U.S. Environmental Protection Agency

News from the Endocrine Disruptor Screening Program

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Office of Chemical Safety and Pollution Prevention

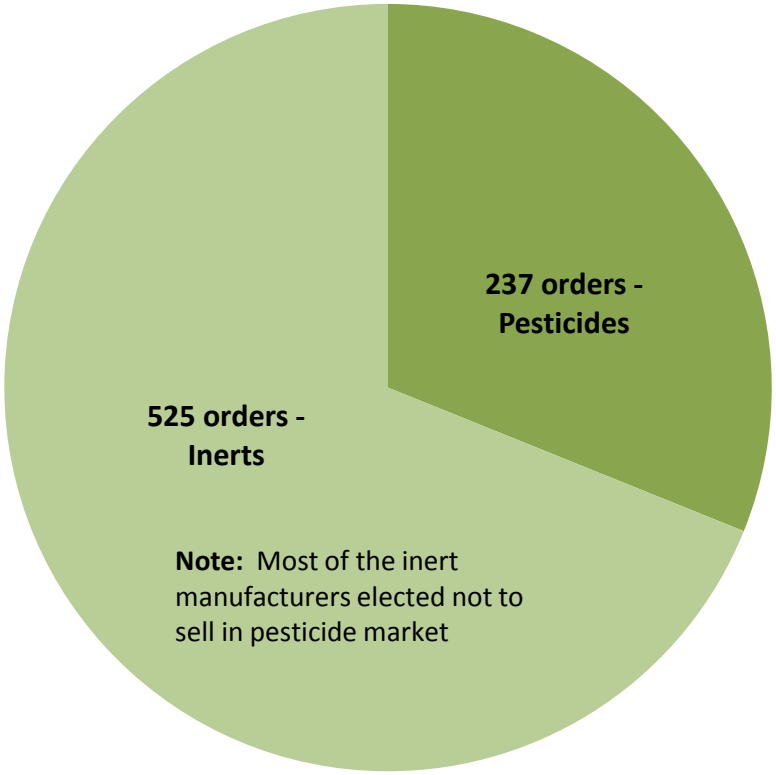
CHE Fertility Working Group call
June 30, 2015

EDSP Update on List 1 Chemicals

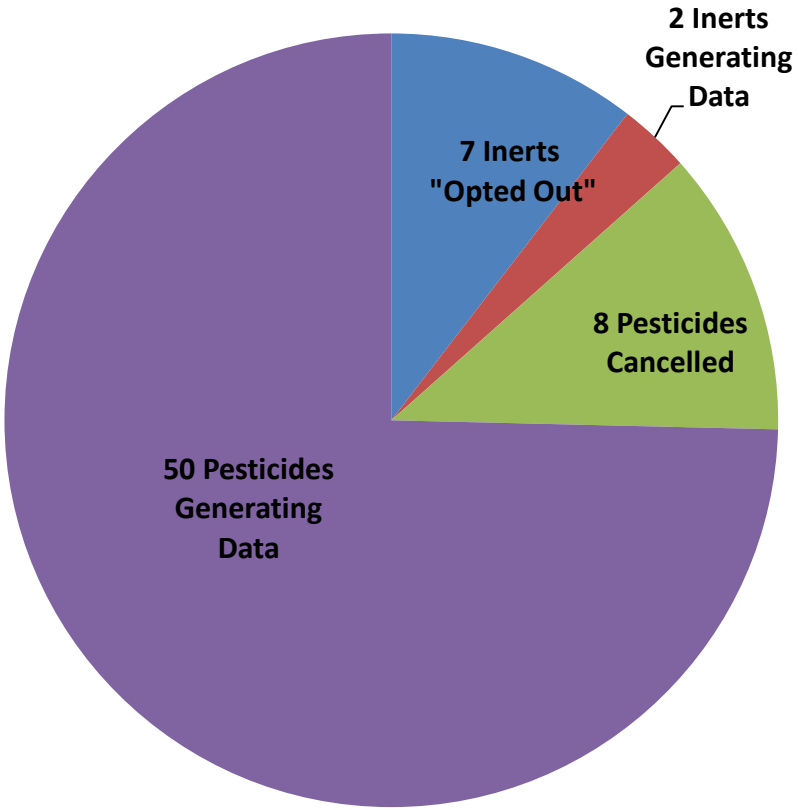
Overview:

- Brief History
- Tier 1 battery interpretation
- Weight of Evidence Assessments
- Tier 2 Study Recommendations/Rationale

762 Orders Issued on 67 Chemicals



General Responses for the 67 Chemicals



EDSP List 1 Process History

Oct 2009-Feb 2010	Jan 2010-Dec 2010	Jan 2012-Oct 2013	Nov 2013–Dec 214	Jan 2015-June 2015
Issued 762 Test Orders for 67 Chemicals	**EDRT - OSRI review**	**Review & Preparation of 504 DERS**	**Weight of Evidence Assessments 52 Chemicals**	**Review, Refine, Revise 52 WoEs**

Data Review

HED (9 Assays/Chem)
- Aromatase
- Estrogen Receptor
- Androgen Receptor
- ERTA
- Hershberger
- Female pubertal
- Male pubertal
- Steroidogenesis
- Uterotrophic

EFED (2 Assays /Chem)
- Amphibian Metamorphosis
- Fish Short-Term Reproduction

SAP Reviews Related to List 1

May, 2013: Tier 1 Assay/Battery Performance
June, 2013: Tier 2 validation
July, 2013: WoE Approach

Assay Review Process

- Primary Review/ Secondary Review of DERs
- T1 Assay Review Committee (T1ARC)
  - EFED, HED, RD, OSCP, ORD Consultation
  - Ensure consistency in the interpretation of endpoints and assay conclusions across chemicals
  - Unacceptable assay(s): 1- FSTRA & 2 Pubertals
- Finalize the 504 assay Data Evaluation Records

Assay Review Process

- **T1 Assay Weight of Evidence Review Committee (T1WoERC)**
  - Secondary review of WoE document by HED/EFED staff
  - Staff presented the document to the TiWoERC
  - Ensure consistency in the WoE assessments across chemicals / pathways / recommendations

- QA/QC - Trifecta Review, Refine and Revise WoE Documents to ensure consistency in the conclusions
  - Resulted in reconsidering recommendations of 2 chemicals, thus far.

Outline of the Individual WOE Documents for Estrogen, Androgen and Thyroid Pathways (based on USEPA 2011 Guidance Document)

I. Introduction

II. Source of Scientific Data and Technical Information

III. Weight of Evidence (WoE) Evaluation

- A. EDSP Tier 1 Screen Assays
- B. Effects on Hypothalamic-Pituitary-Gonadal (HPG) Axis
  1. Effects on Estrogen Pathway
  2. Effects on Androgen Pathway
- C. Effects on Hypothalamic-Pituitary-Thyroidal (HPT) Axis

IV. Committee's Assessment of Weight off Evidence

- A. Systemic/Overt toxicity in the Tier 1 and OSRI Studies
  1. Tier 1 *in vivo* assays
  2. OSRI
- B. Estrogen Pathway
- C. Androgen Pathway
- D. Thyroid Pathway
- E. Conclusions

V. EDSP Tier 2 Testing Recommendations

SAP Recommendations for WOE

The 2013 SAP stated that , “In summary, the Panel agreed that little, if any, weight should be placed on signs of endocrine disruption in the presence of overt toxicity. All effects in endocrine sensitive tissues should be evaluated in terms of primary interactions with the endocrine system vs. secondary effects related to toxicity in non-endocrine organs or overall disruptions in homeostasis”

Overt Toxicity

Overt toxicity for the *in vivo* Tier 1 and OSRI studies are:

- mortality;
- tremors, ataxia, and abnormal swimming (fish and amphibians);
- body weight decreases of ≥10% in mammals.
- other clinical signs (*e.g*, lethargy) especially if the effects were extreme.
- morphological (e.g., organ weights/histopathology), clinical pathology (e.g, hematology, blood chemistry, MOA)
- In some instances, one parameter (i.e., death or >10% decrease in mammalian body weight) was sufficient to consider a dose/concentration to be overtly toxic.
- However, in other instances, more than one parameter was needed to determine overt toxicity. For example, in the FSTRA, generally, body weight decreases were considered along with other responses when assessing potential overt toxicity**Systemic toxicity**

Estrogenic/Anti-Estrogenic Pathway

Lines of Evidence Indicating Potential Interaction with the Estrogenic/Anti-Estrogenic Pathway for Chemical X[1]															
Study Type / Literature Citation	ER Binding	ER Activation	Steroidogenesis	Sex Steroid Hormones	Uterine Weight	Ovarian Weight / GSI	Gonadal Staging and Histopathology	Pituitary Weight	Estrous Cyclicity	Age & Weight at VO	2° Sex Characteristics	Fertility (Frt)/ Fecundity (Fcd)	Vitellogenin	Systemic Toxicity Observed[2]	Overt Toxicity Observed[3]
EDSP Tier 1 Assays															
ER Binding	E														
ERTA		N													
Aromatase			N												
Steroidogenesis			P												
Uterotrophic					N									N	N
Female Pubertal Rat					N	N	N	N	N	N				↑AW[5] (H)	N
FSTRA				NE		N	N				N	Fcd: ↓44% (M); Frt: ↓1.3% (H)	N		N
OSRI															
Part 158 Studies/Literature studies							N							X (M, MH, H)	N

Tier 2 Study Recommendations

- **Human Health**
  - Opted to focused studies
    - Comparative Thyroid Assay (CTA)
    - Male reproductive toxicity
    - Studies more focused to assess specific target organ toxicity – Thyroid & Male Reproduction

- **Wildlife**
  - T2 Medaka Extended One Generation Reproduction Test (*MEGORT*): 12 chemicals
  - T2 Larval Amphibian Growth and Development Assay (*LAGDA*): 6 chemicals

Evidence of Potential Interaction, but no Tier 2 studies Recommended

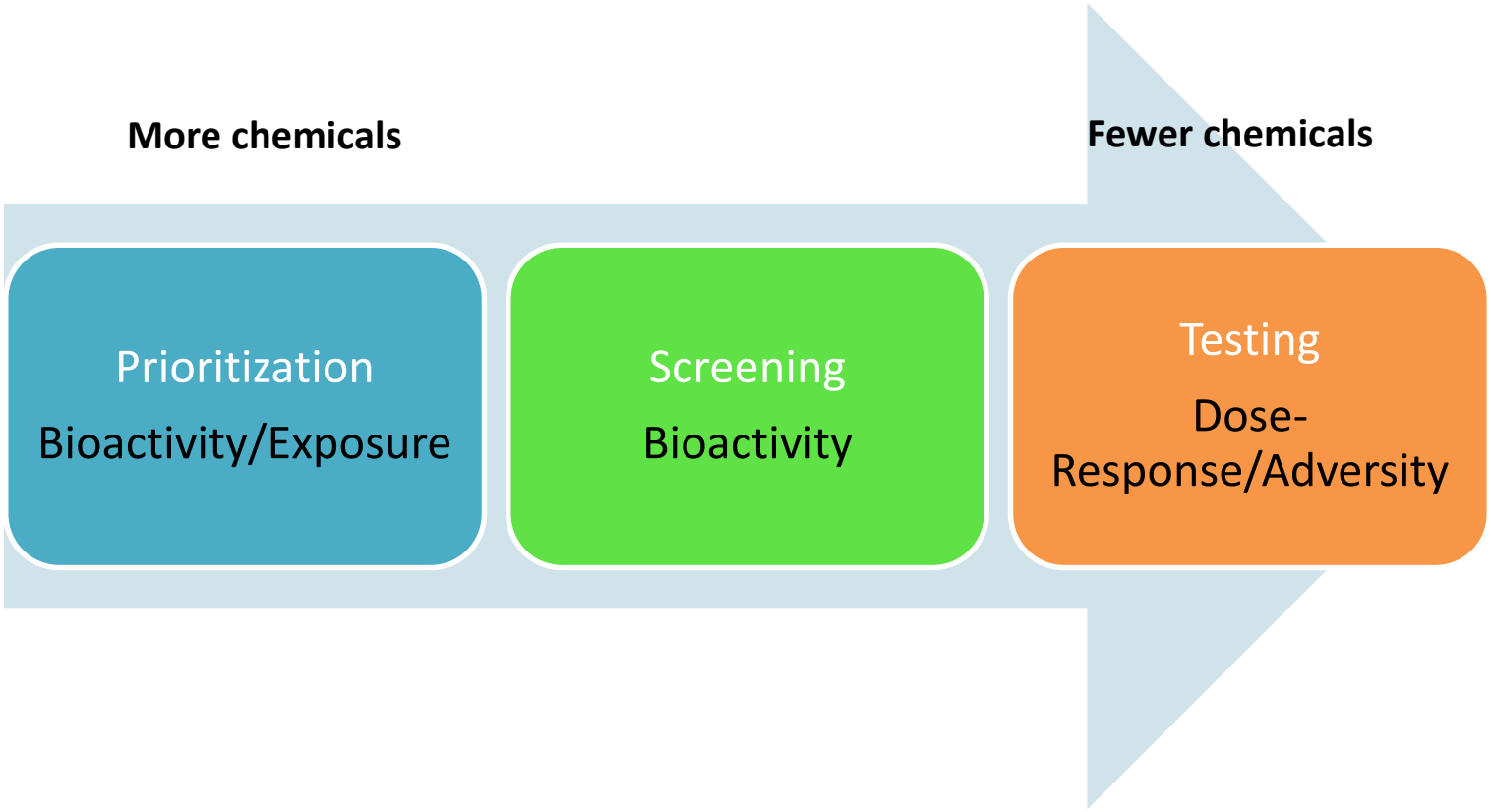
- For human health, PODs/RfDs/MOEs based on more sensitive endpoint(s) [*neuro, hepatic, developmental, or reproductive toxicity*] are considerably lower than the dose(s) that caused E, A or T-mediated effects in the Tier 1 assays

- For wildlife, current NOAECs or Tier 2-like data are available for regulatory purposes.

- Therefore, additional testing would not impact the current EPA established regulatory endpoints for human or ecological risk assessments.

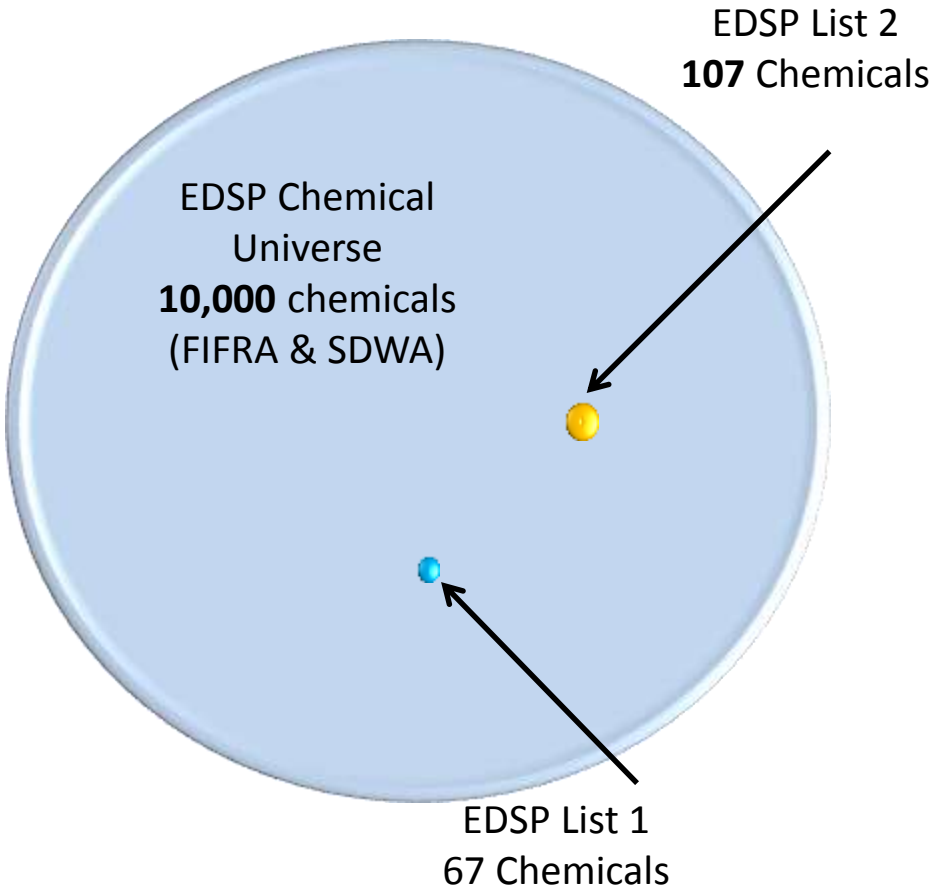
EDSP Update on New High Throughput Screening Tools

EDSP Prioritization, Screening & Testing



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

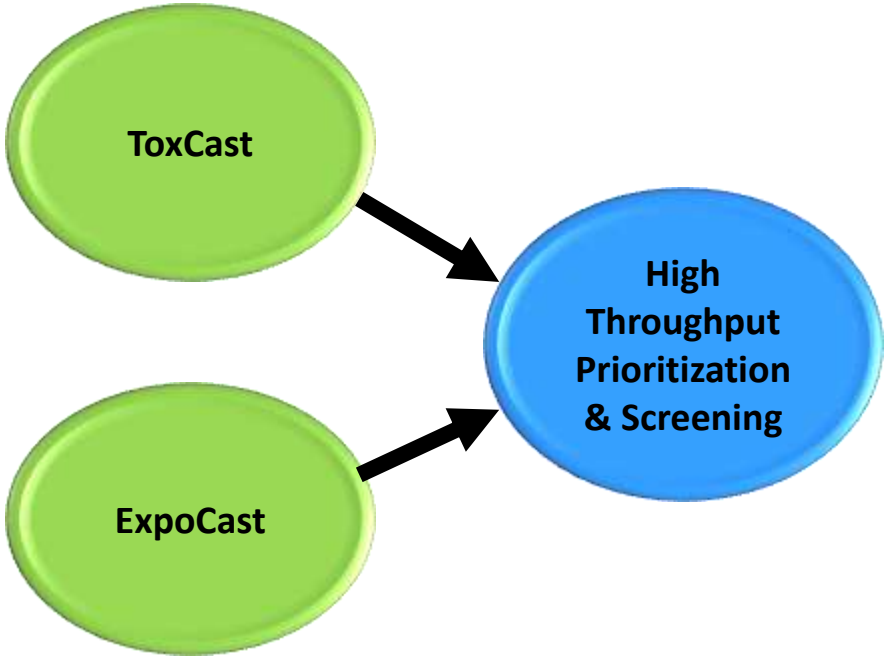
Evolution of EDSP- the Pivot



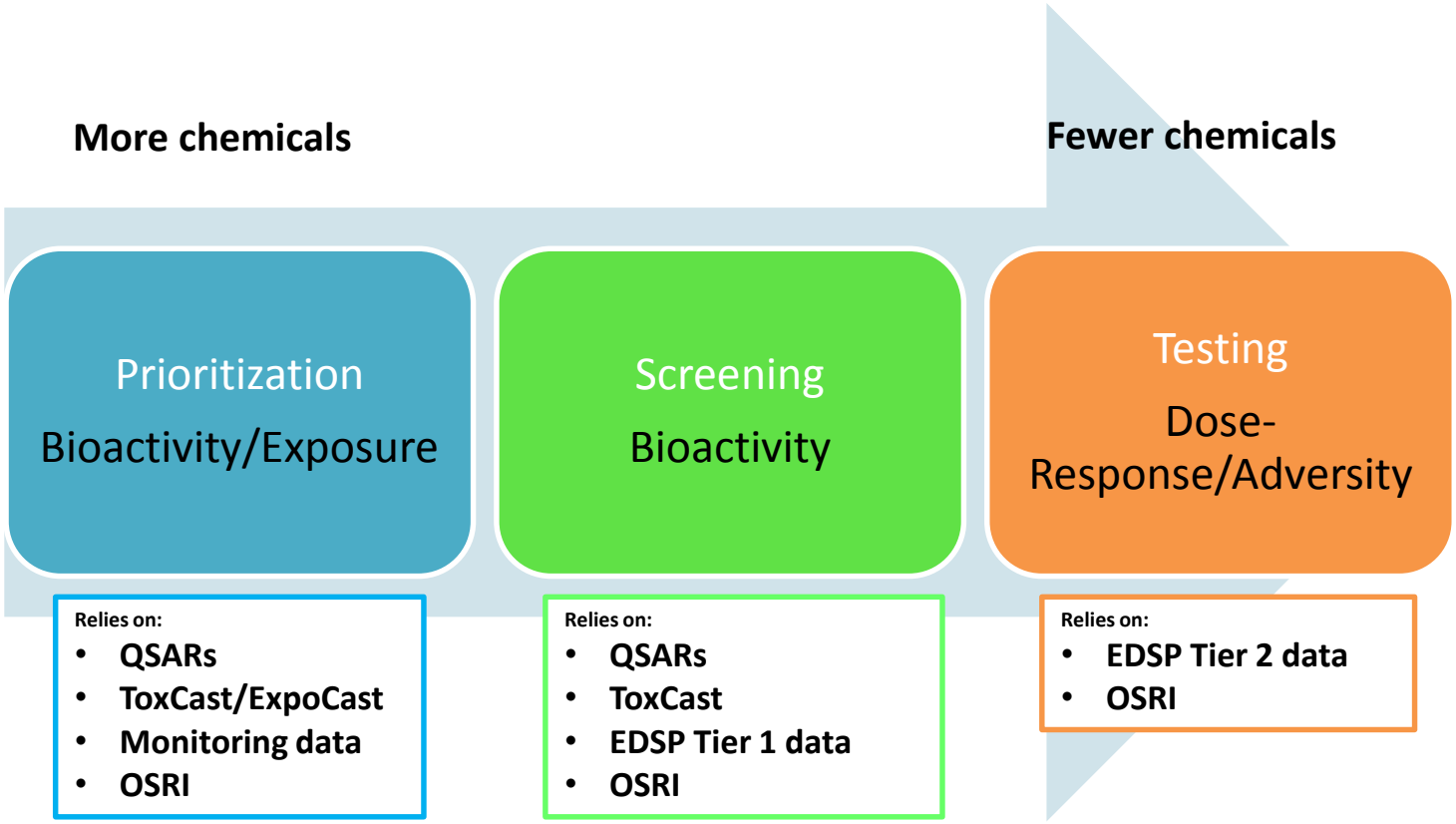
- Based on current pace it could take decades to screen all 10,000 chemicals in EDSP Universe
- Pivot: use high throughput assays and computational models to rapidly screen chemicals for potential bioactivity and exposure

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
Testing for dose-response and adverse effects

EDSP Pivot Goals

Use computational tools and models in the EDSP framework to:

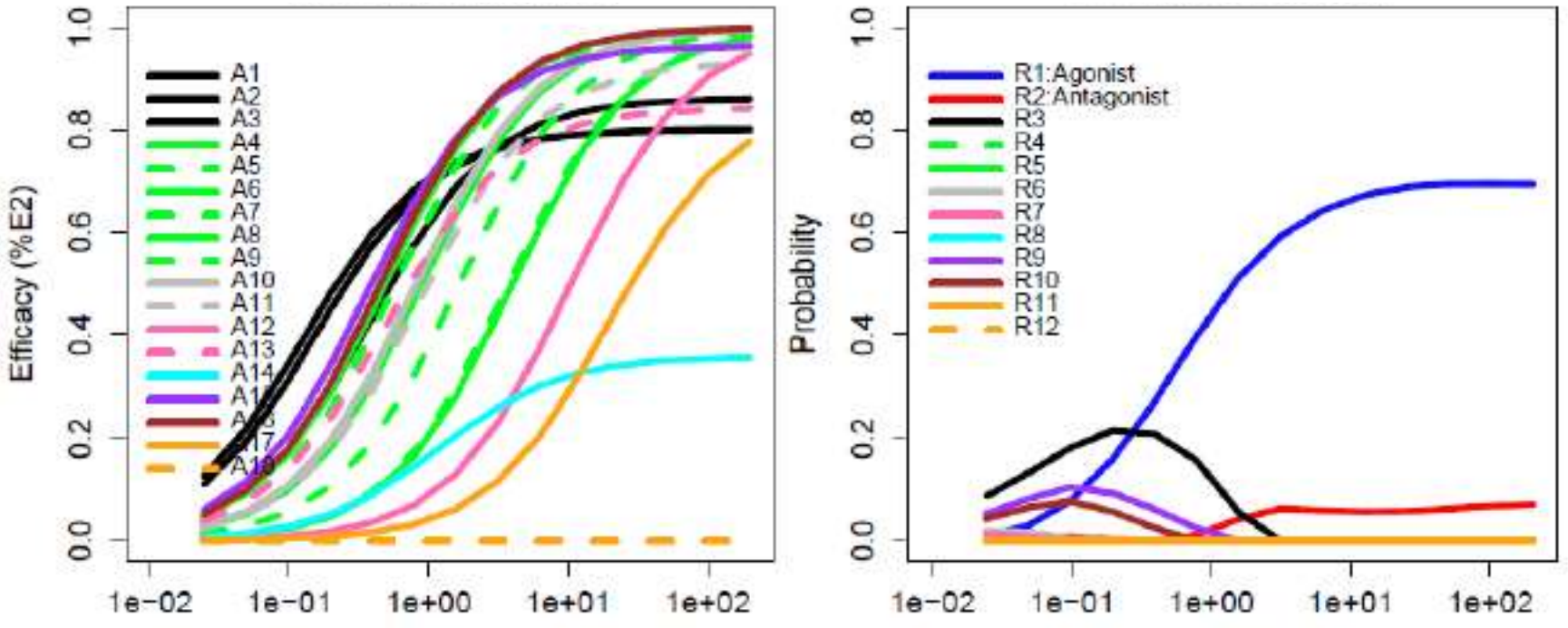
1. Prioritize chemicals for further EDSP screening and testing based on estimated bioactivity and exposure
2. Contribute to the weight of evidence evaluation of a chemical's potential bioactivity
3. Substitute for specific endpoints in the EDSP Tier 1 battery

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
- AR bioactivity model
  - 9 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
  - AR antagonists

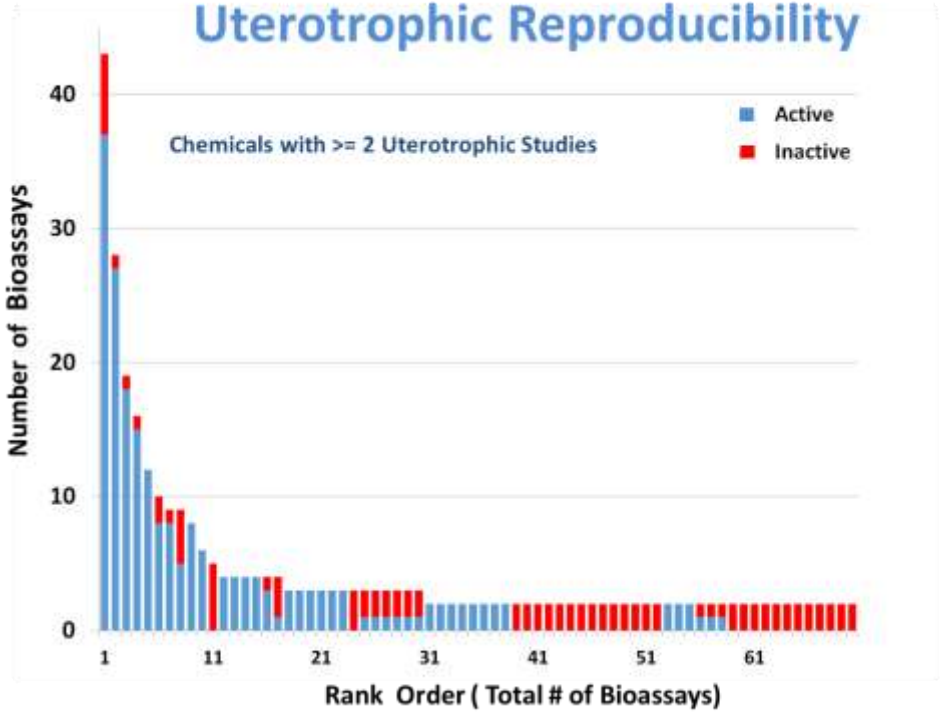
High Throughput Assays Integrated Into A Pathway Bioactivity Model



Judson et al. 2013 SOT

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



Environmental Science & Technology

Article

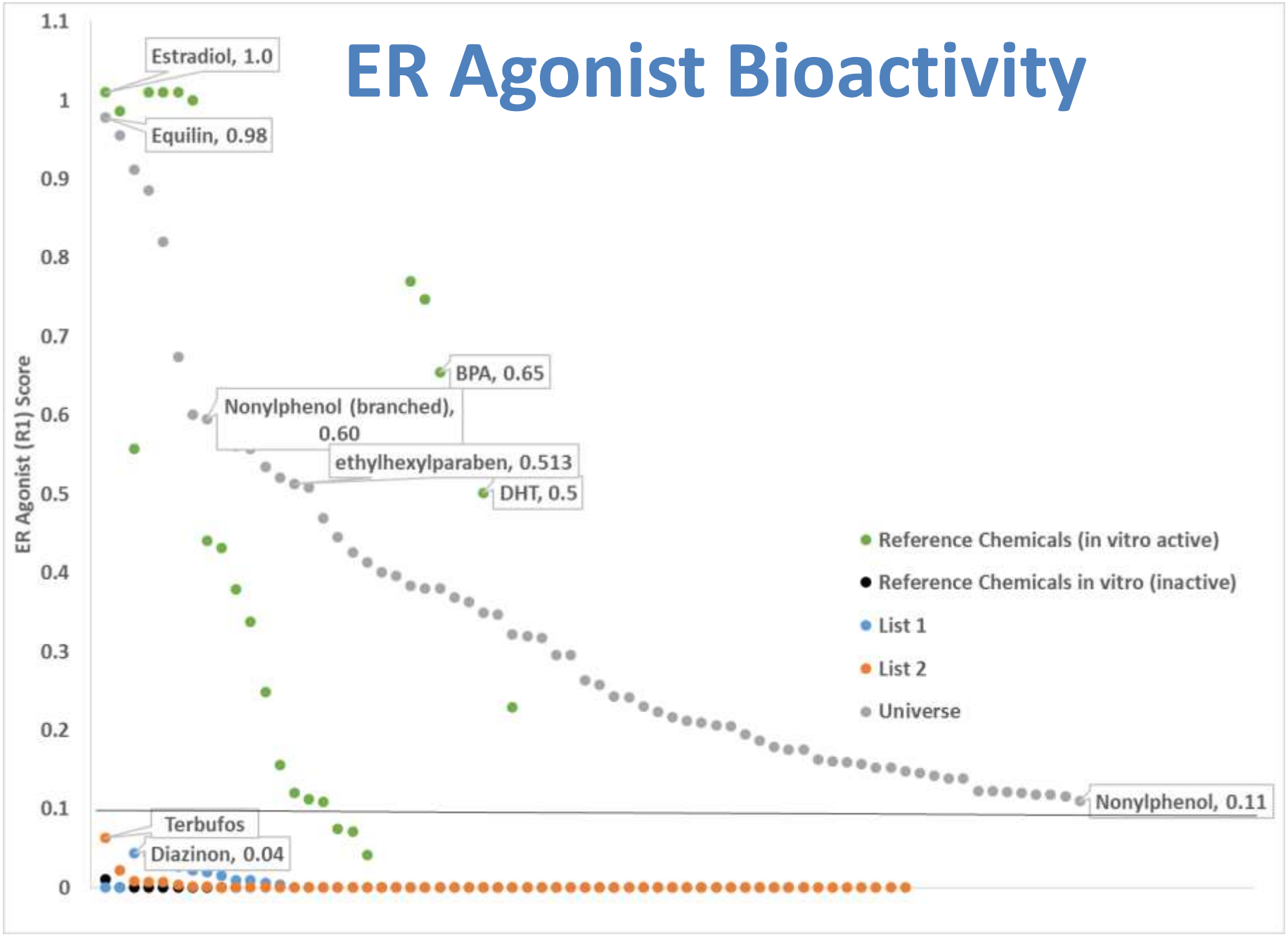
SCREENING CHEMICALS FOR ESTROGEN RECEPTOR BIOACTIVITY USING A COMPUTATIONAL MODEL

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

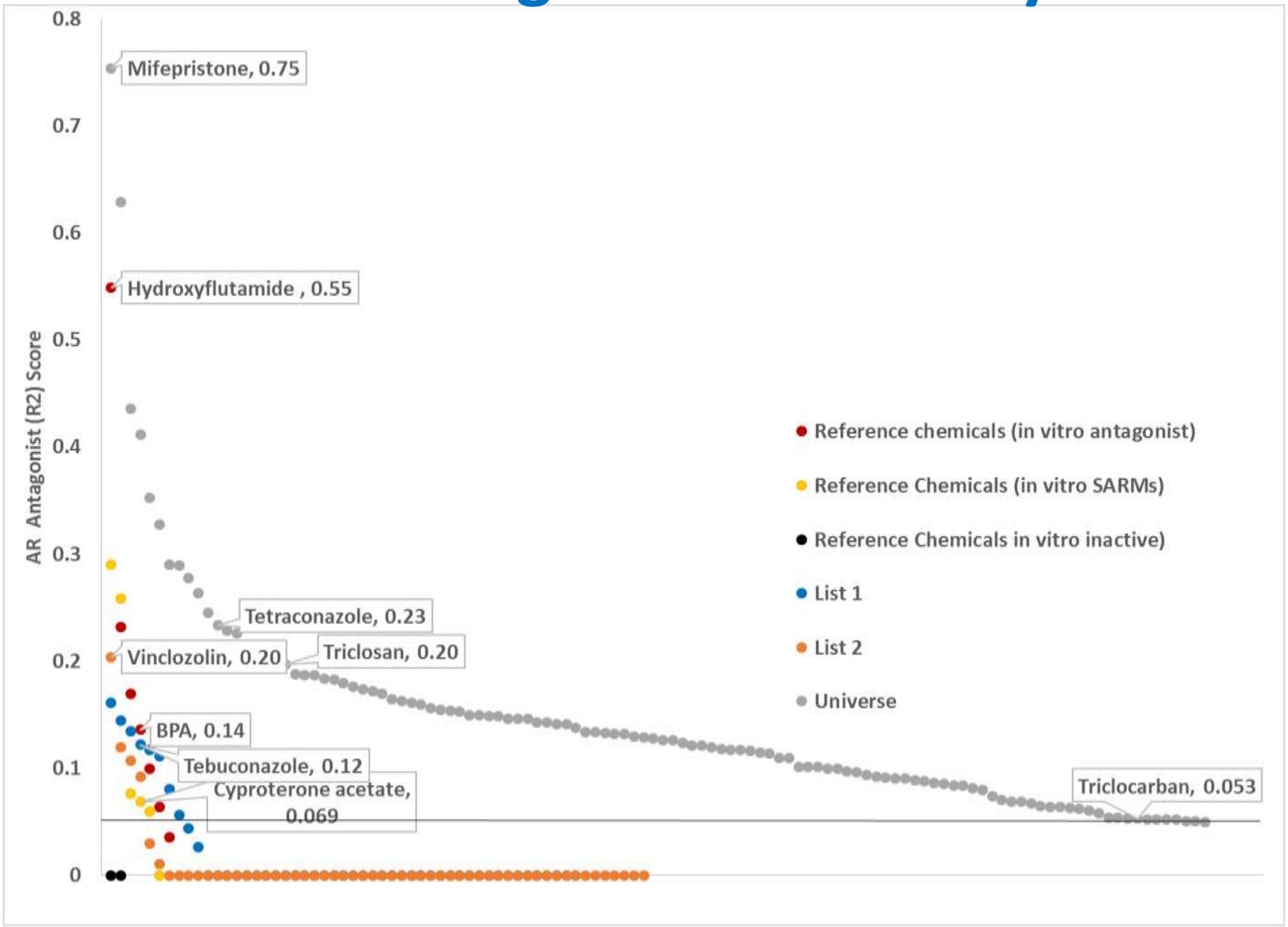
Environ. Sci. Technol., **Just Accepted Manuscript** • DOI: 10.1021/acs.est.5b02641 • Publication Date (Web): 12 Jun 2015

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

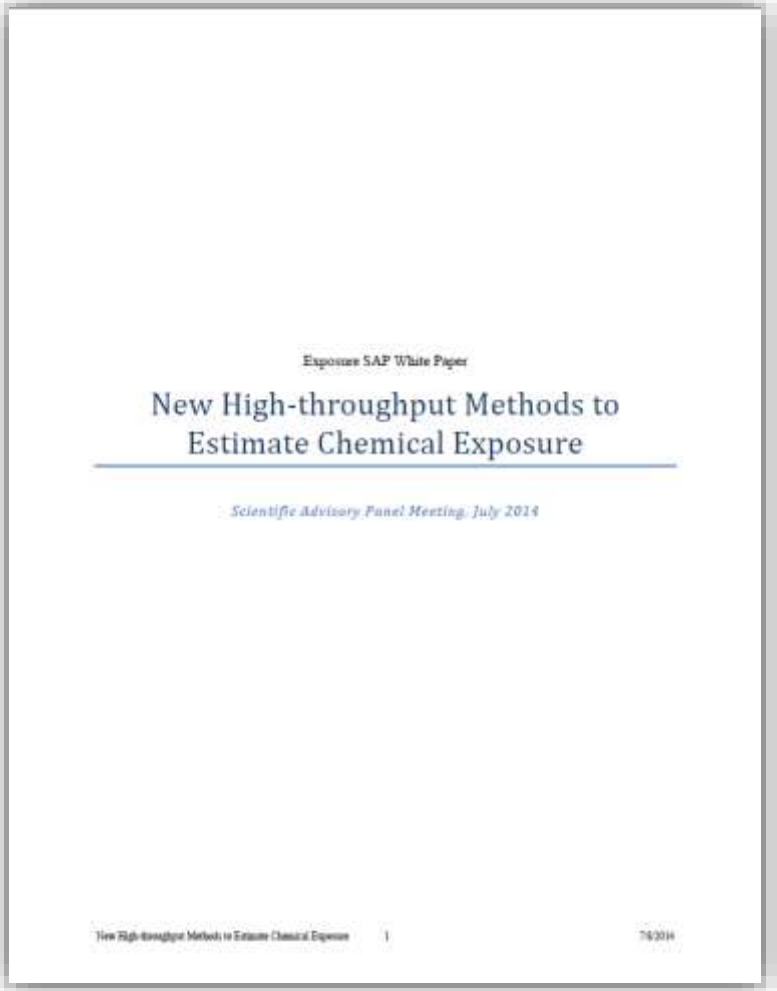
ER Agonist Bioactivity



AR Antagonist Bioactivity



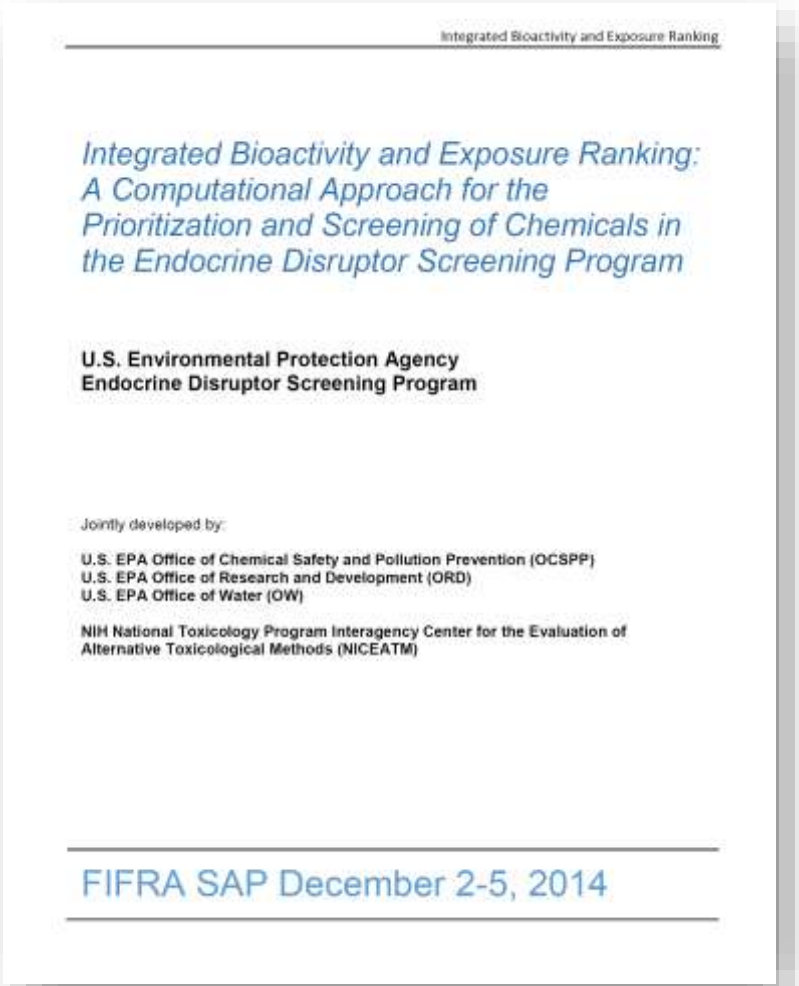
Building Scientific Confidence – Peer Review



Exposure SAP White Paper

New High-throughput Methods to Estimate Chemical Exposure

Scientific Advisory Panel Meeting, July 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)

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