EPA United States Environmental Protection Agency

Case Study on the Use of an Integrated Approach to Testing and Assessment (IATA) for Identifying Androgen Receptor Active Chemicals

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Office of Research and Development
National Center for Computational Toxicology

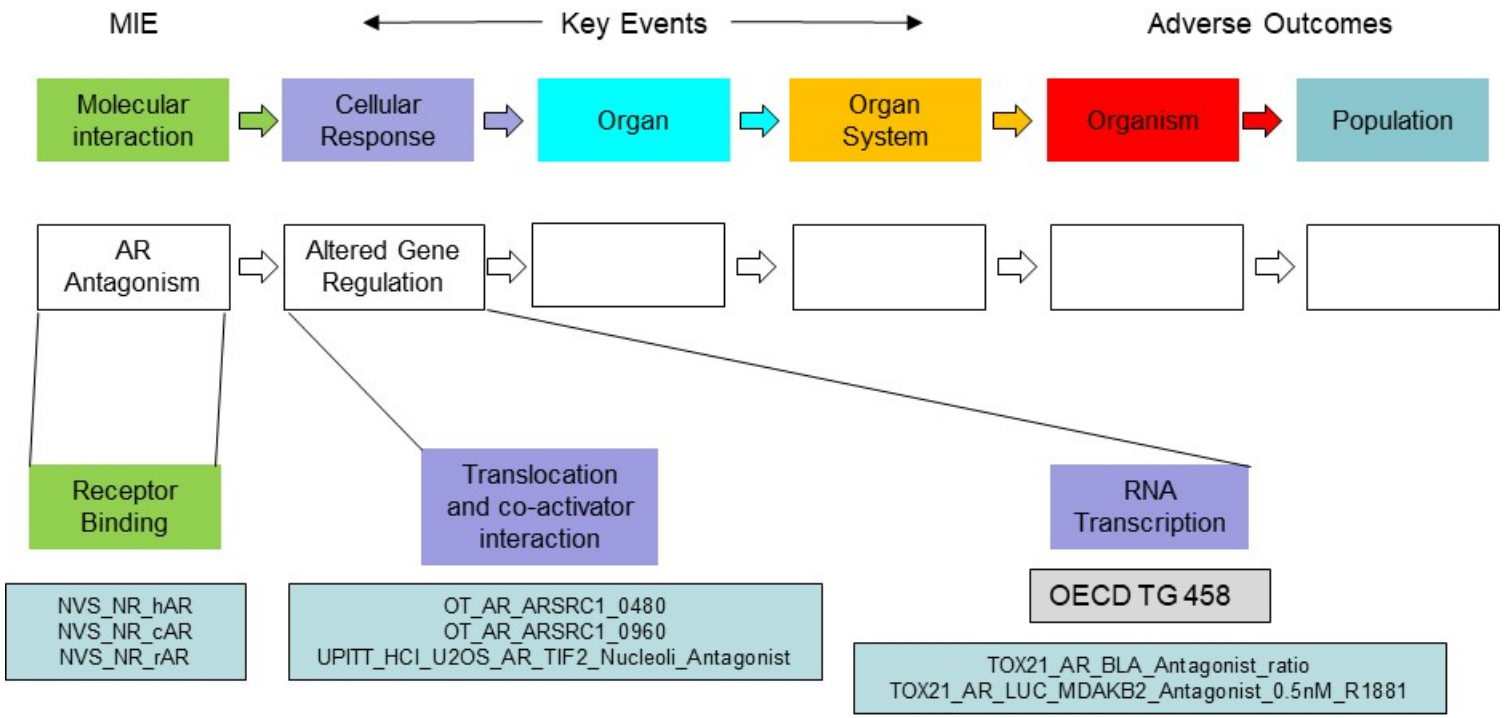
The views expressed in this presentation are those of the author and do not necessarily reflect the views or policies of the U.S. EPA

Intended Application

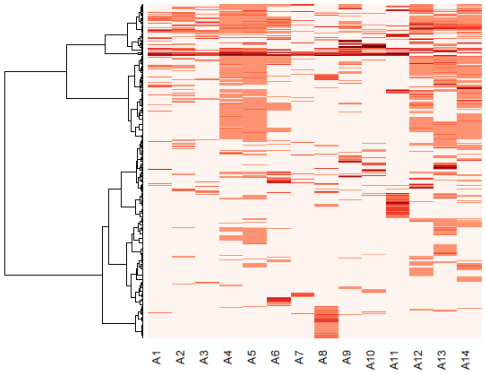
- The intended application of this IATA is for:
  - Screening of environmental chemicals based on their AR antagonist activity .
  - Determining whether further evaluation of endocrine-related activity in higher tier *in vivo* tests (e.g., male pubertal assay, two generation reproductive toxicity study) is needed.
  - Provide mechanistic information on endocrine activity.

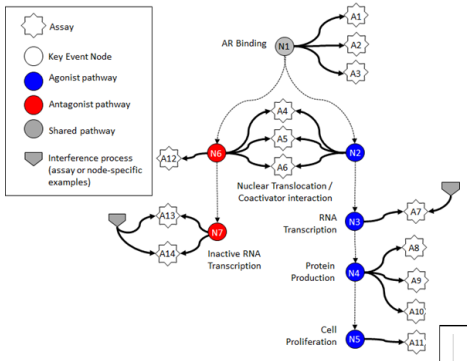
Purpose

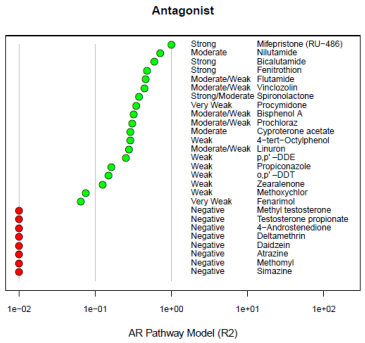
- To use a combination of 5 or more *in vitro* high throughput screening (HTS) assays and a computational biologically-based model for androgen receptor (AR) antagonist activity, as an alternative to a single low throughput *in vitro* transactivation assay for AR antagonist activity which may have imperfect sensitivity and specificity (TG 458).



Overall Approach







Internal

External

AR High-Throughput Screening Data

In vitro Reference Chemicals

AR Pathway Computational Model

Curated by NICEATM

Model Performance Evaluation

Validated Model for Chemical Screening

IATA Process

Run Assays
(Assays 1 - n, cytotoxicity)

- Choose at least 5 in vitro HTS assays that fit the criteria as described. Briefly, the assays chosen must probe diverse points in the AR pathway and use diverse assay reporting technologies and cell types.
- The possible combinations of 5 assays that can be used together are listed in Appendix.

Import Data and Run Mode

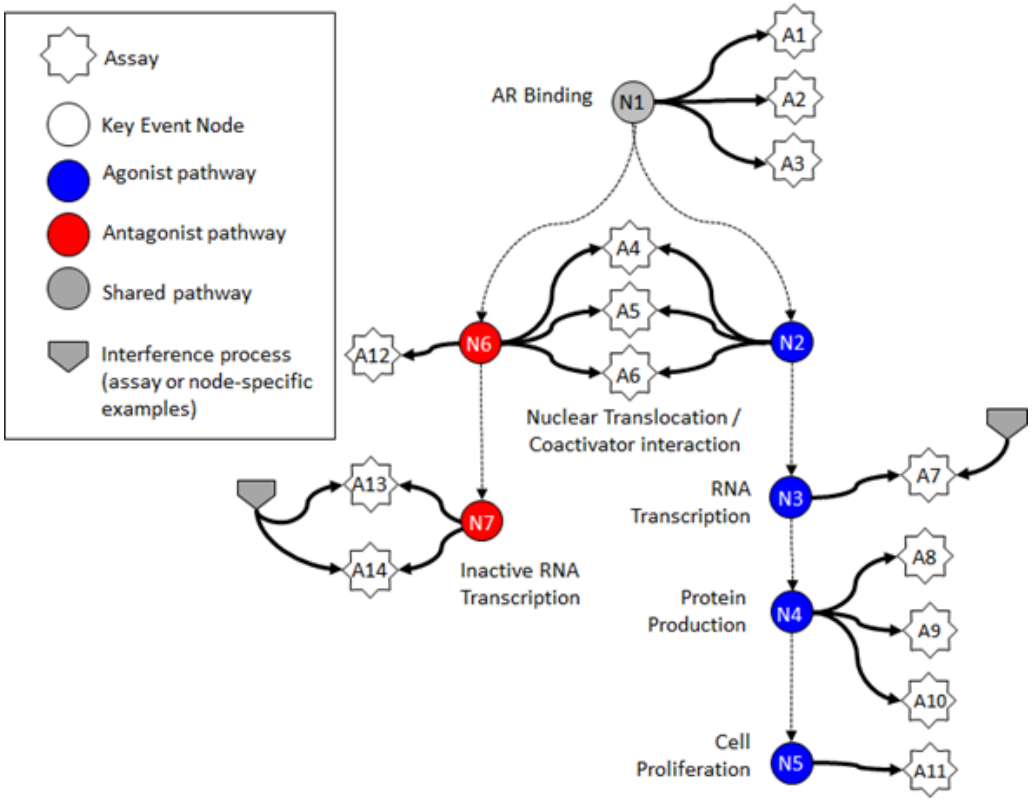
- The next step is to collect data from the assays into a format that the computational model's R-code can import and analyse. Ideally, this process will be performed automatically (electronically) to reduce the chance of user-input error.
- Run the biologically-based model and get an AUC value

Generate Report

- The report should conform to the usual report format of executive summary, methods, results, and discussion. The report should include the raw data to allow the regulatory agencies to analyze the data themselves. It should also include summary tables with the AUC and AC50 values. Figures can be included when needed. The report should also include the results from concurrent cytotoxicity assays.
- Any departure from the methodology of the AR pathway model as presented in this document must be thoroughly described along with the reason for the departure and the proposed impact on the screening results.

In Vitro Androgen Receptor Model

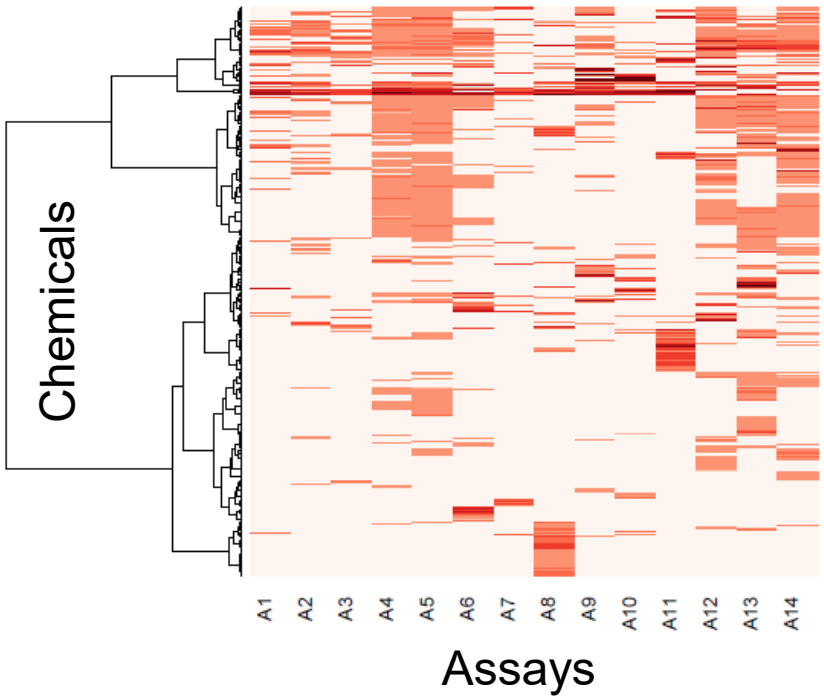
- No *in vitro* assay is perfect
  - Assay Interference
  - Noise
  - No xenobiotic metabolism
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- Use model to integrate assays
- Evaluate model against reference chemicals



Kleinstreuer et al: "Development and Validation of a Computational Model for Androgen Receptor Activity" (Chem Res Tox 2017)

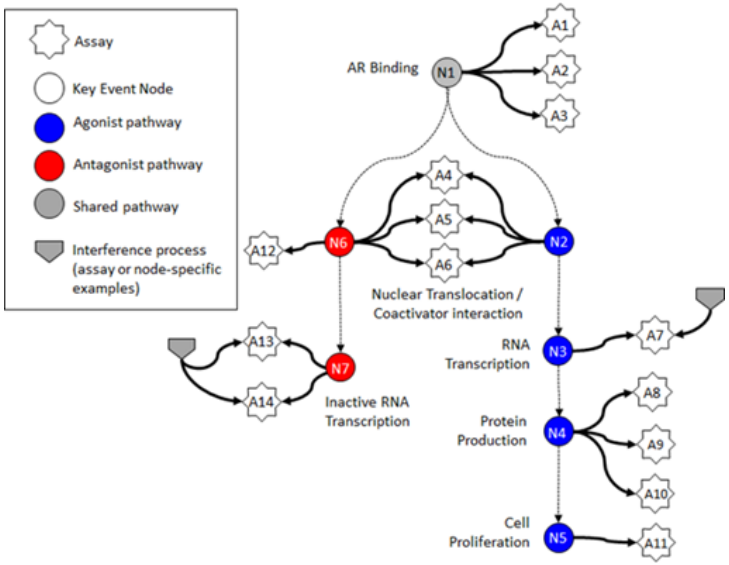
All *in vitro* assays have false positives and negatives

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



Much of this "noise" is reproducible
- "assay interference"
- Result of interaction of chemical with complex biology in the assay

Chemical universe is structurally diverse
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



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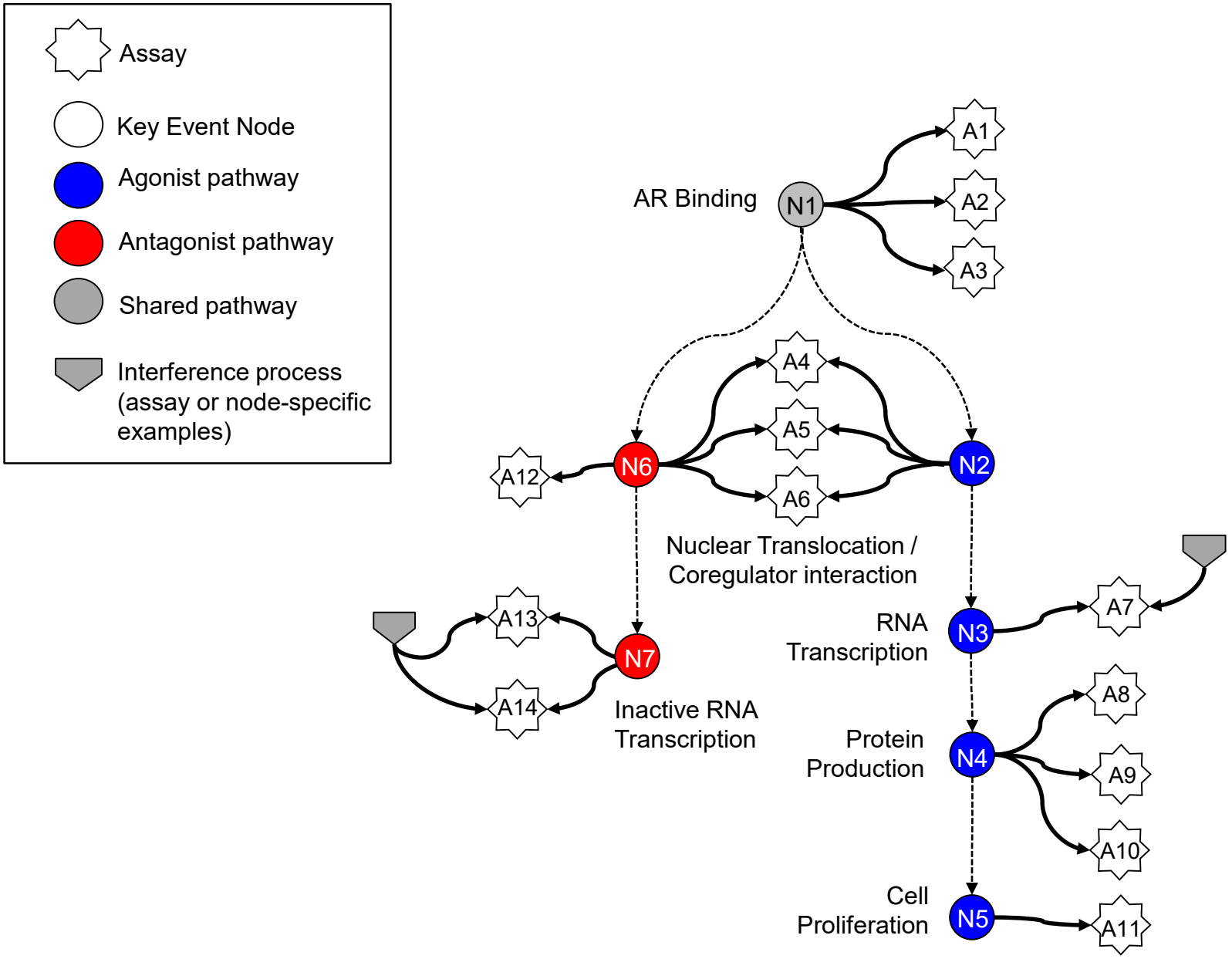
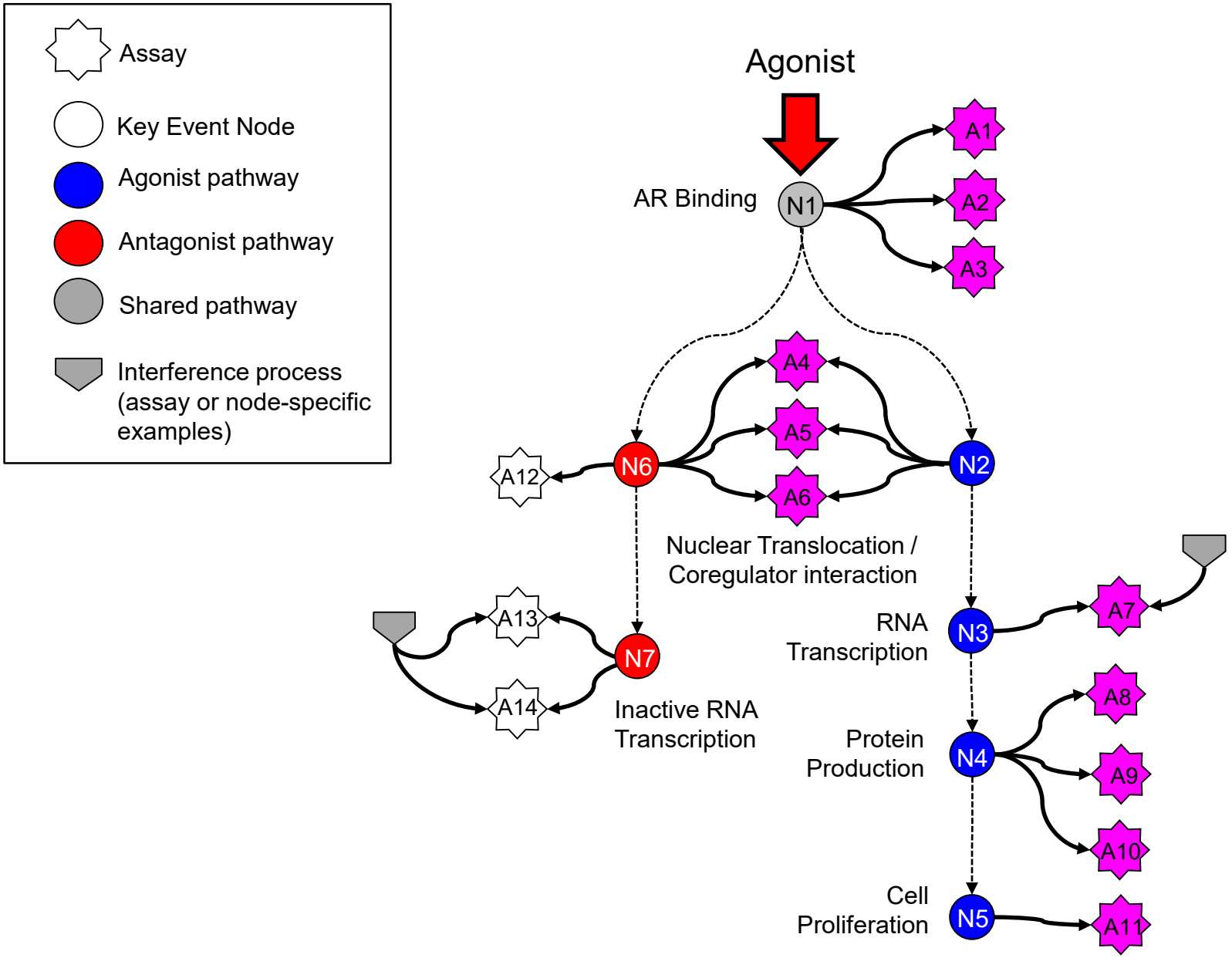
What Does the Model Do?

- 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


Assay
Key Event Node
Agonist pathway
Antagonist pathway
Shared pathway
Interference process (assay or node-specific examples)
AR Binding
N1
A1
A2
A3
A4
A5
A6
A12
N6
N2
Nuclear Translocation / Coregulator interaction
A13
N7
A14
Inactive RNA Transcription
RNA Transcription
N3
A7
Protein Production
N4
A8
A9
A10
Cell Proliferation
N5
A11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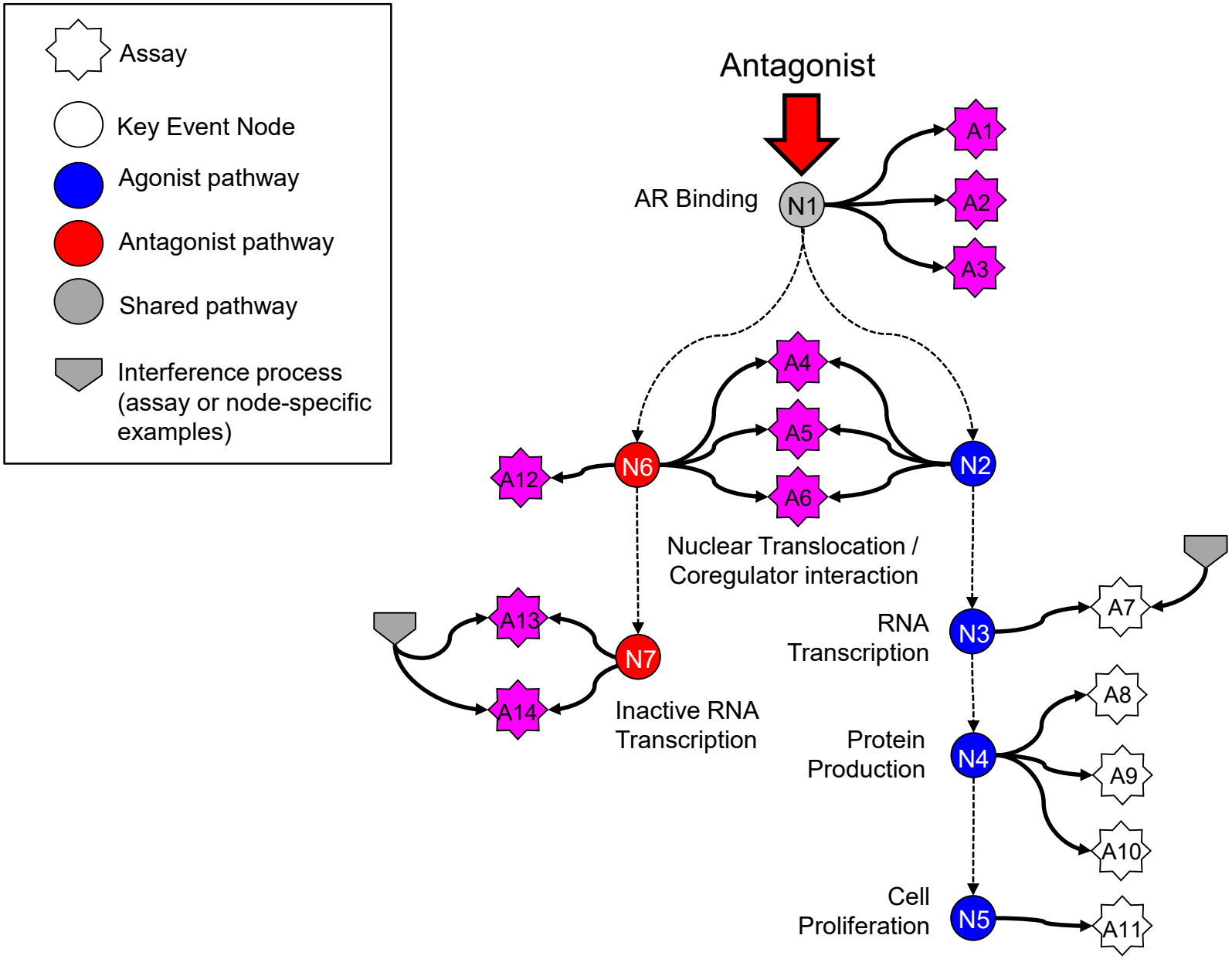
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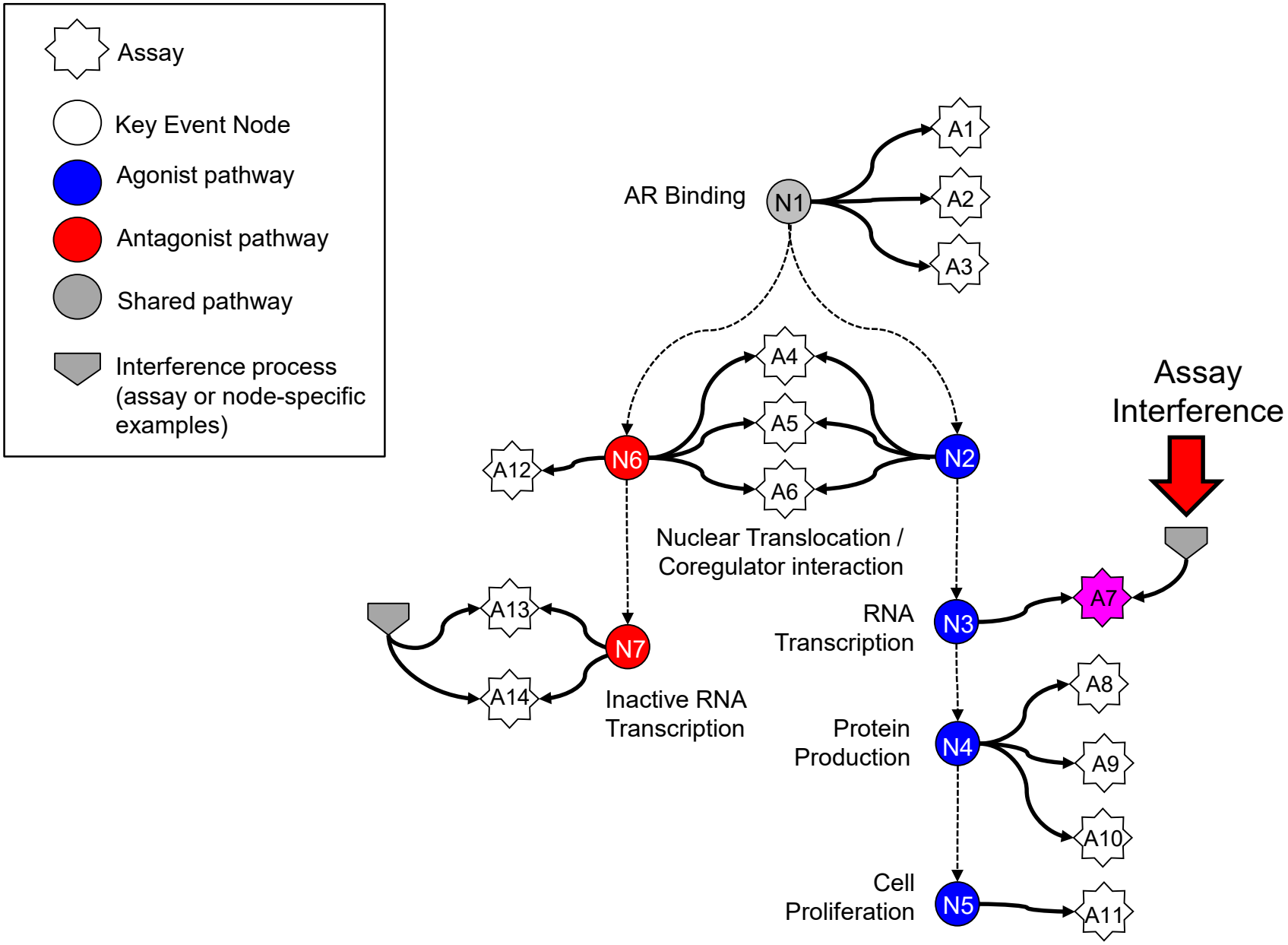
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A10
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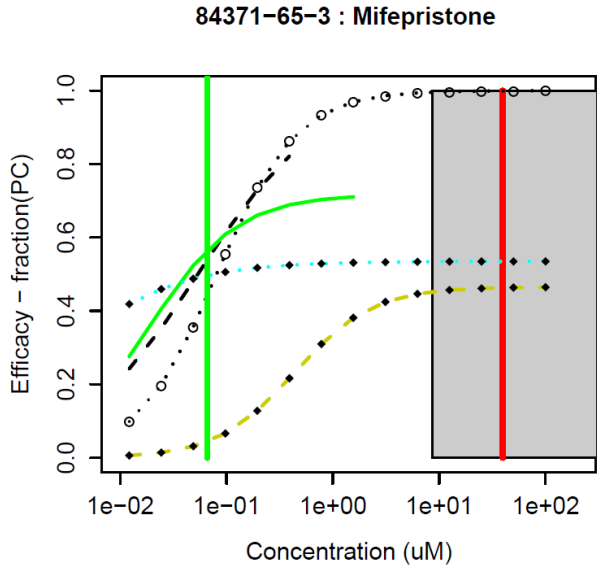
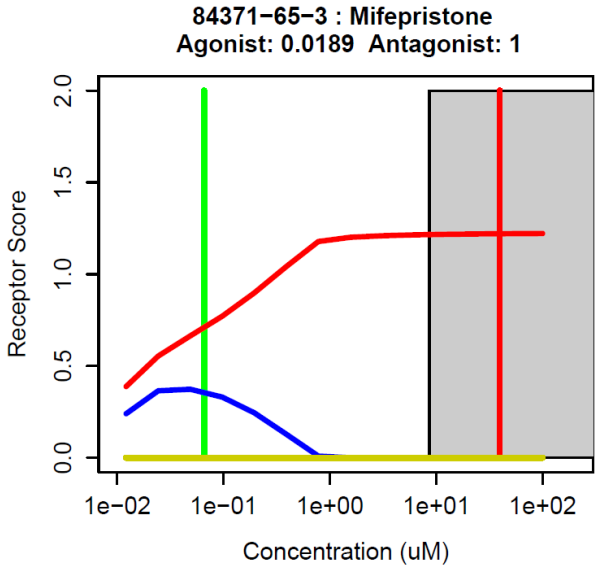
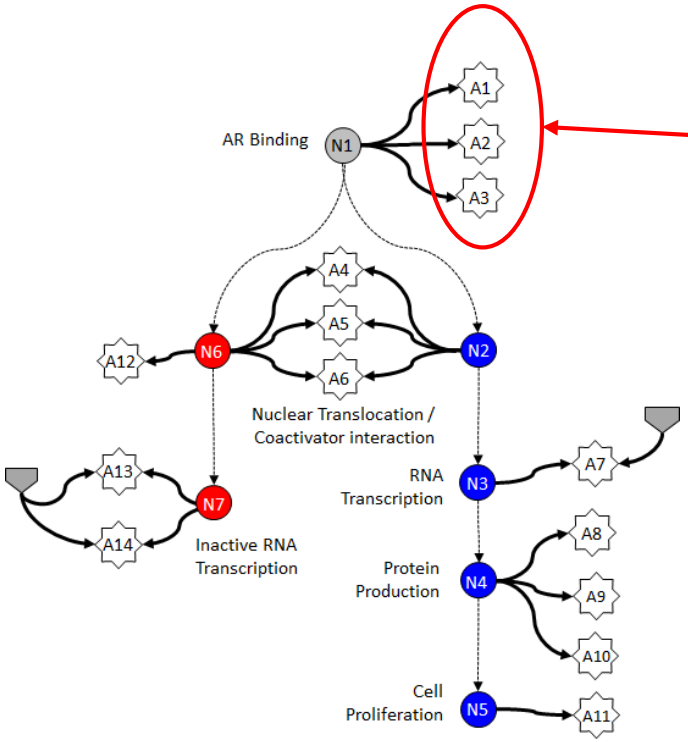
A11

Example chemicals: Observe quantitative uncertainty

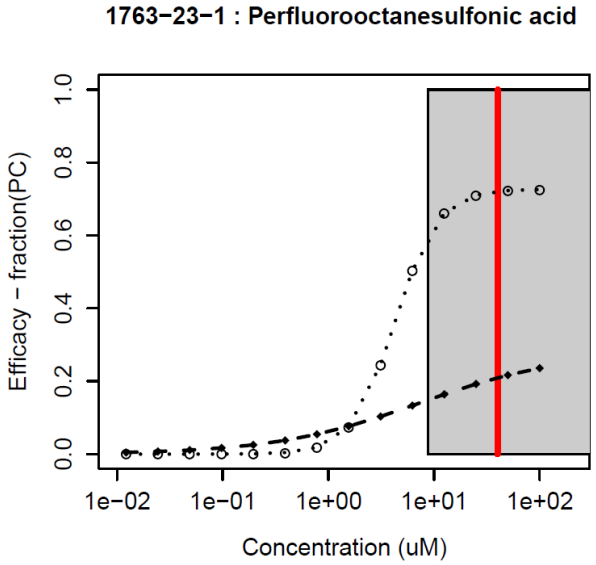
True Antagonist

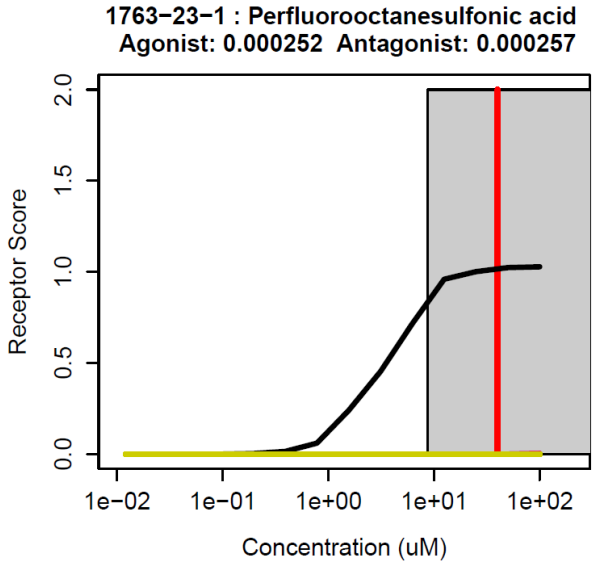






Assay Interference Example “R3”





Subset Model

- Assume that the “full model” (9 antagonist assays, 14 in full model) provides acceptable prediction of AR antagonist activity
  - Model based on detailed biology
  - Validated against in vitro reference chemicals that were developed for this model
- Build simple “subset models” using fewer assays
  - Validate against the full model over 1820 chemicals and in vitro reference chemicals
- Input to the model are assay-chemical concentration-response curves

Reference Chemicals

- No acceptable reference chemical set was available for AR activity
- Kleinstreuer et al. performed a systematic literature survey to identify potential reference chemicals
  - Chemicals were run in multiple labs in multiple assays of different cell types and readout technologies
  - Chemicals gave consistent results (positive and negative)
  - Positive chemicals spanned a range of potencies
  - Total of 28 antagonist mode reference chemicals identified
- Kleinstreuer et al: “Development and Validation of a Computational Model for Androgen Receptor Activity” (Chem Res Tox 2017)

IATA Process

Run Assays
(Assays 1 - n, cytotoxicity)

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- The possible combinations of 5 assays that can be used together are listed in Appendix.

Import Data and Run Mode

- The next step is to collect data from the assays into a format that the computational model's R-code can import and analyse. Ideally, this process will be performed automatically (electronically) to reduce the chance of user-input error.
- Run the biologically-based model and get an AUC value

Generate Report

- The report should conform to the usual report format of executive summary, methods, results, and discussion. The report should include the raw data to allow the regulatory agencies to analyze the data themselves. It should also include summary tables with the AUC and AC50 values. Figures can be included when needed. The report should also include the results from concurrent cytotoxicity assays.
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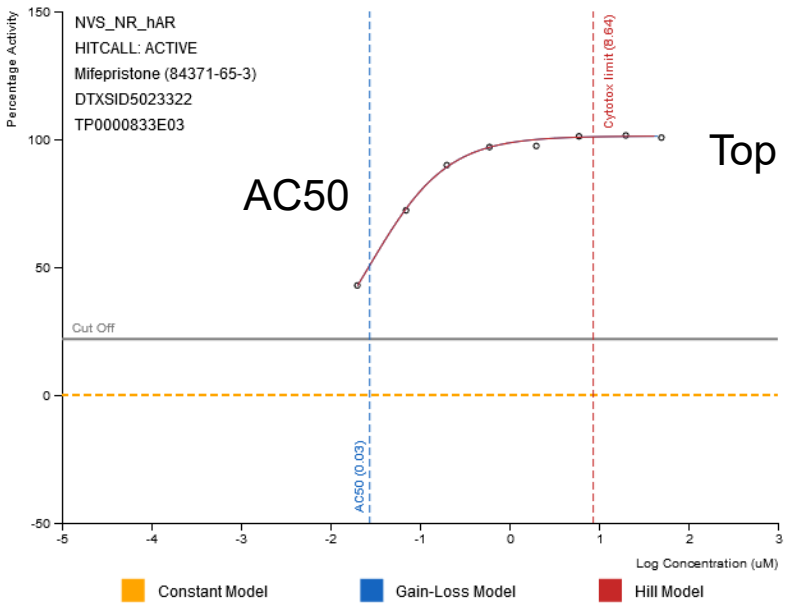
Subset Model Input

- Inputs to model are chemical-assay values: AC50 and Top
- Run assay in concentration-response mode
- Fit to model (e.g. Hill model)
- Calculate
  - AC50 (concentration at half maximum)
  - Top (top of the Hill curve)



Winning Model	Model	AIC	RMSE	Top	AC50	Slope
	Constant	105.28	89.9	-	-	-
	Gain-Loss	32.79	1	101.24	0.03	1
✓	Hill	28.79	1	101.24	0.03	1

Example curve for Mifepristone

Data from:

EPA CompTox Chemicals Dashboard

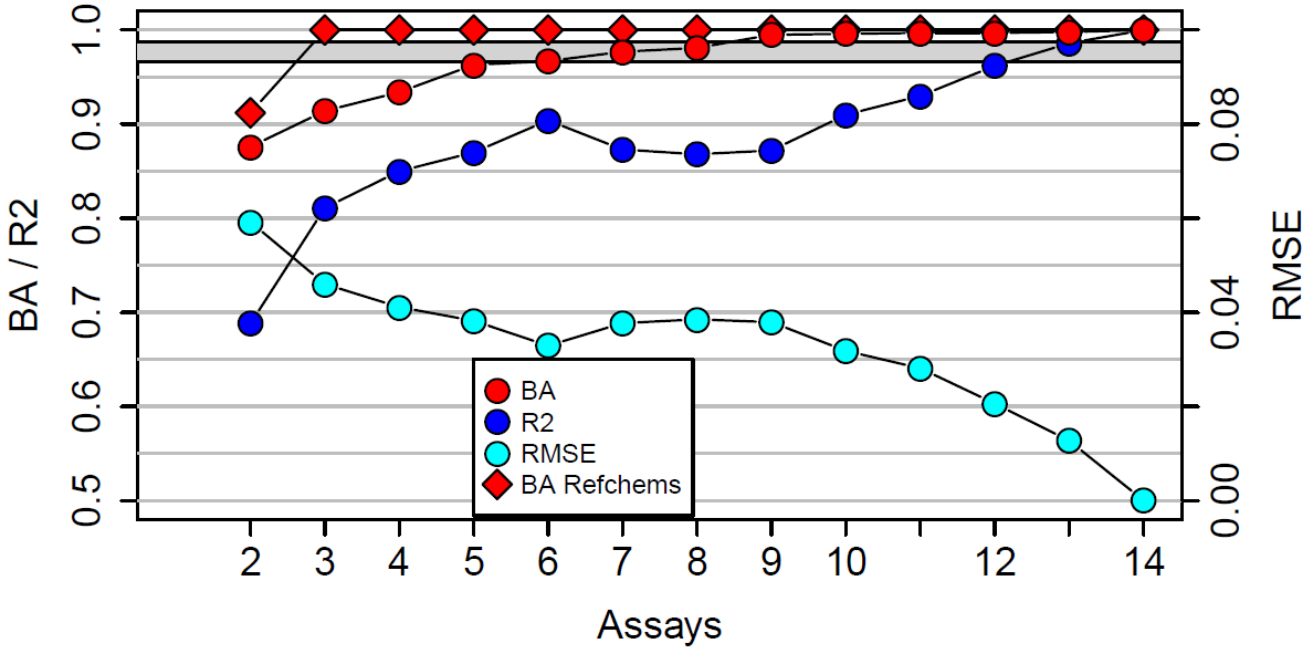
EPA United States Environmental Protection Agency

Building and Evaluating the Model

- For each set of assays (all combinations from 2 to 14), build the complete biologically-based AR model, including both agonist and antagonist activity
- Calculate several statistics on each subset pathway model
  - Sensitivity, specificity, balanced accuracy for all chemicals
  - Sensitivity, specificity, balanced accuracy for in vitro reference chemicals
    - Balanced accuracy is average of sensitivity and specificity
- Allows a user to select any model (i.e. any subset of assays) that provides high enough sensitivity, specificity, balanced accuracy

Statistical Results

Results for Antagonist Mode



Balanced Accuracy, R^2 and RMSE for all chemical and BA for in vitro reference chemicals

Observe that with as few as 5 assays, there are subset models where reference chemical balanced accuracy is perfect and other subset models where all-chemical balanced accuracy is above 95%

Assay Availability Issue

- Commercially available
  - Attagene (RNA-based transactivation assay). Company currently offers these assays
  - Novascreen (cell free binding assays) no longer offered by Novascreen, but other vendors can provide equivalent assays

- Not commercially available
  - Odyssey Thera (protein complementation assays). Company is out of business, no known commercial source of these assays; possible academic lab source
  - Tox21 (cell-based transactivation assays) These are produced by a US Government lab, and are not available as a service; cell line available
  - OECD TG 458 AR STTA

- Academic lab source for nuclear translocation/coactivator interaction

- Variants of these assays could be developed by independent laboratories
  - Specific aspects of assays may be patent protected, but basic technology generally is not
  - Cell lines are available

In Vivo Hershberger Assay Issue

- The in vivo Hershberger is used in the US as an adjunct to the AR transactivation assay to determine AR activity, however this IATA does not include comparison to the Hershberger assay nor validation against it for the following reasons:
  - While it is an OECD harmonised test guideline, the US is the only country that has a data requirement for the Hershberger.
  - The US is considering using the AR model discussed in this IATA for prioritizing chemicals or replacing the AR TA and Hershberger assays.
  - Most environmental chemicals of concern are expected to act as anti-androgens, but the execution of the assay in the anti-androgenic mode requires co-administration of testosterone propionate (TP) with the test chemical. TP is a liver inducer in its own right, so it is quite possible that co-administration of test chemical may further contribute to hepatic liver induction, thus increasing TP clearance and resulting in an "anti-androgenic" effect simply because the activating androgen is being cleared.
  - In practice, it has been difficult to reproduce results for individual chemicals and difficult to execute in general.
  - It is a 10 day assay, which is probably too long to evaluate unconfounded "mechanistic effects" in an animal with a functional liver.

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Summary of Proposed Case Study

- Outline the curation of lists of reference chemicals for *in vitro* and *in vivo* AR activity and the uncertainty and variability associated with the guideline studies
- Integrate results from multiple *in vitro* and *in silico* assays using pathway-based AR computational model as a IATA
- Evaluate performance of the IATA using the curated lists of reference chemicals
- Evaluate issues with metabolism
- Characterize the uncertainty associated with the *in vitro* assays and computational model
- Demonstrate equivalent performance for a subset of *in vitro* assays
- Discuss application to regulatory decisions

Acknowledgements

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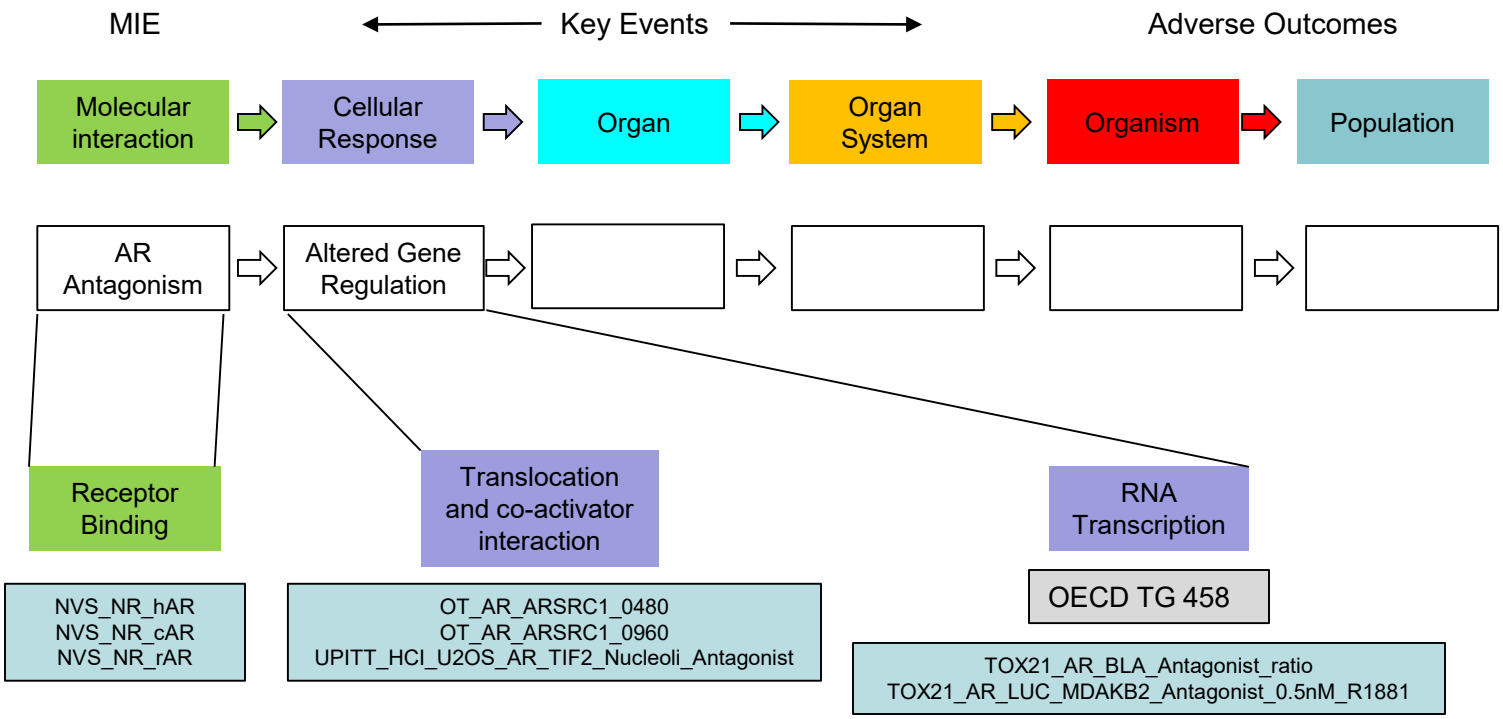
  - Dr. Nicole Kleinstreuer, NICEATM


MIE
Key Events
Adverse Outcomes
Molecular interaction
Cellular Response
Organ
Organ System
Organism
Population
AR Antagonism
Altered Gene Regulation
Receptor Binding
Translocation and co-activator interaction
RNA Transcription
NVS_NR_hAR
NVS_NR_cAR
NVS_NR_rAR
OT_AR_ARSRC1_0480
OT_AR_ARSRC1_0960
UPITT_HCI_U2OS_AR_TIF2_Nucleoli_Antagonist
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