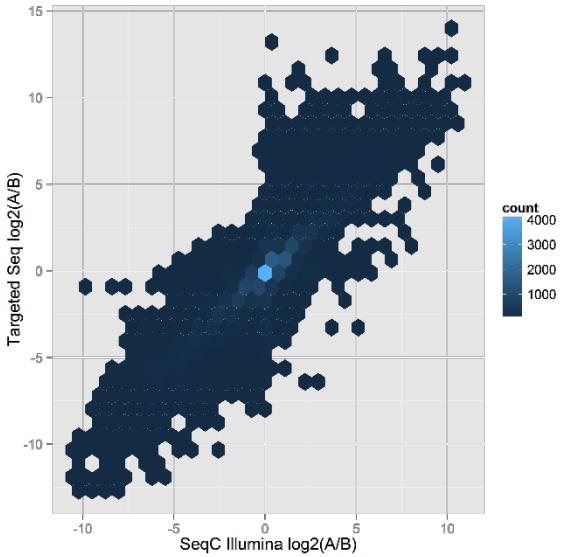
Low Coverage

r^2 0.83



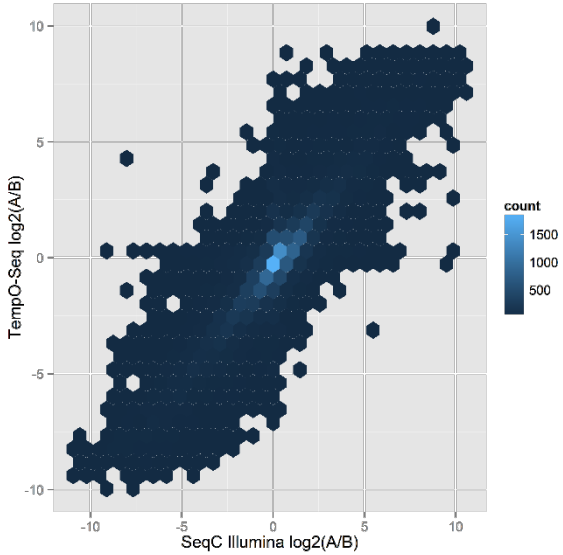
TruSeq

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**

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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