Use of a Defined Approach for Identifying Estrogen Receptor Active Chemicals

Maureen R. Gwinn (gwinn.maureen@epa.gov)
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

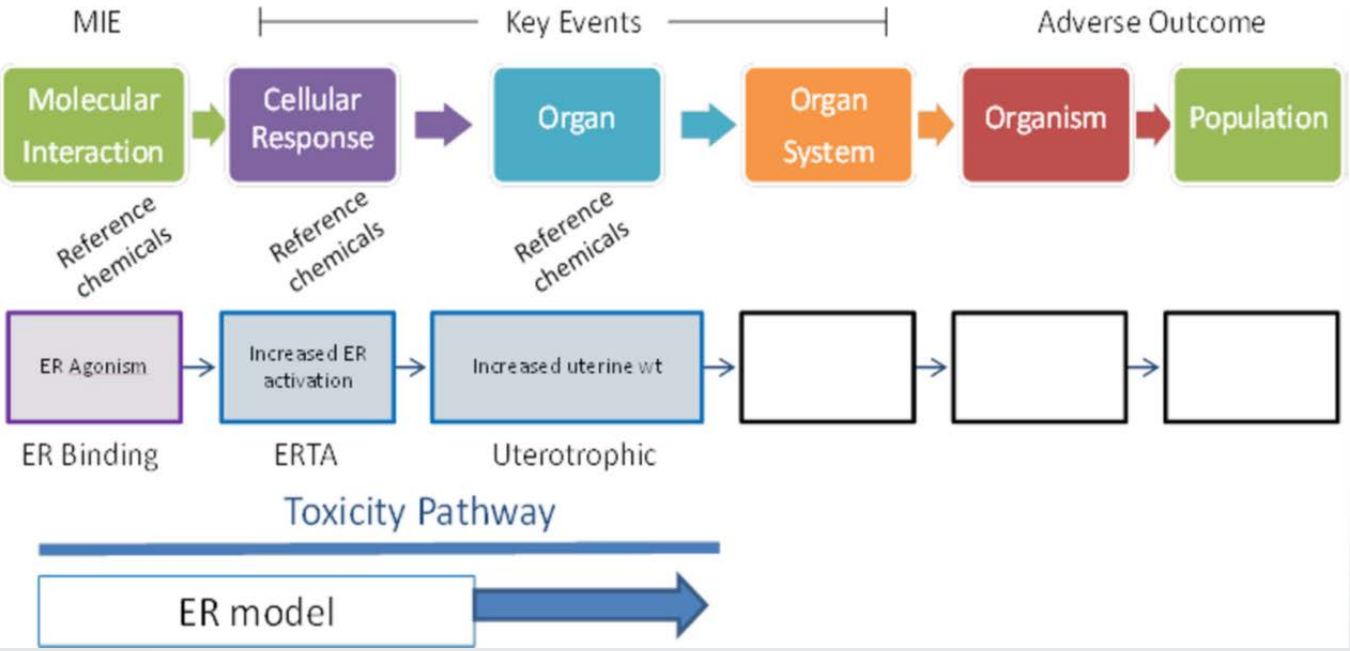
The views of this presentation are those of the author and do not necessarily reflect the views of the US Environmental Protection Agency.

Introduction

- Endocrine disrupting chemicals are a diverse set of substances that have the potential to interfere with normal endocrine function and lead to an adverse outcome.
- Regulatory agencies in many countries evaluate endocrine activity of environmental chemicals for specific regulatory endpoints.
- The defined approach (DA) presented in this document describes an integrated testing strategy (ITS) for the identification of endocrine disruption via estrogen receptor agonism by a substance.

Purpose

- A defined approach (DA) that uses a combination of *in vitro* high-throughput screening assays (as few as 4 assays) and computational model of estrogen receptor(ER) activity to serve as an alternative to low- and medium-throughput in vitro and in vivo tests.



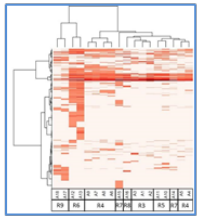
Intended Application

- The intended application of this DA is for
  - screening and priority setting of environmental chemicals based on their ER activity
  - determining if need for further evaluation of endocrine-related activity in higher tier in vivo tests (e.g., female pubertal assay, two generation reproductive toxicity study)

Overall Approach

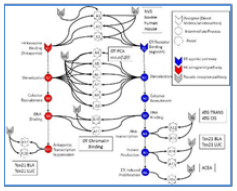
Internal

External



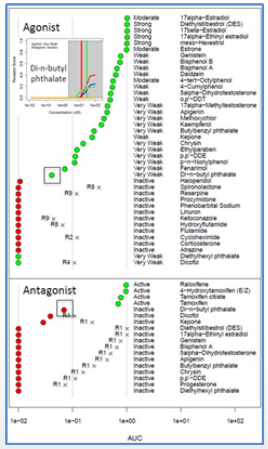
ER High-Throughput Screening Data

In vitro reference chemicals

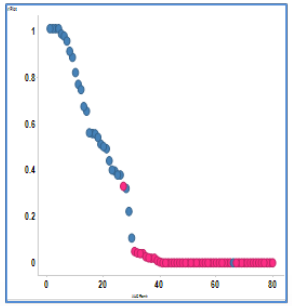


ER Pathway Computational Model

In vivo reference chemicals



Model Performance Evaluation

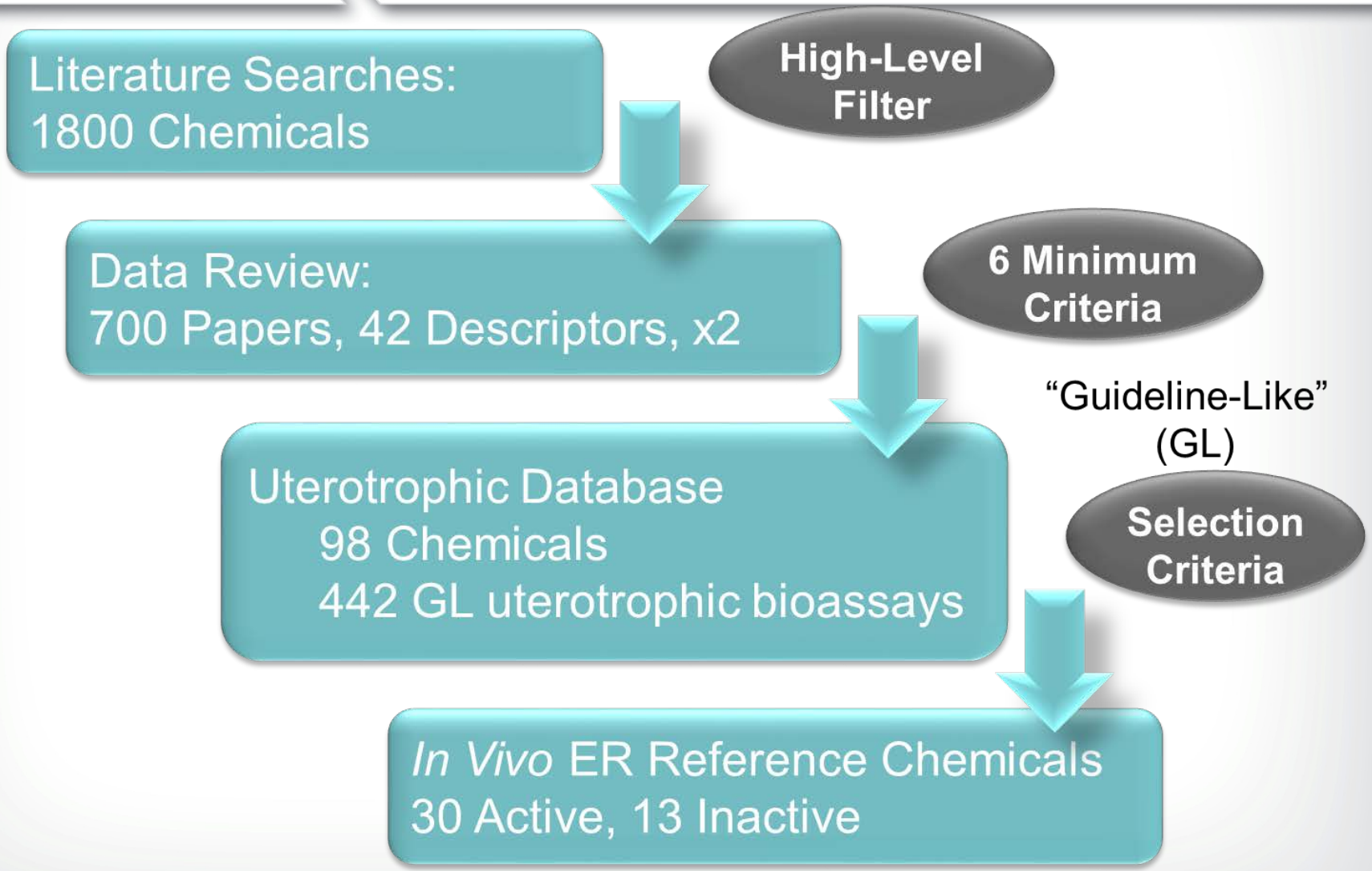


Validated Model for Chemical Screening

Data and Information Gathering: 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)

Data and Information Gathering: 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)

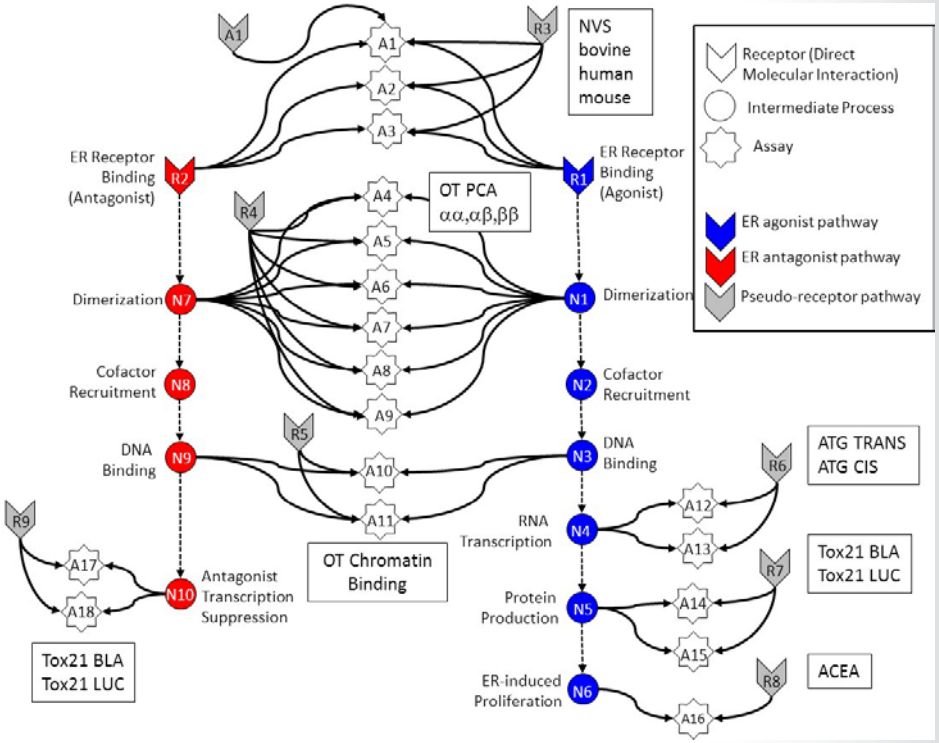
Data and Information Gathering: *In Vitro* Assays

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

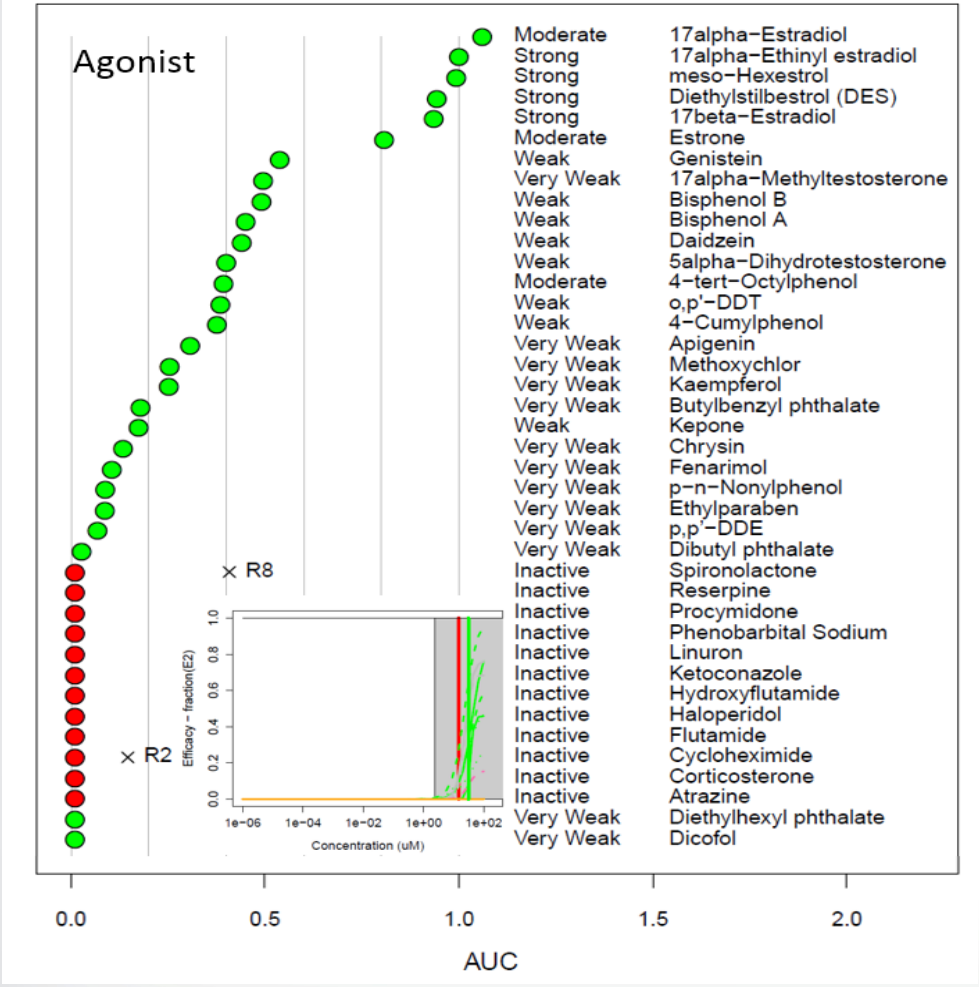
Data and Information Gathering: In Vitro Estrogen Receptor Model

- 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)

Application of DA: Characterizing Performance



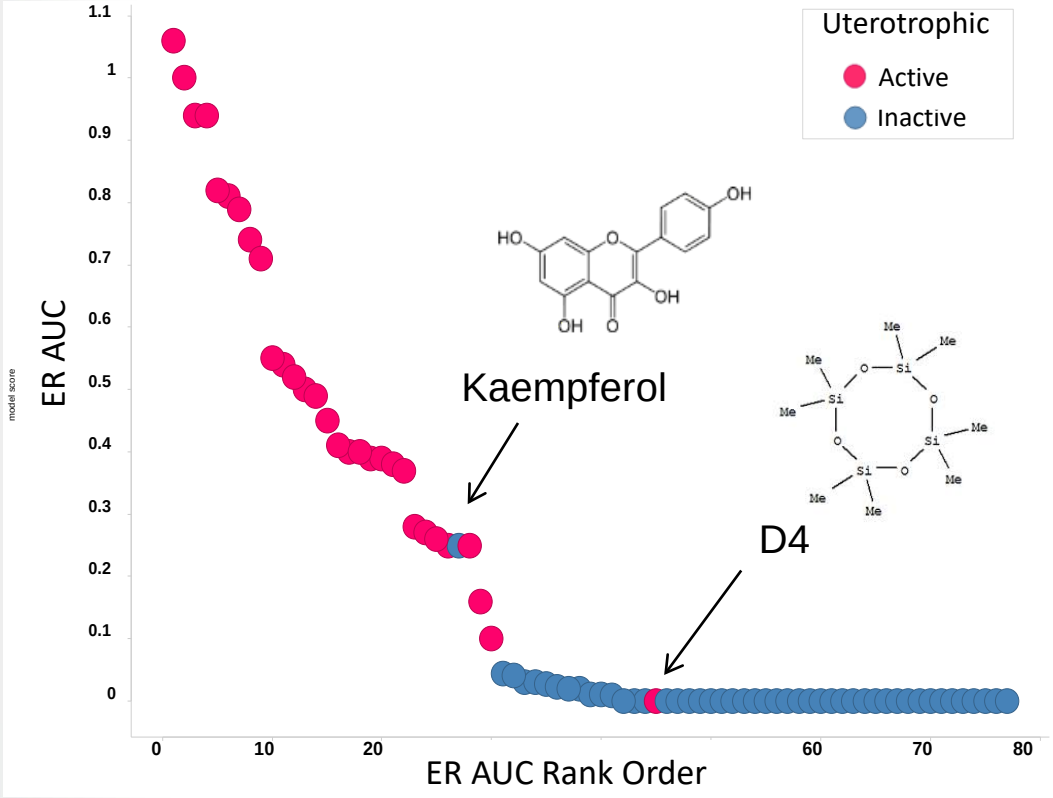
In Vitro Reference Chemicals*

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)**

*Values in parentheses exclude inconclusive chemicals

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

Application of DA: Characterizing Performance

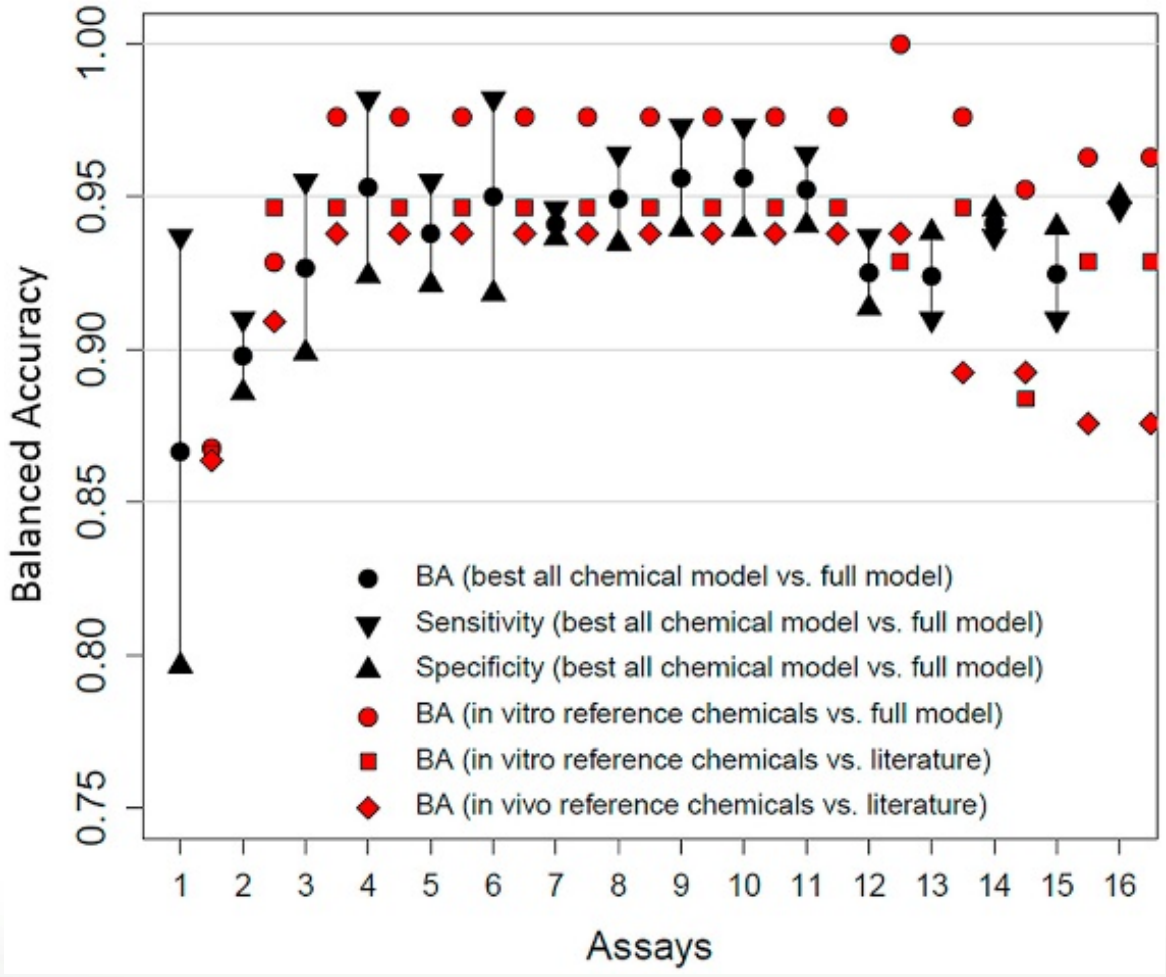


In Vivo Reference Chemicals

True Positive	29
True Negative	46
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.97**

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

Equivalent Performance Observed for Subsets of *In Vitro* Assays



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

Equivalent Performance Observed for Subsets of *In Vitro* Assays

- Results of this analysis demonstrate that one could use one of multiple subset models to accurately predict estrogenic potential of a chemical.
- Subsets of as few as 4 of the original 16 agonist assays have acceptable performance against the full model, and the in vitro and in vivo reference chemicals.
- The acceptable subsets all have assays that:
  - probe diverse points in the ER pathway
  - use diverse assay reporting technologies
  - use diverse cell types

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

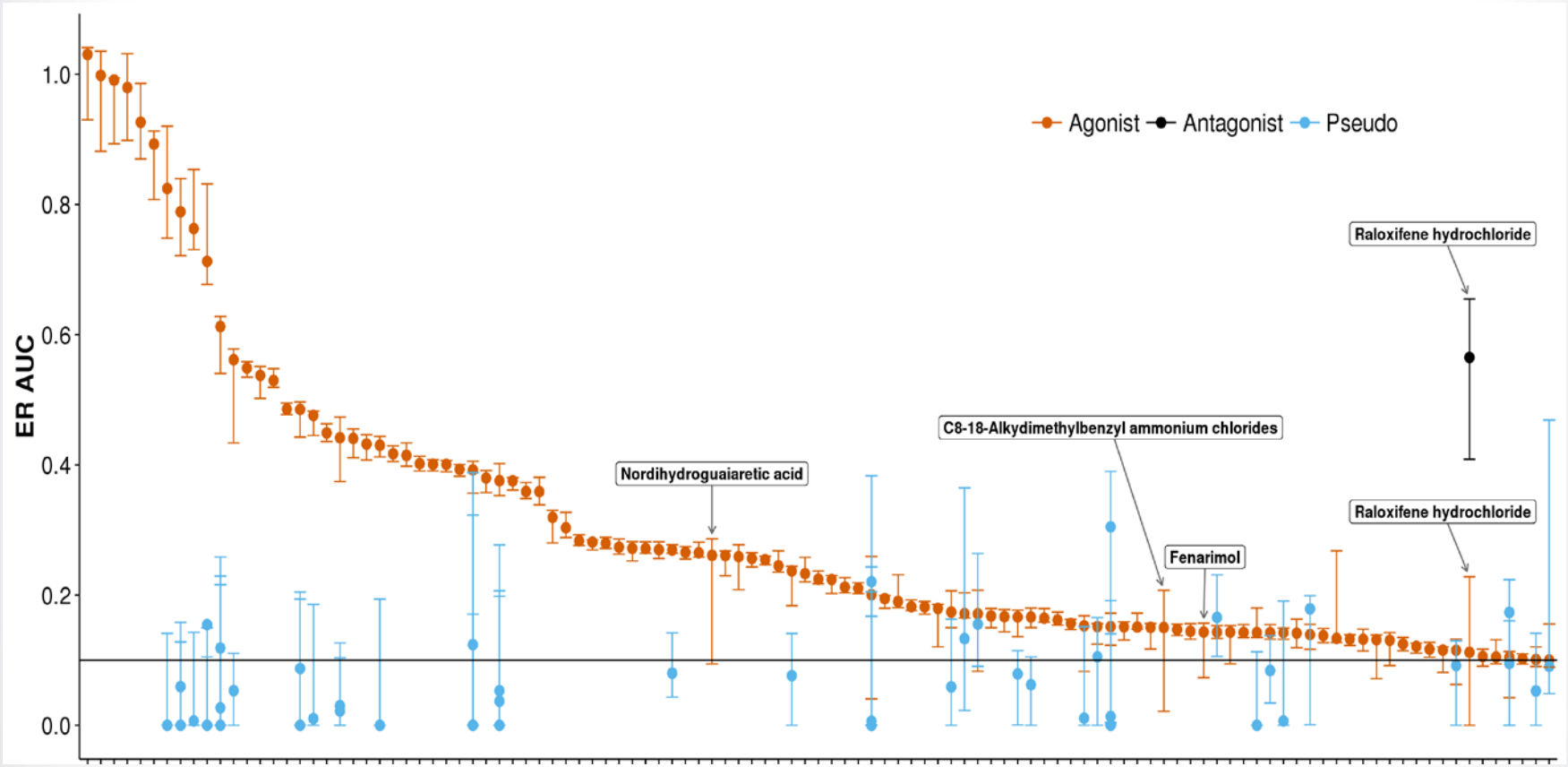
Areas of Potential Uncertainty

- ER Pathway: Pathway frameworks are built upon assumptions of a relationship between the MIE, subsequent KEs and the proposed apical effect or adverse outcome and may not consider that there are unknown pathways that may impact the decisions made about a specific substance.

- Metabolic Competence: Lack metabolic competence in in vitro HTS Assays may lead to over- or underestimation of chemical hazard.

- In Vitro HTS Assays and the Pathway Model Analysis: In the analysis of the HTS assays, there is a need to establish uncertainty bounds around potency and efficacy values.

Evaluation of Uncertainty



Watt and Judson, submitted

Summary of Proposed Case Study

- Outlines the curation of lists of reference chemicals for *in vitro* and *in vivo* ER activity and the uncertainty and variability associated with the guideline studies
- Integrates results from multiple *in vitro* assays using pathway-based ER computational model as a defined approach
- Evaluates performance of the defined approach using the curated lists of reference chemicals
- Demonstrates equivalent performance for subsets of *in vitro* assays
- Characterizes the uncertainty associated with the *in vitro* assays and computational model
- Discusses potential application to regulatory decisions

Co-Authors

- **US Environmental Protection Agency**
  - Russell S. Thomas
  - Richard Judson
- **US National Toxicology Program**
  - Nicole Kleinstreuer
  - Warren Casey