Use of a Defined Approach for Identifying Estrogen Receptor Active Chemicals

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The views of this presentation are those of the authors and do not necessarily reflect the views of the US Environmental Protection Agency.

Endocrine Disrupting Chemicals (EDCs)

- Endocrine Disrupting Chemicals (EDCs)
  - a diverse set of substances that have the potential to interfere with normal endocrine function (e.g., estrogen receptor activity).
  - exposure may lead to adverse outcomes (e.g., impaired reproduction)
  - evaluated by regulatory agencies in many countries using internationally harmonised tools (e.g., IATA)

Integrated Approach to Testing and Assessment (IATA)

- Integrated Approach to Testing and Assessment (IATA)
  - a framework for hazard identification, hazard characterisation and/or safety assessment of a chemical or group of chemicals
  - based on multiple information sources
  - integrates and weights all relevant existing evidence and guides the targeted generation of new data where required
  - informs regulatory decision-making regarding potential hazard and/or risk
  - may include Defined Approaches(DA)

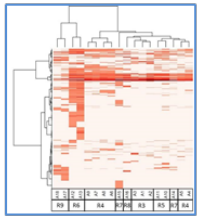
The ER Pathway Model Defined Approach

- Purpose:
  - Use an integrated battery of *in vitro* high-throughput screening assays (4 – 18 assays) and computational model of ER pathway activity as a case study in the development, performance-based evaluation, and regulatory application of a defined approach for endocrine disruption.

Overall Approach

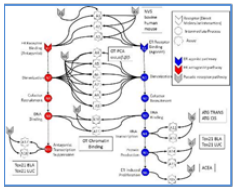
Internal

External



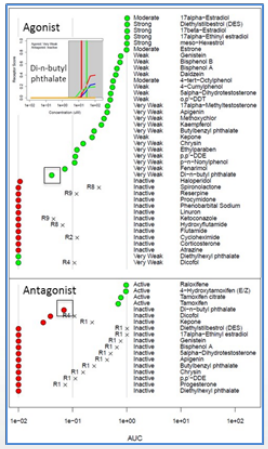
ER High-Throughput Screening Data

In vitro reference chemicals

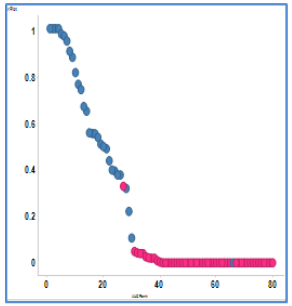


ER Pathway Computational Model

In vivo reference chemicals



Model Performance Evaluation

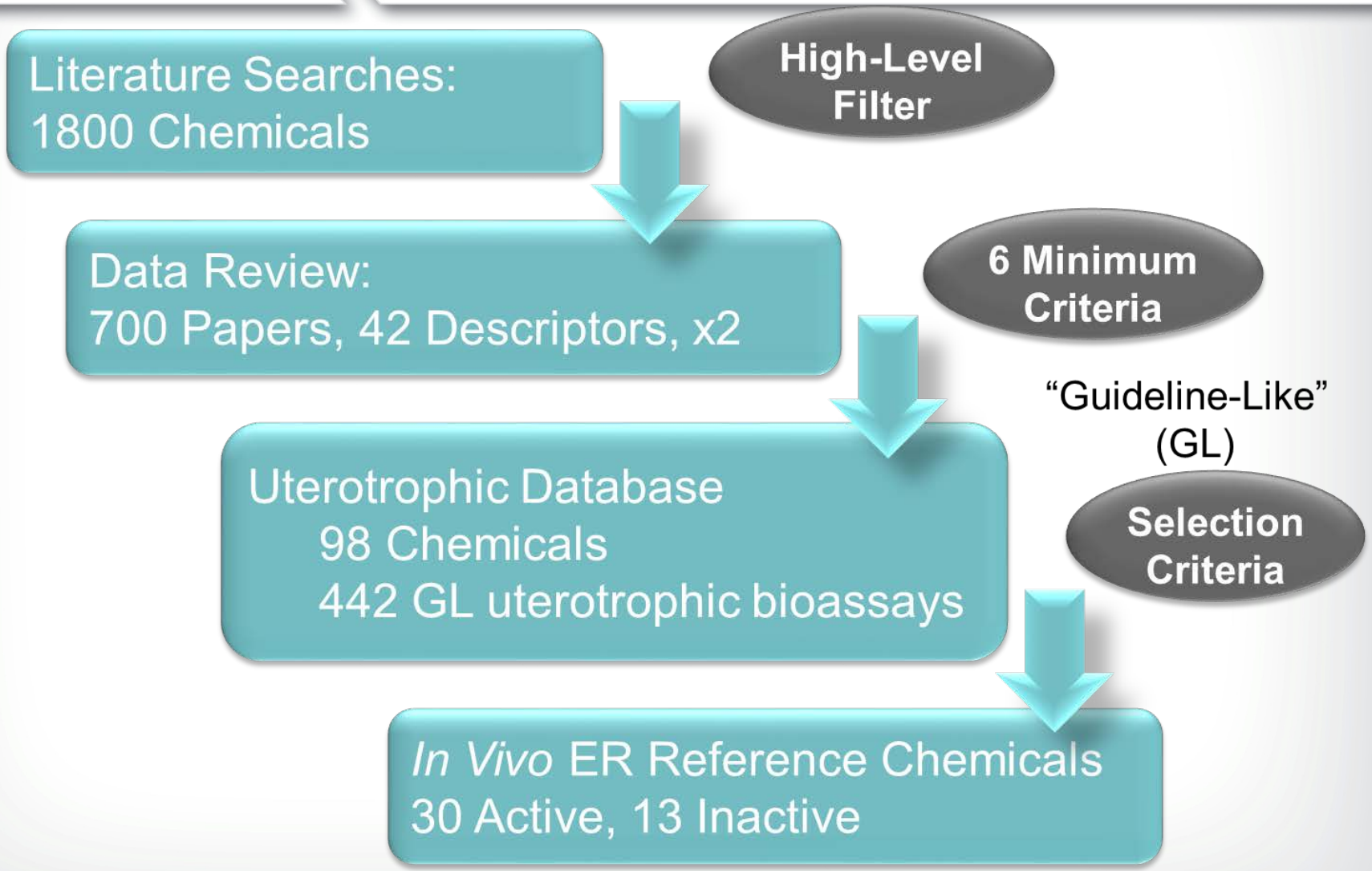


Validated Model for Chemical Screening

Curation of Reference Chemicals

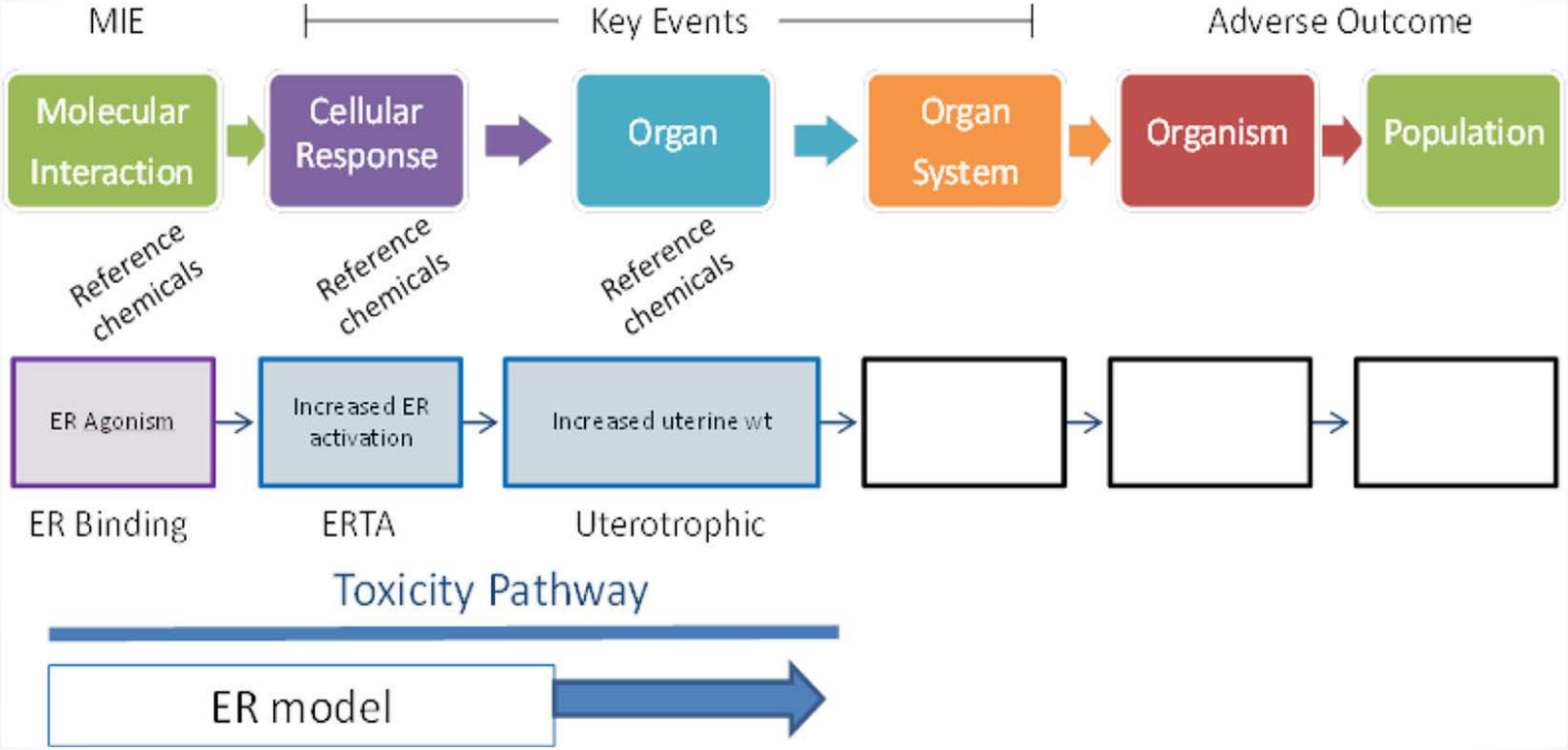
- **In Vitro Reference Chemicals**
  - Identified by ICCVAM and OECD using multiple validated low throughput in vitro ER assays
  - Forty chemicals total (28 agonists and 12 inactive)
- **In Vivo Reference Chemicals**
  - Identified by NICEATM from scientific literature search for rodent uterotrophic data on 1800 ToxCast chemicals
  - Data extracted and data quality reviewed based on minimum guideline-like study criteria
  - Forty-three chemicals total (30 active, 13 inactive)

Curation of In Vivo Reference Chemicals



Browne et al. "Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model" (ES&T 2015)
Kleinstreuer et al: "A Curated Database of Rodent Uterotrophic Bioactivity" (EHP 2016)

ER Pathway


MIE
Key Events
Adverse Outcome
Molecular Interaction
Cellular Response
Organ
Organ System
Organism
Population
Reference chemicals
Reference chemicals
Reference chemicals
ER Agonism
Increased ER activation
Increased uterine wt
ER Binding
ERTA
Uterotrophic
Toxicity Pathway
ER model

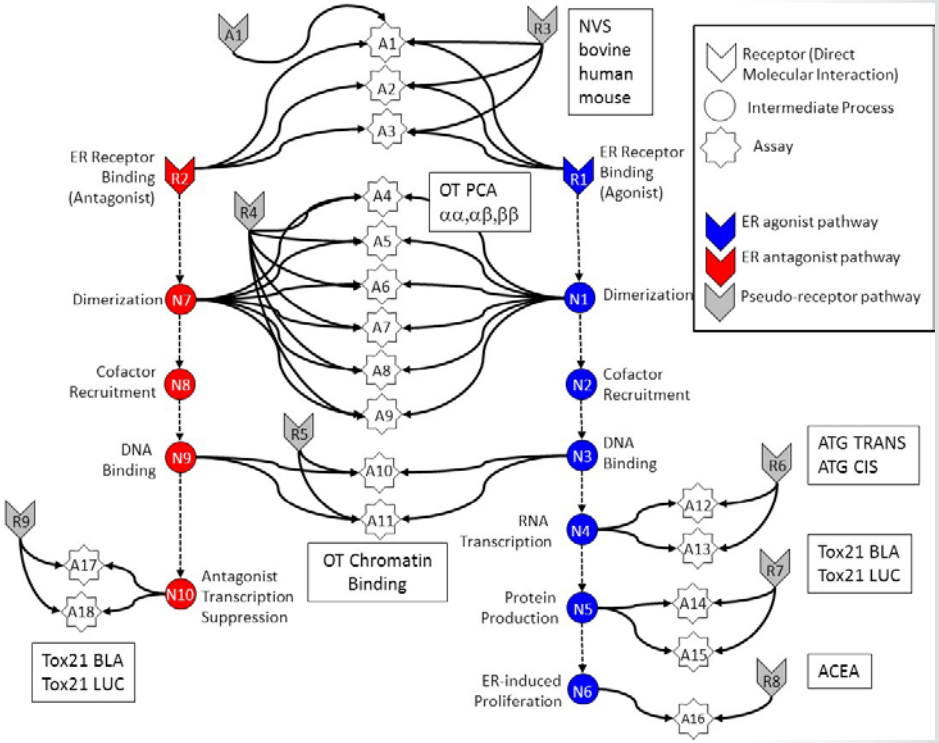
In Vitro Assays

Model

assay ID	assay	biological process	detection	organis	tissue	cell line
A1	NVS_NR_bER	receptor binding	radioligand	bovine	uterus	NA
A2	NVS_NR_hER	receptor binding	radioligand	human	NA	NA
A3	NVS_NR_mERa	receptor binding	radioligand	mouse	NA	NA
A4	OT_ER_ERaERa_0480	protein	fluorescence	human	kidney	HEK293
A5	OT_ER_ERaERa_1440	protein	fluorescence	human	kidney	HEK293
A6	OT_ER_ERaERb_0480	protein	fluorescence	human	kidney	HEK293
A7	OT_ER_ERaERb_1440	protein	fluorescence	human	kidney	HEK293
A8	OT_ER_ERbERb_0480	protein	fluorescence	human	kidney	HEK293
A9	OT_ER_ERbERb_1440	protein	fluorescence	human	kidney	HEK293
A10	OT_ERa_EREGFP_0120	protein production	fluorescence	human	cervix	HeLa
A11	OT_ERa_EREGFP_0480	protein production	fluorescence	human	cervix	HeLa
A12	ATG_ERa_TRANS_up	mRNA induction	fluorescence	human	liver	HepG2
A13	ATG_ERE_CIS_up	mRNA induction	fluorescence	human	liver	HepG2
A14	Tox21_ERa_BLA_Agonist_	protein production	fluorescence	human	kidney	HEK293
A15	Tox21_ERa_LUC_BG1_Ag	protein production	bioluminescence	human	ovary	BG1
A16	ACEA_T47D_80 h_Positive	cell proliferation	electrical	human	breast	T47D
A17	Tox21_ERa_BLA_Antagoni	protein production	fluorescence	human	kidney	HEK293
A18	Tox21_ERa_LUC_BG1_An	protein production	bioluminescence	human	ovary	BG1

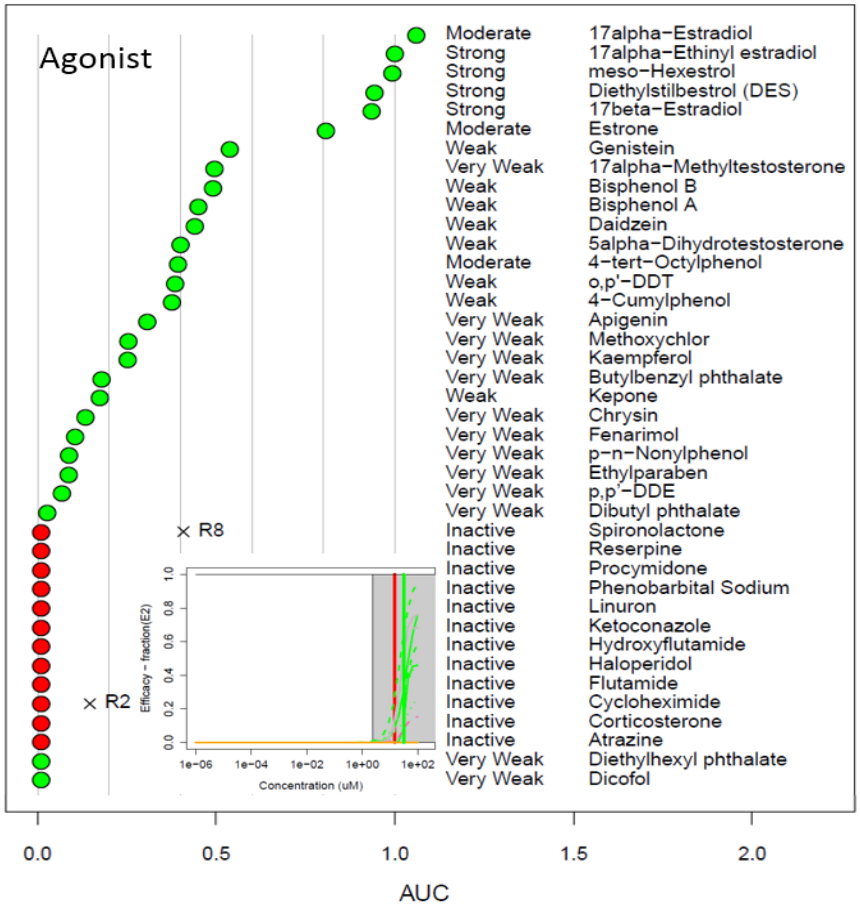
In Vitro Estrogen Receptor Model

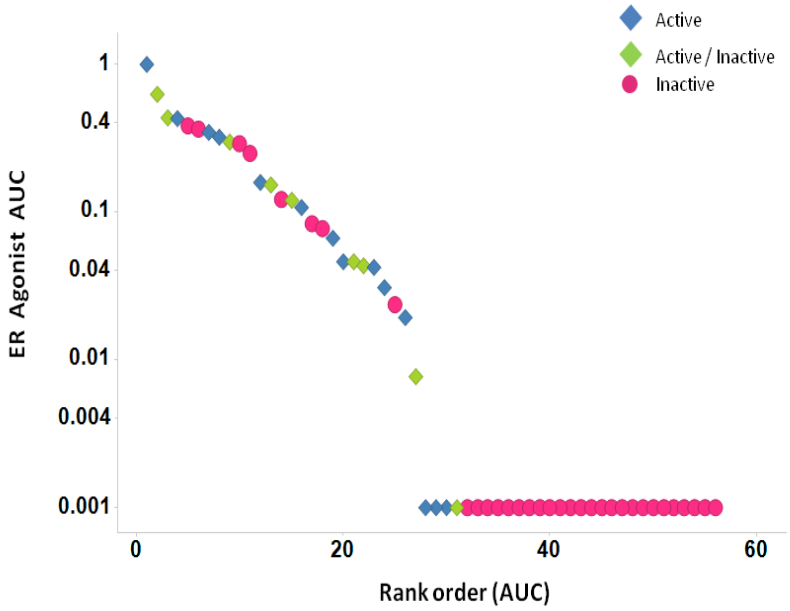
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Model creates a composite dose-response curve for each chemical to summarize results from all assays
  - Used to calculate performance metrics for chemicals with any indication of ToxCast ER agonist bioactivity (AUC > 0.1), inconclusive (0 < AUC < 0.1) or no activity (AUC = 0).



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)

Characterizing Performance of the Defined Approach





Browne *et al., ES&T.* 2015; US EPA 2014

Characterizing Performance of the Defined Approach

In Vitro Reference Chemicals*

True Positive	26 (25)
True Negative	11 (11)
False Positive	1 (0)
False Negative	2 (2)
Accuracy	**0.93 (0.95)**
Sensitivity	**0.93 (0.93)**
Specificity	**0.92 (1.0)**

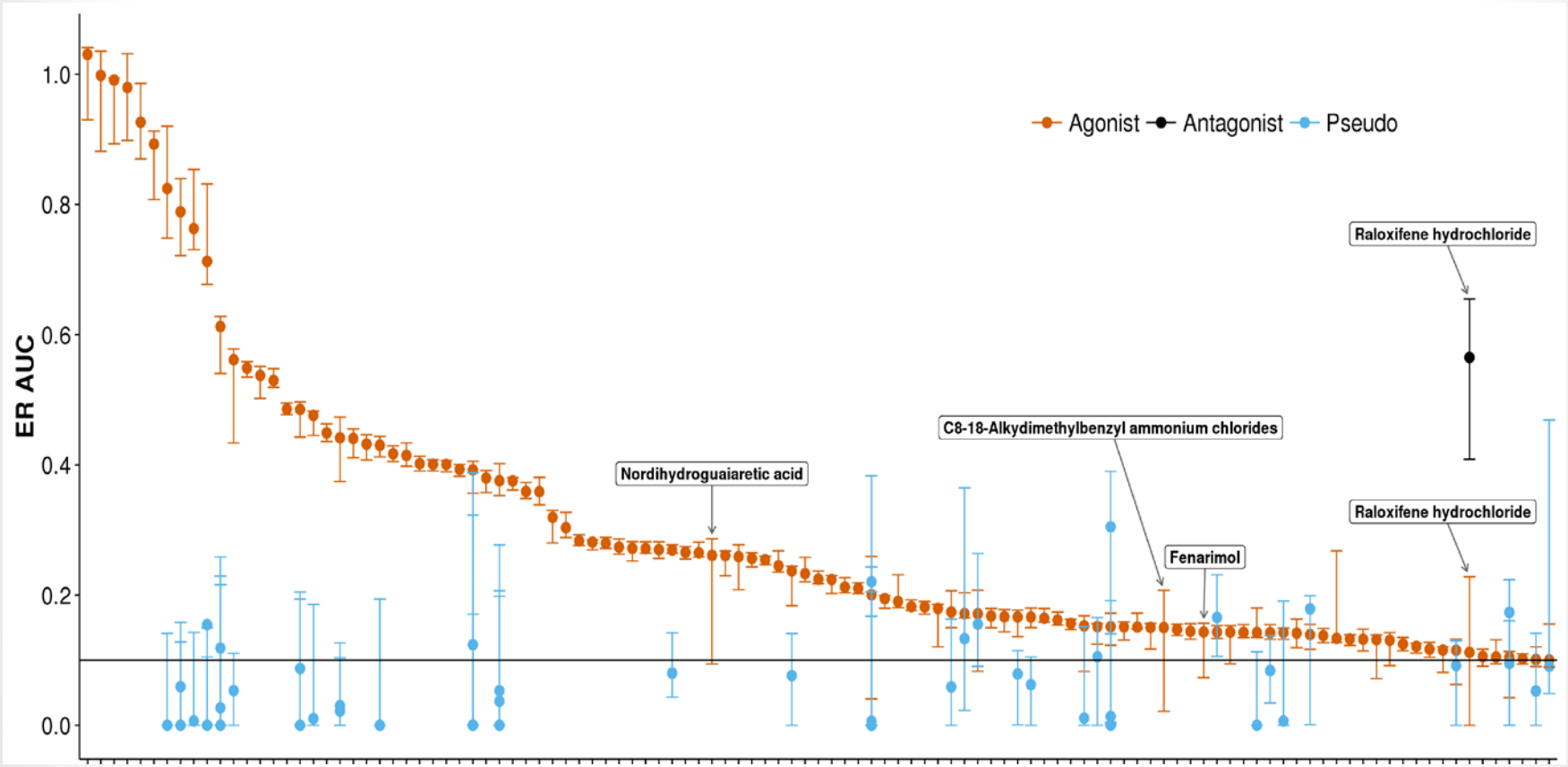
In Vivo Reference Chemicals*

True Positive	29 (29)
True Negative	8 (8)
False Positive	5 (1)
False Negative	1 (1)
Accuracy	**0.86 (0.95)**
Sensitivity	**0.97 (0.97)**
Specificity	**0.67 (0.89)**

*Values in parentheses exclude inconclusive chemicals

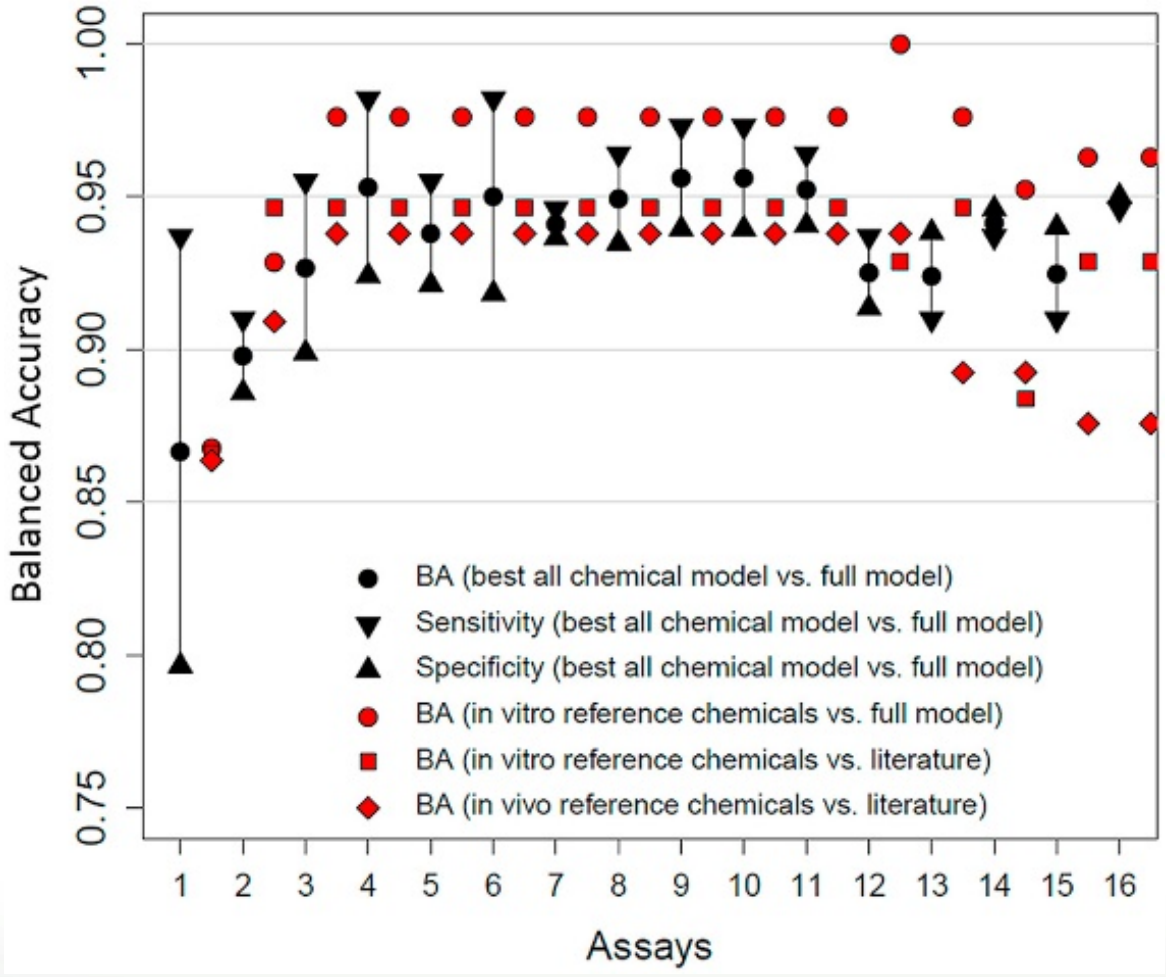
Browne *et al.*, *ES&T.* 2015

Evaluation of Uncertainty



Watt and Judson, submitted

Equivalent Performance Observed for a Subset of *In Vitro* Assays



Judson et al., Reg. Tox. Pharm. (2017)

Conclusions

- Summarized the proposed ER Pathway Model Defined Approach
- A DA can provide predictable outcomes that can either be used on their own or considered together with other sources of information in the context of an IATA.
- DA described here has been demonstrated to predict ER bioactivity of both in vitro and in vivo reference chemical with accuracy ranging from 84 – 93%.
- The results of the analysis of this DA gives scientific support for the potential use in regulatory decisions related to estrogen bioactivity.

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