U.S. Environmental Protection Agency

Endocrine Disruptor Screening Program

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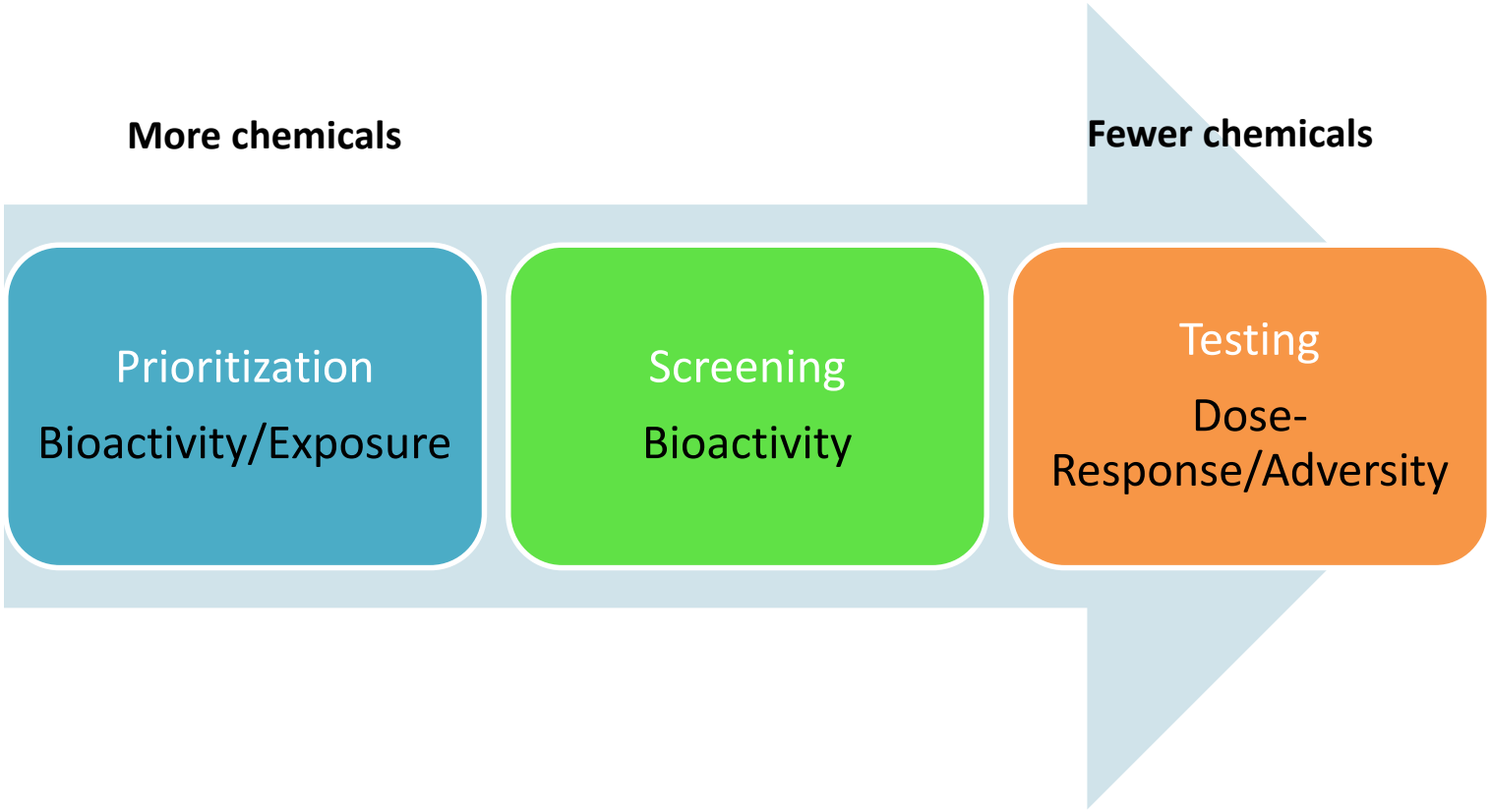
EPA's Computational Toxicology Communities of Practice
April 23, 2015

EDSP Prioritization, Screening & Testing



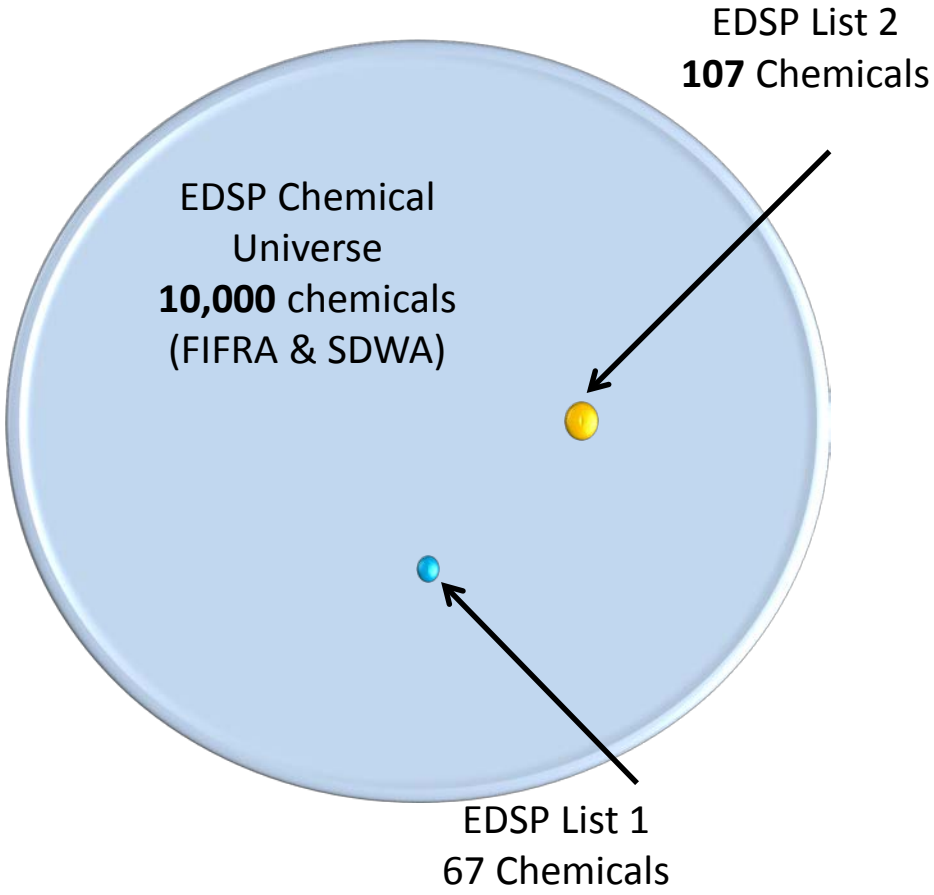
Prioritization and Screening for bioactivity
Testing for dose-response and adverse effects

Screening – Tier 1

Tier 1 Screening Battery	Endocrine Pathway					
	E +	E -	A +	A -	HPG Axis	HPT Axis
In vitro						
ER Binding	■	■				
ERα Transcriptional Activation*	■					
AR Binding			■	■		
Steroidogenesis H295R*	■	■	■	■		
Aromatase Recombinant		■				
In vivo						
Uterotrophic*	■					
Hershberger*			■	■		
Pubertal Male	■	■	■	■	■	■
Pubertal Female	■	■	■	■	■	■
Amphibian Metamorphosis*						■
Fish Short-term Reproduction (male & female)*	■	■	■	■	■	

*OECD harmonized guidelines

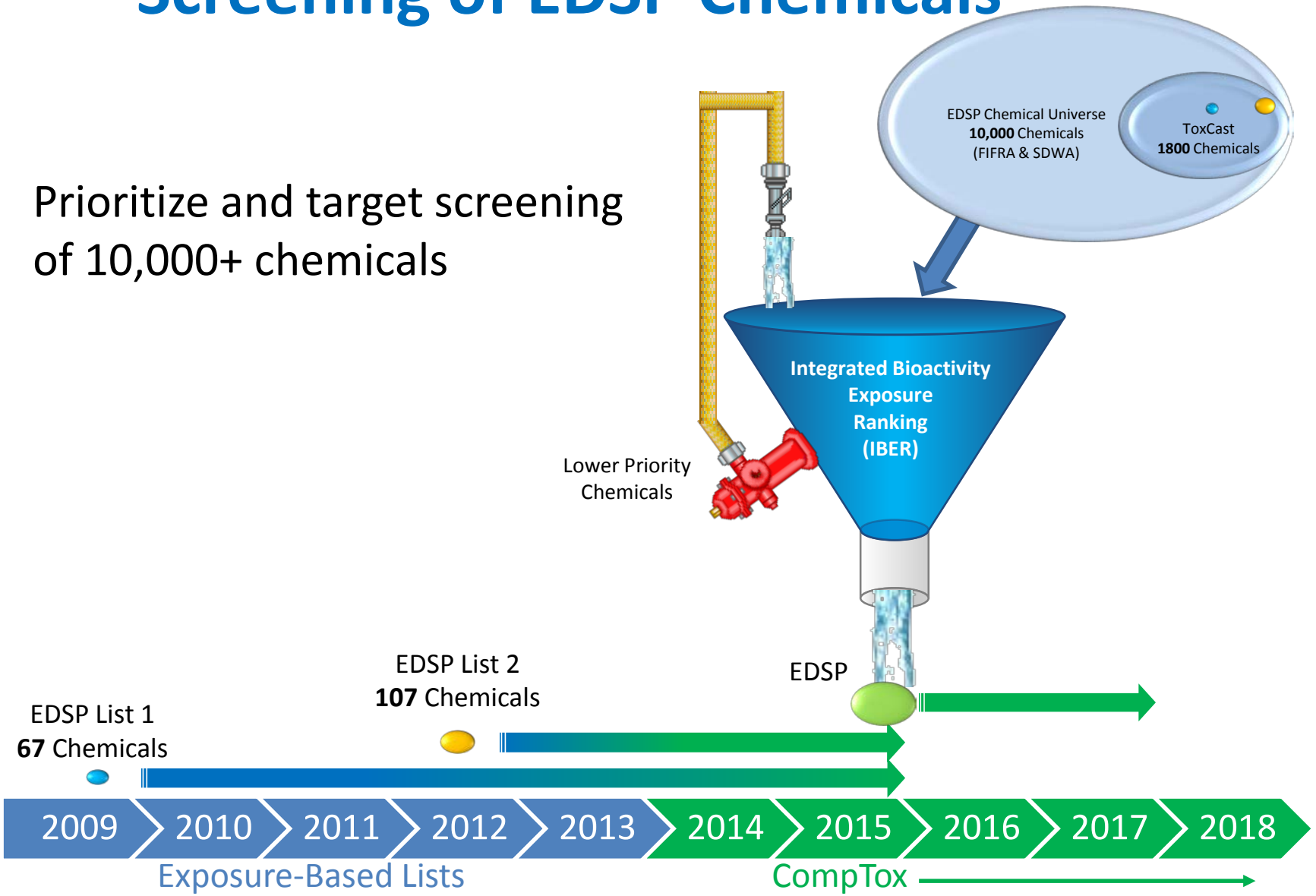
Evolution of EDSP- the Pivot



- 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

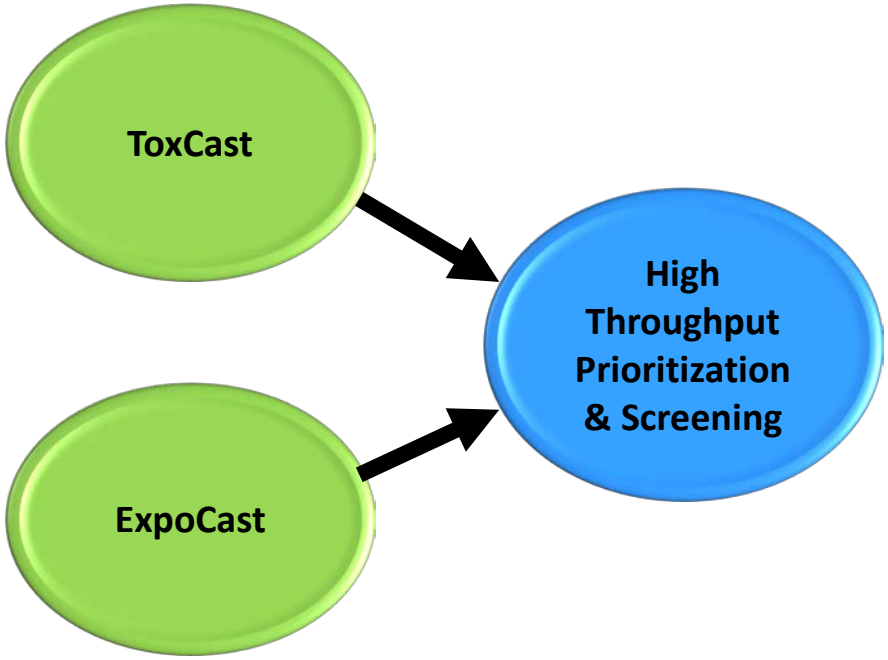
Pivot: High Throughput Prioritization & Screening of EDSP Chemicals

- Prioritize and target screening of 10,000+ chemicals

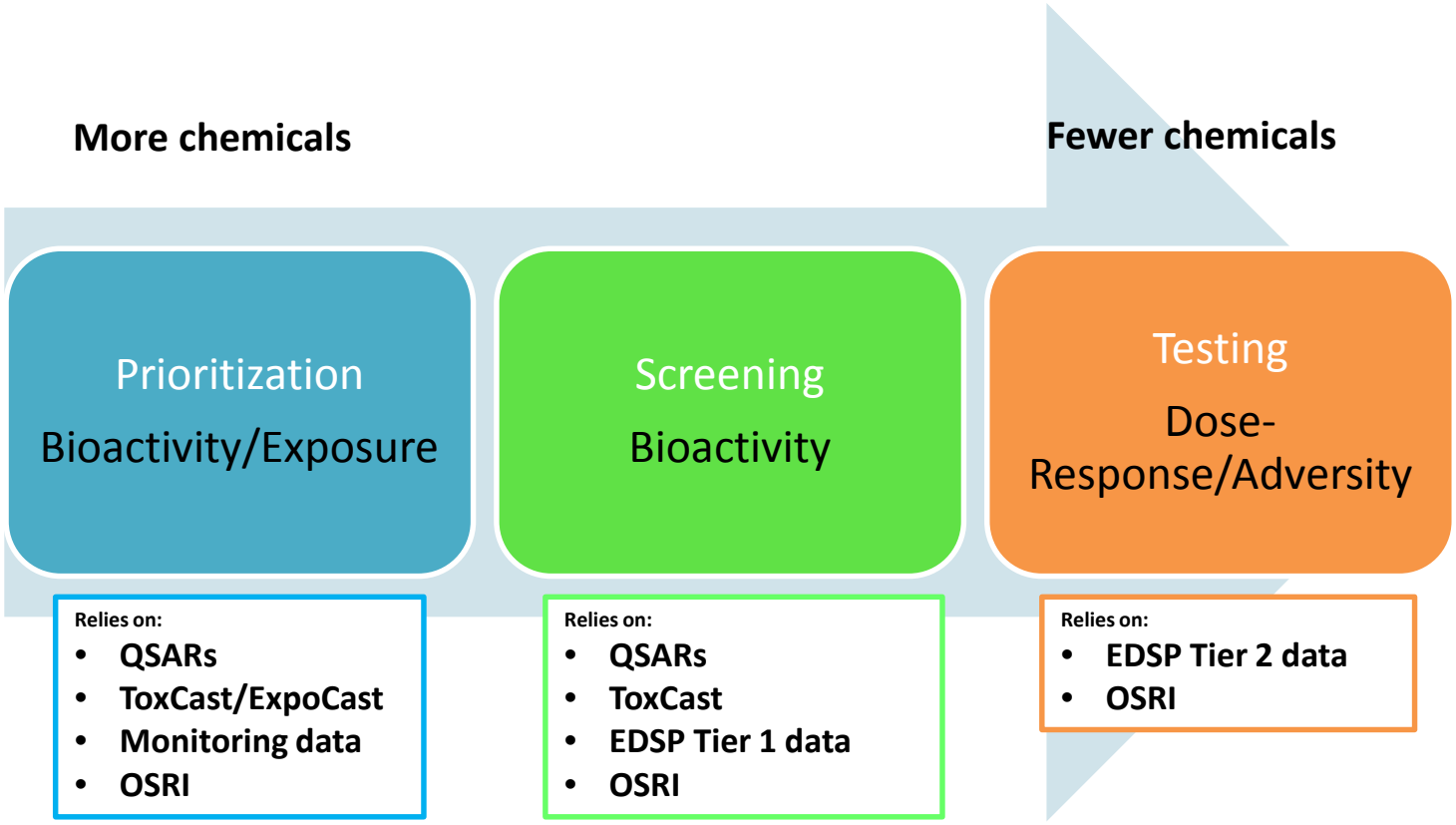


Computational Tools

- 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:

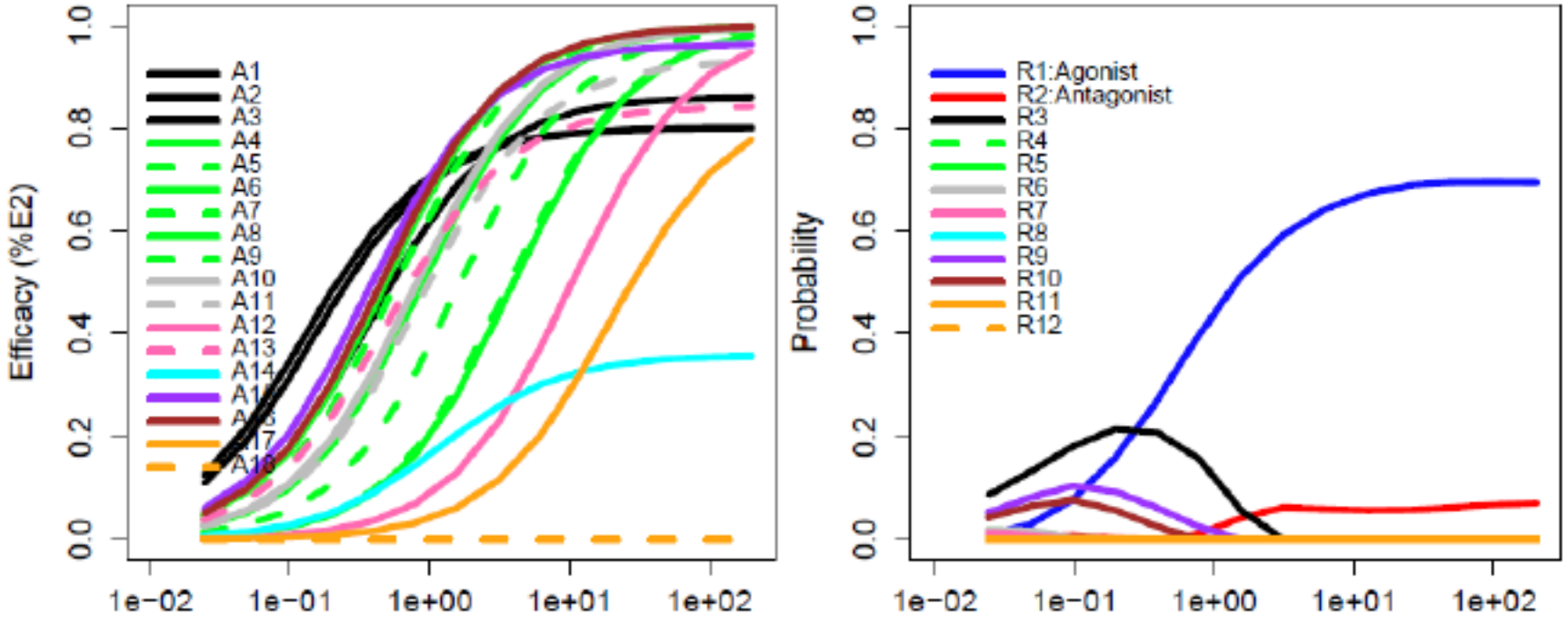
1. Prioritize chemicals for further EDSP screening and testing based on estimated bioactivity and exposure
2. Contribute to the weight of evidence evaluation of a chemical's potential bioactivity
3. Substitute for specific endpoints in the EDSP Tier 1 battery

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

Performance Based Approach to Establish Scientific Confidence

- Reference chemical set that includes a range of structures and potencies that are accurately detected
  - *in vitro* reference chemicals
  - *In vivo* reference chemicals
- New methods compared with current methods
  - Bioactivity model versus Tier 1 results

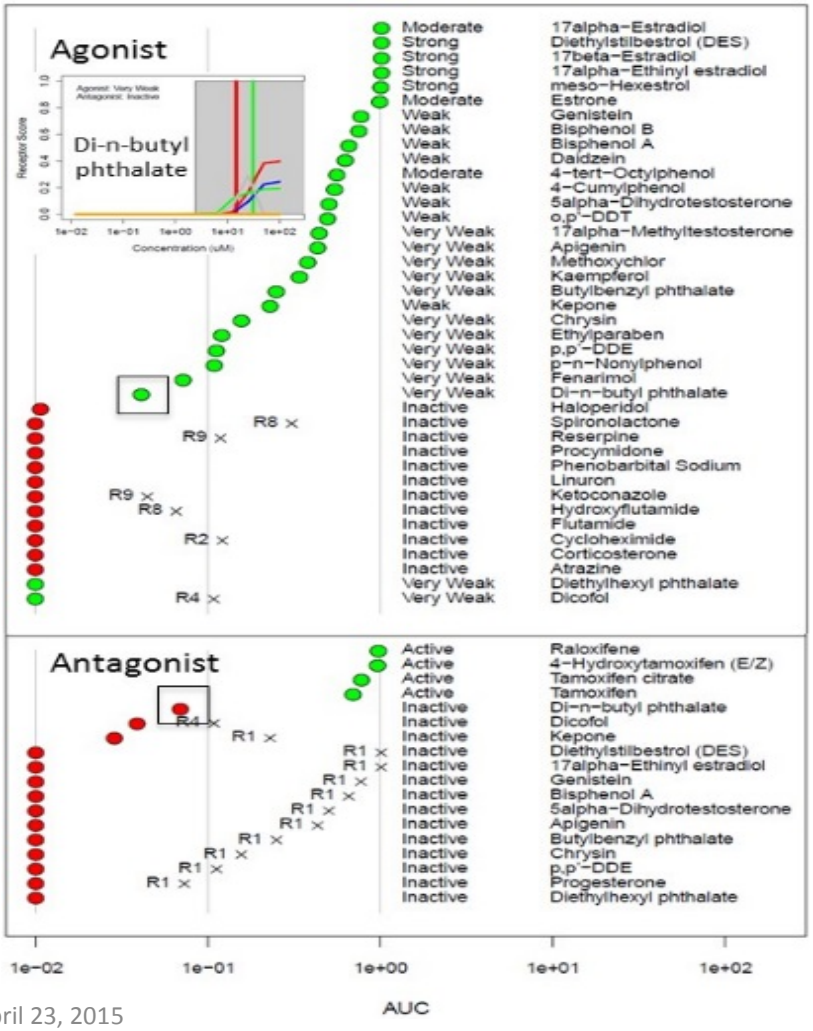
Evaluated by independent, external peer review

- FIFRA Scientific Advisory Panel meetings

ER Bioactivity Model: *in vitro* Reference Chemicals

- Excellent performance of ER model against *in vitro* reference chemicals

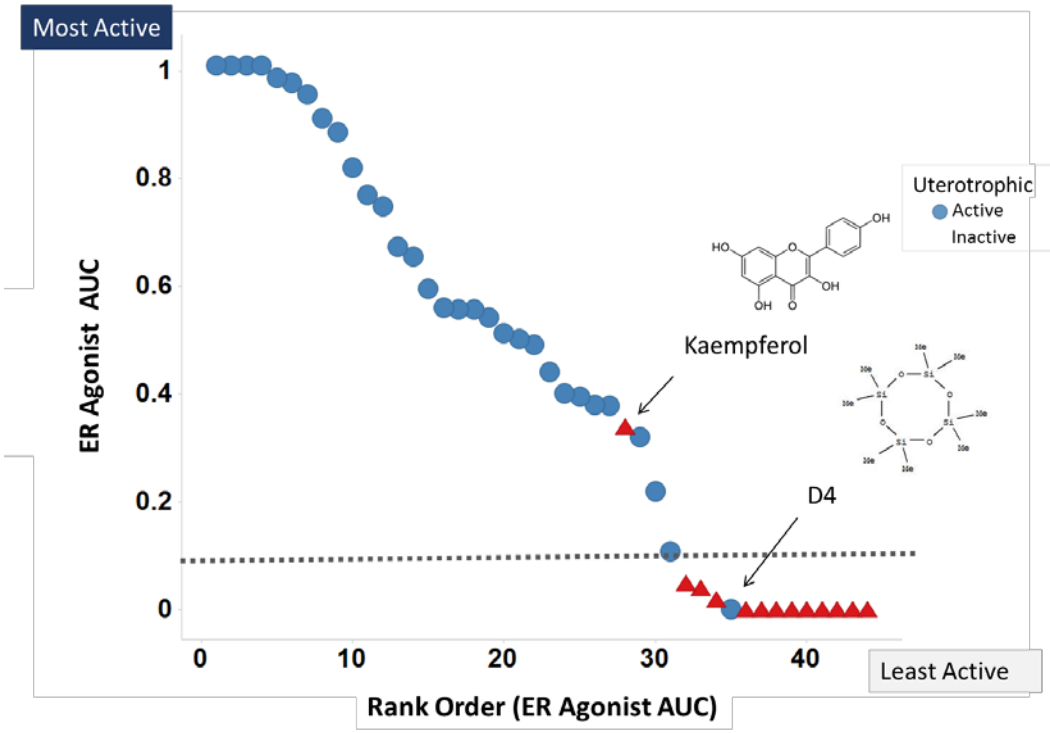
# True Pos	28
# True Neg	12
# False Pos	0
# False Neg	4
PPV	1.0
NPV	0.75
BA	0.94
Sensitivity	0.88
Specificity	1.0



ER Bioactivity Model: *in vivo* Reference Chemicals

- Excellent performance of ER model against i*n vivo* reference chemicals

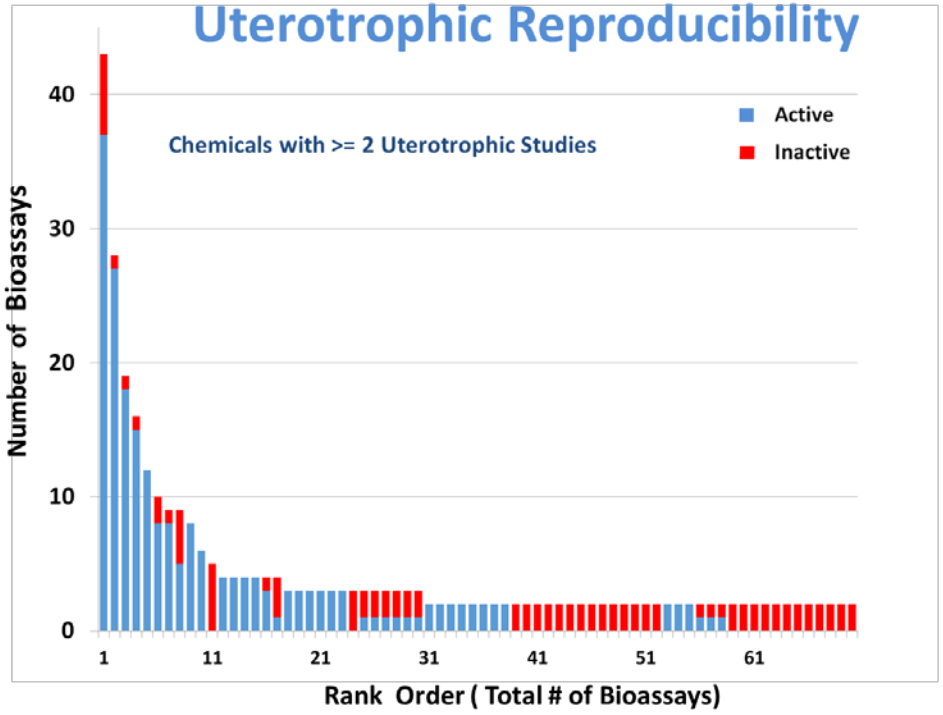
# True Pos	28
# True Neg	12
# False Pos	1
# False Neg	1
PPV	0.97
NPV	0.92
BA	0.95
Sensitivity	0.97
Specificity	0.92



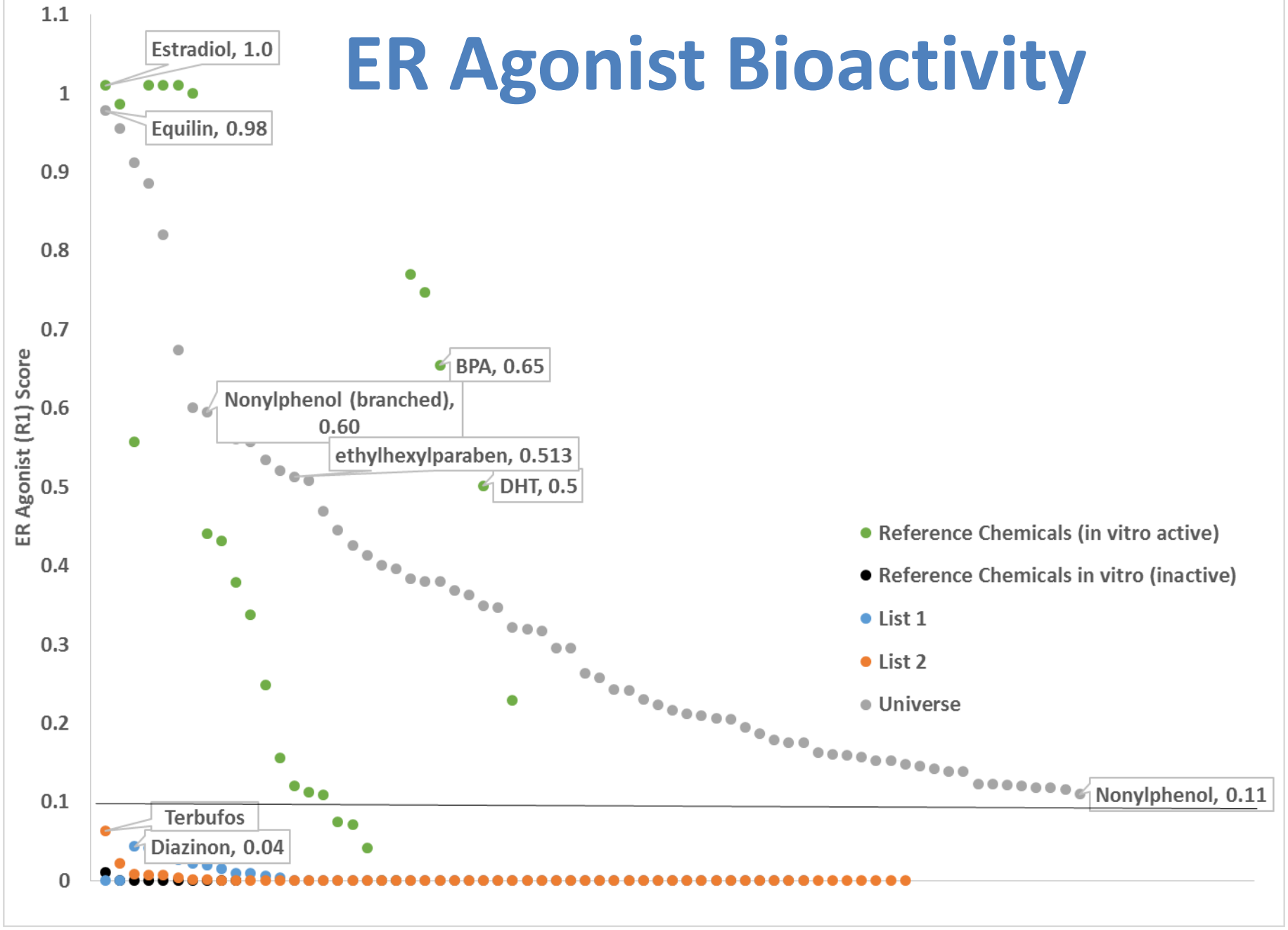
- Poster 2641 Thursday morning

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



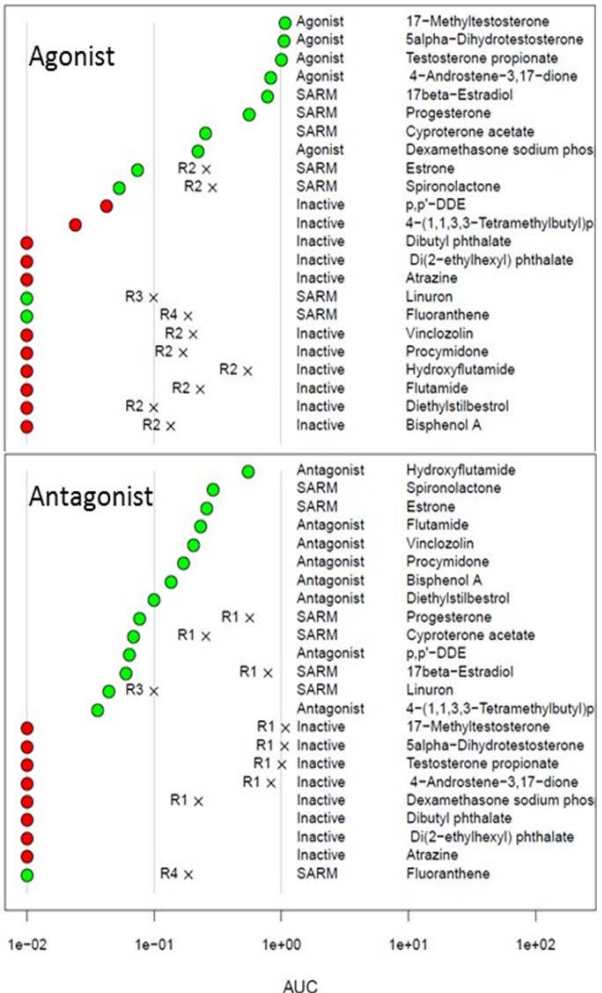
ER Agonist Bioactivity



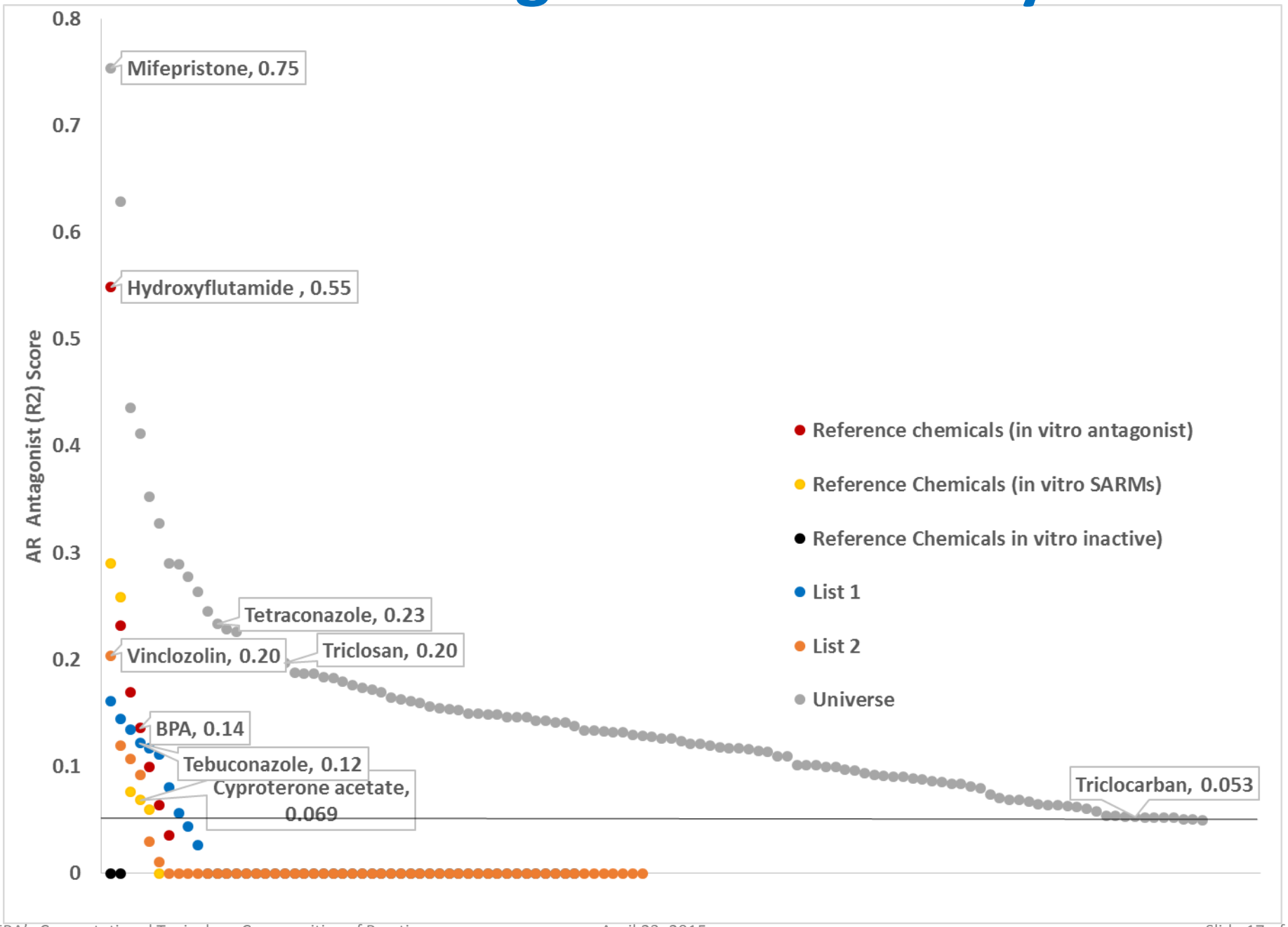
AR Bioactivity Model For Reference Chemicals

- Excellent performance of AR model against *in vitro* reference chemicals
  - AR model evaluated with 23 reference chemicals
  - T1 AR binding: 10
  - HB comparison underway

# True Pos	14
# True Neg	8
# False Pos	0
# False Neg	1
PPV	1.0
NPV	0.89
BA	0.97
Sensitivity	0.93
Specificity	1.0



AR Antagonist Bioactivity

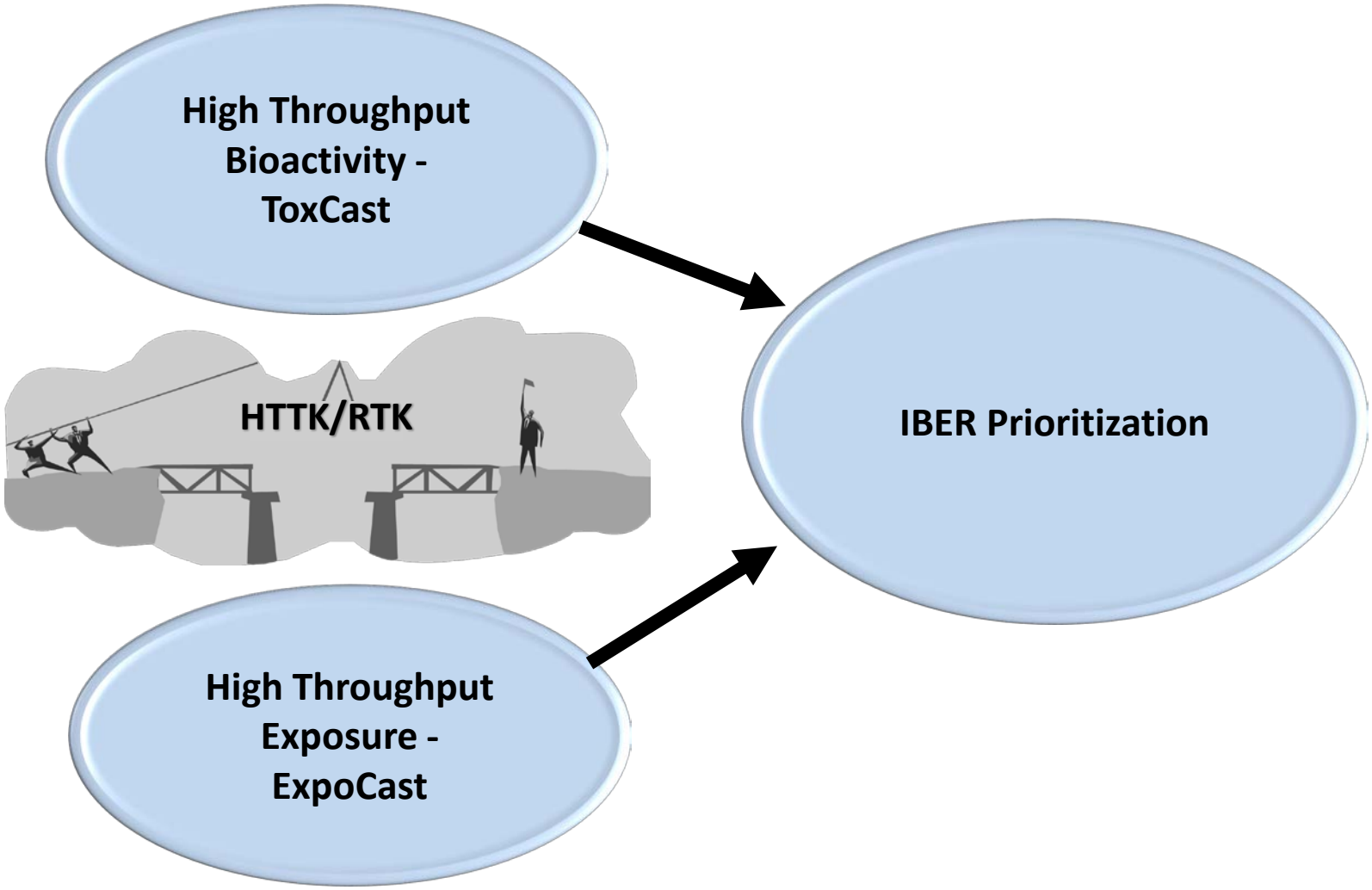


ER & AR Ranking

(less pharmaceuticls)

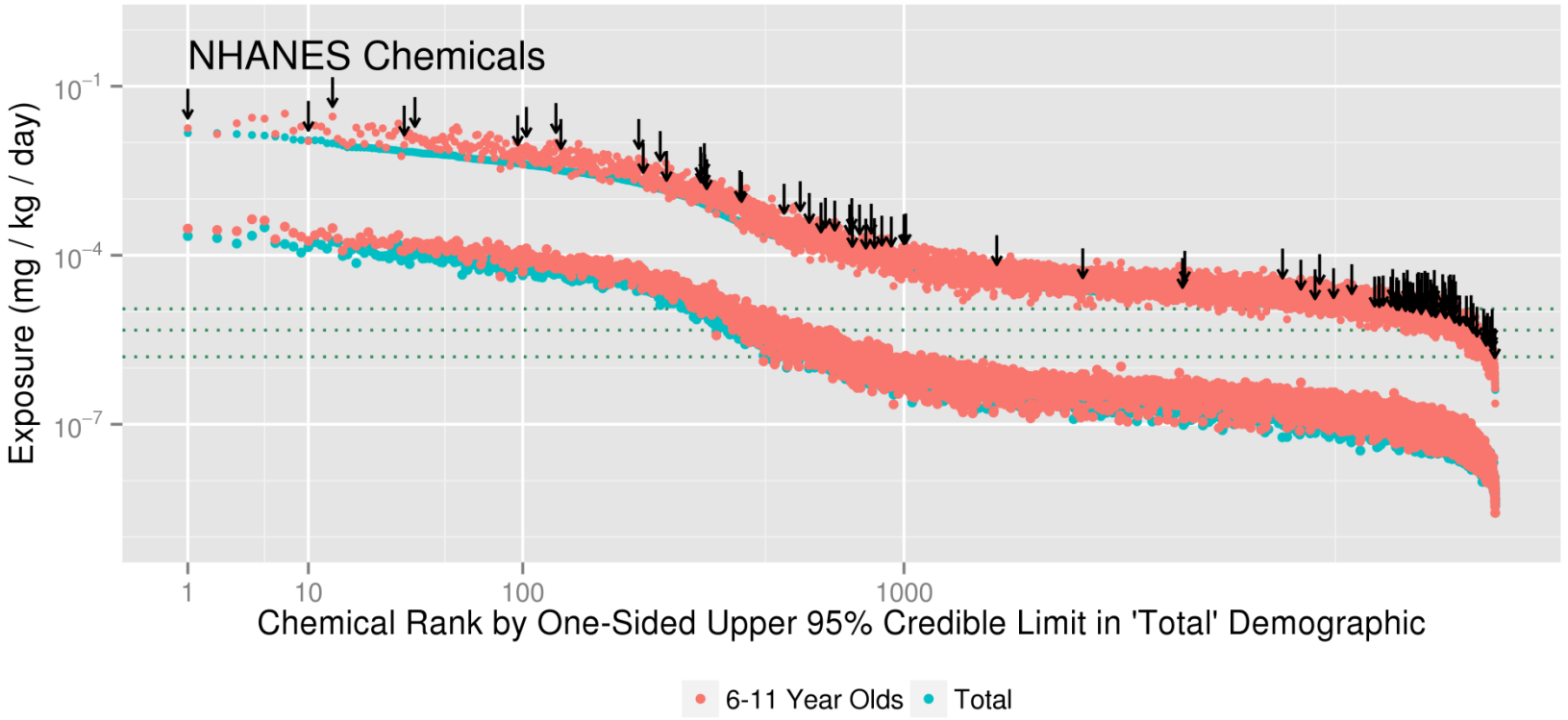
Master CASRN	Master Name	EDSPList1	Final List 1	EDSPList2	ERAgonistAUC	ERAntagonistAUC	ER max bioactivity	ARAUCAgonist	ARAUCAntagonist	AR max bioactivity	Bioactivity Ranking	OPP Active ingredient	OPP Food INERT	OPP Fragrance INERT
50-28-2	17beta-Estradiol	0	0	0	1.01	0	1.01	1.43	0.109	1.43	1.43	0	0	0
52806-53-8	Hydroxyflutamide	0	0	0	0	0	0	0	0.998	0.998	0.998	0	0	0
1478-61-1	Bisphenol AF	0	0	0	0.821	0.00383	0.821	0.00136	0.296	0.296	0.821	0	0	0
446-72-0	Genistein	0	0	0	0.77	0	0.77	0	0	0	0.77	0	0	0
2390-60-5	Basic Blue 7	0	0	0	0	0.0647	0.0647	0	0.749	0.749	0.749	0	0	0
77-40-7	Bisphenol B	0	0	0	0.748	0.00306	0.748	0	0.244	0.244	0.748	0	0	0
80-05-7	Bisphenol A	0	0	0	0.655	0	0.655	0	0.247	0.247	0.655	0	0	0
486-66-8	Daidzein	0	0	0	0.623	0	0.623	0	0	0	0.623	0	0	0
5137-55-3	Methyltrioctylammonium chloride	0	0	0	0.00476	0.0396	0.0396	0	0.596	0.596	0.596	0	0	0
84852-15-3	4-Nonylphenol, branched	0	0	0	0.595	0	0.595	0.0418	0.062	0.062	0.595	0	0	0
104-43-8	4-Dodecylphenol	0	0	0	0.561	0	0.561	0	0	0	0.561	0	0	0
131-55-5	2,2',4,4'-Tetrahydroxybenzophenone	0	0	0	0.558	0	0.558	0.117	0.175	0.175	0.558	0	0	0
140-66-9	4-(1,1,3,3-Tetramethylbutyl)phenol	0	0	0	0.557	0	0.557	0.0439	0.0651	0.0651	0.557	0	0	0
599-64-4	4-Cumylphenol	0	0	0	0.542	0	0.542	0	0.271	0.271	0.542	0	0	0
27193-86-8	Dodecylphenol	0	0	0	0.535	0	0.535	0	0	0	0.535	0	0	0
2971-36-0	2,2-Bis(4-hydroxyphenyl)-1,1,1-trichlo	0	0	0	0	0.143	0.143	0	0.527	0.527	0.527	0	0	0
52-01-7	Spironolactone	0	0	0	0	0	0	0	0.527	0.527	0.527	0	0	0
58-72-0	Triphenylethylene	0	0	0	0.521	0	0.521	0	0	0	0.521	0	0	0
5153-25-3	2-Ethylhexylparaben	0	0	0	0.513	0.00599	0.513	0	0	0	0.513	0	0	0
789-02-6	o,p'-DDT	0	0	0	0.492	0	0.492	0	0	0	0.492	0	0	0
25155-18-4	Methylbenzethonium chloride	0	0	0	0.0171	0.105	0.105	0	0.48	0.48	0.48	1	0	0
491-80-5	Biochanin A	0	0	0	0.47	0	0.47	0	0	0	0.47	0	0	0
27955-94-8	4,4',4-Ethane-1,1,1-triyltriphenol	0	0	0	0.445	0.0385	0.445	0	0.256	0.256	0.445	0	0	0
520-36-5	Apigenin	0	0	0	0.432	0	0.432	0.00091	0.182	0.182	0.432	0	0	0
548-62-9	Gentian Violet	0	0	0	0	0.0000533	0.0000533	0	0.425	0.425	0.425	0	0	0
112281-77-3	Tetraconazole	0	0	0	0	0.0119	0.0119	0	0.411	0.411	0.411	1	0	0
80-46-6	4-(2-Methylbutan-2-yl)phenol	0	0	0	0.401	0	0.401	0	0	0	0.401	1	0	0
131-56-6	2,4-Dihydroxybenzophenone	0	0	0	0.396	0	0.396	0	0.106	0.106	0.396	0	0	0
7173-51-5	Didecyldimethylammonium chloride	0	0	0	0.00319	0.0897	0.0897	0.0195	0.391	0.391	0.391	1	1	0
122-14-5	Fenitrothion	0	0	0	0	0	0	0.0158	0.387	0.387	0.387	1	0	0
1085-12-7	Heptylparaben	0	0	0	0.384	0	0.384	0	0	0	0.384	0	0	0
66-81-9	Cycloheximide	0	0	0	0	0.121	0.121	0	0.382	0.382	0.382	0	0	0
61-73-4	Methylene blue	0	0	0	0.0016	0.0781	0.0781	0	0.381	0.381	0.381	0	1	0
1219-38-1	Octylparaben	0	0	0	0.38	0	0.38	0	0	0	0.38	0	0	0
94-26-8	Butylparaben	0	0	0	0.38	0	0.38	0	0	0	0.38	0	0	0
72-43-5	Methoxychlor	0	0	0	0.379	0	0.379	0	0.0603	0.0603	0.379	1	0	0
50471-44-8	Vinclozolin	0	0	1	0	0	0	0	0.371	0.371	0.371	0	0	0
17696-62-7	Phenylparaben	0	0	0	0.369	0.0167	0.369	0	0	0	0.369	0	0	0
77-09-8	Phenolphthalein	0	0	0	0.363	0	0.363	0	0	0	0.363	0	0	0
3380-34-5	Triclosan	0	0	0	0	0	0	0.016	0.358	0.358	0.358	1	0	0

IBER: Integrated Bioactivity-Exposure Ranking



High Throughput Exposure: ExpoCast

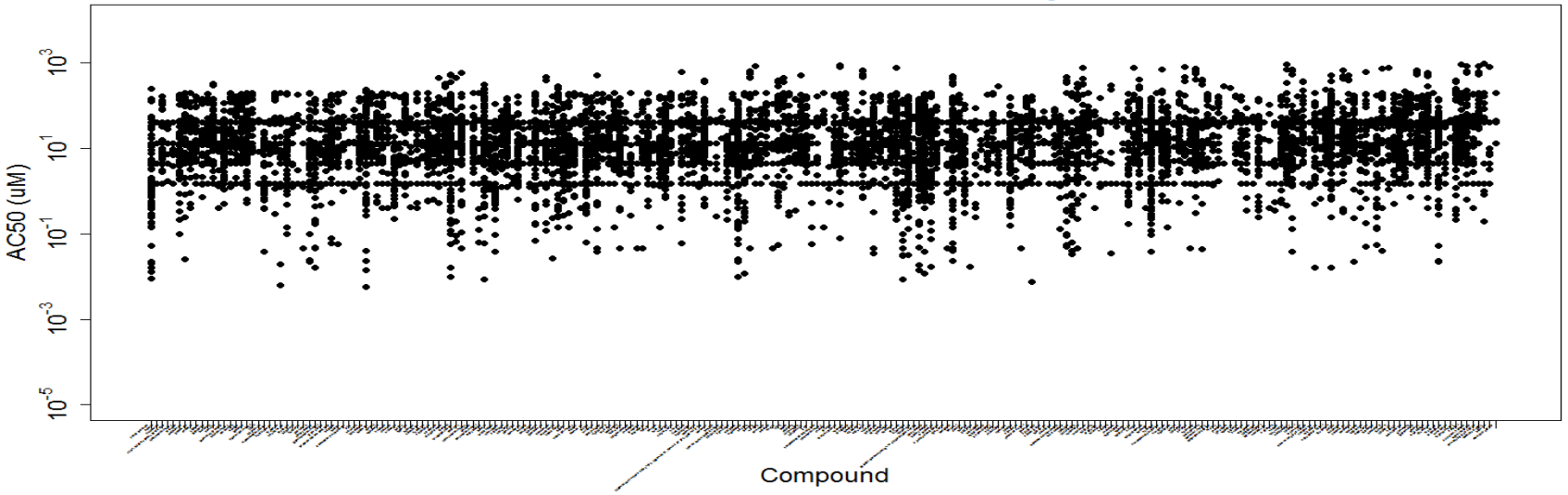
Predictions for 7968 Chemicals



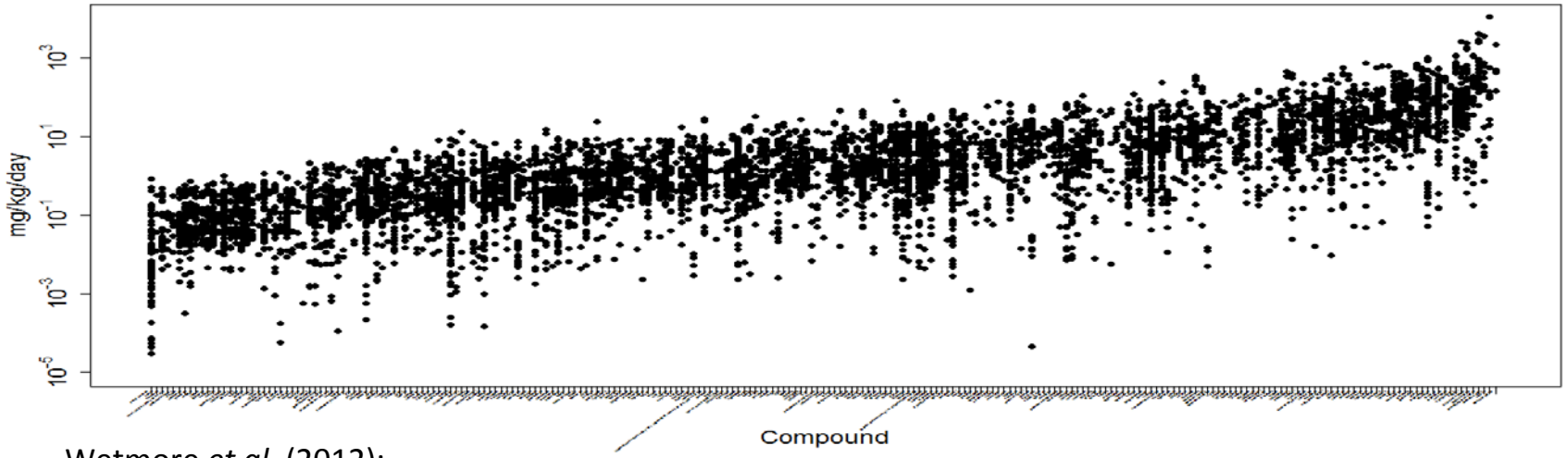
NHANES LoD

High Throughput Exposure Forecasting session Thursday 9-11:45

IVIVE: AC50s to Oral Equivalents

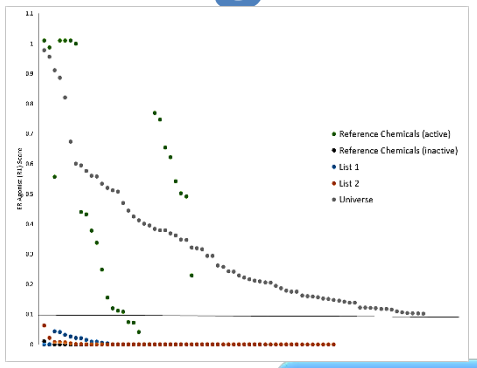


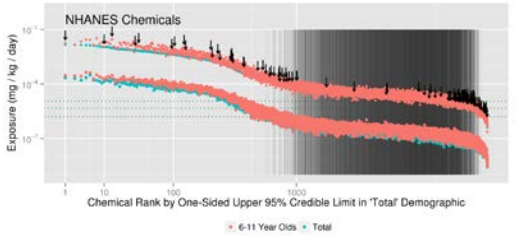
Bioactive *in vitro* concentration converted into estimated steady-state, oral equivalent *in vivo* doses – allow discrimination of chemical potencies.



Wetmore *et al*. (2012);

Integrated Bioactivity Exposure Ranking



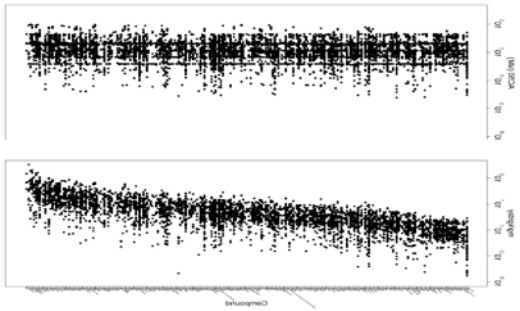


Bioactivity
ToxCast

Exposure
ExpoCast

HTTK

Integrated Bioactivity Exposure Ranking (IBER)



Prioritization

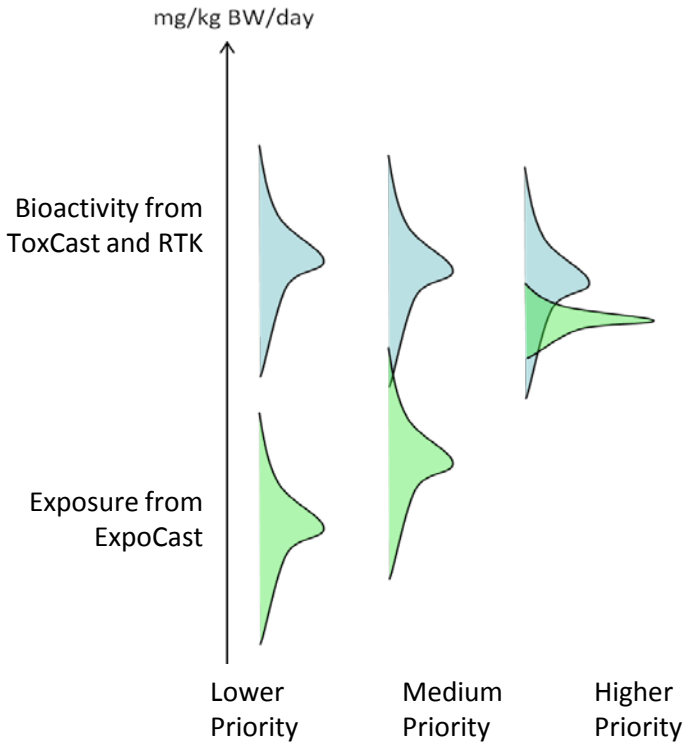
Screening

Some or All
EDSP Tier 1 Assays

Testing

Some or All
EDSP Tier 2 Assays

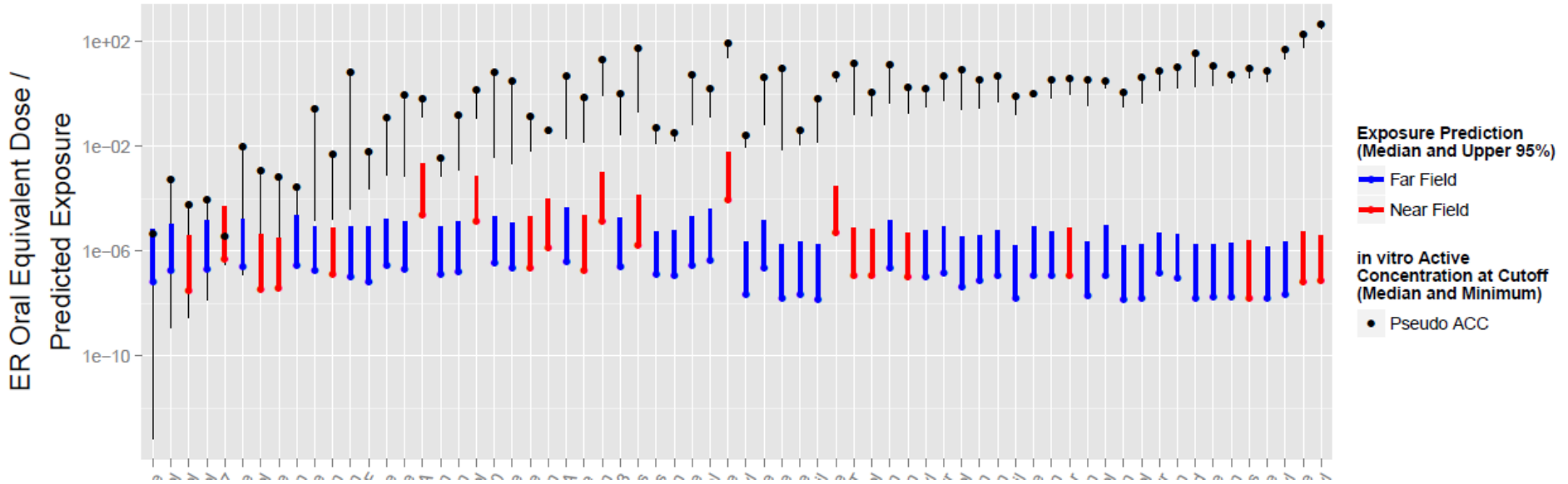
Integrating Bioactivity and Exposure



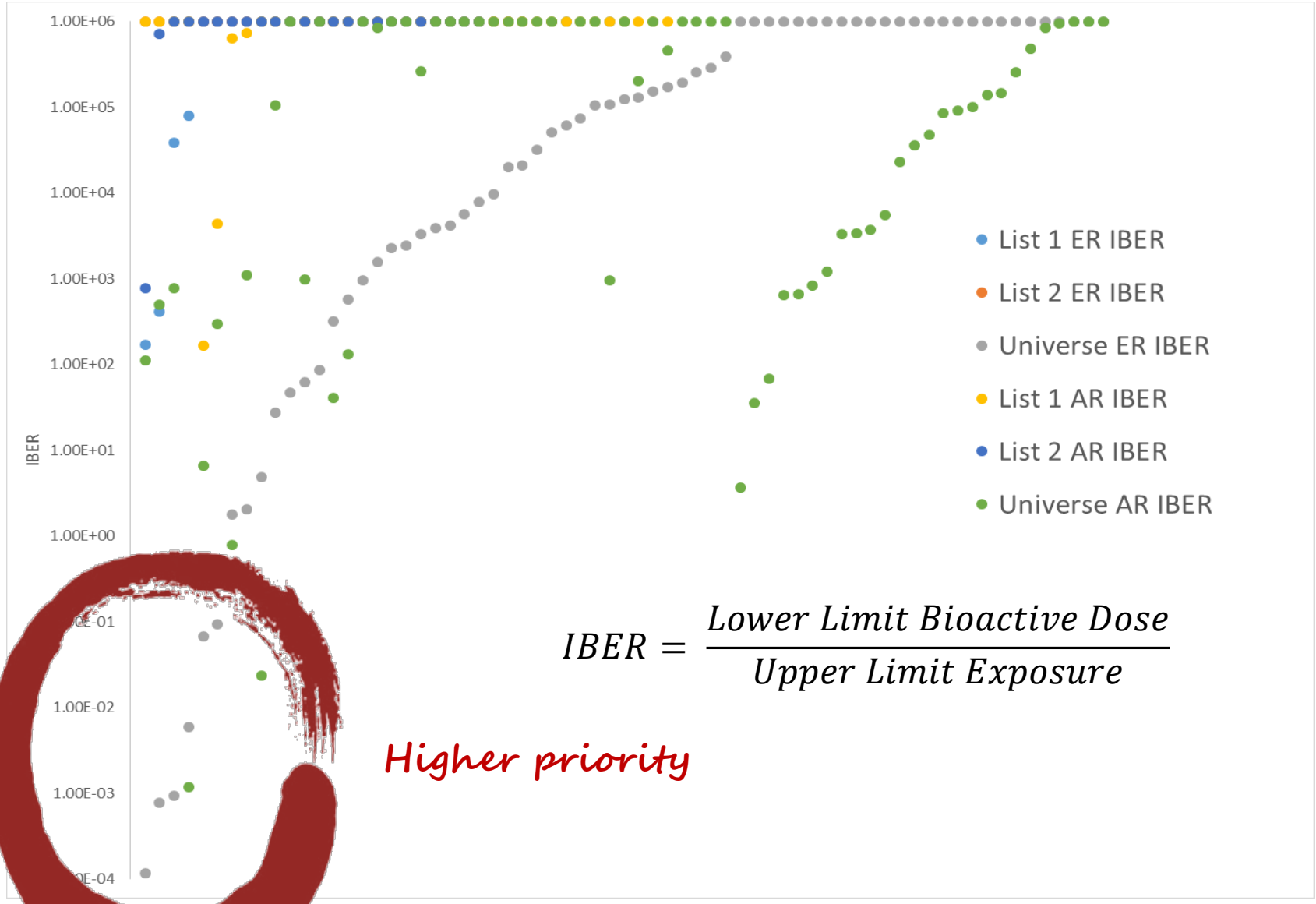
- *in vitro* chemical dose-response HTP bioactivity data are used to identify potential biological targets
- RTK methods are then employed to determine the human dose needed for each chemical to activate these targets *in vivo*
- putative bioactive doses are then directly compared to HTE predictions to estimate likelihood of exposures that cause bioactive doses

Chemicals where the putative human bioactive dose is comparable to HTE predictions become targets for further investigation

Integrated Bioactivity Exposure



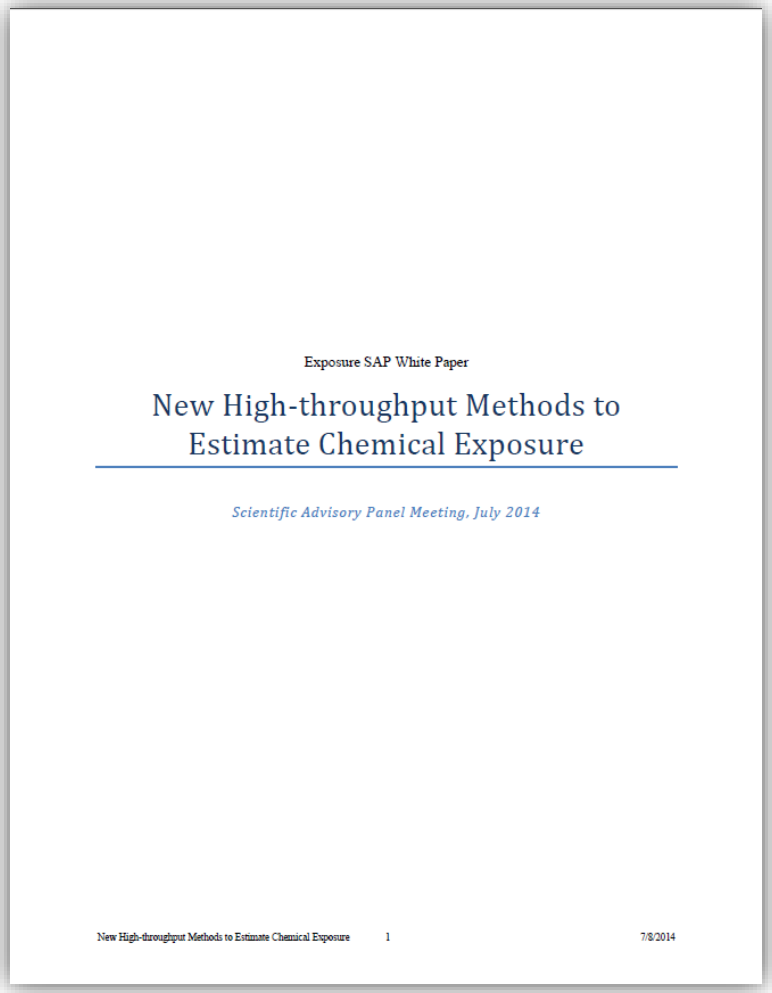
Integrated Bioactivity Exposure Ranking (IBER) Method



$$IBER = \frac{Lower\ Limit\ Bioactive\ Dose}{Upper\ Limit\ Exposure}$$

Higher priority

Building Scientific Confidence – Peer Review

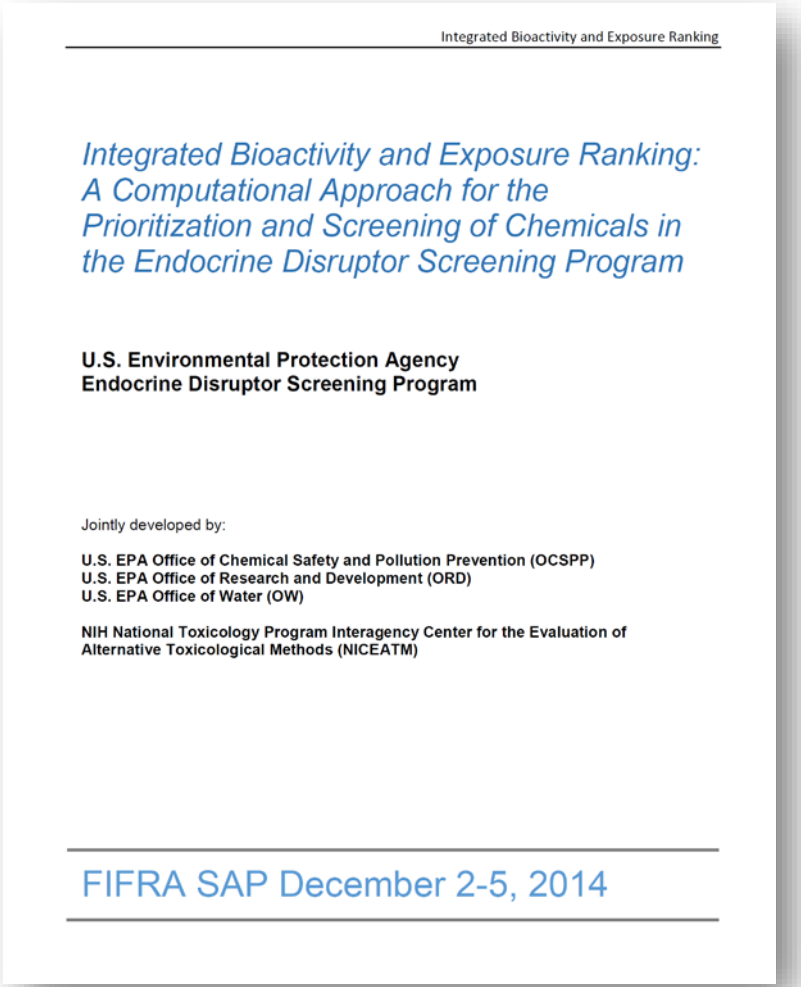


Exposure SAP White Paper

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:

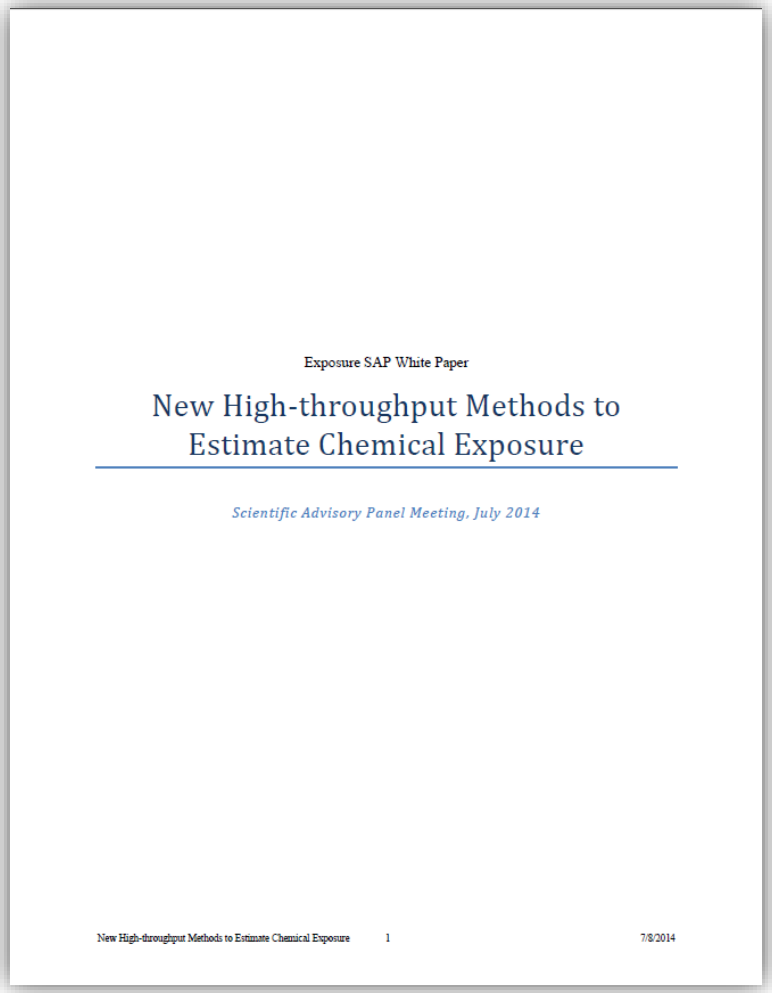
U.S. EPA Office of Chemical Safety and Pollution Prevention (OCSPP)
U.S. EPA Office of Research and Development (ORD)
U.S. EPA Office of Water (OW)

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

The July 2014 FIFRA SAP was charged with advising the Agency in the following 3 topic areas:



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

- **The Systematic Empirical Evaluation of Models (SEEM) Framework for Exposure**
- **High Throughput Toxicokinetics (HTTK) and Reverse Toxicokinetics (RTK)**
- **Future Direction**

July 2014 FIFRA SAP - Highlights from Panel Comments and Recommendations

- SEEM appears scientifically sound and suitable for high throughput exposure (HTE) methods to assess relative risks of chemical exposure for diverse groups of chemicals.
    - Further effort in measuring and minimizing uncertainty within the SEEM framework is needed prior to implementation in the EDSP or other Programs.
- With respect to RTK, the main Panel conclusions were that the EPA is going in the right direction and that there were no other existing viable approaches.
    - Effort should be focused on understanding the failure of the model to better predict the *in vivo* Css.
    - *In vivo* data for additional chemicals should be generated to assist in the calibration.
    - There was no consensus on whether the predictive approach could be used for prioritization and/or screening.

Exposure Modeling Future Direction / Path Forward

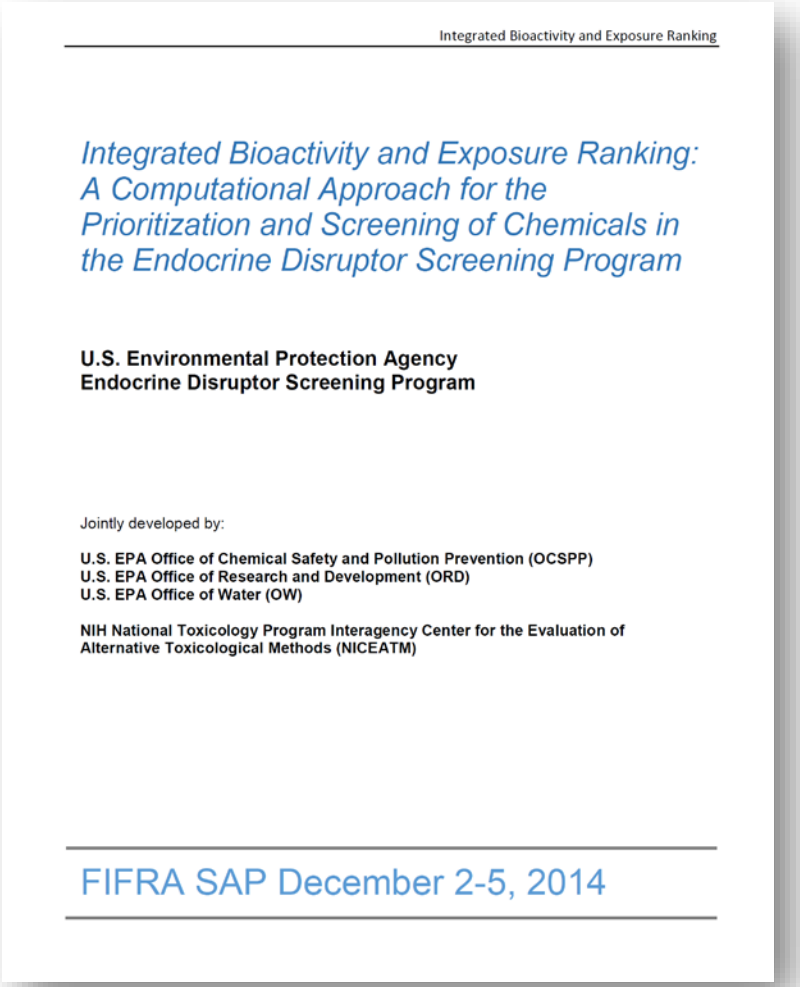
Recommendations from FIFRA SAP Peer Reviews are under consideration; path forward includes:

- Next generation models that include:
  - new exposure models and data (e.g., SHEDS-HT),
  - additional sources of exposure (e.g., ground water and drinking water),
  - dermal and inhalation routes of exposure,
  - exposures other than steady state, and
  - extrapolations to ecological species (e.g., fish)

- Work to expand # of chemicals with biomonitoring data

- Work to expand # of chemicals with reverse toxicokinetic data

The December 2014 FIFRA SAP was charged with advising the Agency in the following 3 topic areas:


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

- **Estrogen receptor (ER) bioactivity model**
- **Androgen receptor (AR) bioactivity model**
- **Integrated Bioactivity Exposure Ranking (IBER) approach**

December 2014 FIFRA SAP - Highlights from Panel Comments and Recommendations on IBER

Strengths

- Agency captured "worst-case scenarios" aimed to account for uncertainty and variability in both chemical bioactivity and population exposure.
- Model is complex enough to capture potential sources of variability yet simple enough to allow for straightforward scientific interpretation, model validation, and further development.
- "Good starting point" (need to further address variability and uncertainty).

Limitations

- Need further model development to account for sources of uncertainty and variability and model them jointly
- Exposure dataset was more limited than data available for bioactivity.
- Concerned that specific human populations such as agricultural workers, chemical formulators and pregnant women, who may have the highest exposure levels for specific compounds were not always taken into account.

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

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

Acknowledgements

- US EPA Office of Chemical Safety and Pollution Prevention
- US EPA Office of Research and Development
- US EPA Office of Water
- National Institutes of Health
  - National Toxicology Program Interagency Center for the Evaluation of Alternative Toxicological Methods (NICEATM)