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

Richard Judson
U.S. EPA, National Center for Computational Toxicology
Office of Research and Development

ECHA Read-Across Workshop

19-20 April 2016, Helsinki

20 minutes

The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

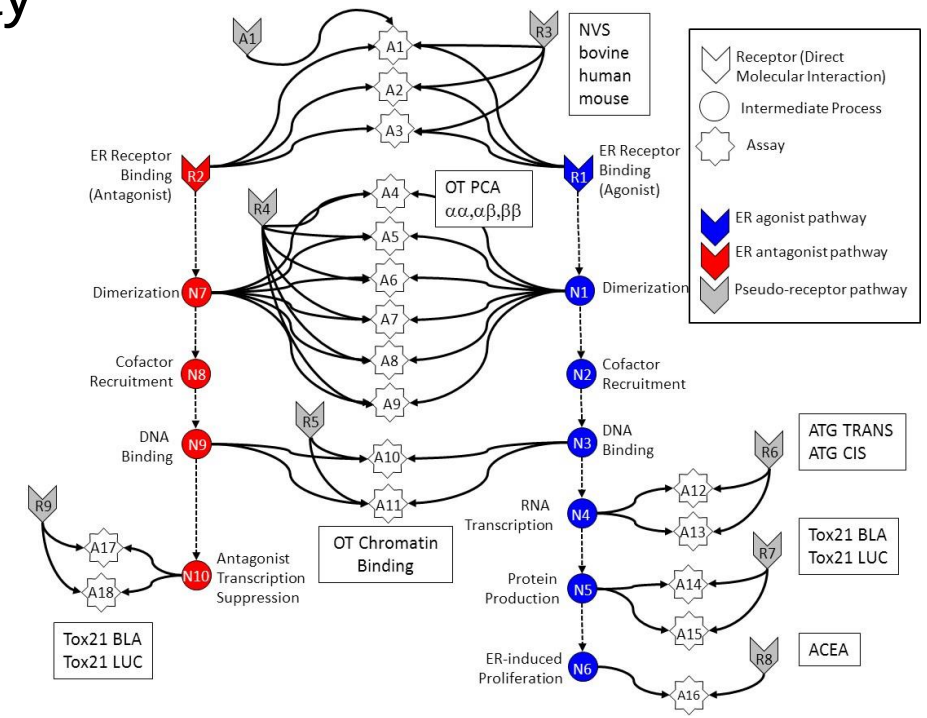
Major Points

- 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

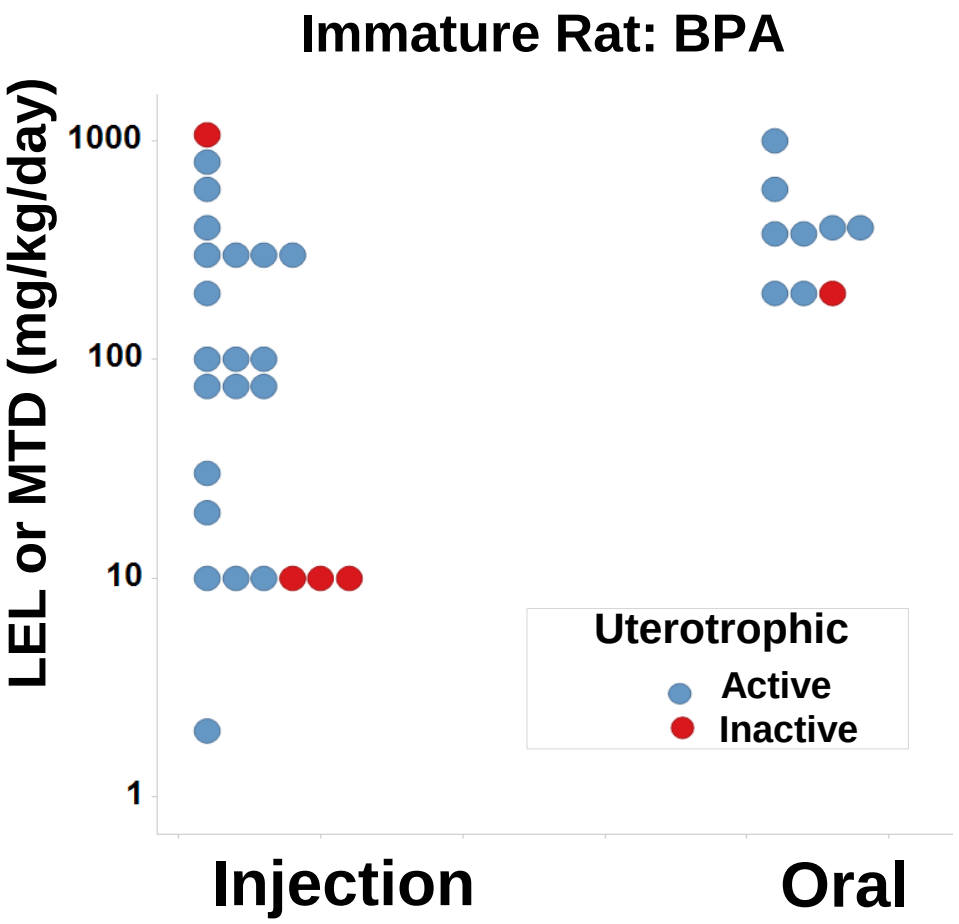
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



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)

In vivo guideline study uncertainty

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



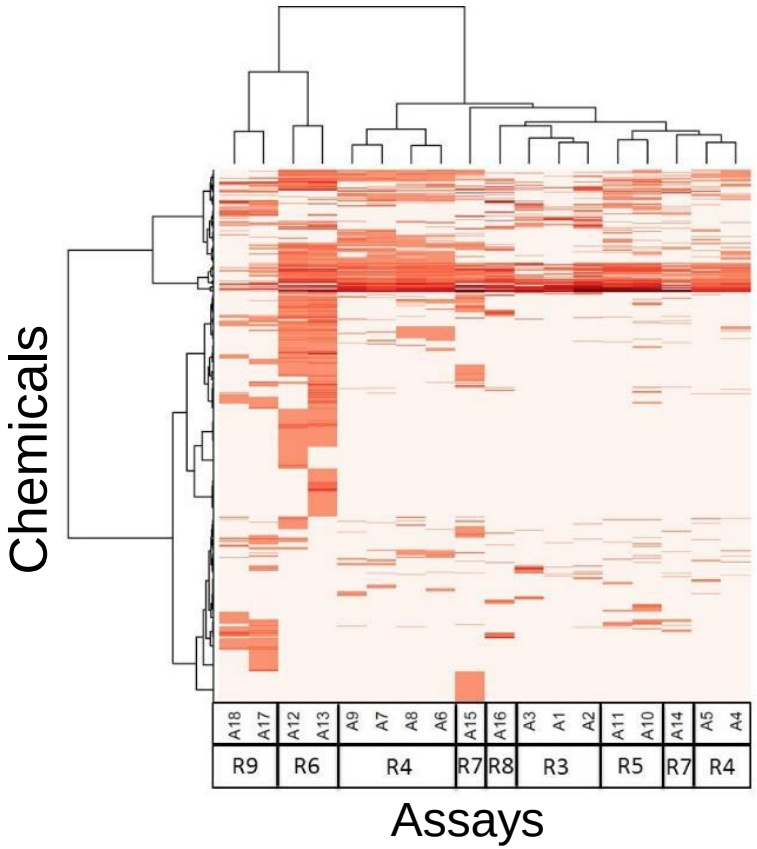
Kleinstreuer et al. EHP 2015

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

In vitro assays also have false positives and negatives

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

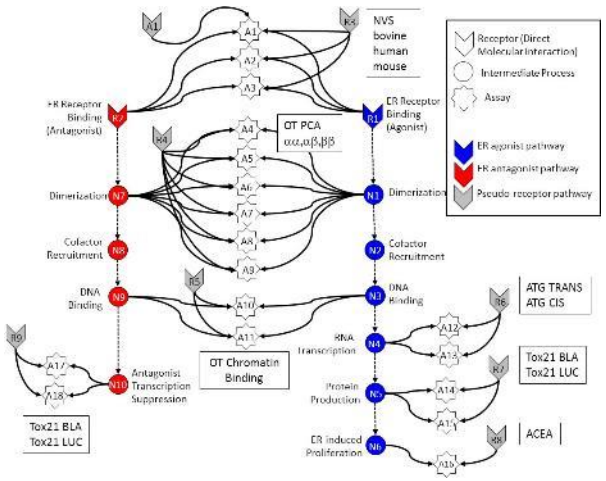


Much of this “noise” is reproducible

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

EDSP chemical universe is structurally diverse

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



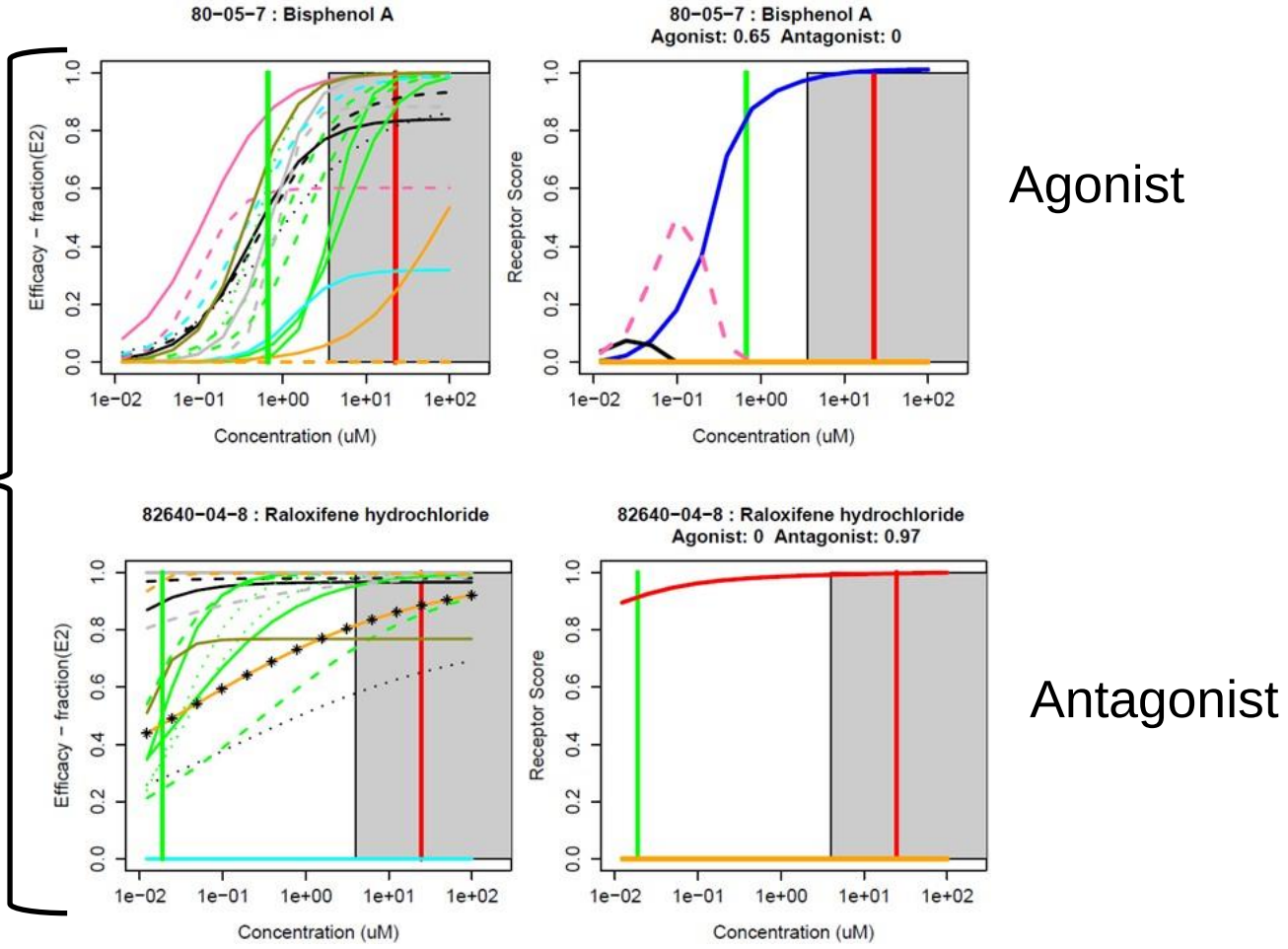
Judson et al: ToxSci (2015)

Assay-to-assay variation

Assay Data | Integrated Model

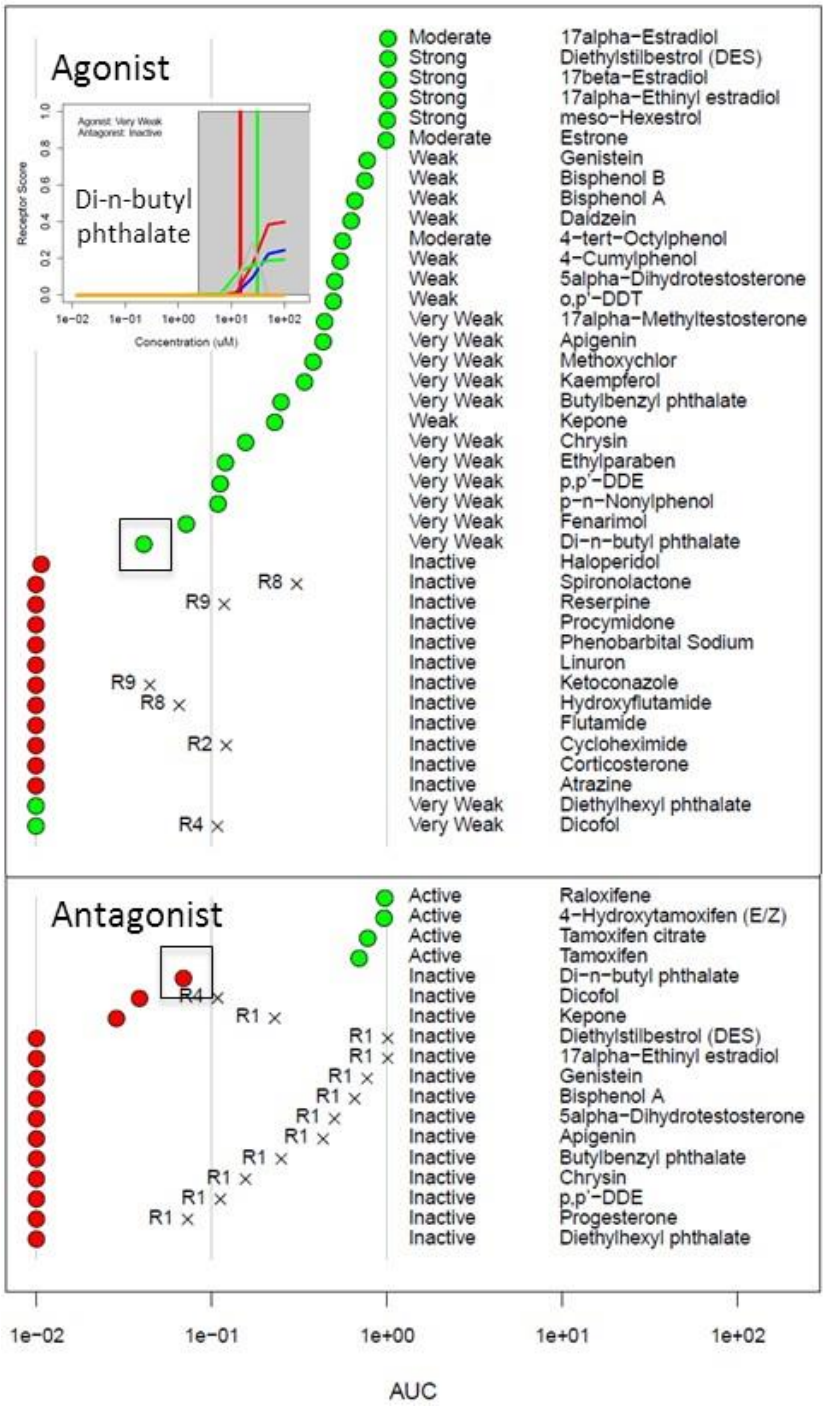
All appropriate assays are active but efficacy and potency vary

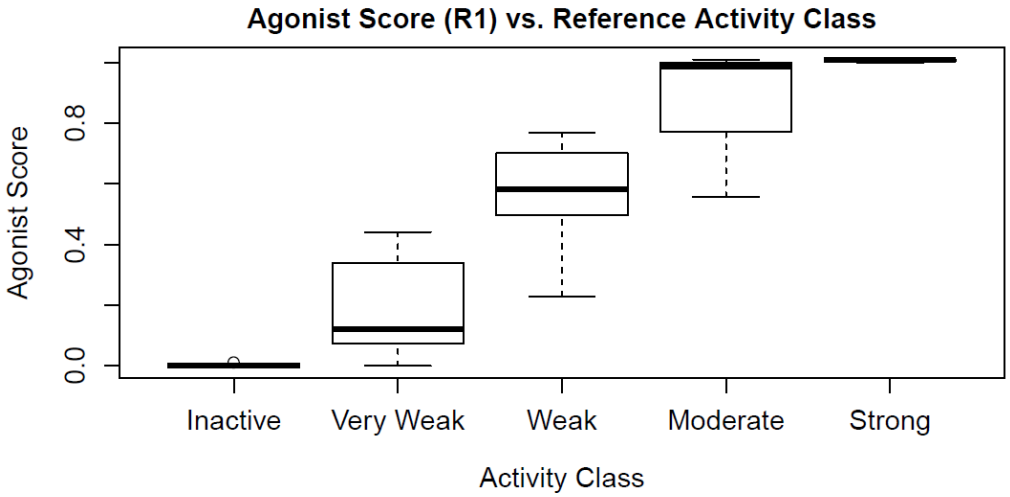
“Noise” or real variation in biology between cell types?



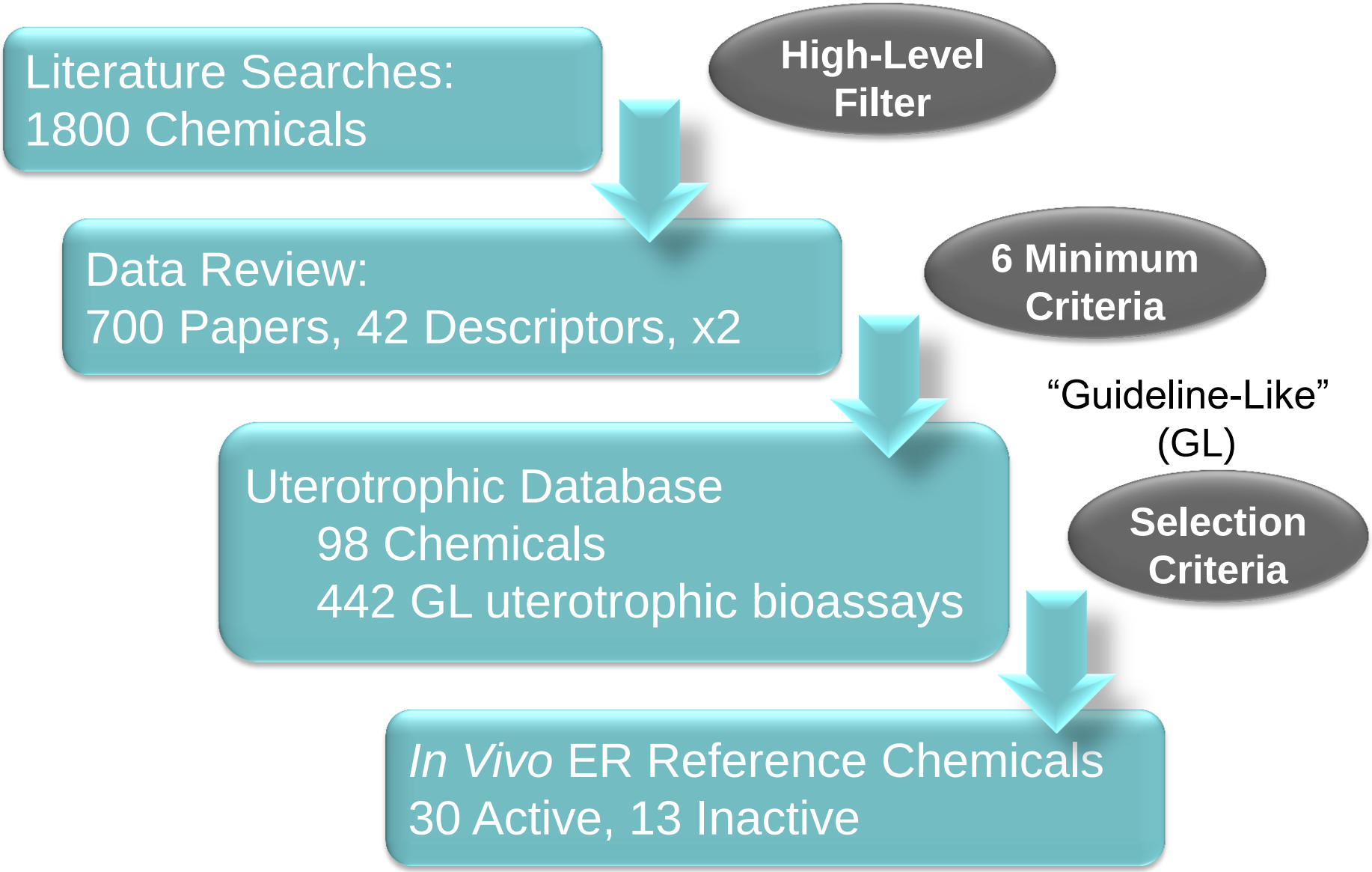
Judson et al: ToxSci (2015)

In Vitro Reference Chemical Performance





Identifying Uterotrophic Reference Chemicals from the Literature

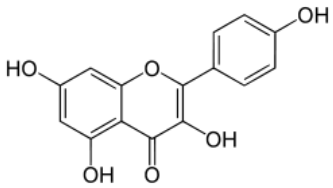


Kleinstreuer et al: "A Curated Database of Rodent Uterotrophic Bioactivity" (submitted)

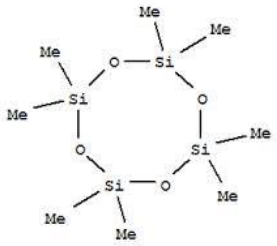
Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic

Restrict to chemicals with consistent results from the literature

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



Kaempferol



D4

Uterotrophic
Inactive
Active

ER Agonist AUC

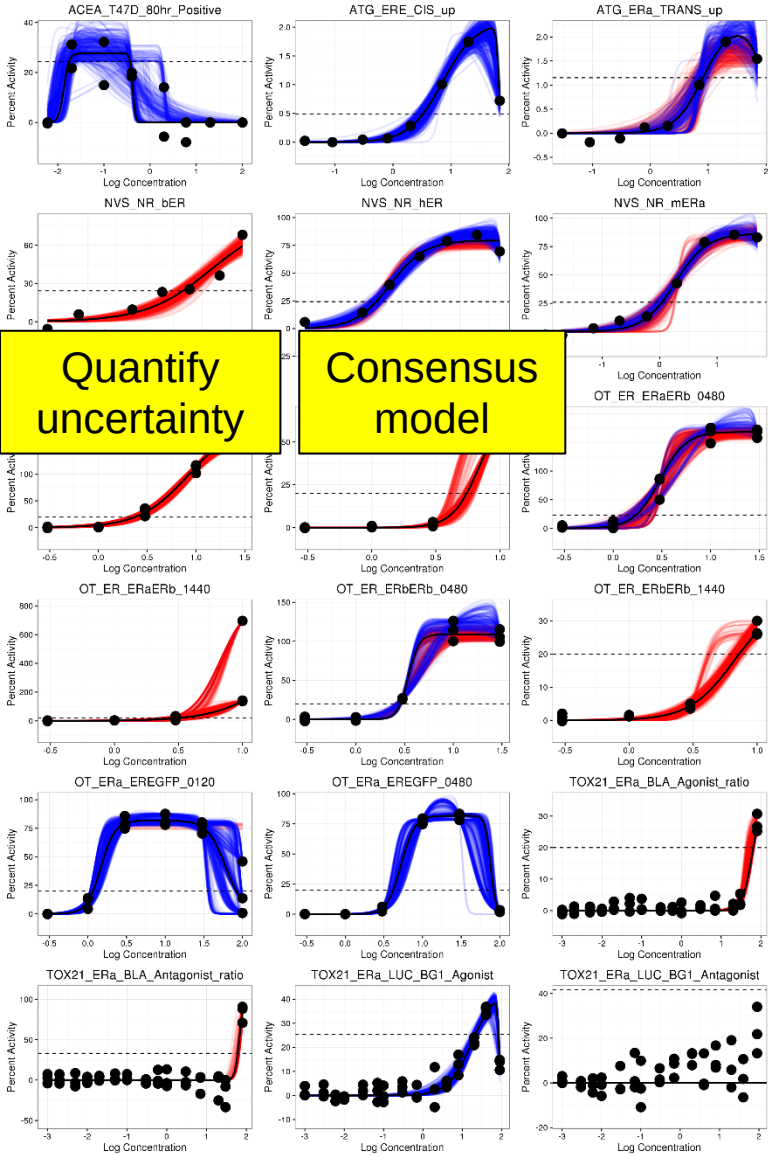
0.8
0.6
0.4
0.2
0

0
20
40
60
80

Rank Order (ER Agonist AUC)

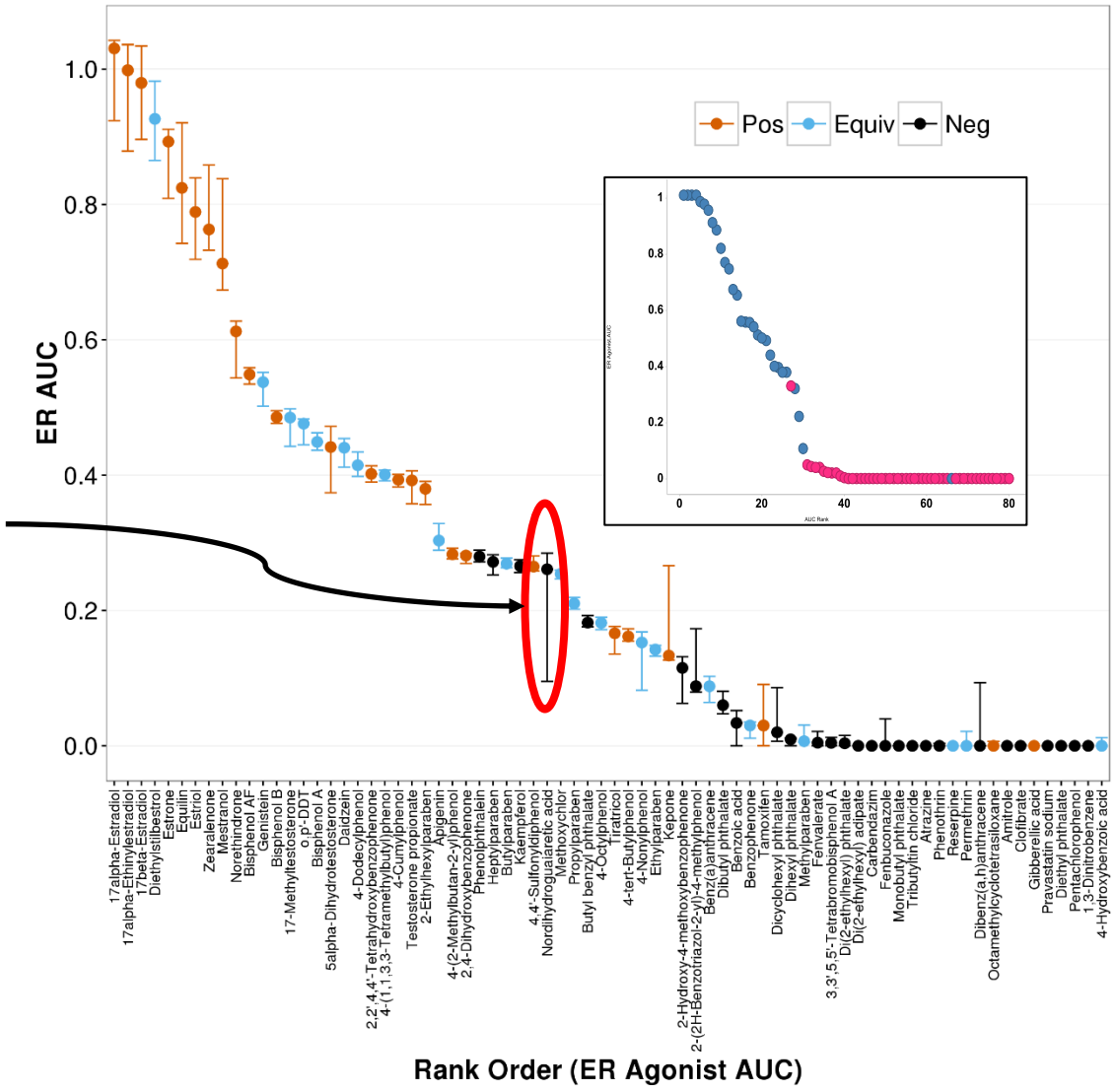
Browne et al. ES&T (2015)

Explicitly Add Uncertainty to *In Vitro* Assay Data



Quantify uncertainty

Consensus model



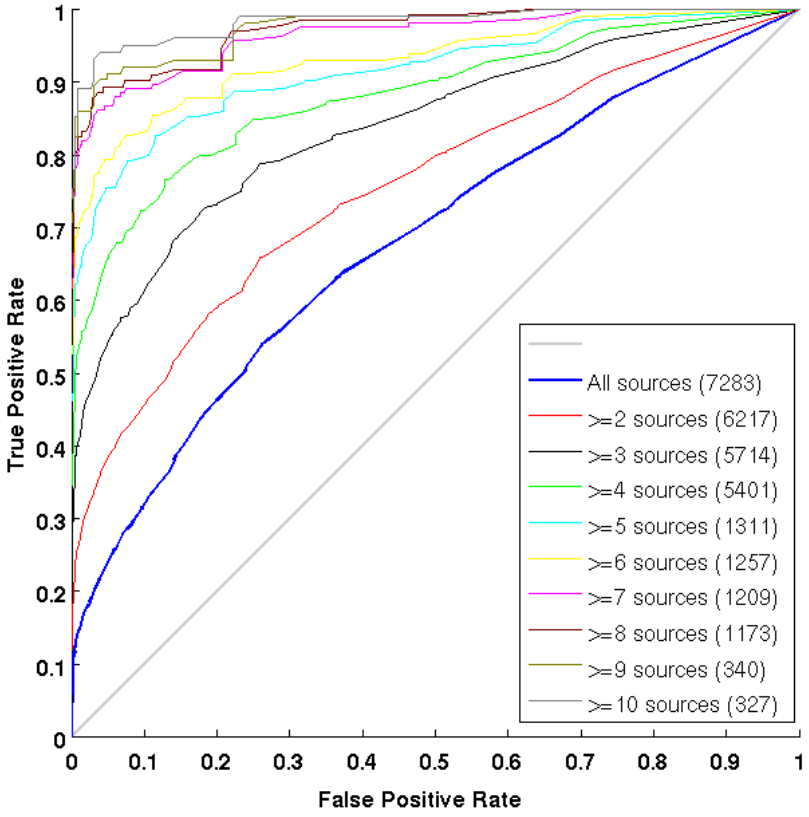
Watt et al. (in prep)

CERAPP: using QSAR for further prioritization

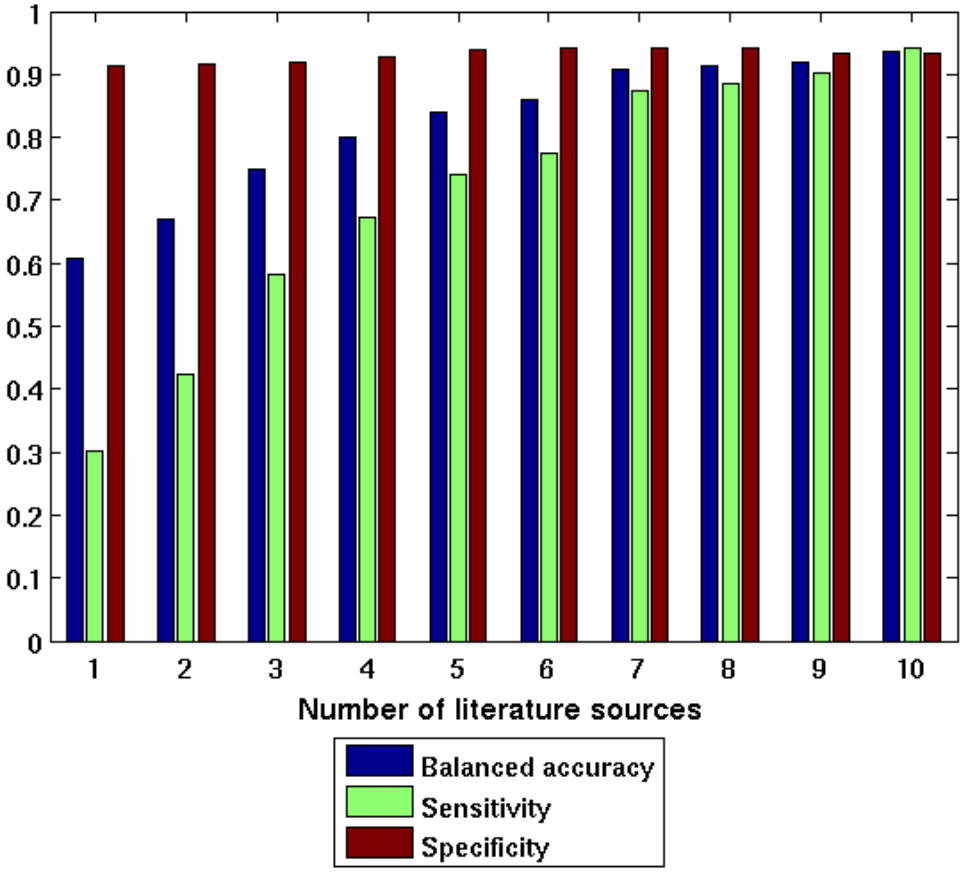
- Collaborative Estrogen Receptor Activity Prediction Project
- Goals:
  - Use ToxCast ER score (or other data) to build many QSAR models
  - Use consensus of models to prioritize chemicals for further testing
- Assumptions
  - ToxCast chemicals cover enough of chemical space to be a good "global" training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

~32K unique structures

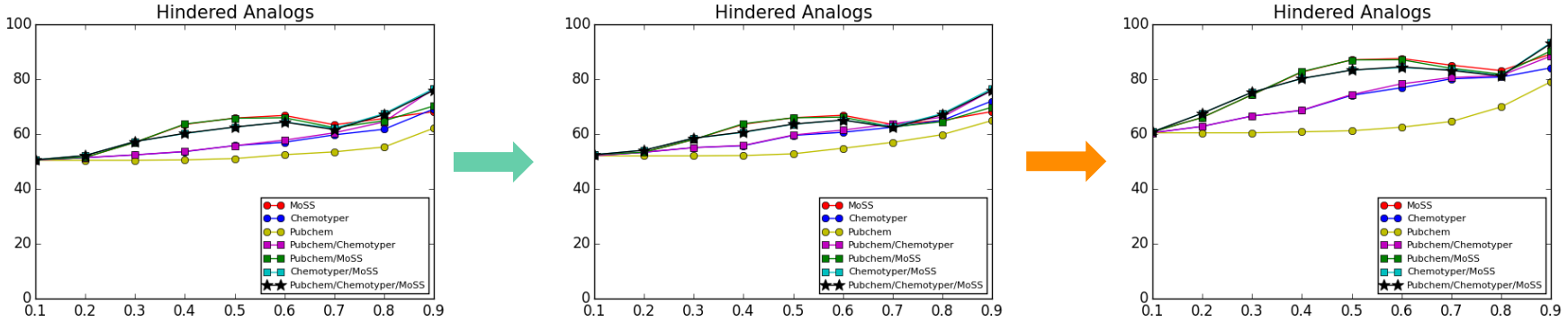
5-10% predicted to be ER-active

Prioritize for further testing

ER Phenol Read-Across Model

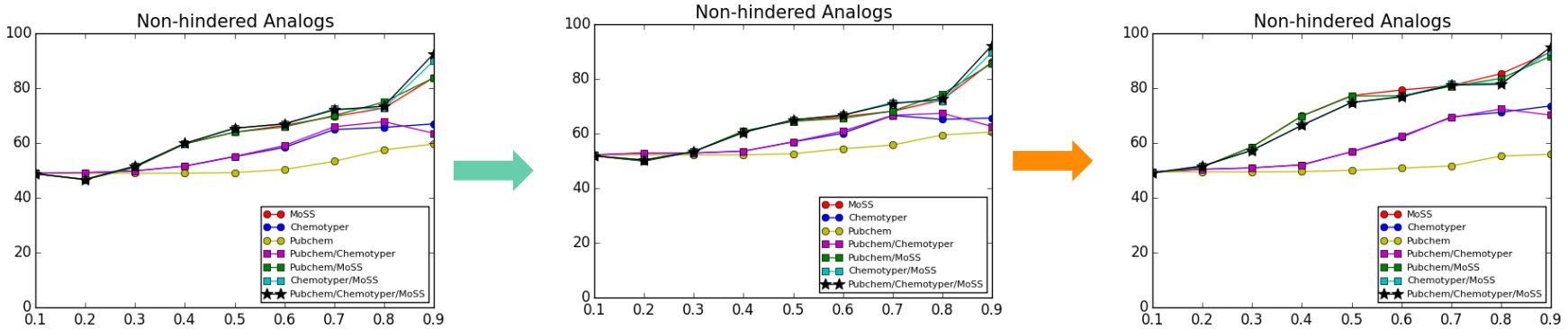
Accuracy increases as

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



Filtering 1 (Log P_{kow} & MV)

Filtering 2 (No. of Literature Sources >= 3)



Pradeep et al. (in prep)

Moving Towards Regulatory Acceptance From FIFRA SAP, December 2014

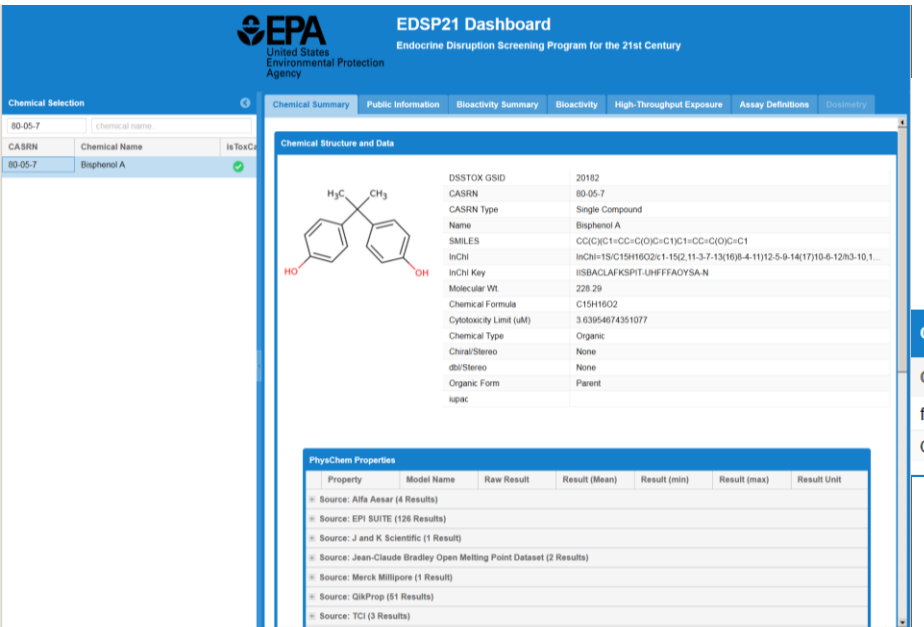
- Can the ER Model be used for prioritization?
  - "… the ER AUC appears to be an **appropriate tool for chemical prioritization** for … the EDSP universe compounds."

- 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."**

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, ...)

Acknowledgements

Kamel Mansouri
Nicole Kleinstreuer
Eric Watt
Prachi Pradeep
Patience Browne

NCCT Staff Scientists
Rusty Thomas
Kevin Crofton
Keith Houck
Ann Richard
Richard Judson
Tom Knudsen
Matt Martin
Grace Patlewicz
Woody Setzer
John Wambaugh
Tony Williams
Steve Simmons
Chris Grulke
Jim Rabinowitz

NCCT Postdocs
Todor Antonijevic
Audrey Bone
Kristin Connors
Danica DeGroot
Jeremy Fitzpatrick
Jason Harris
Dustin Kapraun
Agnes Karmaus
Max Leung
Kamel Mansouri
LyLy Pham
Prachi Pradeep
Caroline Ring
Eric Watt

NCCT
Nancy Baker
Jeff Edwards
Dayne Filer
Parth Kothiya
Doris Smith
Jamey Vail
Sean Watford
Indira Thillainadarajah

NIH/NCATS
Menghang Xia
Ruili Huang
Anton Simeonov

NTP
Warren Casey
Nicole Kleinstreuer
Mike Devito
Dan Zang