Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)

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PCRM DyNAMic Discussions Webinar Series

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Outline

- EDSP Context
- The EDSP Whitepaper overview
- The full ER and AR pathway models
- Subset models
- Plans to use the subset models to test ~500 additional chemicals

Brief History of the Endocrine Disruptor Screening Program (EDSP)

- 1996 Food Quality Protection Act passed: first call to screen chemicals for endocrine-related effects
- 1998 EDSTAC formed to develop testing strategy
- 2005 EPA published final approach to initial screening (Tier 1)
- 2007 First list of chemicals to be screened published
- 2009 Final Tier 1 battery published, List 1 was finalized, and test orders issued
- 2010 List 2 published
- 2012 EDSP Universe (the 10,000) published, pesticidal ingredients and drinking water contaminants
- 2013 Review of Tier 1 List 1 data
- 2014 Reviews of potential high-throughput alternatives to some Tier 1 assays
- 2015 ER Pathway Model published
- 2015 List 1 Tier 1 data released (52 chemicals)
- 2015 Tier 2 Guidelines finalized
- 2017 SAP on Steroidogenesis and AR pathway model
- 2023 Proposal to use NAMs as potential replacements for some Tier 1 assays published

Whitepaper Topics

- "Availability of New Approach Methodologies (NAMs) in the Endocrine Disruptor Screening Program (EDSP)"
  - The Estrogen Receptor Pathway Model (ER)
  - The Androgen Receptor Pathway Model (AR)
  - QSAR Models for ER and AR
  - Combining bioactivity and exposure in a risk context
  - Interspecies extrapolation using SEQAPASS
  - Thyroid AOP Framework
  - Steroidogenesis
  - NAMS for other Tier 1 tests
- Covers 8-9 years of work of many people – can't cover it all in 25 minutes
- Focus on ER and AR because they have actionable recommendations

EDSP Tier 1 and Proposed Alternatives

Current EDSP Tier 1 battery of assays	Alternative high throughput assays and computational model for EDSP Tier 1 battery
Amphibian Metamorphosis	THY Model *(Future).*
Androgen Receptor (AR) Binding	AR Model *(Alternative).*
Aromatase	STR Model *(Future).*
Estrogen Receptor (ER) Binding	ER Model (*Alternative*).
Estrogen Receptor Transactivation (ERTA)	ER Model (*Alternative*).
Female Rat Pubertal	ER, STR, and thyroid (THY) Models *(Future).*
Fish Short Term Reproduction	ER, AR, and STR Models *(Future).*
Hershberger	AR Model *(Future).*
Male Rat Pubertal	AR, STR, and THY Models *(Future).*
Steroidogenesis (STR)	STR Model *(Future).*
Uterotrophic	ER Model (*Alternative*).

EDSP White Paper

Main Conclusion for ER and AR Models

Given the strengths discussed in Section III. C. and the uncertainties and limitations discussed in Section III. D.

Conclusion 1: EPA has determined that the ER pathway model based on the full 18 high throughput ER *in vitro* assays (Browne et al., 2015; Judson et al., 2015) may be used as an **alternative** method, when appropriate, to the following EDSP Tier 1 screening assays:

- ER binding *in vitro* assay (OCSPP 890.1250)
- ER transcriptional activation *in vitro* assay (ERTA; OCSPP 890.1300)
- *In vivo* Uterotrophic assay (rat) (OCSPP 890.1600)

Conclusion 2: EPA has determined that the AR pathway model based on the 11 *in vitro* assays (Kleinstreuer et al., 2017) may be used as an **alternative** method, when appropriate, to the EDSP Tier 1 AR binding assay (U.S. EPA, 2009a)

Conclusion 3: The existing ER and AR pathway model data for >**1,800** chemicals may be taken as **alternatives** for the four assays listed above in EDSP Tier 1 screening **WoE** evaluations of a chemical's potential for estrogen and androgen bioactivity.

Performance Based Model Validation

- Historically, validation has included generation of data in expensive, multi-year "ring trials" to demonstrate ability of multiple labs to use the same assay protocol.
- Some NAMs require specialized equipment, expertise, or intellectual property considerations (i.e., transferability not prerequisite of validation).
- Newer, performance-based validation approaches supplant the need for ring trials with more flexible, fit-for-purpose approaches, including demonstration of reproducibility over time and assessments that consider expanded reference chemical sets.
- Steps to support validation conclusion
  - Models have been published
  - Compared to *in vitro* and *in vivo* reference chemicals
  - Subject to multiple SAP reviews
  - ER model subject to OECD IATA review

Validation: Model and Assays

From OECD GD 34: GUIDANCE DOCUMENT ON THE VALIDATION AND INTERNATIONAL ACCEPTANCE OF NEW OR UPDATED TEST METHODS FOR HAZARD ASSESSMENT (*guiding principles, not strict criteria: intended to be flexible*)

+ A rationale for the test method should be available. This should include a clear statement of scientific need and regulatory purpose.

+ The relationship of the endpoint(s) determined by the test method to the in vivo biological effect and to the toxicity of interest should be addressed. The limitations of a method should be described, e.g., metabolic capability.

+ A formal detailed protocol must be provided and should be readily available in the public domain. It should be sufficiently detailed to enable the user to adhere to it, and it should include data analysis and decision criteria. Test methods and results should be available preferably in an independent peer reviewed publication. In addition, the result of the test should have been subjected to independent scientific review.

- Met for the full model, but some individual assays were proprietary, but they were published in the peer-reviewed literature at the level of detail that another lab could copy. Note that proprietary assays are not excluded from OECD or EPA validation. OECD 211 assay descriptions are posted online and includes all the technical details, intra-test and intra-lab performance characteristics, and reference chemical performance

+ Intra-test variability, repeatability and reproducibility of the test method within and amongst laboratories should have been demonstrated. Data should be provided describing the level of inter- and intra-laboratory variability and how these vary with time.

- Intra-lab (within lab) repeatability and reproducibility data is available. No inter-lab (between lab) testing was done (ring trials). The pathway models explicitly compare results from one lab to many more but with different assays, for 1800 chemicals. For reference chemicals, the results from lab to lab largely agree

+ The test method's performance must have been demonstrated using a series of reference chemicals preferably coded to exclude bias.

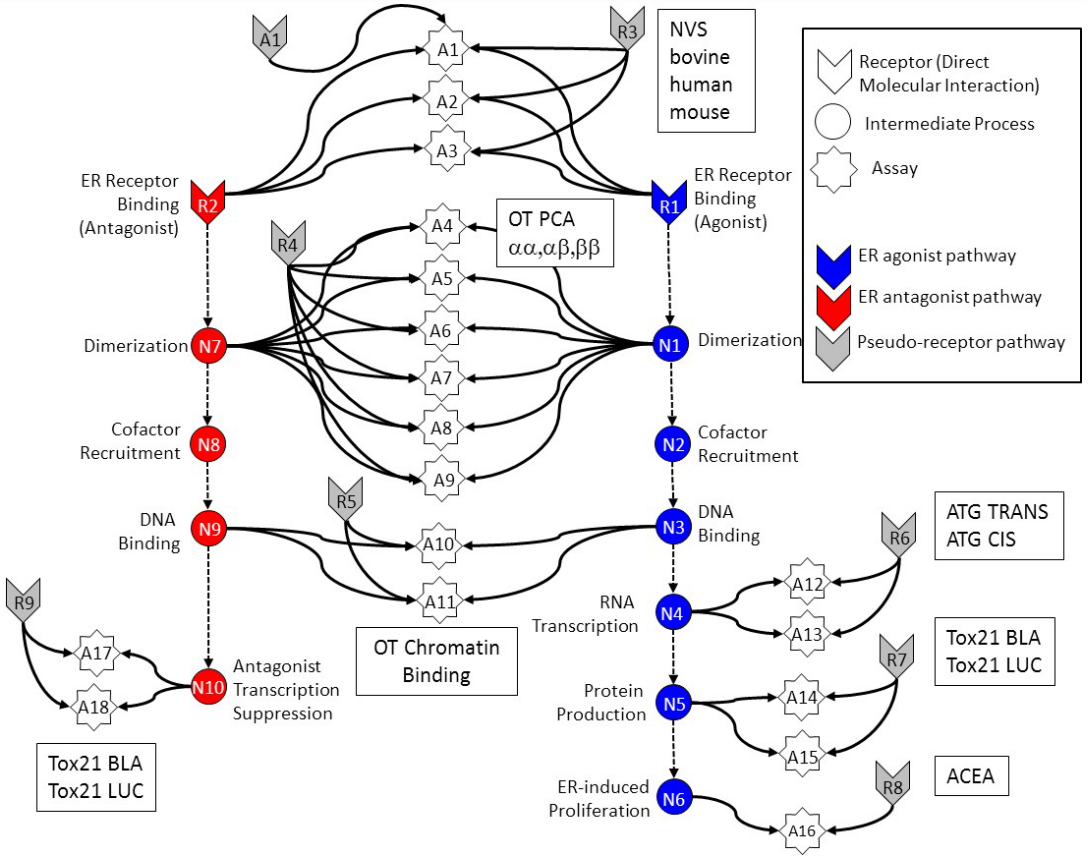
+ The performance of test methods should have been evaluated in relation to existing relevant toxicity data as well as information from the relevant target species.

+ All data supporting the assessment of the validity of the test methods including the full data set collected in the validation study must be available for review.

ER Pathway Model

ToxCast/Tox21 ER Pathway Model

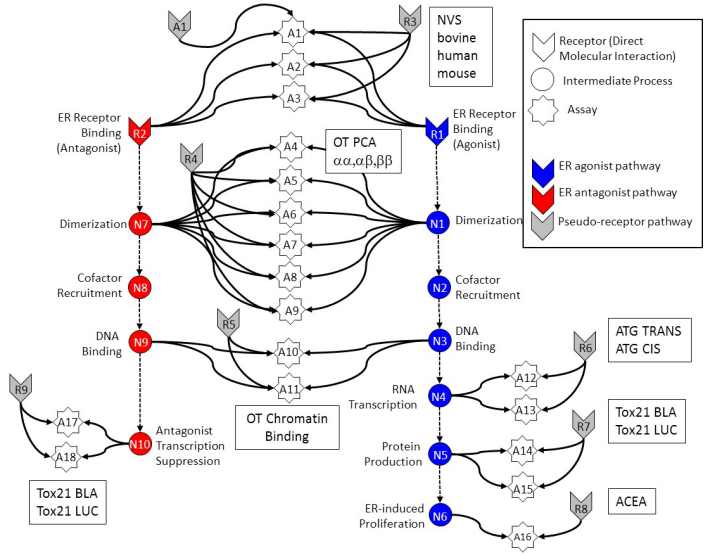
- Use multiple in vitro assays mapped to estrogen receptor pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals



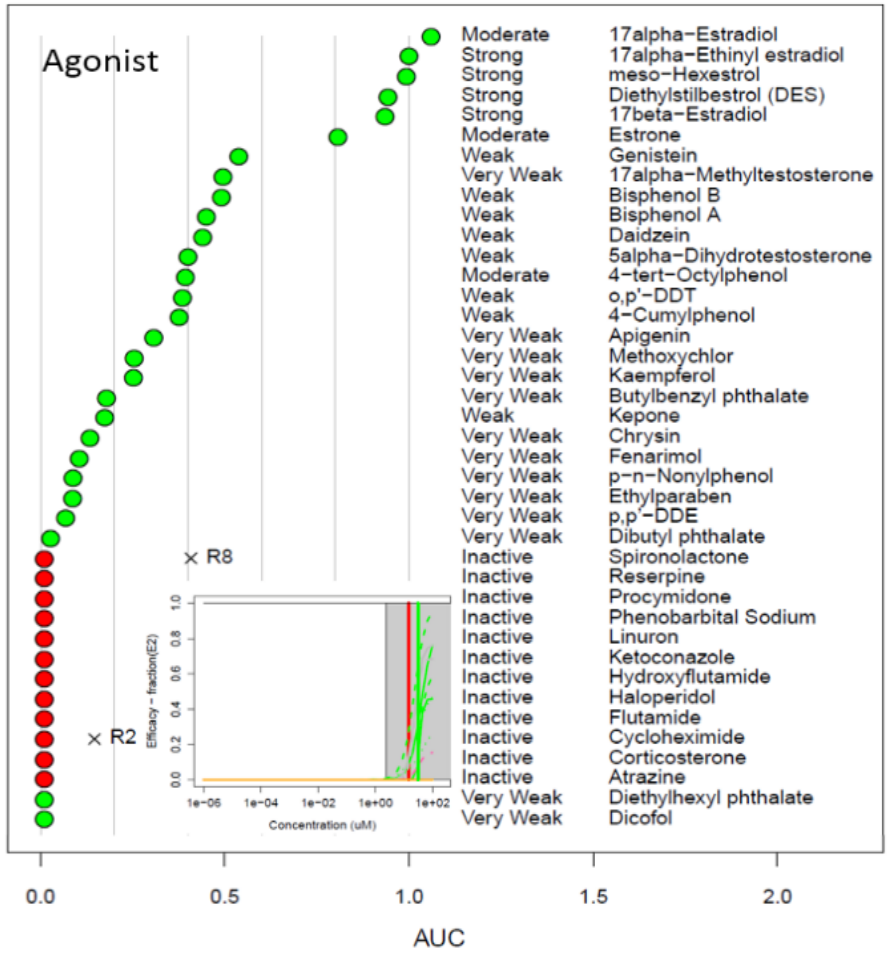
Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)

What Does the Model Do?

- For every concentration, look at the pattern of activity across the assays
  - If pattern is consistent with agonist activity, classify the chemical as an agonist
  - If pattern is consistent with antagonist activity, classify the chemical as an antagonist
  - Else, classify the chemical as acting through some technology or cell-type specific interference process (could be cytotoxicity)



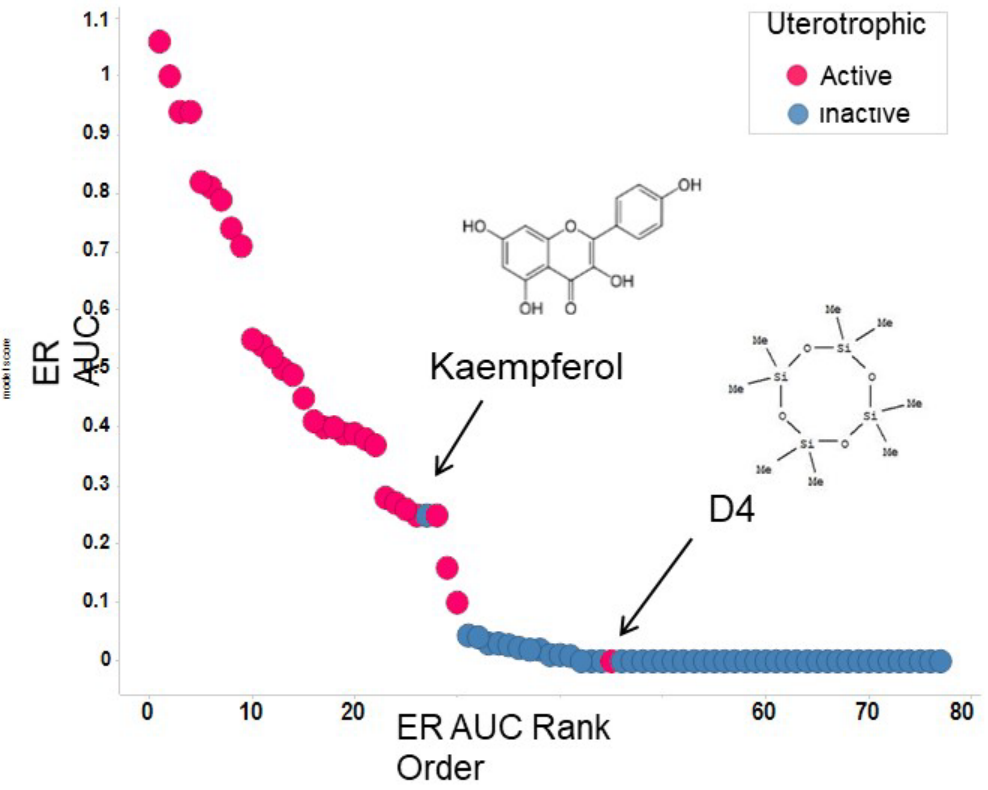
ER agonist model performance: *in vitro* reference chemicals



True Positive	26
True Negative	12
False Positive	0
False Negative	2
Accuracy	0.95
Sensitivity	0.93
Specificity	1

Very weak compounds can be hard to detect

ER agonist model performance: *in vivo* reference chemicals



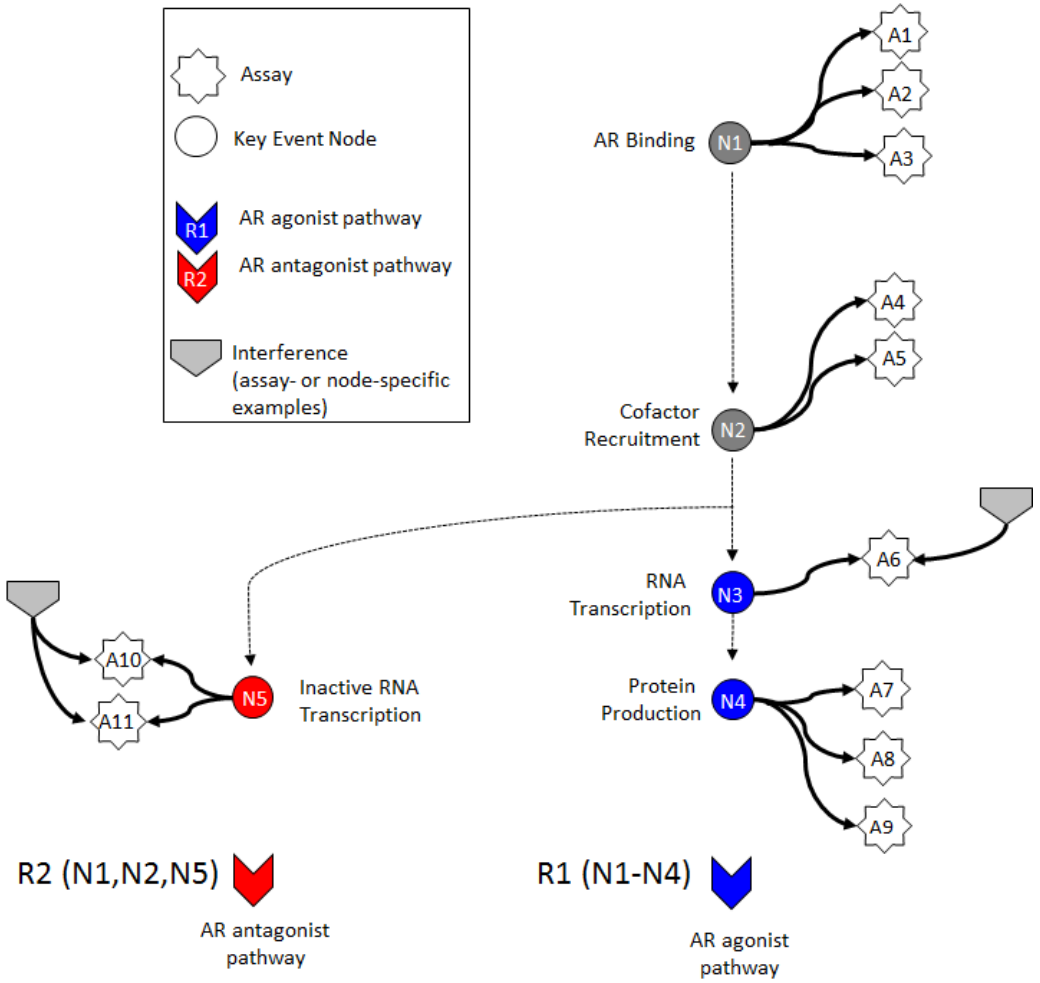
True Positive	29
True Negative	46
False Positive	1
False Negative	1
Accuracy	0.97
Sensitivity	0.97
Specificity	0.98

- D4 is volatile, probably not in experimental well
- Kaempferol is metabolized (deactivated) in vivo

AR Pathway Model

Tox21/ToxCast AR Pathway Model

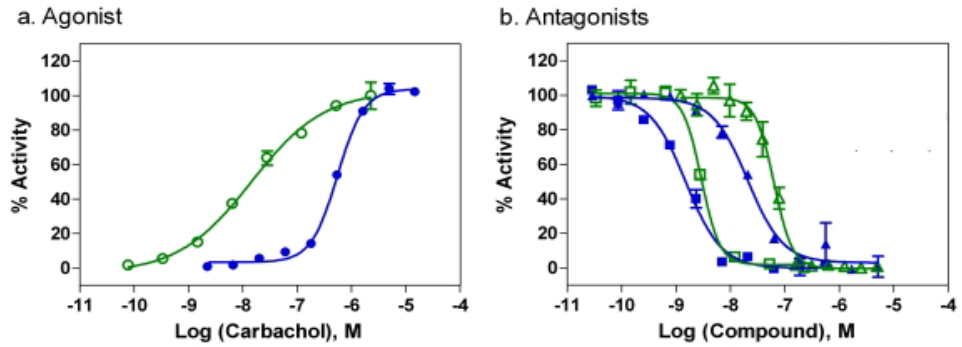
- Orthogonal assays on pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Same mathematical model structure as ER model to integrate assays and calculate AUC values (agonist/antagonist/interference)
- Antagonist Confirmation Data:
  Tox21 MDABK2 Luc antagonist assay (A11) was run twice (2 diff. conc. of R-1881)



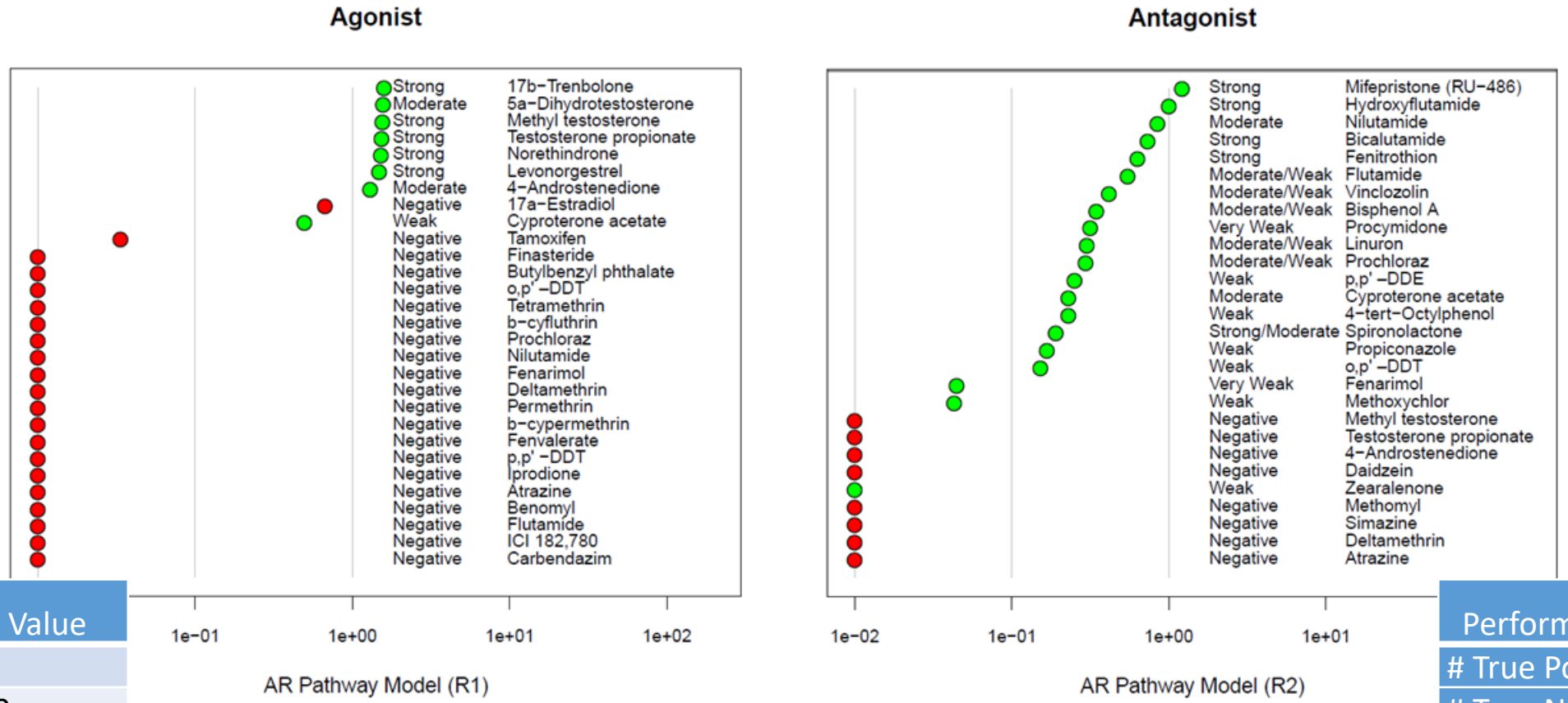
Kleinstreuer et al. 2017 CRT

Special Consideration for Antagonists

- Most environmental chemicals showing activity against the AR pathway are potential antagonists (agonism largely confined to well characterized pharmaceuticals)
- Agonist assays are “gain of signal”; as more agonist is added, the signal increases
- Antagonist assays are just agonist assays with a specified amount of a reference agonist added and then measure competitive inhibition by test chemical
  - Signal starts high, and as an antagonist is added, the signal decreases
  - If a higher amount of reference agonist is added, more antagonist is needed see a decrease in signal
  - Cytotoxicity can also cause signal to decrease
  - For a true antagonist, the shift will be seen. For a cytotoxic compound, there will be no shift.



AR Pathway Model Performance: *in vitro* reference chemicals



Performance	Value
# True Pos	8
# True Neg	19
# False Pos	2
# False Neg	0
Accuracy	0.95
Sensitivity	1.00
Specificity	0.90

The one “false negative” was identified by confirmation assay results.

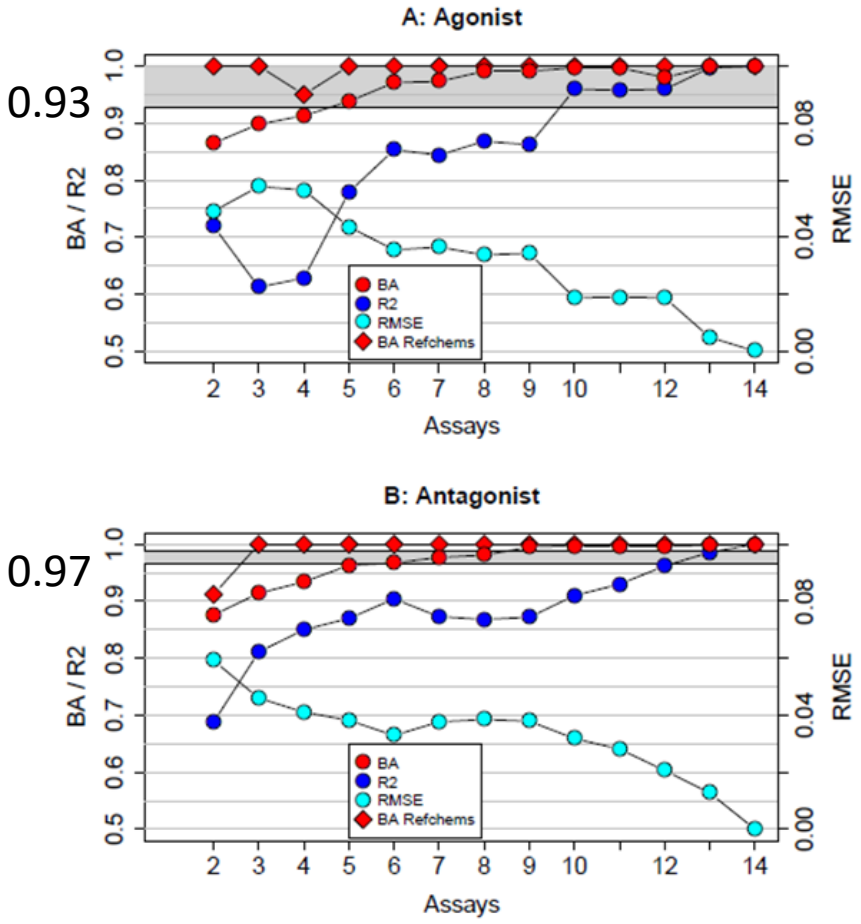
Performance	Value
# True Pos	19
# True Neg	8
# False Pos	0
# False Neg	1
Accuracy	0.975
Sensitivity	0.95
Specificity	1.00

Subset Models

Subset Models

- The full ToxCast ER and AR pathway models are proposed as alternatives to Tier 1 assays
- However …
  - These models would be very expensive to run and some of the assays are no longer commercially available
- Projects were carried out to assess how well subsets of the assays would perform against:
  - Full set of 1800 chemicals
  - Reference chemicals
  - Used existing data
- All subsets of assays from 2 to the complete set were evaluated

New Model Expected Performance: AR example



Performance against reference chemicals is usually better than against the full set of 1800 ToxCast chemicals

Reference chemical vs. full chemical set performance

- ToxCast chemicals usually have more evidence for activity / inactivity than many of the reference chemicals (12 assays vs. 4+)
- The reference chemicals were broken down into inactive, very weak, weak, moderate, and strong, but may not have as many in each category as the full chemical set
- The ToxCast chemicals cover a much broader range of chemical space, providing a larger domain of applicability
- Using subsets with poor all-chemical performance may lead more to misclassification of chemicals in a diverse library like the inerts, relative to models with better performance
- Basing performance on the full model leads to a circular validation approach since the full model was validated based on performance of the reference chemicals

Incorporating variability when comparing performance of the model

- The full model was rebuilt including data variability (ToxBoot).
- The range of performance (BA) of the full but variable model is given by the gray band
- Any subset model with performance within the gray band performs as well as the full model, given its performance variability

Summary of Subset Models

- ER Subset Model:
  - **agonist** batteries of as few as **four assays** to achieve equivalent full chemical performance and **three assays** for reference chemicals
    - Key events: binding, dimerization, transactivation, proliferation

- AR Subset Model:
  - **agonist** batteries of as few as **five assays** to achieve equivalent full chemical performance and **two assays** for reference chemicals
    - Key events: binding, RNA transcription/protein production, proliferation
  - **antagonist** batteries of as few as **five assays** to achieve equivalent full chemical performance and **three assays** for reference chemicals
    - Key events: binding, cofactor recruitment, RNA transcription inhibition

Testing New Chemicals

Testing new chemicals

- OCSPP has requested ORD to test ~500 additional chemicals in ER and AR subset models
- Phase 1: implement multiple assays in house or contract labs and test against reference chemicals and selected set “representative chemicals” from the 1800 to enable characterization of full chemical set performance
  - Where available use guideline (or guideline-like) assays
  - Select a well-performing subset of assays
  - Results will be subject to peer review and public comment
- Phase 2: Use the selected subsets to test ~500 additional chemicals

Status of New Testing Project

- Multiple assays are being developed within EPA ORD for ER and AR
  - Transactivation
  - Protein dimerization
  - DNA binding
  - Proliferation
- QSAR models (COMPARA and CERAPP) may be part of battery
- A commercial lab will conduct radioligand binding assays
- “Validation” chemicals have been selected and procured
  - ~100 each for ER and AR, including the original reference chemicals
- Issues of assay validation and assay transferability are being addressed
- New chemicals to test are being selected

Summary

- EPA has proposed that the ER and AR pathway models can be used as alternatives to specific EDSP Tier 1 assays
  - These high-throughput in vitro-based models can evaluate hundreds of chemicals at a time
- Current data on 1800 chemicals could be used for this purpose
- New assay batteries are being developed to test new chemicals
  - These batteries will need to be validated before new chemicals are tested
- This approach is currently open for public comment
- https://www.regulations.gov/document/EPA-HQ-OPP-2021-0756-0002

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Whitepaper
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