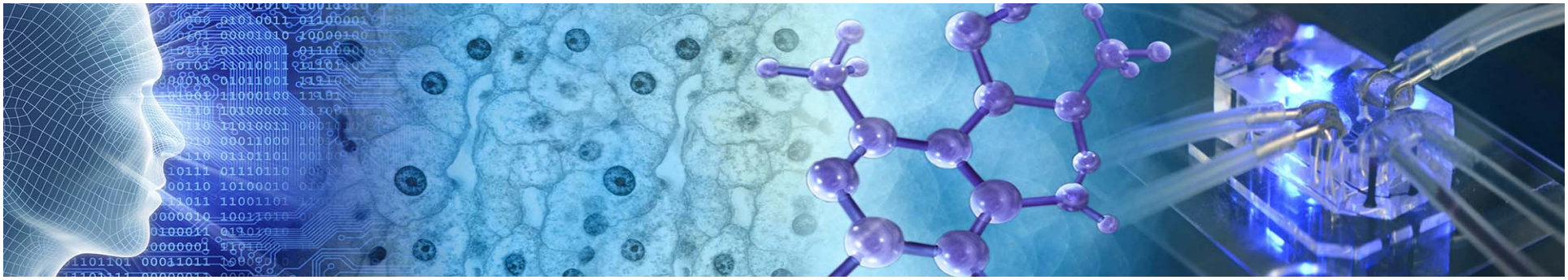
NTP
National Toxicology Program



Case Study 1
Predictive Modeling of Endocrine Disruption Pathways

Nicole Kleinstreuer, PhD
Deputy Director, NICEATM
National Toxicology Program, National Institute of Environmental Health Sciences
PI, Computational Toxicology Group, DIR/BCBB

NAS Workshop on Evidence Integration in Chemical Assessments
4th June, 2019

NICEATM

National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM), organized as an office under the NTP Division, part of NIEHS

National Toxicology Program
NTP

UNITED STATES
ICCVAM
Advancing Alternatives to Animal Testing

ICCVAM

- Interagency Coordinating Committee for the Validation of Alternative Methods
- H.R. 4281 (106th): ICCVAM Authorization Act of 2000
- To establish, wherever feasible, guidelines, recommendations, and regulations that promote the regulatory acceptance of new and revised toxicological tests that protect human and animal health and the environment while reducing, refining, or replacing animal tests and ensuring human safety and product effectiveness.

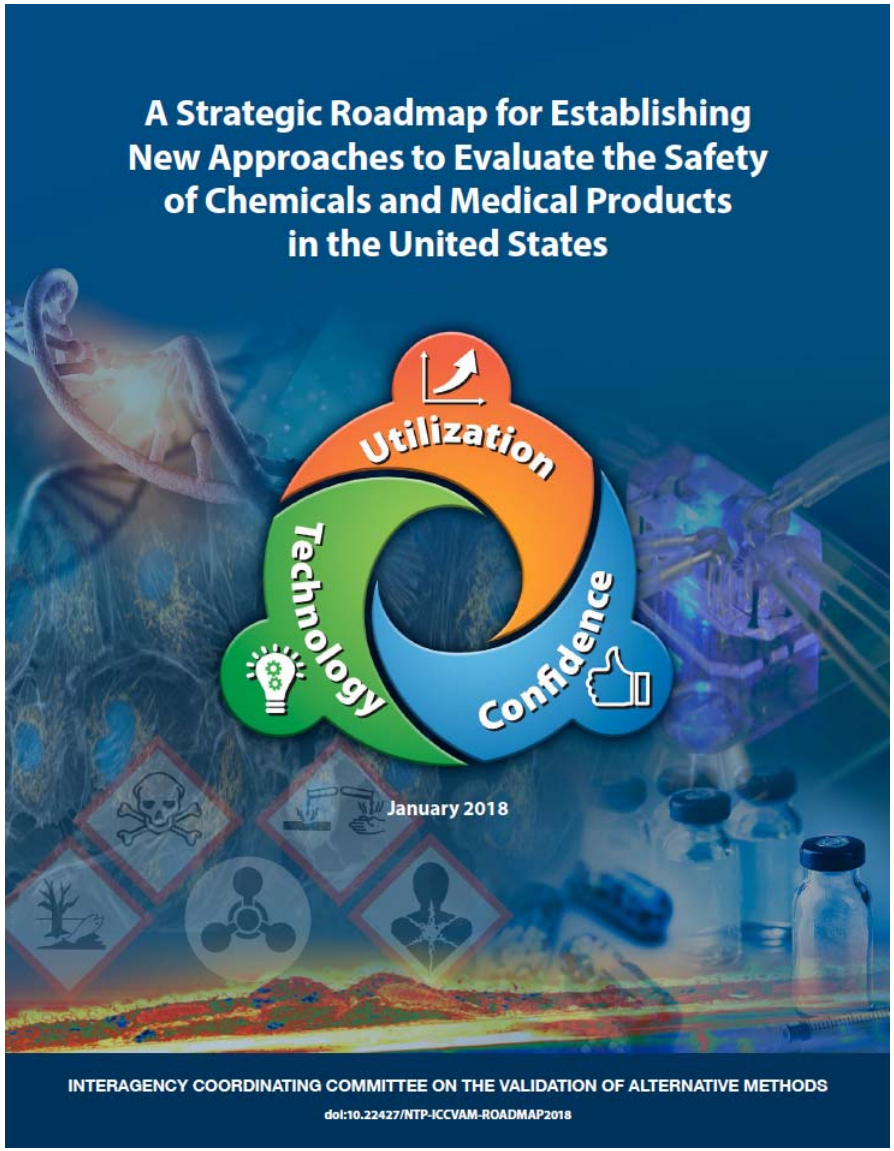
7 Regulatory Agencies

Consumer Product Safety Commission
Department of Agriculture
Department of the Interior
Department of Transportation
Environmental Protection Agency
Food and Drug Administration
Occupational Safety and Health Administration

9 Research Agencies

Agency for Toxic Substances and Disease Registry
National Institute for Occupational Safety and Health
National Cancer Institute
National Institute of Environmental Health Sciences
National Library of Medicine
National Institutes of Health
Department of Defense
Department of Energy
National Institute of Standards and Technology

- Other participants include: NCATS , Tox21 Representatives



https://ntp.niehs.nih.gov/go/natl-strategy

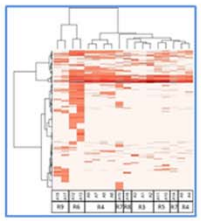
Tox21: From Assays to Pathways

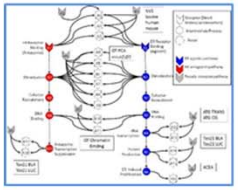
- Identify targets or pathways linked to toxicity/adverse outcomes
- Run corresponding high-throughput screening (HTS) or *in vitro* assays on thousands of chemicals
- Develop predictive systems models: *in silico/in vitro → in vivo*
- Use predictive models (qualitative):
  - Prioritize chemicals for targeted testing
  - Suggest / distinguish possible AOPs
- Use predictive models (quantitative):
  - Screen chemicals for hazard
  - Green chemistry design

http://www.ncats.nih.gov/

NICEATM provides **computational toxicology** and **validation** support to Tox21

Endocrine Project Workflow





Internal

ER High-Throughput Screening Data

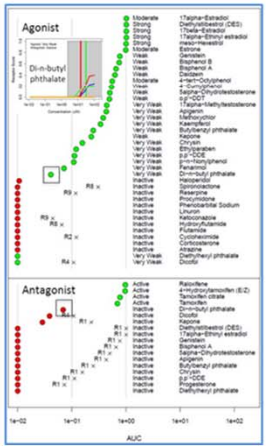
ER Pathway Computational Model

External

In vitro reference chemicals

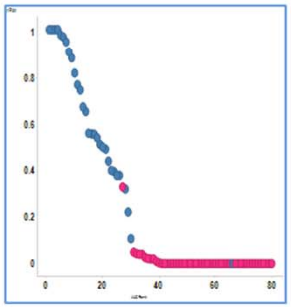
OECD
BETTER POLICIES FOR BETTER LIVES

In vivo reference chemicals



Model Performance Evaluation

Validated Model for Chemical Screening



Endocrine Disruptor Screening Program

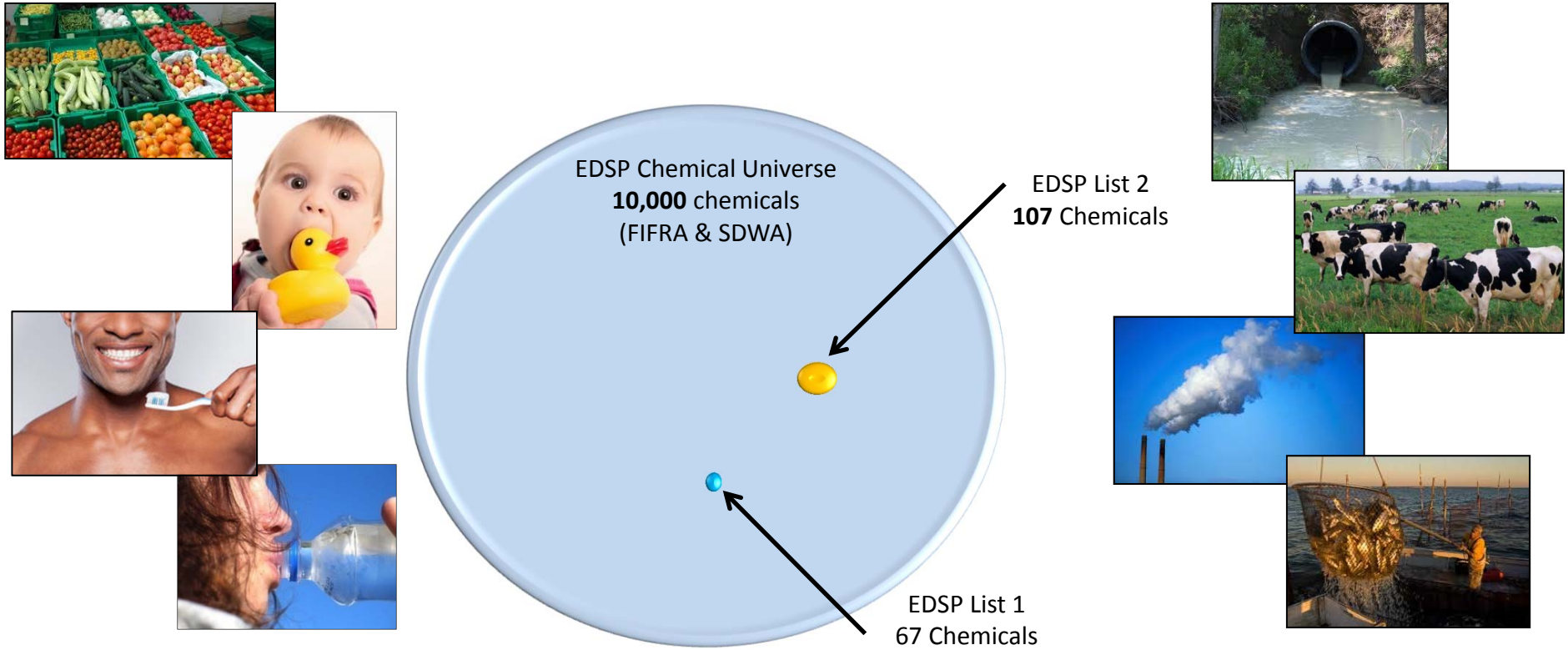
- Concern over environmental chemical disruption of endocrine hormone signaling (e.g. reproductive and developmental consequences, contribution to chronic disease, metabolic syndrome)

- Congressionally mandated, multiple EDSP testing tiers

- EDSP Tier 1 Testing: for the purposes of prioritization and screening, identify chemicals with the potential to disrupt estrogen, androgen, or thyroid hormone receptor signaling.

- There is a mismatch between resources needed for EDSP Tier 1 testing and the number of chemicals to be tested
  - 10-30,000 chemicals in EDSP Universe
  - ~$1M per chemical for Tier 1
    - 11 low-throughput & animal based tests
  - 50-100 year backlog

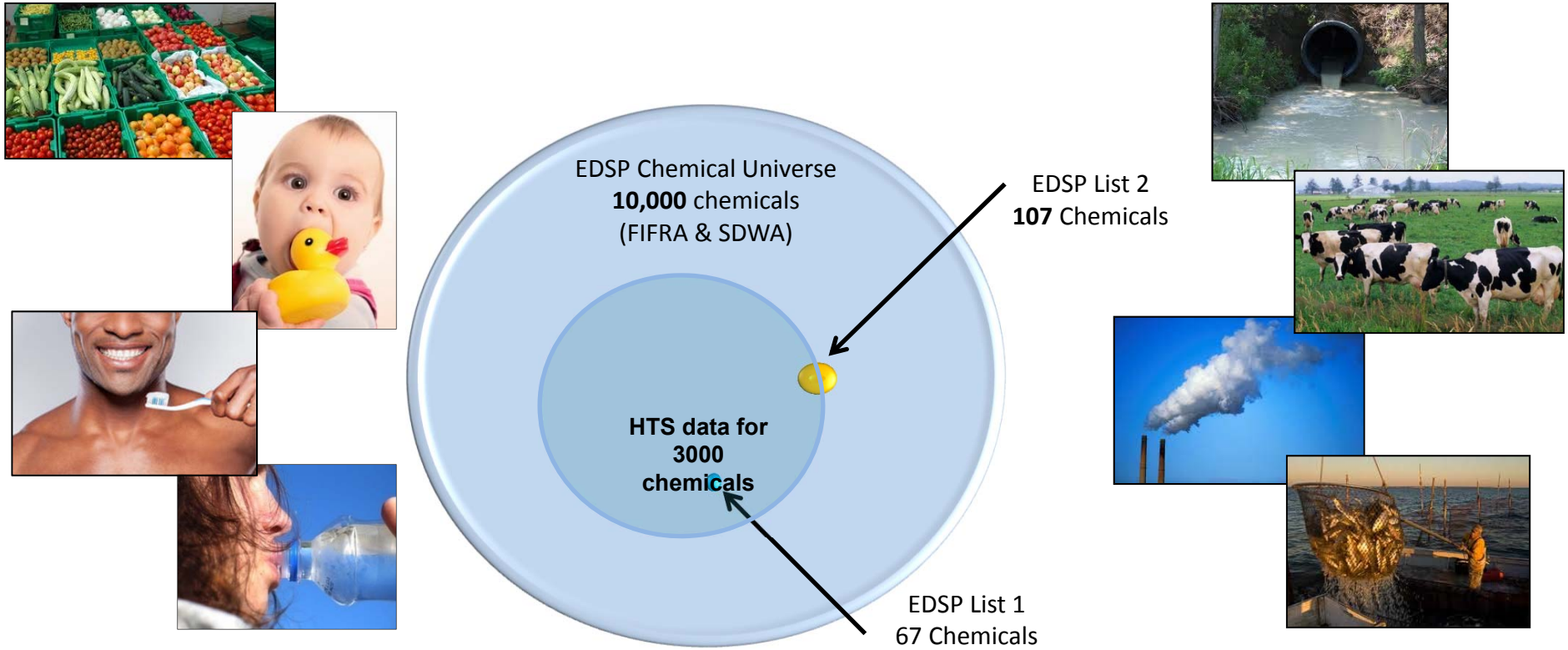
Evolution of the EDSP



New Approach: **EDSP + Tox21 = EDSP21**

- Pathway-based predictive models
- Multiple high-throughput *in vitro* assays
- Validate to replace selected Tier 1 screening assays

Evolution of the EDSP



New Approach: **EDSP + Tox21 = EDSP21**

- Pathway-based predictive models
- Multiple high-throughput *in vitro* assays
- Validate to replace selected Tier 1 screening assays

Validation: Fit for Purpose

OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Validation is a process by which the reliability and relevance of a test method are established for a specific purpose.

EDSP Tier 1

For the purposes of prioritization and screening, identify chemicals with the potential to disrupt estrogen, androgen, or thyroid receptor signaling.

OECD
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Validation: Performance Based

OECD GD 34, Validation and International Acceptance of New or Updated Test Methods

Relevance and reliability should be characterized against data generated with a list of reference chemicals tested in the original method accepted by regulatory agencies.

Reference chemicals: Chemicals selected for use in the validation process, for which responses in the *in vitro* or *in vivo* reference test system or the species of interest are already known.

OECD
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Performance Based Validation Approach

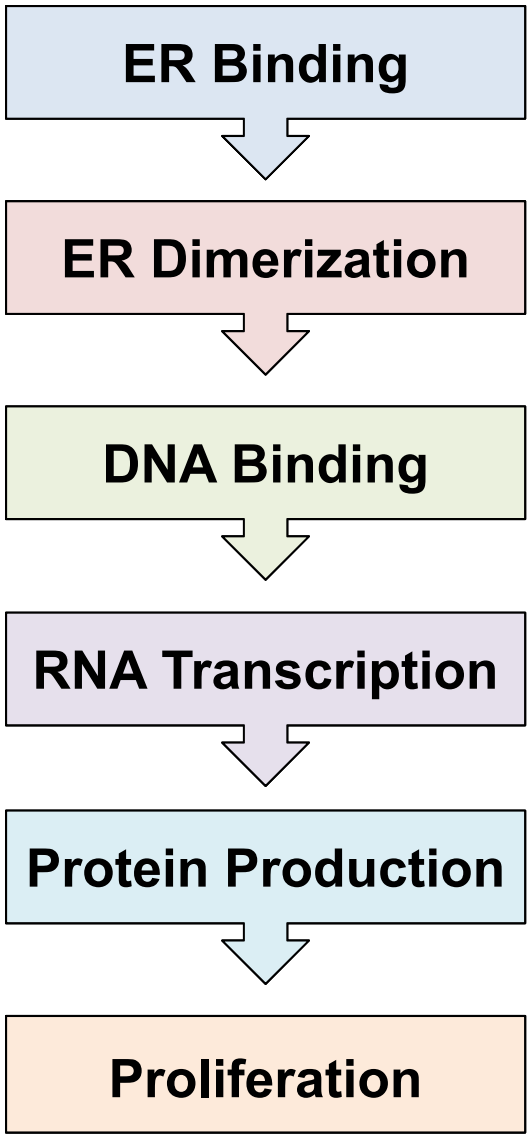
Estrogen Receptor Pathway Model: Fit for Purpose

Judson et al. 2015, Tox Sci: “Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor"

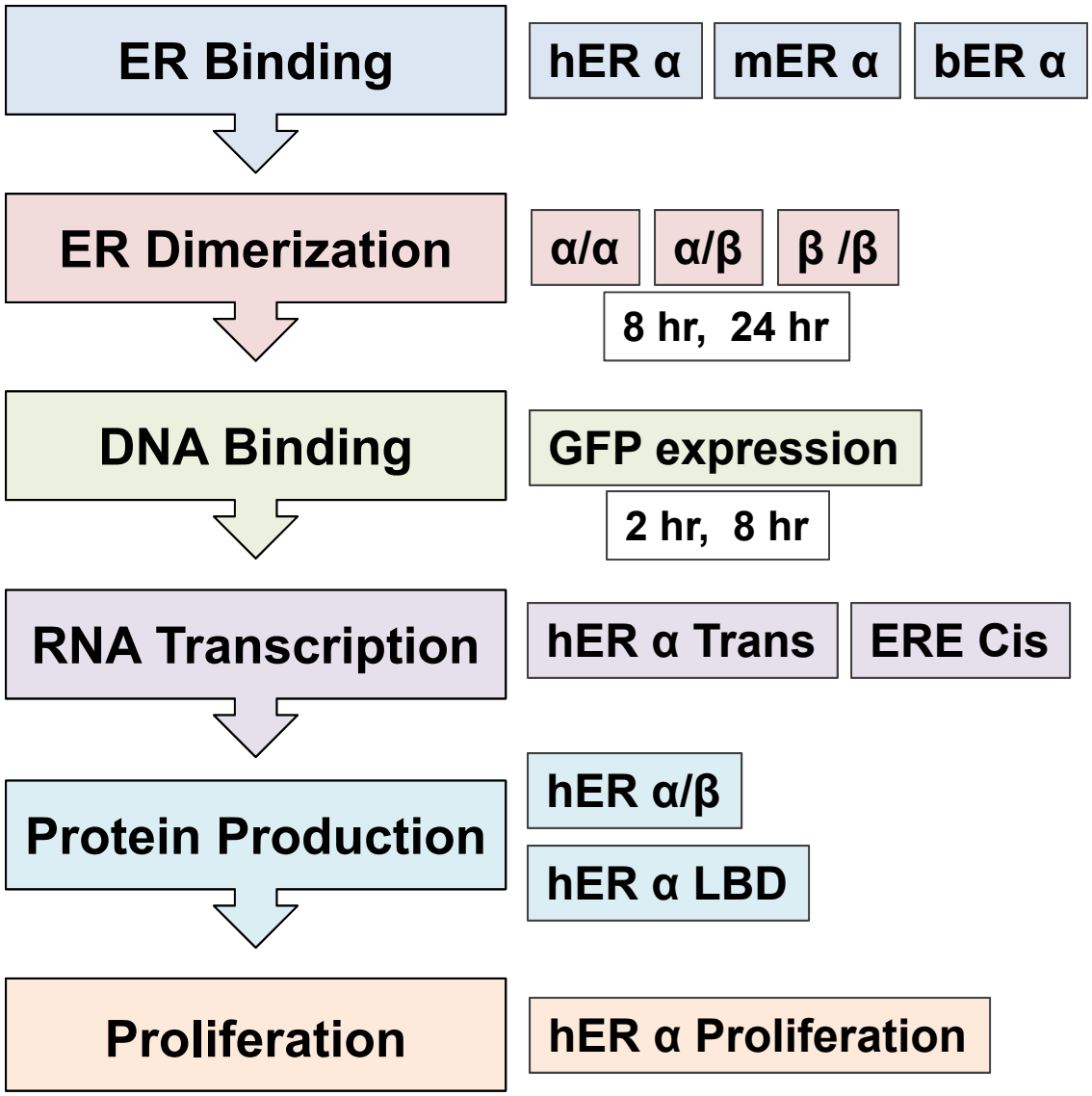
Kleinstreuer et al. 2015, EHP: “A Curated Database of Rodent Uterotrophic Bioactivity"

Browne et al. 2015, ES&T: “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model"

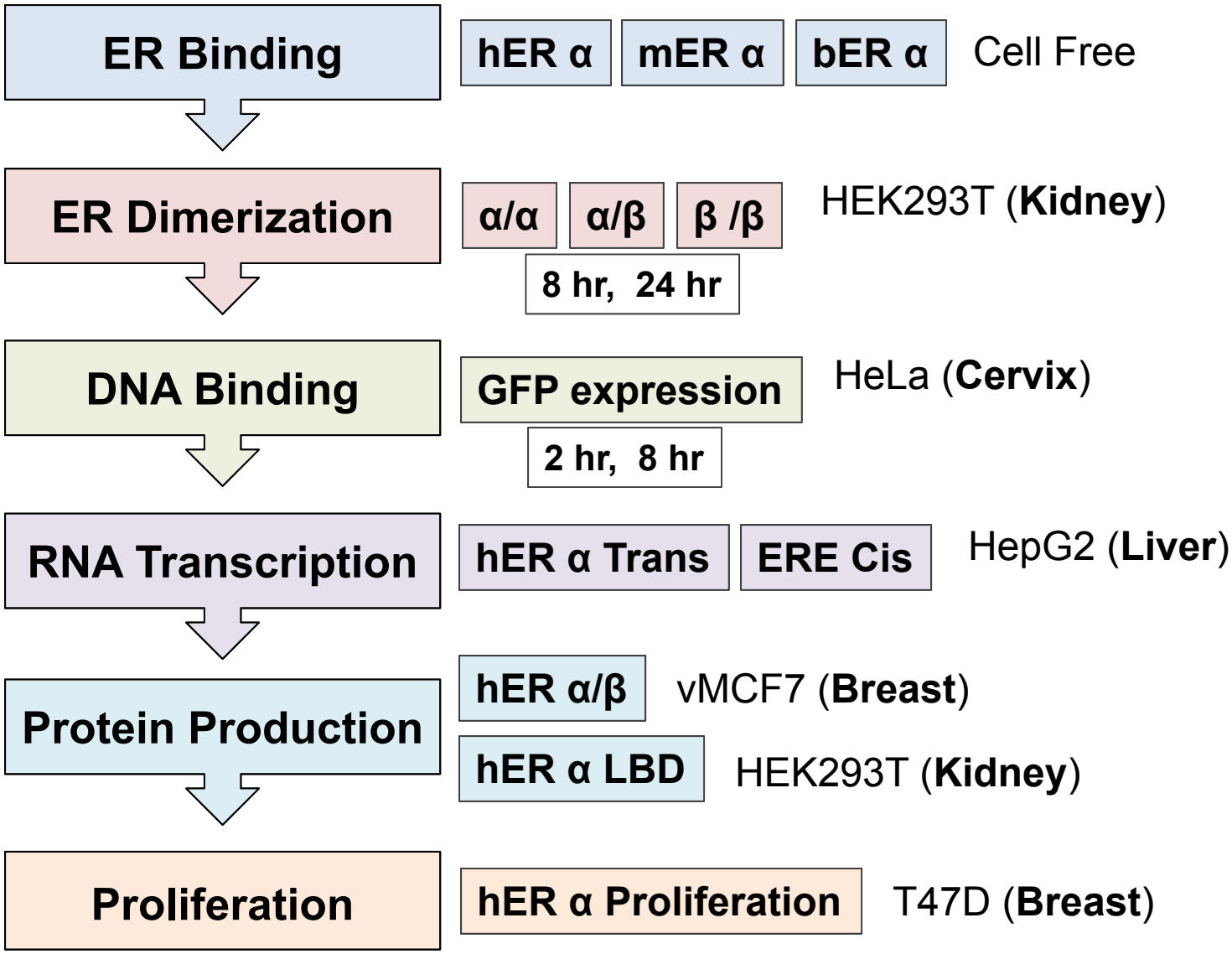
Estrogen Receptor Signaling Assays



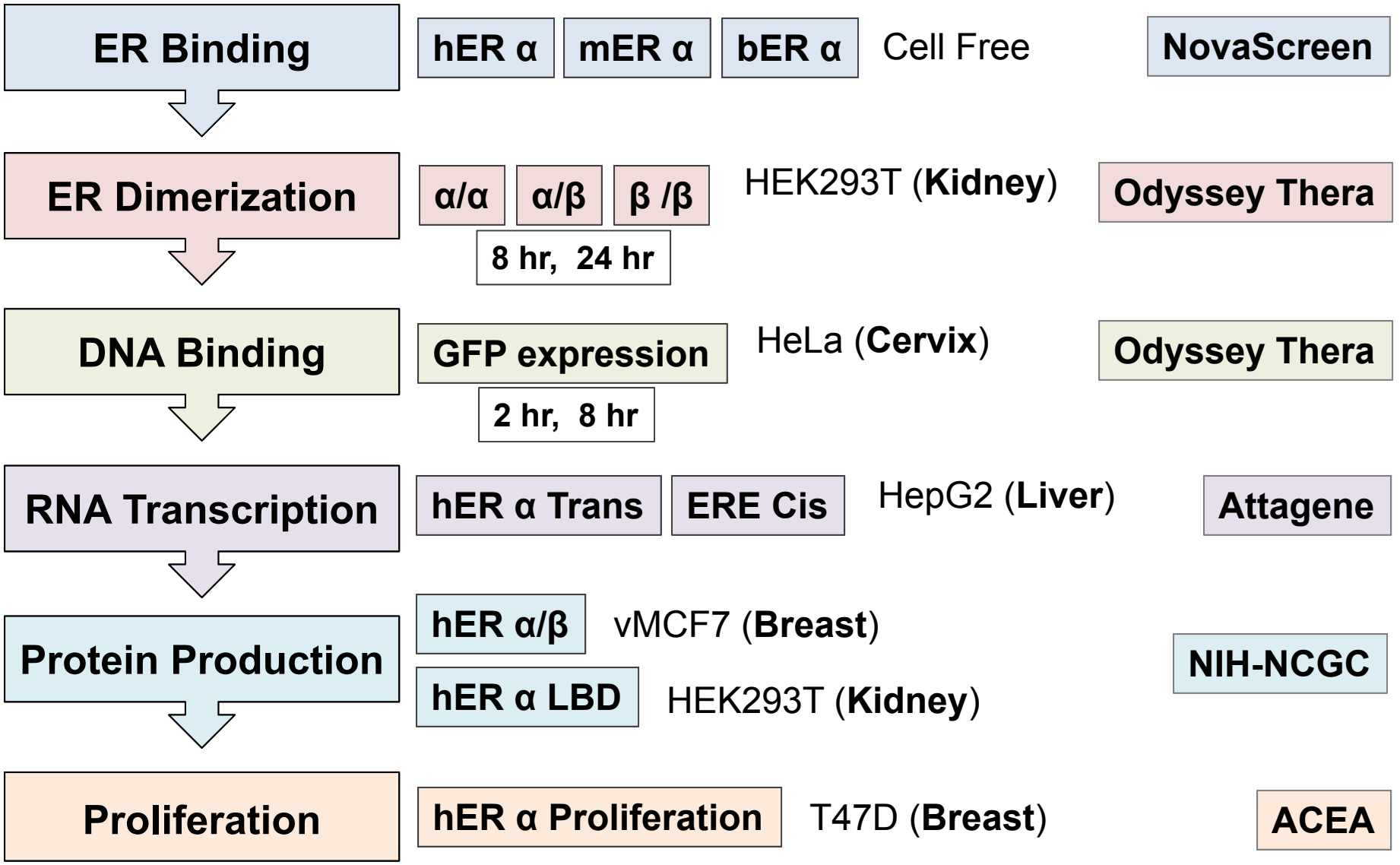
Estrogen Receptor Signaling Assays



Estrogen Receptor Signaling Assays



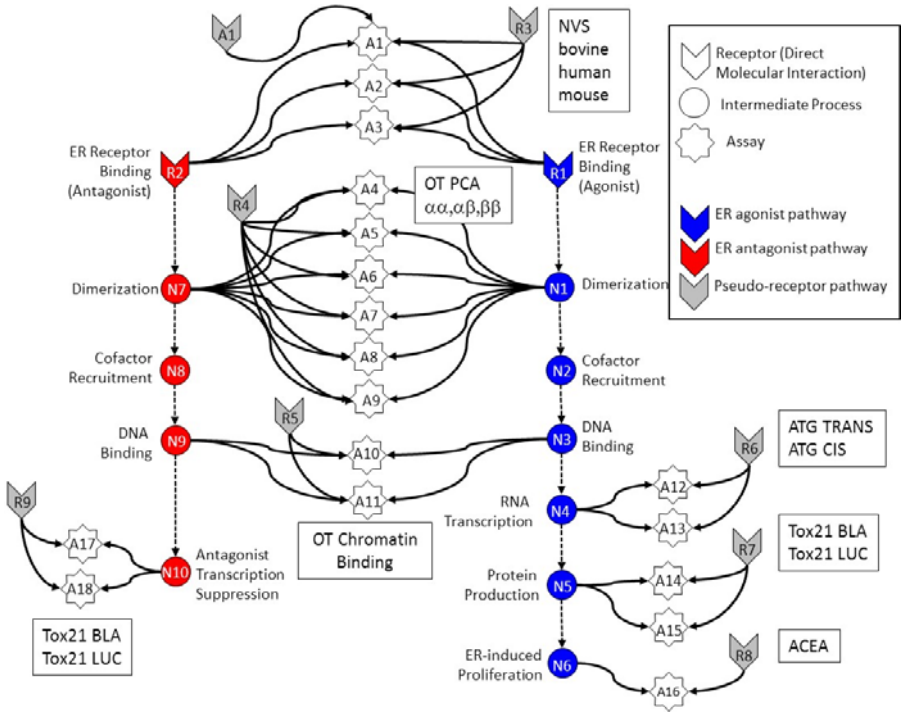
Estrogen Receptor Signaling Assays



Tox21/ToxCast ER Pathway Model

Combine results from multiple ToxCast in vitro assays

- Orthogonal assays on pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use mathematical model to integrate assays



- For each chemical, the model summarizes results from all assays with a composite dose-response curve, which is used to calculate an AUC relative to 17β-estradiol

Judson et al. 2015 Tox Sci

Mathematical Model

$$A_i = \sum_j F_{ij} R_j$$

***A**_i* is the efficacy of the assay at a given concentration
***R**_j* is the "true" efficacy which is unobservable
F links receptors to assays

$$\varepsilon^2 = \sum_i (A_i^{pred} - A_i^{meas})^2 + penalty(\vec{R})$$

Solve a constrained least-squares problem to minimize difference between the measured and predicted assay values

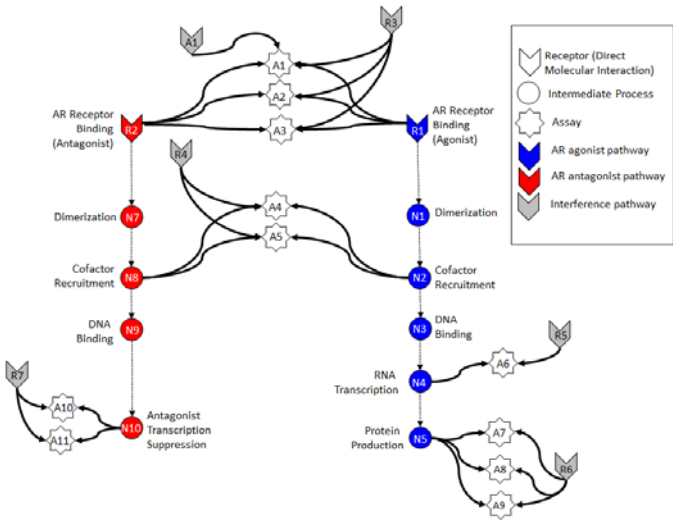
$$A_i^{pred} \in [1,0]$$

$$penalty(\vec{R}) = \alpha \frac{SR^2}{SR^2 + SR_0{}^2}$$

Penalty enforces physical assumption that chemical will not hit many targets simultaneously

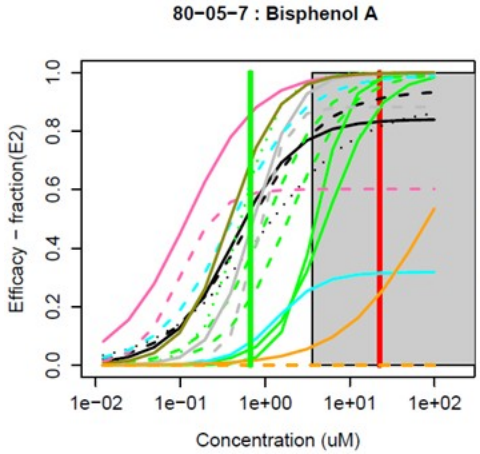
$$AUC_j = \frac{1}{N_{conc}} \sum_{i=1}^{N_{conc}} sign(slope) \times R_j(conc_i)$$

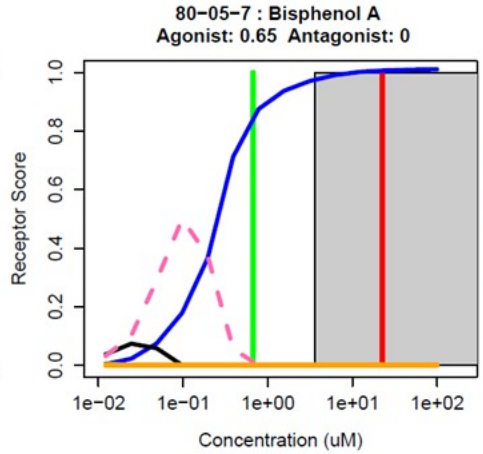
AUC Summarizes results normalized to positive control



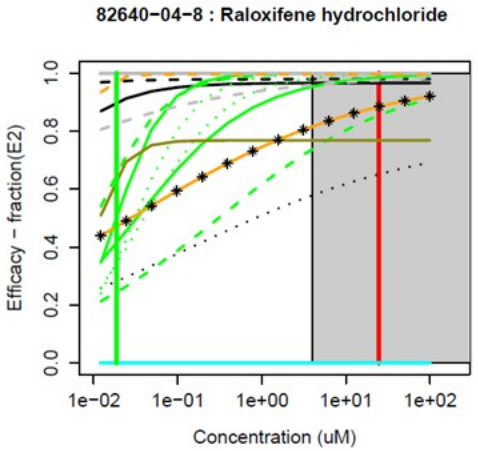
Example curves

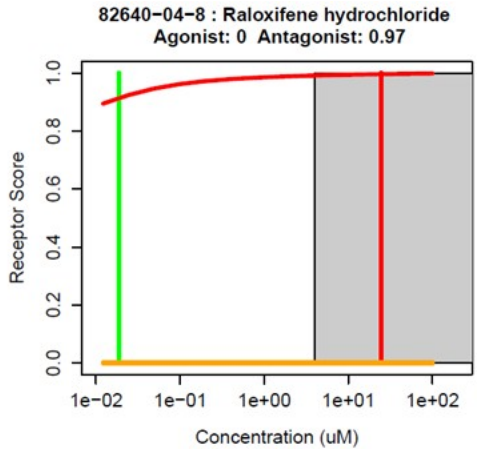
True Agonist



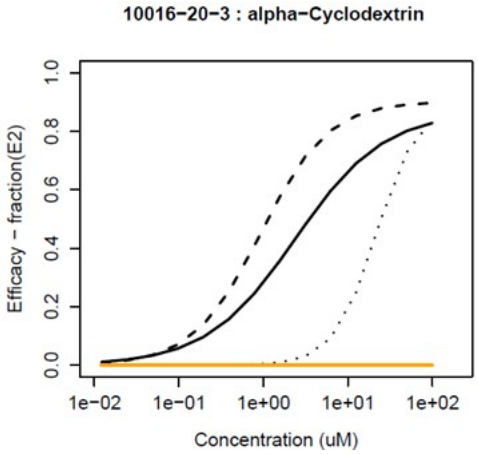


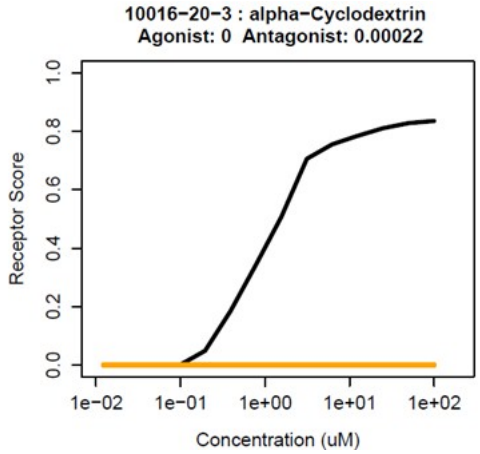
True Antagonist





Negative-Narrow Assay Interference



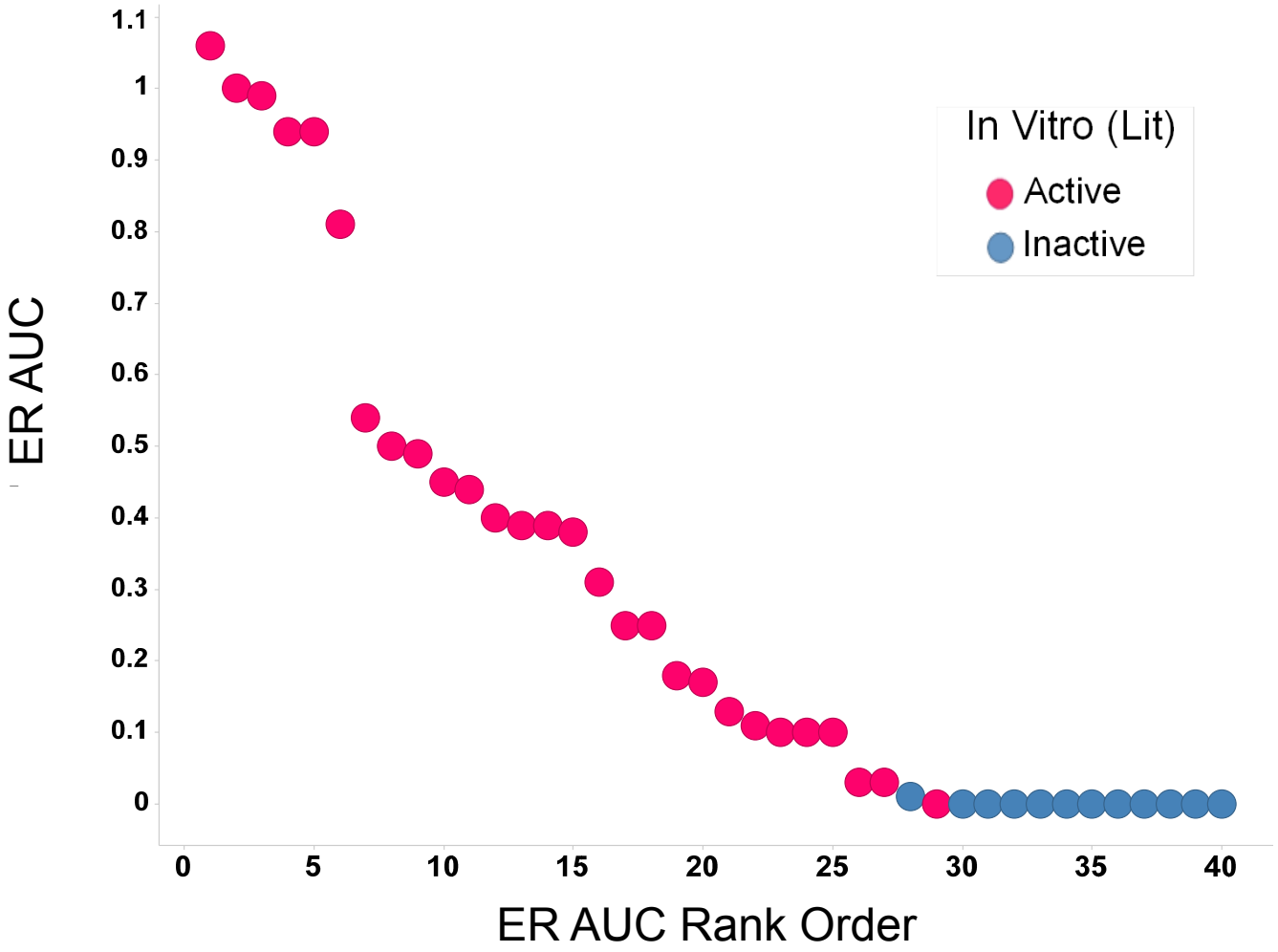


Performance-based Validation

- *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)

ER Agonist Model Performance

In Vitro Reference Chemicals



True Positive	25
True Negative	12
False Positive	0
False Negative	3
Accuracy	**0.95**
Sensitivity	**0.89**
Specificity	**1.00**

Judson et al. 2015 Tox Sci

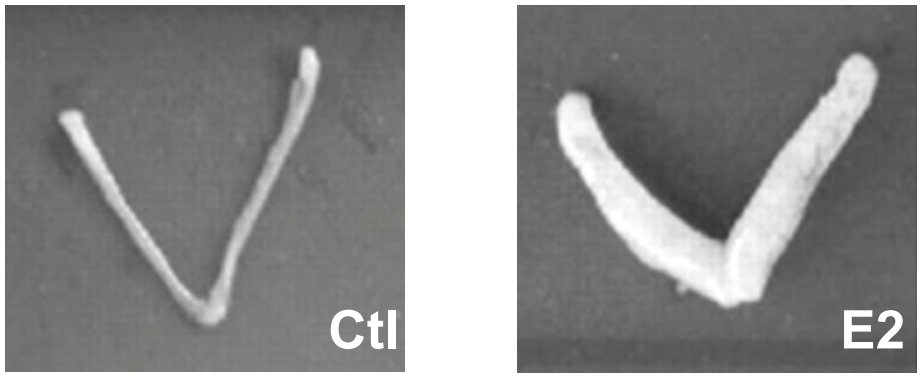
Rodent Uterotrophic Bioassay

Purpose

- Short term *in vivo* screen to evaluate the ability of a chemical to elicit a biological response similar to that of natural estrogens

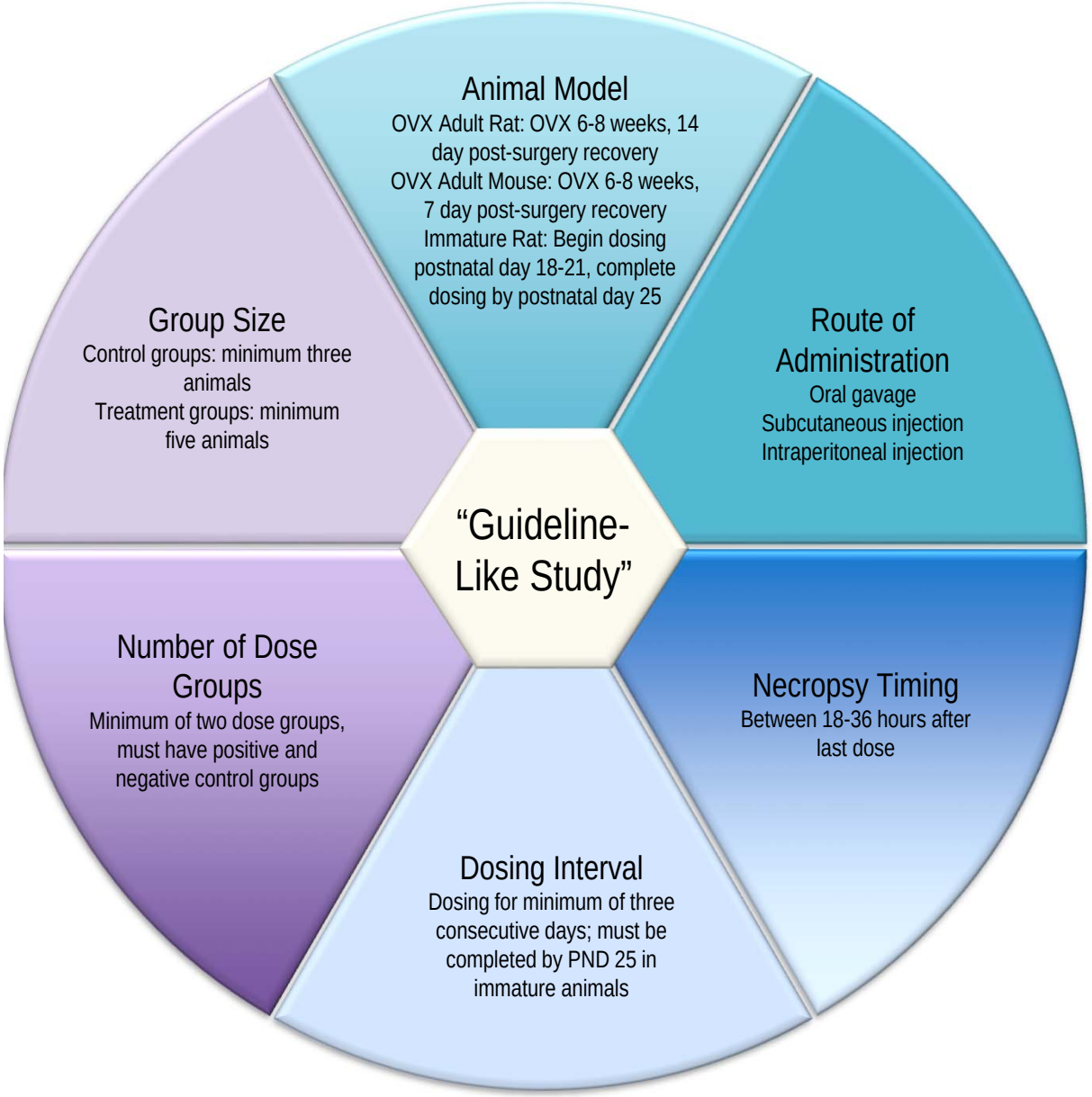
Principle

- Uterus is under the control of estrogens to stimulate growth
- Production of endogenous estrogens is prevented
  - Ovariectomized (OVX)
  - Immature (Imm)
- Uterus becomes sensitive to external estrogenic substances



Billon-Galés A et al. PNAS 2011

Identifying *In Vivo* Reference Chemicals

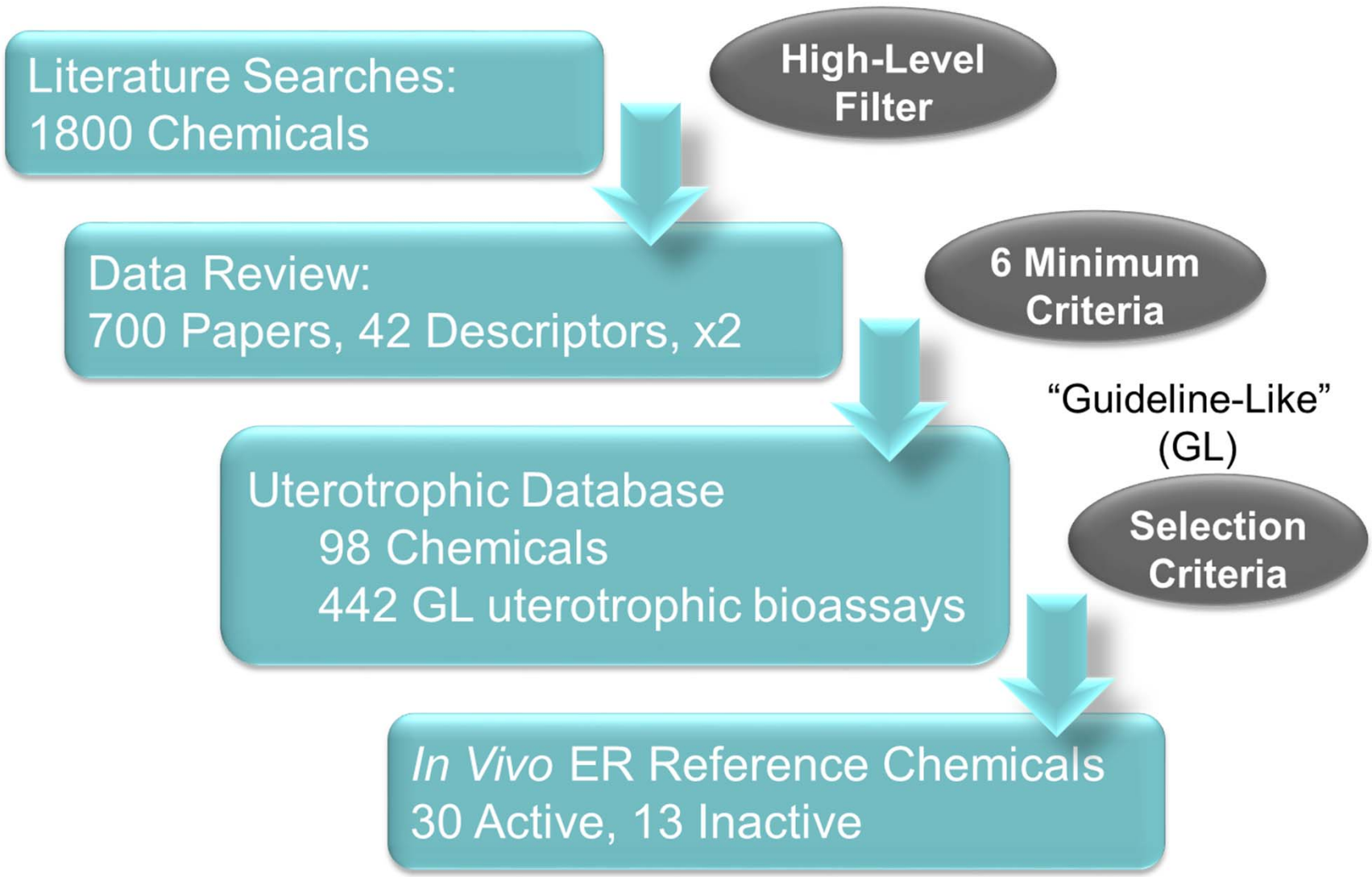


Leverage existing *in vivo* uterotrophic data

- Systematic literature search of publically available data (e.g. PubMed, Scopus)
- Identify chemical activities measured in "guideline-like" uterotrophic studies
- Identify a subset of *in vivo* reference chemicals
  - Active chemicals verified in ≥2 independent studies
  - Inactive chemicals verified in ≥2 independent studies (with no positive results in any study)

Kleinstreuer et al. 2015 EHP

Identifying *In Vivo* Reference Chemicals

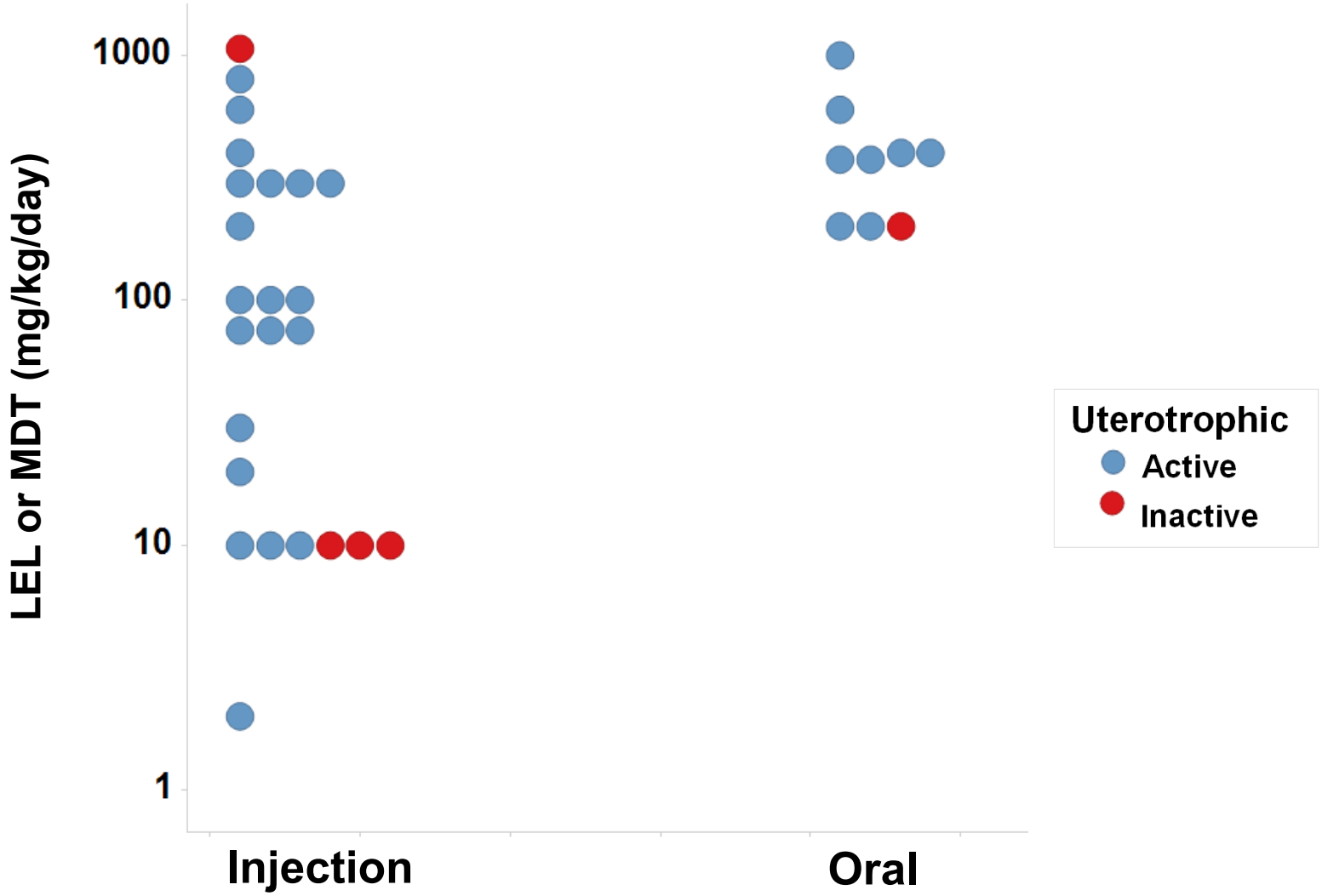


Browne et al. "Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model" (ES&T 2015)
Kleinstreuer et al: "A Curated Database of Rodent Uterotrophic Bioactivity" (EHP 2016)

M. Gwinn, US EPA

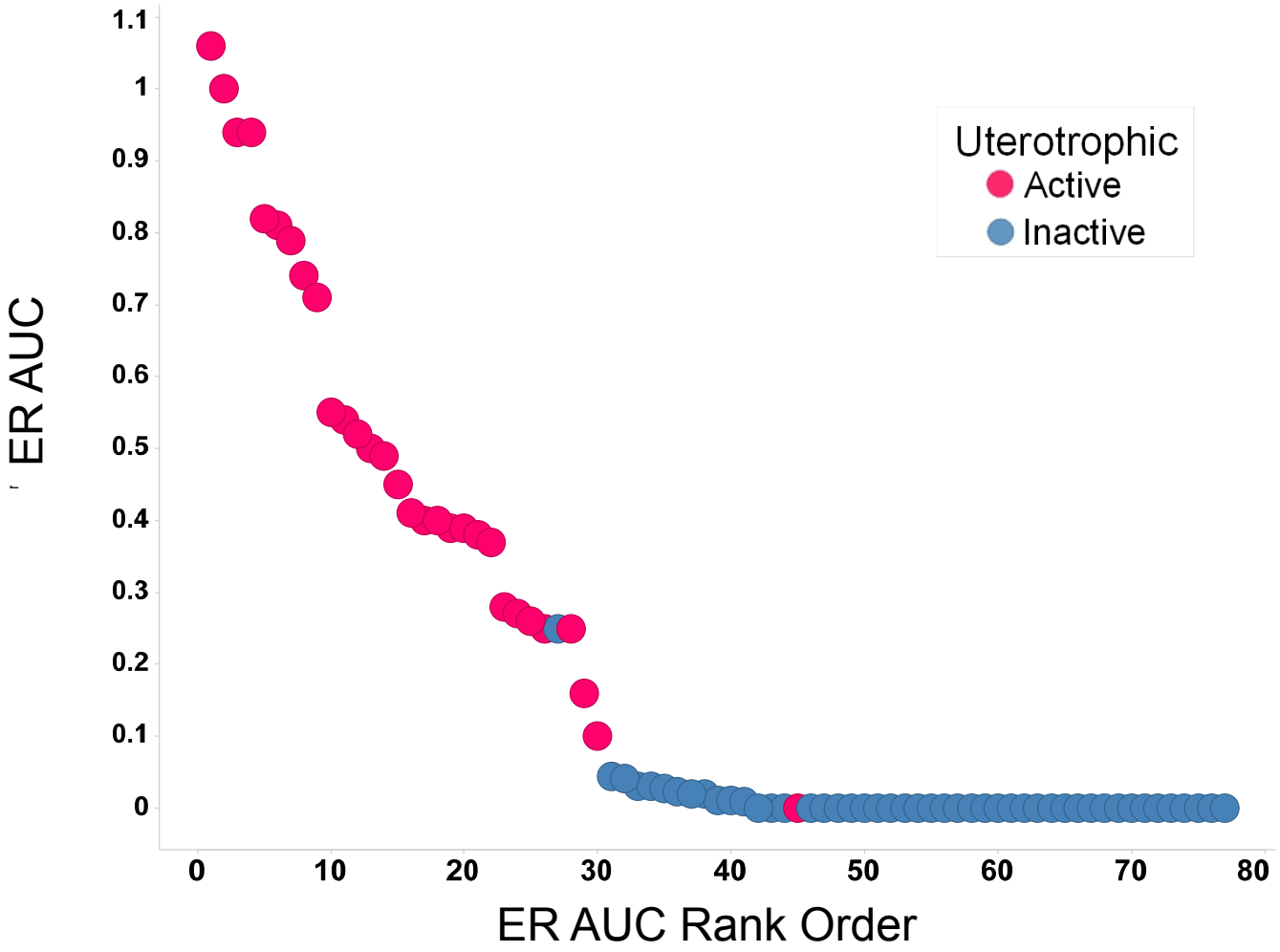
Uterotrophic Reproducibility

Same Study Design (Immature Rat): BPA



ER Agonist Model Performance

In Vivo Reference Chemicals

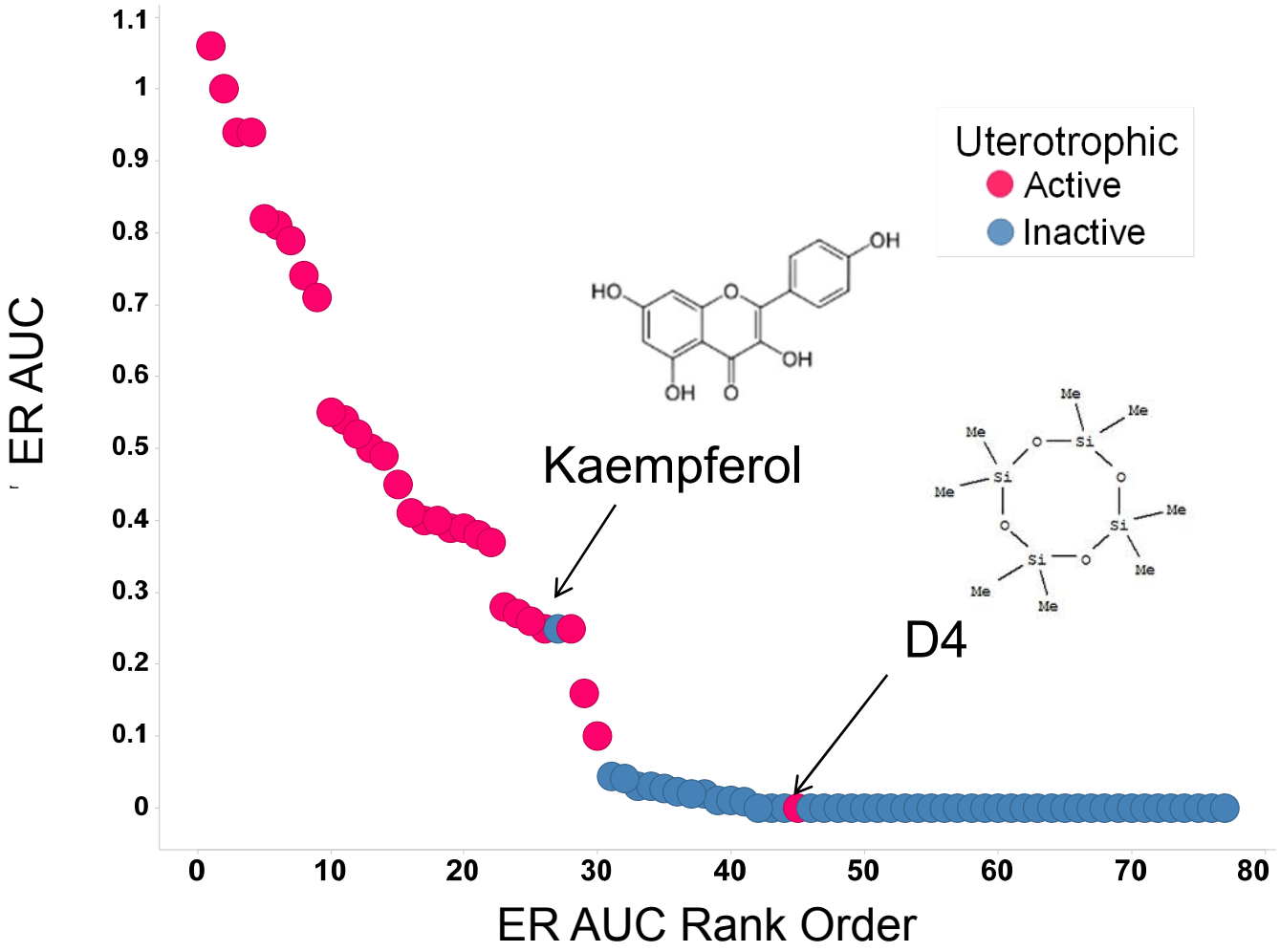


True Positive	29
True Negative	46
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.97**

Browne et al. 2015 ES&T

ER Agonist Model Performance

In Vivo Reference Chemicals



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Adopting Alternative EDSP Assays

EDSP Tier 1 Battery of Assays	Model Alternative Development
Estrogen Receptor (ER) Binding ★	ER Model FY 2015
Estrogen Receptor Transactivation (ERTA) ★	ER Model FY 2015
Rodent Uterotrophic ★	ER Model FY 2015
Androgen Receptor (AR) Binding	AR Model FY 2017
Rodent Hershberger	AR Model FY 2017
Aromatase	STR Model FY 2017
Steroidogenesis (STR)	STR Model 2017
Female Rat Pubertal	ER, STR & THY Models FY 2018
Male Rat Pubertal	AR, STR & THY Models FY 2018
Fish Short Term Reproduction	ER, AR & STR Models FY 2018
Amphibian Metamorphosis	THY Model FY 2018

FEDERAL REGISTER
The Daily Journal of the United States Government

June 19, 2015
FRL-9928-69
"Use of High Throughput Assays and Computational Tools; Endocrine Disruptor Screening Program; Notice of Availability and Opportunity for Comment"

Adopting Alternative EDSP Assays

EDSP Tier 1 Battery of Assays	Model Alternative Development
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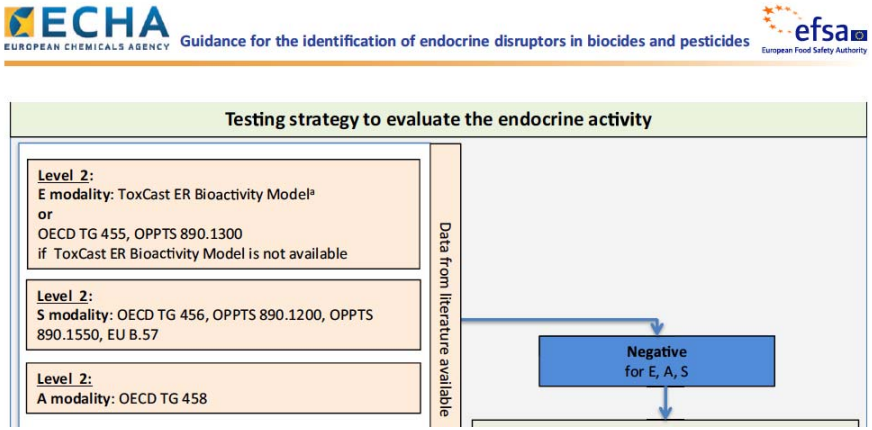
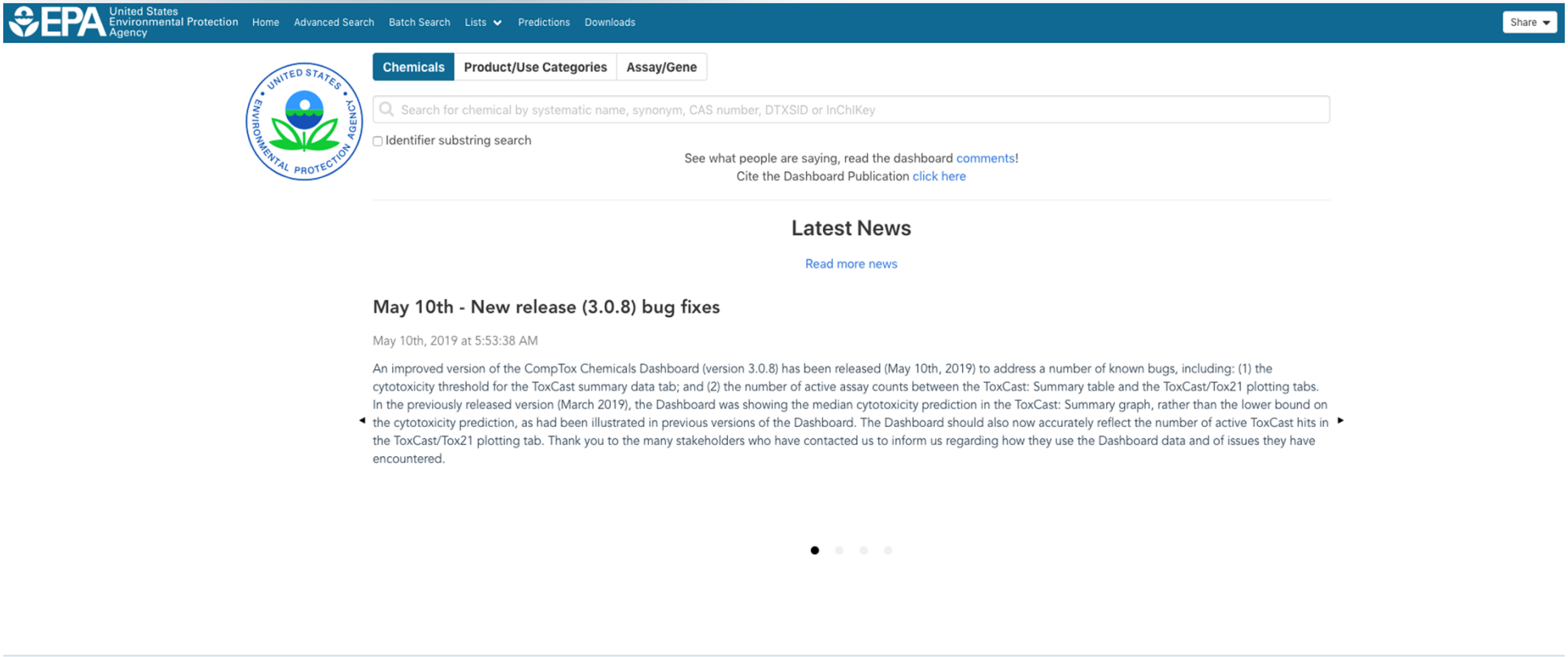
Androgen Receptor (AR) Binding	AR Model FY 2017
Rodent Hershberger	AR Model FY 2017
Aromatase	STR Model FY 2017
Steroidogenesis (STR)	STR Model 2017
Female Rat Pubertal	ER, STR & THY Models FY 2018
Male Rat Pubertal	AR, STR & THY Models FY 2018
Fish Short Term Reproduction	ER, AR & STR Models FY 2018
Amphibian Metamorphosis	THY Model FY 2018

July 2018

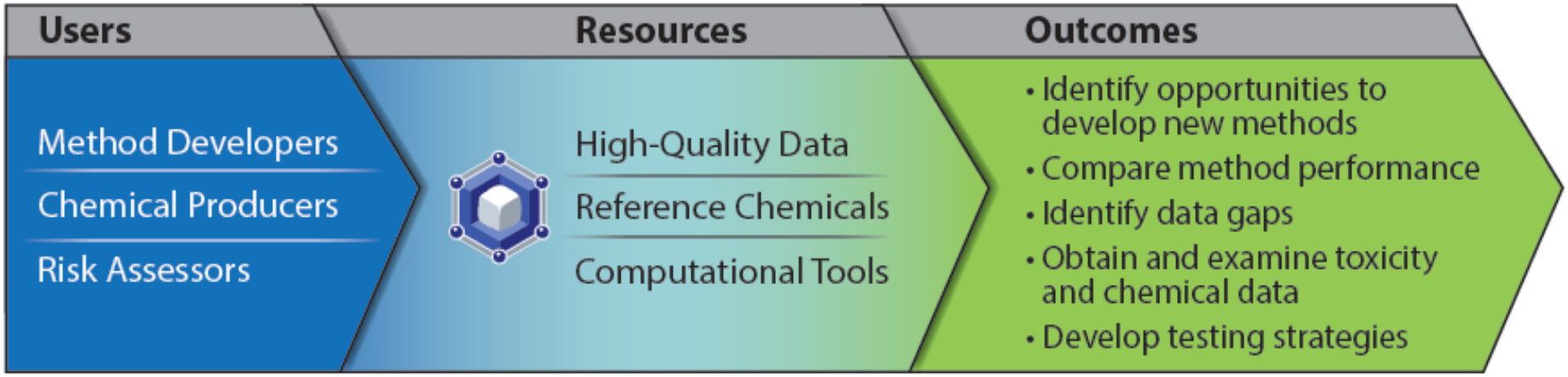


CompTox Chemistry Dashboard

https://comptox.epa.gov/dashboard



Integrated Chemical Environment: ICE



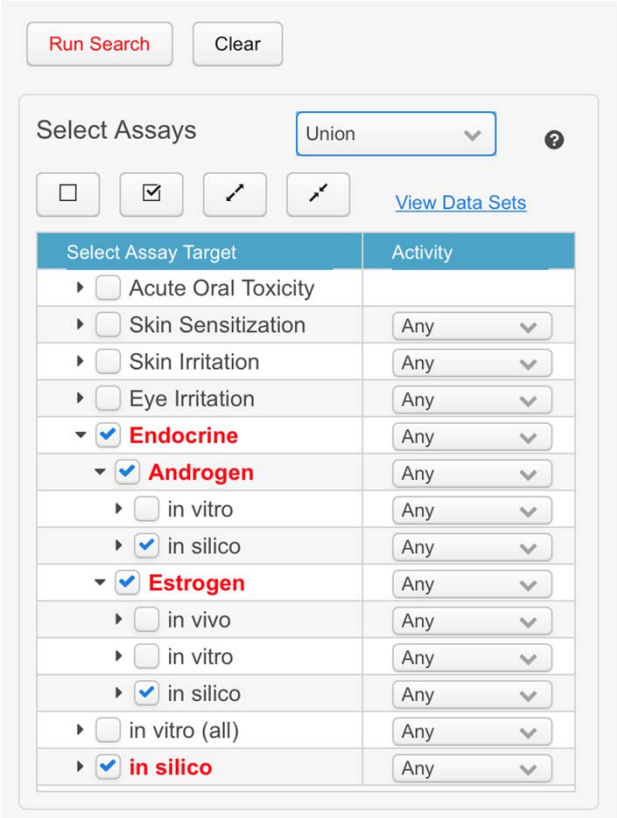
https://ice.ntp.niehs.nih.gov/

Bell et al. 2017 EHP

- Data integrator:
  - Structured format designed for ease of use
  - Allows access to data for multiple regulatory endpoints
  - Query by CASRN or established reference chemical lists
  - Flexible, exportable results
- Workflows:
  - Property predictions, Chemical space characterization, IVIVE, Mechanistic models, AOP mapping

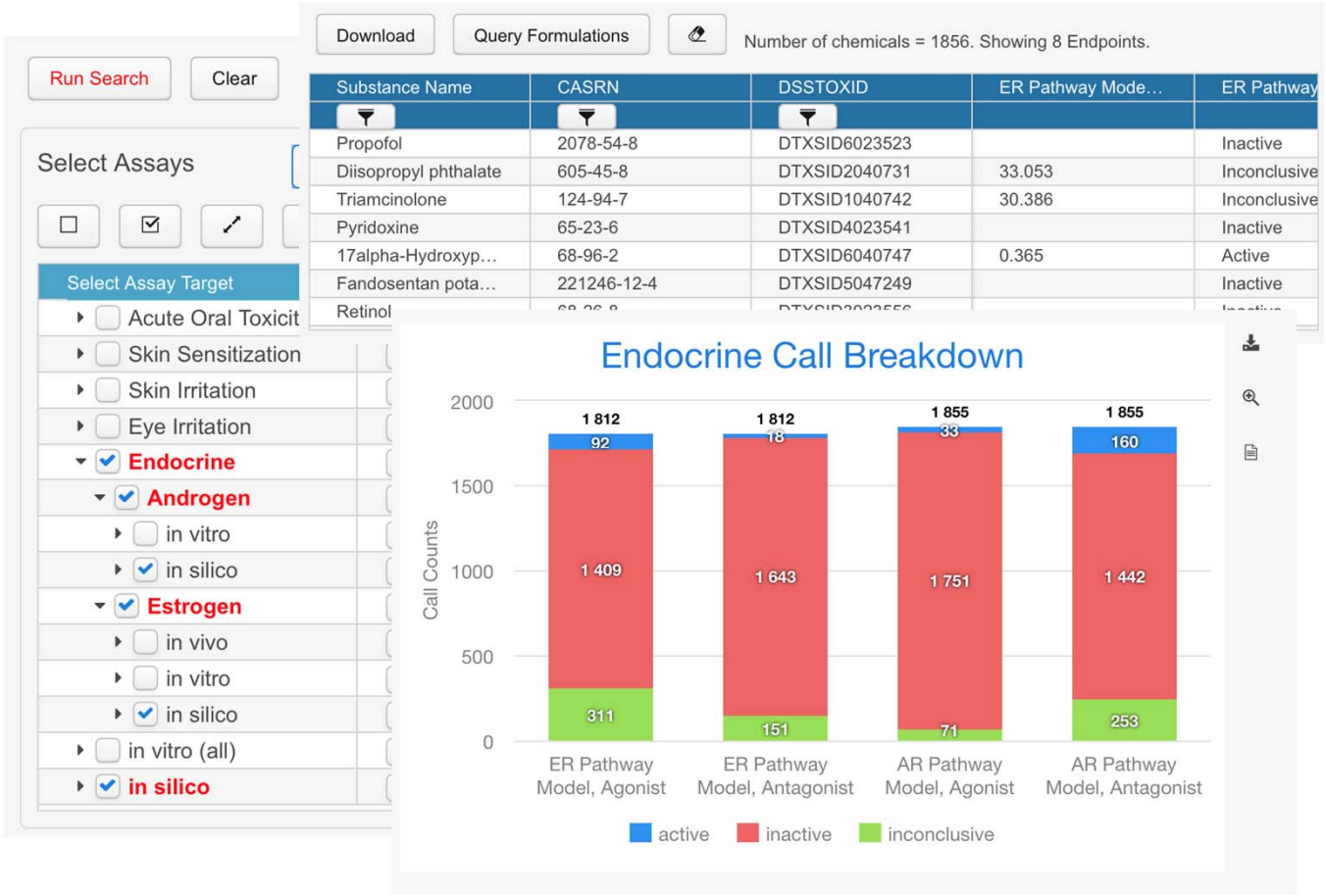
Integrated Chemical Environment: ICE

Endocrine Pathway Models



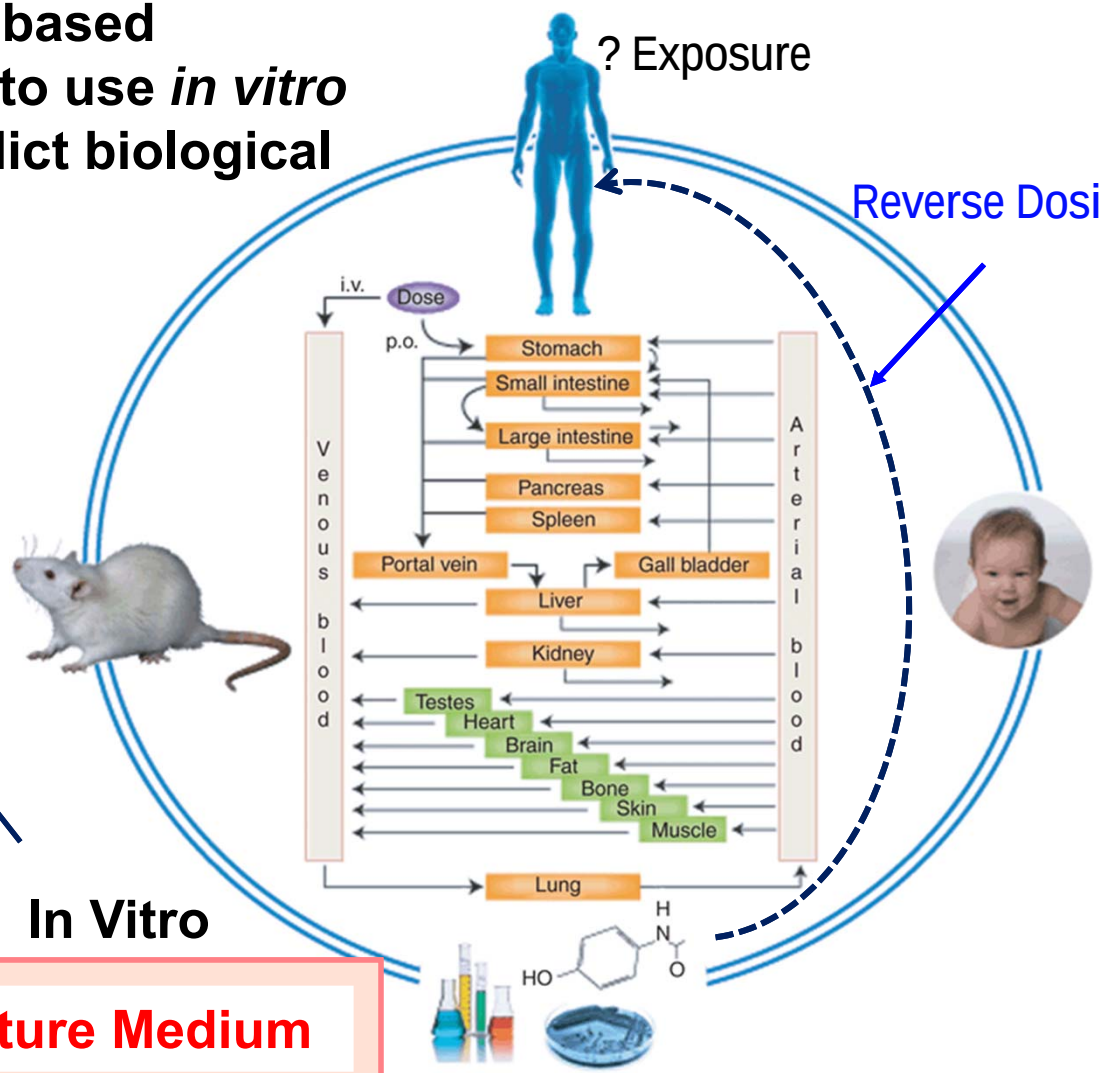
Integrated Chemical Environment: ICE

Endocrine Pathway Models



In Vitro to *In Vivo* Extrapolation (IVIVE)

Applying physiologically based pharmacokinetic models to use *in vitro* experimental data to predict biological effects *in vivo*



In Vivo

Capillary Blood

Tissue

Reverse Toxicokinetics:
PK/PBPK Models

In Vitro

Culture Medium

Cells

IVIVE for Quantitative Comparison

Research

A Section 508–conformant HTML version of this article is available at https://doi.org/10.1289/EHP1655.

Evaluation and Optimization of Pharmacokinetic Models for *in Vitro* to *in Vivo* Extrapolation of Estrogenic Activity for Environmental Chemicals

Warren M. Casey,[1] Xiaoqing Chang,[2]* David G. Allen,[2] Patricia C. Ceger,[2] Neepa Y. Choksi,[2] Jui-Hua Hsieh,[4] Barbara A. Wetmore,[3] Stephen S. Ferguson,[1] Michael J. DeVito,[1] Catherine S. Sprankle,[2] and Nicole C. Kleinstreuer[1]*

[1]National Toxicology Program Division, National Institute of Environmental Health Sciences, National Institutes of Health, Research Triangle Park, North Carolina, USA
[2]Integrated Laboratory Systems, Inc., Morrisville, North Carolina, USA
[3]ScitoVation, Research Triangle Park, North Carolina, USA
[4]Kelly Government Solutions, Research Triangle Park, North Carolina, USA

Background: To effectively incorporate *in vitro* data into regulatory use, confidence must be established in the quantitative extrapolation of *in vitro* activity to relevant end points in animals or humans.

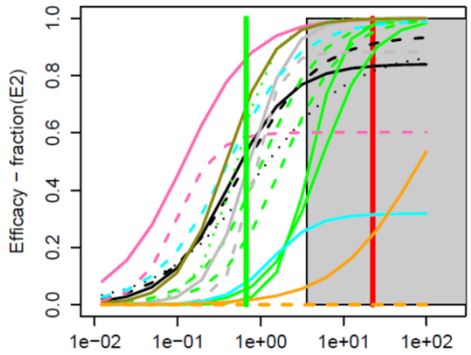
Objective: Our goal was to evaluate and optimize *in vitro* to *in vivo* extrapolation (IVIVE) approaches using *in vitro* estrogen receptor (ER) activity to predict estrogenic effects measured in rodent uterotrophic studies.

Methods: We evaluated three pharmacokinetic (PK) models with varying complexities to extrapolate *in vitro* to *in vivo* dosimetry for a group of 29 ER agonists, using data from validated *in vitro* [U.S. Environmental Protection Agency (U.S. EPA) ToxCast™ ER model] and *in vivo* (uterotrophic) methods. *In vitro* activity values were adjusted using mass-balance equations to estimate intracellular exposure via an enrichment factor (EF), and steady-state model calculations were adjusted using fraction of unbound chemical in the plasma (f_u) to approximate bioavailability. Accuracy of each model-adjustment combination was assessed by comparing model predictions with lowest effect levels (LELs) from guideline uterotrophic studies.

Results: We found little difference in model predictive performance based on complexity or route-specific modifications. Simple adjustments, applied to account for *in vitro* intracellular exposure (EF) or chemical bioavailability (f_u), resulted in significant improvements in the predictive performance of all models.

Conclusion: Computational IVIVE approaches accurately estimate chemical exposure levels that elicit positive responses in the rodent uterotrophic bioassay. The simplest model had the best overall performance for predicting both oral (PPK_EF) and injection (PPK_f_u) LELs from guideline uterotrophic studies, is freely available, and can be parameterized entirely using freely available *in silico* tools. https://doi.org/10.1289/EHP1655

***In vitro* ER activity**



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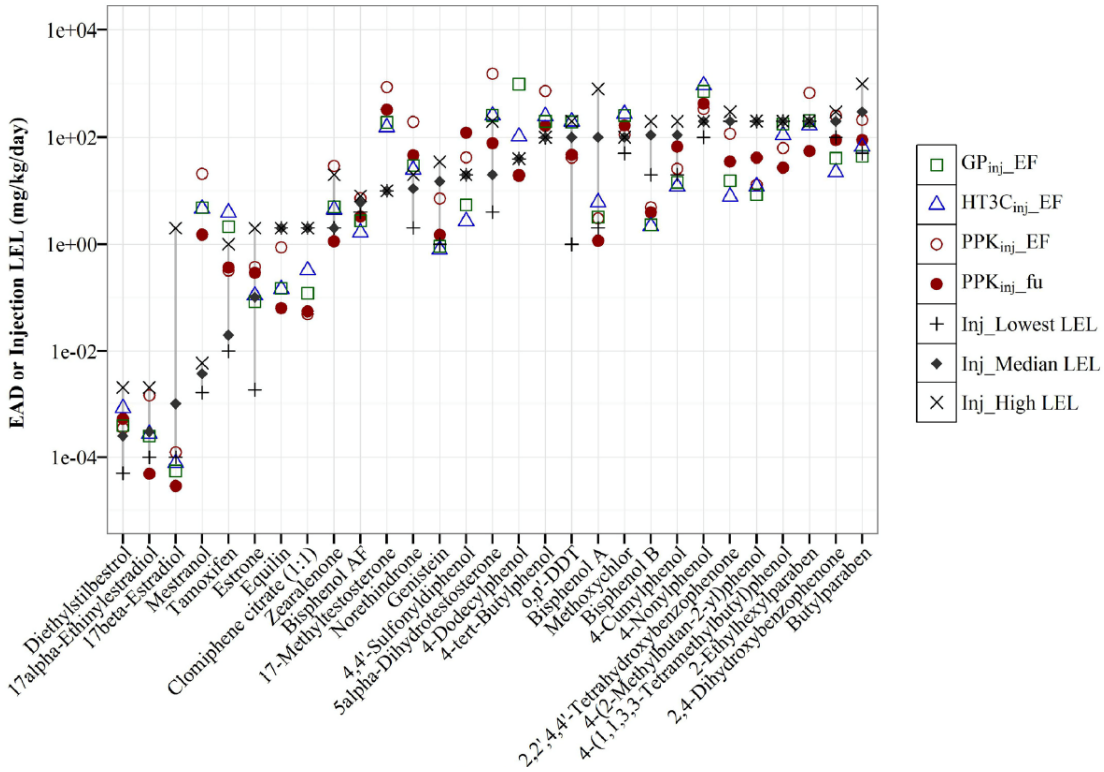
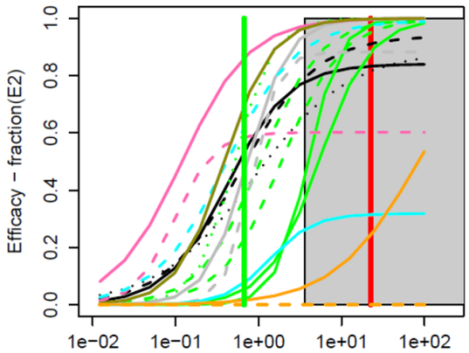
Bioactivity in Rat / Mouse uterus

IVIVE for Quantitative Comparison



In vitro ER activity

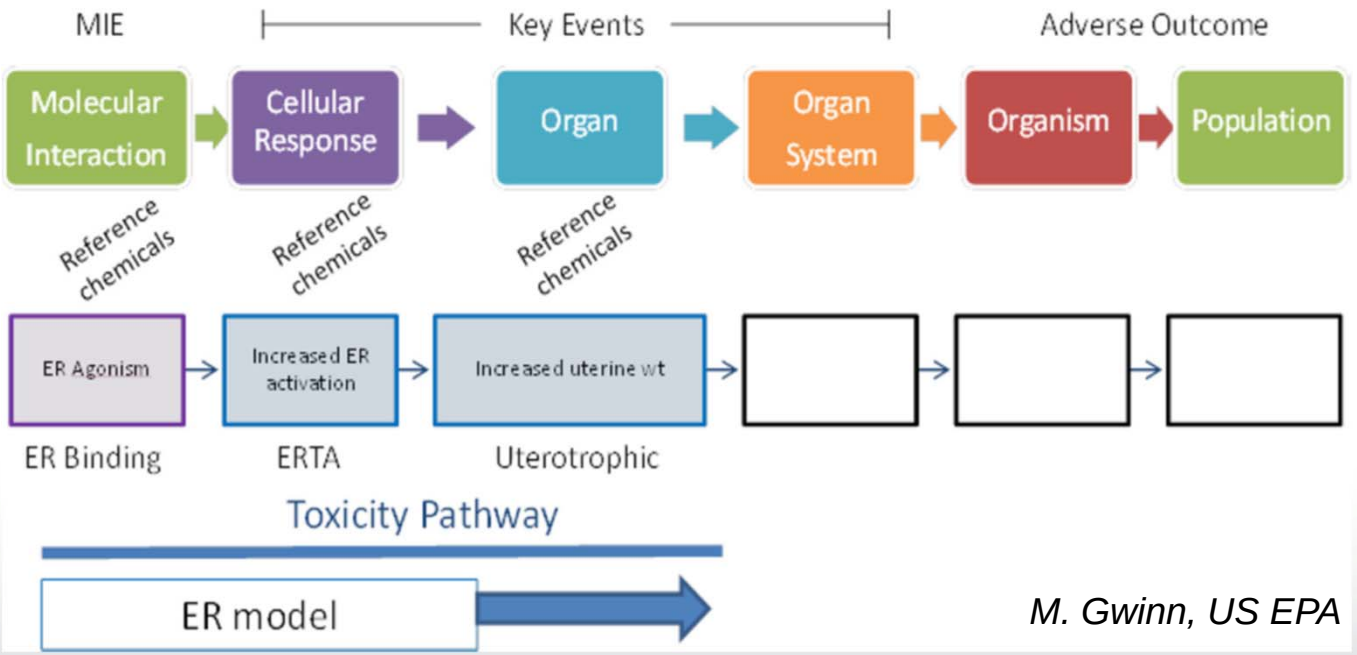


≈

Bioactivity in Rat / Mouse uterus

OECD IATA Case Study

Use 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.

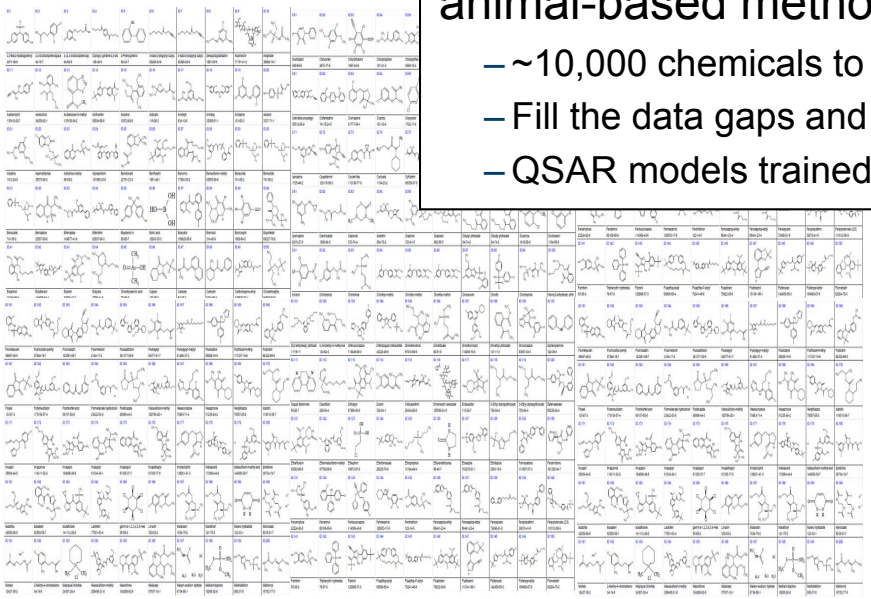


M. Gwinn, US EPA

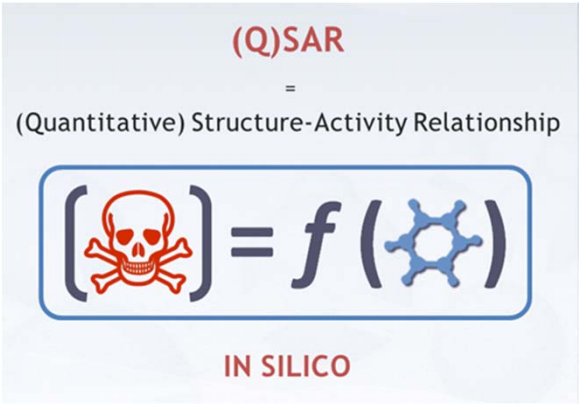
CERAPP: QSAR Modeling

Far too many chemicals to test with standard animal-based methods or even *in vitro* HTS

- ~10,000 chemicals to be tested for EDSP, >50,000 for TSCA
- Fill the data gaps and bridge the lack of knowledge
- QSAR models trained on ToxCast ER pathway data



Alternative



CERAPP

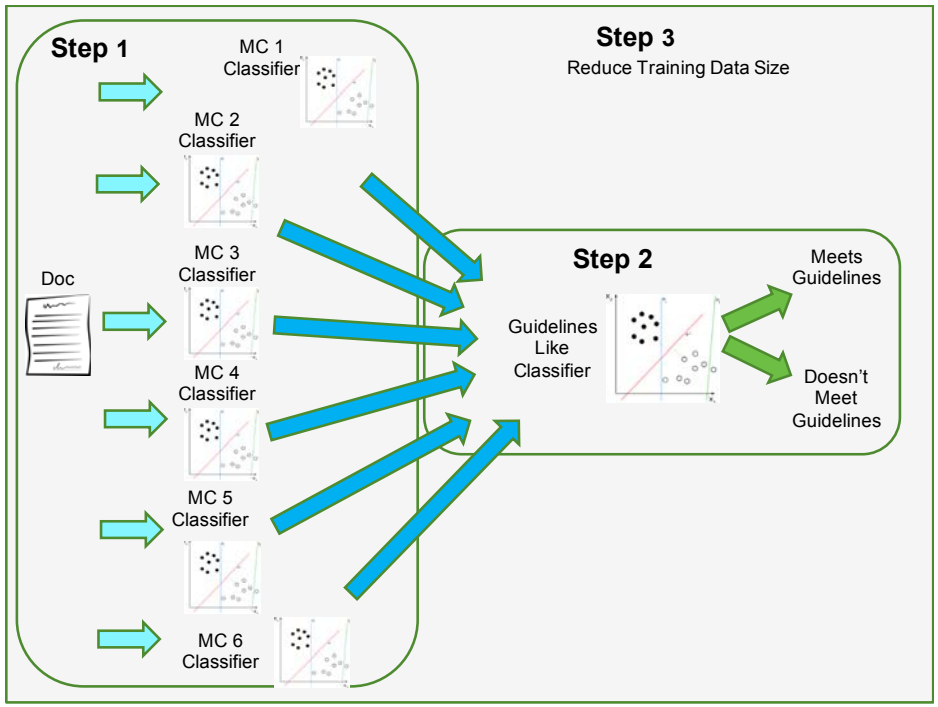
Collaborative Estrogen Receptor Activity Prediction Project

Mansouri et al. EHP (2017)

OPERA
OPEn (q)saR App

https://github.com/NIEHS/OPERA

Automating Reference Data Identification



- Project with Oak Ridge National Labs (ORNL) and FDA CFSAN to apply text-mining (NLP) approaches & ML to identify high-quality data
- Semi-automated retrieval and evaluation of published literature (trained on uterotrophic database)
- Apply to developmental toxicity studies (with ICCVAM DARTWG)
  - Define literature search keywords, identify corpus
  - Extract/characterize study protocol details from regulatory guidelines: minimum criteria
  - Apply ML algorithms to identify high-quality studies, expert check

Acknowledgments

- ILS/NICEATM
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- EPA/ORD
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