

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

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ECHA Red-Across Workshop

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20 minutes

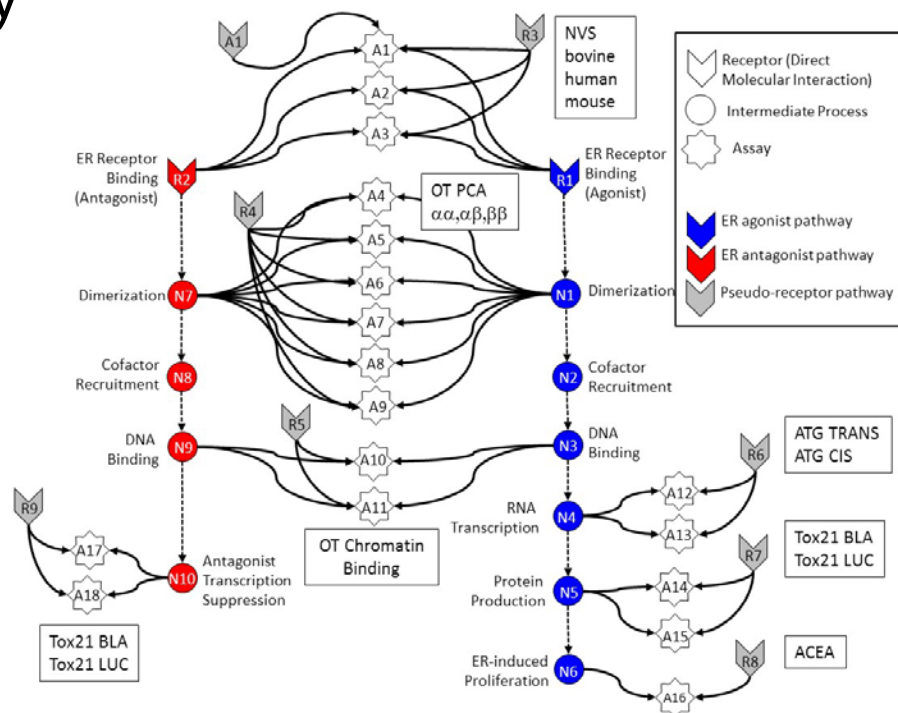
# Major Points

- 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* Estrogen Receptor Model

## Combines results from multiple in vitro assays

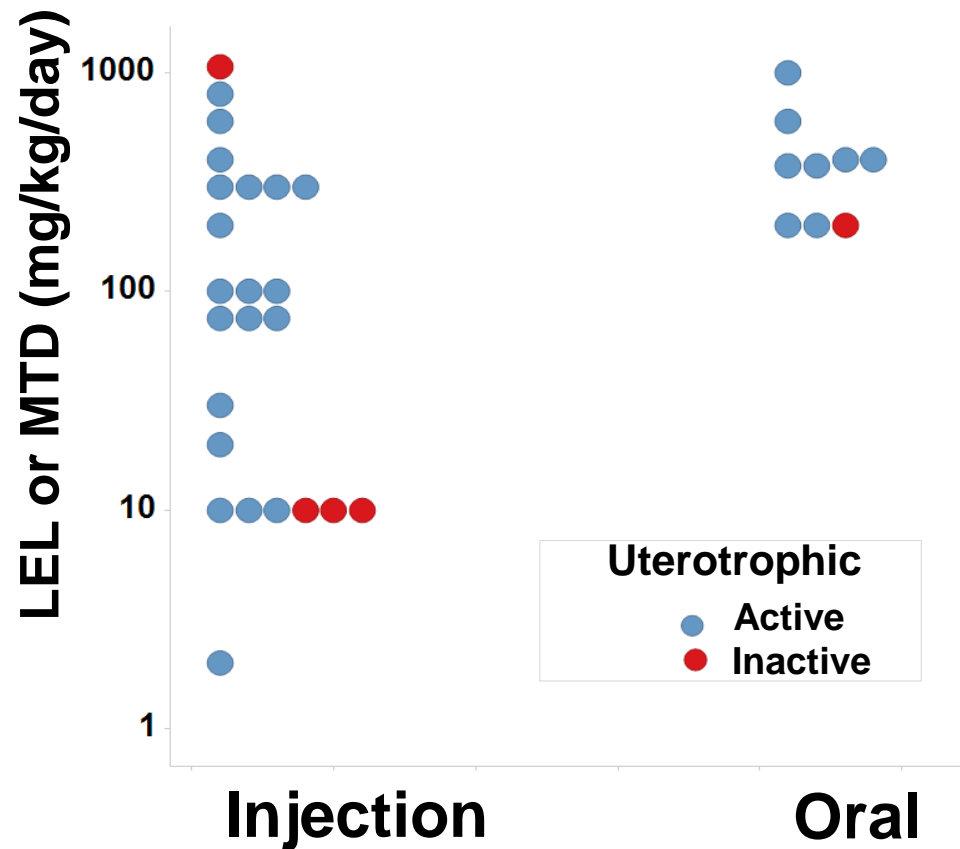
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



# *In vivo* guideline study uncertainty

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

### Immature Rat: BPA



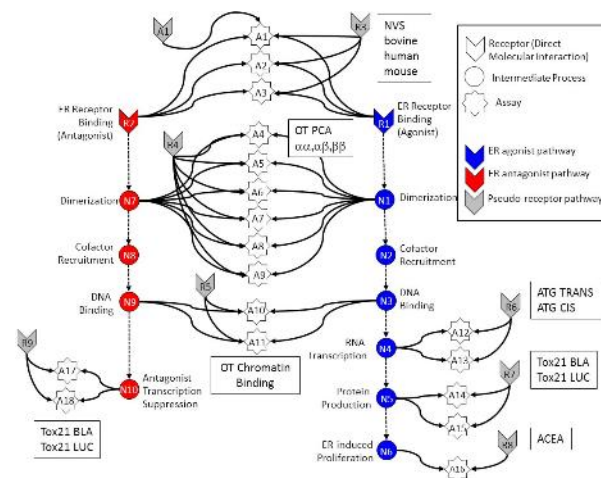
### Phenotype X

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

Much of this “noise” is reproducible

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

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



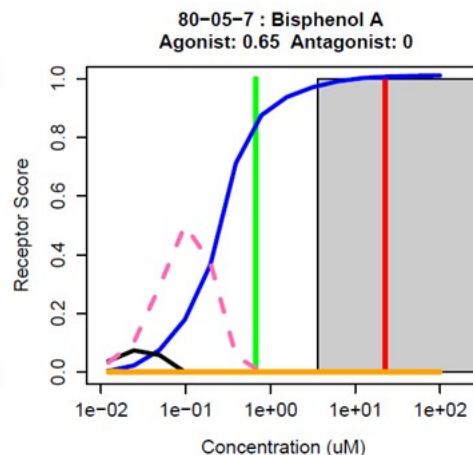
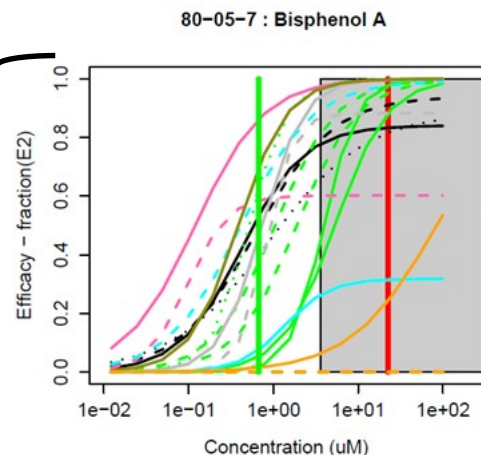
# Assay-to-assay variation

Assay Data

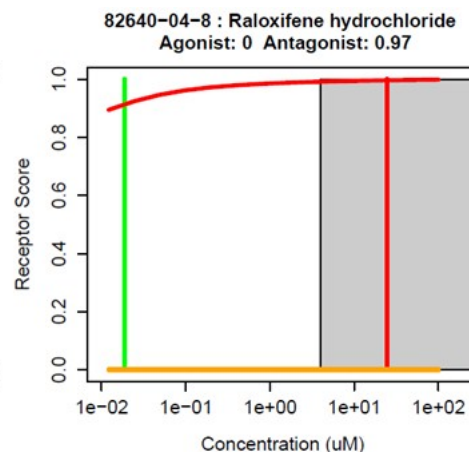
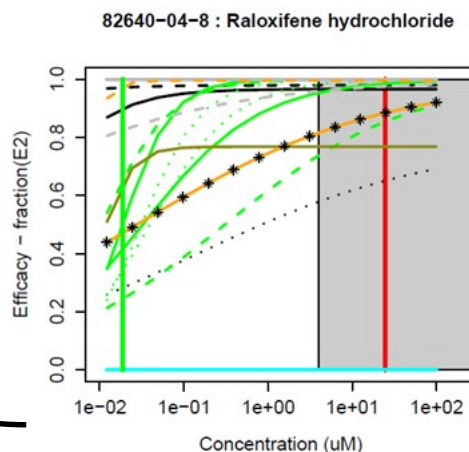
Integrated Model

All appropriate  
assays are active  
but efficacy and  
potency vary

“Noise” or real  
variation in biology  
between cell types?



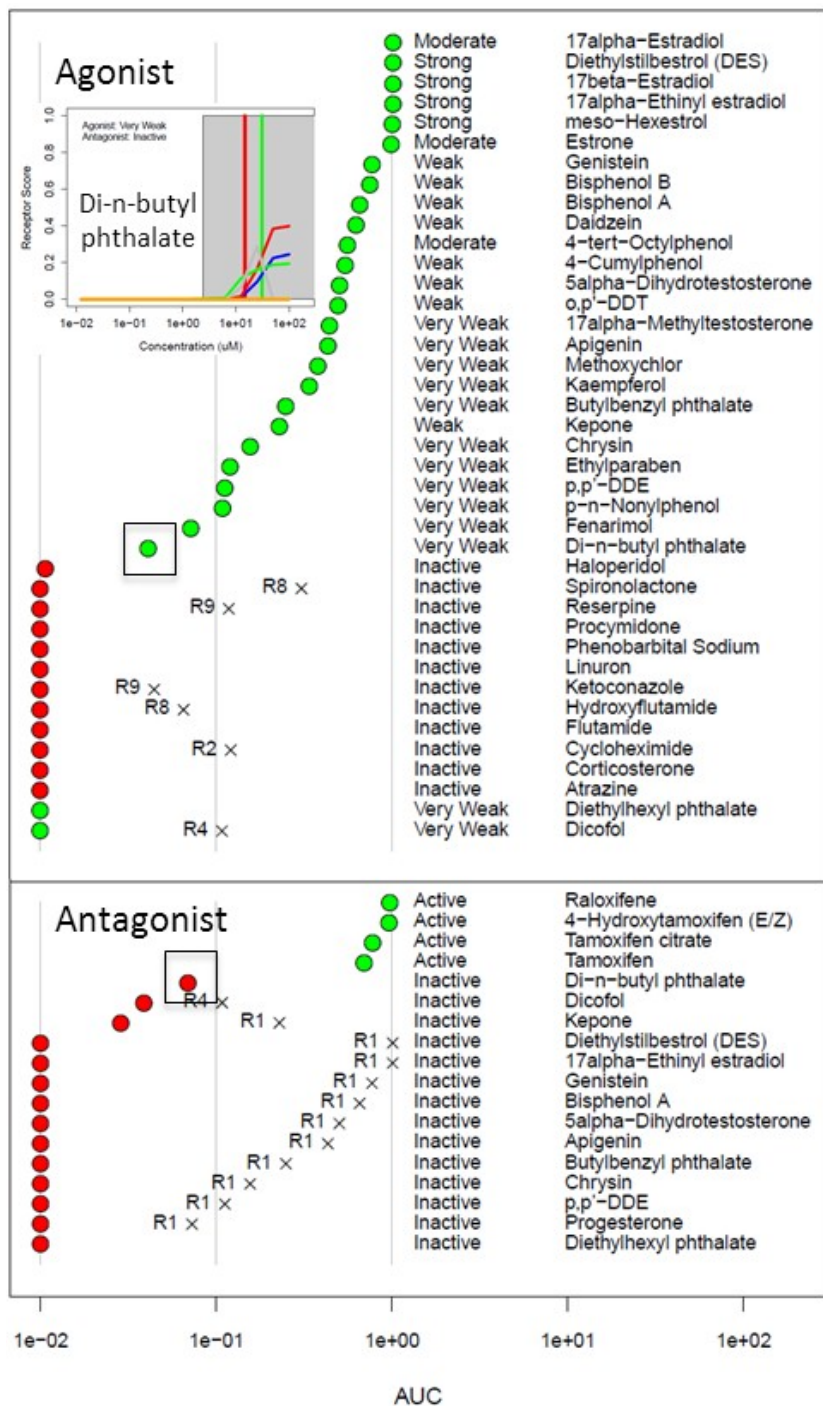
Agonist



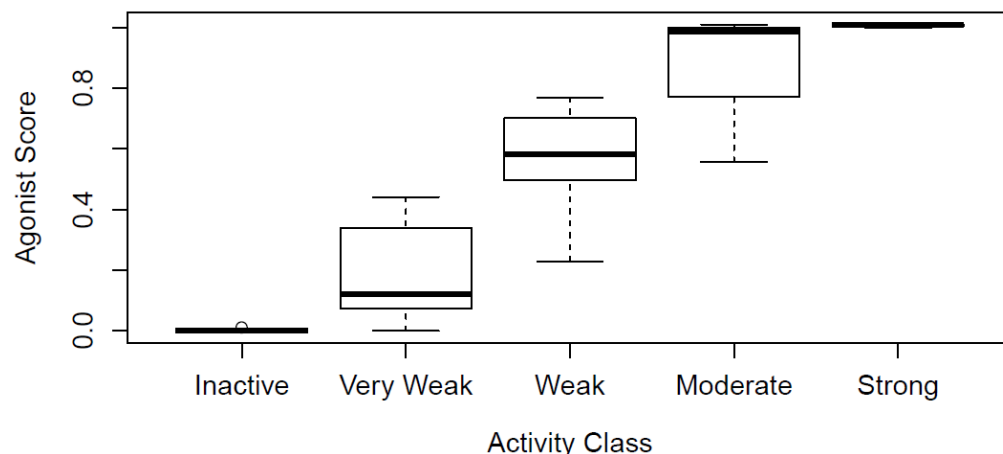
Antagonist



## *In Vitro* Reference Chemical Performance



### Agonist Score (R1) vs. Reference Activity Class



# Identifying Uterotrophic Reference Chemicals from the Literature

Literature Searches:  
1800 Chemicals

High-Level  
Filter

Data Review:  
700 Papers, 42 Descriptors, x2

6 Minimum  
Criteria

Uterotrophic Database  
98 Chemicals  
442 GL uterotrophic bioassays

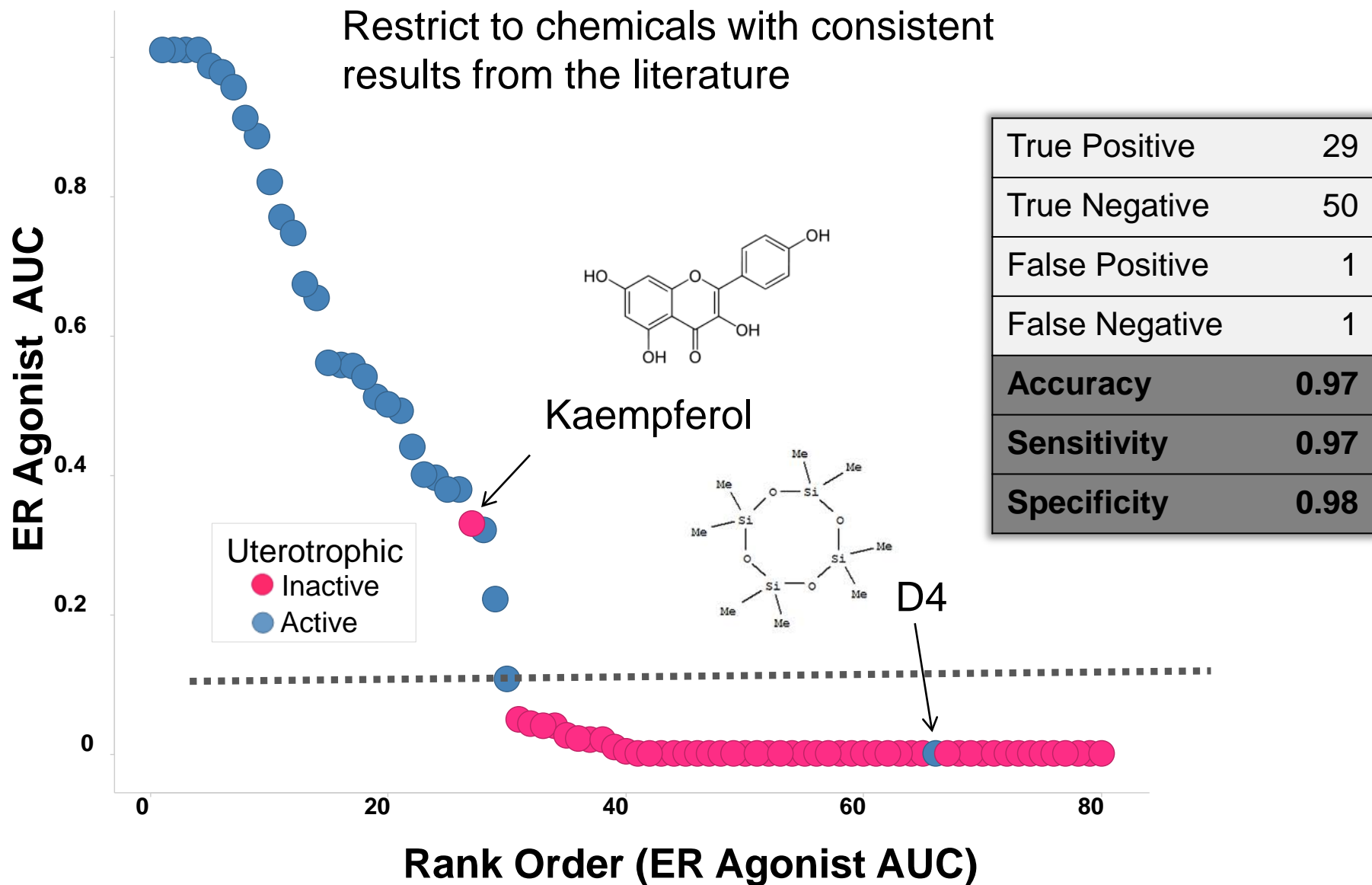
“Guideline-Like”  
(GL)

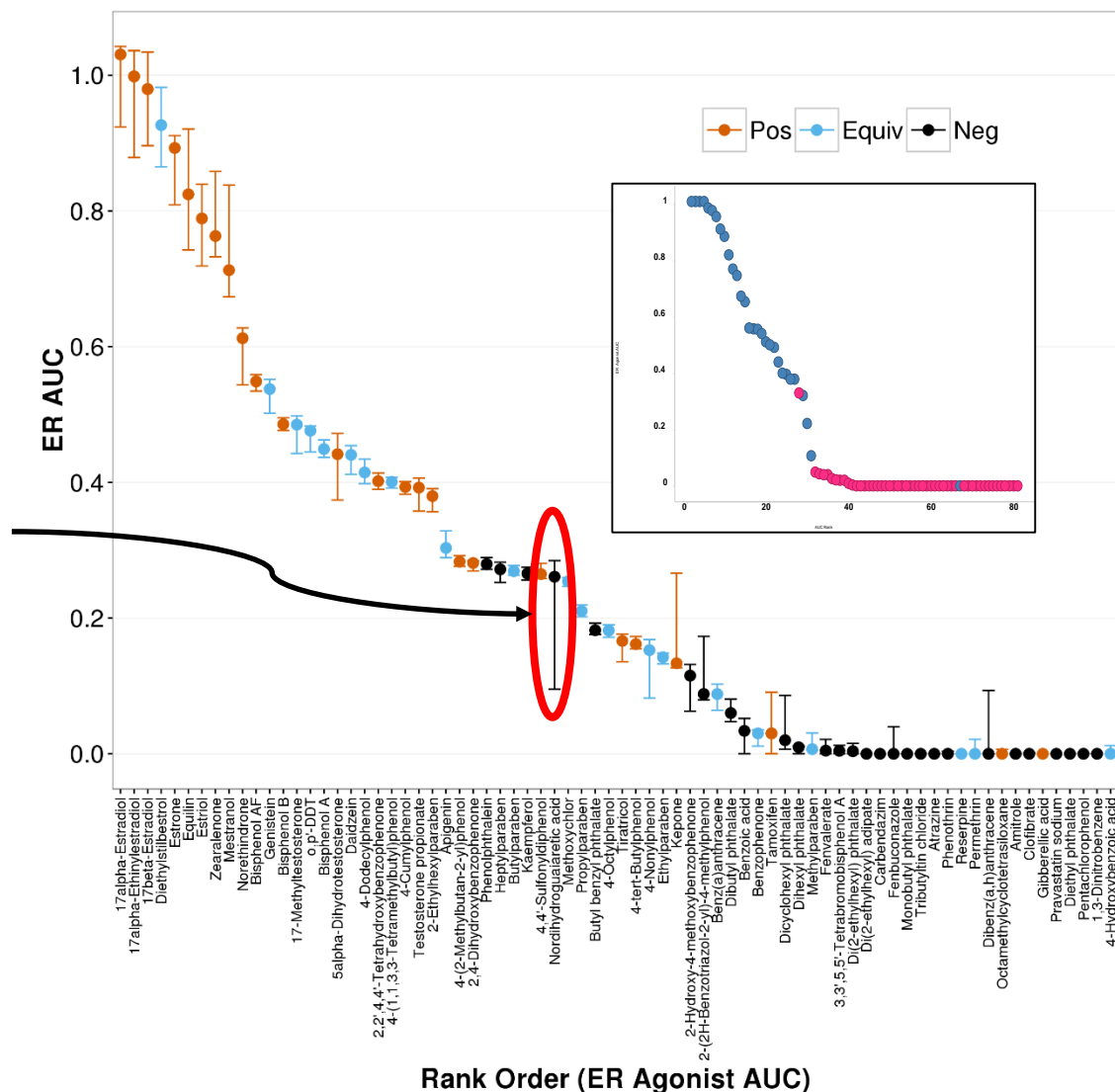
Selection  
Criteria

*In Vivo* ER Reference Chemicals  
30 Active, 13 Inactive



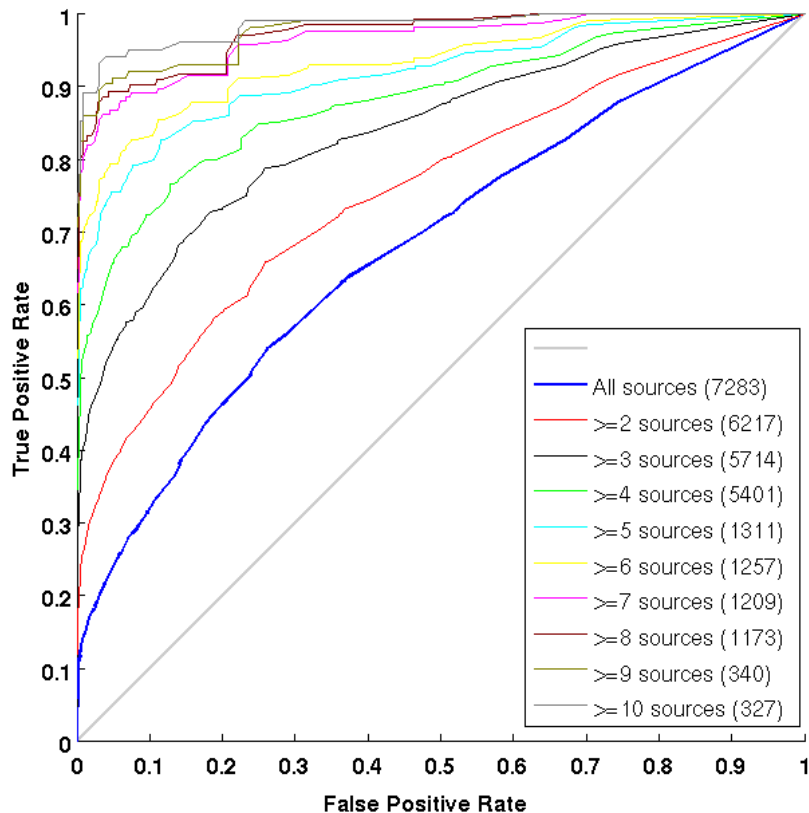
# Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic





- 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

# CERAPP Consensus evaluation



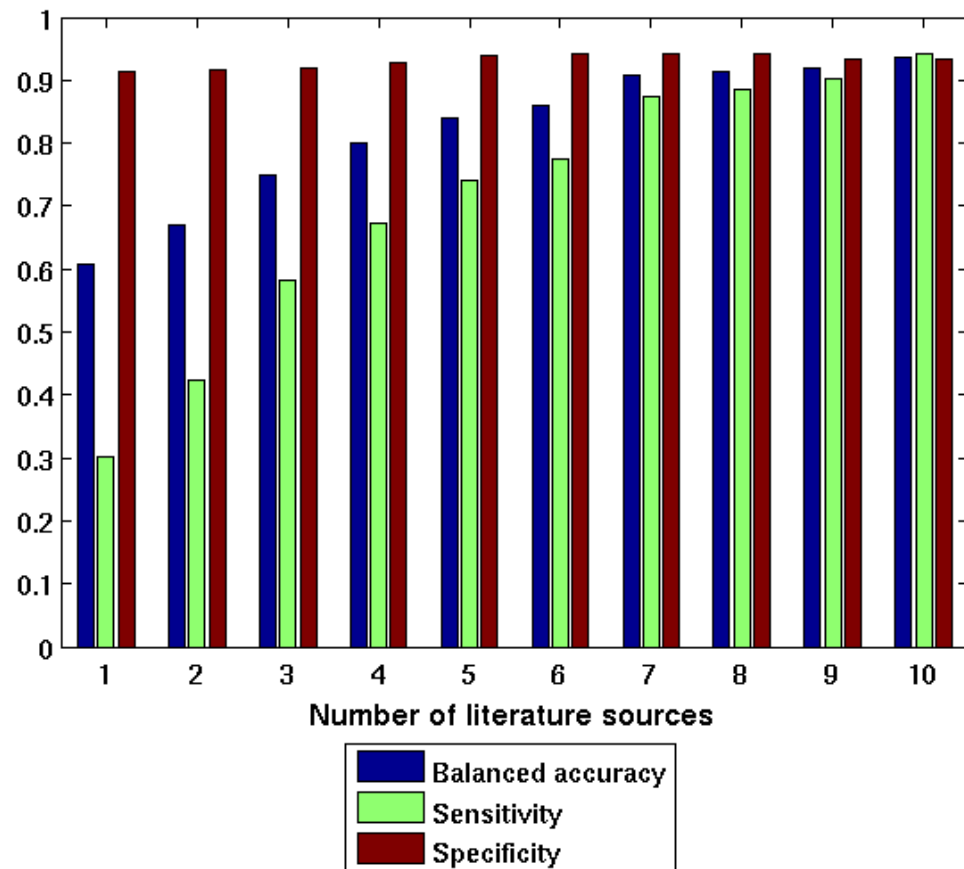
Total Database

Binders: 3961

Agonists: 2494

Antagonists: 2793

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



# 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**

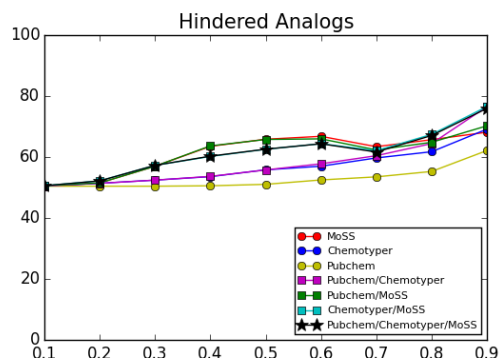
**5-10% predicted to be ER-active**

**Prioritize for further testing**

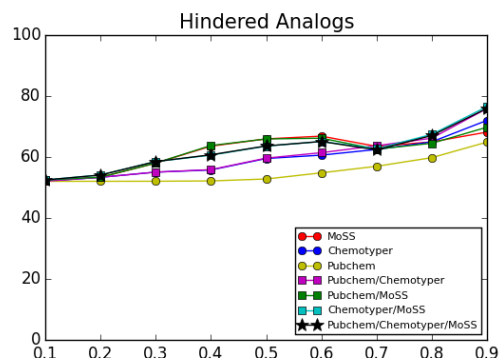
# ER Phenol Read-Across Model

Accuracy increases as

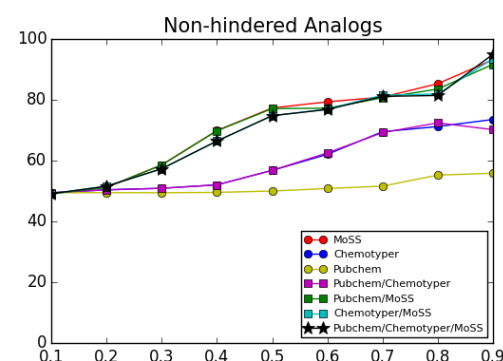
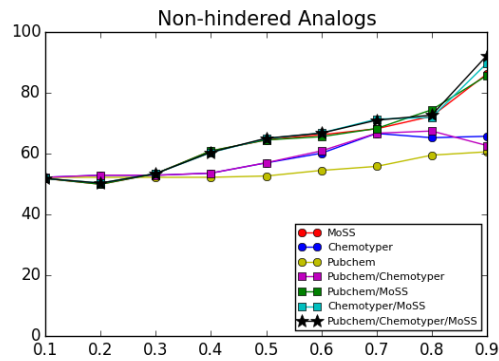
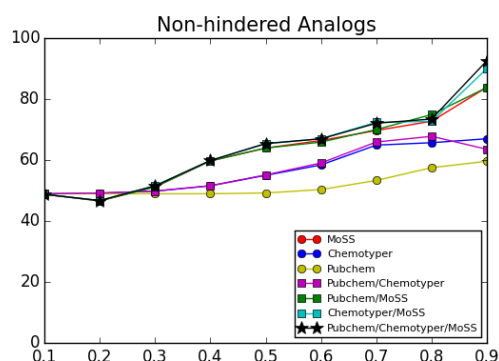
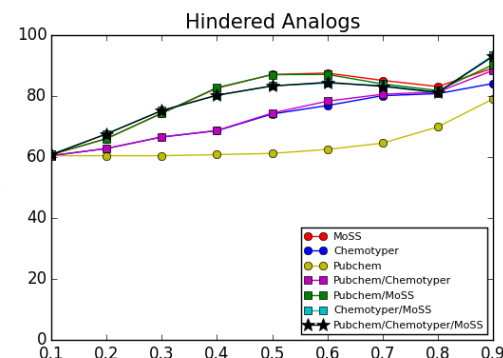
1. Better data is used in the evaluation
2. Neighbors are closer (structure and physchem)



Filtering 1 (Log  $P_{\text{kow}}$  & MV)



Filtering 2 (No. of Literature Sources  $\geq 3$ )





## 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.”

- 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>

The screenshot displays the EDSP21 Dashboard interface. On the left, a sidebar shows a list of chemicals with 'Bisphenol A' selected. The main panel is titled 'EDSP21 Dashboard' and 'Endocrine Disruption Screening Program for the 21st Century'. It features several tabs: 'Chemical Selection', 'Chemical Summary', 'Public Information', 'Bioactivity Summary', 'Bioactivity', 'High-Throughput Exposure', 'Assay Definitions', and 'Download'. The 'Chemical Summary' tab is active, showing 'Chemical Structure and Data' for Bisphenol A. The chemical structure is shown as two phenol rings connected by a central carbon atom with two methyl groups. Below the structure, various properties are listed: DISTOX GUID (20192), CASRN (80-05-7), CASRN Type (Single Compound), Name (Bisphenol A), SMILES (CC(C)(C1=CC=C(C=C1)C=C2C=CC(=C2)O)C3=CC=C(C=C3)O), InChI, InChI Key, Molecular Weight (228.29), Chemical Formula (C15H16O2), Cytotoxicity Limit (uM) (3.63954074351077), Chemical Type (Organic), Chiral/Stereo (None), dR/Stereo (None), Organic Form (Parent), and upac. At the bottom, a 'PhysChem Properties' table lists various properties and their sources.

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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