



SOT FDA Colloquia on Emerging Toxicological Science Challenges in Food and Ingredient Safety

Gaining Confidence in Replacing Animal Tests: A Case Study of the Endocrine Disruption Program at the US EPA

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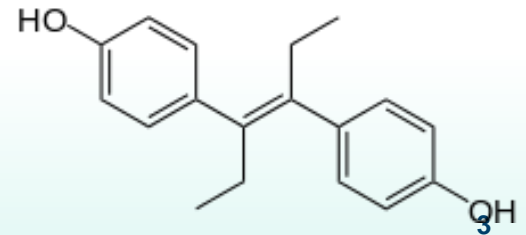
Conflict of Interest Statement

- The author has no conflicts of interest



DES – one origin of the EDSP

- Diethylstilbesterol – DES
 - Synthetic estrogen
 - From about 1940 to 1971, DES was given to pregnant women in the mistaken belief it would reduce the risk of pregnancy complications and losses
 - Shown to cause reproductive organ cancers and other reproductive problems in women exposed in utero
 - Time from exposure to adverse effect was 20 years or more
 - “Chemical time bomb”



The Endocrine Disruptor Screening Program (EDSP)

- As a result, Congress passed laws leading to the formation of the EDSP (1996)
 - FIFRA (Pesticide regulation)
 - SWDA (Drinking water regulations)
 - Requires all pesticide ingredients and potential drinking water contaminants to be tested for potential to be endocrine disruptors
- Original regulations focused on estrogen mimics
- Subsequent work by EPA EDSTAC expanded scope to androgen and thyroid (1998)

EDSP is a 2 Tier Testing Program

- Tier 1
 - “Screening”: Hazard assessment
 - 11 *in vitro* and *in vivo* assays
 - Assays development and validation finalized in 2009
 - Test orders for initial 73 chemicals issued in 2009
 - Tests run in starting in 2012
 - Evaluation of results of 52 tested chemicals in 2015
 - 19 years after law passage
- Tier 2
 - “Testing”: Quantitative risk assessment
 - No test orders issued to date

EDSP Tier 1 battery

Complementary Modes of Action Among Screening Assays in the EDSP Tier 1 Battery

Screening Assays	Modes of Action							
	Receptor Binding				Steroidogenesis		HPG ³ Axis	HPT ³ Axis
	E ²	Anti-E	A ²	Anti-A	E ²	A ²		
<i>In vitro</i>								
ER Binding ¹	■	■ ⁴						
ERα Transcriptional Activation	■							
AR Binding ¹			■	■				
Steroidogenesis H295R					■	■		
Aromatase Recombinant					■			
<i>In vivo</i>								
Uterotrophic	■							
Hershberger			■	■		■		
Pubertal Male			■	■		■	■	■
Pubertal Female	■	■ ⁴			■		■	■
Amphibian Metamorphosis								■
Fish Short-term Reproduction (male & female)	■	■ ⁴	■	■	■	■	■	

Issues with EDSP Tier 1

- Total EDSP Universe is ~10,000 chemicals
 - Cost of Tier 1: ~\$1M / chemical
 - Throughput: 50 chemicals / year
- Implications: \$B's, 100-200 years to complete
- Need new approaches
 - Prioritize chemicals
 - Replace some or all low-throughput Tier 1 assays
- Origin of EDSP21 Program (2012)

ToxCast and Tox21

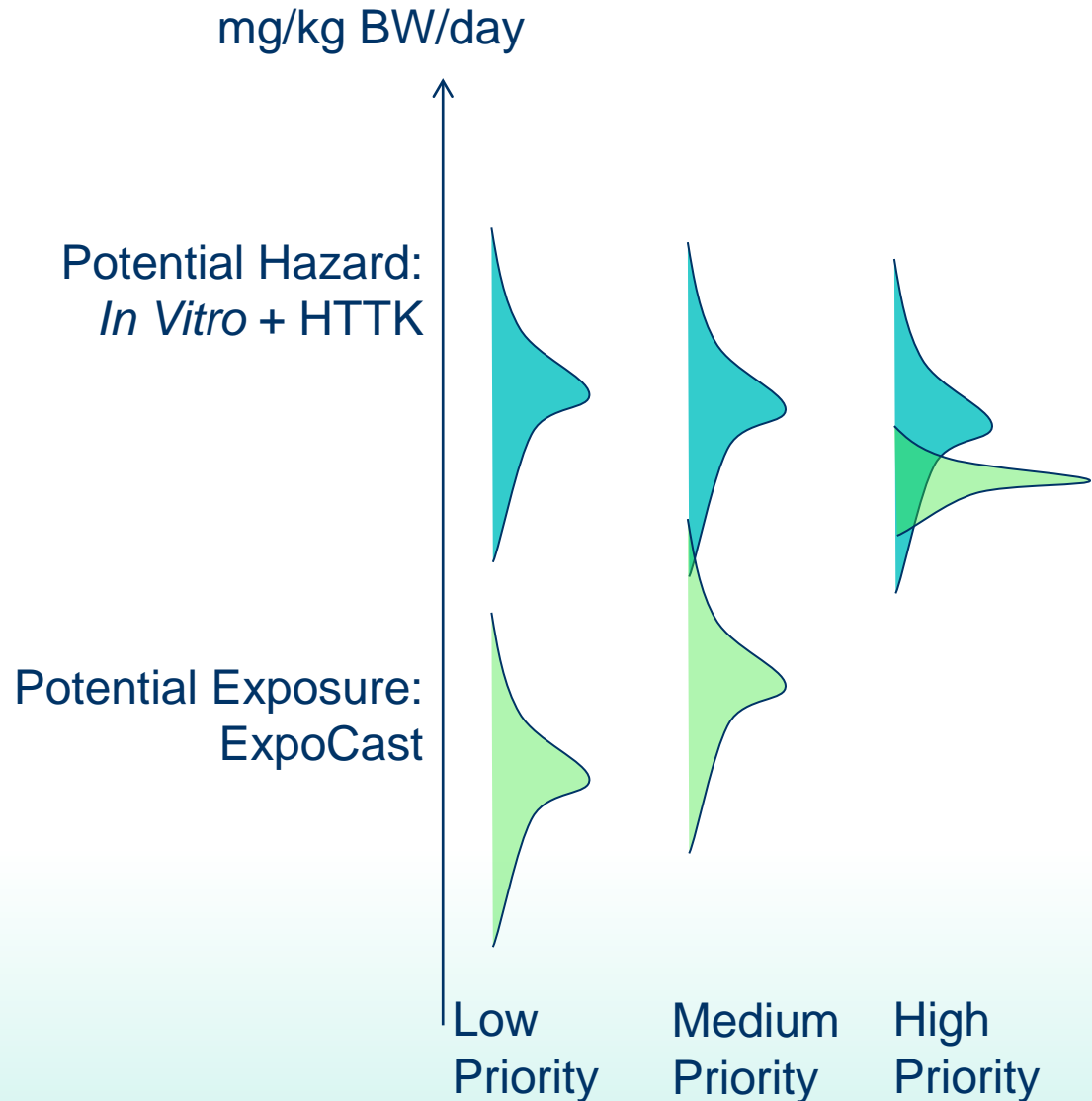
High-Throughput Toxicology

- Started in 2007
- EPA, NTP, NCATS, FDA
- Screening up to 10,000 chemicals (Tox21 library)
- ToxCast has ~3000 chemicals in up to 800 *in vitro* assays
 - Cell-based (cell lines, primary cells)
 - Model organisms (zebrafish, *c. elegans*)
 - Organotypic systems
- EDSP21 is one example of Tox21 data and models

Tox21 Aims to Perform High-Throughput Risk Assessment

Semi-quantitative
In Vitro to *In Vivo*

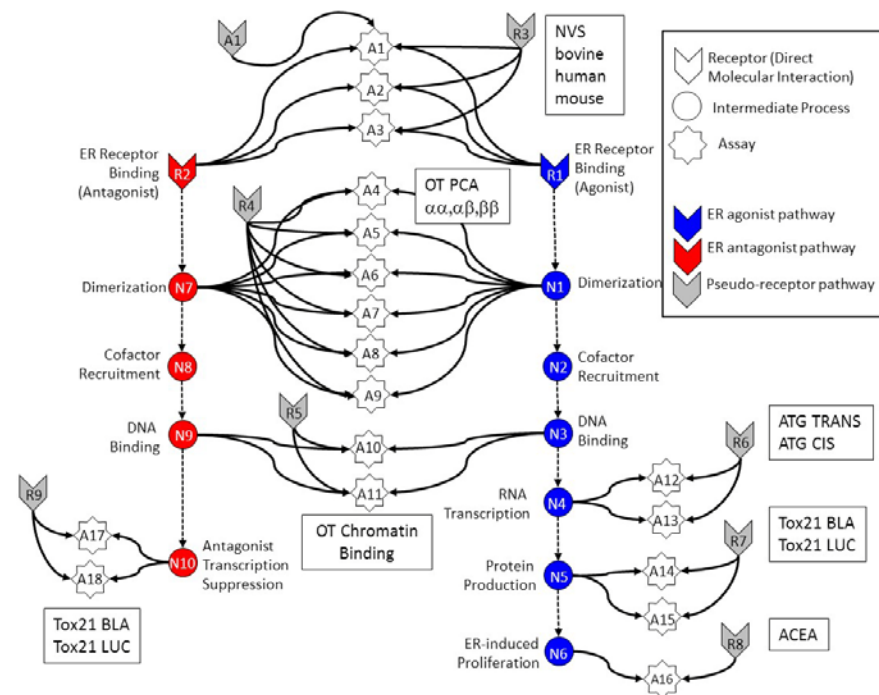
- Hazard
- Exposure
- Uncertainty



In Vitro Estrogen Receptor Model

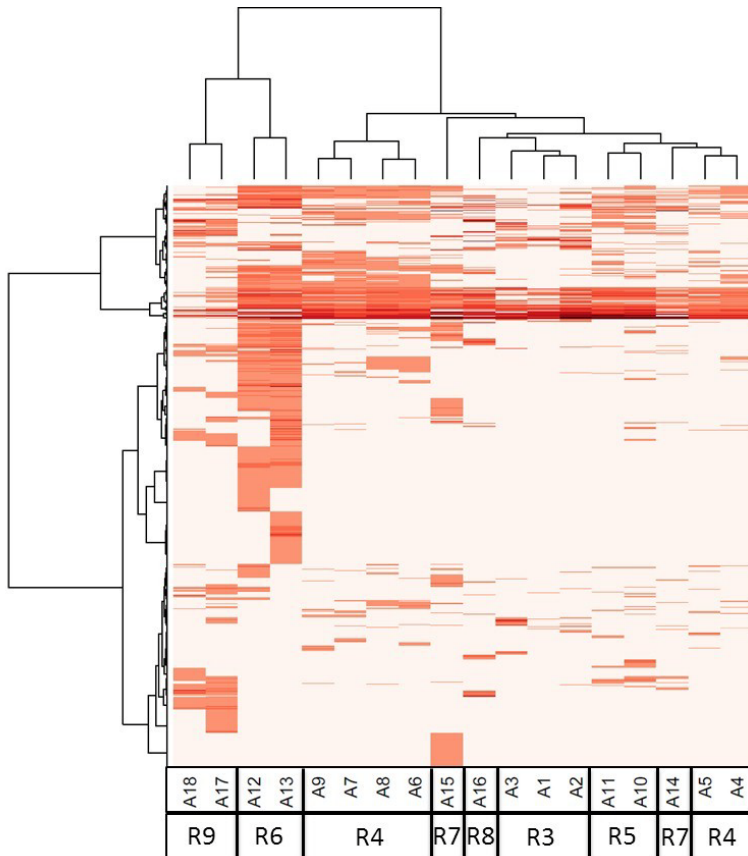
Combines results from multiple in vitro assays

- Use multiple assays per pathway
 - Different technologies
 - Different points in pathway
- No assay is perfect
 - Assay Interference
 - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



Major theme – all assays have false positives and negative

Assays cluster by technology, suggesting technology-specific non-ER bioactivity

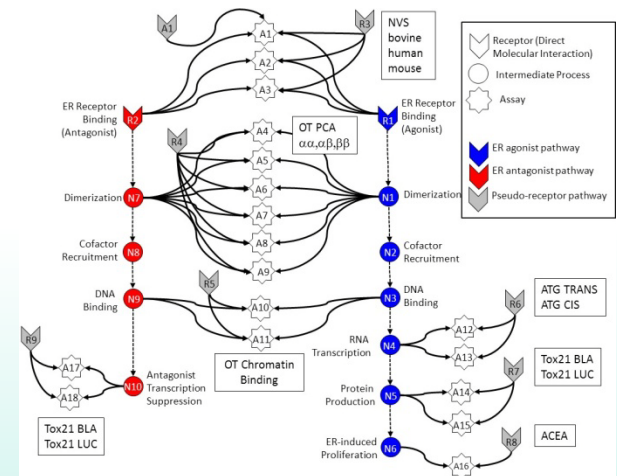


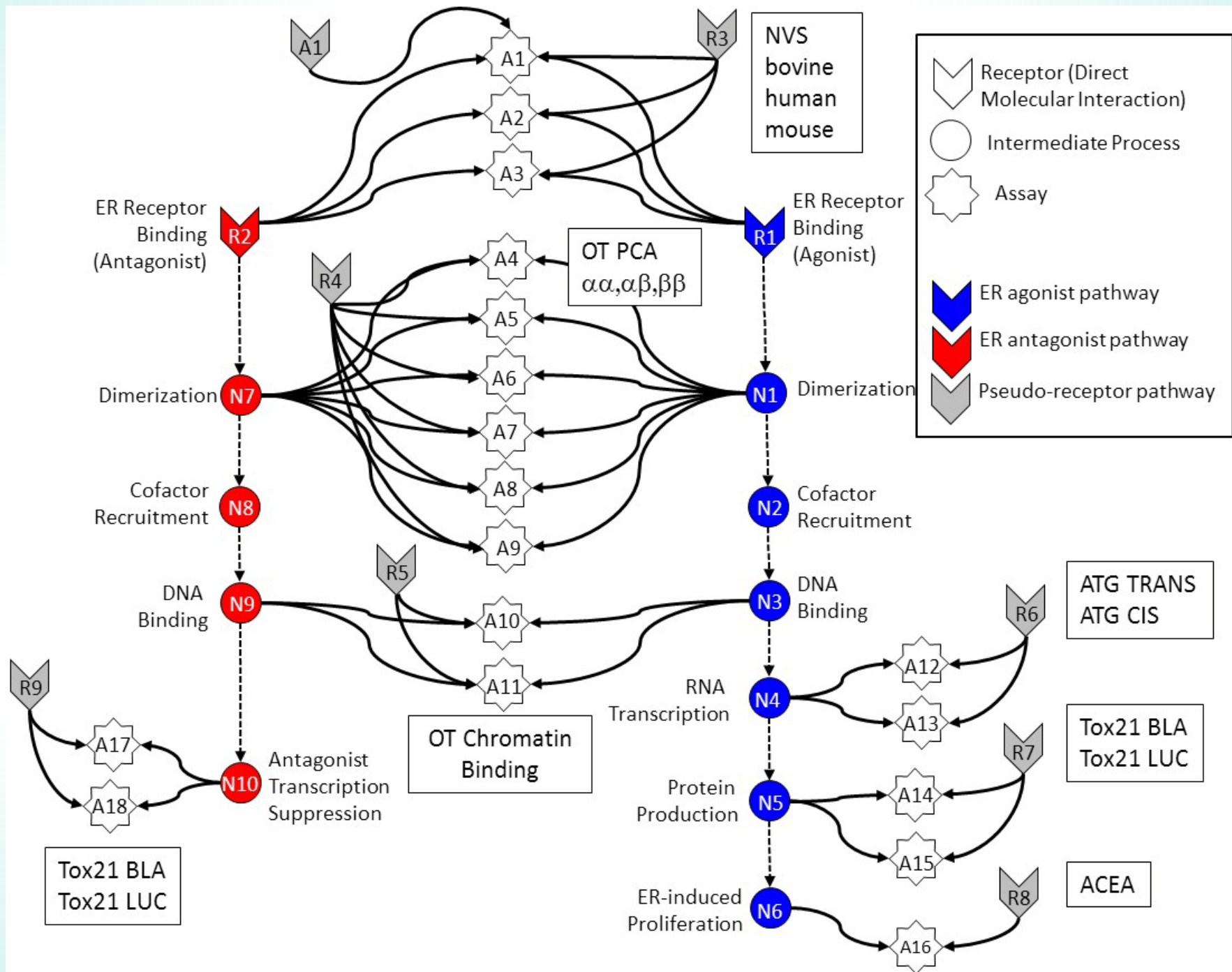
Much of this “noise” is reproducible

- “assay interference”
- Result of interaction of chemical with complex biology in the assay

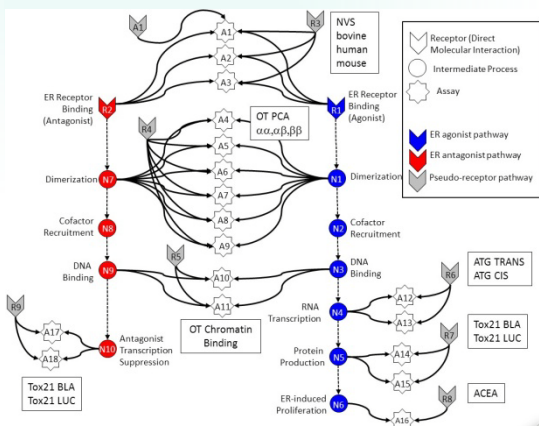
EDSP chemical universe is structurally diverse

- Solvents
- Surfactants
- Intentionally cytotoxic compounds
- Metals
- Inorganics
- Pesticides
- Drugs





Validate the *in vitro* ER model against the *in vivo* version: the Uterotrophic Assay



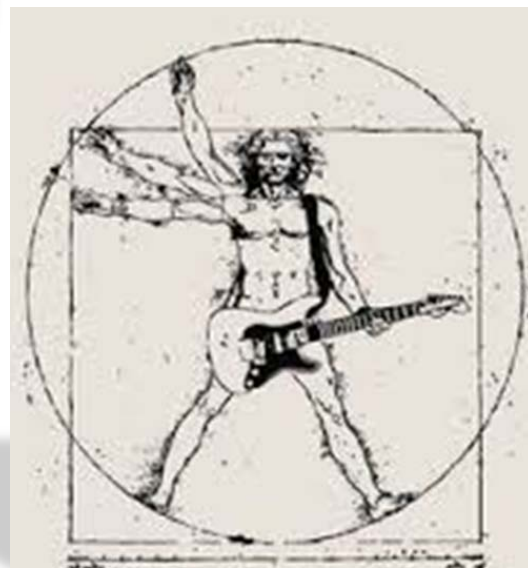
In vitro hER activity:

- Human Breast
- Human Ovary
- Human Uterus
- Human Cervix
- Human Liver
- Human ER (cell free)



ER-Bioactivity

- Rat or Mouse uterus (guideline uterotrophic)



Human Relevance

Identifying Uterotrophic Reference Chemicals from the Literature

Literature Searches:
1800 Chemicals

High-Level
Filter

Data Review:
700 Papers, 42 Descriptors, x2

Minimum
Criteria

Uterotrophic Database
98 Chemicals
442 GL uterotrophic bioassays

“Guideline-Like”
(GL)

Selection
Criteria

In Vivo ER Reference Chemicals
30 Active, 13 Inactive

Uterotrophic Assay Outline

Requirement for report to be “guideline-like”

- Remove ovaries: intrinsic source of estrogen & driver of uterine growth
- Test a chemical’s ability to restore uterine growth through ER activity
- Closely follows EPA and OECD guidelines
 1. Adult rats or mice: Ovariectomy: 6-8 weeks of age; treatment: 14+ days post-surgery (rats), 7 days (mice)
 2. Immature rats: start dosing between PND 18 and PND 21, and finish by PND 25
 3. Control groups: 3 animal min
 4. Test groups: 5 animal min; minimum of 2 test groups
 5. Oral gavage, subcutaneous injection, and intraperitoneal injection
 6. Dosing over a minimum of 3 days
 7. Necropsy: 18–36 hours after the last dose

Adding Tier 1 / List 1 chemicals to the Literature DB: 81 Guideline Chemicals

Uterotrophic Literature
“Guideline-Like” studies:
Chemicals with more
than one consistent test

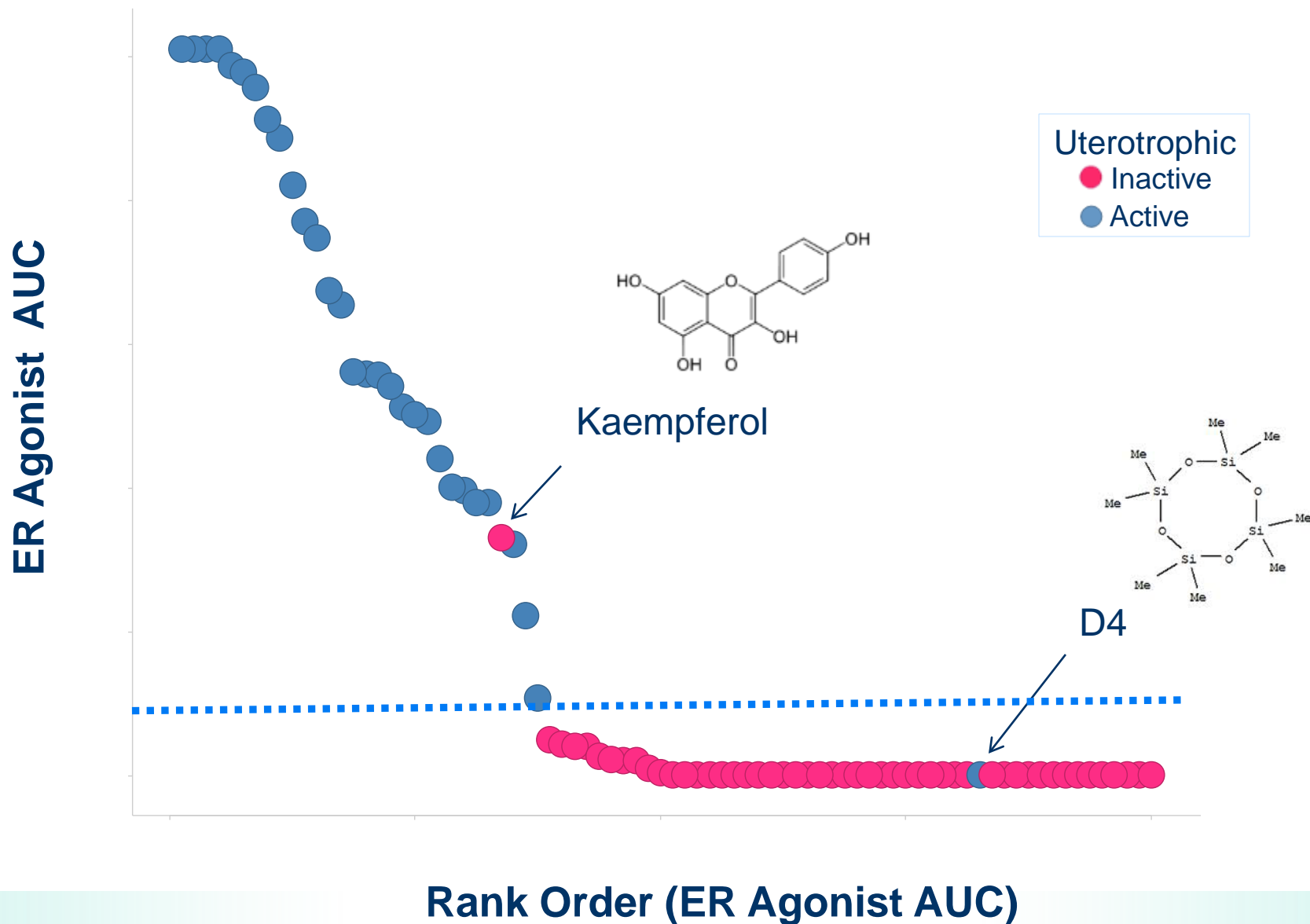
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EDSP List 1 Uterotrophic
“Guideline” Studies



Uterotrophic Reference Chemicals:
30 Active, 51 Inactive

ER Agonist AUC vs. Uterotrophic Outcomes



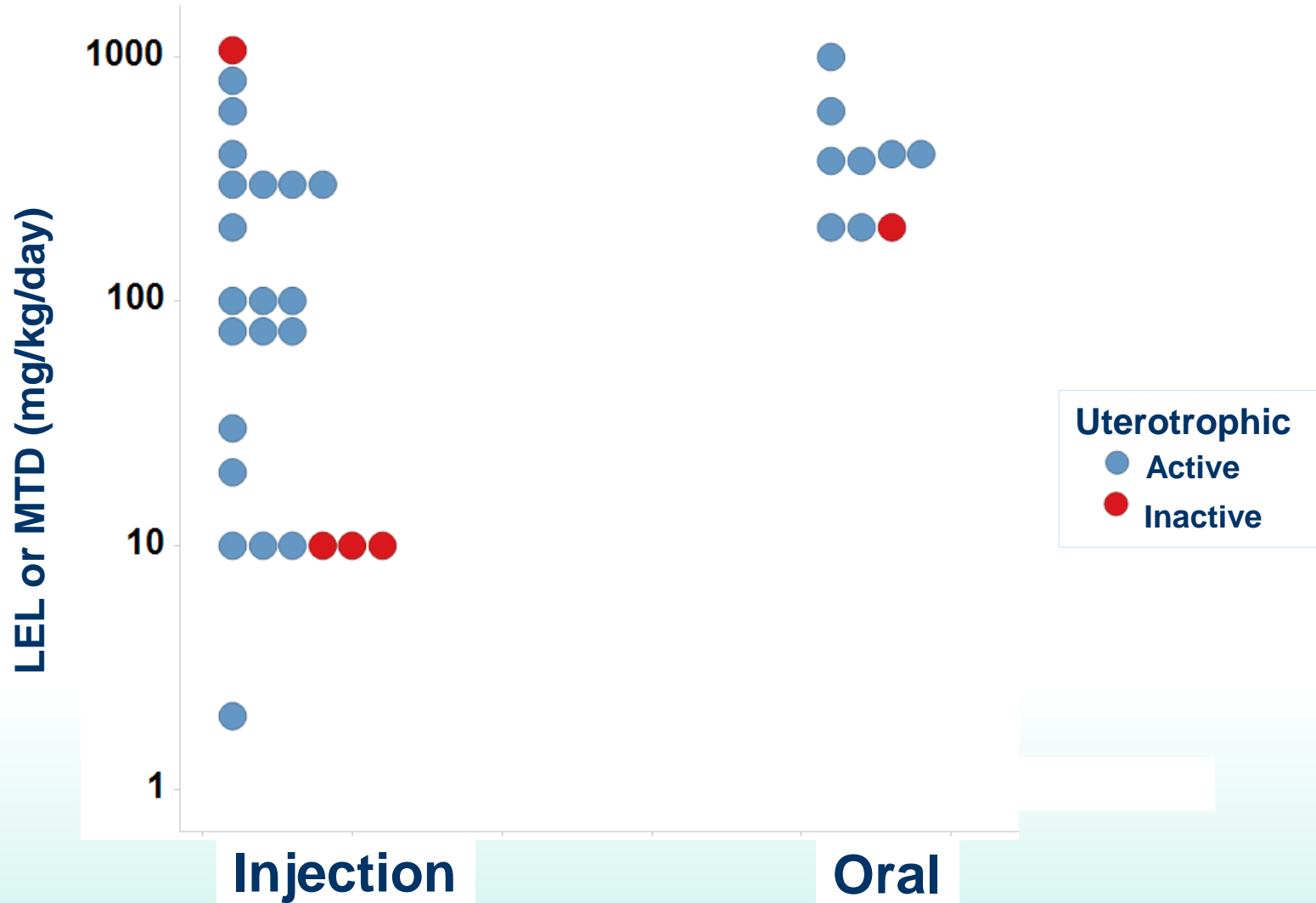
In Vitro Activity vs. Uterotrophic Outcomes

ER Agonist Model AUC

True Positive	29
True Negative	50
False Positive	1
False Negative	1
Accuracy	0.97
Sensitivity	0.97
Specificity	0.98

In vivo guideline studies have the same types of uncertainty as *in vitro*:
26% of chemicals have at least one positive and one negative study

Immature Rat: BPA



EPA Moves to Use ER Model in EDSP

- Federal Register Notice (FRN) June 2015
 - Based on 3 FIFRA Scientific Advisory Panels
 - Publications on ER Model, Uterotrophic Database, Comparison
- “The approach incorporates **validated** high throughput assays and a computational model and, based on current research, can serve as an **alternative** for some of the current assays in the Endocrine Disruptor Screening Program (EDSP) Tier 1 battery.”
- “Use of these alternative methods will **accelerate the pace** of screening, **decrease costs**, and **reduce animal testing**. In addition, this approach advances the goal of providing sensitive, specific, quantitative, and efficient screening using alternative test methods to some assays in the Tier 1 battery to protect human health and the environment.”
- Multiple public comments

Data Transparency: EDSP21 Dashboard

- Goal: To make EDSP-related data easily available to all stakeholders
 - Assay-by-assays concentration-response plots
 - Model scores – AUC agonist and antagonist
 - ER QSAR calls
 - Other relevant data

- <http://actor.epa.gov/edsp21>

ToxCast Model Predictions		
Model	Agonist AUC	Antagonist AUC
ER	0.45	0
AR	0	0.136

Consensus CERAPP QSAR ER Model Predictions			
Class	Agonist (Potency Level)	Antagonist (Potency Level)	Binding (Potency Level)
from Literature	Active (Weak)	-	Active (Weak)
QSAR Consensus	Active (Weak)	Active (Strong)	Active (Weak)

The screenshot displays the EDSP21 Dashboard interface. At the top, it features the EPA logo and the text "EDSP21 Dashboard Endocrine Disruption Screening Program for the 21st Century". Below this is a navigation bar with tabs for "Chemical Selection", "Chemical Summary", "Public Information", "Bioactivity Summary", "Bioactivity", "High-Throughput Exposure", "Assay Definitions", and "Discovery".

The "Chemical Selection" tab is active, showing a search for "80-05-7" which identifies "Bisphenol A". A table below lists the CASRN (80-05-7) and the chemical name (Bisphenol A) with a "Is Toxic" checkbox.

The "Chemical Structure and Data" section shows the chemical structure of Bisphenol A (two phenol rings connected by a central carbon atom bonded to two methyl groups) and a list of identifiers and properties:

- DSSTOX GSID: 20182
- CASRN: 80-05-7
- CASRN Type: Single Compound
- Name: Bisphenol A
- SMILES: CC1=C(C)C(O)C=C1C1=CC=C(O)C=C1
- InChI: InChI=1S/C15H16O2/c1-15/2-11-3-7-13(16)/4-11(12-5-8-14)(17)/10-6-12N3-10,1...
- InChI Key: IISBACLAFKSPIT-UHFFFAOYSA-N
- Molecular Wt: 228.29
- Chemical Formula: C15H16O2
- Cytotoxicity Limit (µM): 3.63954674351077
- Chemical Type: Organic
- Chiral/Stereo: None
- dbl/Stereo: None
- Organic Form: Parent
- Iupac: (not fully visible)

The "PhysChem Properties" section at the bottom shows a table with columns for Property, Model Name, Raw Result, Result (Mean), Result (min), Result (max), and Result Unit. It lists various sources and their corresponding result counts, such as "Source: Alfa Aesar (4 Results)", "Source: EPI SUITE (125 Results)", "Source: J and K Scientific (1 Result)", "Source: Jean-Claude Bradley Open Melting Point Dataset (2 Results)", "Source: Merck Millipore (1 Result)", "Source: QikProp (51 Results)", and "Source: TCI (3 Results)".

Summary

- EDSP21 ER model is the first successful example of using ToxCast/ Tox21 data and models to replace low-throughput animal tests with high-throughput *in vitro* assays
- Requires strong collaboration
 - Assay developers, modelers, assay validators, regulators
- Next steps
 - Androgen receptor: aim to replace Hershberger
 - Steroidogenesis: Move from low to high-throughput *in vitro*
 - Thyroid – assay development and testing underway for several targets (THR, TPO, deiodinases, ...)

Key Publications

- Estrogen Receptor *In Vitro* Model

- Judson et al. “Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor”, *Toxicol. Sci.* (2015) doi: 10.1093/toxsci/kfv168

- Uterotrophic Database

- Kleinetreuer et al. “A Curated Database of Rodent Uterotrophic Bioactivity” *Environ. Health Persp.* (2015) in press.

- *In Vitro* to *In Vivo* Comparison

- Browne et al. “Screening Chemicals for Estrogen Receptor Bioactivity Using a Computational Model” *Environ. Sci. Technol.*, 2015, 49 (14), pp 8804–8814

- Federal Register Notice

- <https://federalregister.gov/a/2015-15182>

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