Case Study on the Use of an Integrated Approach to Testing and Assessment for Identifying Estrogen Receptor Active Chemicals

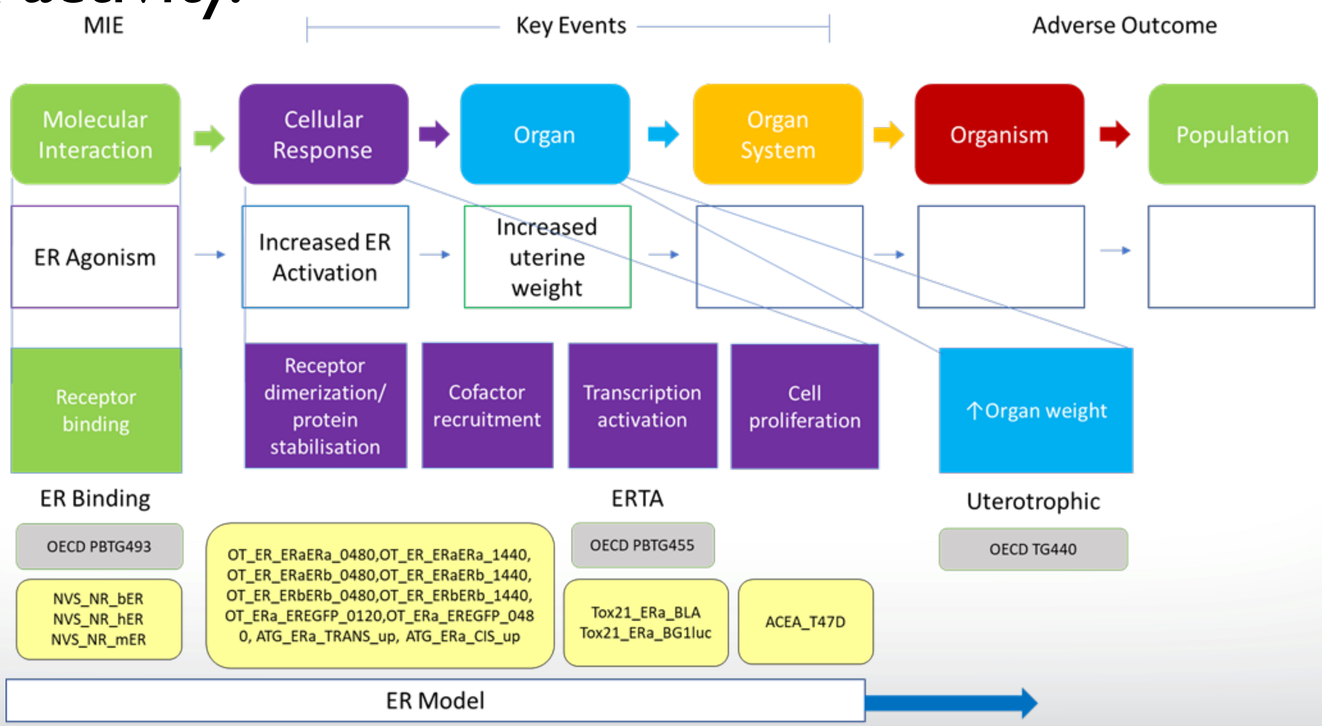
Dr. Maureen R. Gwinn (gwinn.maureen@epa.gov)
U.S. Environmental Protection Agency
Office of Research and Development
Washington, DC

Intended Application

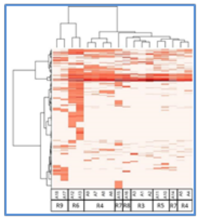
- The intended application of this IATA is for
  - screening of environmental chemicals based on their ER agonist activity
  - determining whether further evaluation of endocrine-related activity in higher tier in vivo tests (e.g., female pubertal assay, two generation reproductive toxicity study) is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

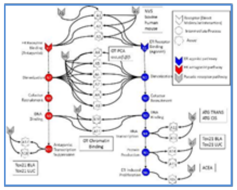
Purpose

- To use a combination of 16 in vitro high throughput screening (HTS) assays and a computational model for estrogen receptor (ER) agonist activity, as an alternative to low and medium throughput in vitro and in vivo tests for ER activity.



Overall Approach





Internal

ER High-Throughput Screening Data

ER Pathway Computational Model

External

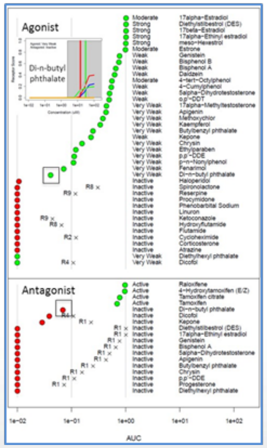
In vitro reference chemicals

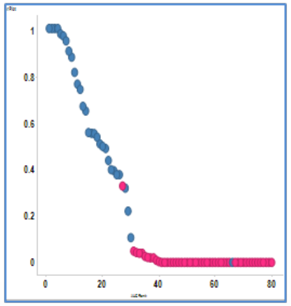
BETTER POLICIES FOR BETTER LIVES

In vivo reference chemicals

Model Performance Evaluation

Validated Model for Chemical Screening





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 activity 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)

Summary of Proposed Case Study

- Outlines the curation of lists of reference chemicals for *in vitro* and *in vivo* ER activity
- Integrates results from multiple *in vitro* assays using pathway-based ER computational model as an IATA
- Evaluates performance of the IATA 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

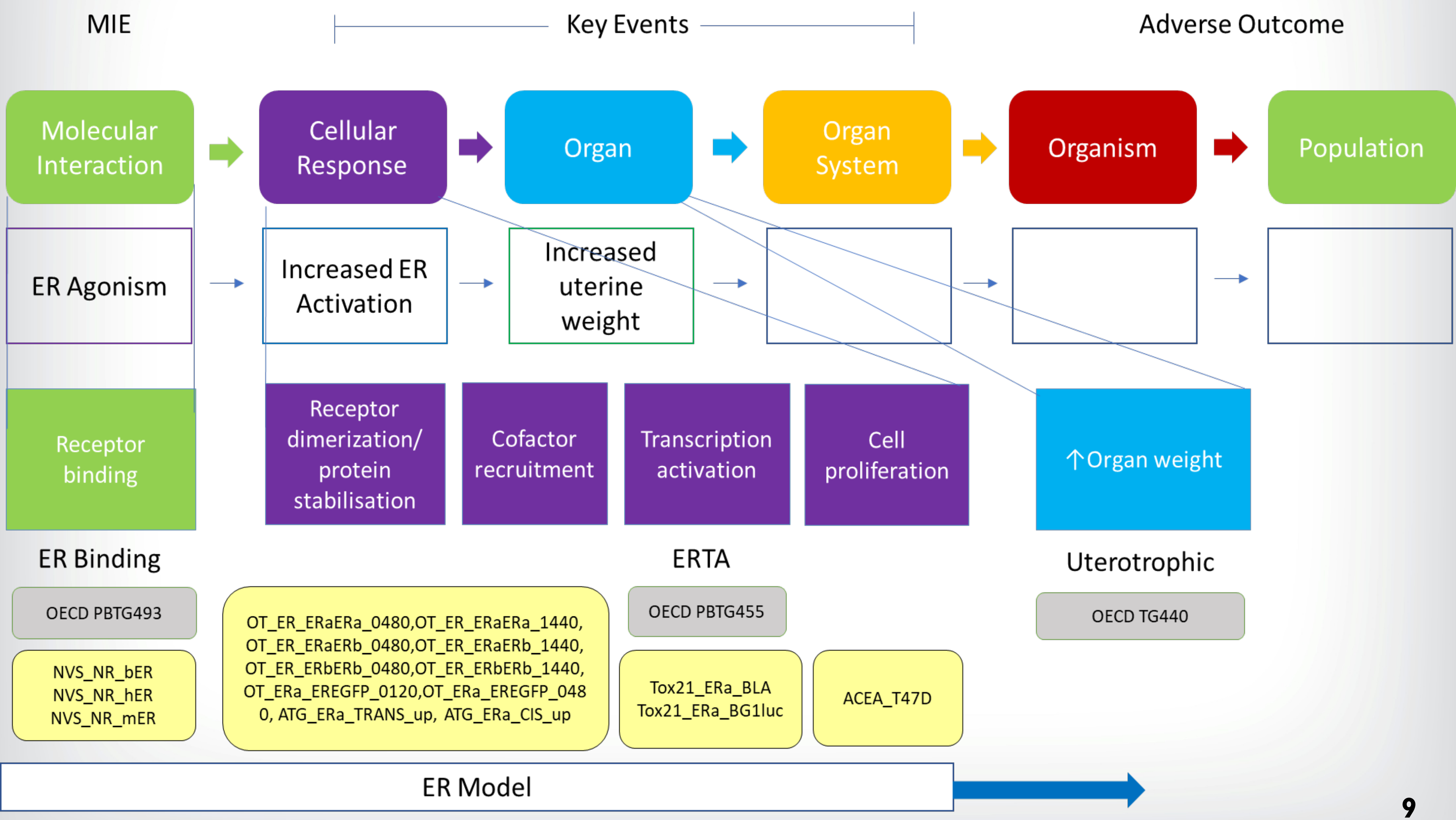
Overview of Main Reviewer Comments

- Comments largely requested clarification on various aspects of the case study:
  - the ‘defined approach’ presented
  - the pathway and key events that are assayed by this approach
  - the process of the approach
  - the use of the subset of in vitro assays

The Defined Approach

- Further clarified that this is an Integrated Approach to Testing and Assessment (IATA) that has elements of a defined approach, but is being submitted as an IATA case study and not a final defined approach.
  - Learning from reviewer input from member countries on this IATA, we plan on a future submission of this as a defined approach via the standard project submission form (SPSF) process to the Working Group of the National Coordinators of the Test Guidelines Programme (WNT).

Estrogen Receptor Pathway



IATA Process

Run Assays

(Assays 1 - n, cytotoxicity)

- Choose at least 4 in vitro HTS assays that fit the criteria as described. Briefly, the assays chosen must probe diverse points in the ER pathway and use diverse assay reporting technologies and cell types.
- The possible combinations of 4 assays that can be used together are listed in Annex III. More than 4 assays can be chosen, in which case the results of all chosen assays must be modelled and the results reported. Concurrently evaluate a phenotypic response, cellular proliferation.

Import Data and Run Model

- 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.
- Although useful, advanced computational expertise is not necessary to run the model. For example, the formula used for the subset model analysis could be implemented in a spreadsheet so limited computational expertise required.

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 the cytotoxicity assay.
- Any departure from the methodology of the ER 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.

Use of a Subset Model

- The IATA has been clarified further to demonstrate that:
  - a subset of as few as 4 assays as an alternative approach to the current guideline
  - the subset of assays can be any assays that fit into specific criteria as described
    - interrogates different points on the ER pathway
    - incorporate different technologies
- Included in this case study is an annex that describes the 9 subset models with 7 or fewer assays that achieve ≥94% balanced accuracy for all chemicals and the in vitro and in vivo reference chemical sets. (Annex III).
- Further articulated the benefits of using a subset of assays in the IATA, specifically the flexibility it gives users by allowing the use of any assays that fit the described criteria.

Additional comments on presentation of information

- We have addressed in the document the following reviewer requests for changes to:
  - the details of the in vitro assays used
  - include more detailed figure descriptions
  - create an uncertainty table similar to that used in other case studies
- Reviewers also requested combination of specific sections
  - these changes were not completed, but clarification was added to sections to address specific issues
  - the authors have requested this be discussed at the meeting as overarching issues with the template

Acknowledgements

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