US EPA Endocrine Disruptor Screening Program – The Pivot

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ICCA-LRI / EPA Workshop “What Will Work?”

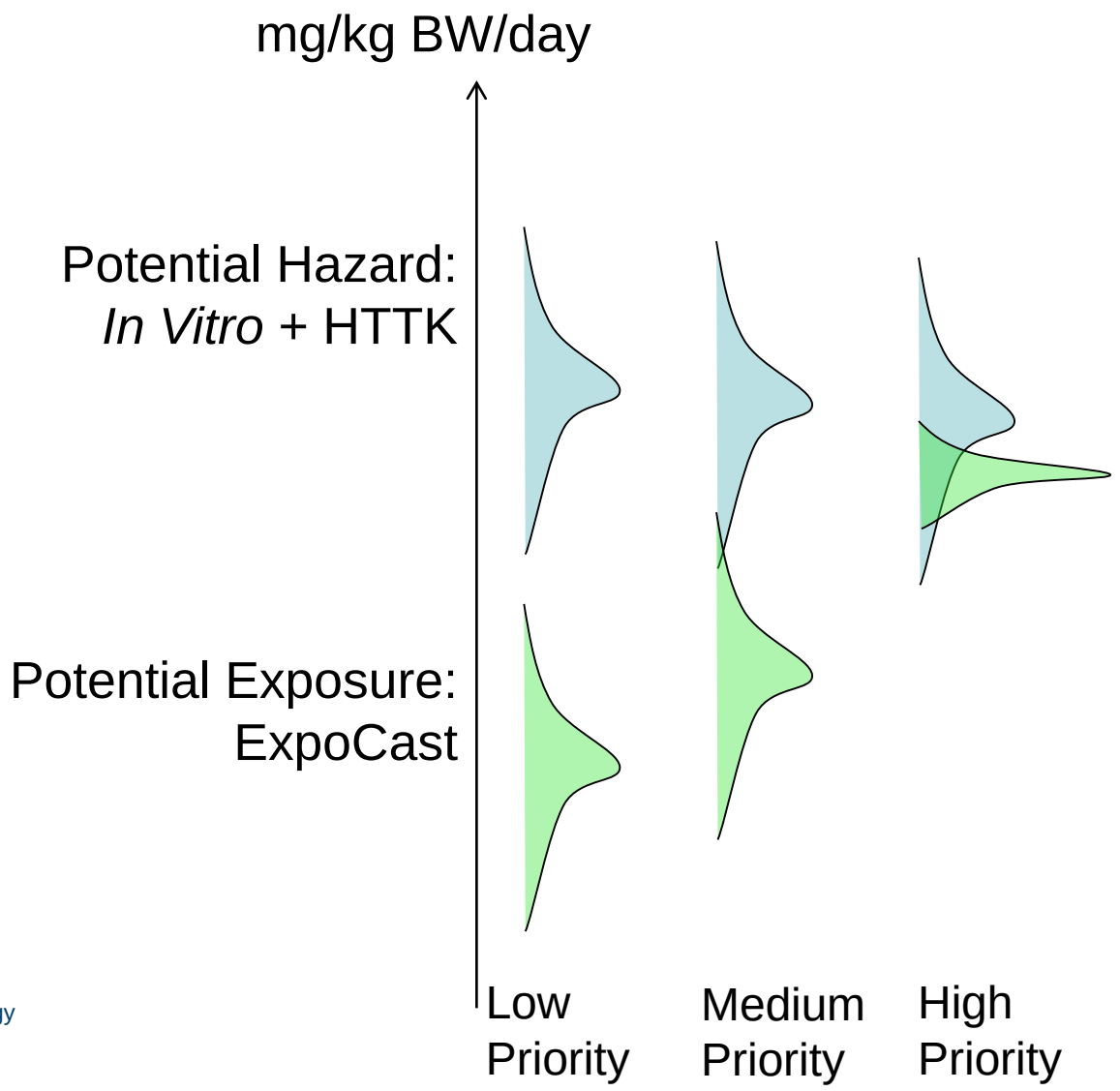
16-17 June 2015, New Orleans

Major Points

- 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
- Need new approach
  - Prioritize chemicals
  - Replace low-throughput assays with high-throughput variants
- Demonstrate new approach: Estrogen receptor
  - Multiple high-throughput in vitro assays
  - Demonstrate use to prioritize chemicals and replace selected Tier 1 assays

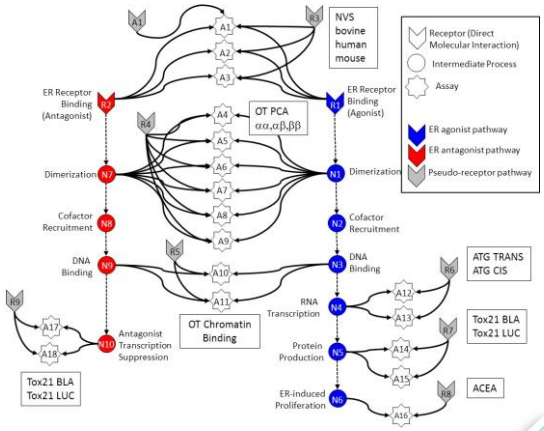
- *In Vitro* assays: Bioactivity Concentration
- Need Bioactivity Dose to compare with exposure
- Convert using High Throughput Toxicokinetics (HTTK)

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



Validate multi-assay consensus against *in vitro* and *in vivo* reference chemicals

United States Environmental Protection Agency



***In vitro hER* activity:**

- **Human Breast**
- **Human Ovary**
- **Human Uterus**
- **Human Cervix**
- **Human Liver**
- **Human ER (cell free)**

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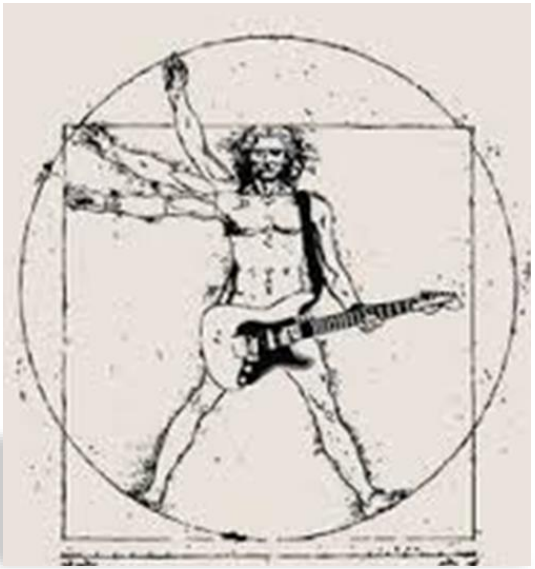
ER-Bioactivity

- **Rat or Mouse uterus (guideline uterotrophic)**

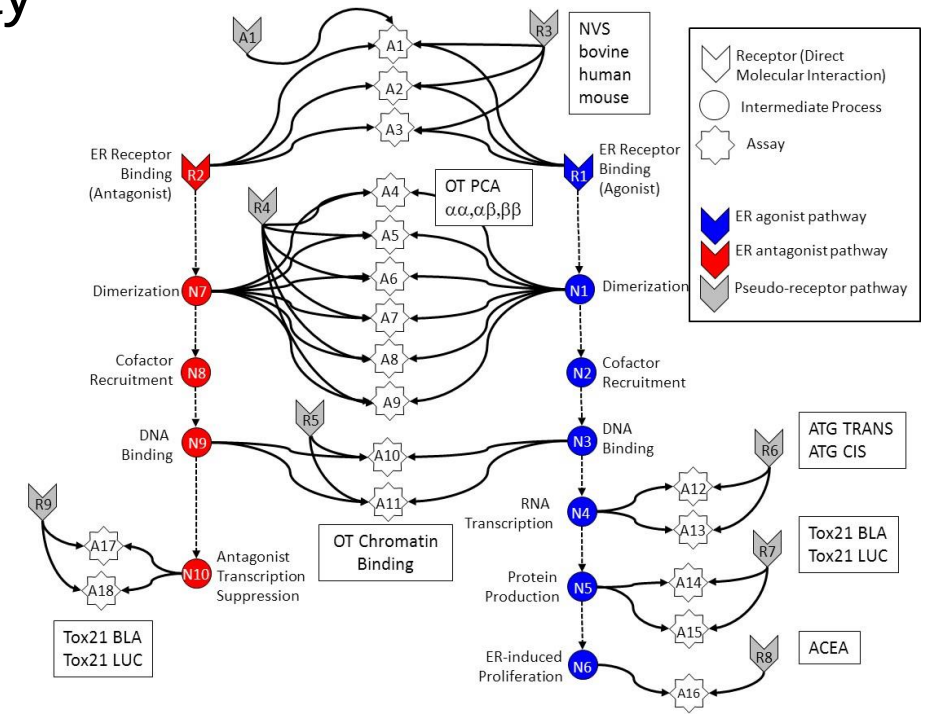


Human Relevance

In Vitro Estrogen Receptor Model

Combines results from multiple in vitro assays

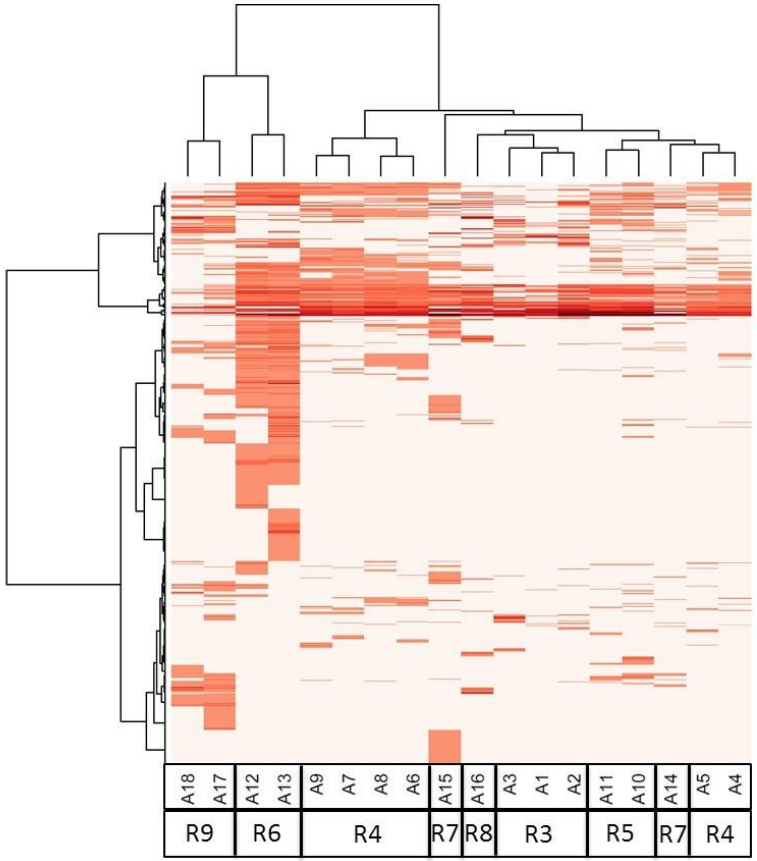
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



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

Major theme – all assays have false positives and negative

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

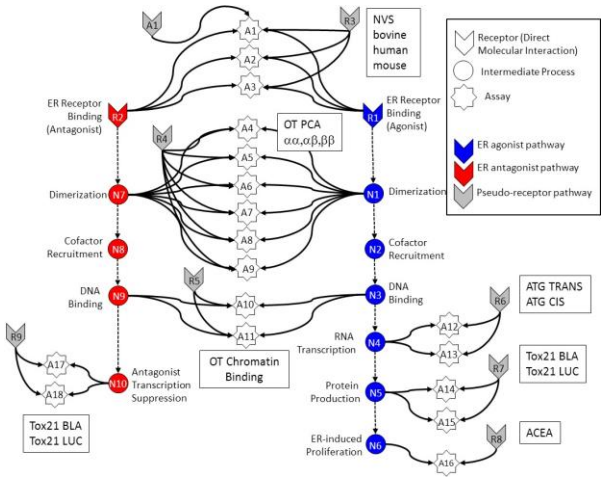


Much of this “noise” is reproducible

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

EDSP chemical universe is structurally diverse

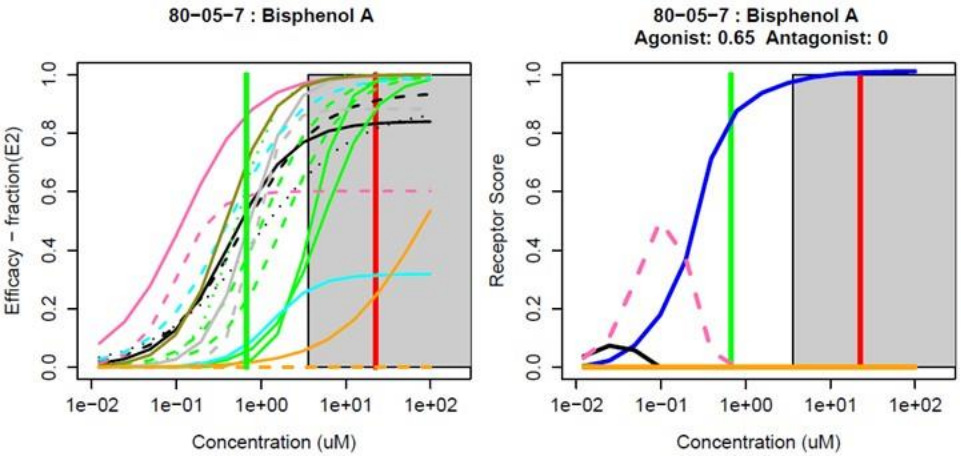
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



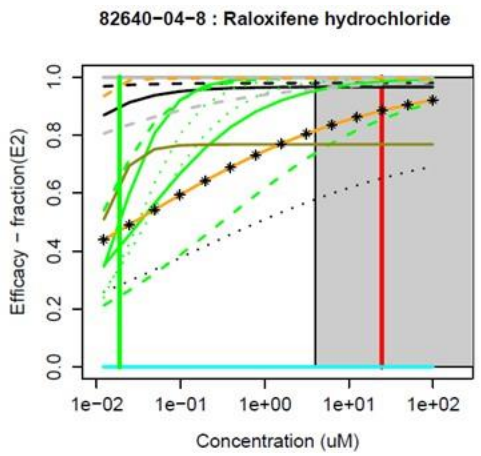
Example curves

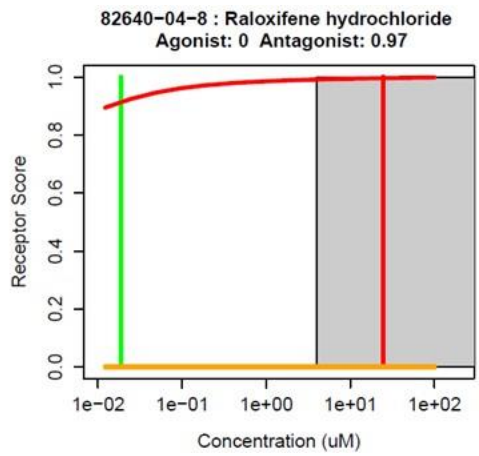
United States Environmental Protection Agency

True Agonist

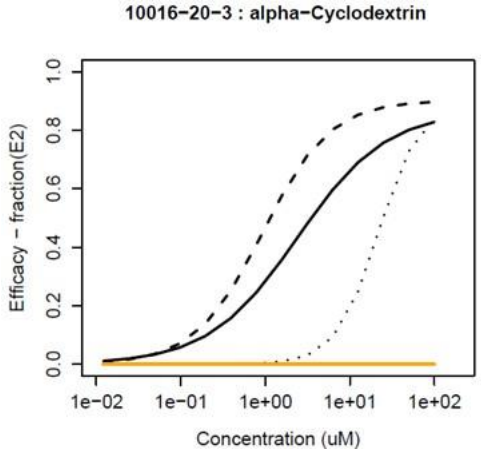


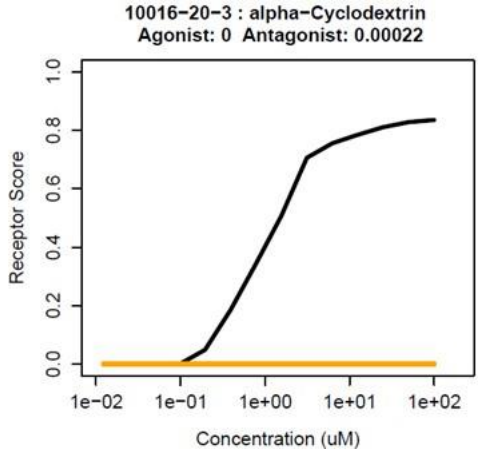
True Antagonist



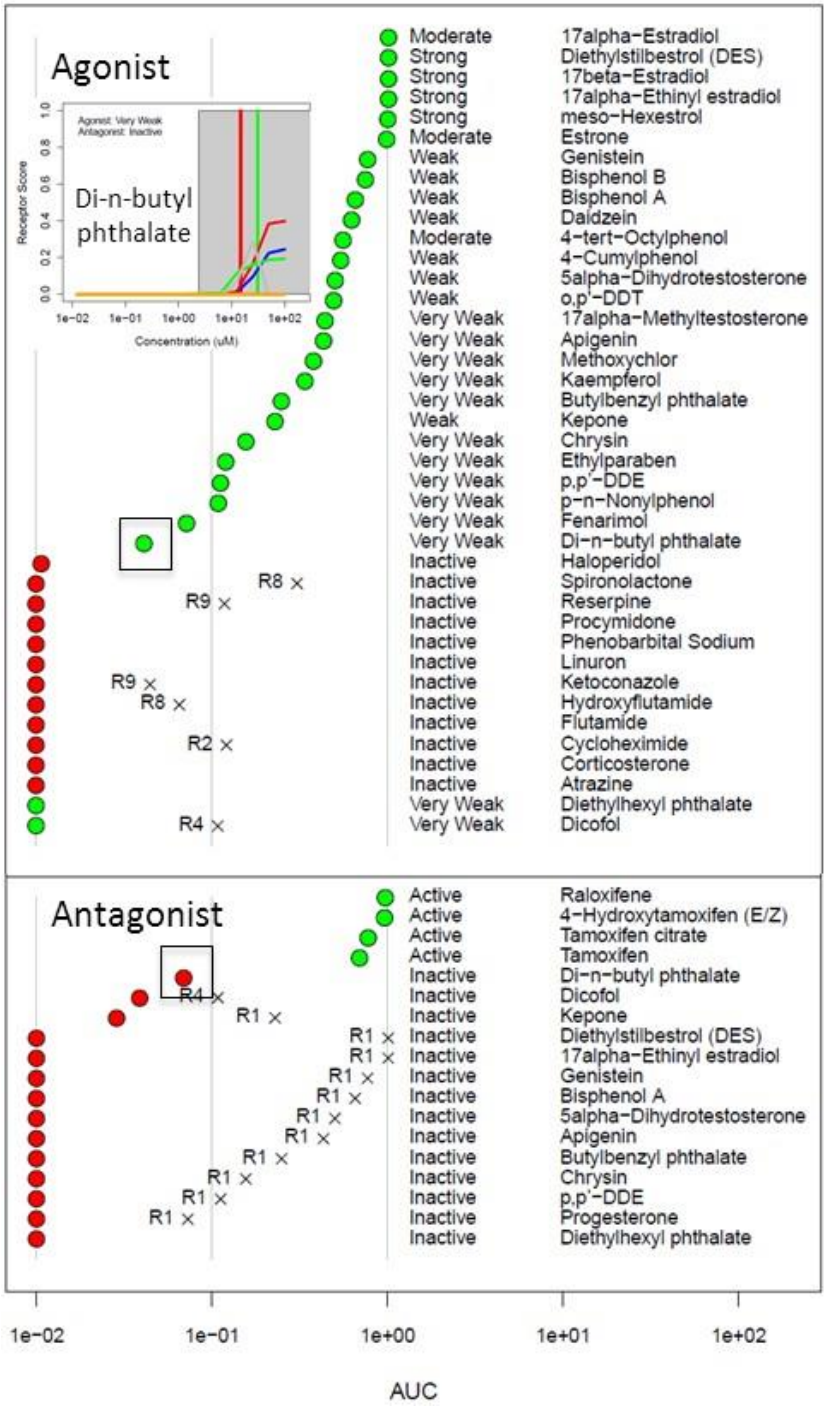


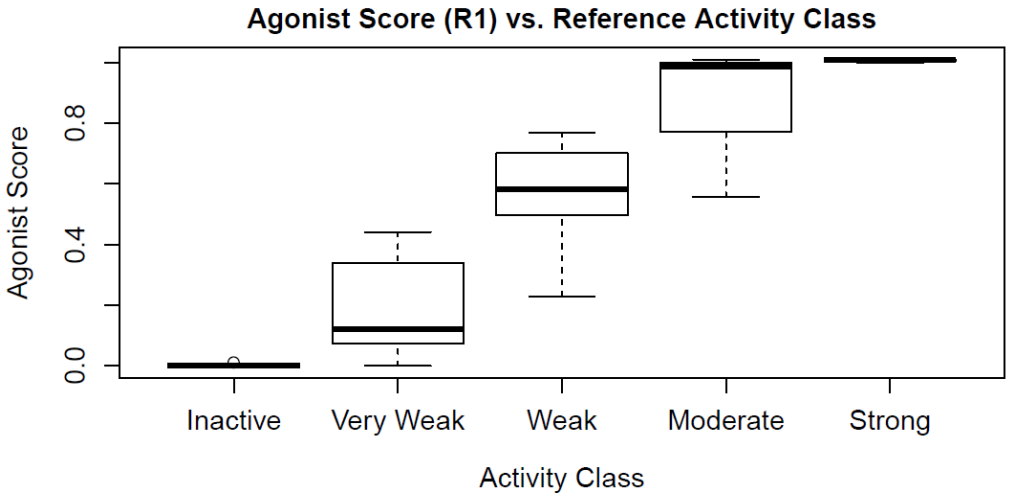
Negative-Narrow Assay Interference



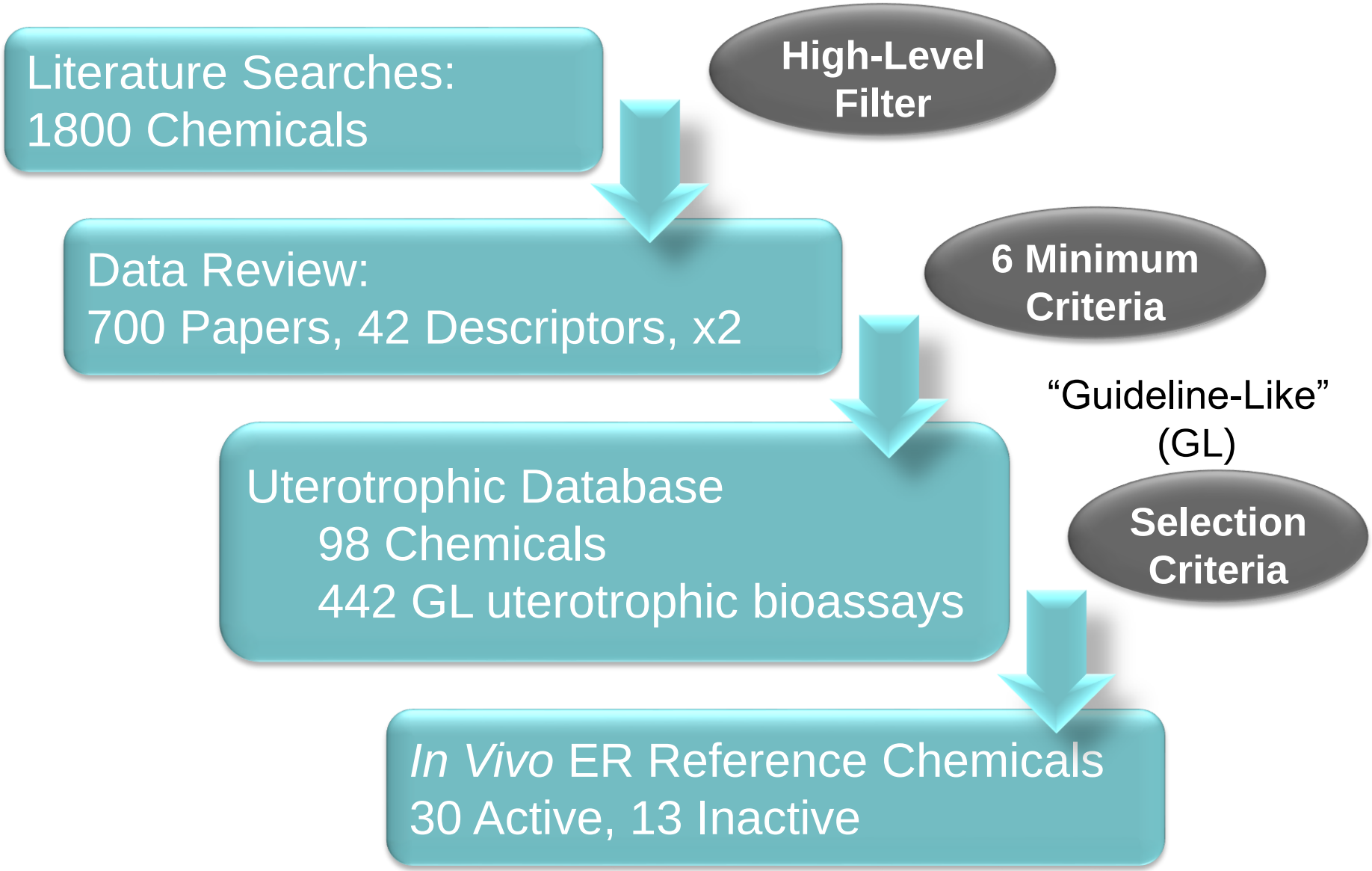


In Vitro Reference Chemical Performance





Identifying Uterotrophic Reference Chemicals from the Literature



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

Adding Tier 1 / List 1 chemicals to the Literature DB: 81 Guideline Studies

Uterotrophic Literature "Guideline-Like" Studies

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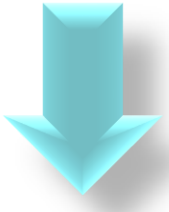
EDSP List 1 Uterotrophic "Guideline" Studies



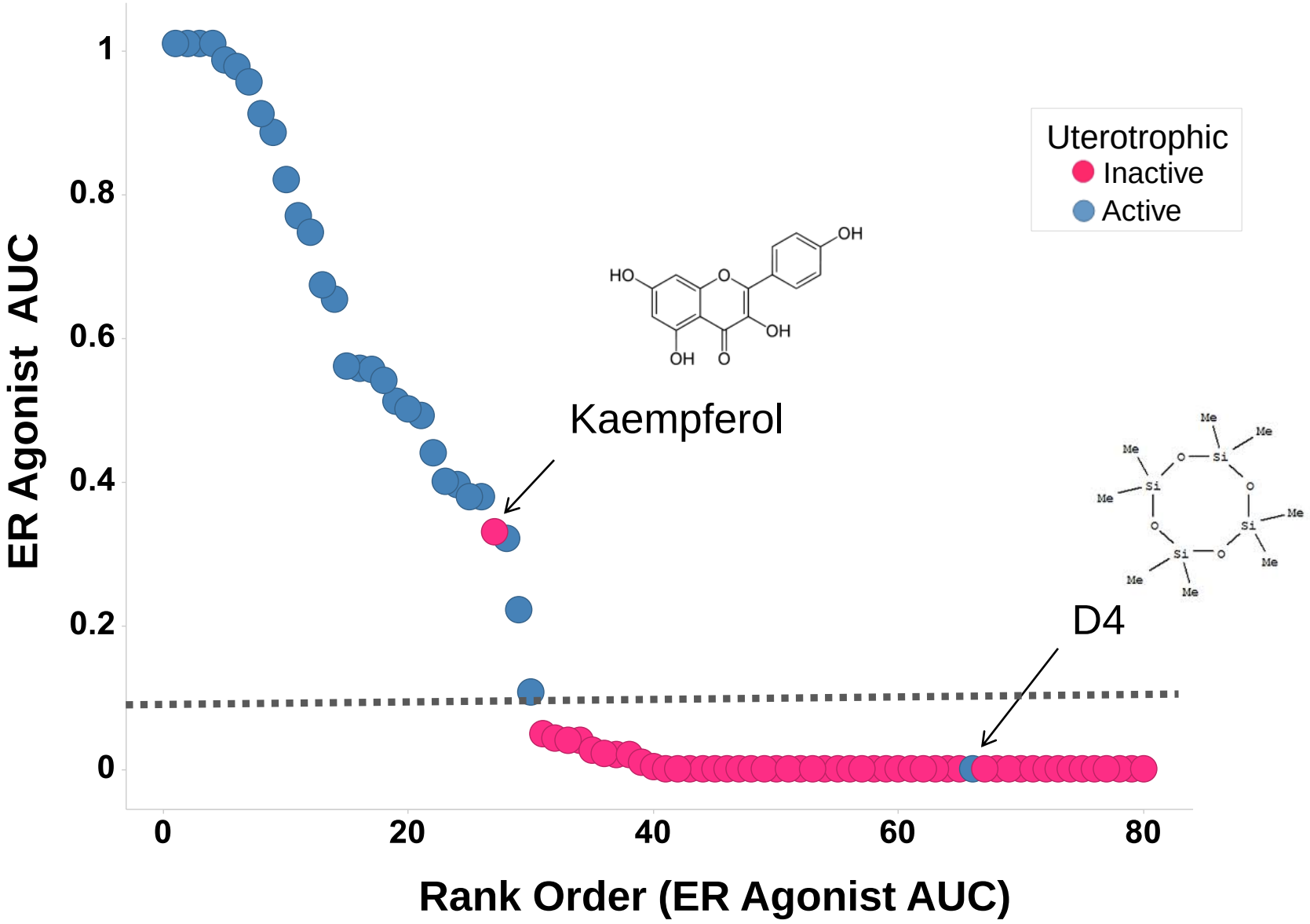
Uterotrophic Reference Chemicals:
30 Active, 51 Inactive

In Vitro Activity vs. Uterotrophic Outcomes

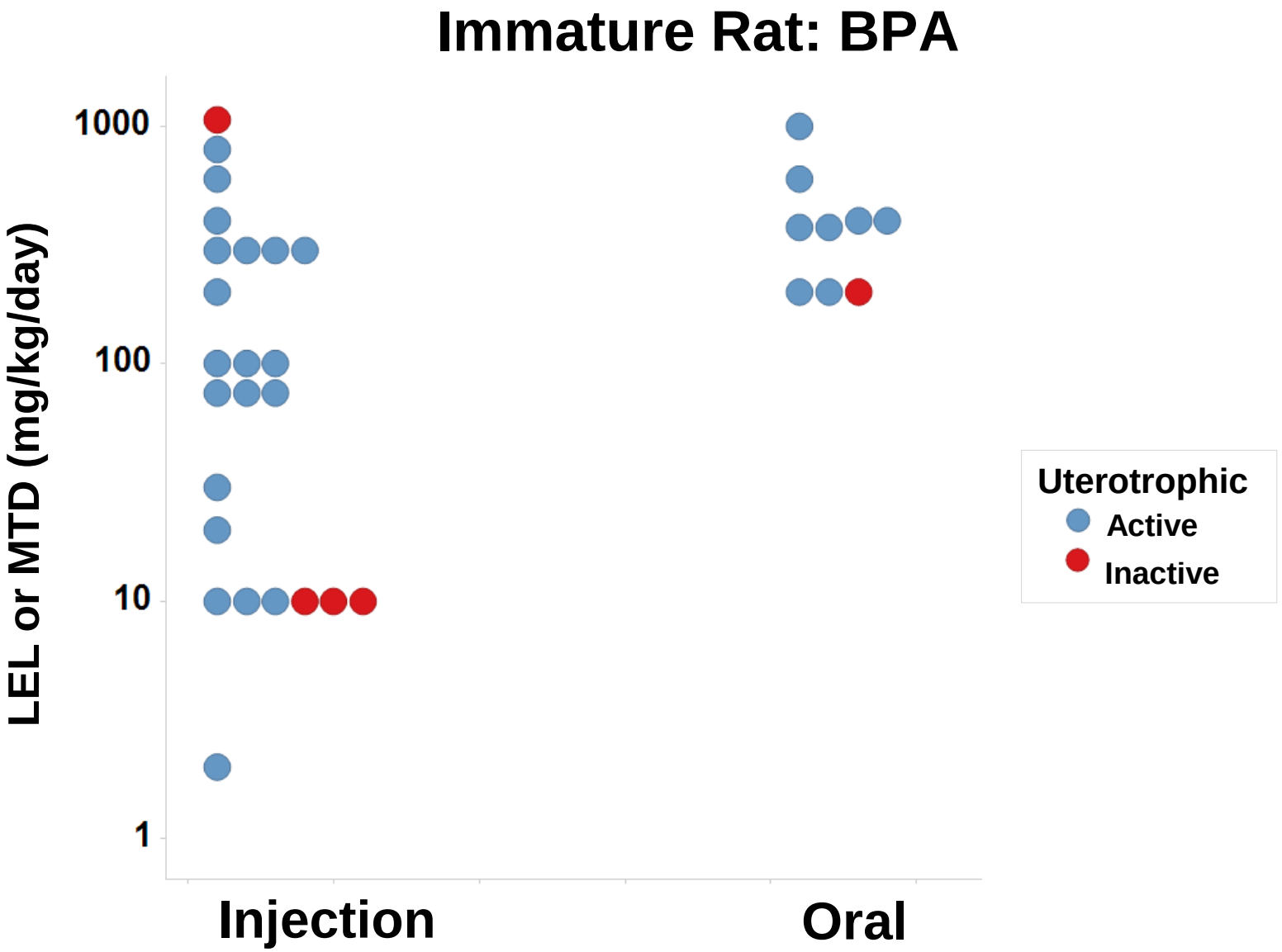
ER Agonist Model AUC

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

ER Agonist AUC vs. Uterotrophic Outcomes



In vivo guideline studies have the same types of uncertainty as *in vitro*

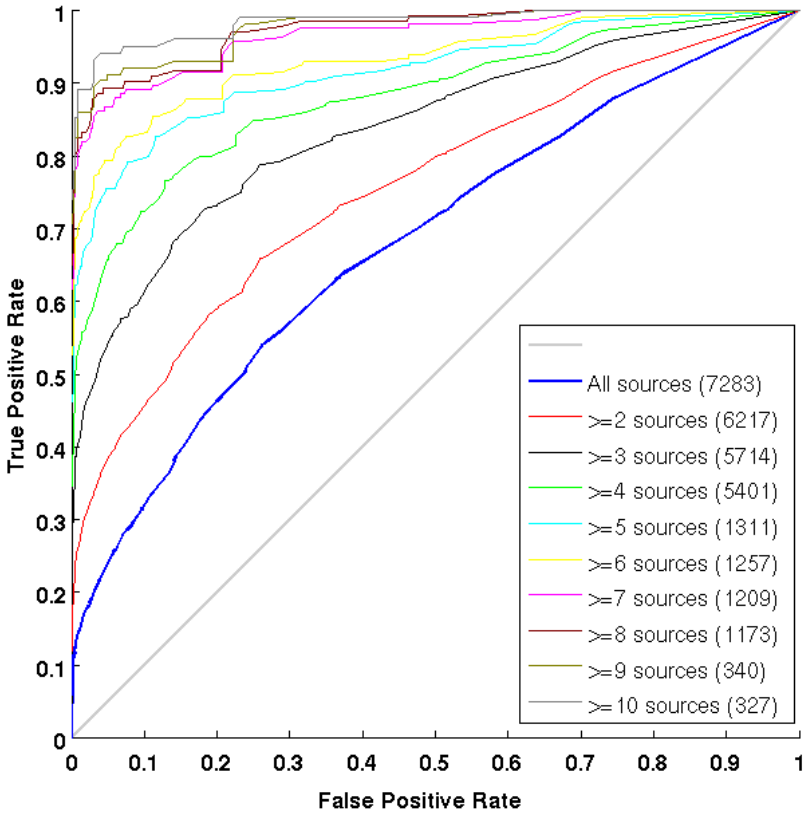


CERAPP: using QSAR for further prioritization

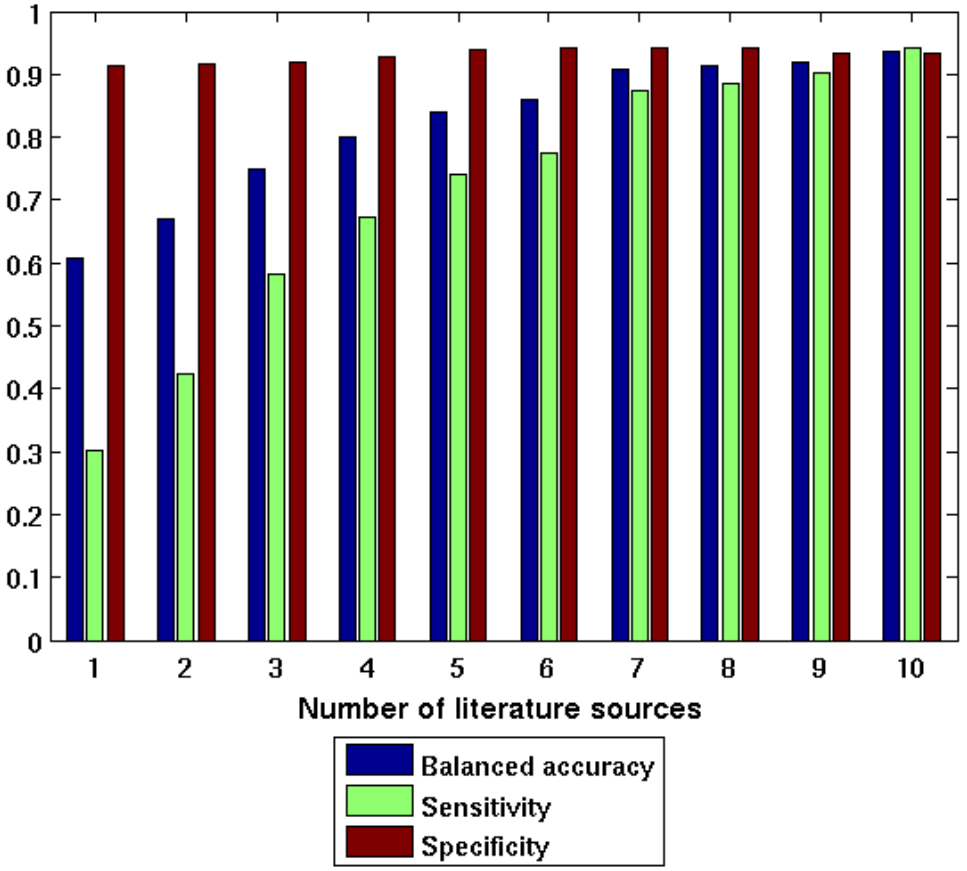
- Collaborative Estrogen Receptor Activity Prediction Project
- Goals:
  - Use ToxCast ER score (or other data) to build many QSAR models
  - Use consensus of models to prioritize chemicals for further testing
- Assumptions
  - ToxCast chemicals cover enough of chemical space to be a good "global" training 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" (In prep)

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)

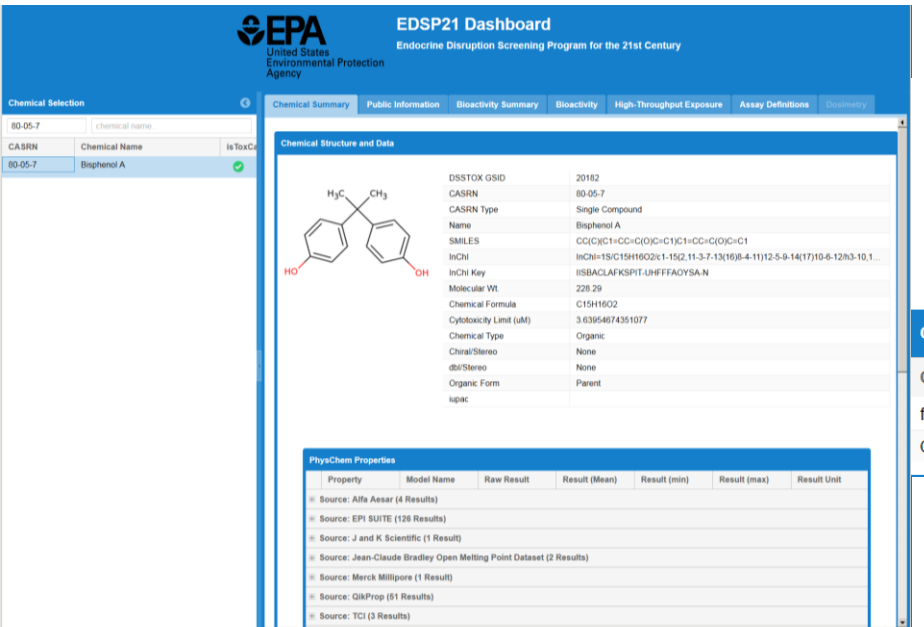
~32K unique structures

5-10% predicted to be ER-active

Prioritize for further testing

Data Transparency: EDSP21 Dashboard

- Goal: To make ER and AR data easily available to all stakeholders
  - Assay-by-assays concentration-response plots
  - Model scores – AUC agonist and antagonist
  - ER QSAR calls
  - Other relevant data
- http://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)

Regulatory Review
FIFRA SAP, December 2014

- Can the ER Model be used for prioritization?
  - "Overall, with minor limitations for compounds that require metabolic activation or have targets other than nuclear receptors, the ER AUC appears to be an appropriate tool for chemical prioritization for List 1, List 2 and the EDSP universe compounds."
- Can the ER model substitute for the Tier 1 ER in vitro and uterotrophic assays?
  - "Overall, because both the ER AUC model and the Tier 1 *in vitro* assays capture either nuclear receptor binding and/or transactivation, replacement of the Tier 1 *in vitro* ER endpoints (ER binding and ERTA) with the ER AUC model will likely be a more effective and sensitive measure for the occurrence of estrogenic activity that occurs through nuclear receptor binding and activation."
  - The Panel found that the data comparing the ER AUC model to the uterotrophic assay were strong for the reference compounds that were clearly estrogenic … or unmistakably not estrogenic …. However, the model outcomes were less straight forward when the ER AUC model for non-reference chemicals was compared to uterotrophic studies where the data were limited or discordant. … This finding suggests a very low risk of false negatives in this data set, but was limited by the fact that there were no chemicals with either high or intermediate AUC model values available for functional comparison. … 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)"
- Results presented here are part of the recommended follow-up

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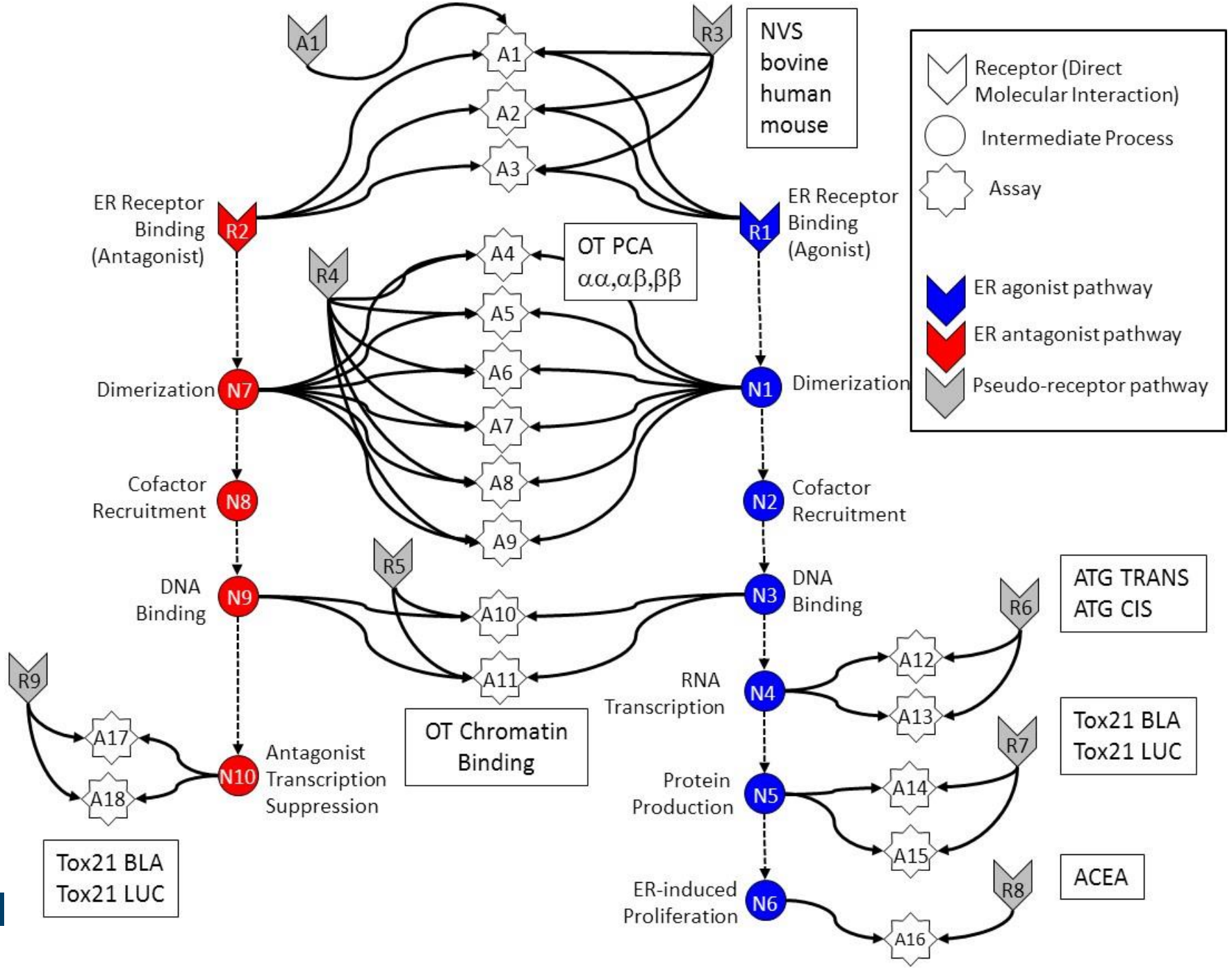
NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
A1
A2
A3
R1
R2
R3
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
OT PCA
αα,αβ,ββ
R4
A4
A5
A6
A7
A8
A9
Dimerization
N7
N1
Dimerization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