Use of High Throughput Assays and Predictive Models by the U.S. Environmental Protection Agency's Endocrine Disruptor Screening Program

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SACATM
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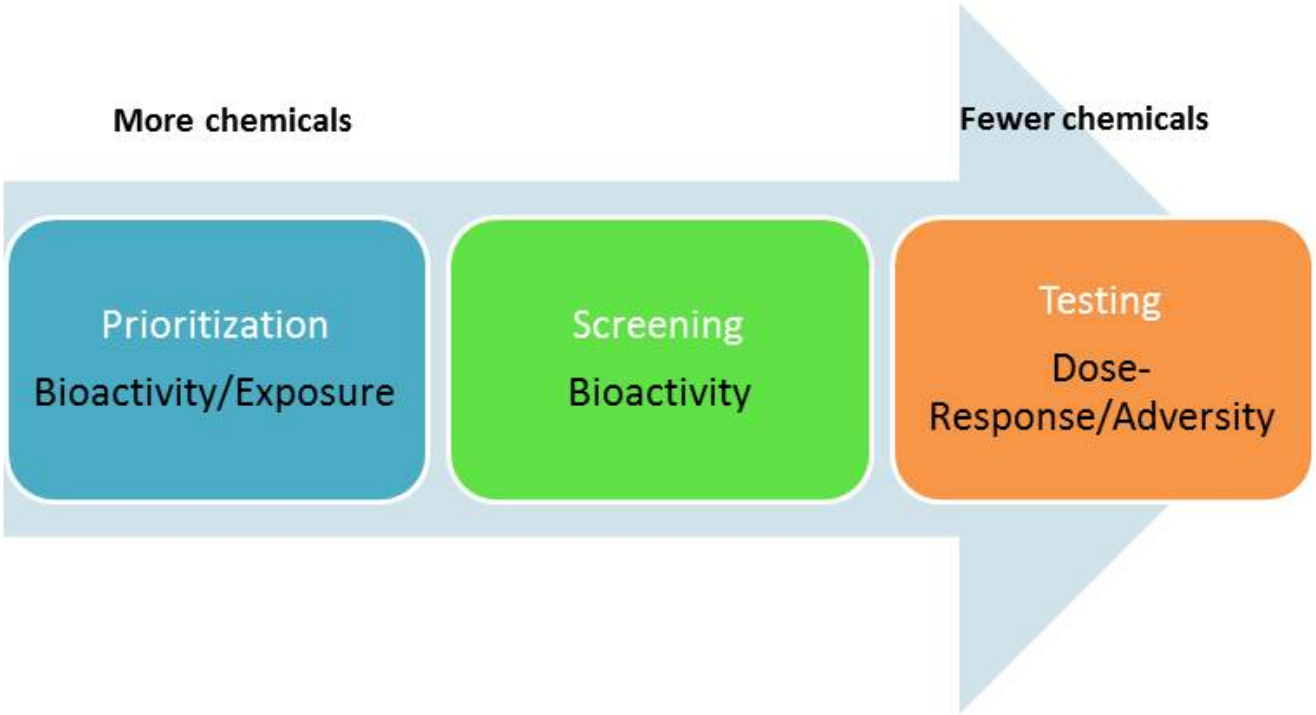
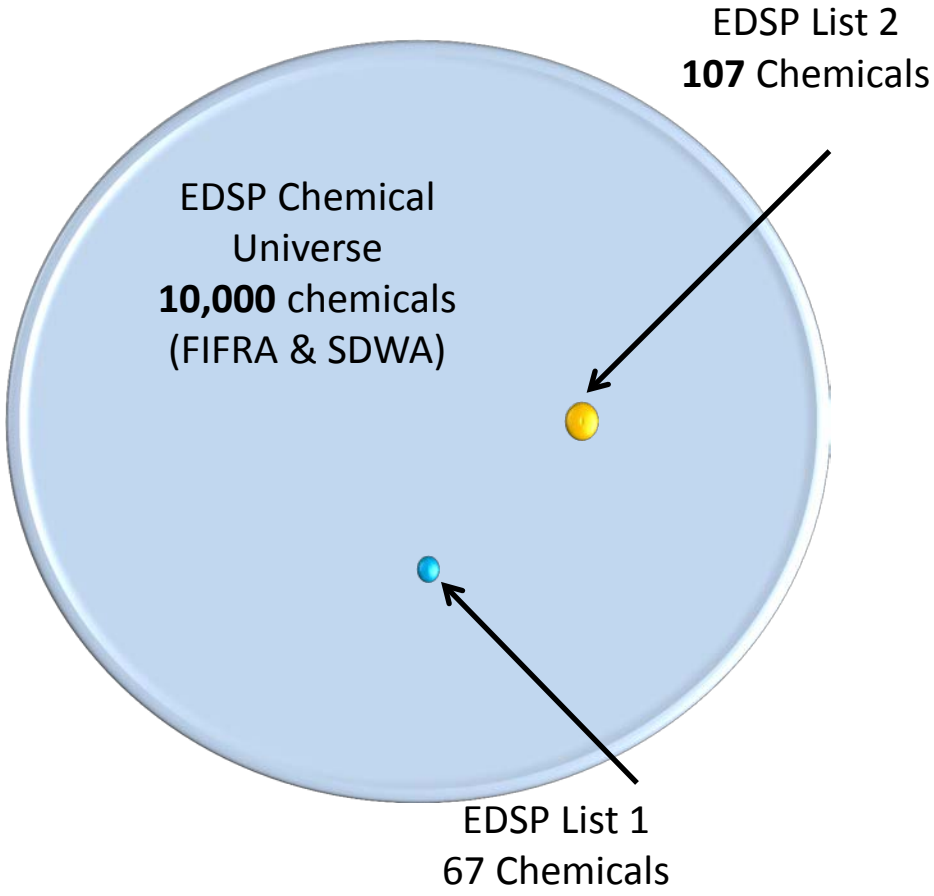
EDSP Prioritization, Screening & Testing



Prioritization and Screening for bioactivity
Testing for dose-response and adverse effects

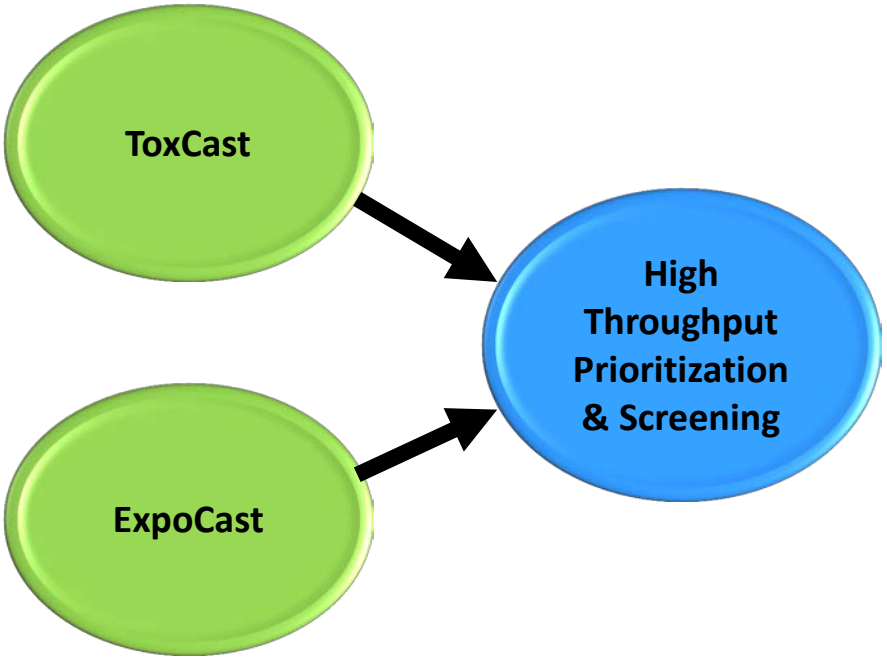
Evolution of EDSP- the Pivot



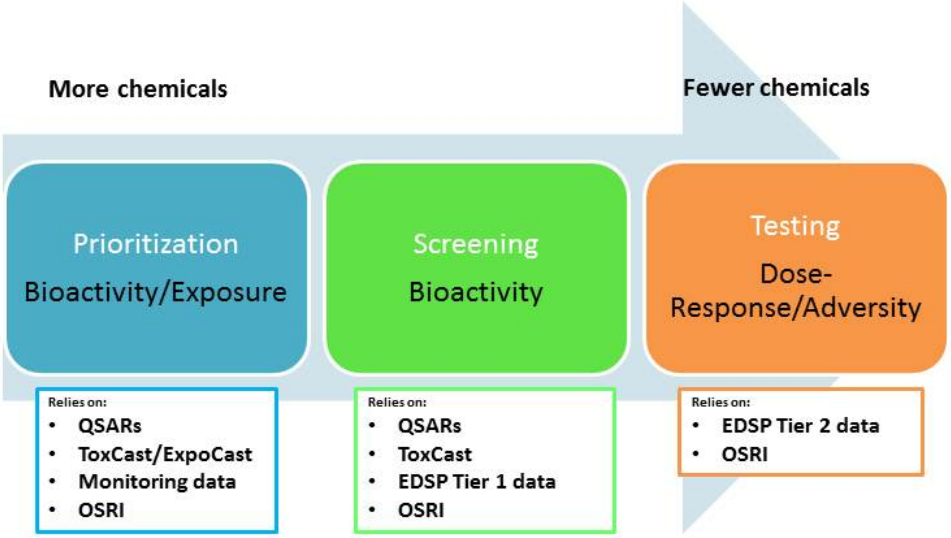
- Based on current pace it could take decades to screen all 10,000 chemicals in EDSP Universe
- Pivot: use high throughput assays and computational models to rapidly screen chemicals for potential bioactivity and exposure

Computational Tools

- ToxCast
  - Hight throughput in vitro assays and in silico models to support prioritization and screening
  - Transparent and collaborative
- ExpoCast
  - Rapid exposure estimation based on readily available chemical use and production data
  - Use toxicokinetics to bridge in vitro, concentration-based ToxCast data to in vivo, dose-based exposures from ExpoCast



EDSP Prioritization, Screening & Testing



Prioritization and Screening for bioactivity
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EDSP Pivot Goals

Use computational tools and models in the EDSP framework to:

1. Prioritize chemicals for further EDSP screening and testing based on estimated bioactivity and exposure
2. Contribute to the weight of evidence evaluation of a chemical's potential bioactivity
3. Substitute for specific endpoints in the EDSP Tier 1 battery

Ultimately, these goals are common to the estrogen, androgen and thyroid pathways, however, estrogen bioactivity is the most mature model and is used to demonstrate the proposed approach. AR and IBER are presented as works-in-progress.

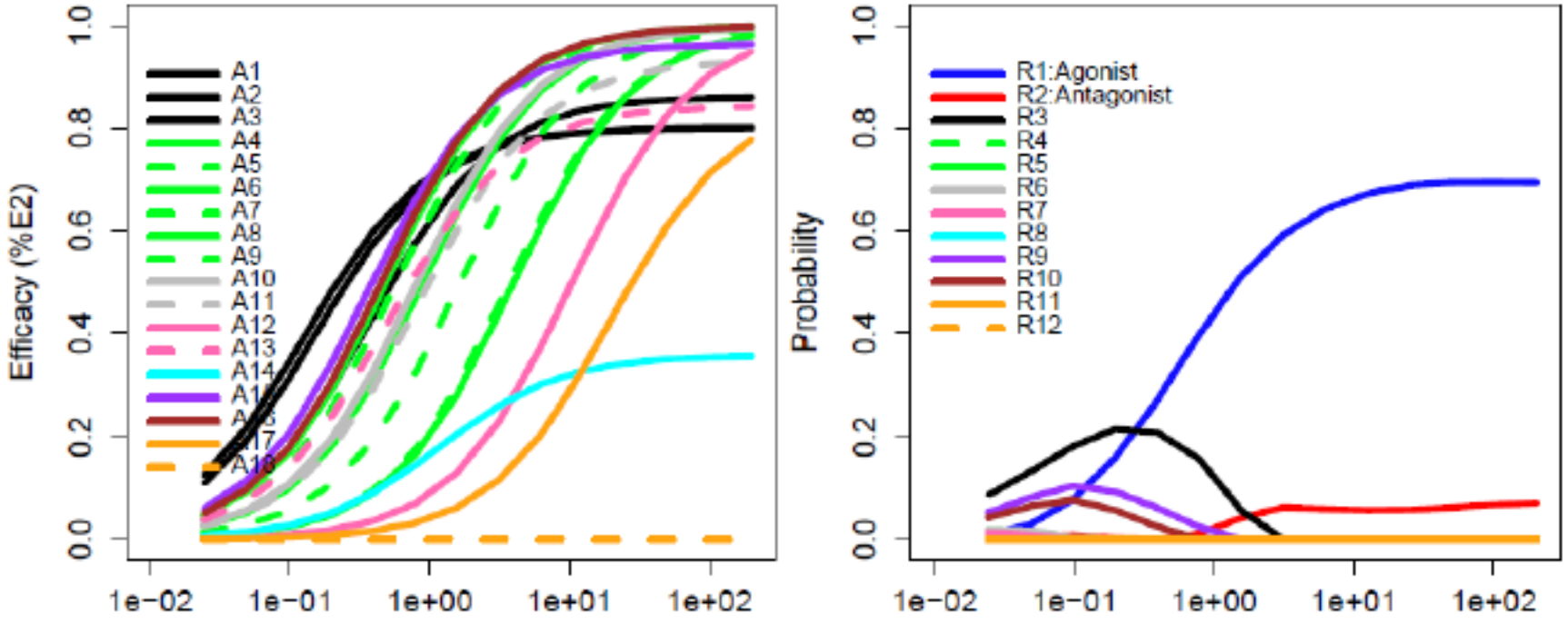
Endocrine Bioactivity Models

- ER bioactivity model
  - 18 HTS assays
- Detect receptor interaction at various points along signaling pathway
- Use a variety of technologies
  - Capable of distinguishing “true” activity from cytotoxicity
- Values range from 0 to 1
  - ER agonists

Judson *et al.* 2015 Toxicological Sciences

"Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor"

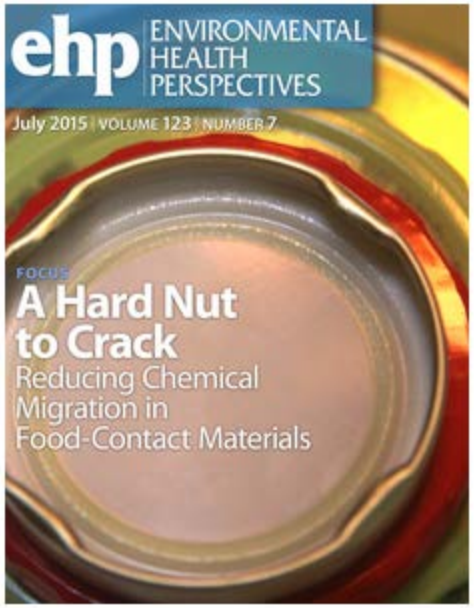
High Throughput Assays Integrated Into A Pathway Bioactivity Model

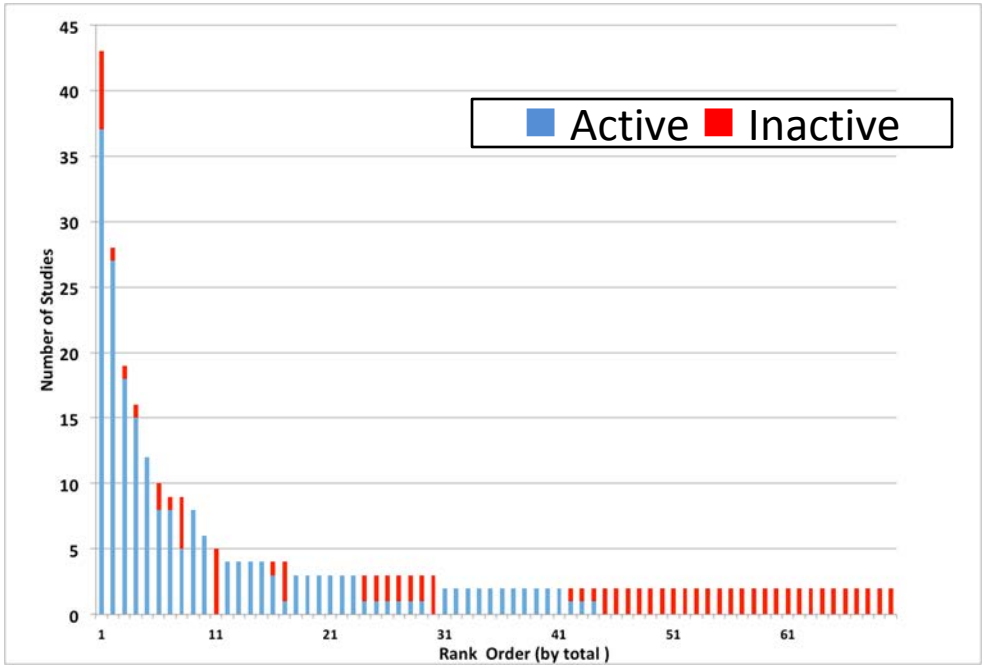


[Judson *et al.* 2015 Tox Sci]

Kleinstreuer et al. 2015 Environmental Health Perspectives

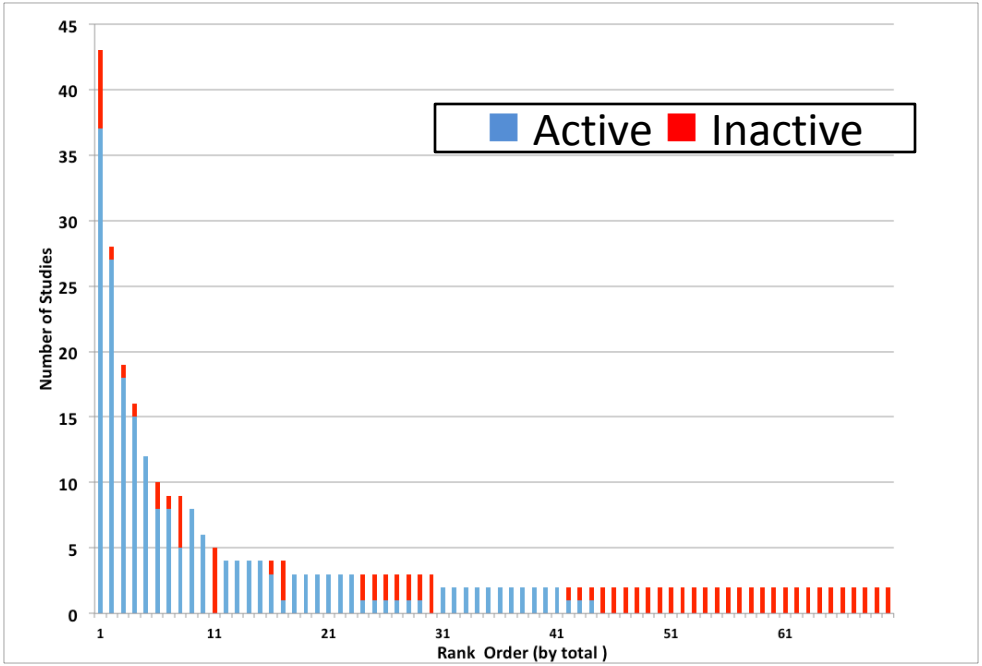
"A Curated Database of Rodent Uterotrophic Bioactivity"





ER Bioactivity Model Versus Tier 1

- ER model performs as well or better than existing methods
- Model evaluated with 45 reference chemicals
  - T1 ER binding: 23 (35% were not were not consistent with expected outcome)
  - T1 ERTA: 12
  - T1 UT: 7
- ER model in 100% agreement with Tier 1 ER, ERTA, and Uterotrophic results for List 1 chemicals (very low or no ER activity)
- ER model may be more sensitive than Tier 1 assays due to redundancy



Results from uterotrophic studies for chemicals that had at least two independent GL studies. Blue bars represent the number of active reports; red bars represent the number of inactive reports. Data from chemicals commonly used as positive controls (i.e., ethinyl estradiol and estradiol) were excluded from this plot.

[Kleinstreuer *et al.* 2015 Environmental Health Perspectives]

Browne *et al.* 2015
Environmental Science & Technology

Article

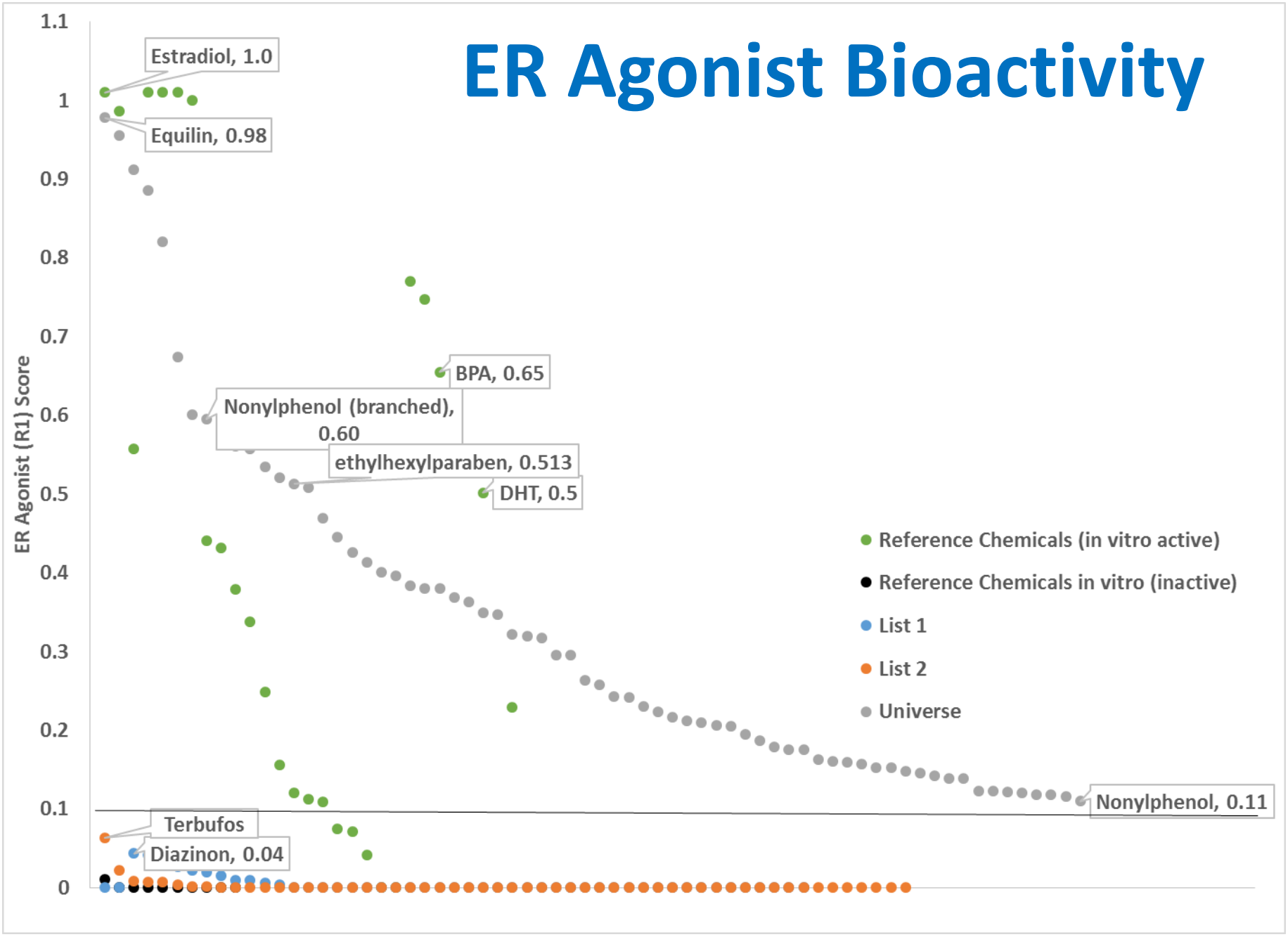
SCREENING CHEMICALS FOR ESTROGEN RECEPTOR BIOACTIVITY USING A COMPUTATIONAL MODEL

Patience Browne, Richard S. Judson, Warren Casey, Nicole Kleinstreuer, and Russell S. Thomas

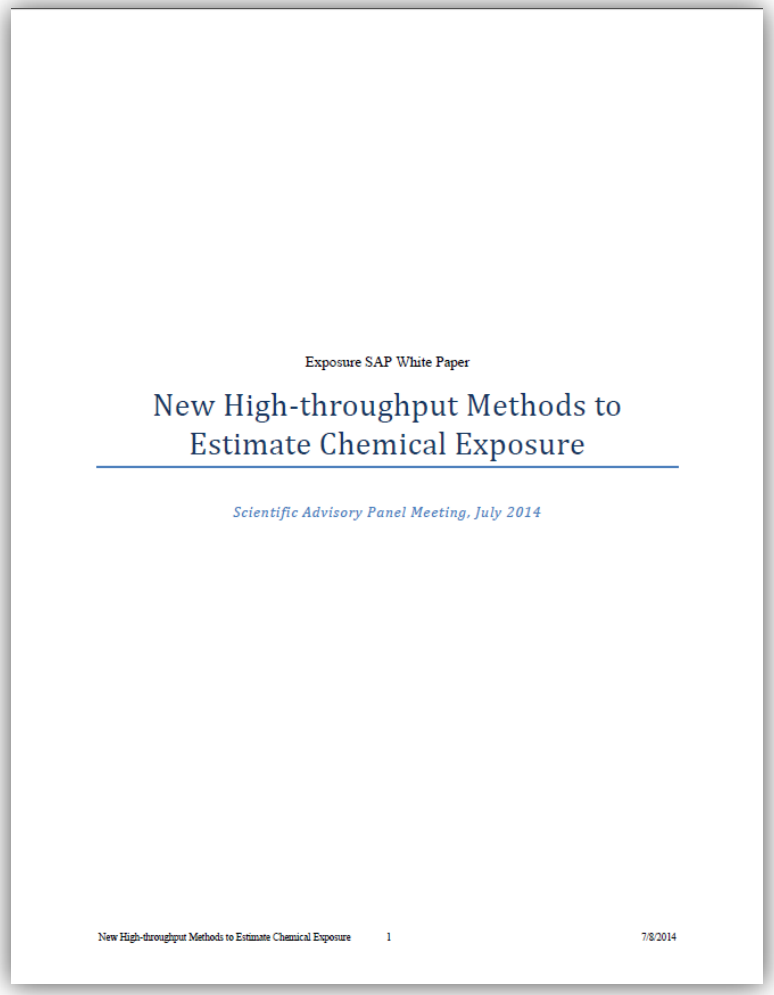
Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.5b02641 • Publication Date (Web): 12 Jun 2015

http://pubs.acs.org/doi/abs/10.1021/acs.est.5b02641

ER Agonist Bioactivity



Building Scientific Confidence – Peer Review

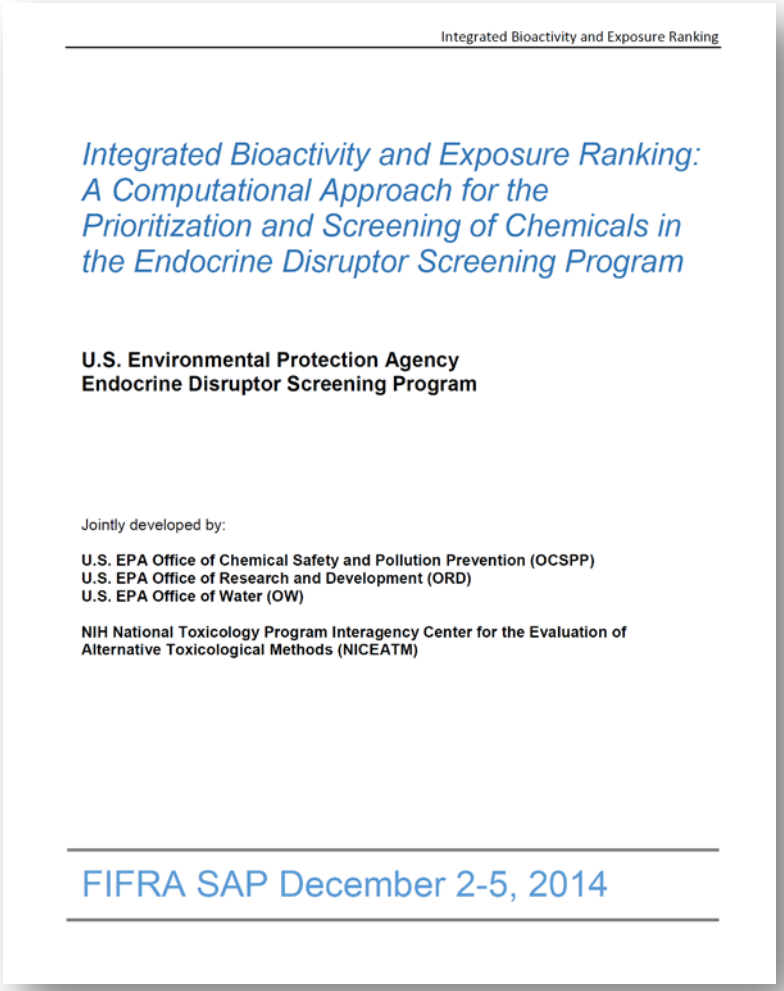


Exposure SAP White Paper

New High-throughput Methods to Estimate Chemical Exposure

Scientific Advisory Panel Meeting, July 2014

New High-throughput Methods to Estimate Chemical Exposure 1 7/8/2014



Integrated Bioactivity and Exposure Ranking

Integrated Bioactivity and Exposure Ranking: A Computational Approach for the Prioritization and Screening of Chemicals in the Endocrine Disruptor Screening Program

U.S. Environmental Protection Agency
Endocrine Disruptor Screening Program

Jointly developed by:

U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP)
U.S. EPA Office of Research and Development (ORD)
U.S. EPA Office of Water (OW)

NIH National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)

FIFRA SAP December 2-5, 2014

http://www.epa.gov/scipoly/sap/meetings/2014/index.html

Recent EDSP Milestones

EPA Solicits Comments on Use of High-Throughput Assays and Computational Tools in Endocrine Disruptor Screening Program

- Federal Register notice describes and solicits comments on how EPA is planning to incorporate scientific advancements and new tools incorporating validated high-throughput assays and a computational model as an alternative for some of the current assays in the EDSP Tier 1 battery.
- The adoption of scientific advancements into the EDSP has been under way and part of the public dialogue about EDSP for several years, and the Agency intends to continue to incorporate in the EDSP new methods involving high-throughput assays and computational toxicology in order to accelerate the pace of screening, add efficiencies, decrease costs and reduce animal testing.
- Currently, EPA has partial screening results for over 1,800 chemicals that have been evaluated using the high-throughput assays and computational model for the estrogen receptor pathway.
- The Federal Register Notice (with information on how to provide comments) can be viewed at http://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPPT-2015-0305-0001.
- The press release related to the publishing of this Federal Register Notice can be viewed at http://yosemite.epa.gov/opa/admpress.nsf/d0cf6618525a9efb85257359003fb69d/77377414ba7ebc5885257e68006ea110!OpenDocument.
- More detailed information on the Endocrine Disruptor Screening Program and its use of computational tools: http://www.epa.gov/endo/ or http://www.epa.gov/endo/pubs/pivot.htm.

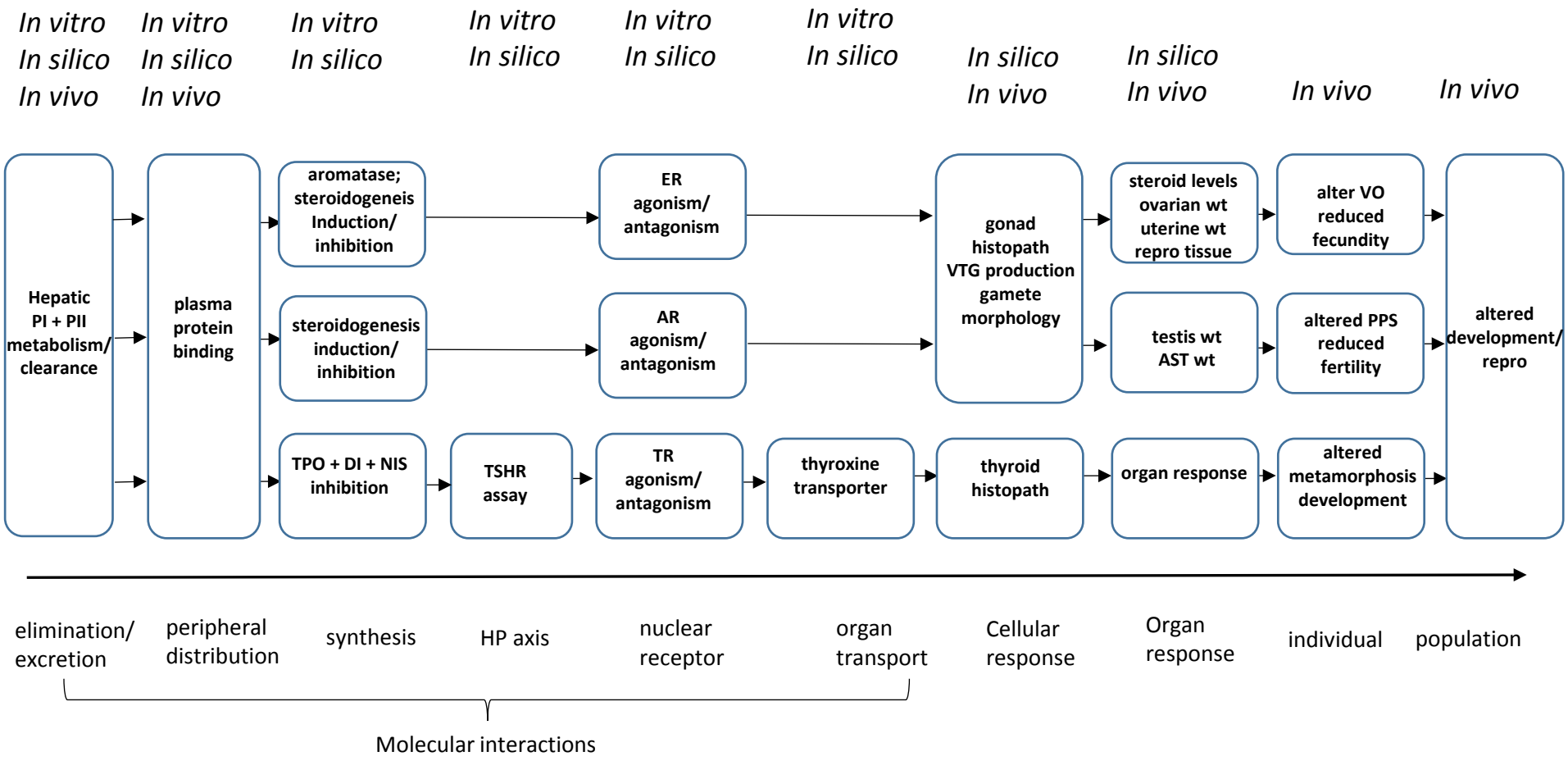
EDSP Path Forward

- Determine how well existing models predict intact animal results
  - Comparison to other Tier 1 endpoints
  - Additional Tier 1 assay substitution?
- Use additional computational tools to develop models for estrogen, androgen, and thyroid pathways
  - Integrate more assays
  - Integrate more key events
- Expand reference chemicals with defined potencies for performance based test guidelines incorporating computational tools
  - Use high quality in vivo data from peer reviewed literature
- Revise IBER for prioritizing and screening chemicals with limited exposure data
  - Revised models for dermal and inhalation exposures
  - Will allow for extrapolation to ecotoxicology

Evolution of Screening in the EDSP

EDSP Tier 1 Battery of Assays (current)	High Throughput Assays and Computational Model Tier 1 Battery Alternatives
Estrogen Receptor (ER) Binding	ER Model (alternative)
Estrogen Receptor Transactivation (ERTA)	ER Model (alternative)
Uterotrophic	ER Model (alternative)
Female Rat Pubertal	ER, STR , and thyroid (THY) Models (Future)
Male Rat Pubertal	AR, STR , and THY Models (Future)
Androgen Receptor (AR) Binding	AR Model (Future)
Hershberger	AR Model (Future)
Aromatase	STR Model (Future)
Steroidogenesis (STR)	STR Model (Future)
Fish Short Term Reproduction	ER, AR, and STR Models (Future)
Amphibian Metamorphosis	THY Model (Future)

Endocrine Pathways



Summary

- Pivot to using high throughput and computational methods in EDSP
- Computational tools have been peer-reviewed by SAP and for publication
- Endocrine pathway models will continue to be revised and improved as more data are available (ER, AR, thyroid…)
  - Provides bioactivity predictions for thousands of chemicals
- Allows resources to be focused on chemicals more likely to have endocrine effects
  - List 1 chemicals have limited estrogen and/or androgen receptor-mediated bioactivity
  - Prioritizes chemicals based on bioactivity (and exposure)
  - Provides alternative to current Tier 1 screening
- Multi-century project becomes multi-year