EPA
United States
Environmental Protection
Agency

A Case Study: Using New Approach Methods to Assess Estrogen Receptor Activity

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Toxicology Track: Integrated Approaches
Summer School 2020: Innovative Science Without Animals
June 22 – 26, 2020

Office of Research and Development
National Center for Computational Toxicology

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

Big Questions

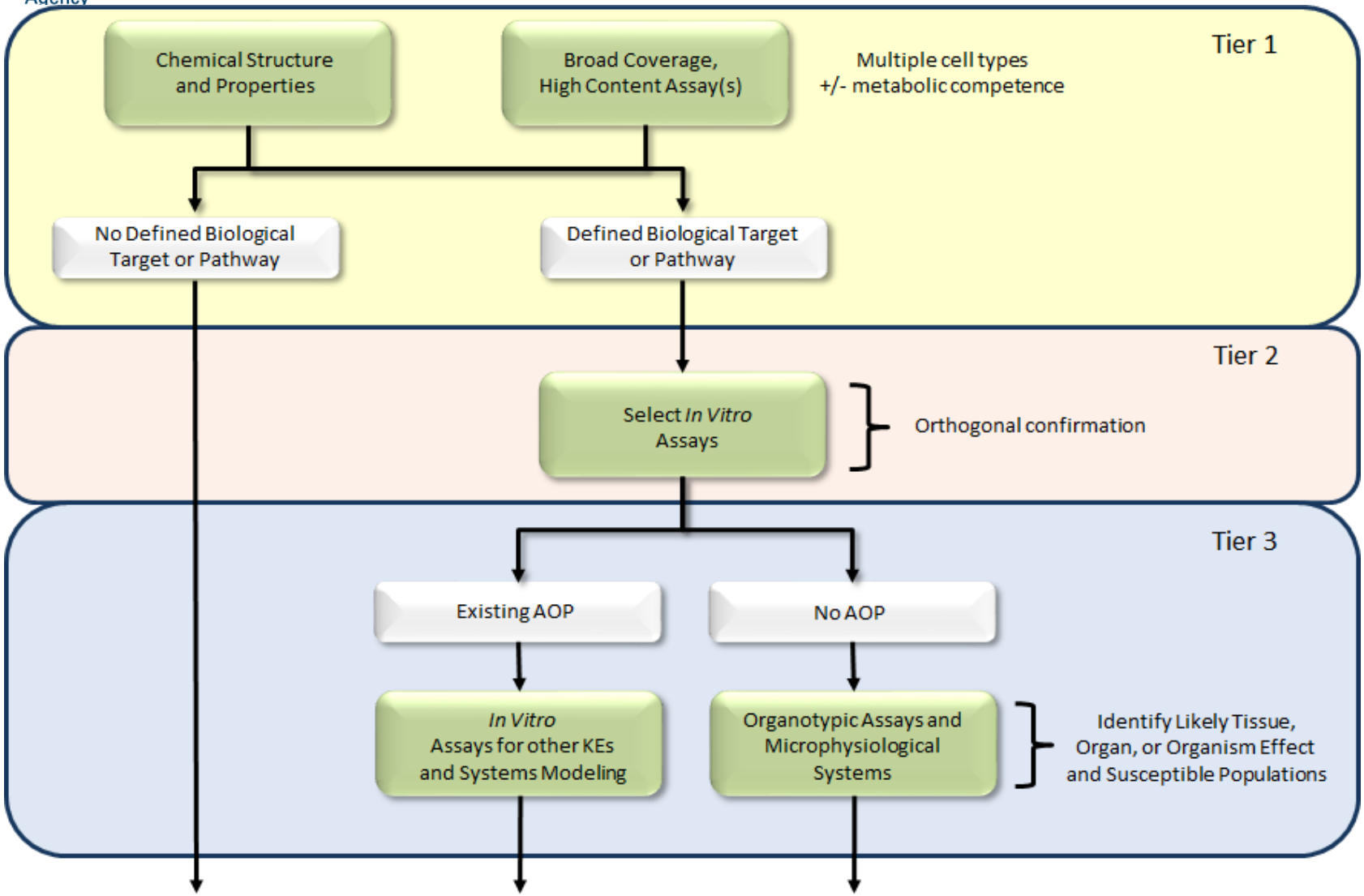
1. At what dose does a chemical cause adverse affects?

2. What effects does the chemical cause?

3. Can we answer 1 and 2 without using animals?

NAMs (New Approach Methodologies) attempt to answer these

Tiered Hazard Evaluation Approach



The NexGen Blueprint of CompTox at USEPA Tox. Sci. 2019; 169(2):317-322

New Approach Methods

- In silico (e.g. QSAR and Read-across)
  - Estimate effects and doses
- In vitro assays
  - Broad / screening (transcriptomics, cell painting)
  - Targeted (receptors, enzymes)
  - In vitro PODs, modes / mechanisms of action
- In vitro Toxicokinetics
  - Allow conversion of an in vitro POD to in vivo (IVIVE)
- Computer models
  - Integrate multiple in silico and in vitro data streams
- Databases of existing traditional toxicology data
  - Enables training and validation of NMA models

Illustrating NAMs

OECD Staging:

1. IATA (Integrated Approach to Testing and Assessment)
2. DA (Defined Approach)
3. TG (Test Guideline)

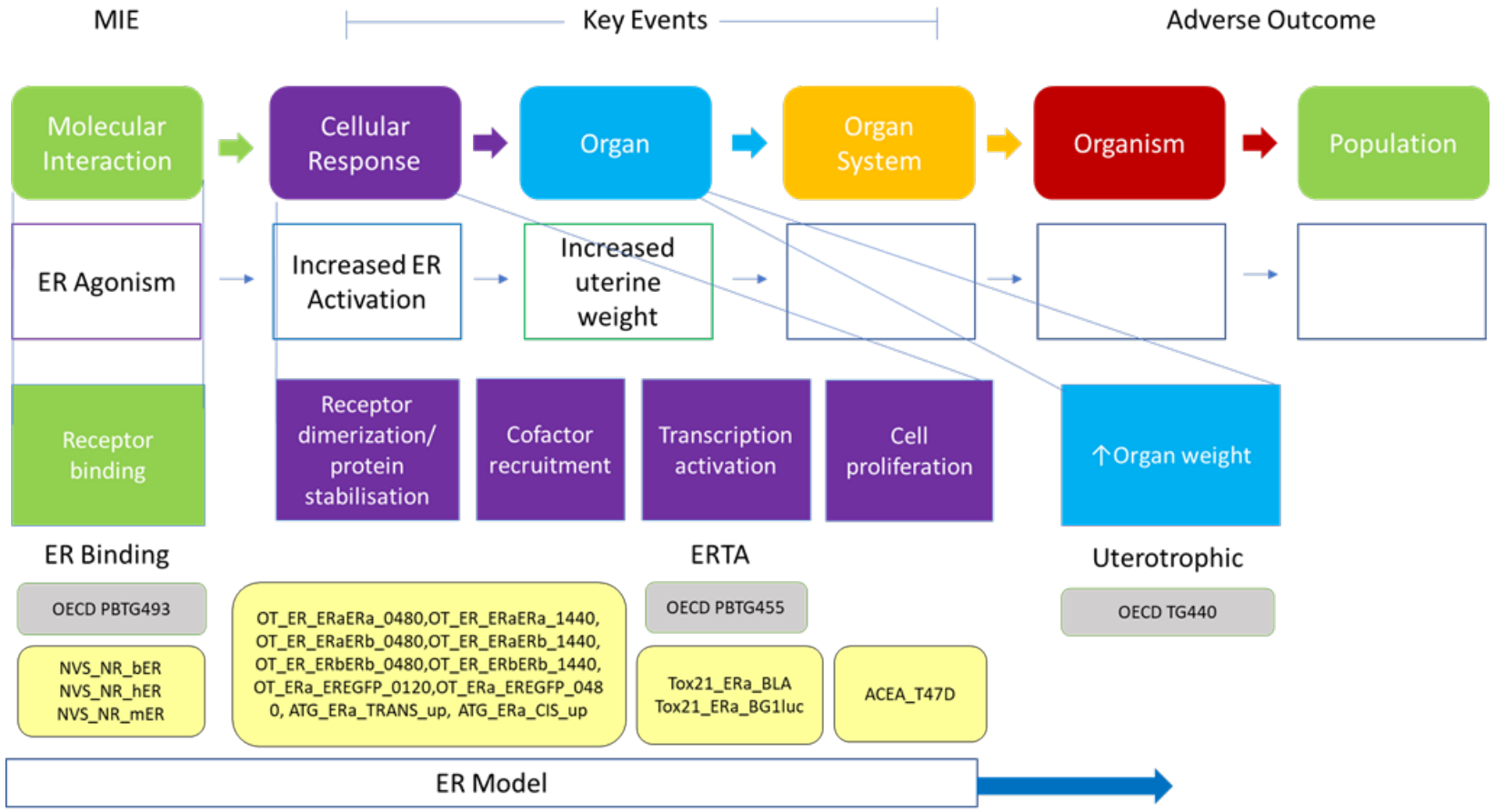
US EPA may accept tests / approaches / models without OECD acceptance

Use example of Estrogen Receptor (ER) activity

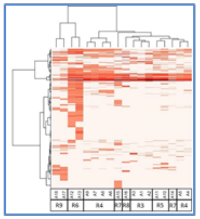
EDC Context

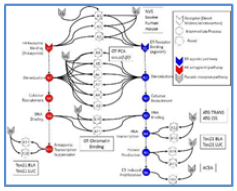
- Endocrine disrupting chemicals are a diverse set of substances that have the potential to interfere with normal endocrine function and lead to an adverse outcome.
- Regulatory agencies in many countries evaluate endocrine activity of environmental chemicals for specific regulatory endpoints.
- The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

Linking *in vitro* to *in vivo* biology using an Adverse Outcome Pathways (AOP)



IATA Overall Approach





Internal

ER High-Throughput Screening Data

ER Pathway Computational Model

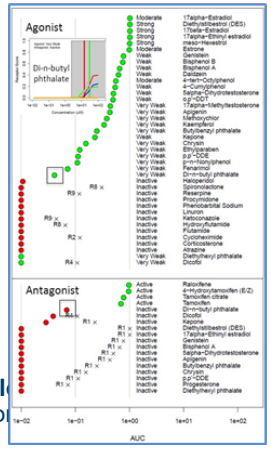
External

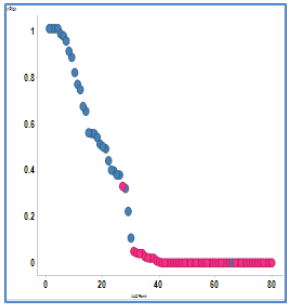
In vitro reference chemicals

In vivo reference chemicals

Model Performance Evaluation

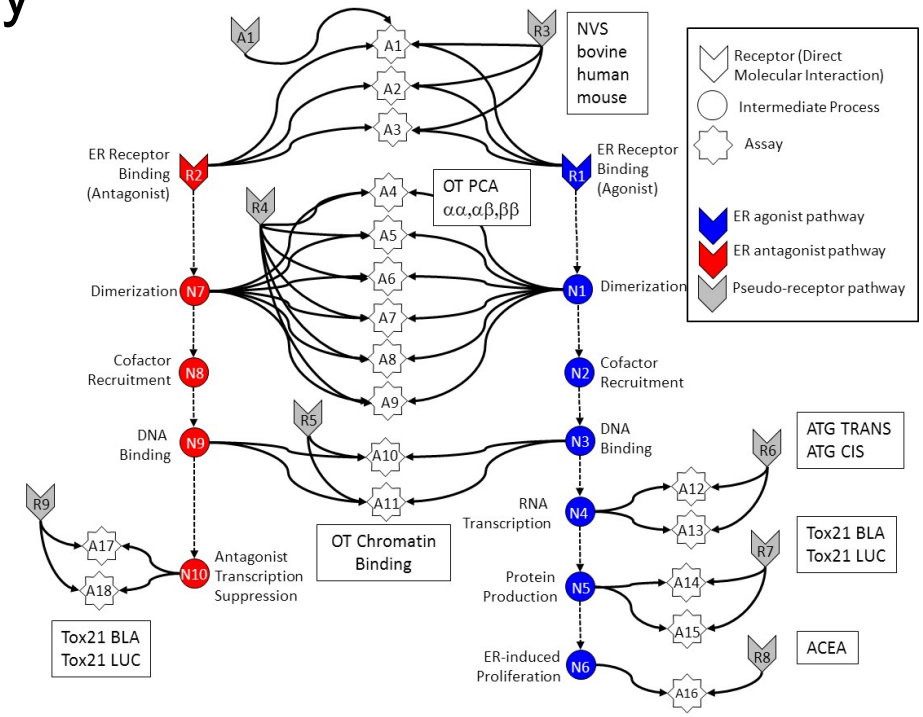
Validated Model for Chemical Screening





In Vitro Estrogen Receptor Model

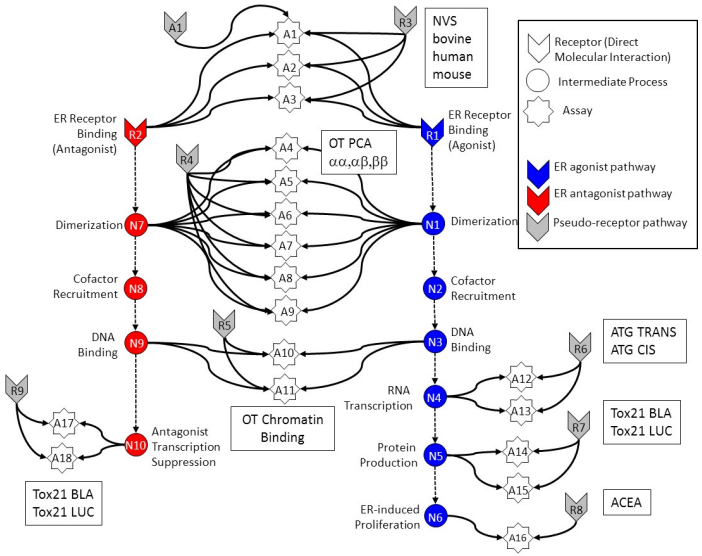
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals

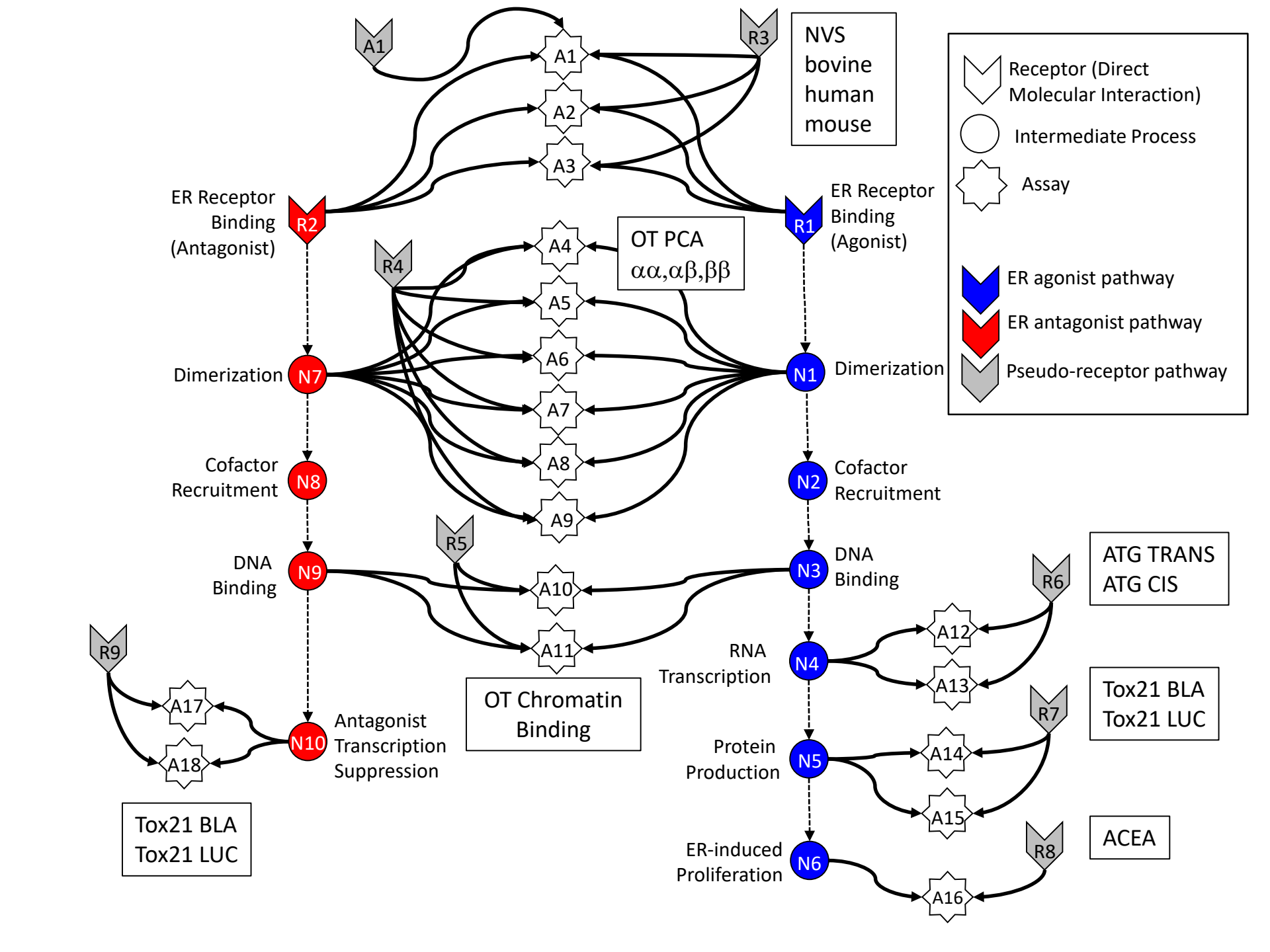


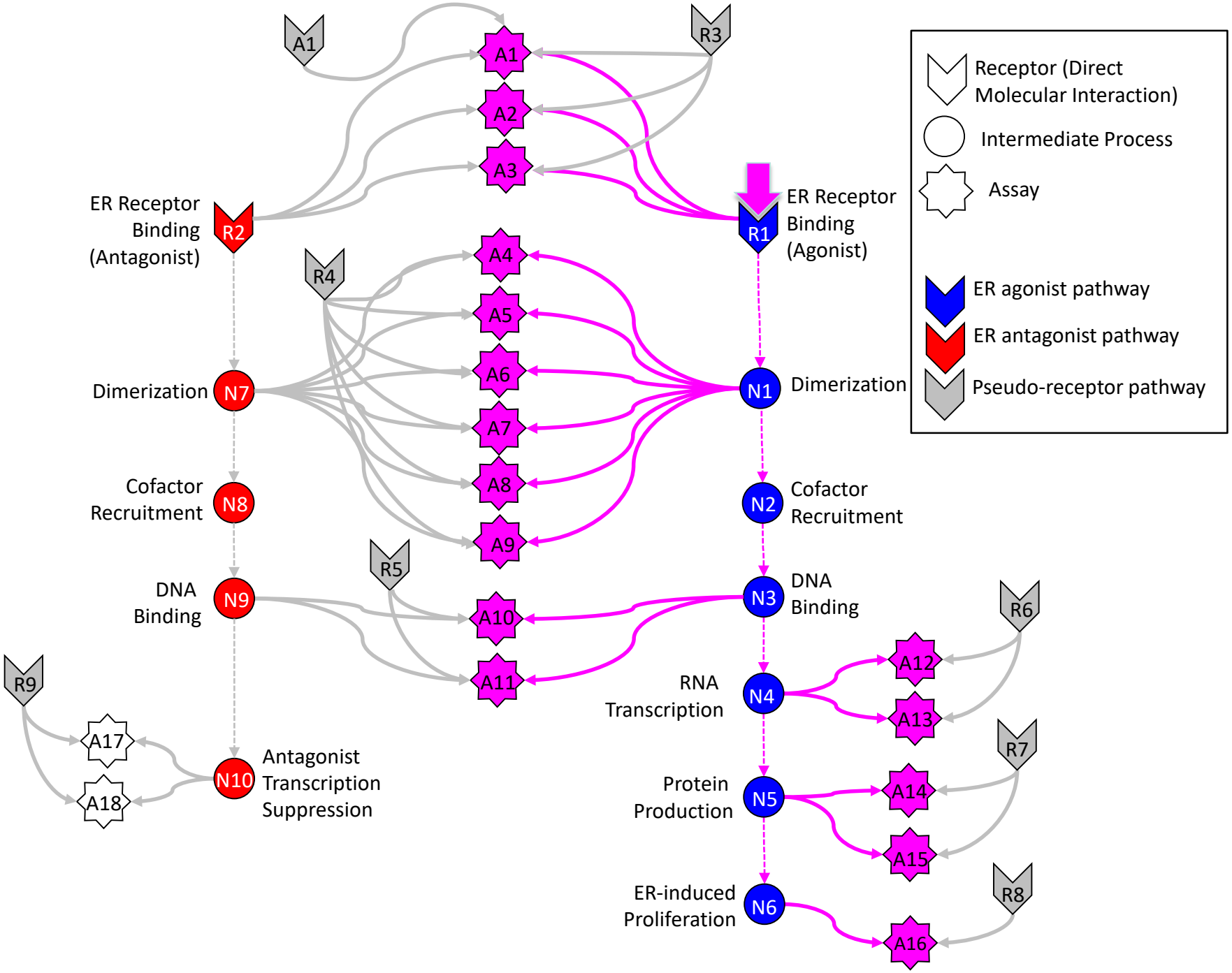
Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)

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




NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
A1
A2
A3
R1
R2
R3
ER Receptor Binding (Antagonist)
ER Receptor Binding (Agonist)
OT PCA
αα,αβ,ββ
R4
A4
A5
A6
A7
A8
A9
Dimerization
N7
N1
Dimerization
Cofactor Recruitment
N8
N2
Cofactor Recruitment
R5
DNA Binding
N9
N3
DNA Binding
A10
A11
OT Chromatin Binding
ATG TRANS
ATG CIS
R6
A12
A13
RNA Transcription
N4
Tox21 BLA
Tox21 LUC
R7
Protein Production
N5
A14
A15
ACEA
R8
ER-induced Proliferation
N6
A16
R9
A17
A18
N10
Antagonist Transcription Suppression
Tox21 BLA
Tox21 LUC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A7
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A9
Dimerization
N7
N1
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R6
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A11
A12
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R7
Protein Production
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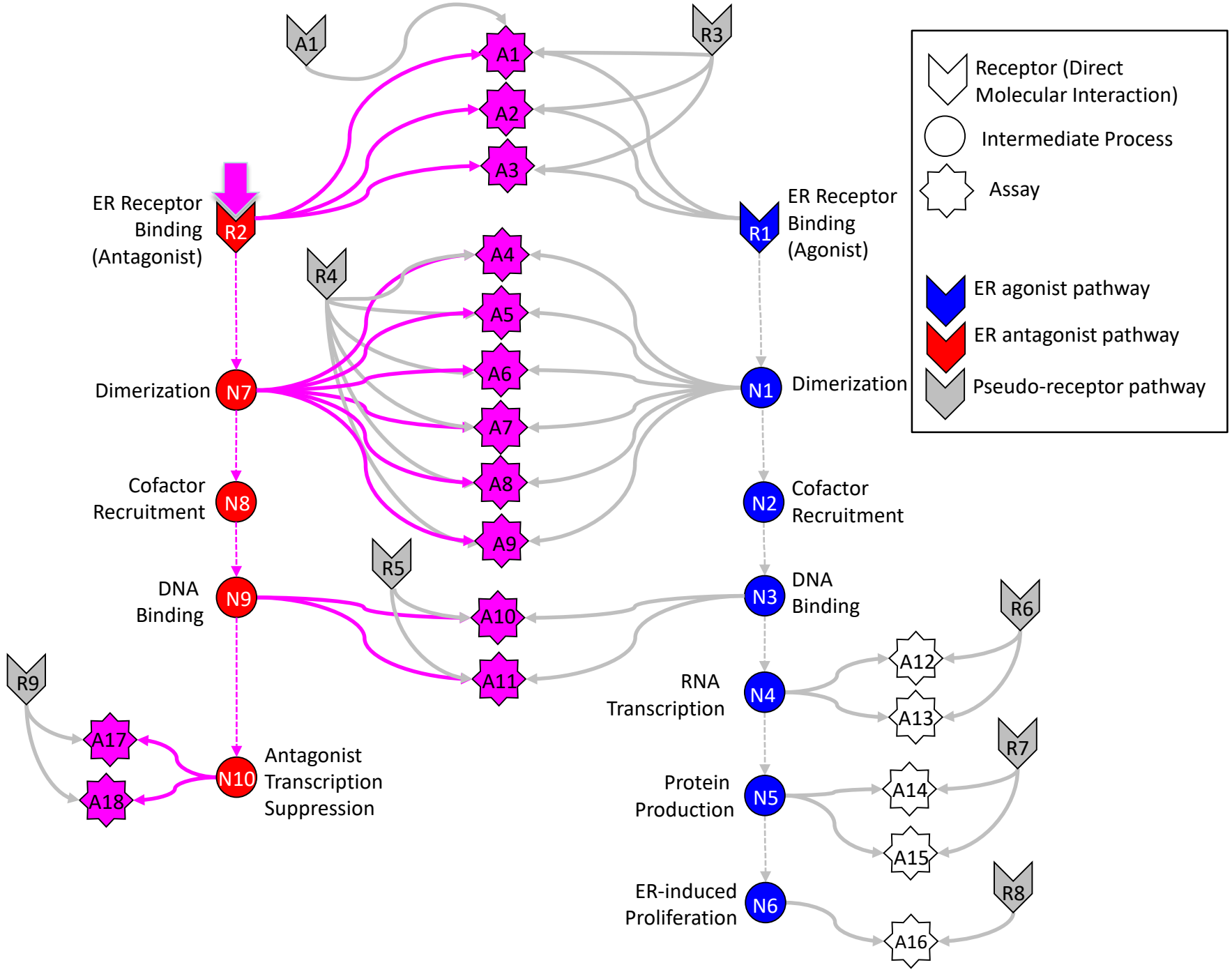
A14
A15
ER-induced Proliferation
N6
R8
A16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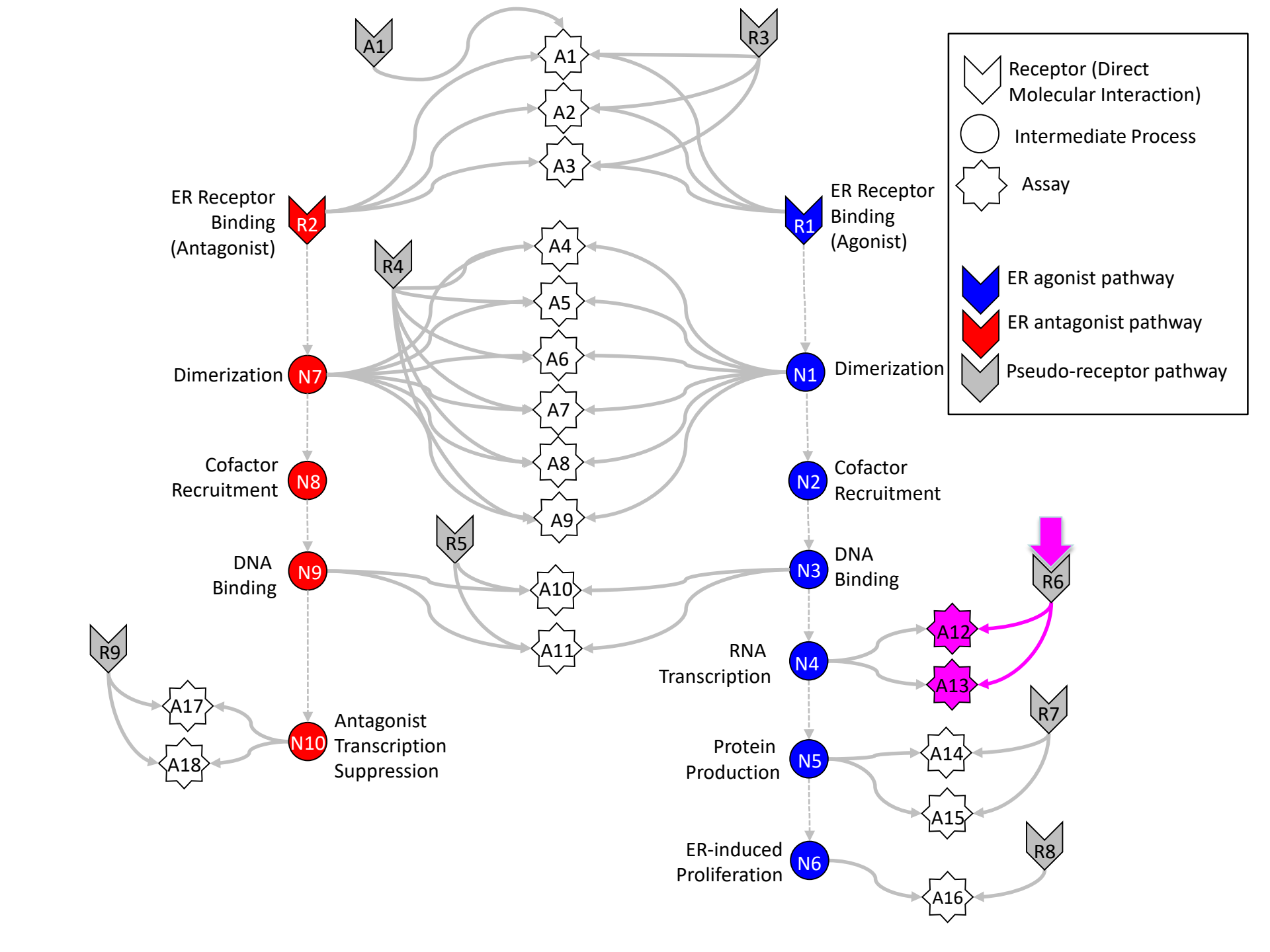
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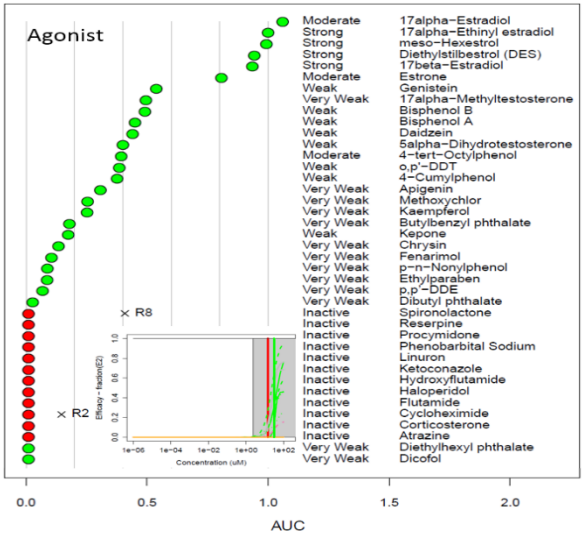
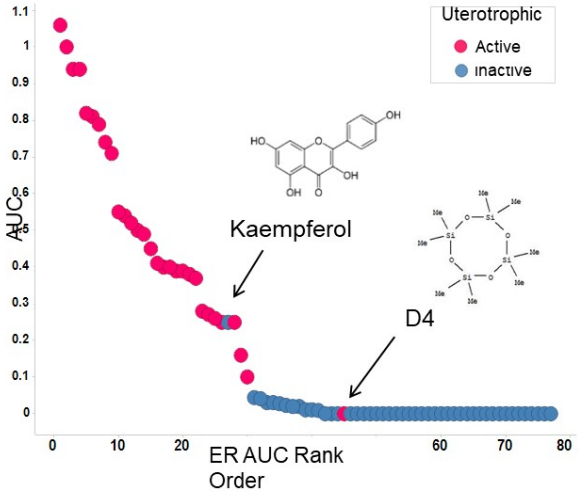
N10

Demonstrate that the full model replicates reference chemical activity



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 Vitro



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

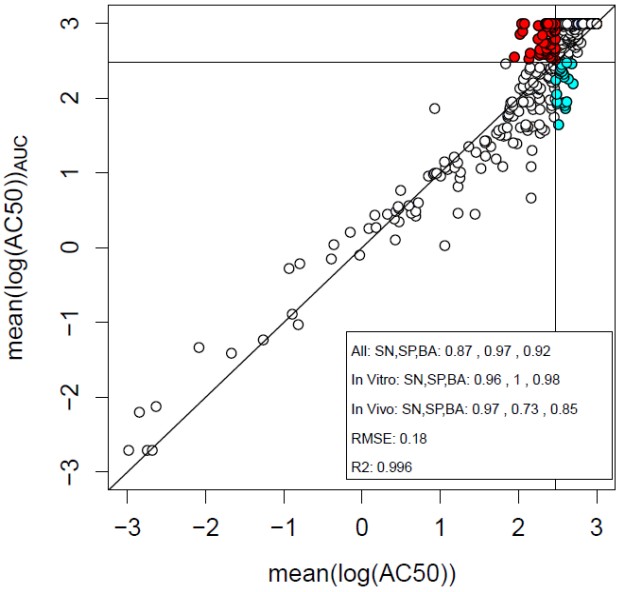
In Vivo

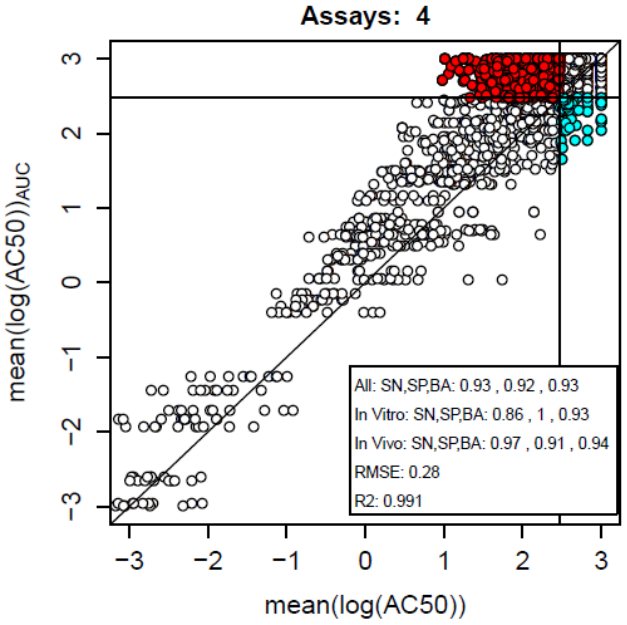
Moving to a Practical Application

- Full model requires …
  - 16 assays in agonist mode, many not commercially available
  - A complex mathematical model
  - But serves as benchmark (not the “truth”) for evaluating simpler models
- “Subset models” perform almost as well
  - Use a subset of as few as 4 assays (one can be a QSAR model)
  - Combining rule uses simple arithmetic (average potency across assays)
- The IATA and DA (defined approach) are built around these simple subset models

Demonstrating the performance of the simple model

- Show that simple arithmetic can reproduce the mathematical model within the uncertainty of the model
  - Does not need to be perfect because current tests are variable
- Show that subsets (including QSAR model) are still accurate within the uncertainty of the model
- In both cases, chemicals that are misclassified are mostly “inactive” or “very weak”, and are ones that current tests may misclassify



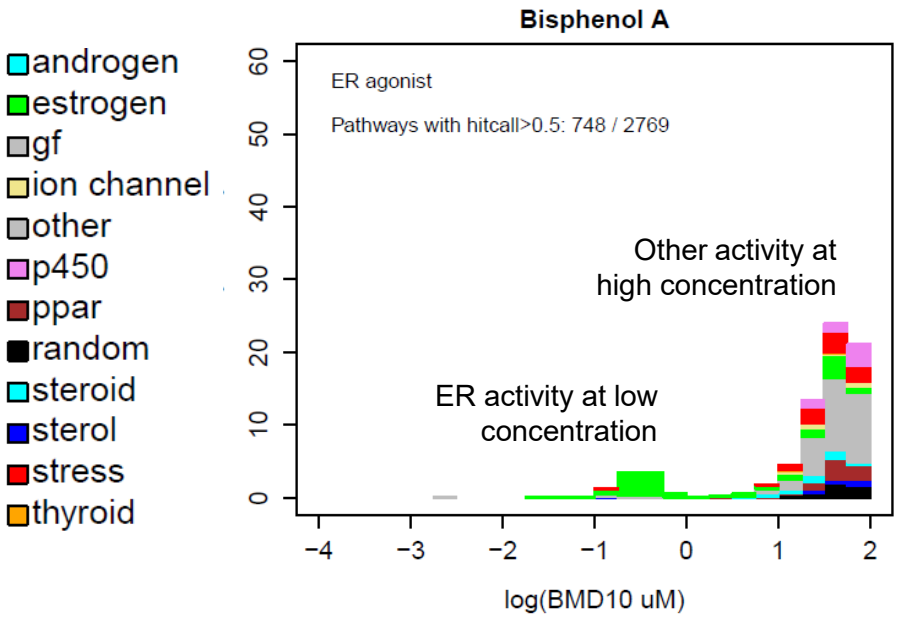


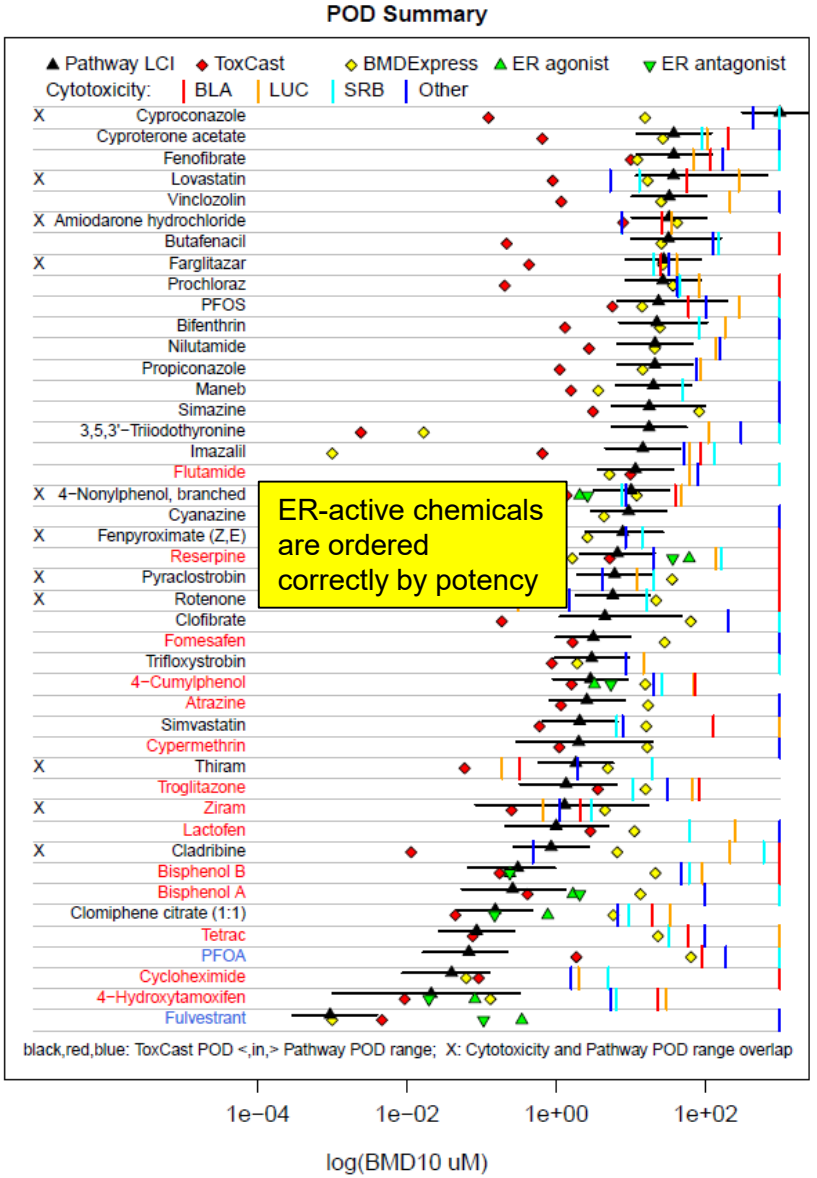
Steps to getting OECD acceptance

- IATA was reviewed and published in 2018-2019
  - Demonstrates an approach of interest
  - 1-2 years
- Defined Approach is to be reviewed in 2020
  - Gives more details of implementation
  - Will include proposal for specific assays that could be generally available and process for “validation” of these
  - 1-2 years
- Test Guideline(s) will likely be needed
  - These would give more specific details on how to run each assay and combine the results
  - Multiple years
- However, EPA and EFSA are already using the full ER model in making regulatory decisions

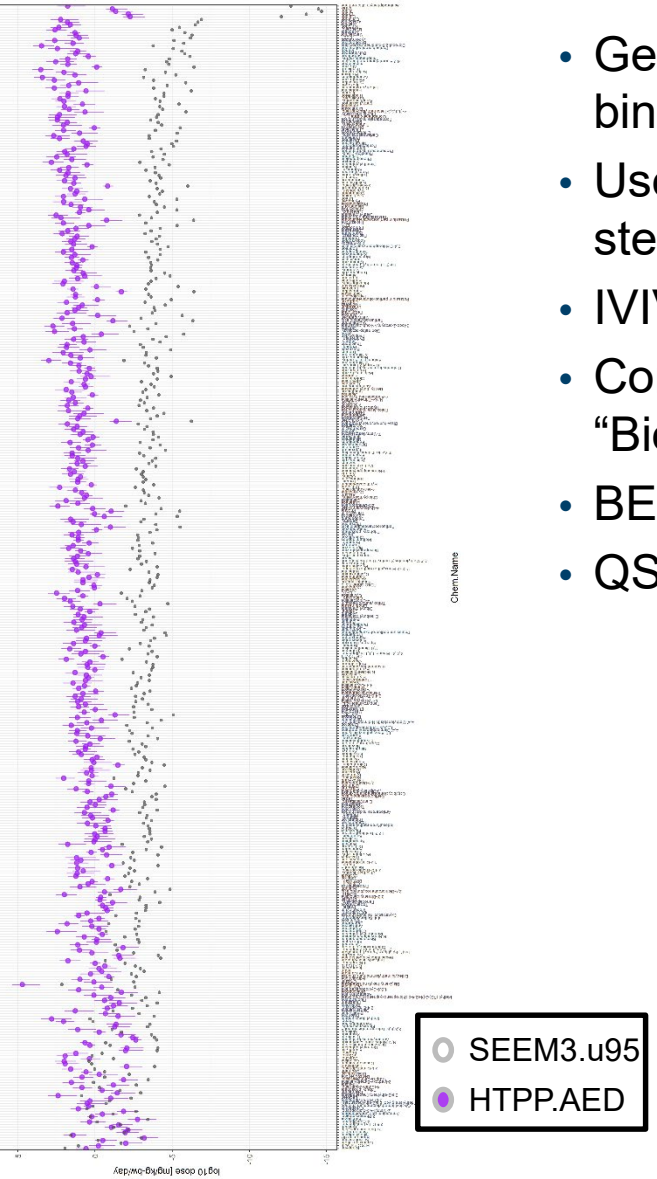
Other tools in the NAM Toolkit
High-throughput transcriptomics

- Measures RNA (gene) changes in all ~20,000 genes at once
- Technology has been around for 10-20 years but cost has dramatically dropped in the last ~2 years without loss of quality
- Pilot results show that chemicals can simultaneously be screened for many mechanisms

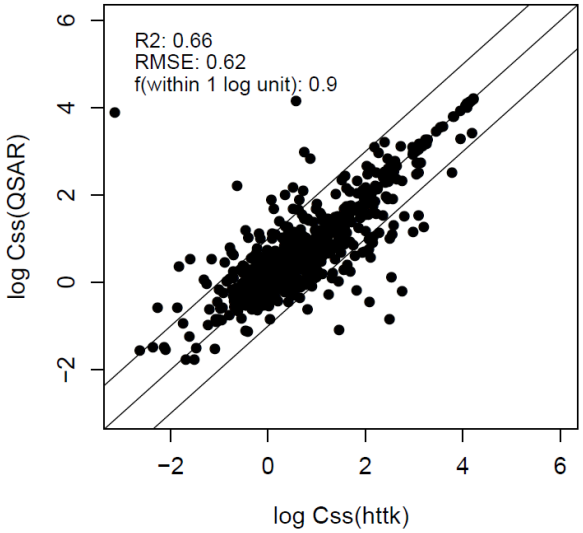




Other tools in the NAM Toolkit
In vitro Toxicokinetics

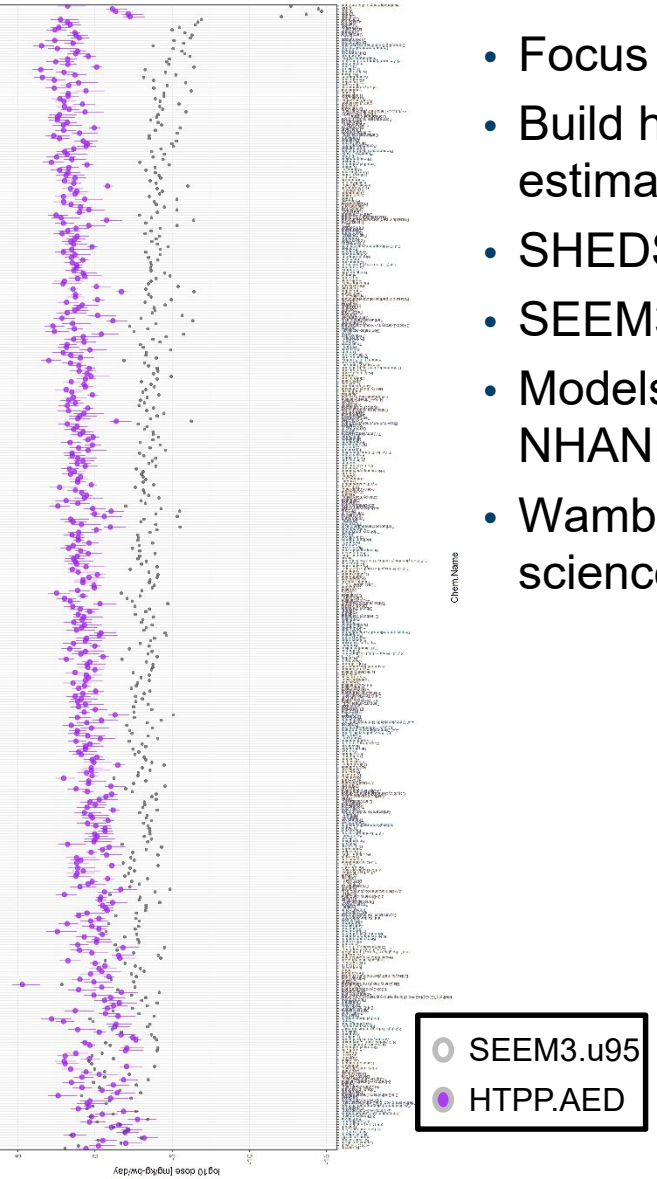


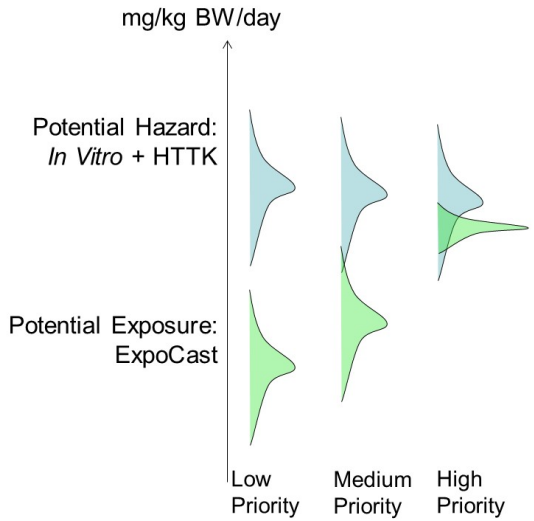
- Generate cell-based measurements of plasma protein binding and intrinsic hepatic clearance
- Use a PBPK model to generate "Css", concentration at steady-state give a 1 mg/kg/day oral dose
- IVIVE POD = in vitro POD / Css
- Compare IVIVE POD to exposure predictions to generate "Bioactivity to Exposure Ratio", BER
- BER << 1 indicates low risk
- QSAR model can give adequate predictions of Css



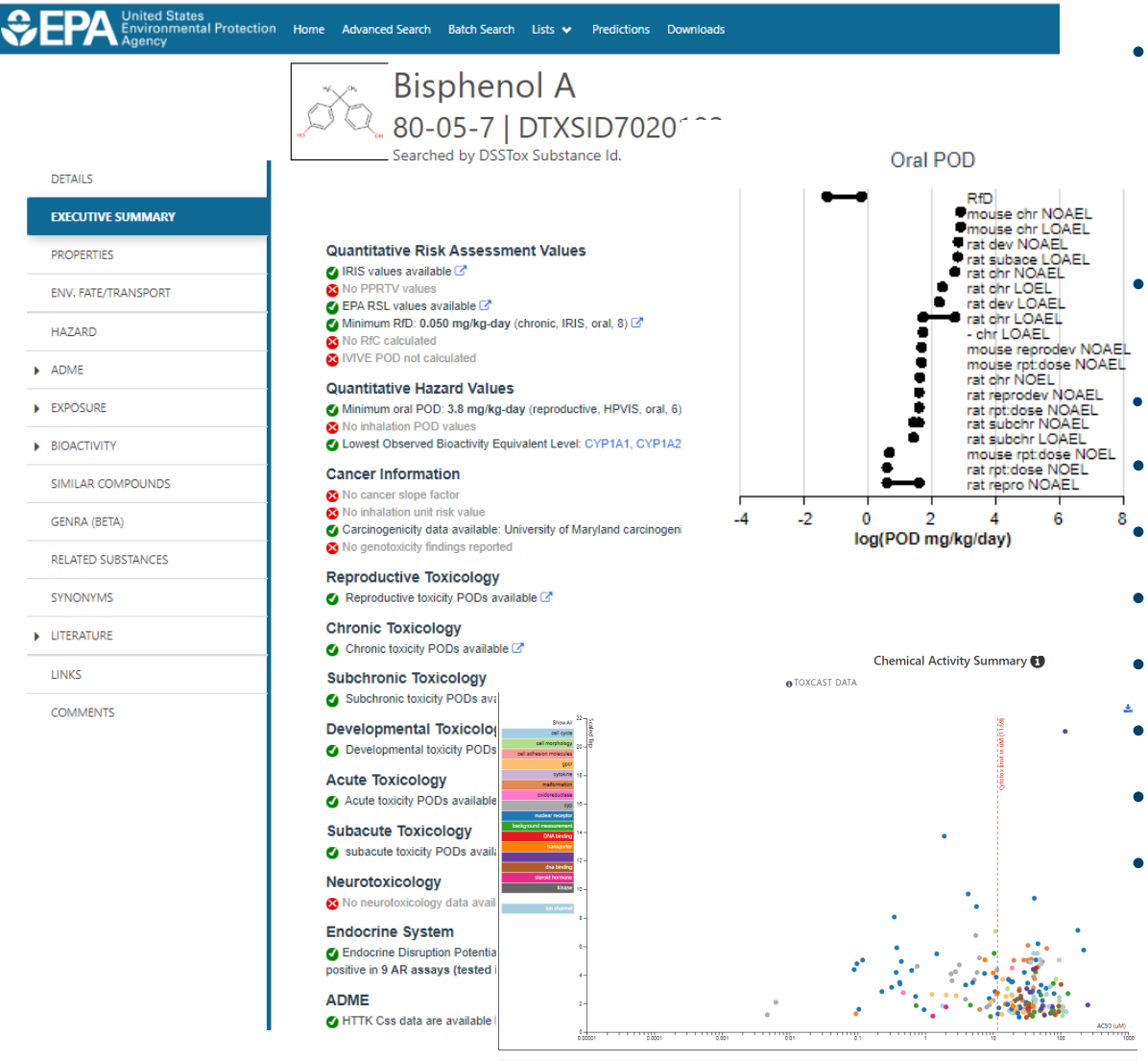
Other tools in the NAM Toolkit
High-throughput exposure estimates

- Focus on Risk: compare hazard to exposure
- Build hierarchical models – exposure for some chemicals can be estimated more accurately than others
- SHEDS-HT – detailed use patterns drive exposure
- SEEM3 – more generic model
- Models are calibrated using measured exposure levels from NHANES
- Wambaugh et al. “New Approach Methodologies for exposure science”, Current Opinions in Toxicology, 15, 76-92 (2019)





Other tools in the NAM Toolkit
Large Databases



- EPA is developing databases and dashboards to make traditional and NAM data widely available and easy to use
- Comptox Chemicals Dashboard is the primary portal
- https://comptox.epa.gov/dashboard
- Chemistry
- Physchem properties
- In vivo hazard
- In vitro bioactivity
- Exposure
- Chemical Use
- Literature

EPA / OCSPP is developing a NAM Plan

1. Identification, Development and Integration of New Approach Methodologies (NAMs)
2. Establishing Scientific Relevance, Reliability and Confidence
3. Importance of Training, Education and Collaboration
4. Implementation of NAMs Under TSCA
   - Commitment of time and resources through the establishment of the TSCA NAM Team (TNT)
   - *"For the purposes of TSCA, EPA recognizes this new term (i.e., NAMs) as encompassing any 'alternative test methods and strategies to reduce, refine, or replace vertebrate animals.'"*

https://www.epa.gov/sites/production/files/2018-06/documents/epa_alt_strat_plan_6-20-18_clean_final.pdf

Summary

- New approach methods (NAMs) are being developed to screen and prioritize chemicals using a combination of in silico and in vitro methods
- Regulatory acceptance requires demonstrating performance against various benchmarks
- Individual agencies (e.g. EPA and EFSA) are beginning to use NAMs, but wide acceptance requires OECD acceptance
  - IATA, DA, TG

Acknowledgments

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