NTP
National Toxicology Program

Assessing the Biological Relevance of *In Vitro* Data: A Case Study Using Estrogen Pathway Signaling

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NTP Board of Scientific Counselors (BSC) Meeting
Dec 9-10, 2014

Interagency Collaboration

- NIEHS / NTP
- EPA OSCP
- EPA NCCT

  - Presented at EPA's FIFRA SAP: Integrated Bioactivity Exposure Ranking, 2-5 Dec 2014.
  - White paper available on SAP website:

http://www.epa.gov/scipoly/sap/meetings/2014/120214meeting.html

Positions or views expressed here do not represent official EPA policy or guidance

Questions

- For the purposes of prioritization and screening, can *in vitro* assays identify chemicals that *have the potential* to interact with the human estrogen receptor? If so, does *in vitro* potency correlate with *in vivo* bioactivity (uterotrophic)?
- If a chemical is not ER-active *in vitro*, what level of confidence do we have that it will not be ER-active *in vivo* (uterotrophic)?

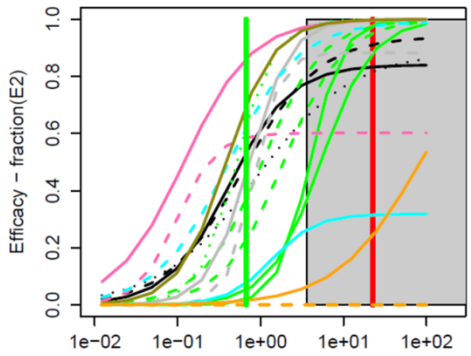
Validation

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.

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

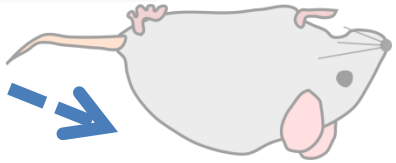
20 K ft. View



***In vitro* activity**

Bioactivity

Toxicity

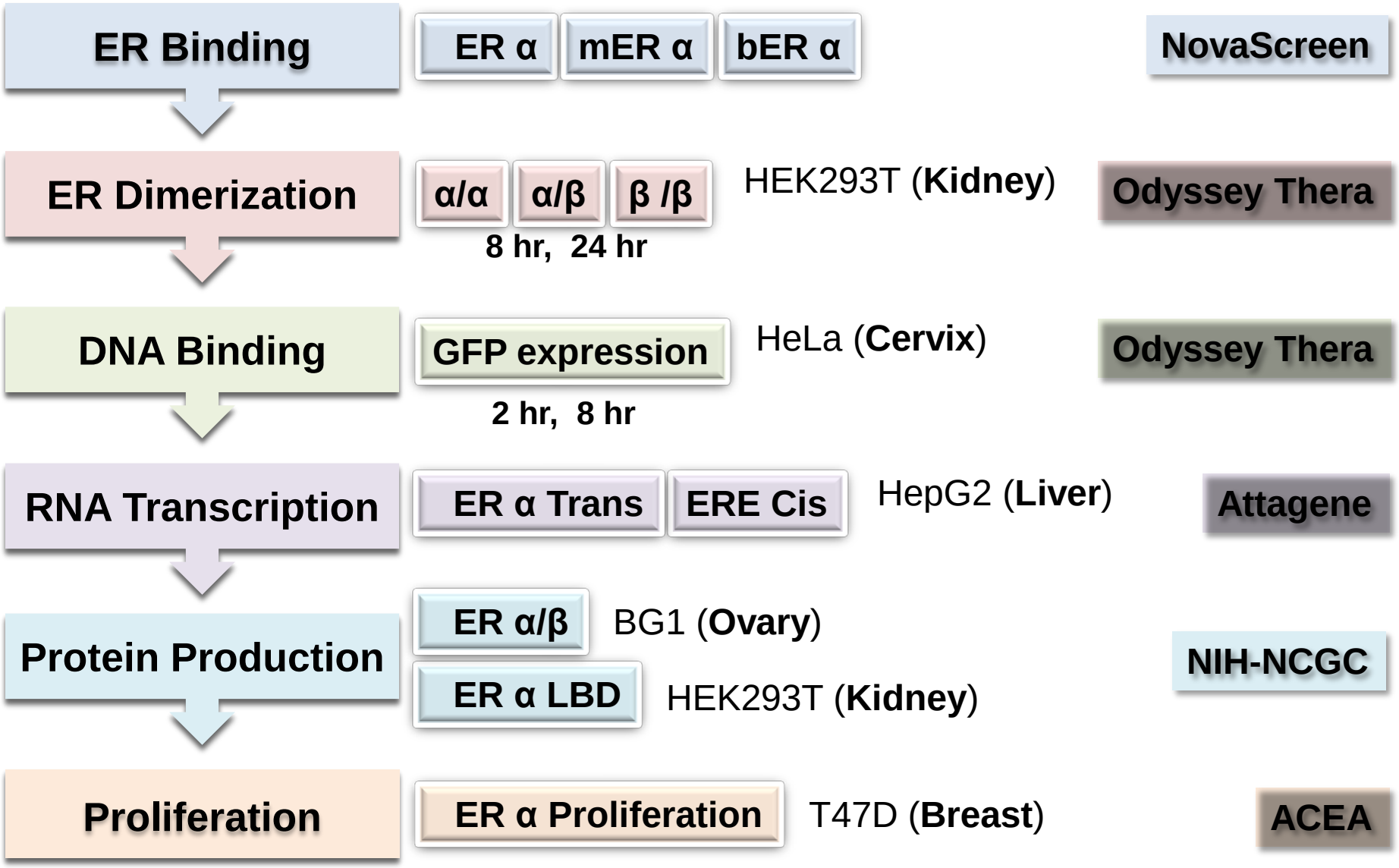


Human Relevance

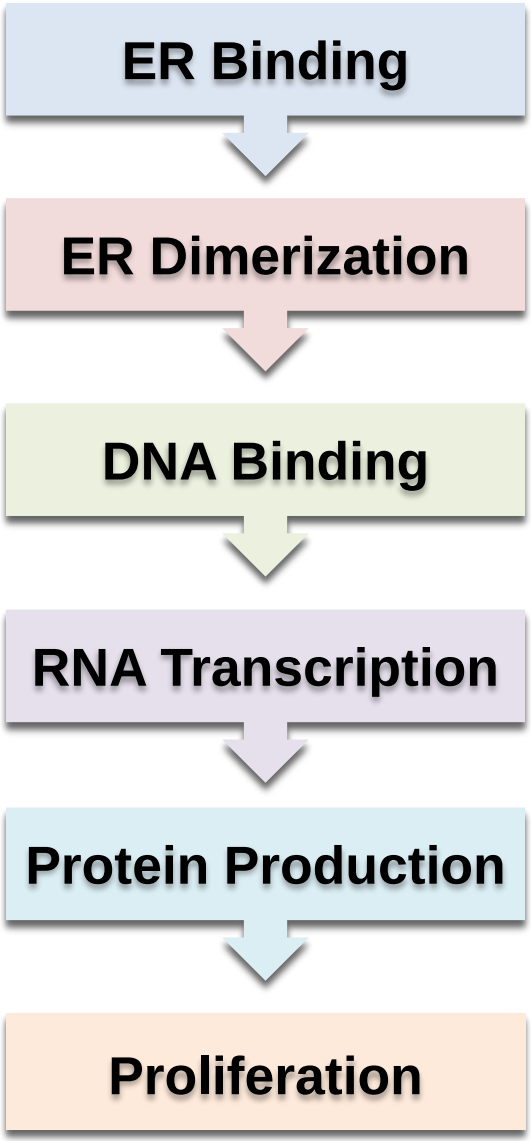
1K ft View

- 1800 Chemicals run 16 Tox21/ToxCast ER Agonist assays (1800 x 16 dose-response curves)
- Mathematical model developed to summarize results of all 16 assays for each chemical (1800 “AUC” values)
- Database of uterotrophic outcomes, developed from the literature, was used to identify *in vivo* (uterotrophic) reference chemicals for validation
- Model results assessed using “real world” data from EPA EDSP List 1 chemicals

In Vitro Assays: ER Agonist Pathway

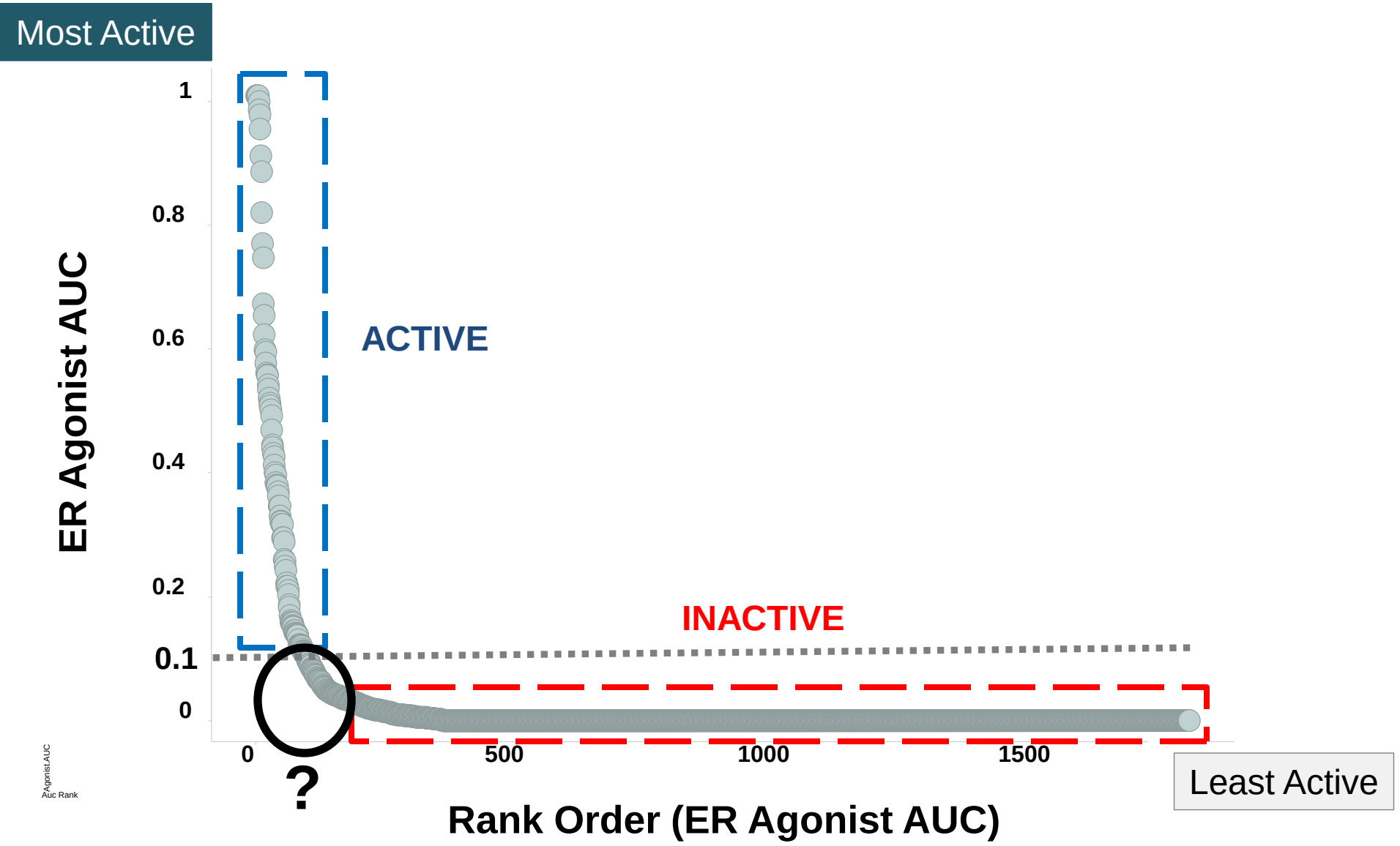


Mathematical Model of the ER Agonist Pathway



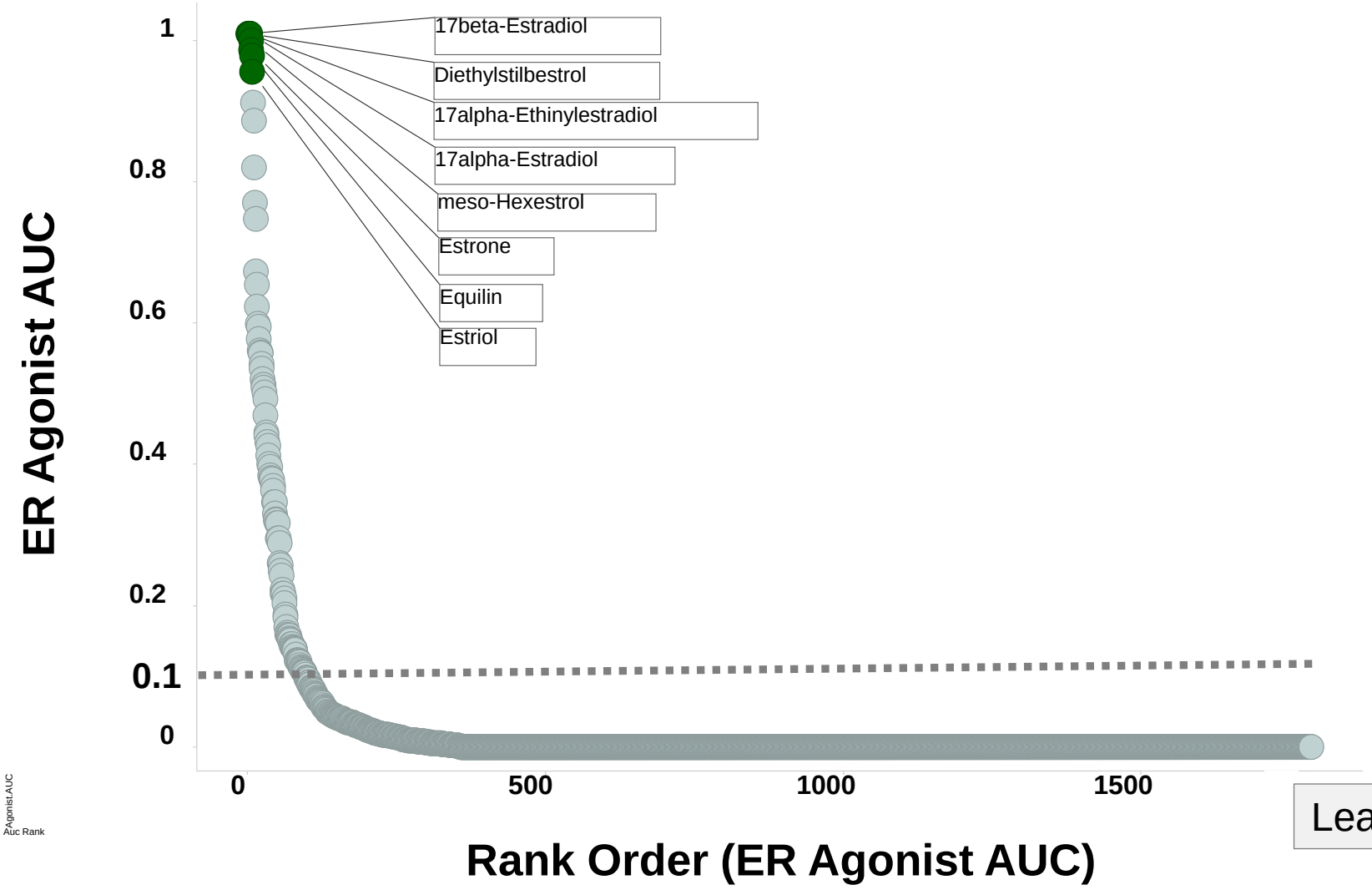
- Built using a strictly mathematical approach (i.e., no data used for training/learning)
- Assesses consistency of response across all assays, discounting assay / technology specific results
- For each chemical, the model summarizes results from all assays with a composite dose-response curve, which is used to calculate an AUC relative to 17β-estradiol (e.g. AUC for E2 = 1.0)
- AUCs range from 1-0, with ~0.1 representing the approximate limit of detection (~100 μM)

ER AUC Values of 1800 ToxCast Chemicals



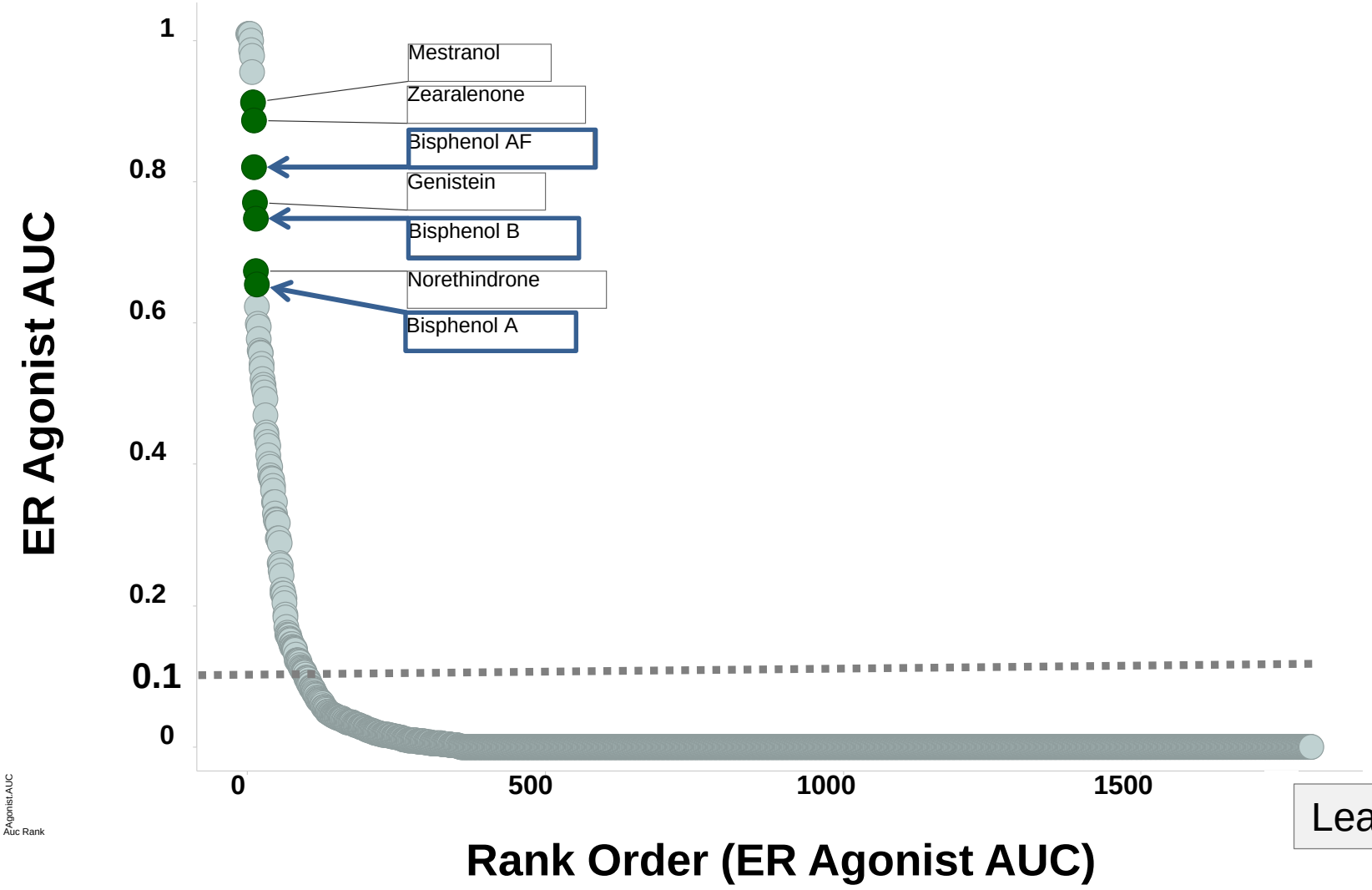
ER AUC Values of 1800 ToxCast Chemicals

Most Active



Least Active

Most Active



Least Active

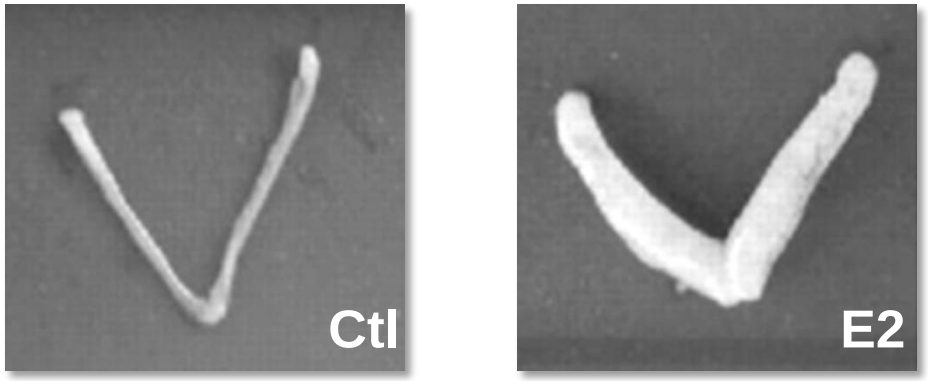
Uterotrophic Bioassay

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

Uterotrophic Bioassay

Validation

Organization of Economic Cooperation and Development (OECD)

Guidelines

OECD TG 440 / OCSPP 890.1600

Rodent models

- OVX Rat
- Imm Rat
- OVX Mouse

Dosing route

- Oral gavage (Oral)
- Subcutaneous injection (Inj)

Identifying Uterotrophic Reference Chemicals

Literature Searches:
1800 Chemicals

High-Level Filter

Data Review:
700 Papers, 42 Descriptors, x2

Minimum Criteria

Uterotrophic Database
98 Chemicals
442 uterotrophic bioassays

Selection Criteria

Reference Chemicals
31 Active, 13 Inactive

Identifying Uterotrophic Reference Chemicals

Chemical Name
CASRN
PMID
Author
Year
Study Type
Species
Strain
Target
Route of Administration
Age at 1st Dose Administration
Age at OVX
Dose/Response (0 no, 1 yes)
# of doses used
Value
Unit Response
Value type
LEL
Max Conc Tested

Descriptors captured for each chemical-bioassay combination
~2400 entries

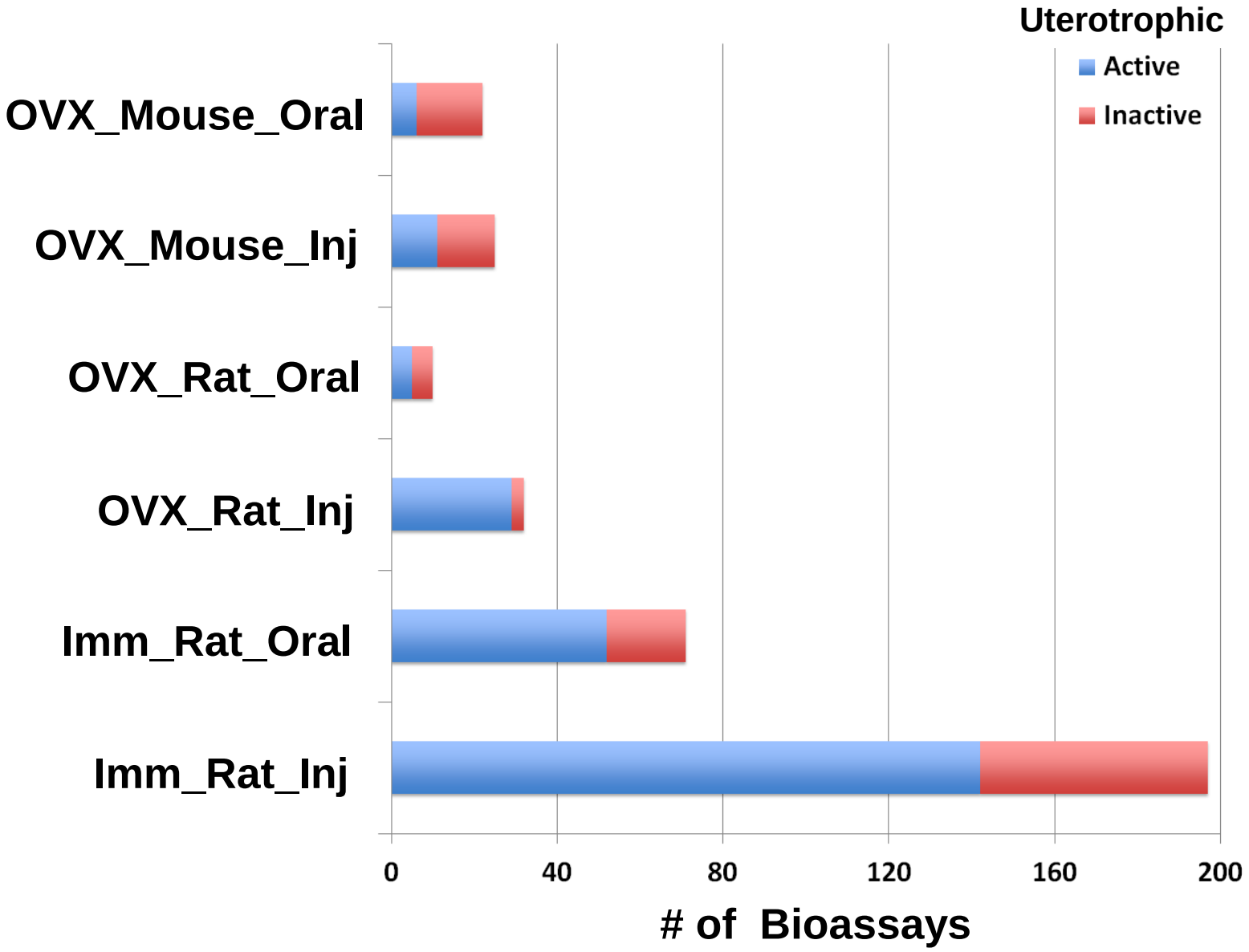
Elapsed time between OVX and RX
Dosing Length
# of doses per day
# of animals in estrogen control group
# of animals in RX group
Reference Estrogen
Vehicle/RX control?
Diet
Indicated that Diet is low-PE?
necropsy time after last dose
Additional Assay Info
Source Name SID
Chemical Tested
Chemical Purity

Identifying Uterotrophic Reference Chemicals

Minimum Criteria for "Guideline-Like" Studies

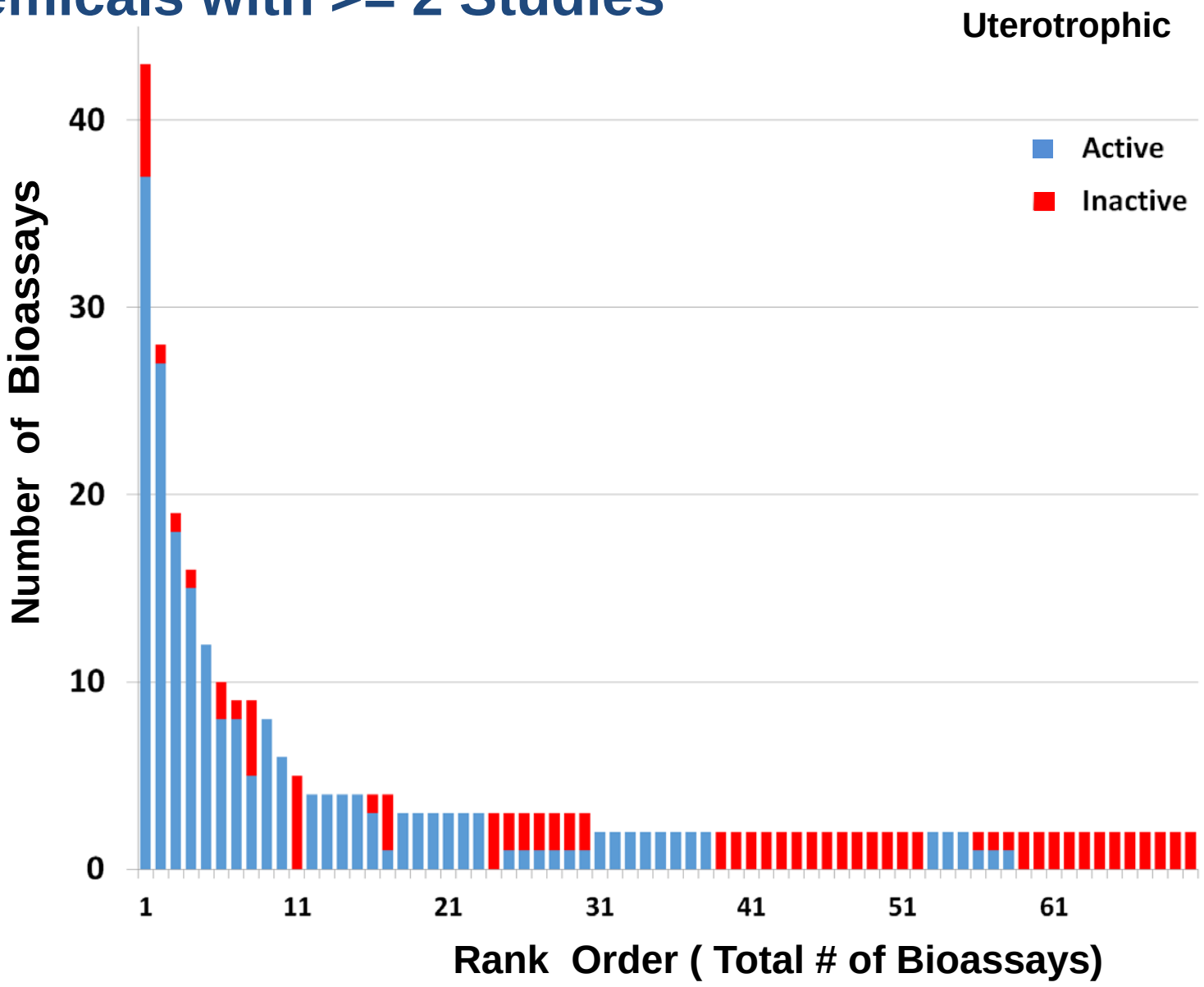
1. **Animal model**
   - Imm rats
   - OVX rats or mice
   - OVX performed between six and eight weeks of age
2. **Group size**
   - Control group n >=3, test group n >=5
3. **Route of administration**
   - Oral gavage, subcutaneous or intraperitoneal injection
4. **Number of dose groups**
   - Minimum of two dose levels plus positive control and vehicle control
5. **Dose timing and duration**
   - Minimum of three consecutive days
   - Immature rats: dosing should begin between post-natal day (PND) 18 and PND 21, and be completed by PND 25
6. **Necropsy timing**
   - Animal necropsy should be carried out 18-36 hours after the last dose

Uterotrophic Study Designs



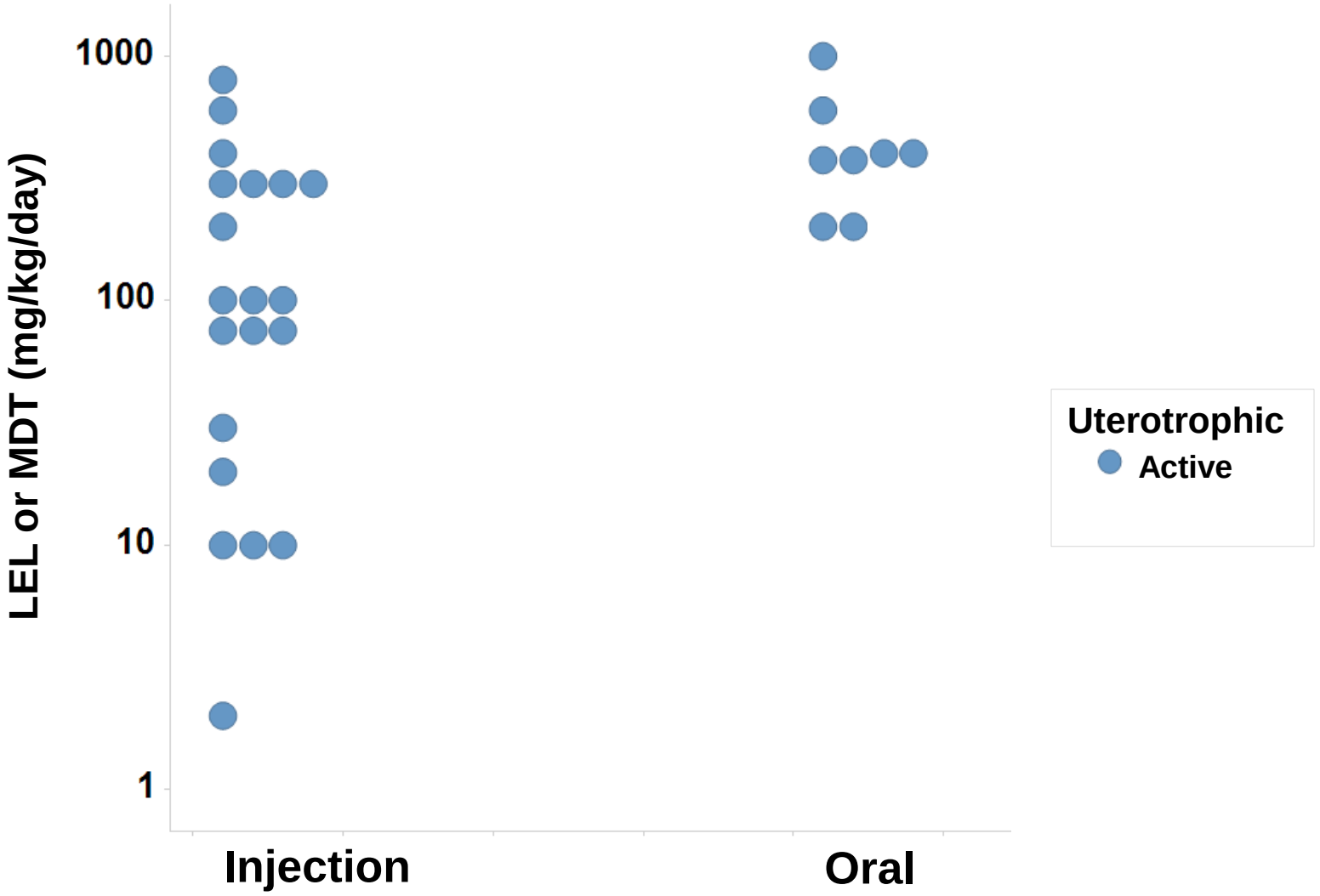
Uterotrophic Reproducibility

Chemicals with >= 2 Studies



Uterotrophic Reproducibility

Same Study Design (Immature Rat): BPA



Selection of Reference Chemicals

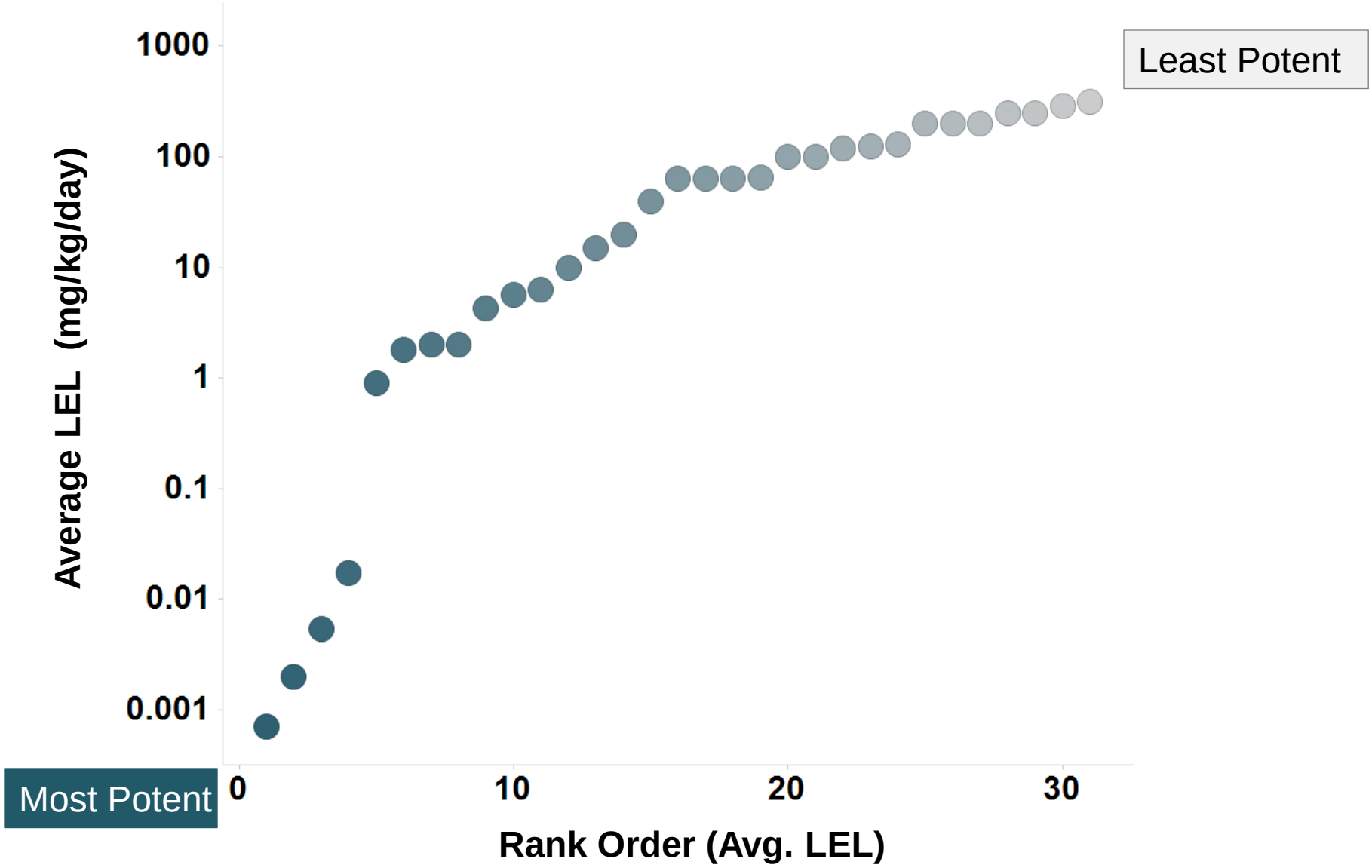
The relevance of a new test method should be characterized against data generated with a list of reference chemicals in the original test method.

Active – reported as active in two or more independent GL bioassays, regardless of the number of inactive results

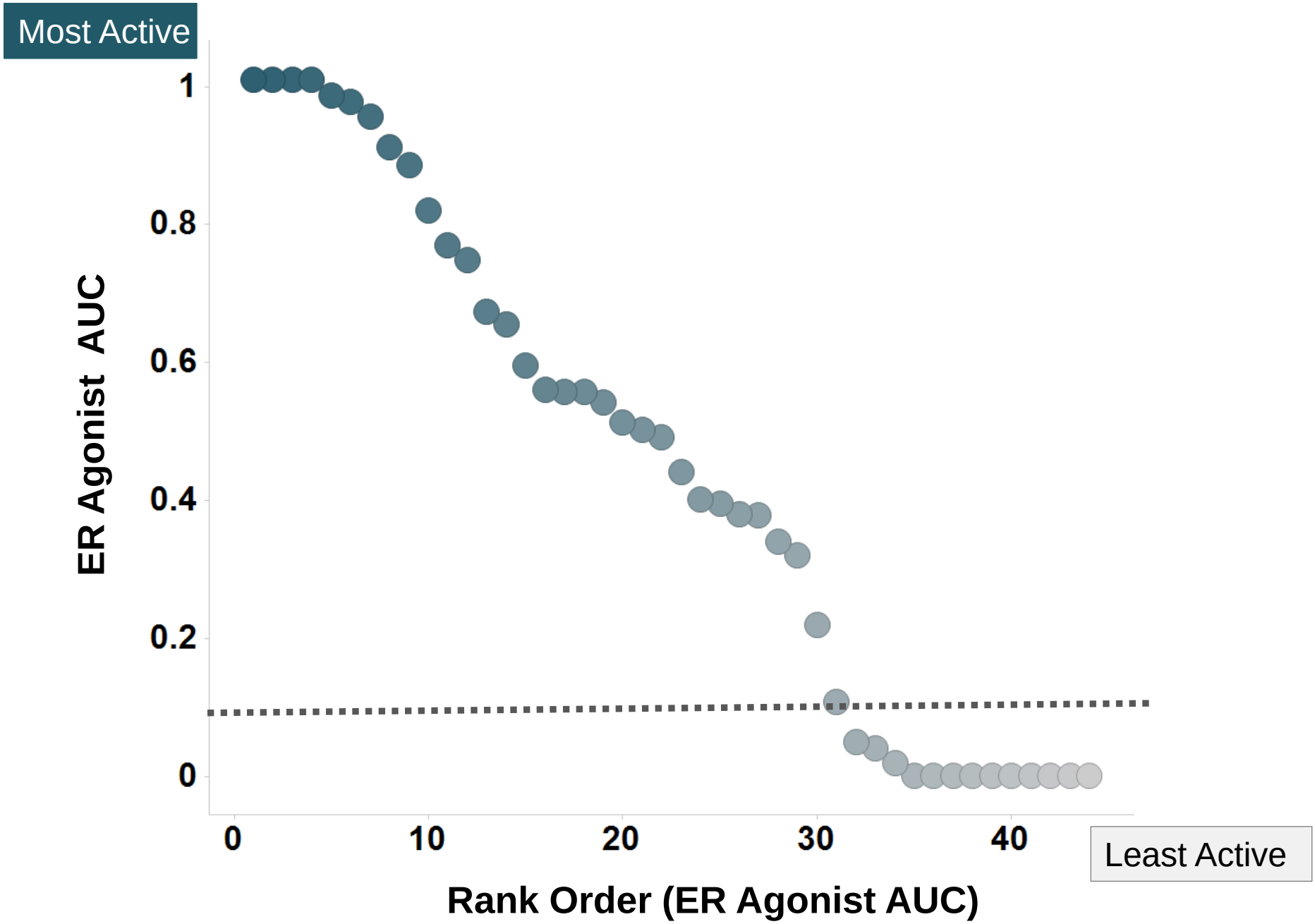
Inactive – reported as inactive in two or more independent GL bioassays, with no GL studies reporting bioactivity

44 Reference Chemicals (31 Active, 13 Inactive)

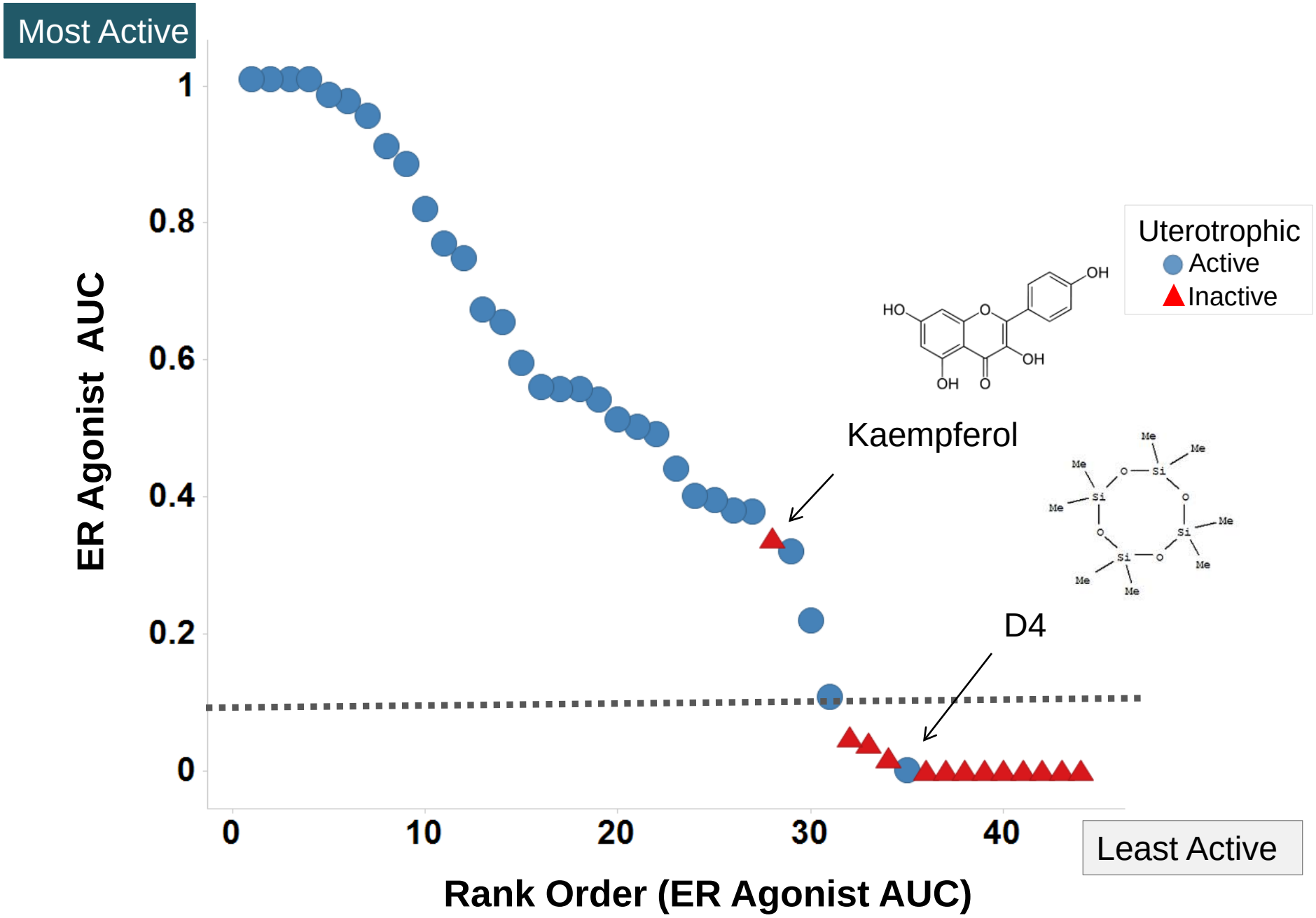
Avg LEL of Reference Chemicals (Uterotrophic)



AUC of Reference Chemicals (*In Vitro*)



AUC of Reference Chemicals (*In Vitro*)



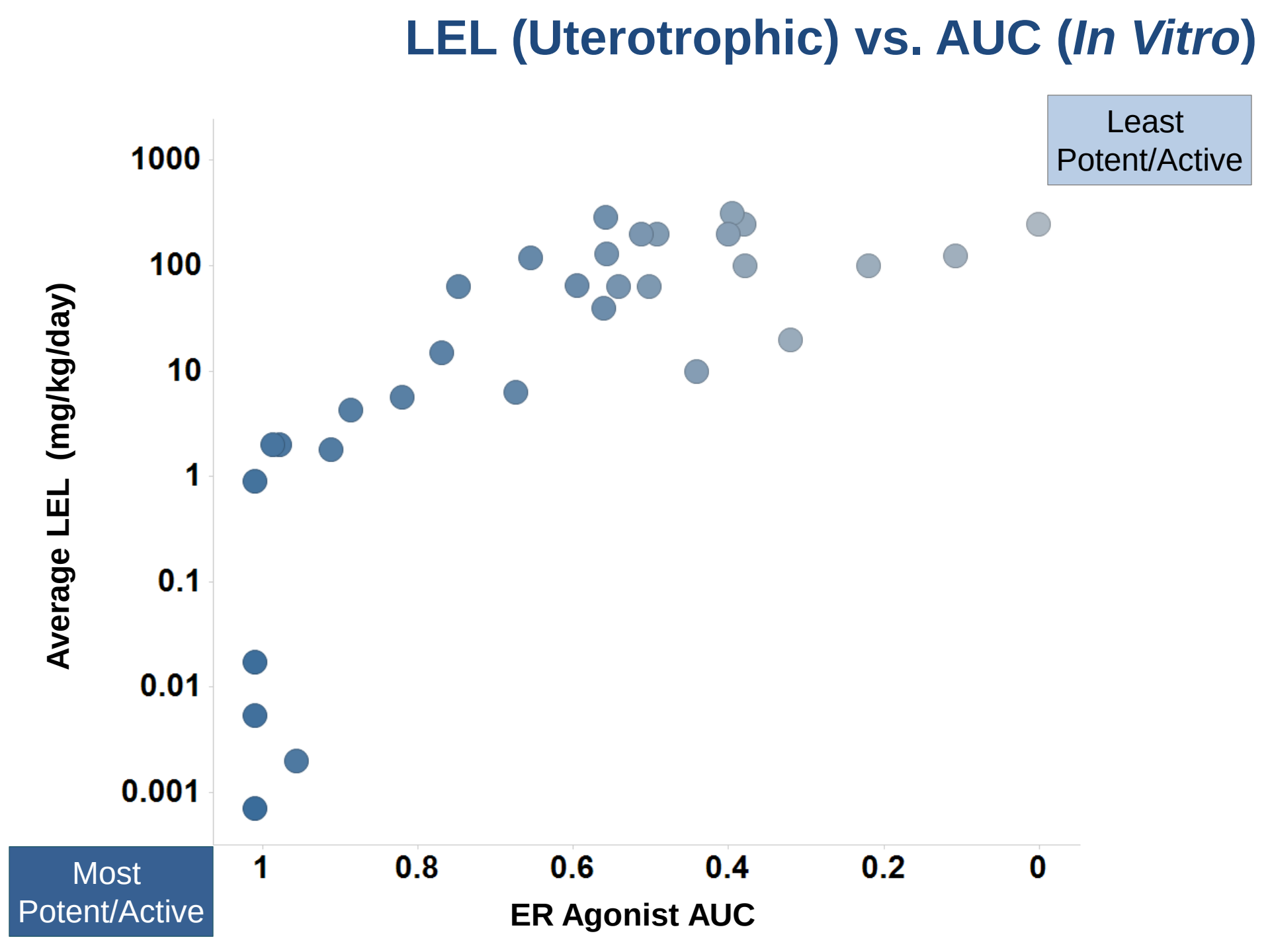
Performance of ER AUC Model

Uterotrophic reference chemicals (31 Active, 13 Inactive)

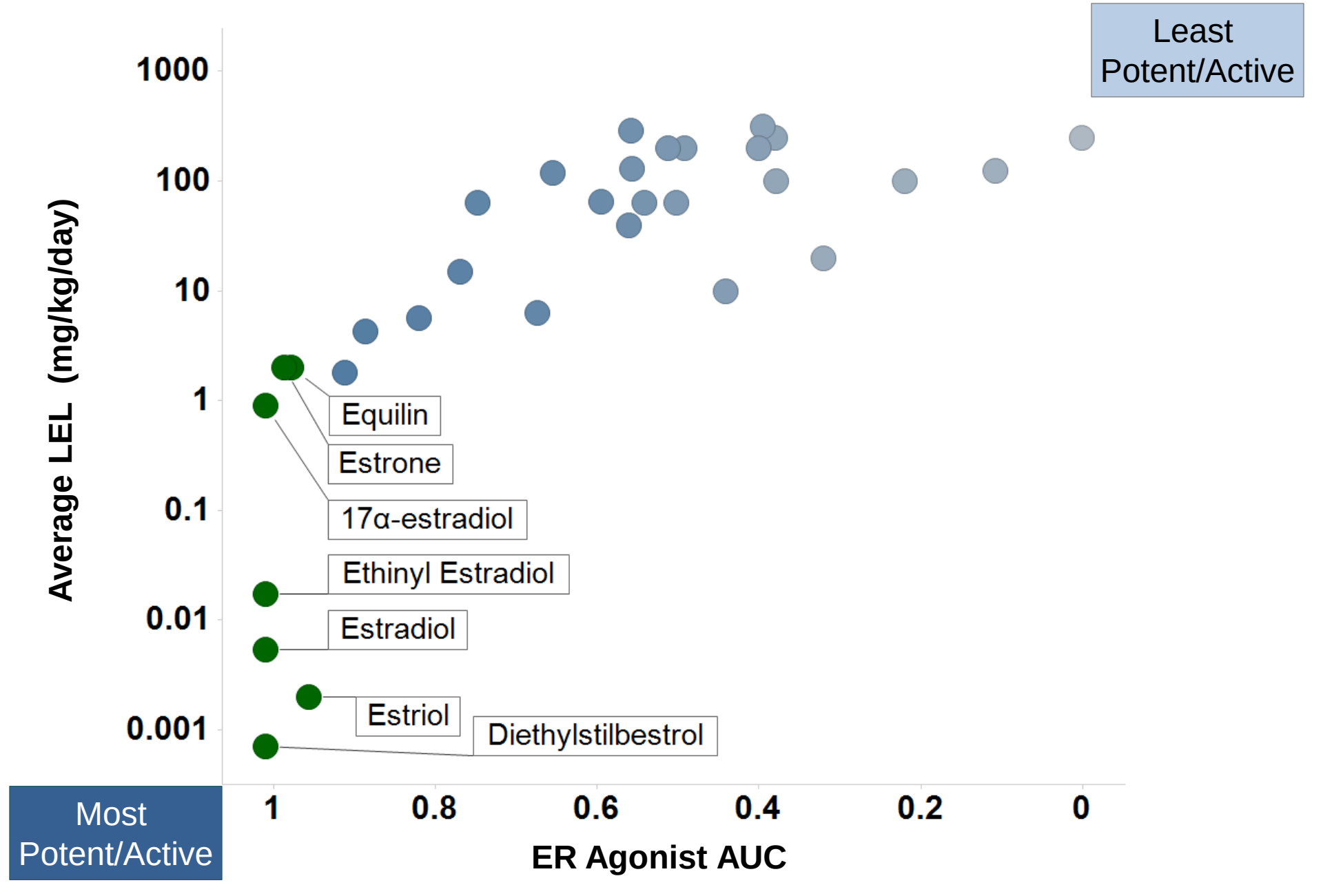
True Positive	30
True Negative	12
False Positive	1
False Negative	1
Accuracy	**0.95**
Sensitivity	**0.97**
Specificity	**0.92**

ER AUC >= 0.1 as Active

LEL (Uterotrophic) vs. AUC (*In Vitro*)



LEL (Uterotrophic) vs. AUC (*In Vitro*)

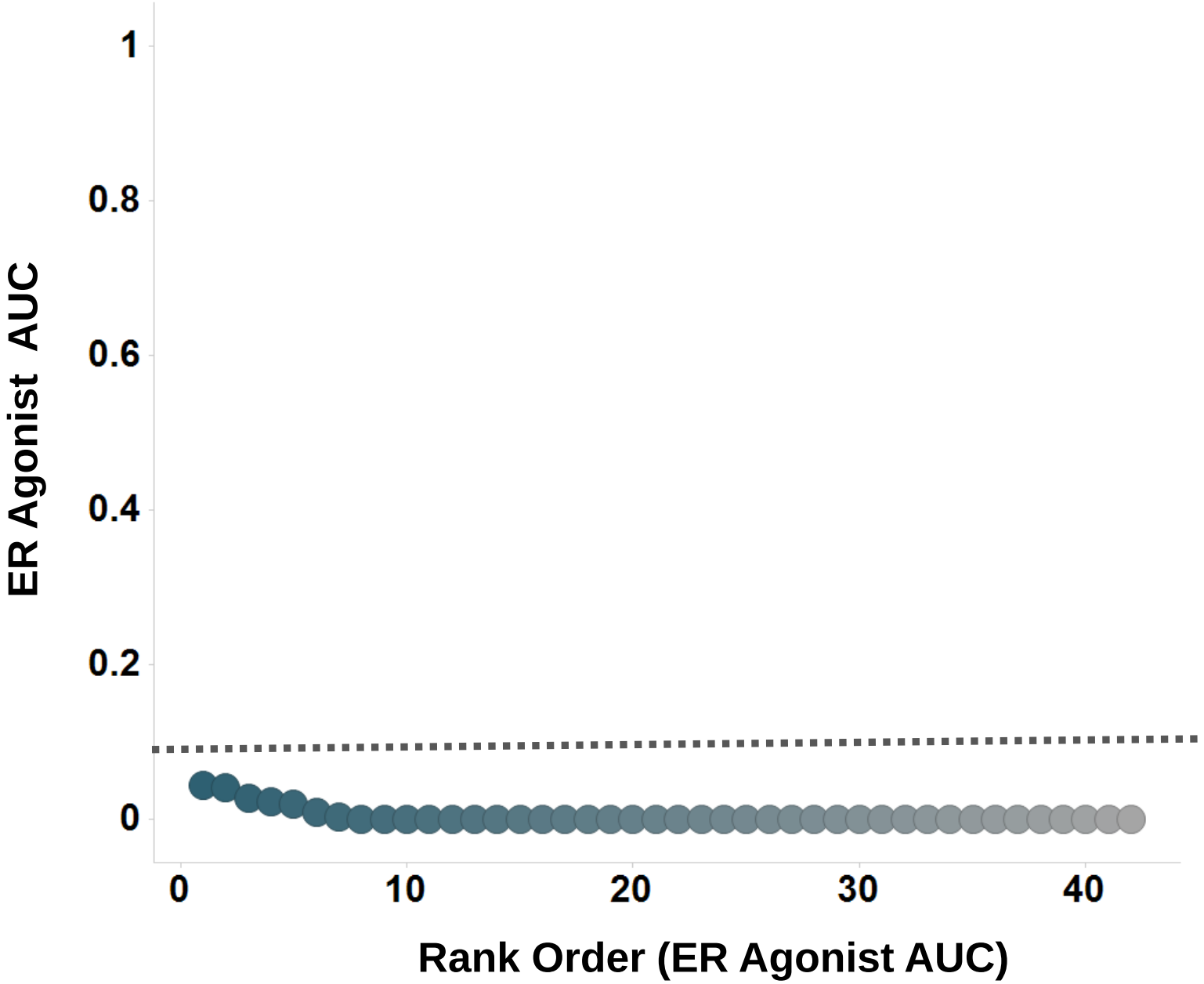


EDSP Tier 1 Battery ER-Specific Endpoints

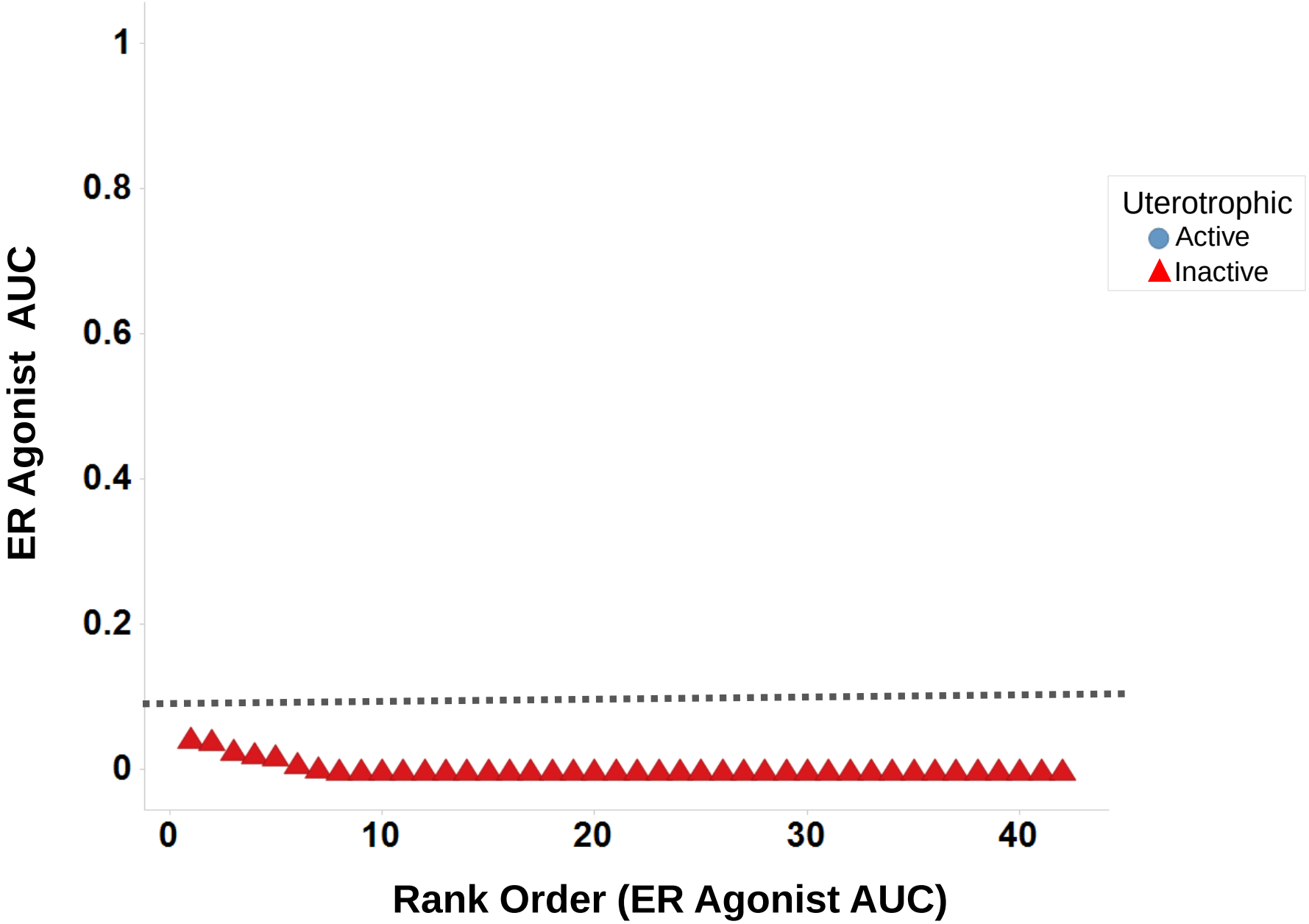
Tier 1 Screening Battery	Endocrine Pathway					
	E +	E -	A +	A -	HPG[1] Axis	HPT[1] Axis
In vitro						
ER Binding	■	■				
ERα Transactivation	■					
In vivo						
Uterotrophic	■					
Pubertal Female	■	■			■	■
Fish Short-term Reproduction (male & female)	■	■	■	■	■	

Note: The full EDSP Tier 1 battery includes five in vitro and six in vivo assays that provide additional information on a chemical's potential to interact with the androgen and thyroid, as well as alter steroidogenesis.

ER AUC of 42 EPA EDSP List 1 Chemicals



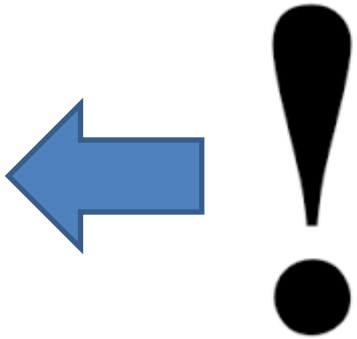
ER AUC of 42 EPA EDSP List 1 Chemicals



Performance of ER AUC Model

Uterotrophic ref. chemicals + EDSP List 1 (31 Active, 55 Inactive)

True Positive	30
True Negative	54
False Positive	1
False Negative	1
Accuracy	**0.97**
Sensitivity	**0.97**
Specificity	**0.98**



ER AUC >= 0.1 as Active

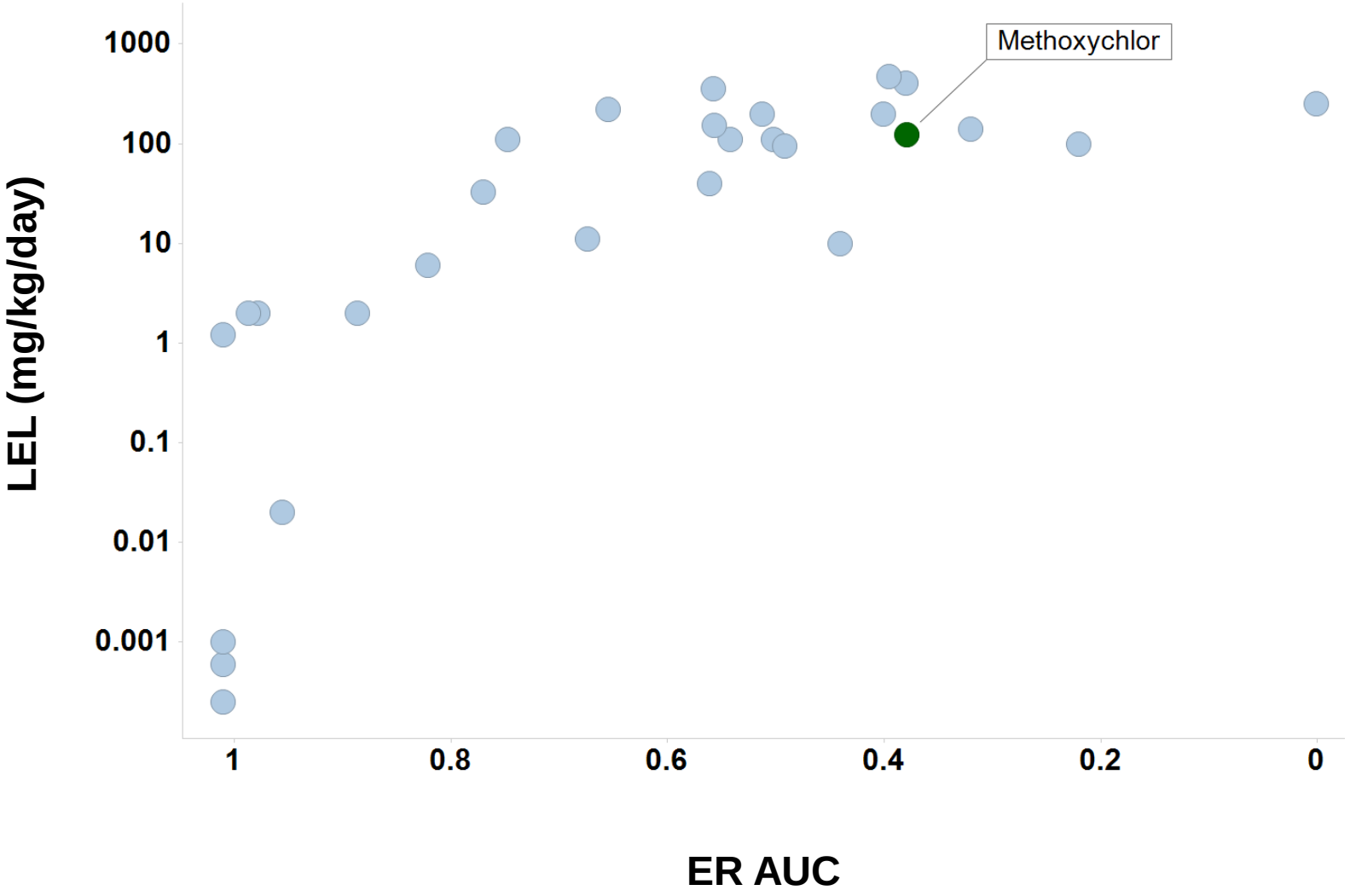
Next Steps

- ER Model Reduction / Optimization
- AR Pathway Model (9 In Vitro Assays / Hershberger)
- Evaluate concordance with Repro/Dev endpoints
- *In Vitro* to *In Vivo* Correlation (IVIVE)

http://ntp.niehs.nih.gov/pubhealth/evalatm/3rs-meetings/commprac-2015/index.html

- **QUESTIONS?**

Methoxychlor (AUC=3.8, LEL=128 mg/kg/day)



Non Monotonic Responses

Benzo(a)pyrene