Application of computational and high-throughput *in vitro* screening for prioritization

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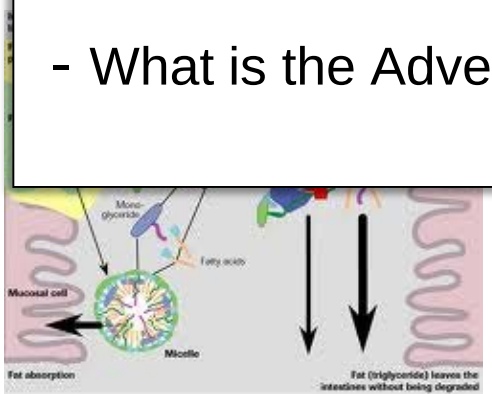
Problem Statement

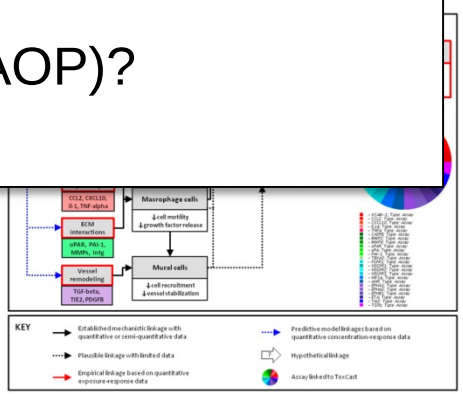
Too many chemicals to test with standard animal-based methods

- Cost, time, animal welfare

Need for better mechanistic data

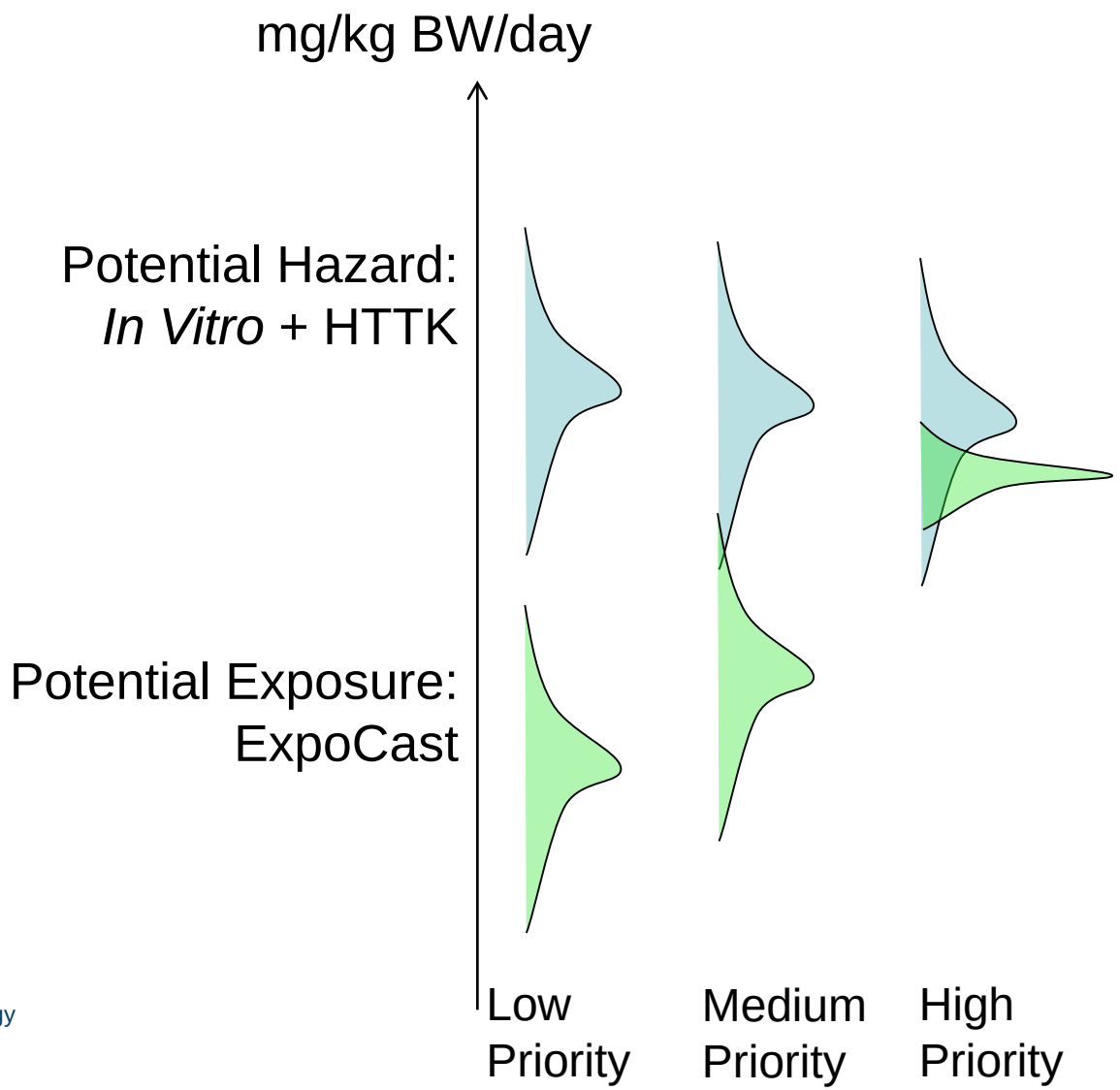
- Determine human relevance
- What is the Adverse Outcome Pathway (AOP)?





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 to test chemicals
- Identify “Human Exposure Chemical Universe” to test
- Develop models that link *in vitro* to *in vivo* hazard
- Use pharmacokinetic models to predict activating doses
- Develop exposure models for all chemicals
- Add uncertainty estimates
- Create high-throughput risk assessments

EDSP21 Project: Major Points

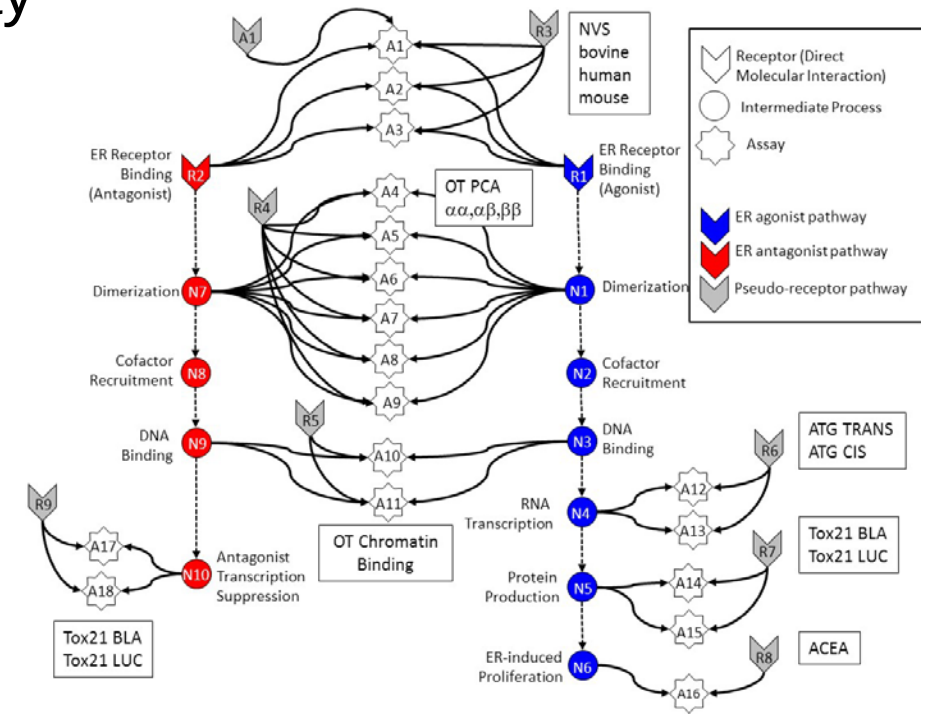
- EDSP: Endocrine Disruptor Screening Program
  - Mandated by U.S. Congress
  - "Tier 1 battery" – 11 in vitro and in vivo assays (estrogen, androgen, thyroid)

- EDSP has a mismatch between resources needed for Tier 1 and number of chemicals to be tested
  - ~10,000 chemicals in EDSP Universe
  - ~$1M per chemical for Tier 1, 50-100 year backlog

- Demonstrate new approach: Estrogen Receptor (ER)
  - Multiple high-throughput *in vitro* assays
  - Prioritize chemicals and replace selected Tier 1 assays

In Vitro Estrogen Receptor Model

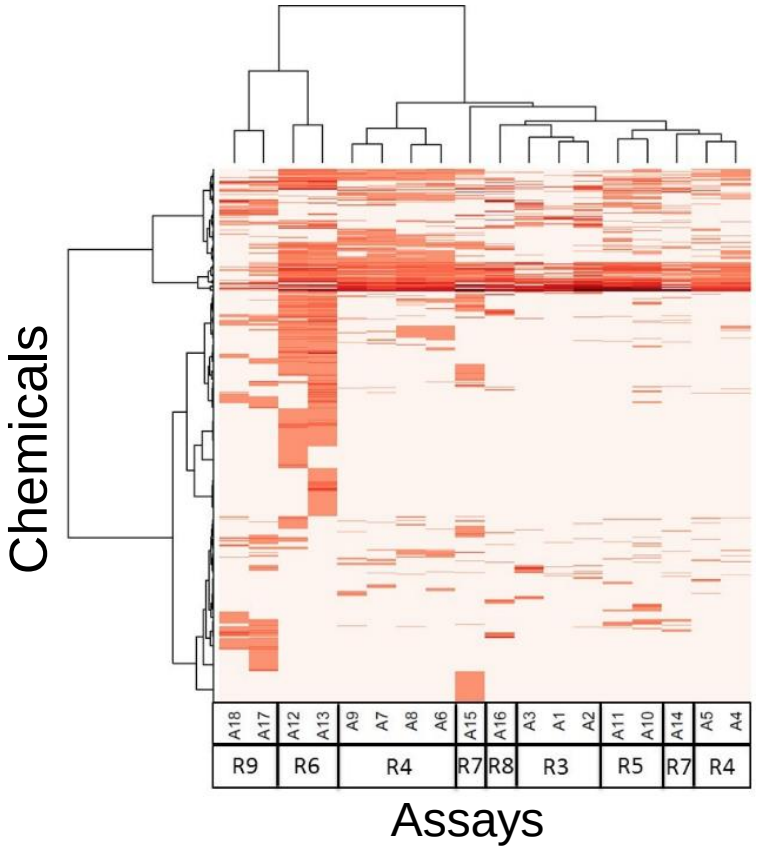
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



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)

All *In vitro* assays have false positives and negatives

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

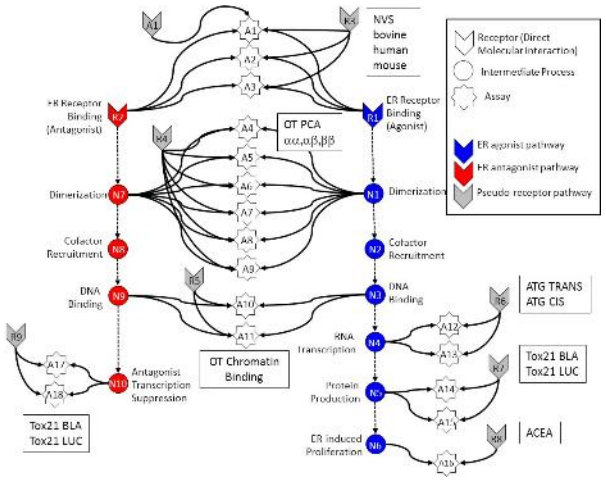


Much of this “noise” is reproducible

- “assay interference”
- Result of interaction of chemical with complex biology in the assay

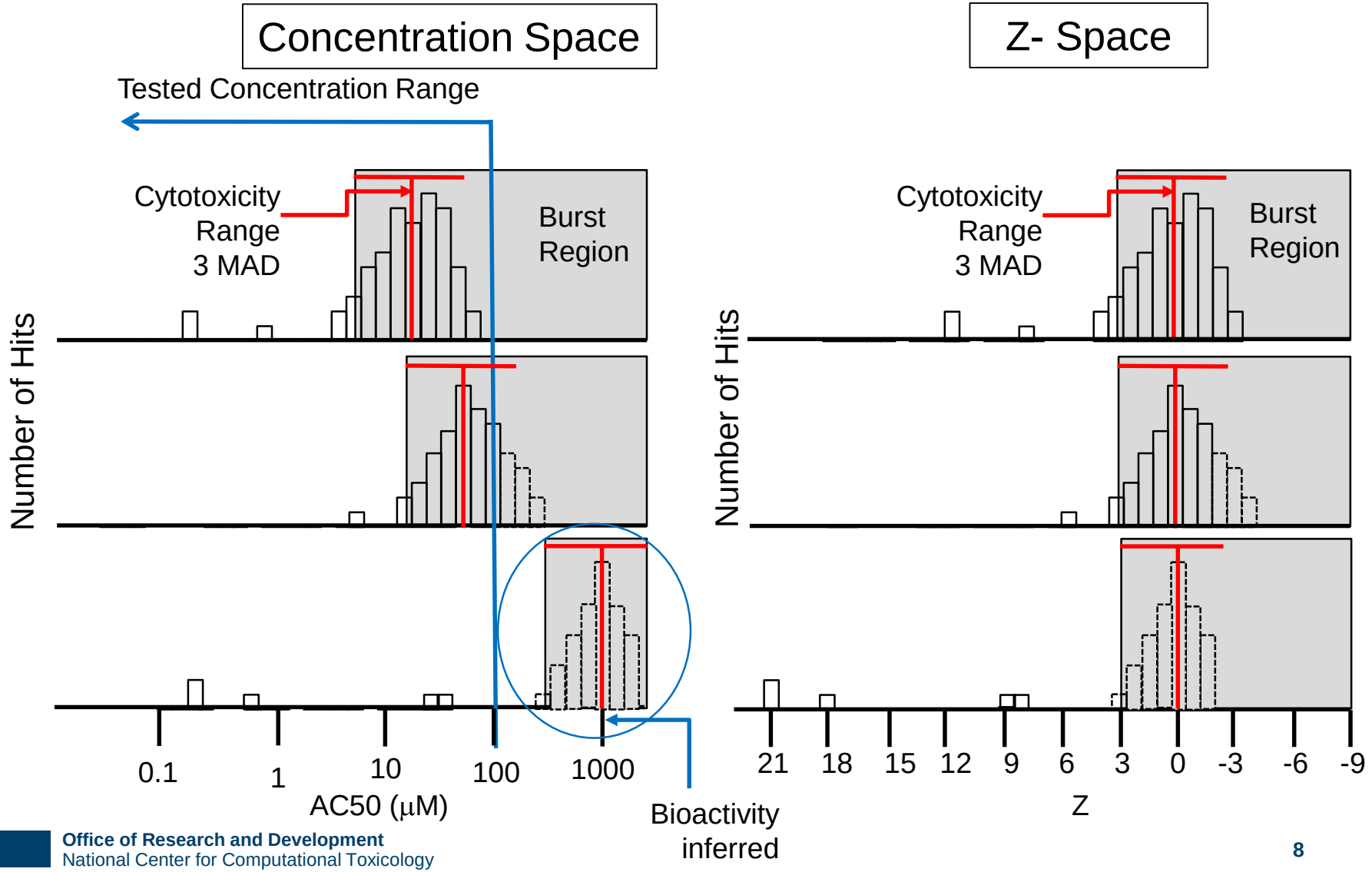
EDSP chemical universe is structurally diverse

-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



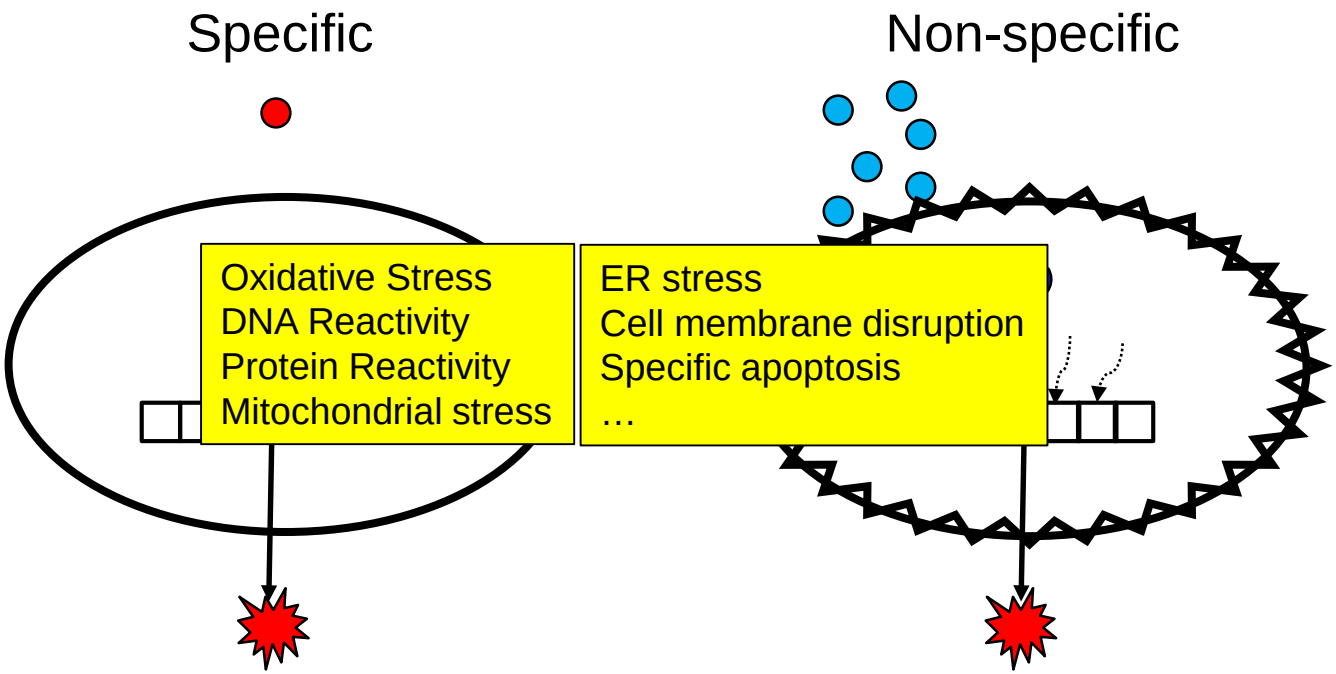
Judson et al: ToxSci (2015)

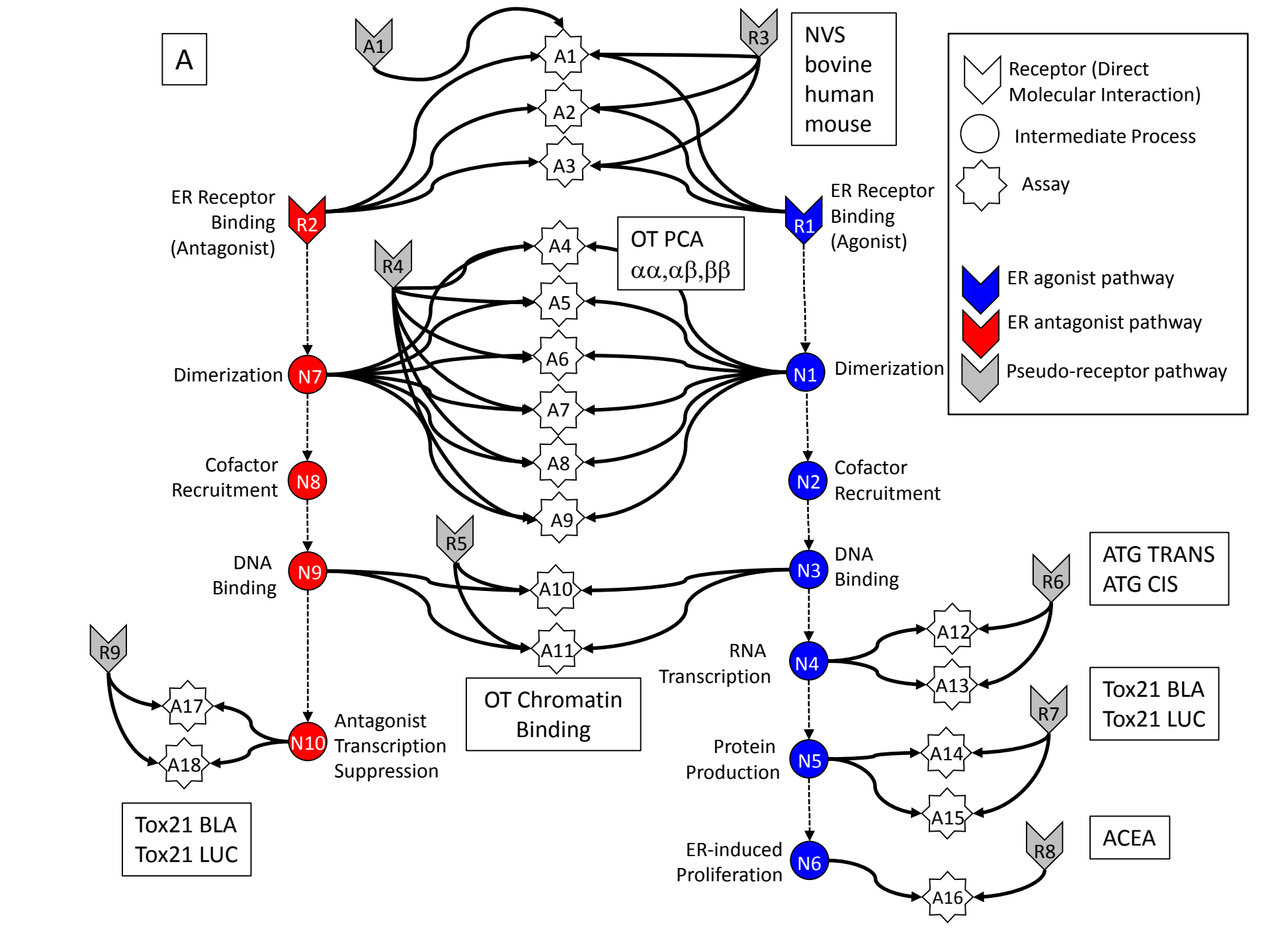
Most chemicals display a “burst” of potentially non-selective bioactivity near cytotoxity concentration



Judson et al. Tox.Sci. in press (2016)

Schematic explanation of the burst




A
NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
OT PCA
αα,αβ,ββ
Dimerization
Dimerization
Cofactor Recruitment
Cofactor Recruitment
DNA Binding
DNA Binding
OT Chromatin Binding
ATG TRANS
ATG CIS
RNA Transcription
Tox21 BLA
Tox21 LUC
Protein Production
Antagonist Transcription Suppression
Tox21 BLA
Tox21 LUC
ER-induced Proliferation
ACEA
A1
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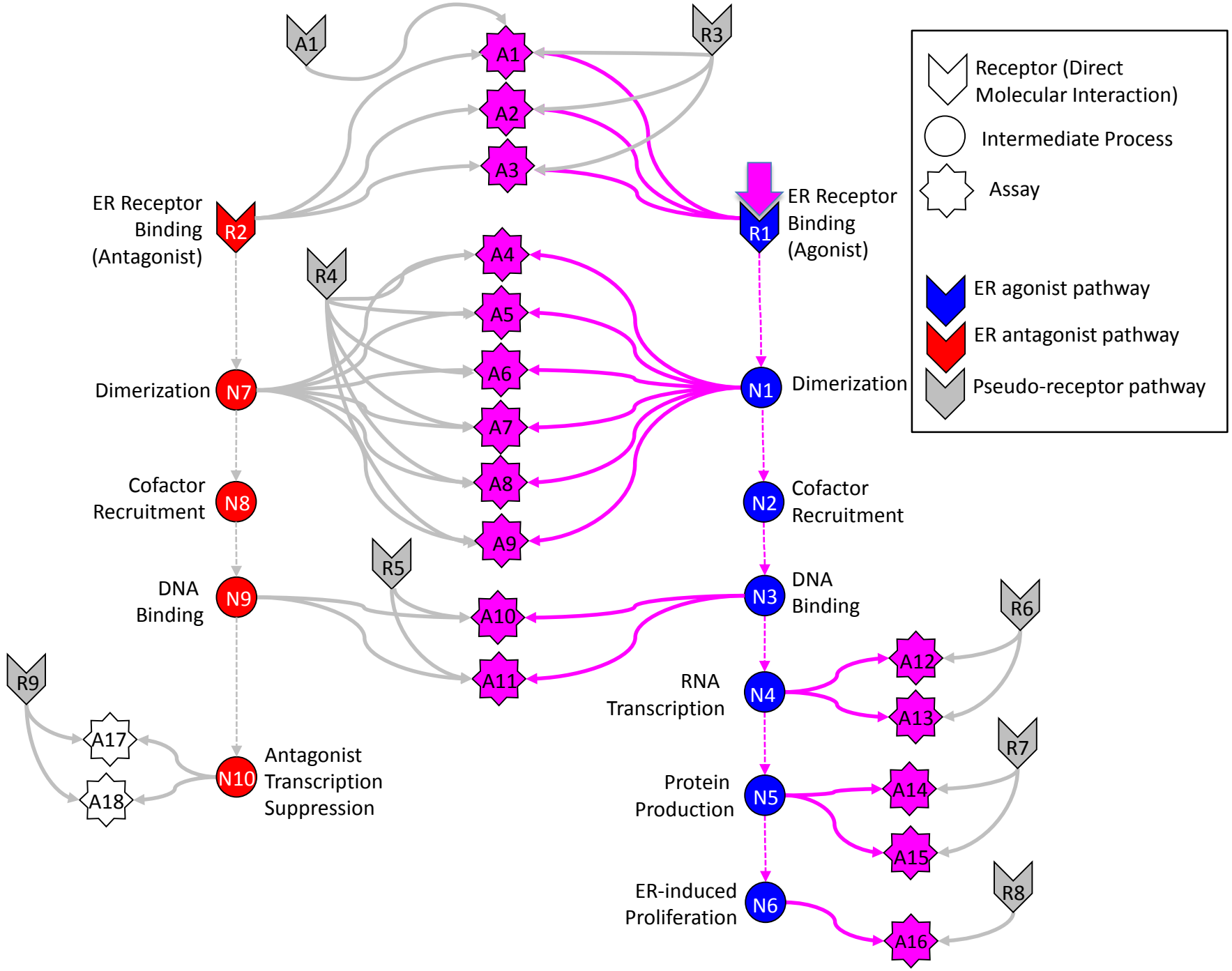
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R1
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R4
R5
R6
R7
R8
R9
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DNA Binding
N4
RNA Transcription
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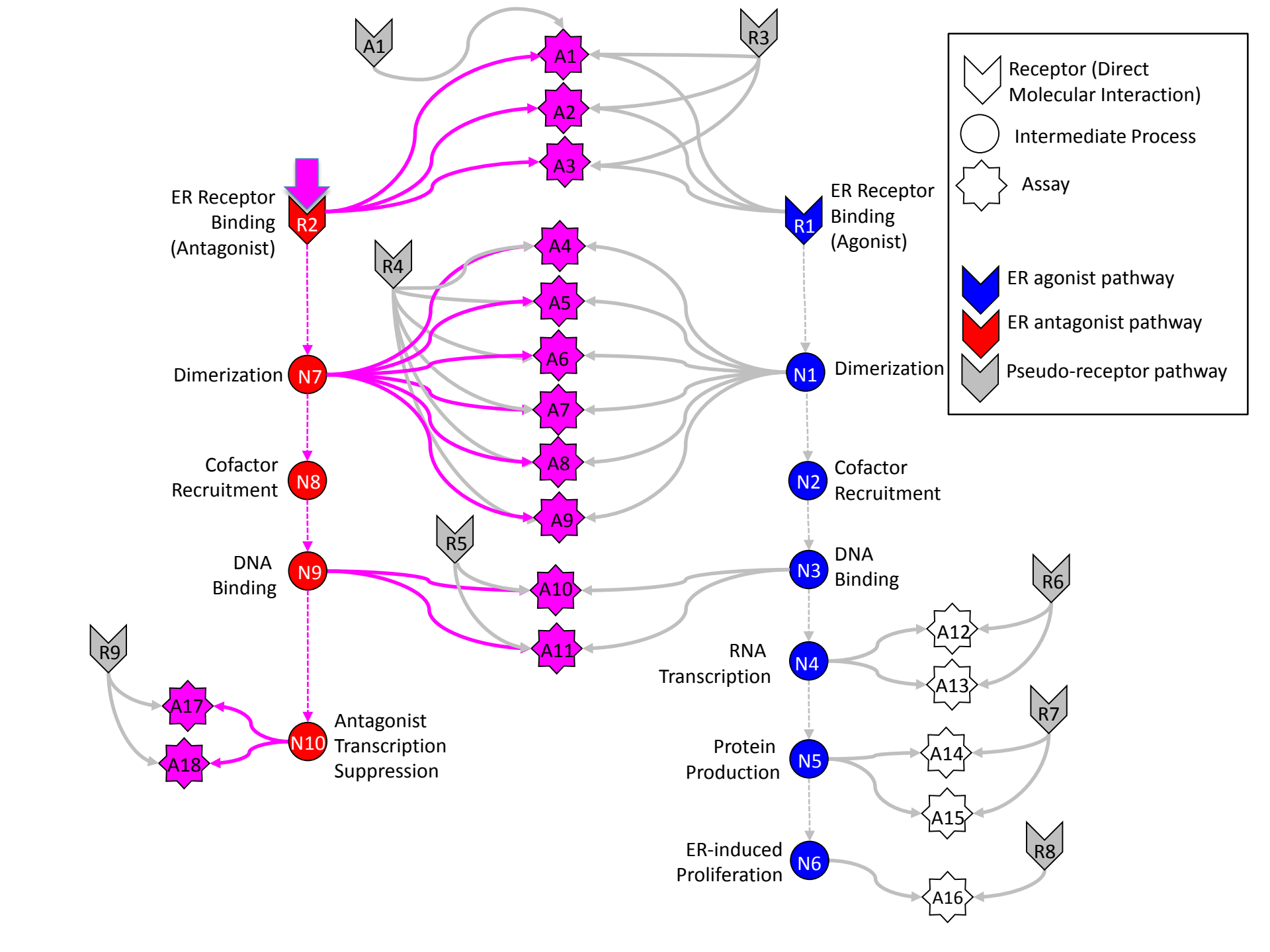
N10
Antagonist Transcription Suppression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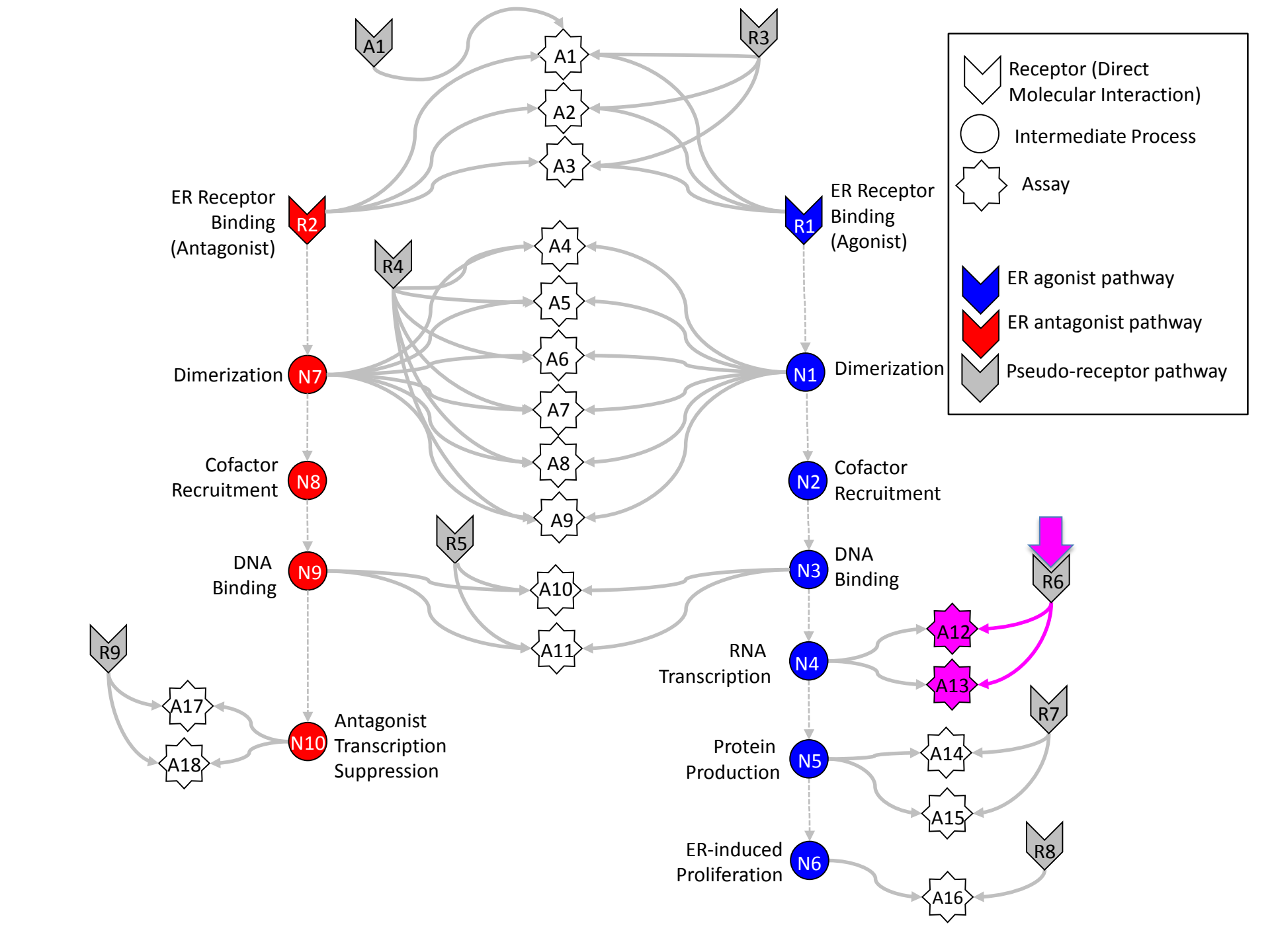
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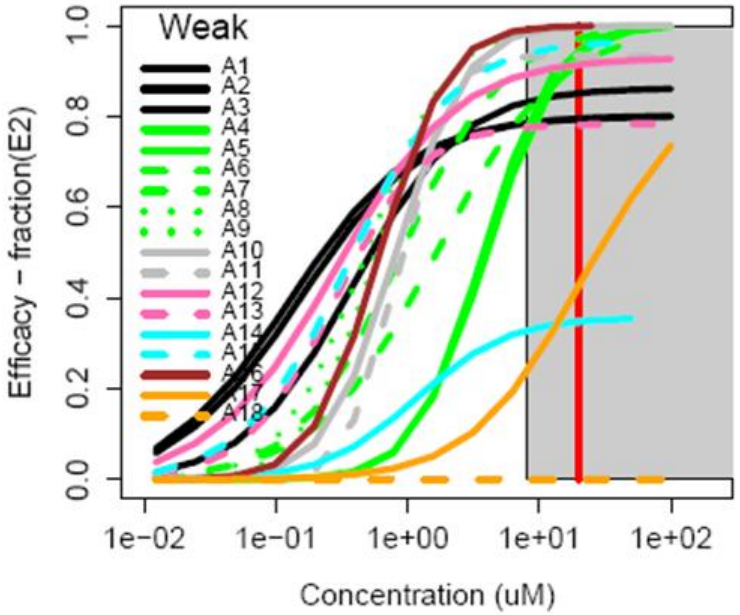
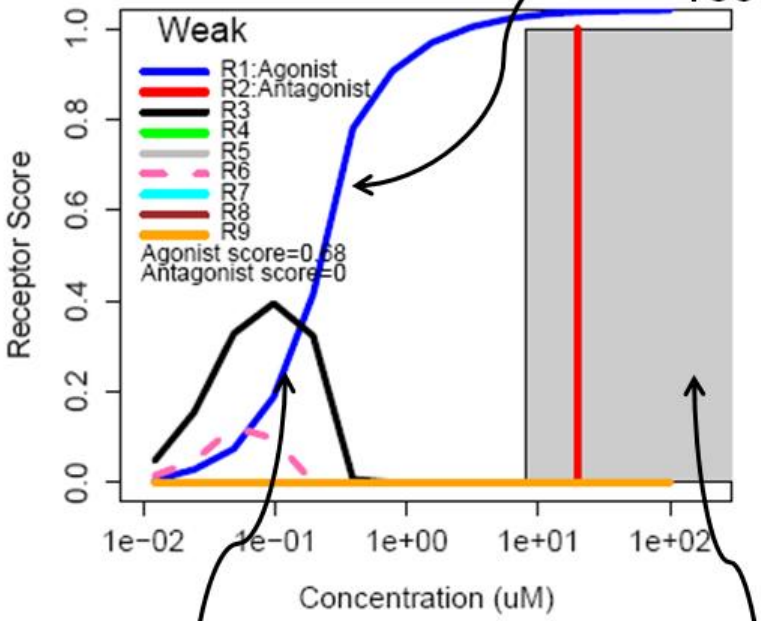
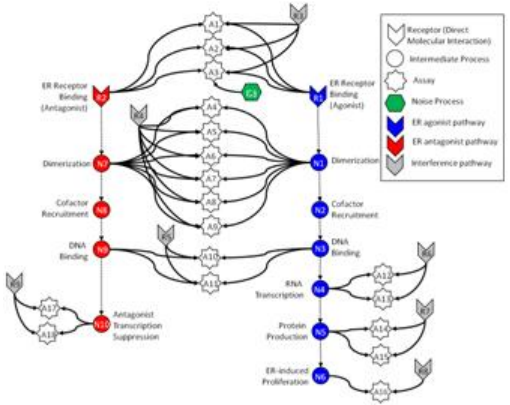
N10
Antagonist Transcription Suppression

Example 1 – BPA: true agonist (AUC=0.66)





Blue: agonist "receptor"



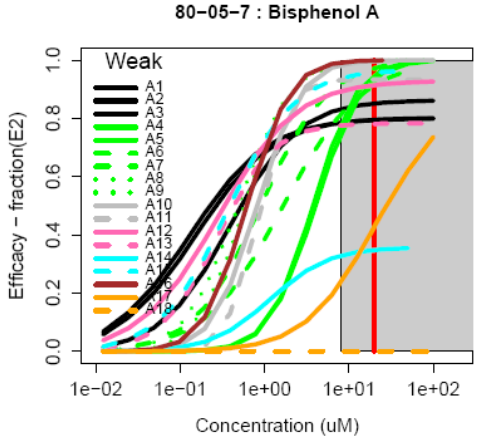
Binding assays active at lowest concentration

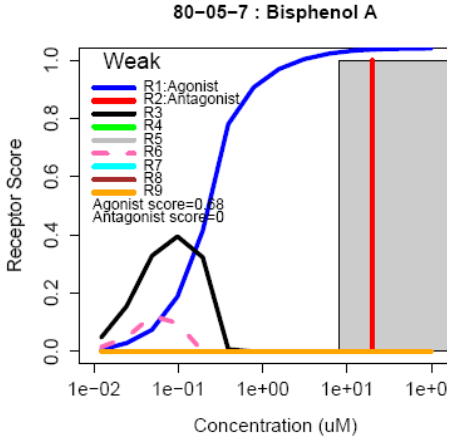
AUC "sign" feature will discount this

Cytotoxicity Region: red line is median cytotox AC50

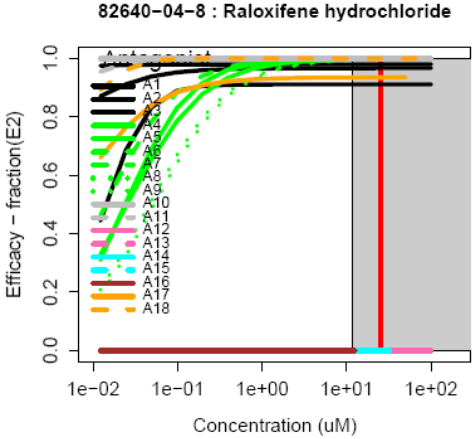
Example curves

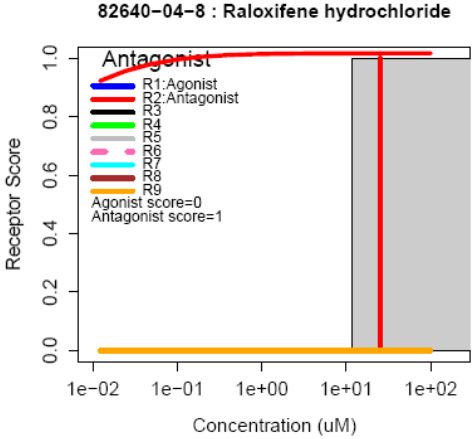
True Agonist



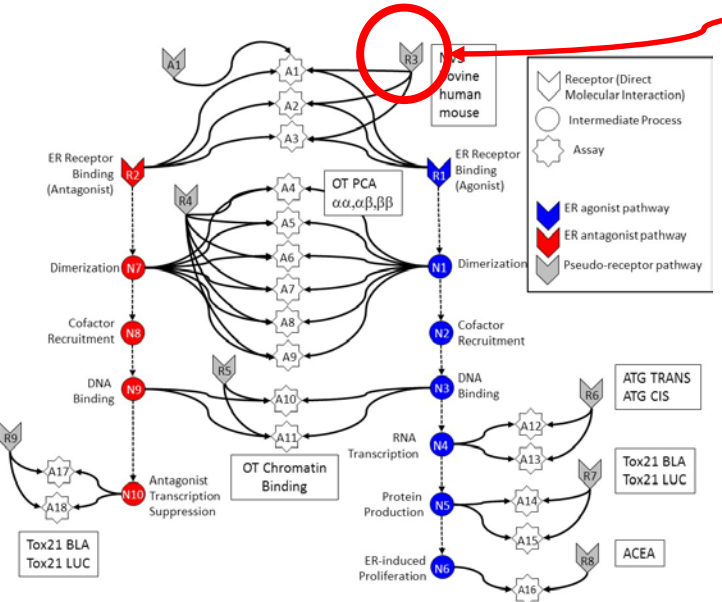


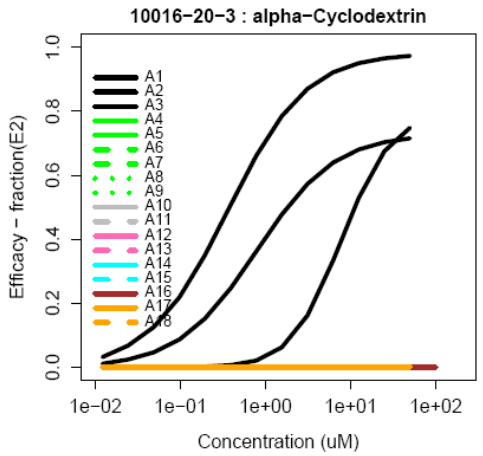
True Antagonist

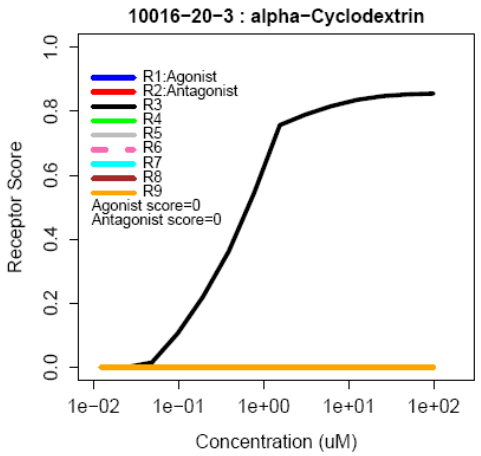




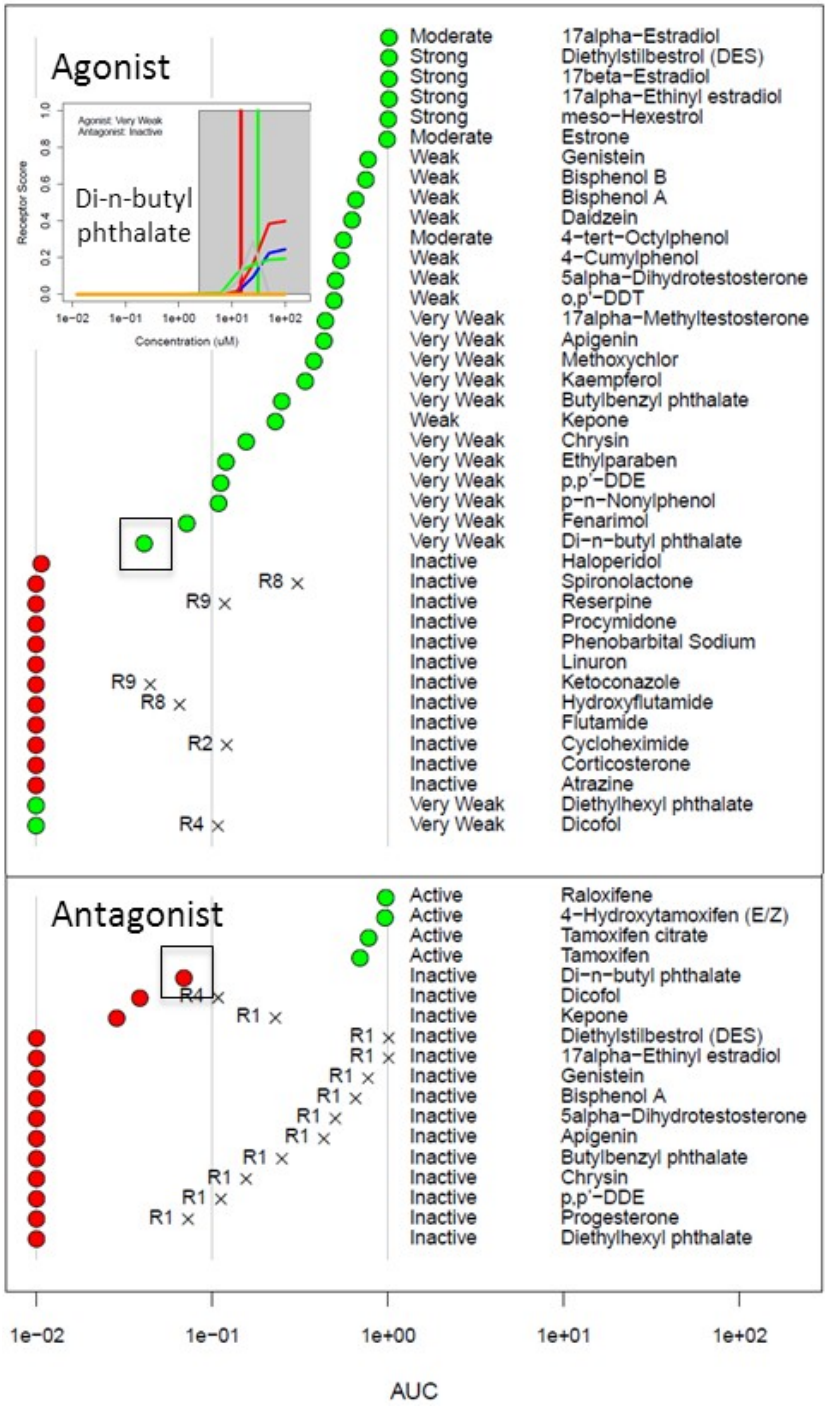
Assay Interference Example “R3”

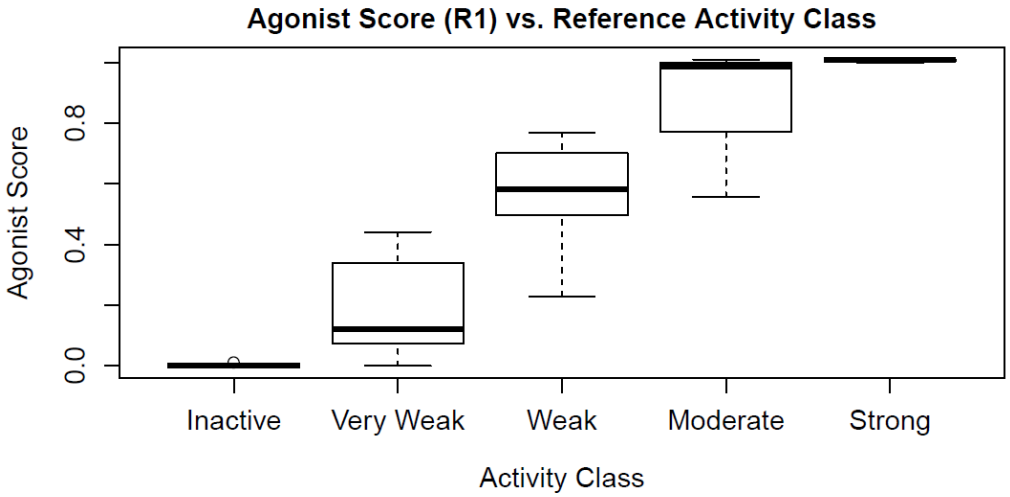




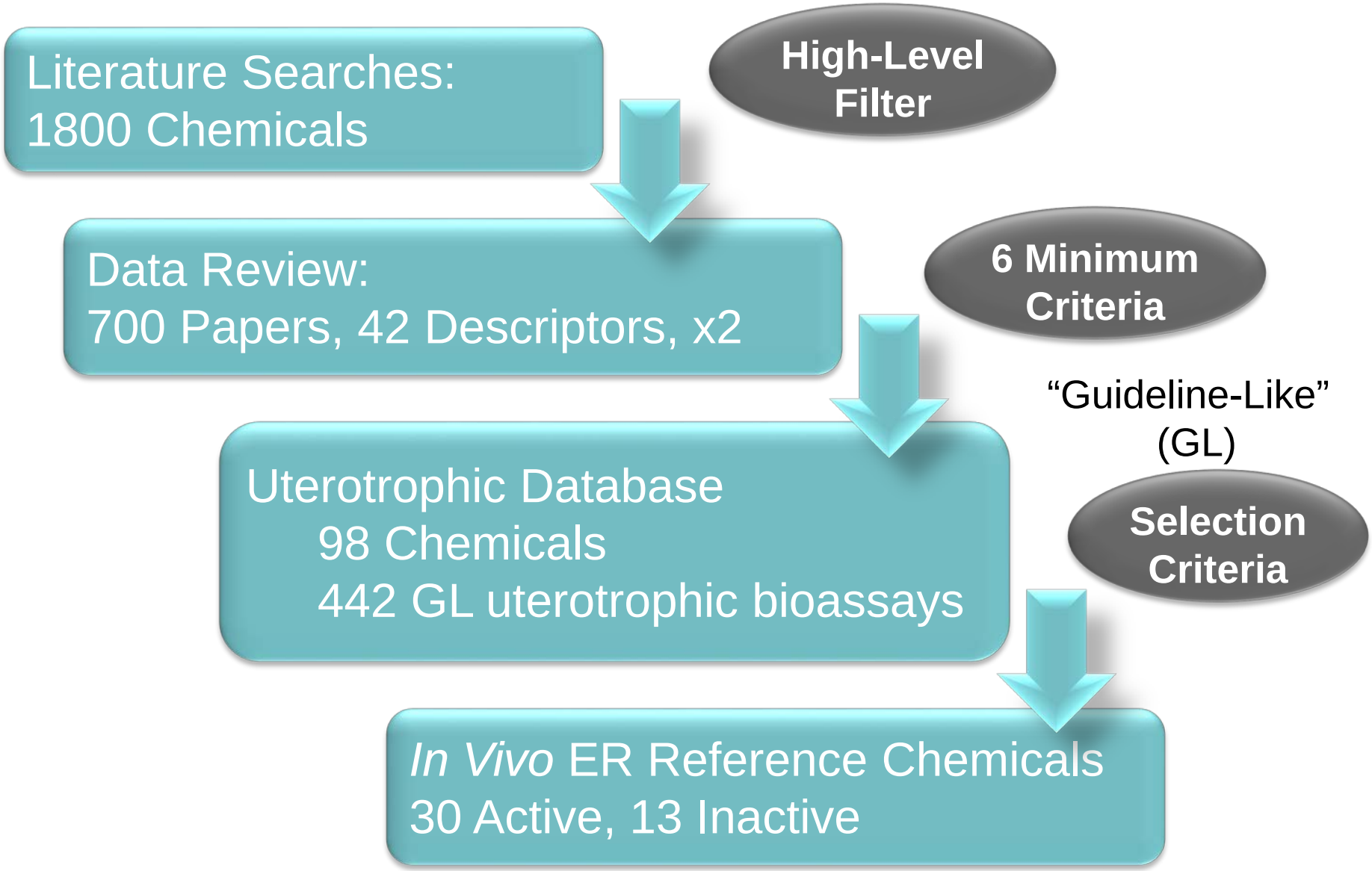


In Vitro Reference Chemical Performance





In vivo validation: Identifying Uterotrophic Reference Chemicals from the Literature

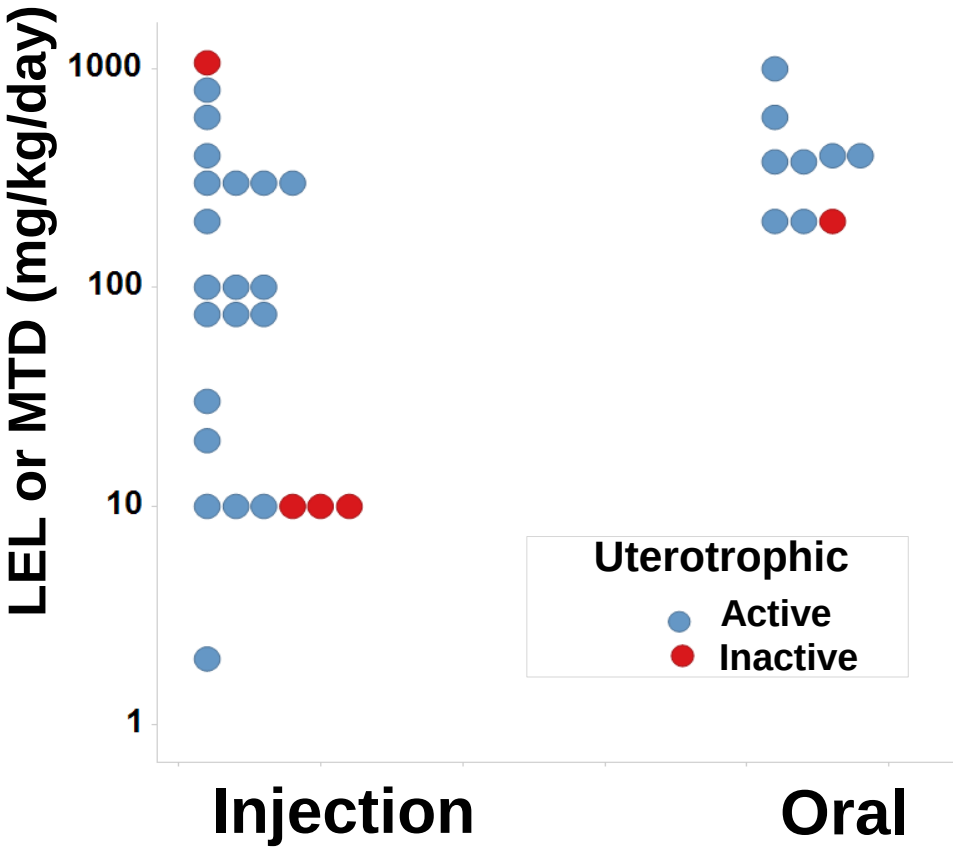


Kleinstreuer et al: "A Curated Database of Rodent Uterotrophic Bioactivity" (EHP 2016)

In vivo guideline study uncertainty

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

United States Environmental Protection Agency



Phenotype X

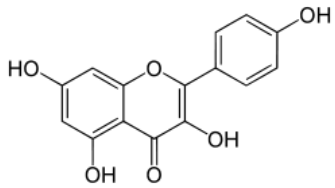
species / study 1	species / study 2	Reproduce	Does Not Reproduce	Fraction Reproduce
rat SUB	rat CHR	18	2	0.90
rat CHR	dog CHR	13	2	0.87
rat CHR	rat SUB	18	4	0.82
rat SUB	rat SUB	16	4	0.80
rat SUB	dog CHR	11	4	0.73
mouse CHR	rat CHR	11	4	0.73
mouse CHR	rat SUB	13	7	0.65
dog CHR	rat SUB	11	6	0.65
dog CHR	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

Kleinstreuer et al. EHP 2016

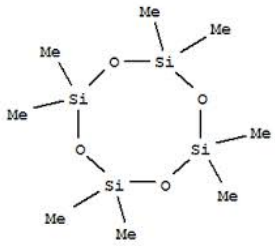
Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic

Restrict to chemicals with consistent results from 2 or more labs

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



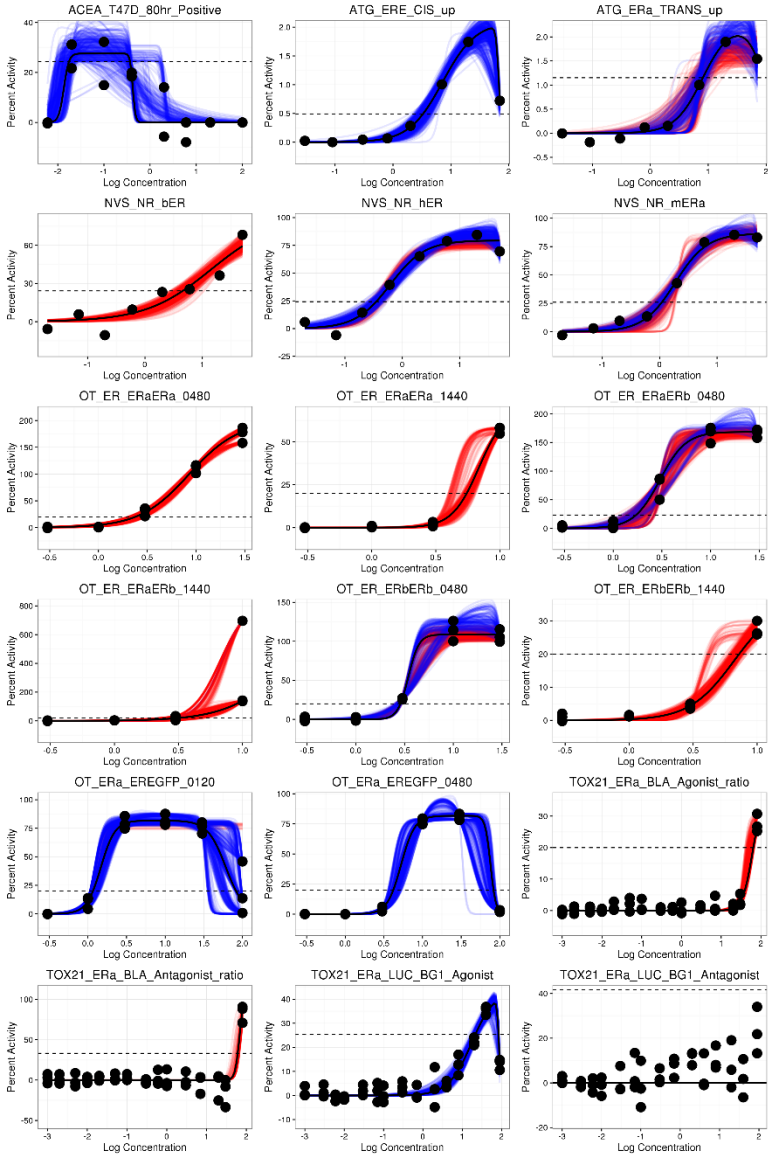
Kaempferol

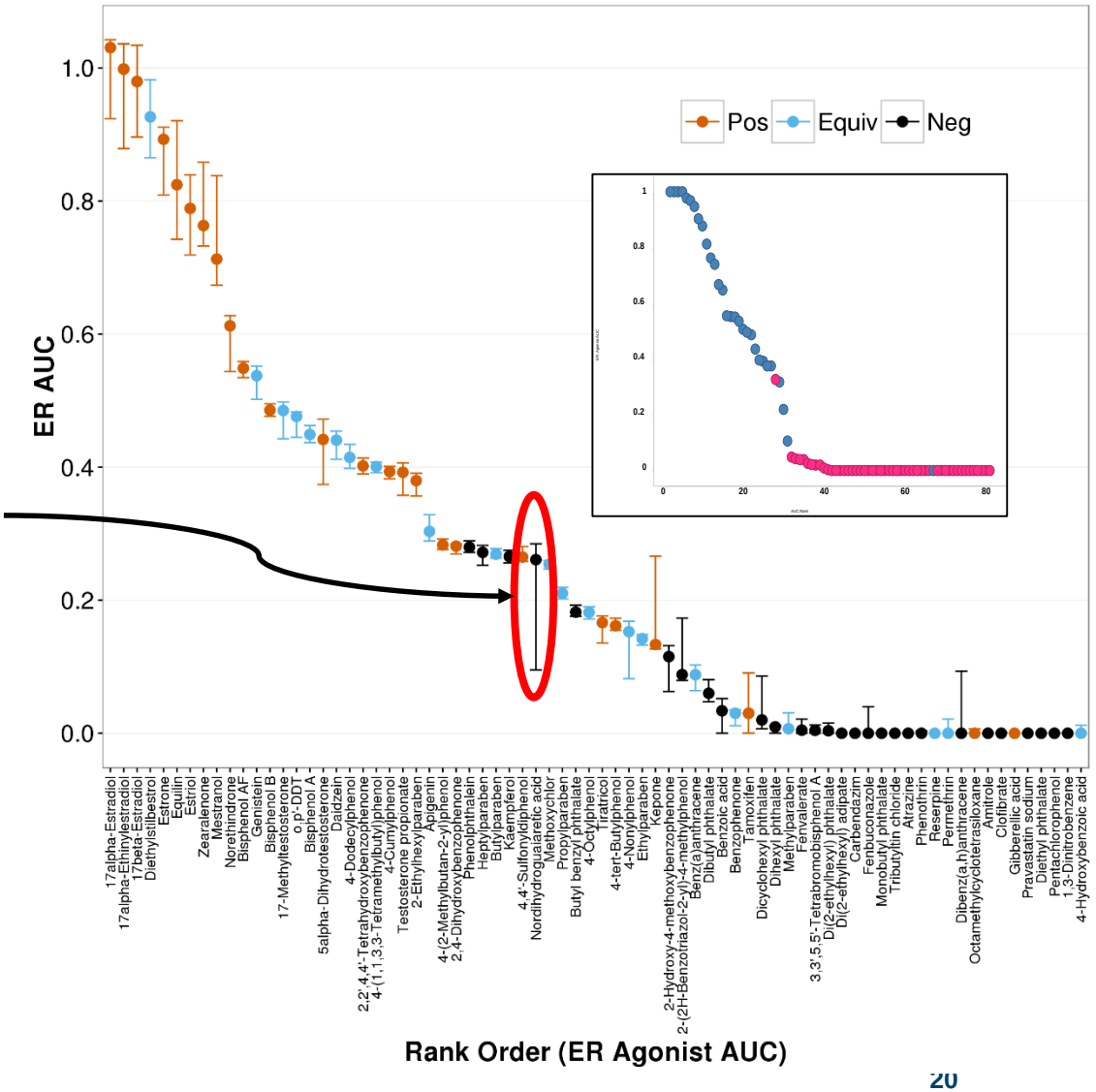


D4

Browne et al. ES&T (2015)

Add Uncertainty to *In Vitro* Assay Data





Watt et al. (in prep)

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."**

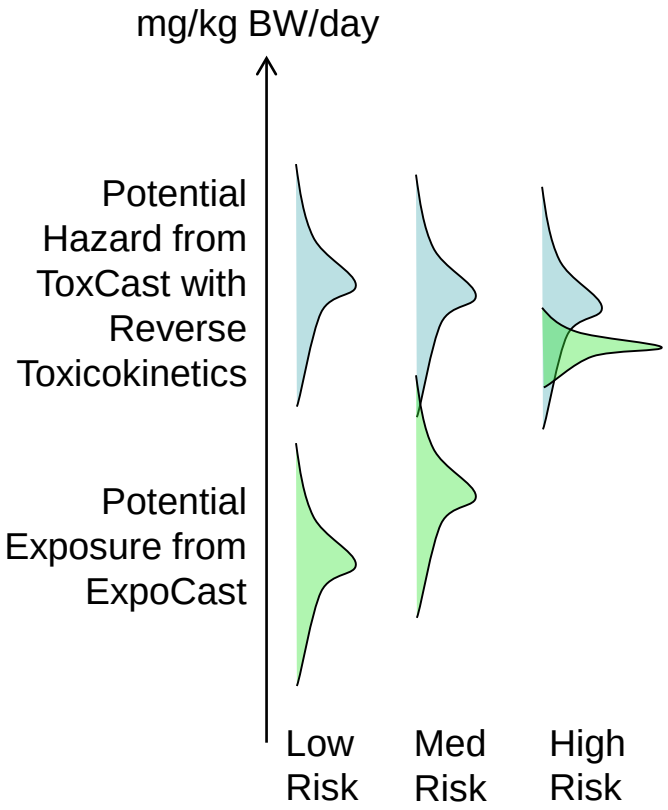
High Throughput Dosimetry and Exposure

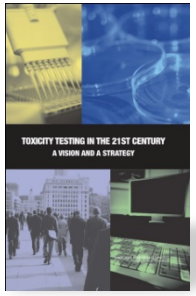
High throughput pharmacokinetic (HTPK*)* *in vitro* methods have been developed by pharmaceutical industry for predicting efficacious doses in clinical trials

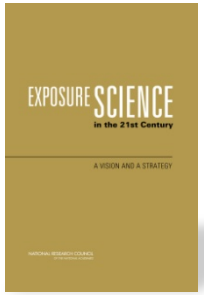
In Wetmore *et al.* (2012) the same methods are used to approximately convert ToxCast *in vitro* bioactive concentrations (µM) into daily doses needed to produce similar levels in a human (mg/kg BW/day)

These doses can then be directly compared with exposure data, ***where available***

Egeghy *et al.* (2012) and National Academy Report: "Exposure Science in the 21st Century" points out that not much exposure information is out there



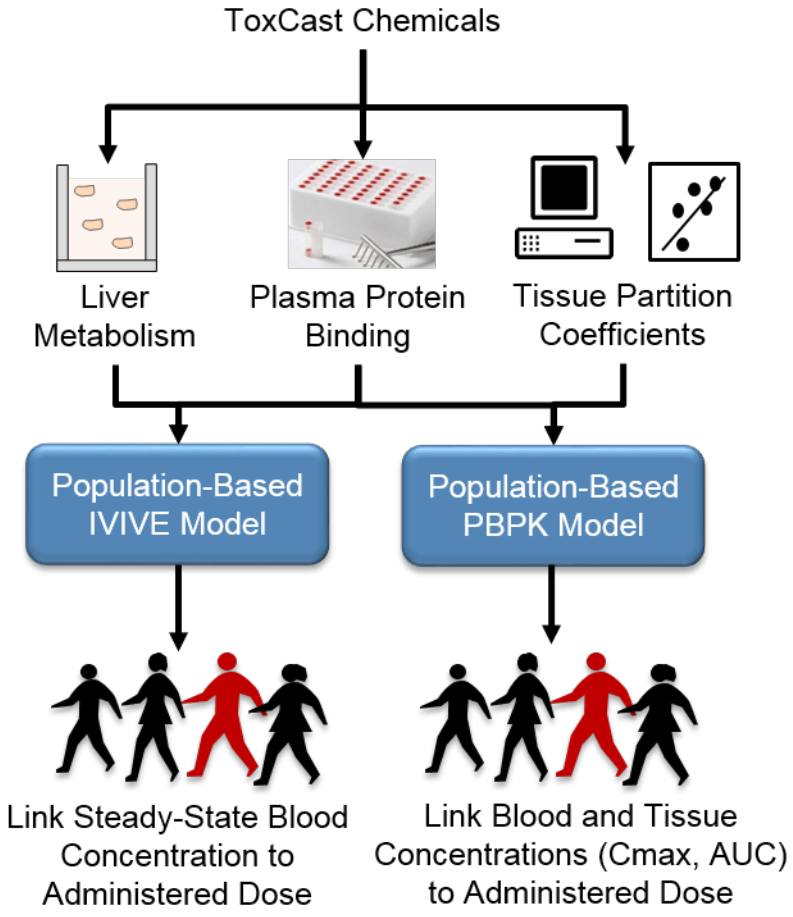


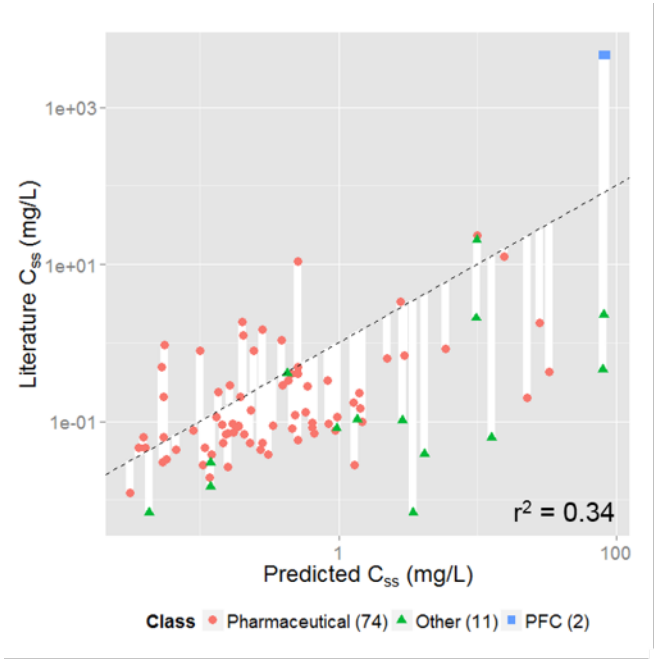


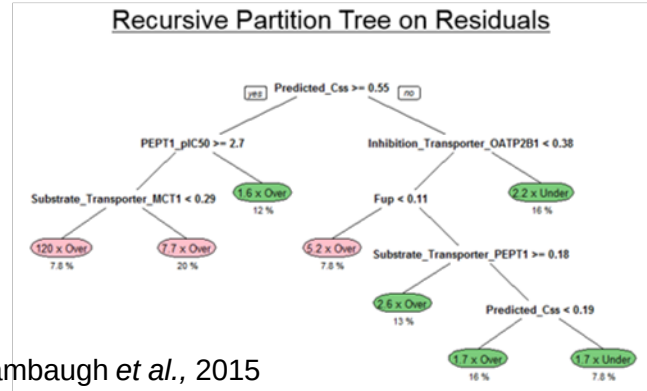
e.g. Judson *et al.*, (2011)

Toxicokinetics Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



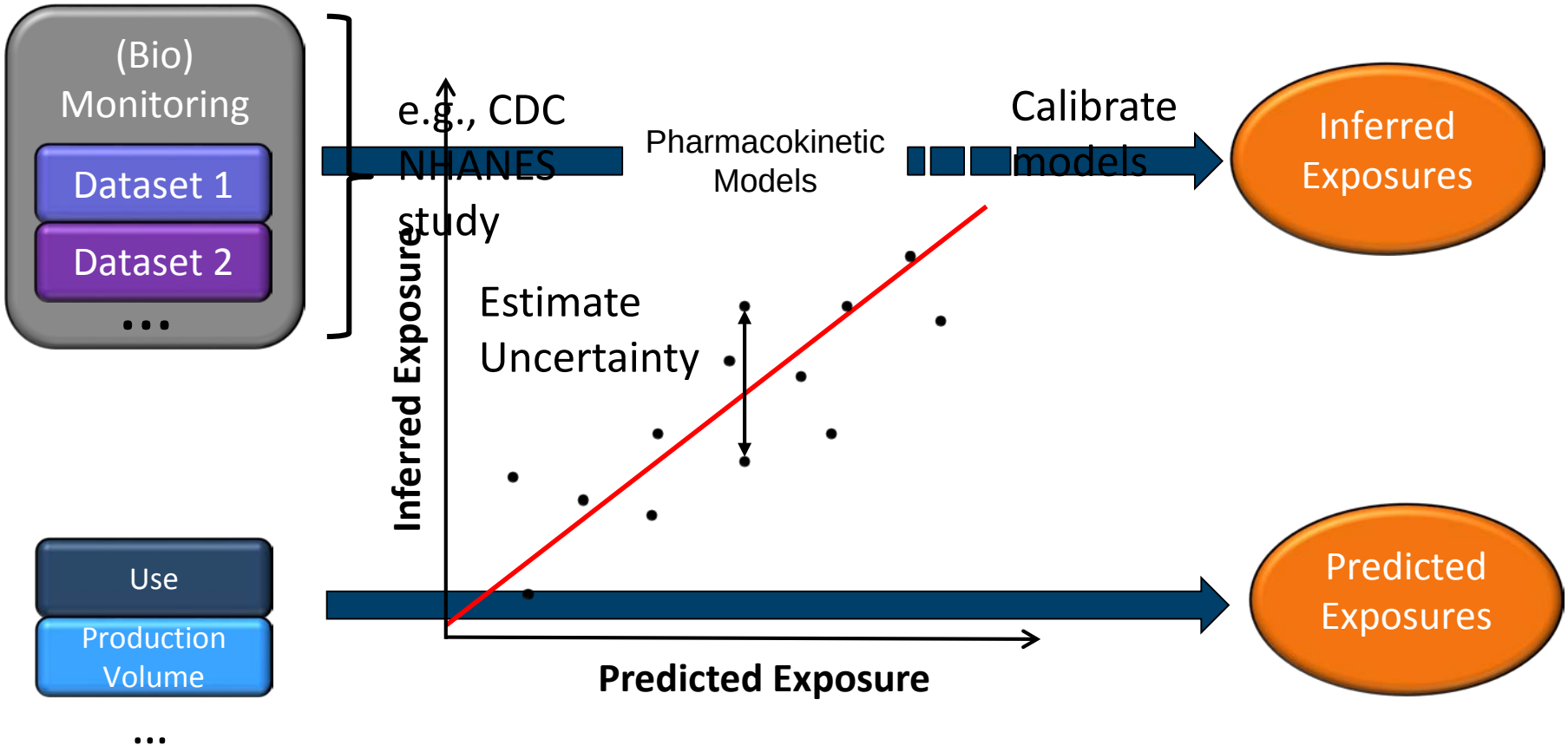




Wambaugh *et al.*, 2015

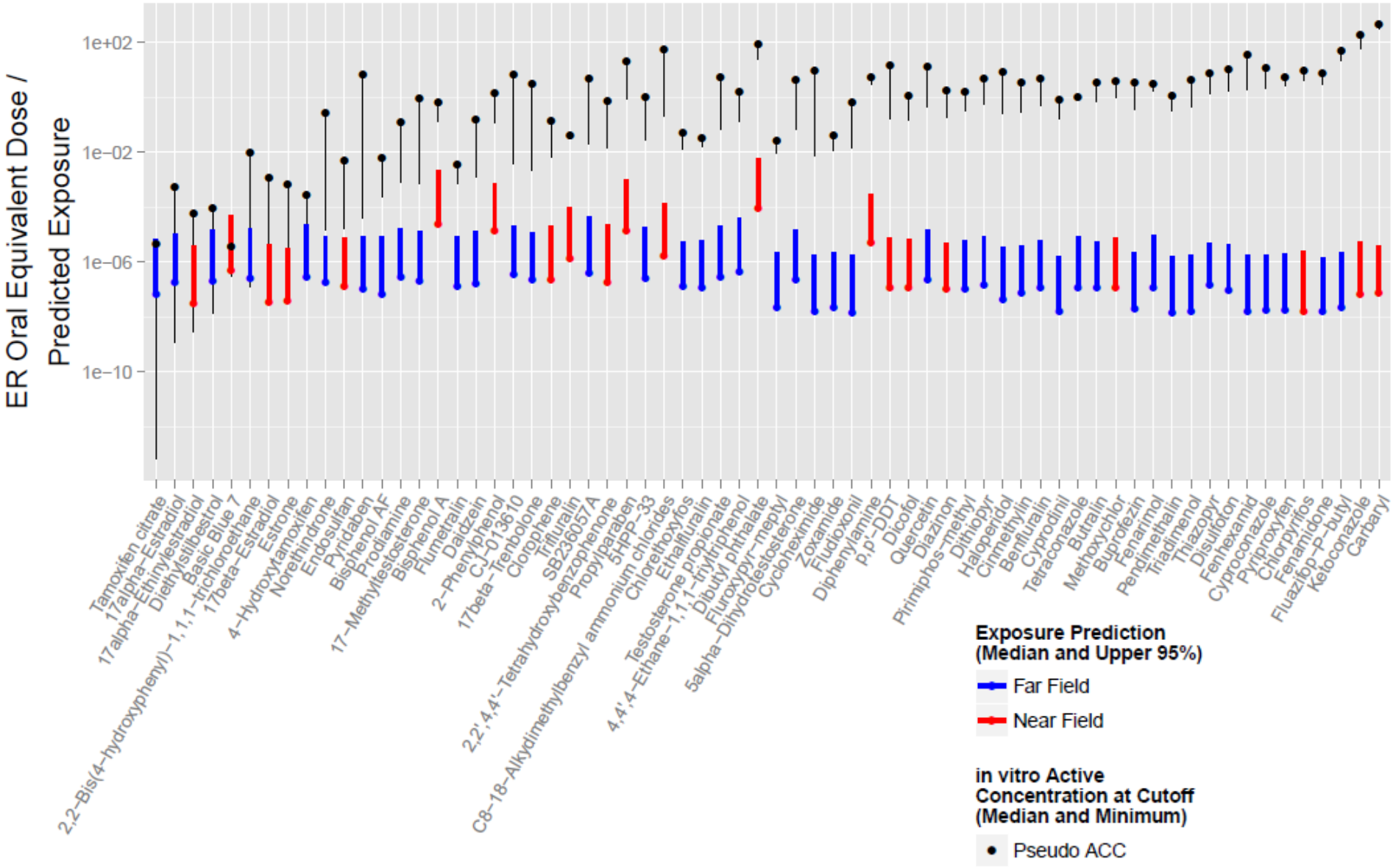
Population and Exposure Modeling

Estimating Exposure and Associated Uncertainty with Limited Data



Wambaugh *et al.*, 2014

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

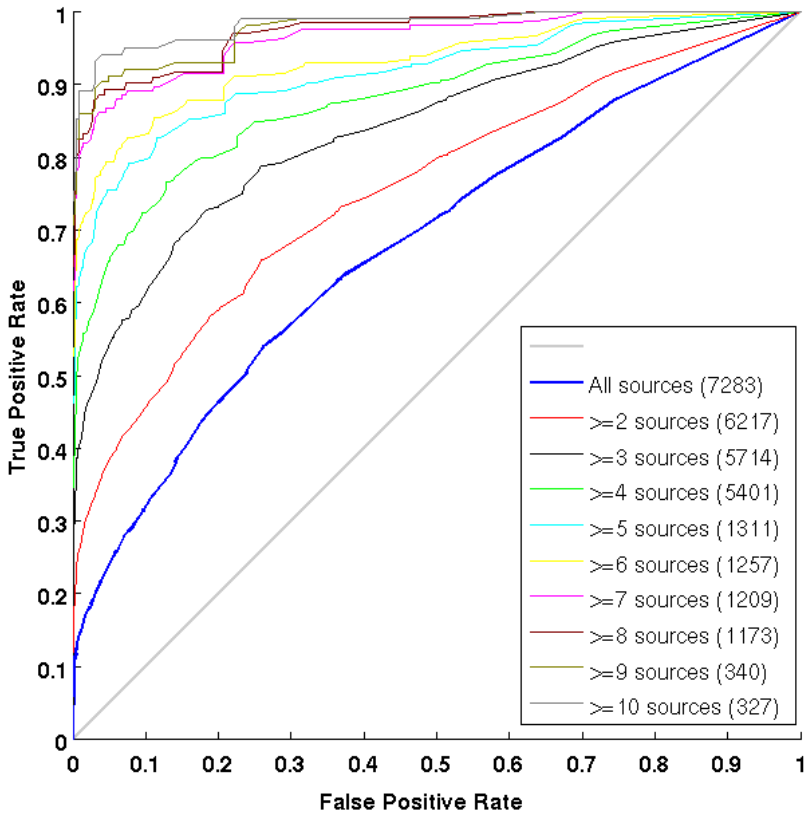


CERAPP: using QSAR for further prioritization

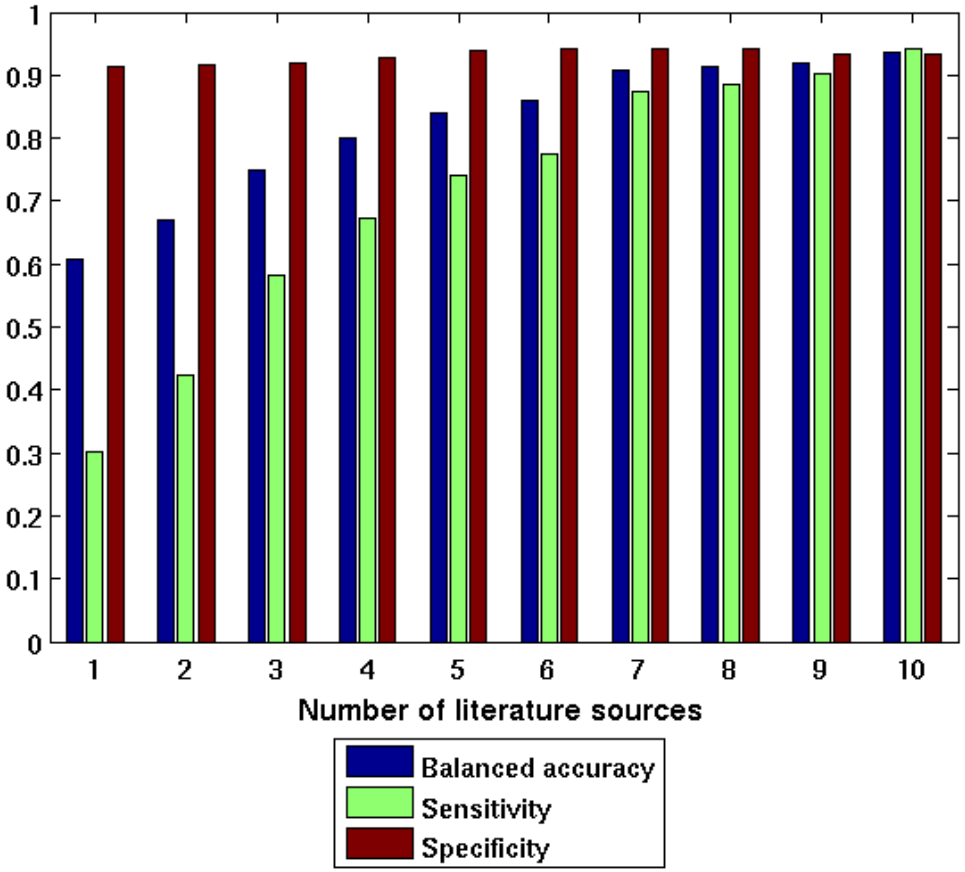
- Collaborative Estrogen Receptor Activity Prediction Project
- Goals:
  - Use ToxCast ER score (or other data) to build many QSAR models
  - Use consensus of models to prioritize chemicals for further testing
- Assumptions
  - ToxCast chemicals cover enough of chemical space to be a good "global" training set
  - Consensus of many models will be better than any one individually
- Process
  - Curate chemical structures
  - Curate literature data set
  - Build many models
  - Build consensus model
  - Evaluate models and consensus

Mansouri et al: "CERAPP: Collaborative Estrogen Receptor Activity Prediction Project" EHP (2016)

CERAPP Consensus evaluation



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



Total Database

Binders: 3961

Agonists: 2494

Antagonists: 2793

CERAPP Summary

- EDSP Universe (10K)
- Chemicals with known use (40K) (CPCat & ACToR)
- Canadian Domestic Substances List (DSL) (23K)
- EPA DSSTox – structures of EPA/FDA interest (15K)
- ToxCast and Tox21 (In vitro ER data) (8K)

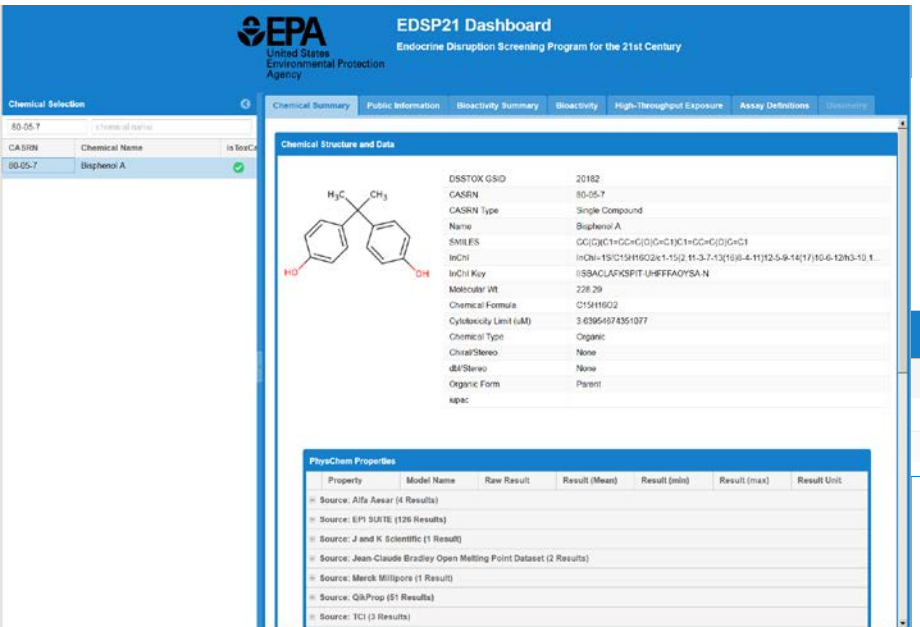
~32,000 unique structures evaluated

5-10% predicted to be ER-active

Prioritize for further testing

Data Transparency: EDSP21 Dashboard

- Goal: To make EDSP21 data easily available to all stakeholders
  - Assay-by-assays concentration-response plots
  - Model scores – AUC agonist and antagonist
  - ER QSAR calls
  - Other relevant data
- https://actor.epa.gov/edsp21



ToxCast Model Predictions

Model	Agonist AUC	Antagonist AUC
ER	0.45	0
AR	0	0.136

Consensus CERAPP QSAR ER Model Predictions

Class	Agonist (Potency Level)	Antagonist (Potency Level)	Binding (Potency Level)
from Literature	Active (Weak)	-	Active (Weak)
QSAR Consensus	Active (Weak)	Active (Strong)	Active (Weak)

Summary

- EDSP is in need of new approach to handle large testing universe
  - Reduce cost, speed throughput
- Estrogen Receptor Model is first example of this
  - 54 chemicals in low-throughput Tier 1 assays
  - 1800 chemicals tested and published in high-throughput
  - 1000 more in queue – 2016 planned release
- Next steps
  - Androgen receptor (1800 chemicals tested, modeling and validation in progress)
  - Steroidogenesis (1000 chemicals with preliminary data)
  - Thyroid – assay development and testing underway for several targets (THR, TPO, deiodinases, ...)

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