Alternative Approaches to Chemical Risk Assessment: Assays, Databases, Models

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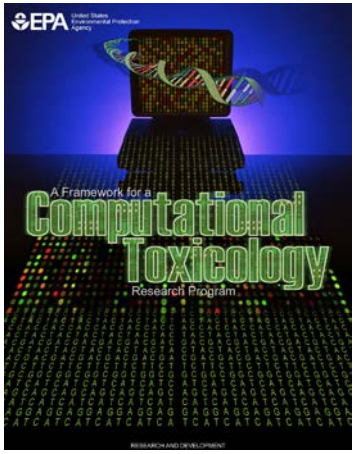
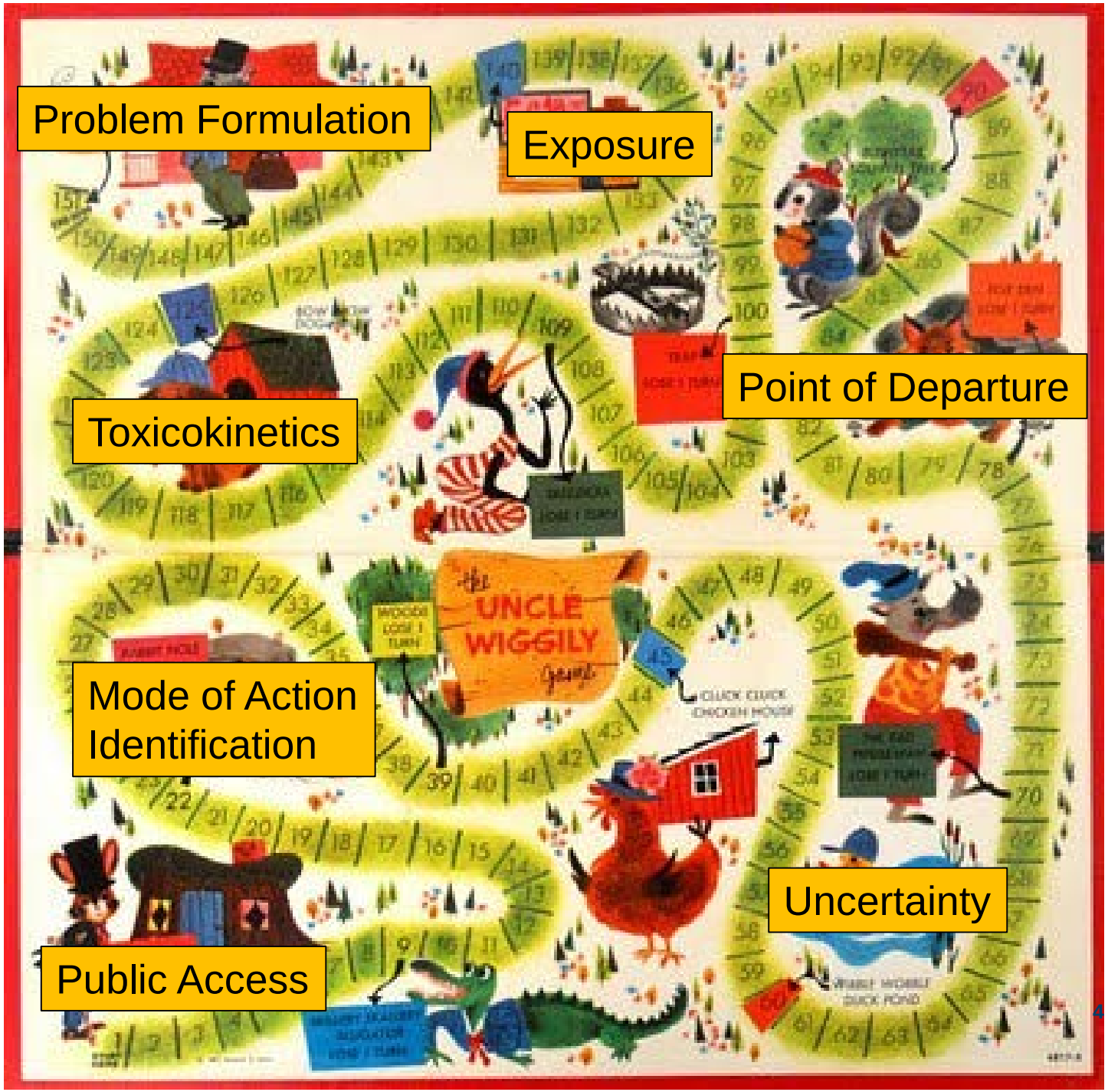
If I seem unduly clear to you, you must have misunderstood what I said

Alan Greenspan

National Center for Computational Toxicology



- National Center for Computational Toxicology established in 2005 to integrate
  - High-throughput and high-content technologies
  - Modern molecular biology
  - Data mining and statistical modeling
  - Computational biology and chemistry
- Currently staffed by ~60 employees
- Exists within the EPA's Office of Research and Development
- Home of the ToxCast and ExpoCast research efforts
- Key partner in U.S. Tox21 federal consortium


Problem Formulation
Exposure
Point of Departure
Toxicokinetics
Mode of Action
Identification
Uncertainty
Public Access
UNCLE WIGGILY

Typical Problem #1

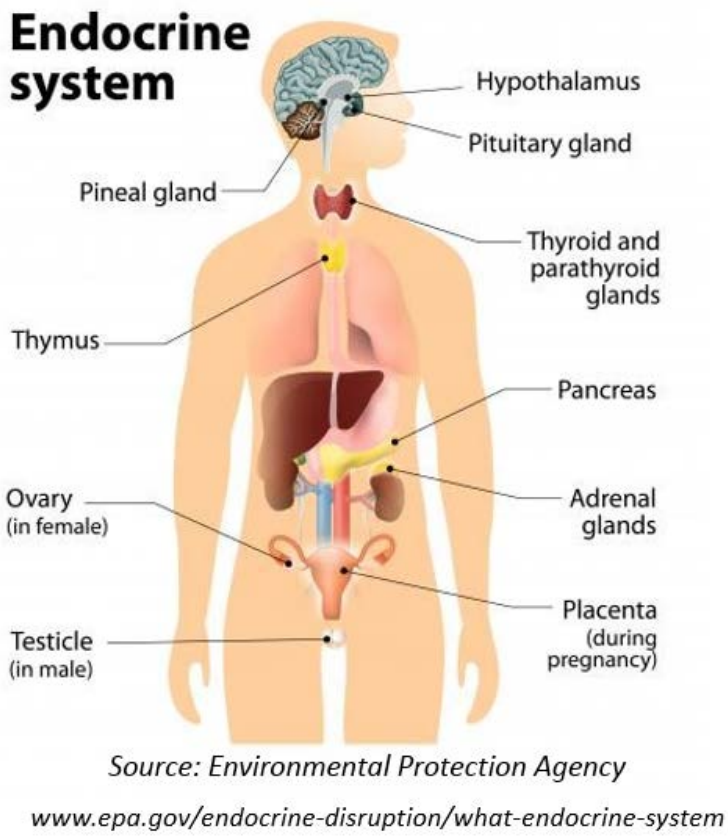
- At SuperFund site, EPA has identified 600 unique chemicals
- 300 of these have “good” reference doses / concentration (RfD / RfC), but 300 do not

- Can we determine “good enough” RfD/RfC to aid in cleanup planning?
- Can we do in it a few months?

Typical Problem #2

- Office of Pesticide Programs (OPP) has been petitioned to perform risk assessments on hundreds of pesticidal “inert ingredients”
- Companies have not typically been required to submit in vivo data on individual inerts
- Can we prioritize which of these chemicals should be the focus of detailed risk assessments?

Typical Problem #3



Source: Environmental Protection Agency

www.epa.gov/endocrine-disruption/what-endocrine-system

- The Endocrine Disruptor Screening Program (EDSP) is required to test an unknown number of chemicals as potential endocrine disruptors
- The EDSP Tier 1 screening battery costs ~$1M per chemical and has a throughput of 100 chemicals every few years

- Can we define the chemical universe subject to EDSP? (yes: ~10,000 chemicals)
- Can we develop approaches to prioritize chemicals and streamline Tier 1?

Common Themes

- Many chemicals (100s to 1000s)
- Many of these are data poor
- People, fish frogs, … are currently exposed

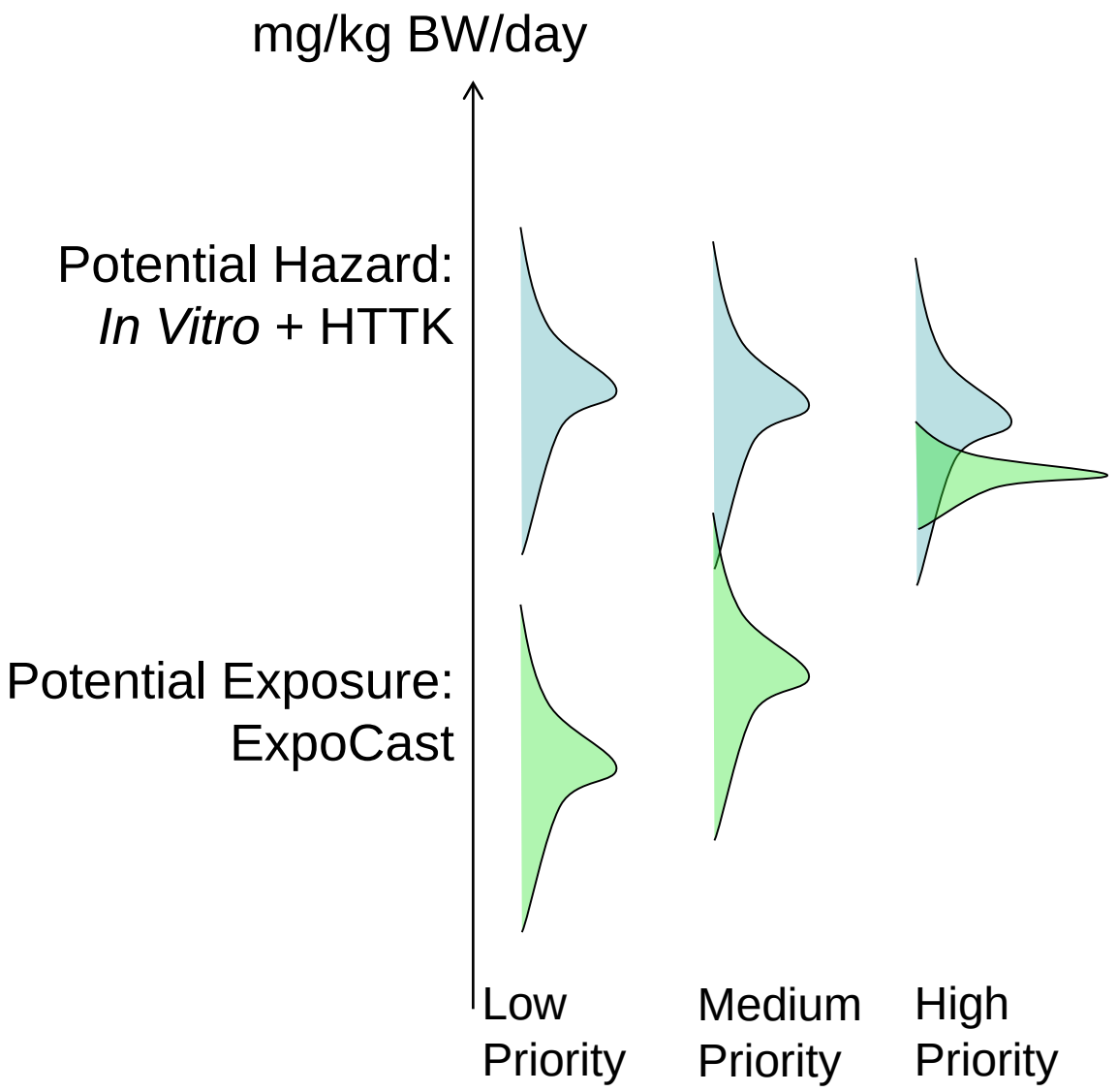
- Decision-makers need tools they can use today
  - They have a willingness to try new approaches
  - Evaluation of new methods can occur in real time on real-world problems
  - Evaluation needs to account for uncertainties in both new and old tools

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

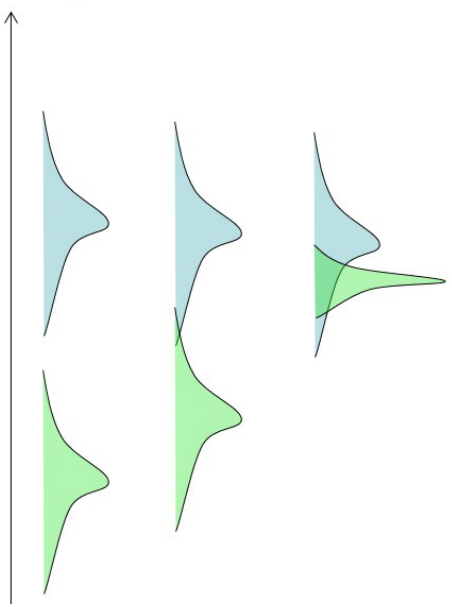
Risk-based Approach
Hazard + Exposure

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



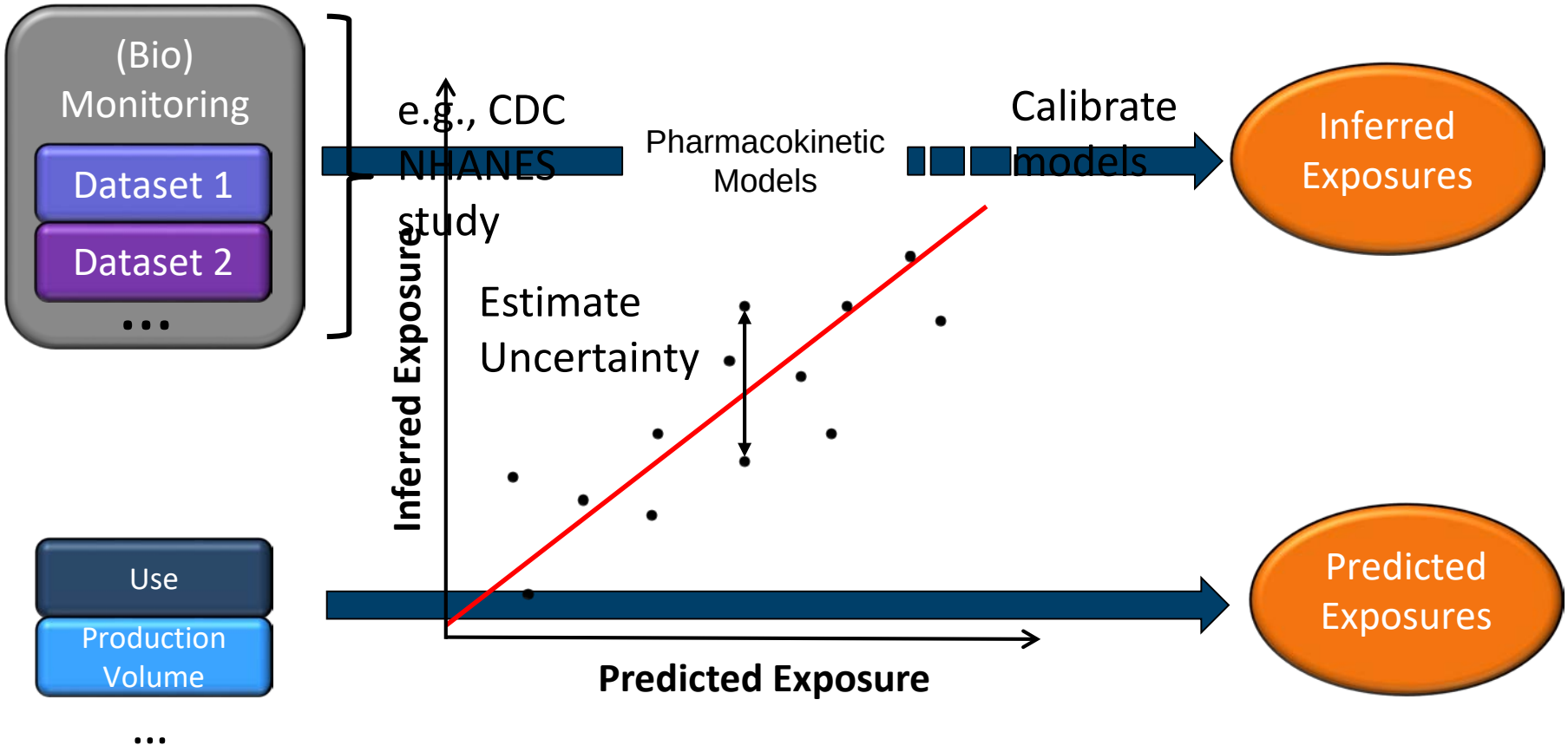
Tools / Models / Data needed

- Exposure information or model
  - Quantify in mg/kg/day
  - Include uncertainties
- Hazard information or model
  - Start in vitro
  - Quantify in uM required to trigger bioactivity
  - Include uncertainties
- Toxicokinetics
  - Use to convert between external dose and internal concentration
  - Include uncertainties



Population and Exposure Modeling

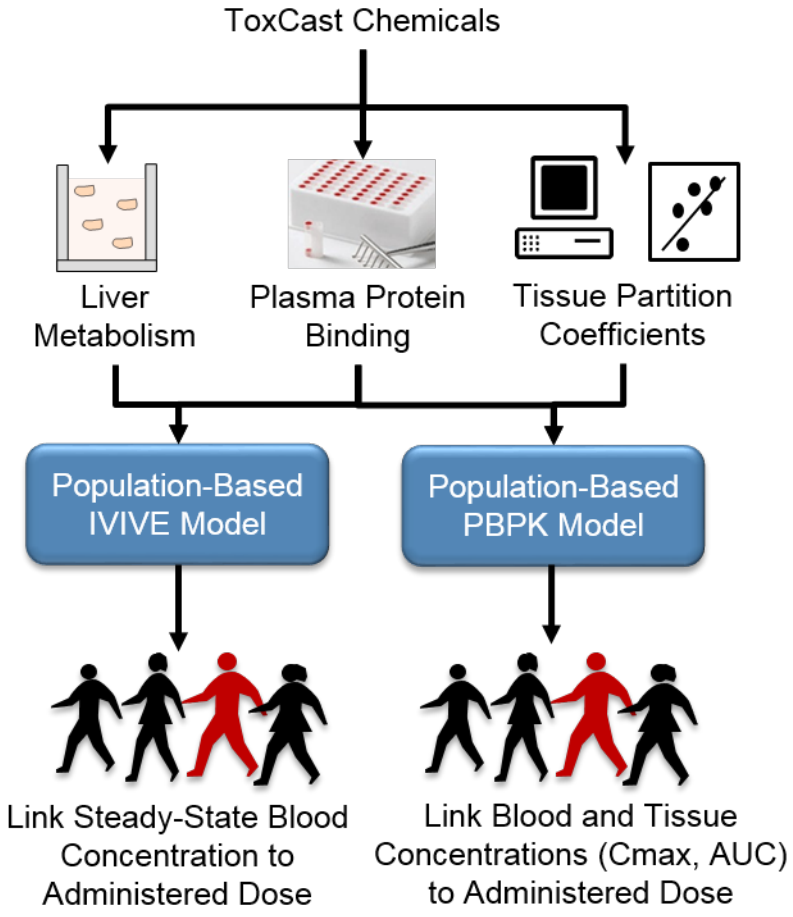
Estimating Exposure and Associated Uncertainty with Limited Data



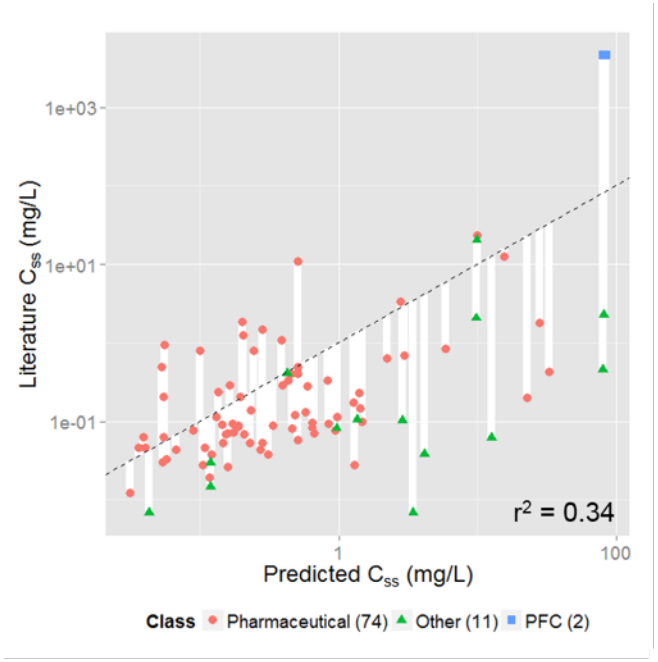
Wambaugh *et al.*, 2014

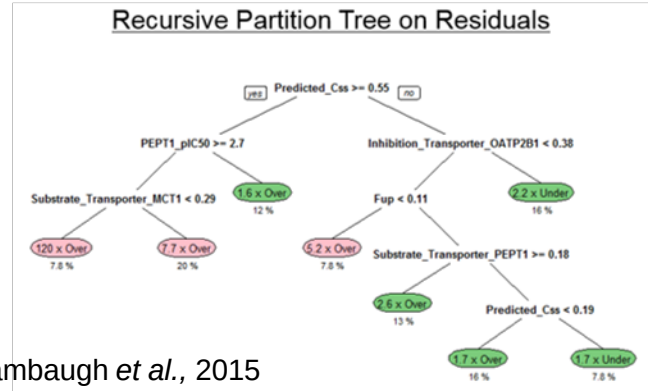
Toxicokinetics Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



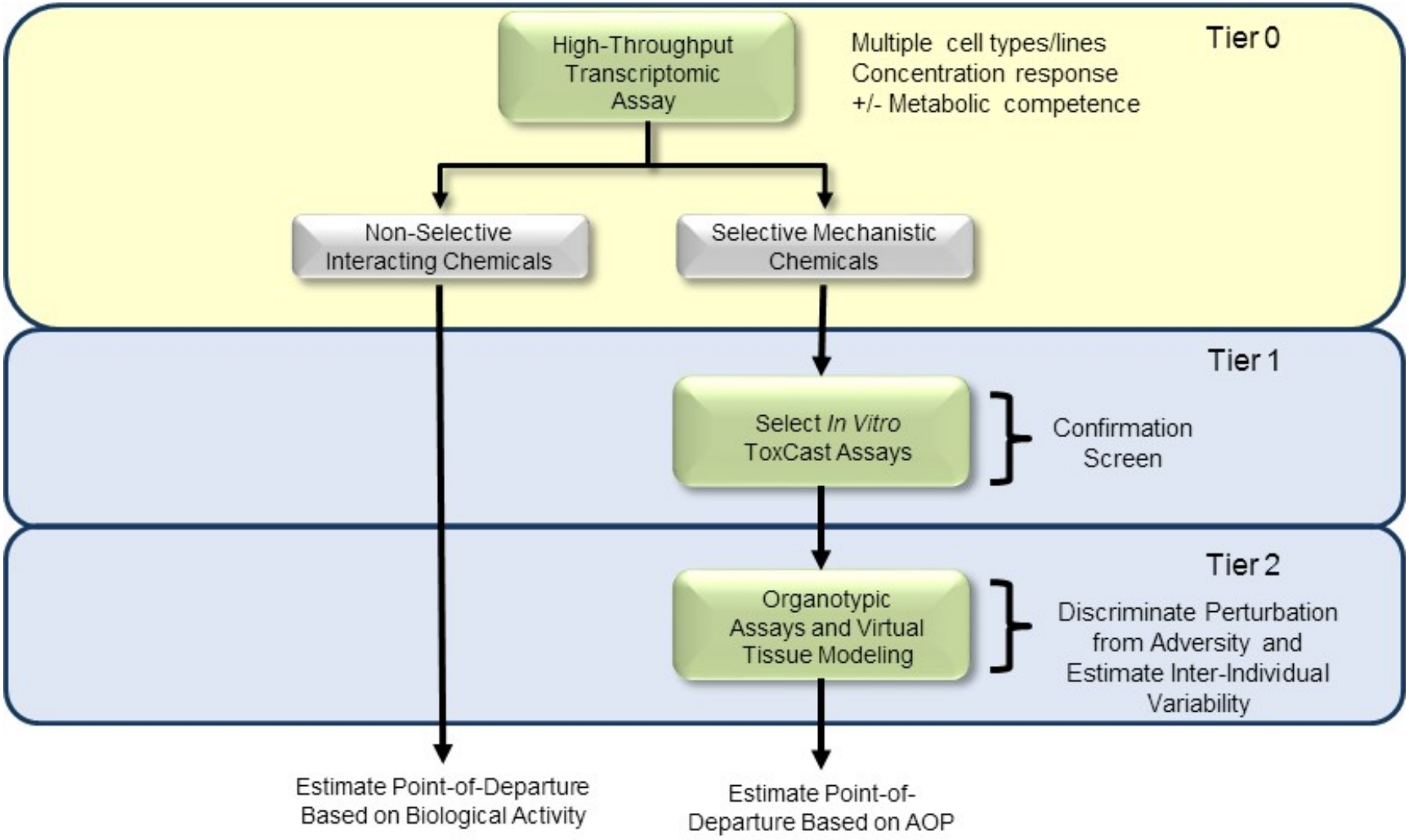
Wetmore et al.





Wambaugh *et al.*, 2015

The “Minimal Hazard Battery”

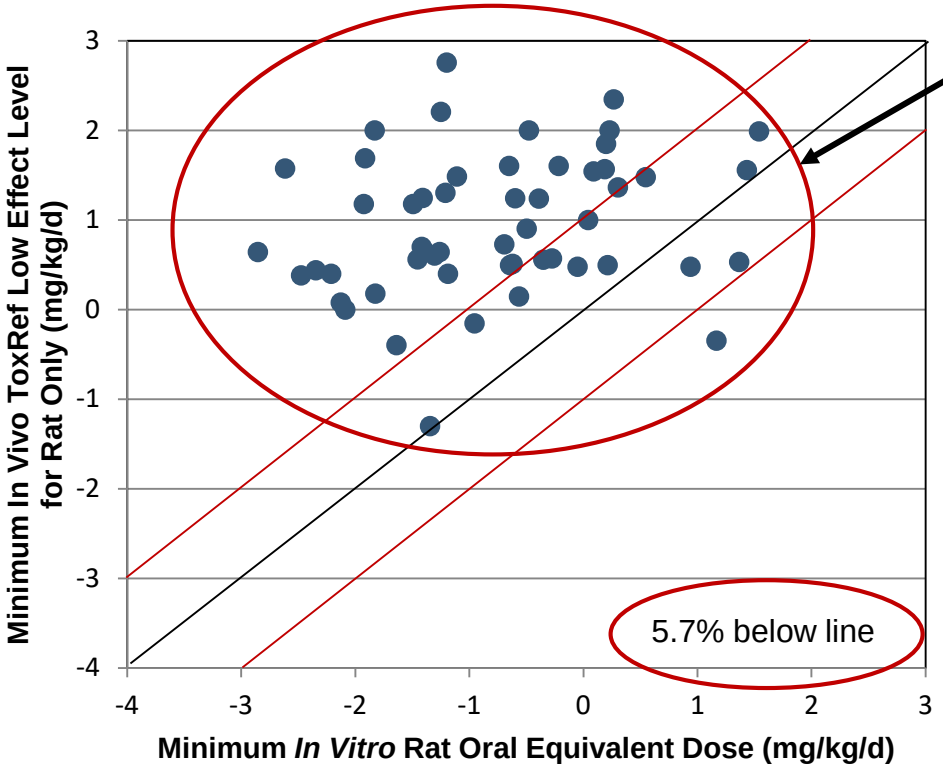


The “Minimal Hazard Battery”

- Tier 1 provides
  - *in vitro* LOAEC / NOAEC
  - Survey of perturbed pathways
  - Concentrations where cell stress may interfere with assays giving false positive signals
  - If expected doses overlap with cell-stress concentrations, then the chemical is probably dangerous
- Tier 2
  - Confirmation of pathways perturbed
- Tier 3
  - More in vivo-like context around findings
- Still in exploratory stage

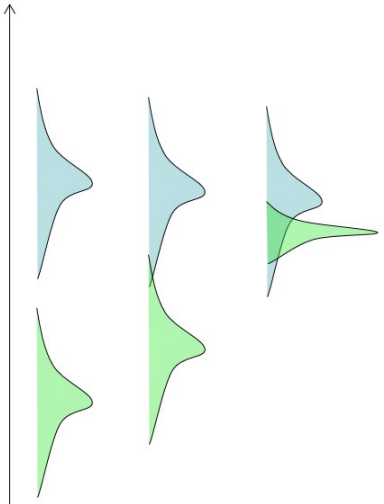
First test: Can the battery predict *in vivo* POD?

Spanned 38 *In Vivo* Endpoints across Multiple Tissues, Organ Systems, and Study Types (Repro, Chronic, and Dev)

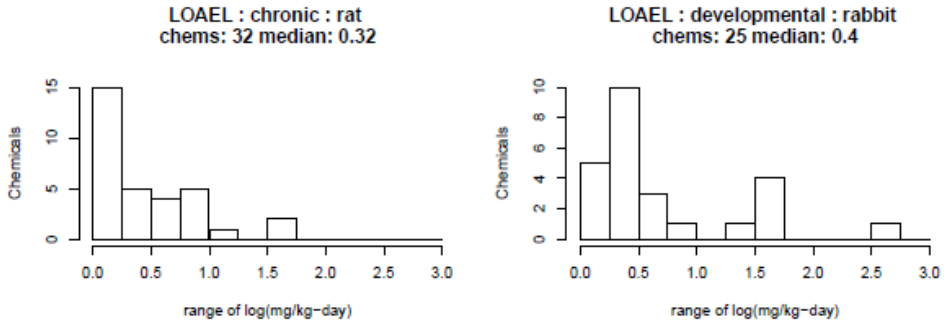


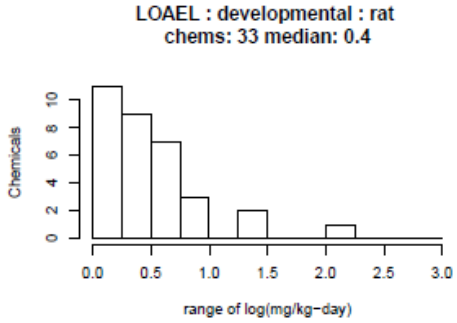
- Start with battery of in vitro assays
- Convert to dose with HT toxicokinetics
- 94% of chemicals have a health-protective prediction of POD

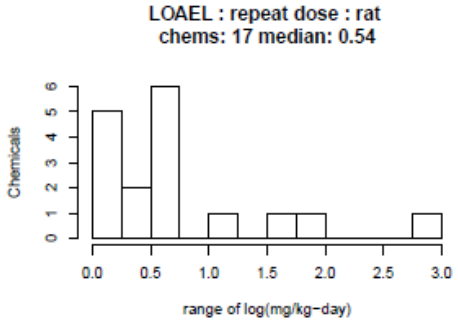
- But: How golden is the gold-standard?

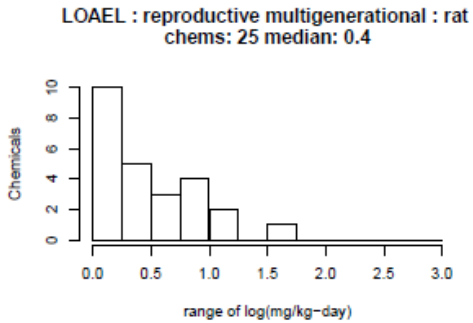


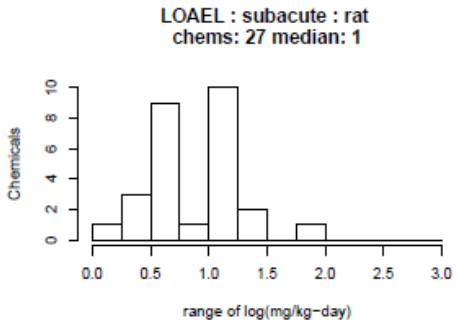
Wetmore *et al., Tox Sci.,* 2013

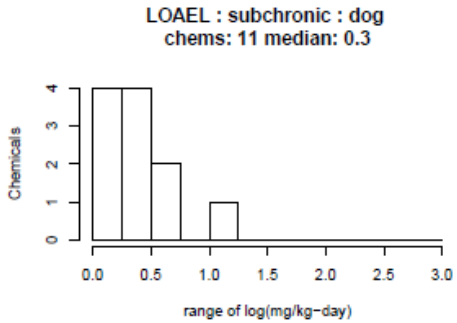


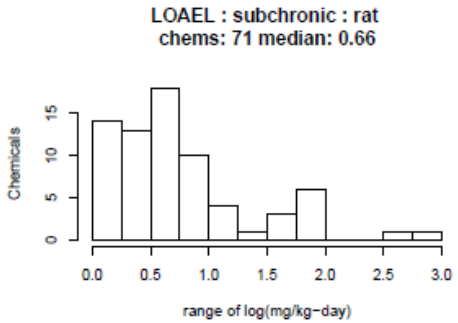












How golden is the gold standard?

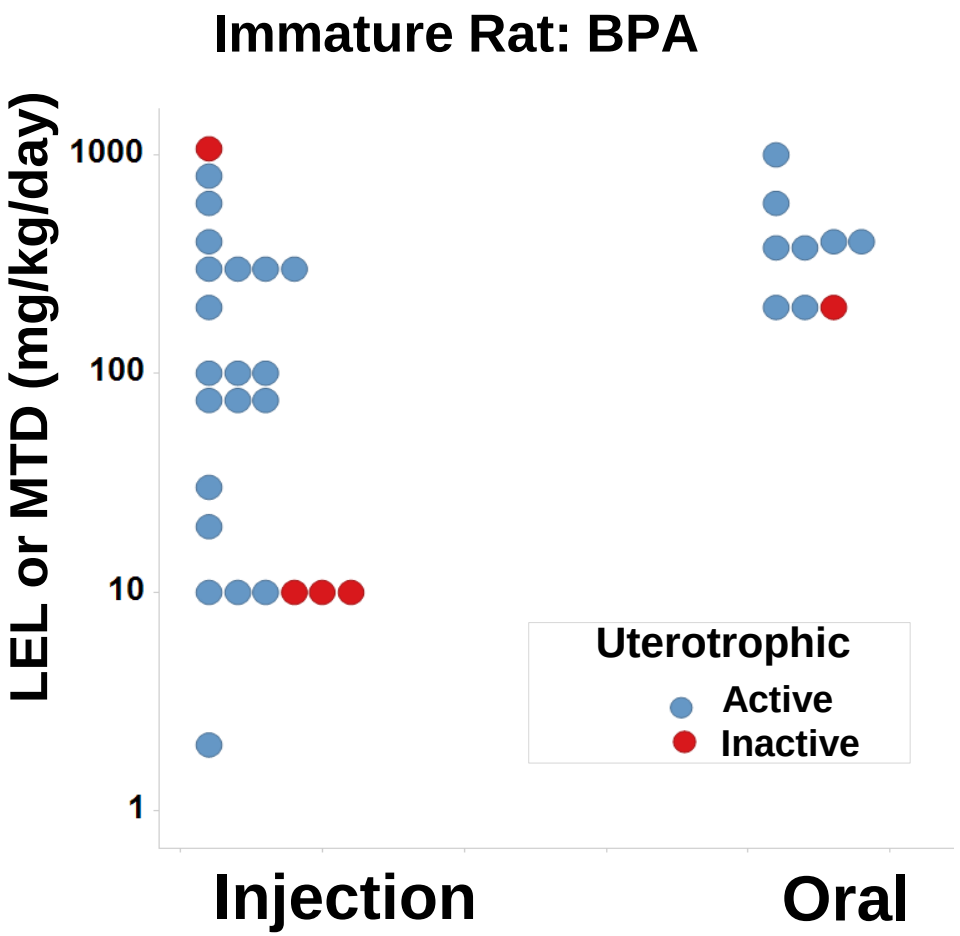
PODs vary from one lab to the next

Median span from lowest to highest LOAEL is 0.3 to 1.0 log units

Data taken from EPA ToxValDB

Uterotrophic guideline study uncertainty

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



Kleinstreuer et al: “A Curated Database of Rodent Uterotrophic Bioactivity” EHP (2015)

Anemia concordance results

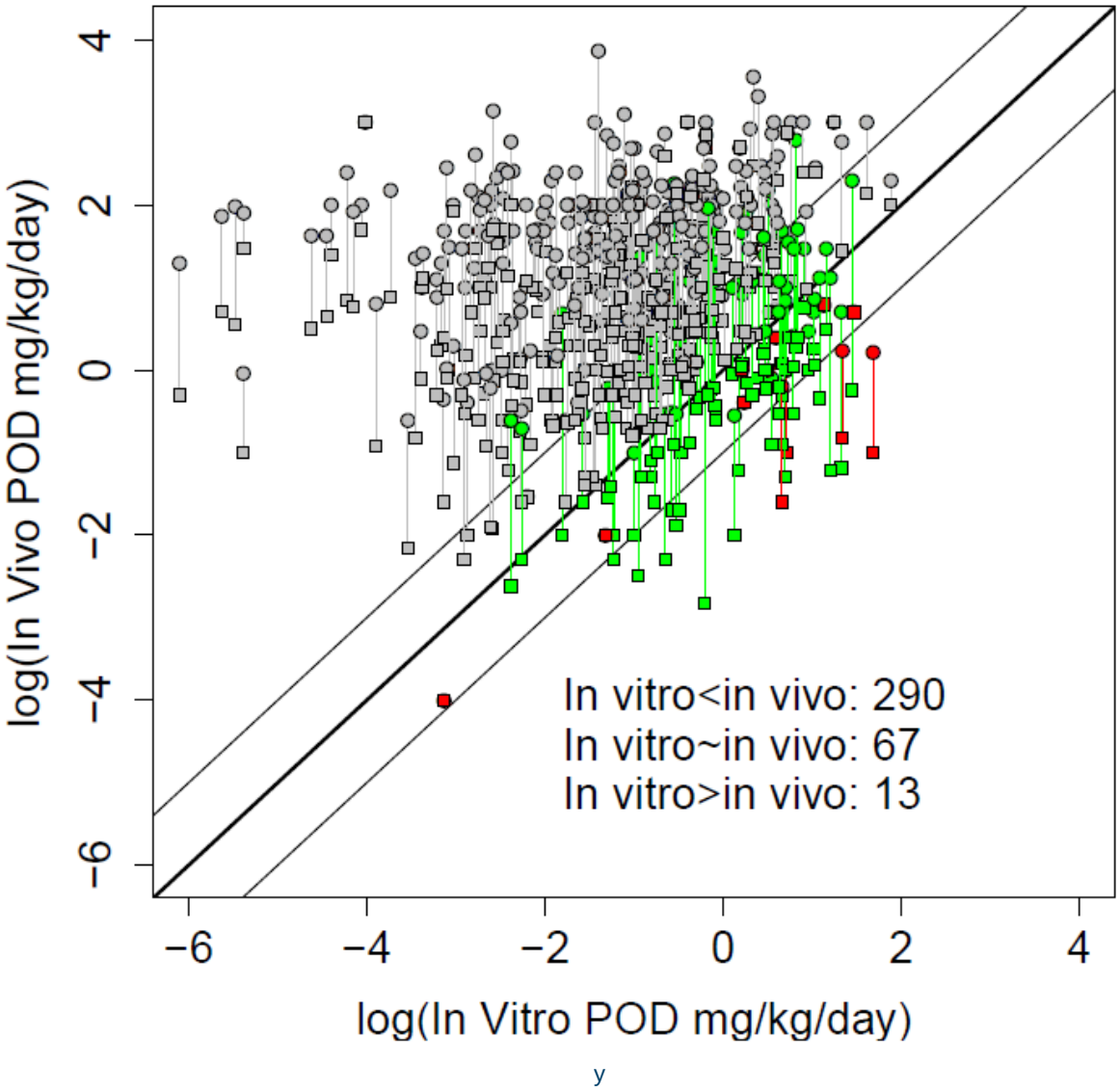
Species / study 1	Species / study 2	Concordant	Not Concordant	Fraction Concordant
rat SUB	rat CHR	18	2	0.90
rat CHR	dog CHR	13	2	0.87
rat CHR	rat SUB	18	4	0.82
rat SUB	**rat SUB**	**16**	**4**	**0.80**
rat SUB	dog CHR	11	4	0.73
mouse CHR	rat CHR	11	4	0.73
mouse CHR	rat SUB	13	7	0.65
dog CHR	rat SUB	11	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. Reg. Tox. Pharm (2017) “Retrospective Mining of Toxicology Data to Discover Multispecies and Chemical Class Effects: Anemia as a Case Study”.

Sources of Uncertainty / Variability In Vivo

- Experimental variability
  - Species, strain, dose range, dose spacing
- Statistical power issues
  - Too few animals to see weak or rare effect
- Reporting bias
  - Was an effect negative or not looked for?
- Observer bias
  - Less severe phenotypes not reported when more severe ones are present
- Diagnostic terminology drift
- Data assimilation and analysis
  - Typos, incomplete transcription

Updated IVIVE, accounting for uncertainty

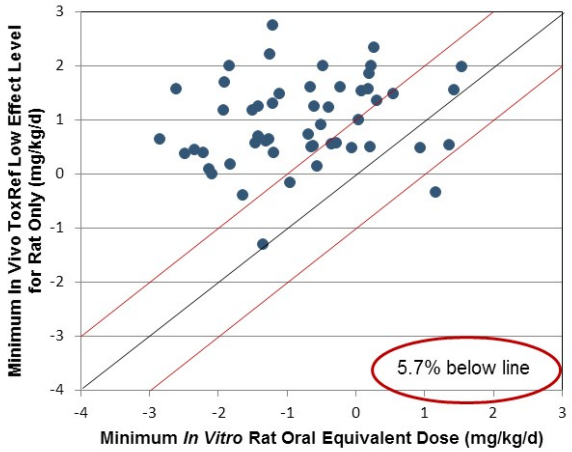


Larger set of chemicals with in vivo, in vitro, TK

~4% have in vitro POD consistently greater than in vivo values

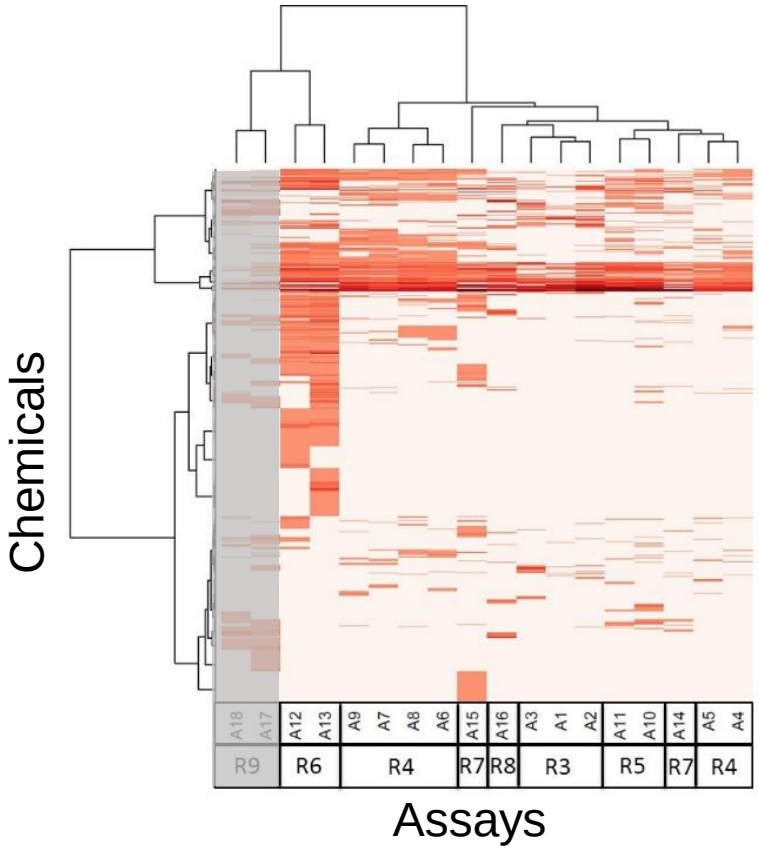
Issue: what is the correct in vitro POD assay?
- Bioactivity vs. adversity

Unpublished



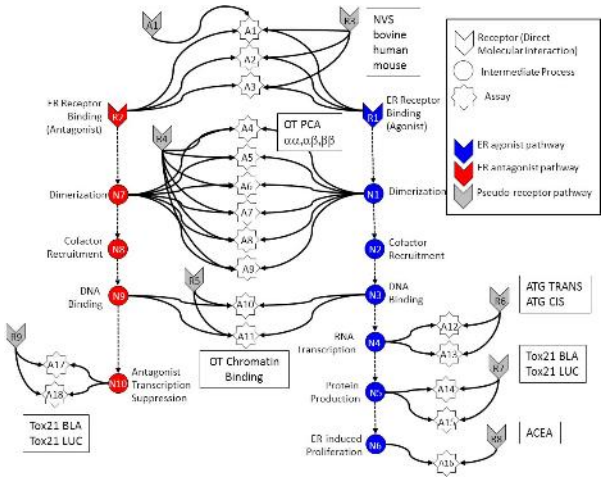
In vitro assays also have false positives and negatives

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



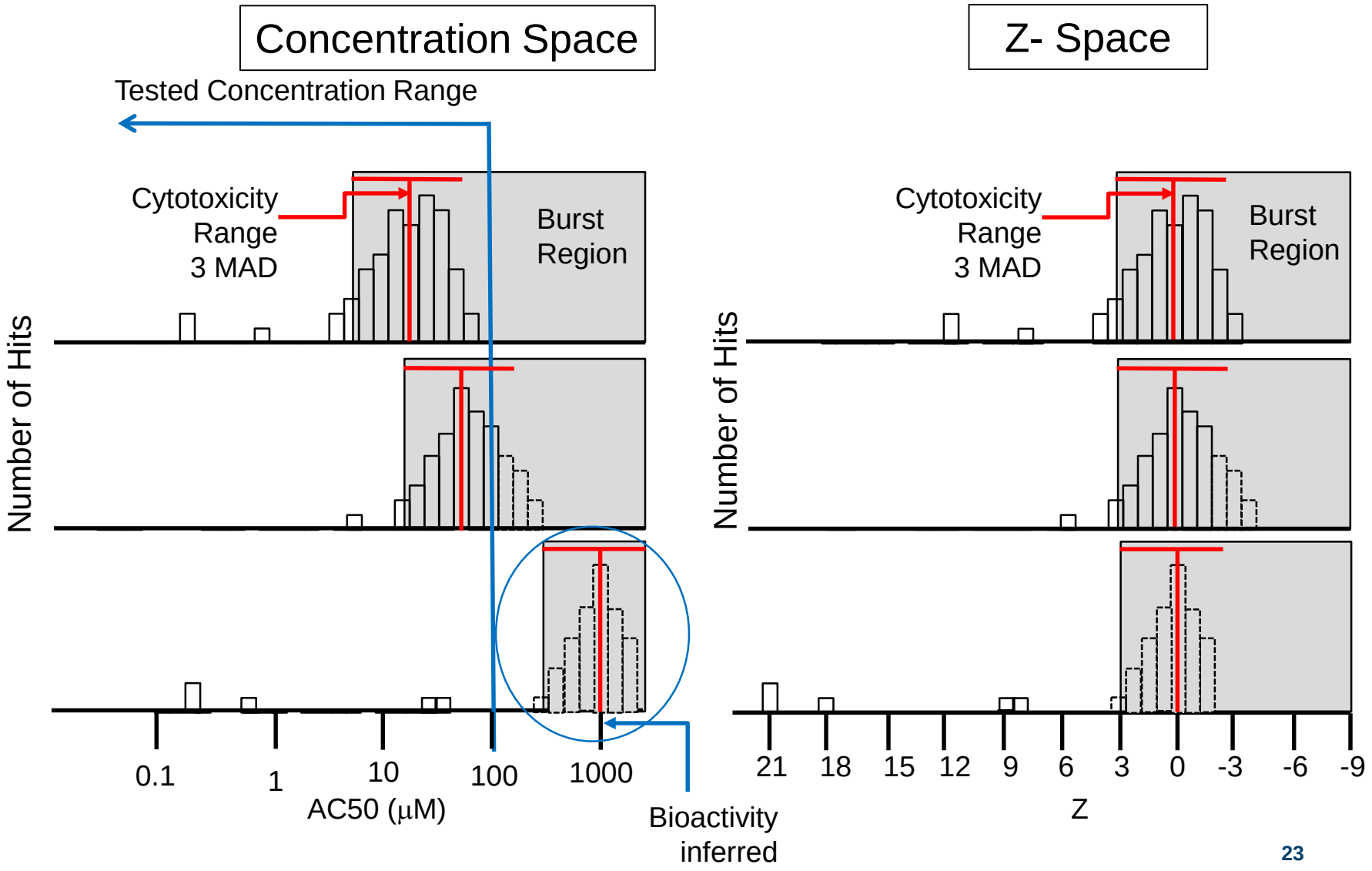
Much of this “noise” is reproducible
- “assay interference”
- Result of interaction of chemical with complex biology in the assay

Chemical universe is structurally diverse
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



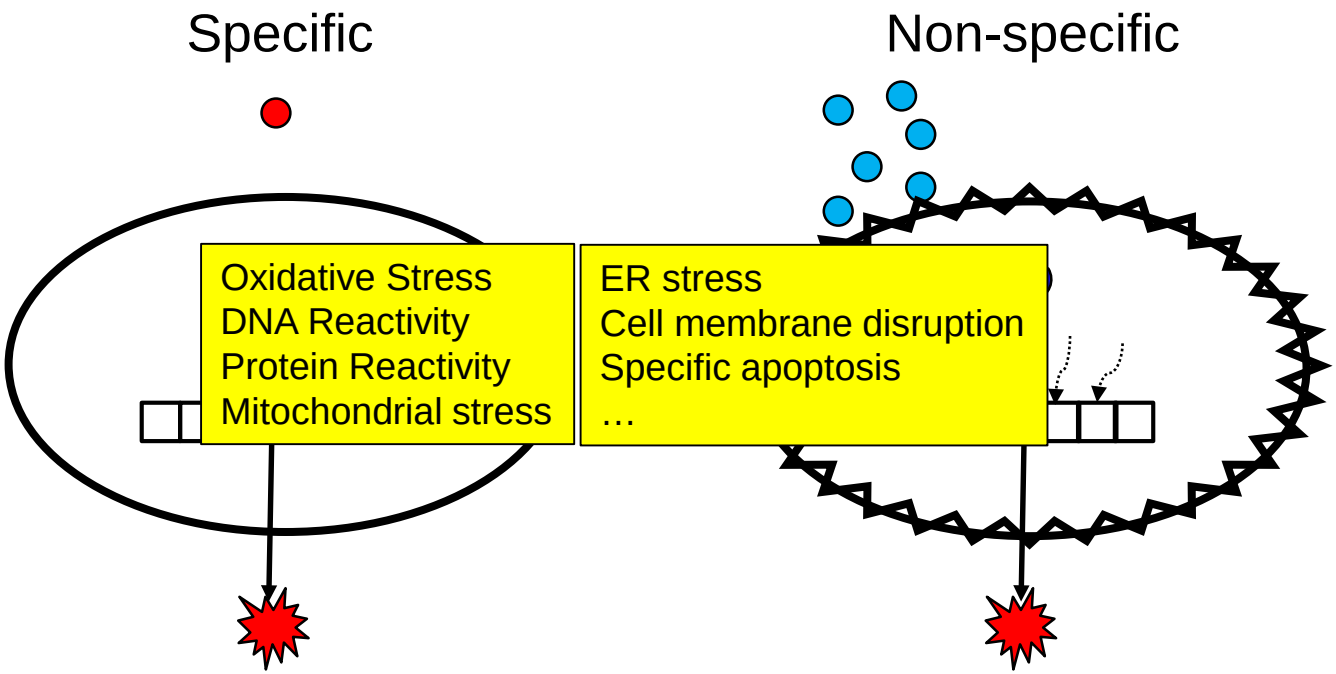
Judson et al: ToxSci (2015)

Most chemicals display a “burst” of potentially non-selective bioactivity near cytotoxity concentration

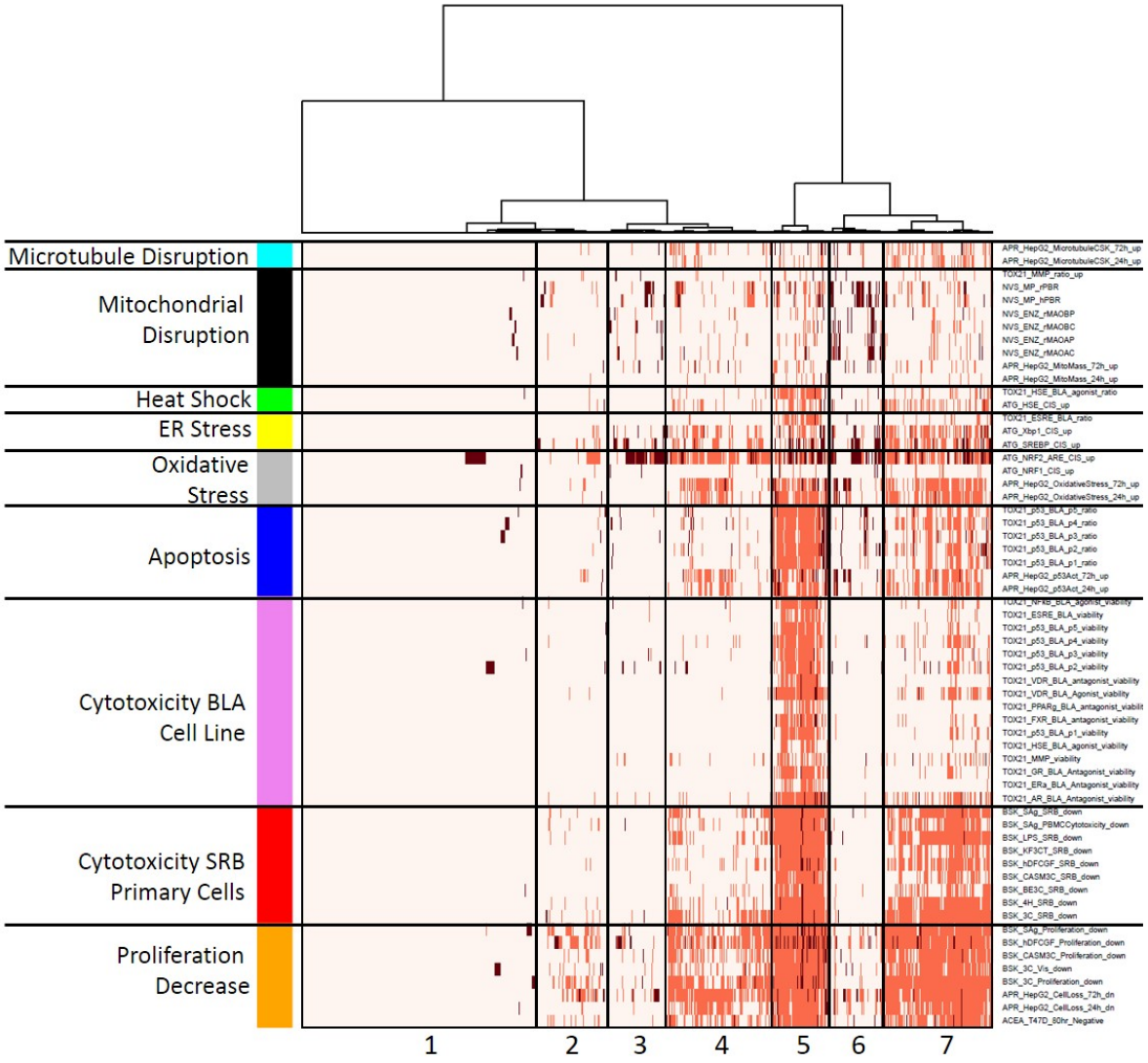


Judson et al. Tox.Sci. (2016)

Schematic explanation of the burst



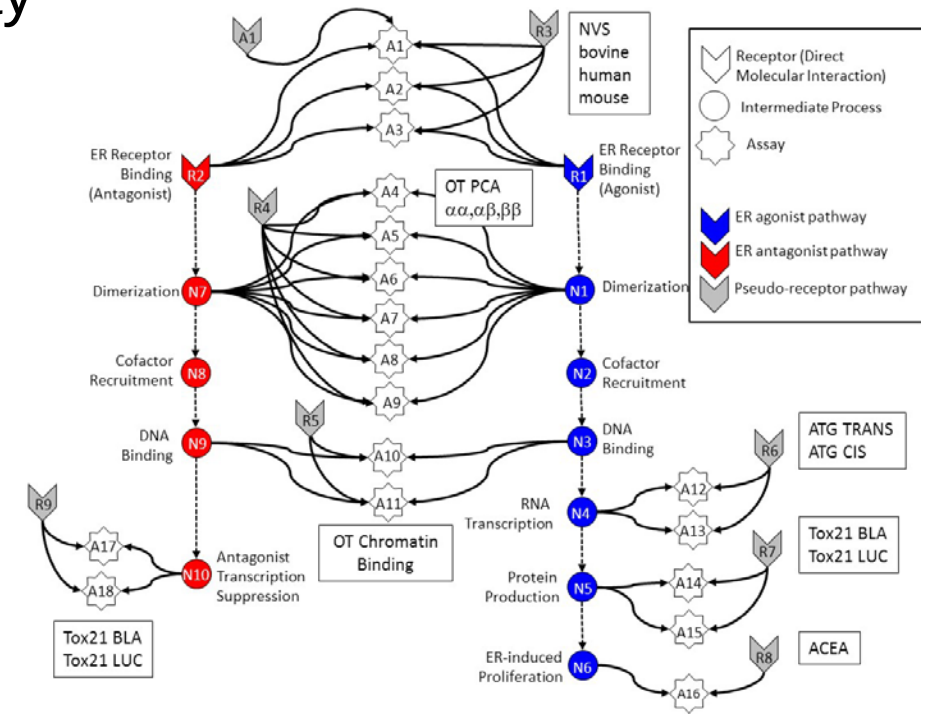
Heatmap of stress and cytotoxicity assays in 1000 chemicals



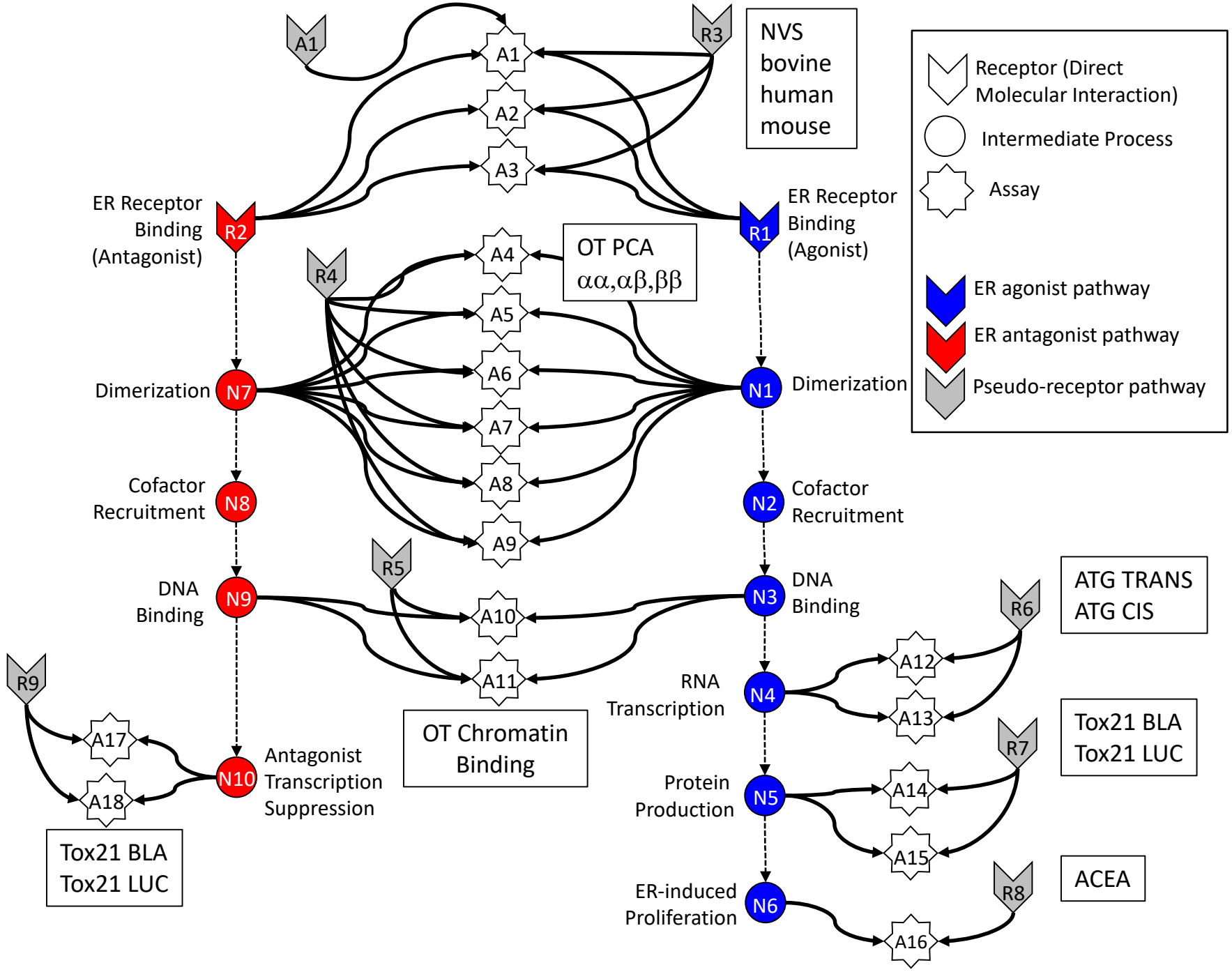
Judson et al. ToxSci (2016)

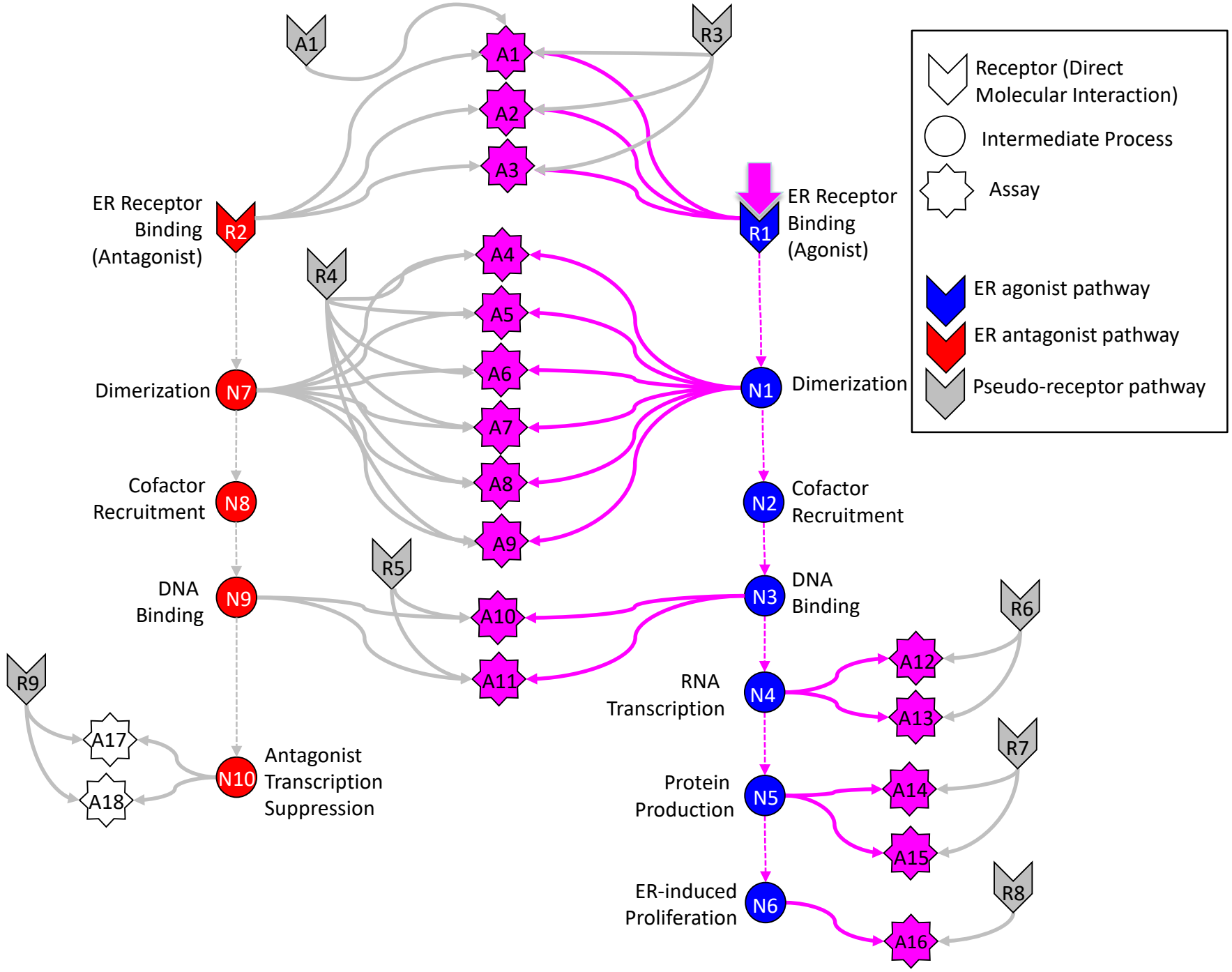
In Vitro Estrogen Receptor Model

- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)


NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
A1
A2
A3
R1
R2
R3
ER Receptor Binding (Agonist)
ER Receptor Binding (Antagonist)
OT PCA
αα,αβ,ββ
A4
A5
A6
A7
A8
A9
R4
N1
N2
N3
N4
N5
N6
N7
N8
N9
N10
Dimerization
Cofactor Recruitment
DNA Binding
R5
A10
A11
OT Chromatin Binding
ATG TRANS
ATG CIS
R6
A12
A13
RNA Transcription
Tox21 BLA
Tox21 LUC
R7
A14
A15
Protein Production
ACEA
R8
A16
ER-induced Proliferation
R9
A17
A18
Antagonist Transcription Suppression
Tox21 BLA
Tox21 LUC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R3
R4
R5
R6
R7
R8
R9
N1
Dimerization
N2
Cofactor Recruitment
N3
DNA Binding
N4
RNA Transcription
N5
Protein Production
N6
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N7
Dimerization
N8
Cofactor Recruitment
N9
DNA Binding
N10
Antagonist Transcription Suppression
A1
A2
A3
A4
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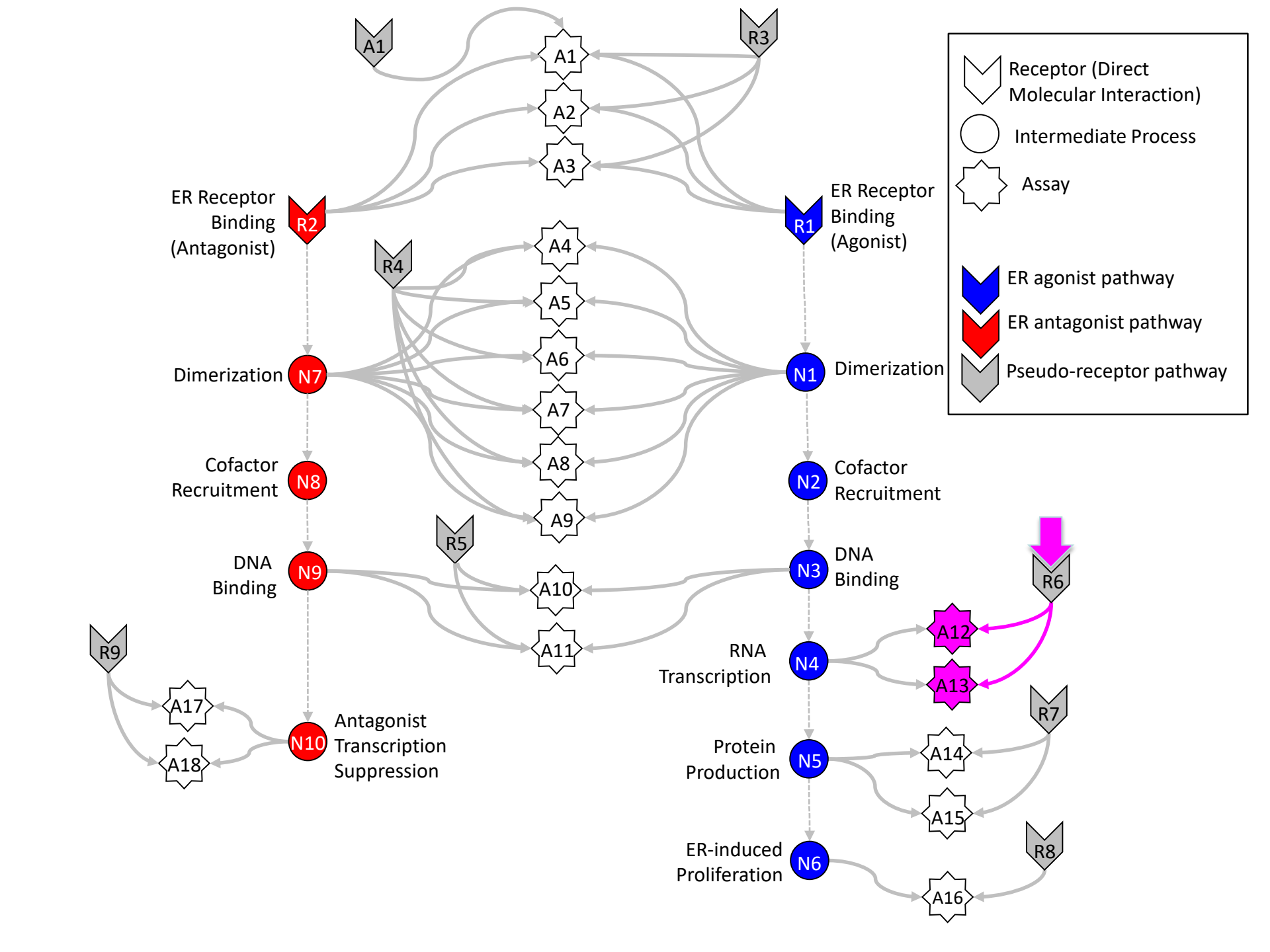
A15
A16
A17
A18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R3
R4
R5
R6
R7
R8
R9
A1
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A14
A15
A16
A17
A18
N1
Dimerization
N2
Cofactor Recruitment
N3
DNA Binding
N4
RNA Transcription
N5
Protein Production
N6
ER-induced Proliferation
N7
Dimerization
N8
Cofactor Recruitment
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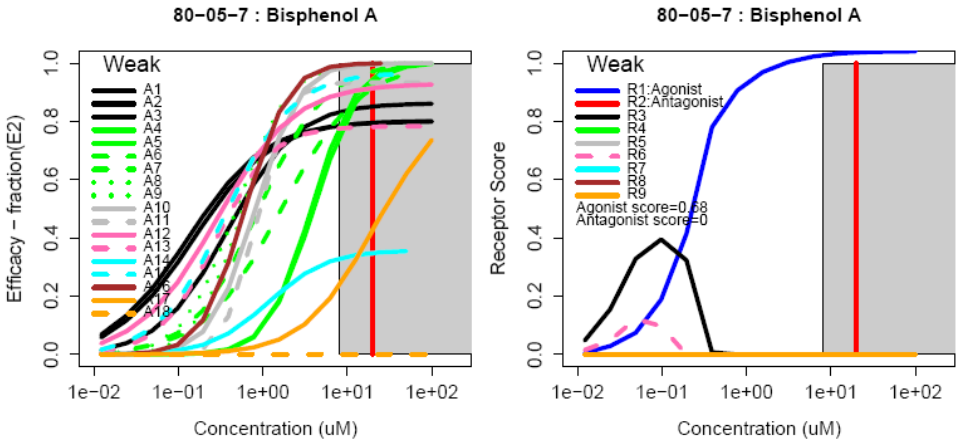
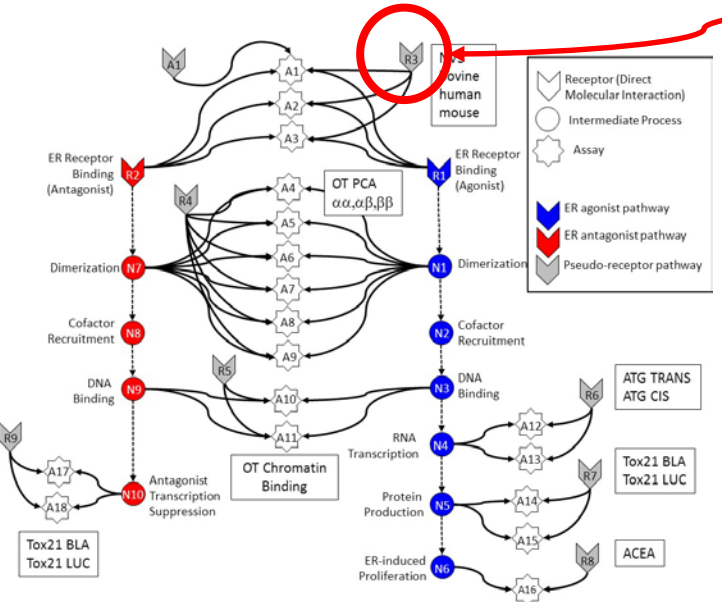
DNA Binding
N10
Antagonist Transcription Suppression

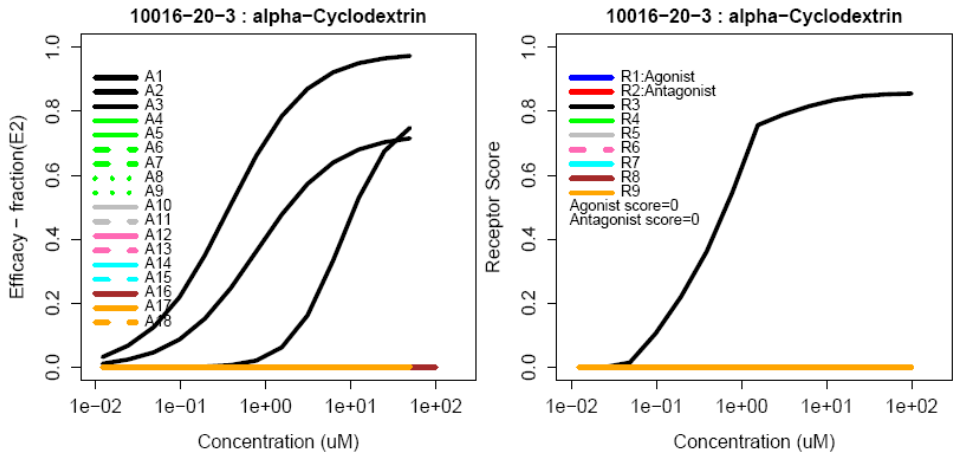
Example curves

True Agonist

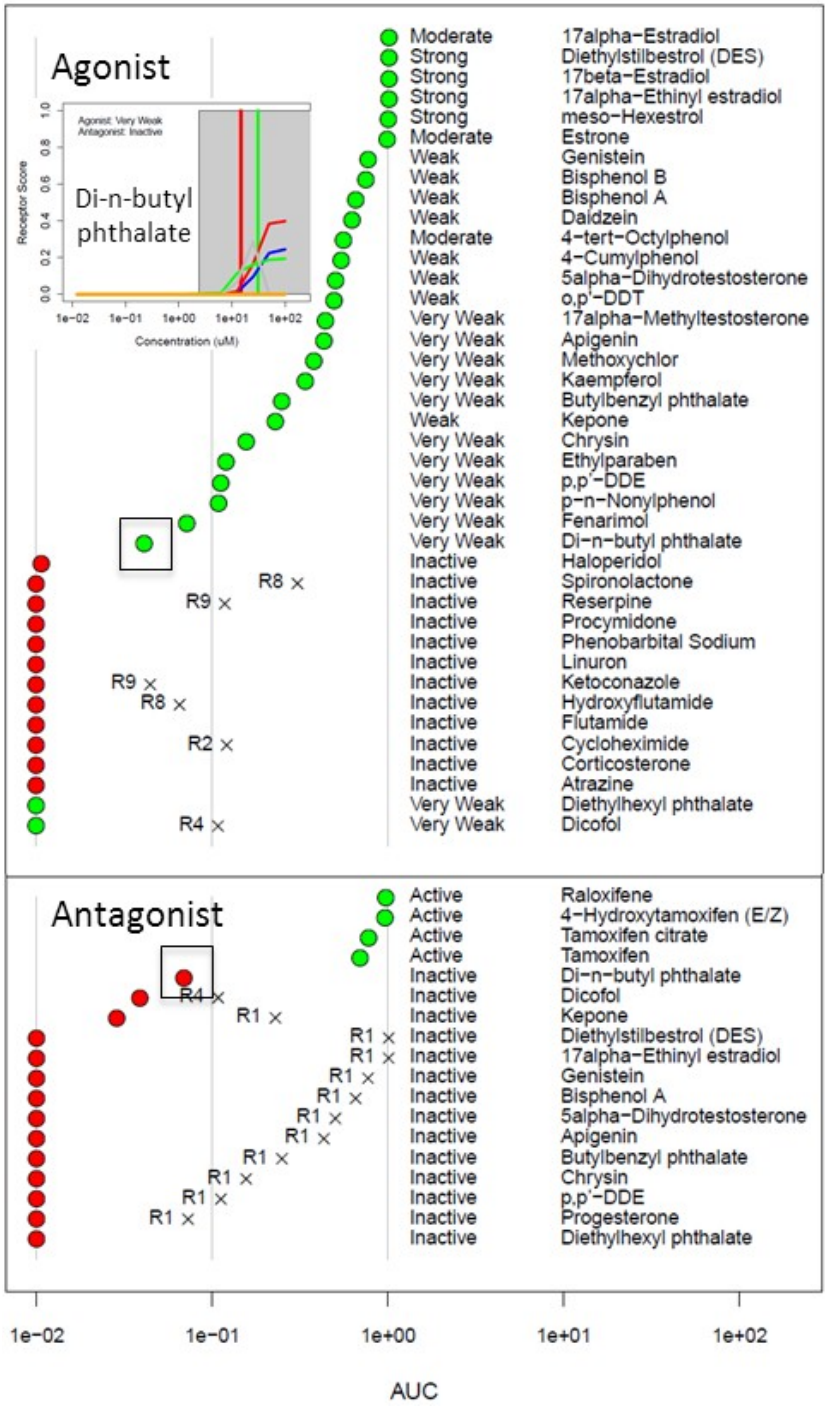


Assay Interference Example “R3”

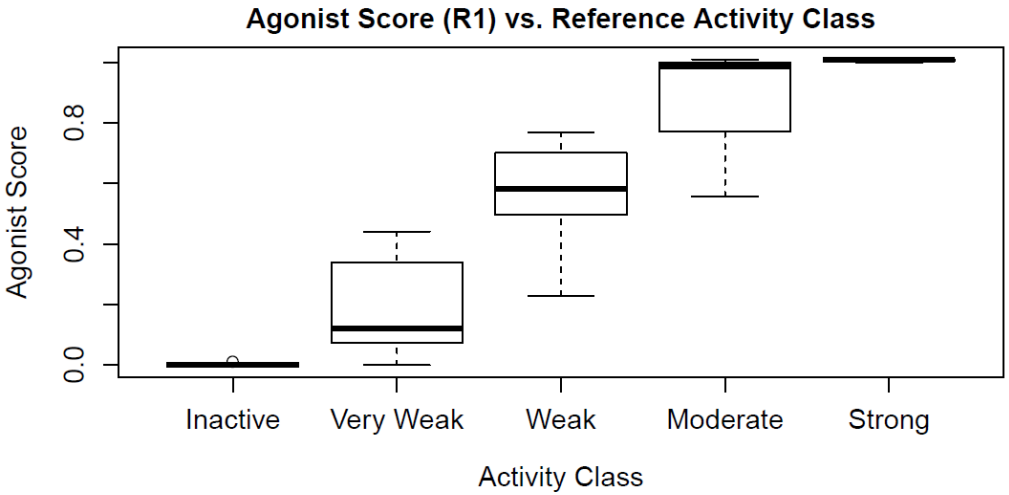




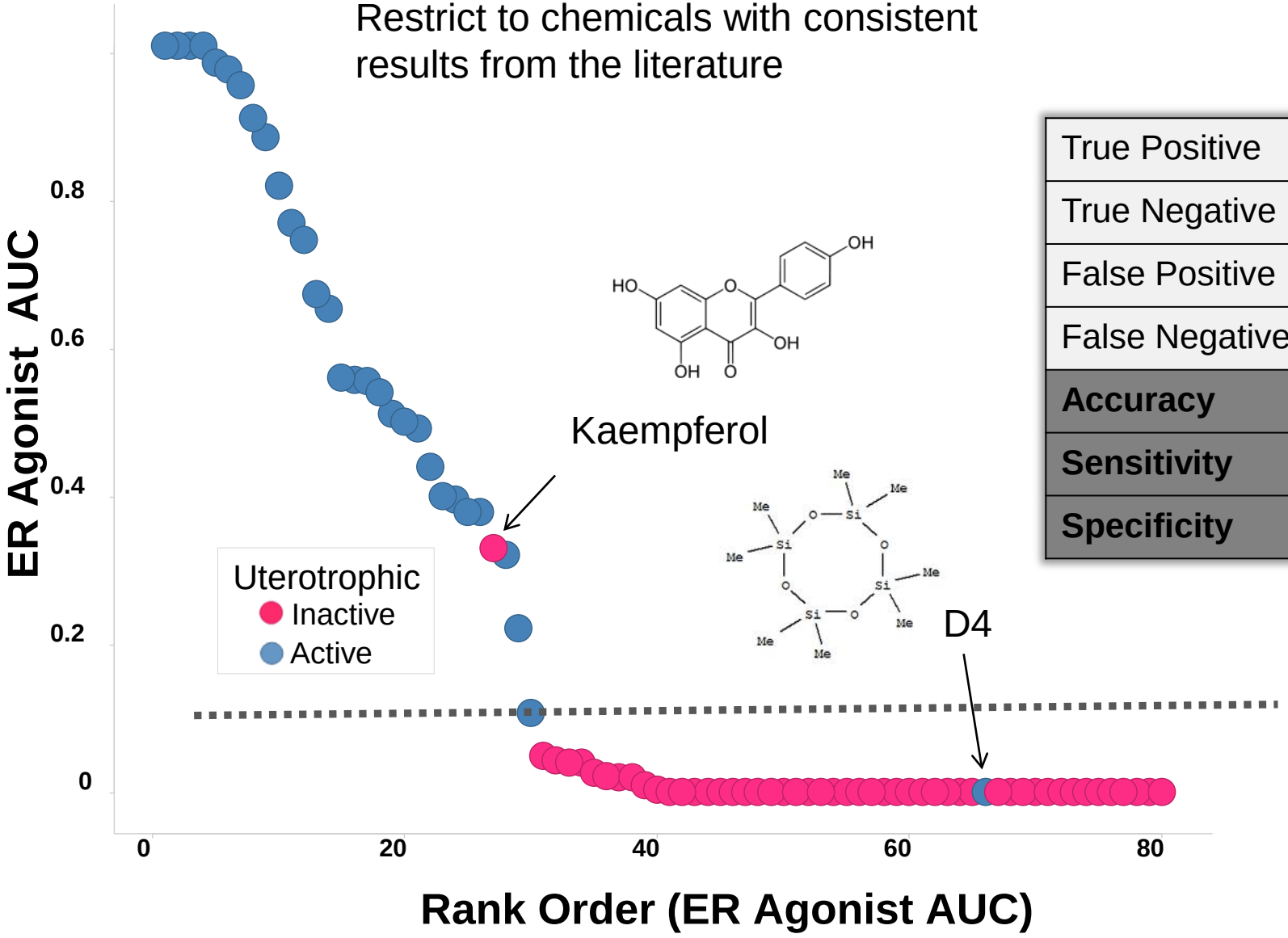
In Vitro Reference Chemical Performance



By using battery of assays and model of noise, we can accurately predict activity



Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic

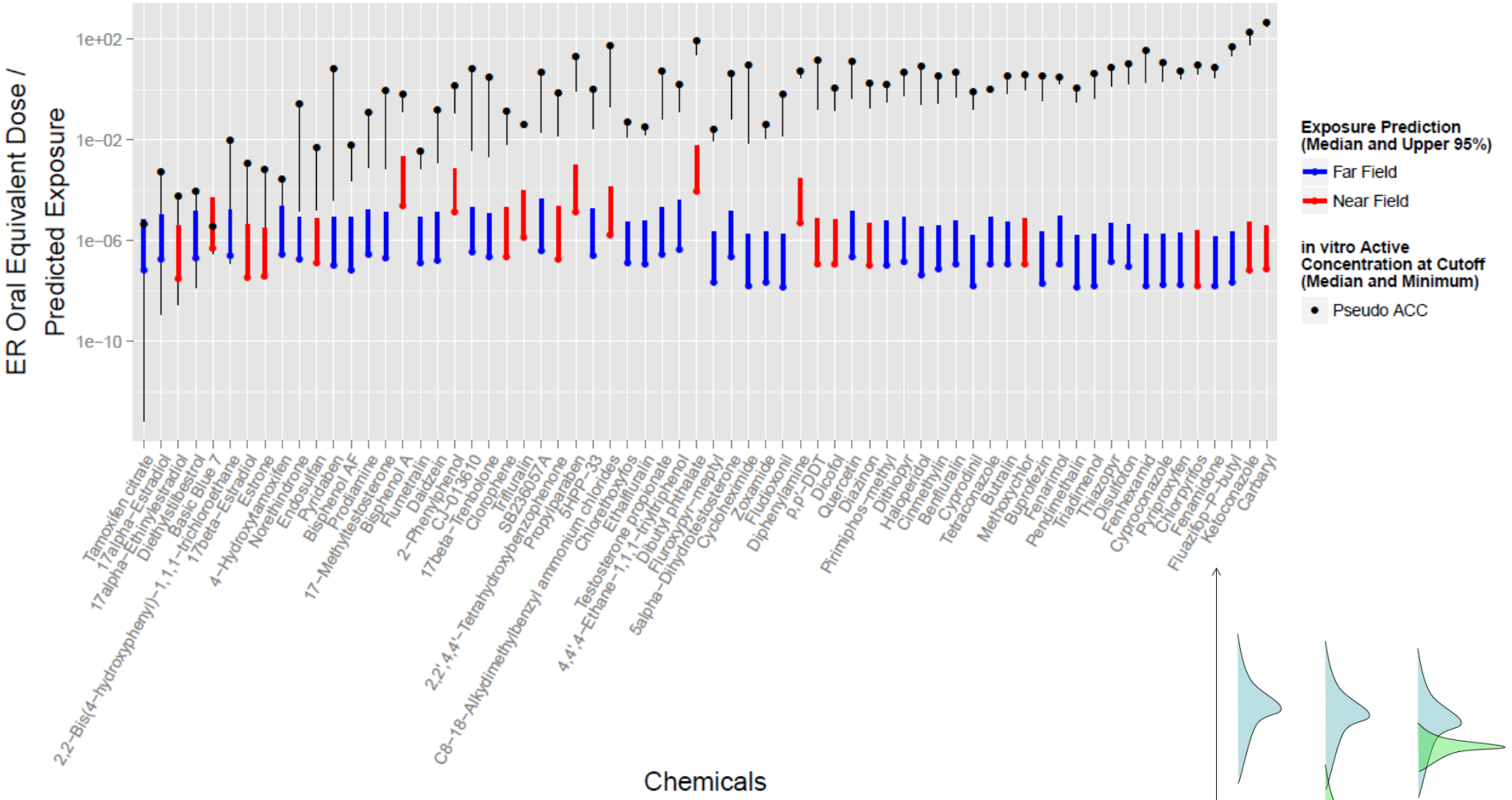


True Positive	29
True Negative	50
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.98**

Browne et al. ES&T (2015)

Prioritization (Replacement) Example

Compare predicted exposure and hazard POD



Compare estrogen receptor assay battery and exposure model

Moving Towards Regulatory Acceptance From FIFRA SAP, December 2014

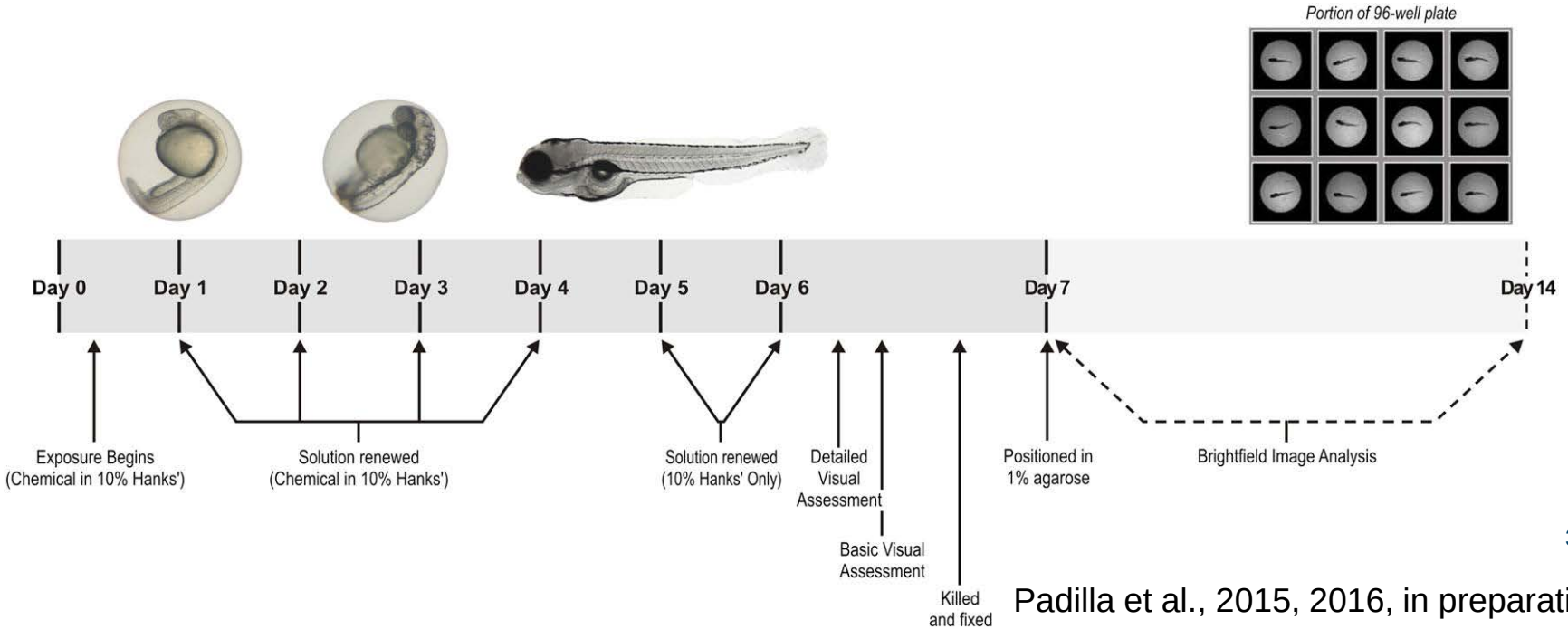
- Can the ER Model be used for prioritization?
  - "… the ER AUC appears to be an **appropriate tool for chemical prioritization** for … the EDSP universe compounds."

- Can the ER model substitute for the Tier 1 ER in vitro and uterotrophic assays?
  - "… **replacement of the Tier 1 *in vitro* ER endpoints …with the ER AUC model will likely be a more effective and sensitive measure for the occurrence of estrogenic activity** …"
  - "… the Panel **did not recommend that the uterotrophic assay be substituted** by the AUC model at this time. The Panel suggested that the EPA considers: 1) conducting limited uterotrophic and other Tier 1 in vivo assay testing, using the original Tier 1 Guidelines (and/or through literature curation)"

- Based on follow-up presented here (FR notice, June 18 2015) …
  - **"EPA concludes that ER Model data are sufficient to satisfy the Tier 1 ER binding, ERTA and uterotrophic assay requirements."**

Zebrafish and Developmental Toxicology

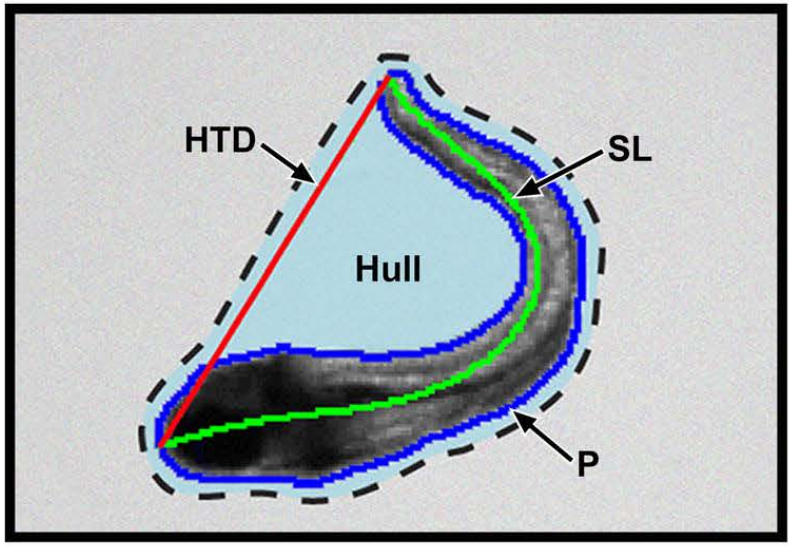
- Goal: Use zebrafish as an *in vivo* model of vertebrate developmental toxicity
- Build *in vitro* to *in vivo* models using ~700 human assays
- ~1000 Chemicals
  - pharmaceuticals, pesticides, industrial chemicals, personal care product chemicals and food ingredients
- Can we combine with ToxCast to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



Padilla et al., 2015, 2016, in preparation

Zebrafish Imaging and scoring

A

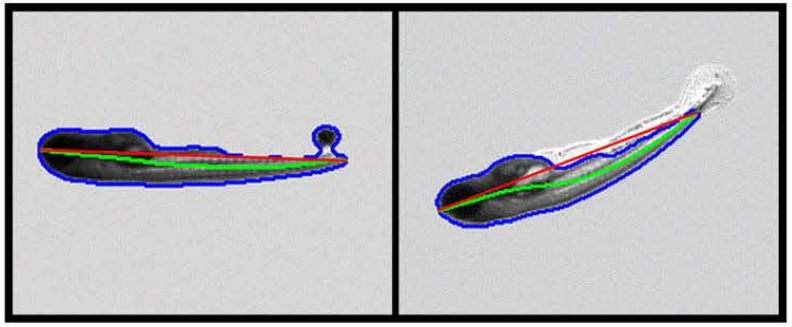


B

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

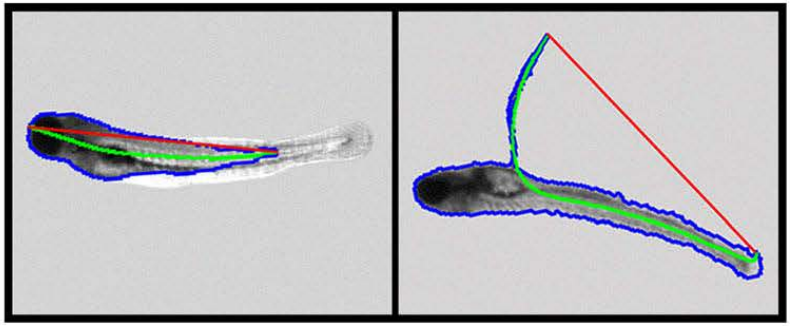
C

Acceptable



D

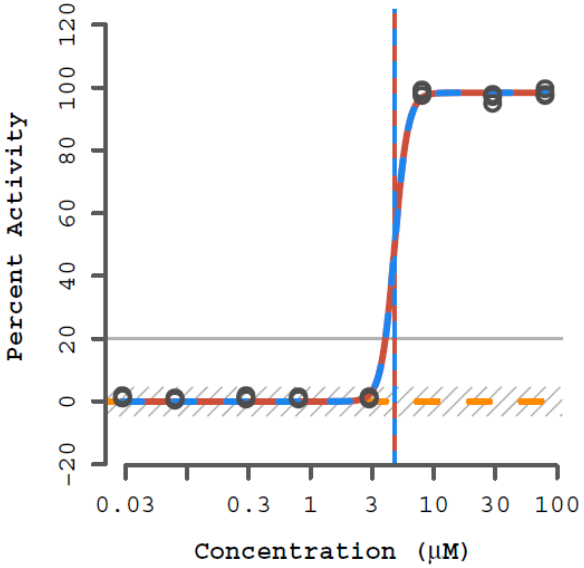
Unacceptable



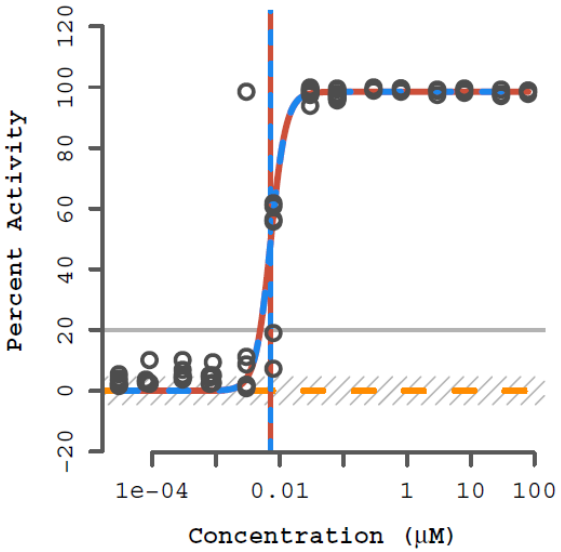
Deal et al. J Applied Tox. 2016

Zebrafish Example Chemicals

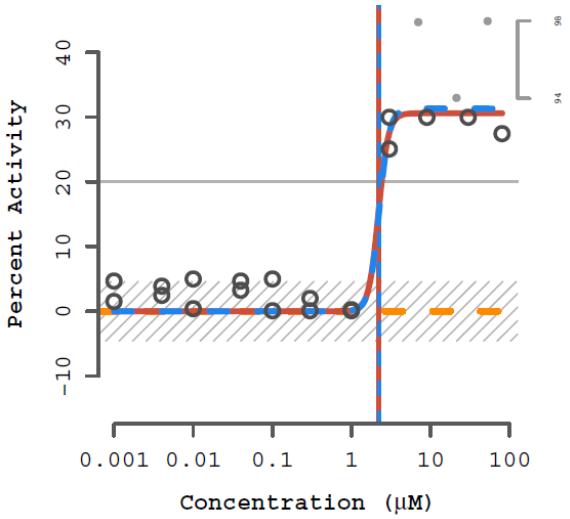
DES



Lovastatin

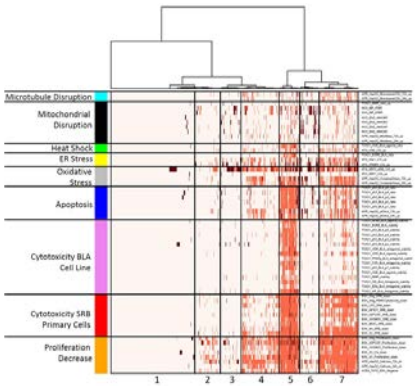


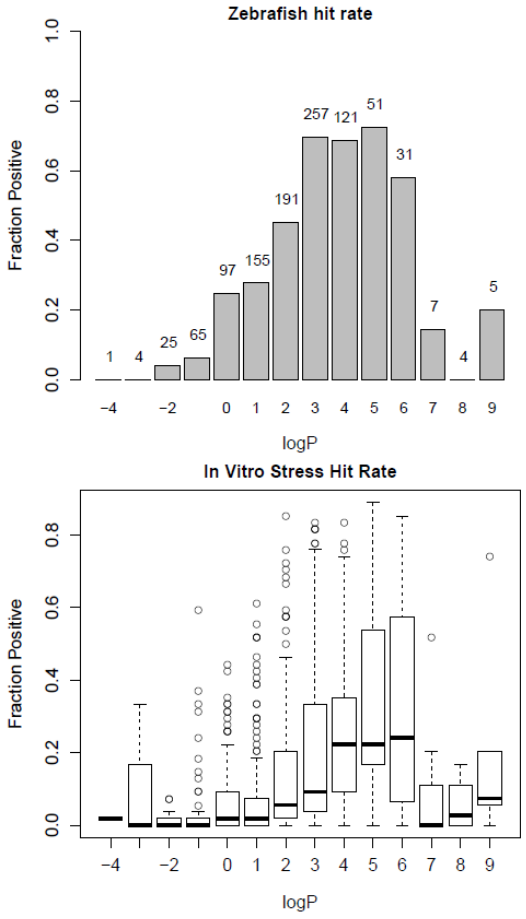
Permethrin

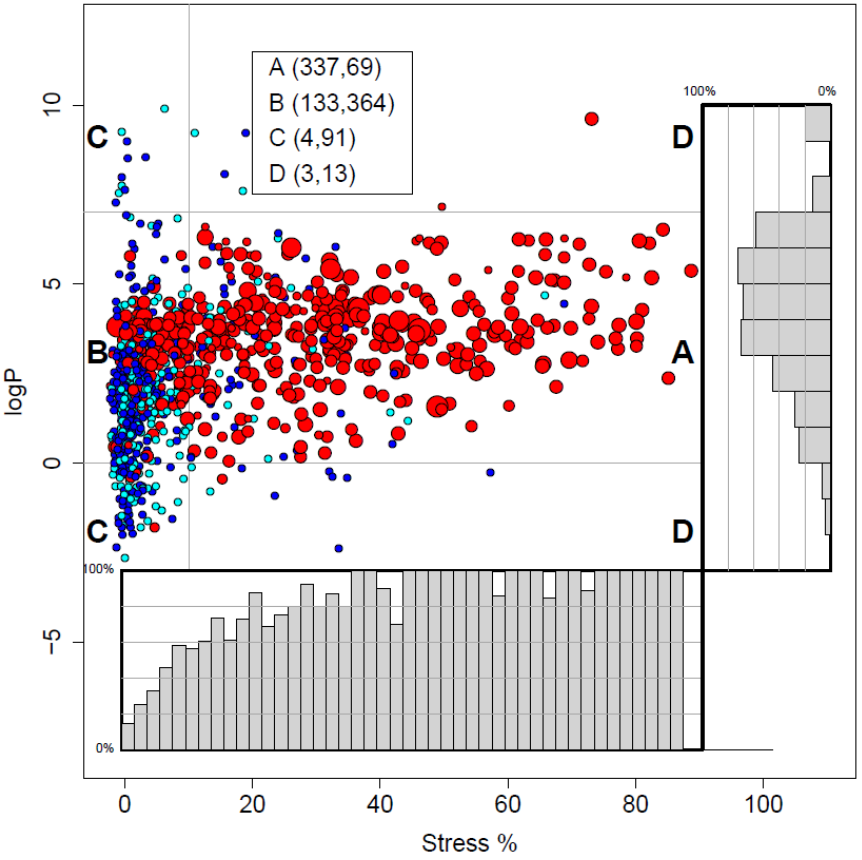


100% = death
<100% = malformations

Stress, logP and zebrafish toxicity are related

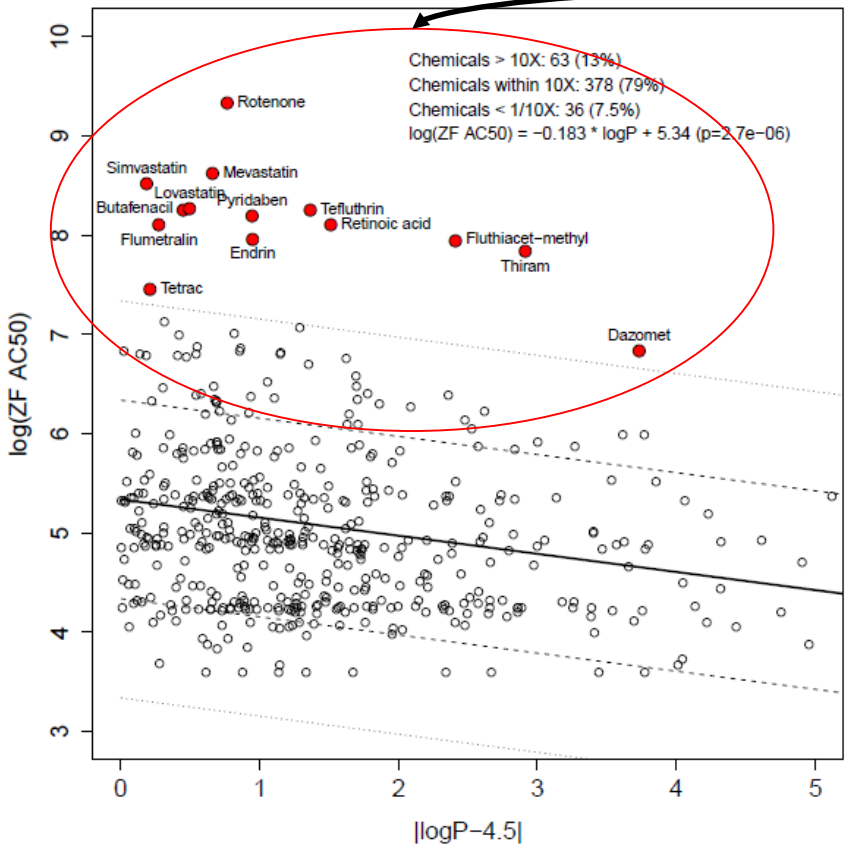


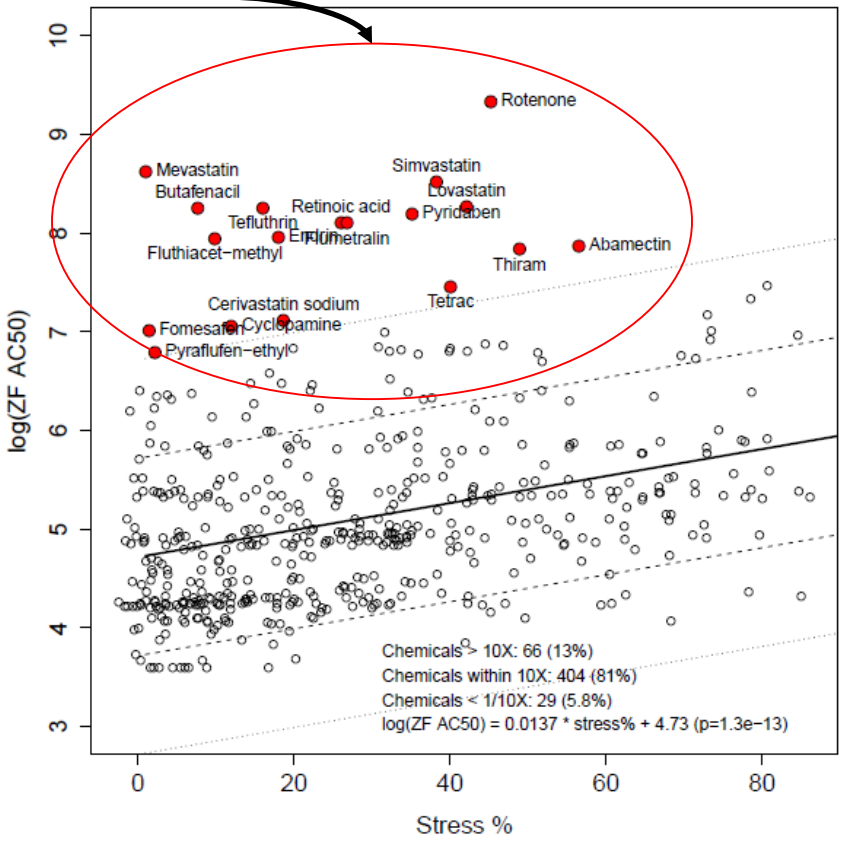




Subset of chemicals are more potent than expected from stress or logP

Chemical showing “excess” toxicity

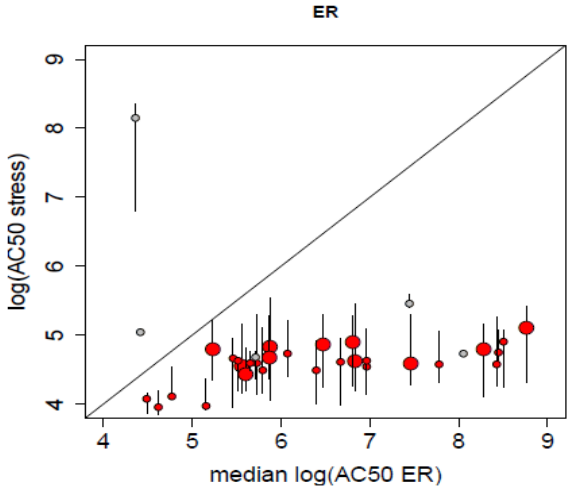


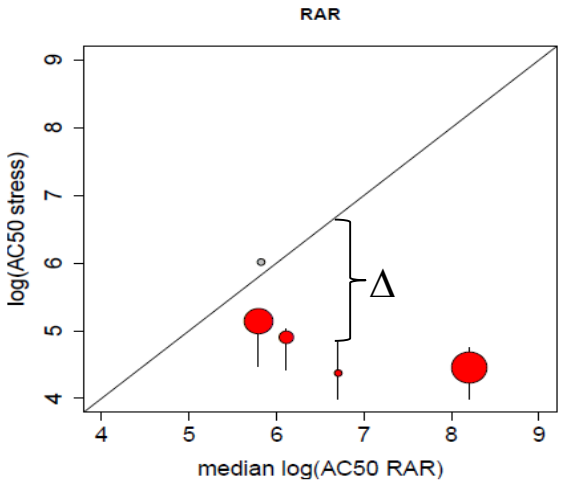


Proposed Mode of Action

- Are target+ chemicals highly likely to be ZF+?
- Does target activity occur below cell stress and cytotoxicity?

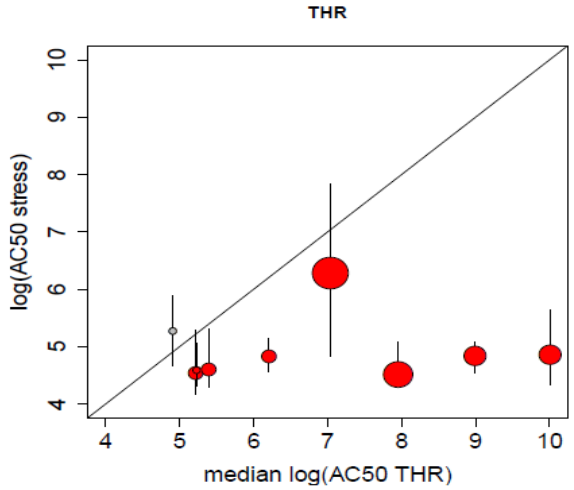
Estrogen Receptor
86% active
(30 / 35)

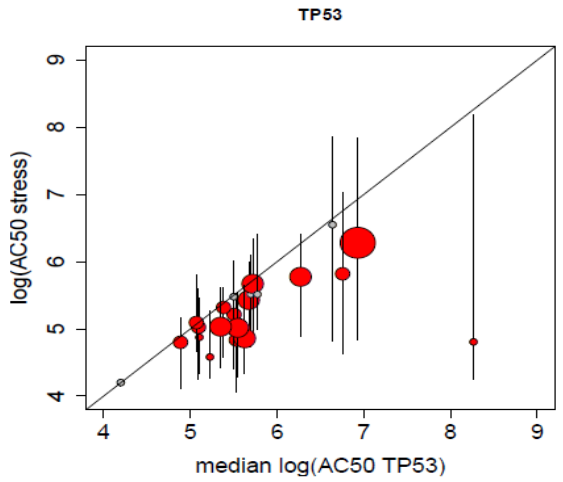




Retinoic Acid Receptor
83% active
(5 / 6)

Thyroid Hormone Receptor
90% active
(9 / 10)





TP53
77% active
(17 / 22)

MOA with in vitro support

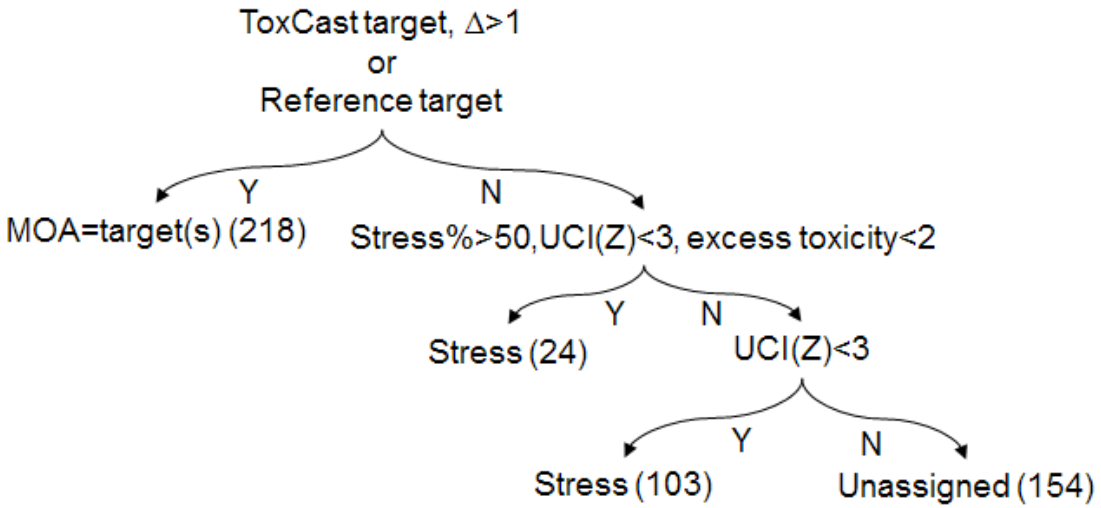
Target	Description	Target-active chemicals	Zebrafish and Target active chemicals	Fraction positive
ADRB	Beta adrenergic receptors	6	6	1.00 [*]
AR	Androgen receptor	18	15	0.83 [**]
CYP1	CYP450, family 1	4	4	1.00 [*]
CYP2	CYP450, family 2	13	13	1.00 [***]
CYP3	CYP450, family 3	24	22	0.92 [***]
DRD	Dopamine receptors	15	12	0.80 [*]
ER	Estrogen receptors	35	30	0.86 [***]
mitochondria	Mitochondria targeting	4	3	0.75
NR1I3	Constitutive androstane receptor (CAR)	13	12	0.92 [**]
PPARG	Peroxisome proliferating receptor gamma	14	11	0.78 [*]
RAR	Retinoic acid receptor	6	5	0.83
THR	Thyroid hormone receptor	10	9	0.90 [**]
TP53	p53, apoptosis	22	17	0.77 [**]

MOA from literature targets

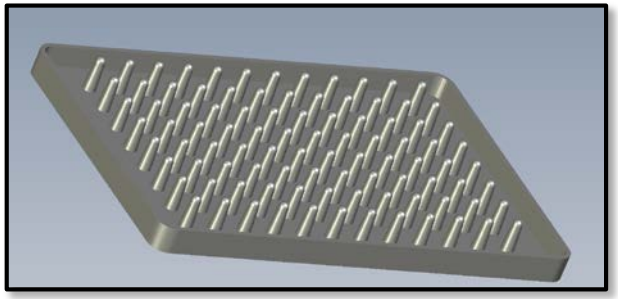
Target	Description	Target-active chemicals	Zebrafish and Target active chemicals	Fraction positive
ACCase	Plant Acetyl CoA Carboxylase (lipid synthesis inhibitors)	11	10	0.91[**]
ACHE	Acetylcholinesterase	55	41	0.75 [***]
AR	Androgen receptor	14	12	0.86 [**]
ER	Estrogen receptor	29	25	0.86 [***]
HMGCR	HMG-coA reductase	8	6	0.75
HTR2A	Serotonin receptor 2A	5	4	0.80
ion channel	General ion channels	33	27	0.82 [***]
ion channel (Na)	Sodium ion channels	22	19	0.86 [***]
lipid synthesis	Lipid synthesis targeting (includes sterol synthesis)	38	30	0.79 [***]
microtubule	Microtubule-targeting	20	18	0.90 [**]
mitochondria	Mitochondria targeting	21	21	1.00 [***]
PGR	Progesterone receptor	5	4	0.80
PPO	Plant Protoporphyrinogen Oxidase (lipid membrane disruption)	13	11	0.85 [**]
sterol synthesis	Sterol synthesis targeting	24	23	0.96 [***]
THR	Thyroid hormone receptor	4	4	1.00 [*]
tubulin	Tubulin (microtubule) targeting	7	7	1.00 [**]

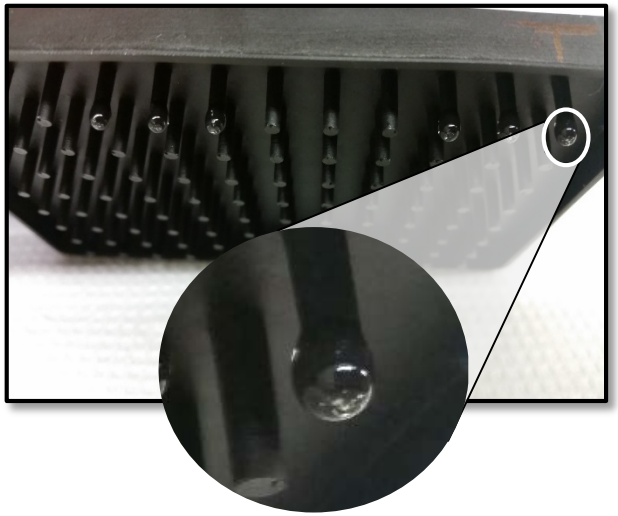
Common MOA Classes

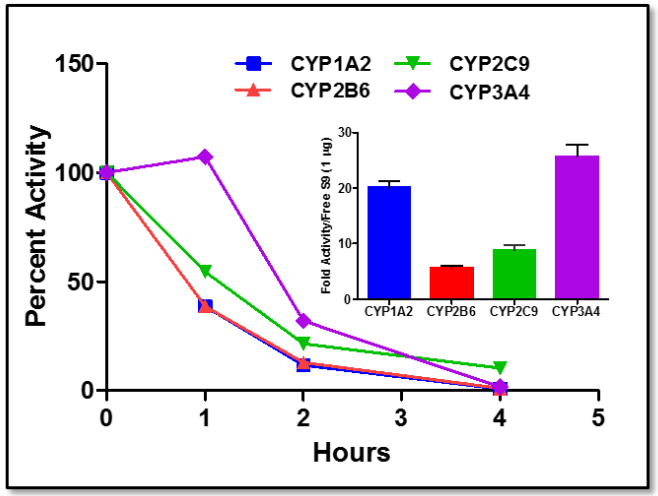
- Endocrine Pathways
- Lipid synthesis (cell membrane) disruptors
  - HMGCR
  - PPO inhibitors (disrupts plant cell membranes)
- ACHE
- Ion channel blockers
- Mitochondrial disruptors
- Microtubule disruptors
- Chemicals reacting with protein SH groups

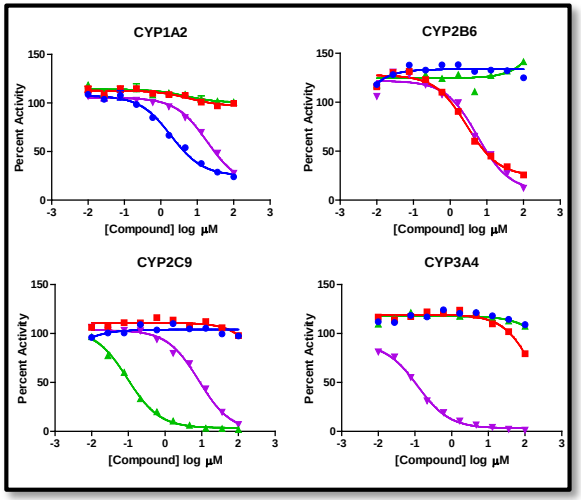


Efforts to Address Metabolism Challenge









Furafylline
Thio-TEPA
Tienilic Acid
Ketoconazole

DeGroot and Simmons, Unpublished

NCCT Software Architecture

Databases

DSSTox: Chemistry
PhysChemDB
ToxRefDB: Hazard Details
ToxValDB: Hazard Summary
InVitroDB: ToxCast
ScrubChem: PubChem
ExpoCastDB: Exposure
CPCat: Chemical Use
ACToR: Multiple Types
PKDB: Toxicokinetics
LitDB: Open Literature

Web Services

Models

Physchem
Exposure
Bioactivity
Pharmacokinetics

Dashboards

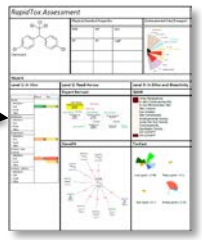
ACToR

CPCat/CPDat

EDSP21

ToxCast

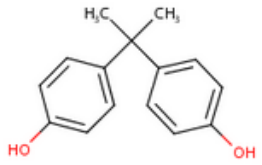
Chemistry



RapidTox

RapidTox Dashboard: Risk Assessment Tool

RapidTox Report: Bisphenol A



Property	Value
CASRN	80-05-7
Preferred Name	Bisphenol A
IUPAC Name	4,4'-propane-2,2-diyldiphenol
SMILES	CC(C)(C1=CC=C(O)C=C1)C1=CC=C(O)C=C1
InCHI Key	IISBACLAFKSPIT-UHFFFAOYSA-N
Formula	C15H16O2

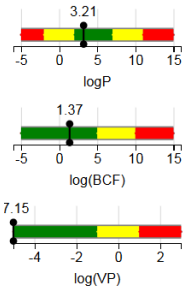
External Links

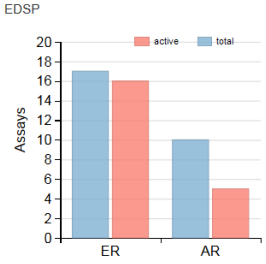
- ACToR
- Chemistry Dashboard
- RapidTox Dashboard
- EDSP21 Dashboard
- ToxCast Dashboard
- CPCat

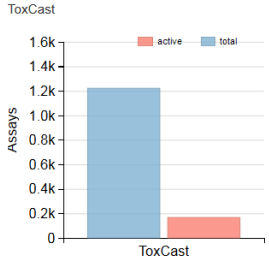
Executive Summary

- **Quantitative Risk Assessment Value**
  - IRIS values available
  - EPA RSL values available
  - Minimum RfD: 0.050 mg/kg-day (IRIS, oral, -, -)
  - Minimum oral POD: 5.0 mg/kg-day (ToxRefDB, LOEL, sub, rat)
  - No RfC calculated
  - No inhlation POD values
  - HTTK Css(95%)= 1.66, Css(median)=0.569 um/(mg/kg-day)
  - IVIVE POD = 0.0536 to 0.156 mg/kg-day, based on NVS_NR_hCAR_Antagonist AC50=0.089 uM
  - Genes with activity below 1 uM: ESR1, ESR2, Esr1, PPARA, NR1I3, ESR1, NR1I2, MMP3, Cyp2c11, Tpo

Summary Plots







Ongoing 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 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

National Center for Computational Toxicology

United States Environmental Protection Agency

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