Identify and Translate Learnings from On-Going Assay Validation Efforts into Standard HTS Testing Practice

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Workshop: State of the Science on Alternatives to Animal Testing and Integration of Testing Strategies for Food Safety Assessments

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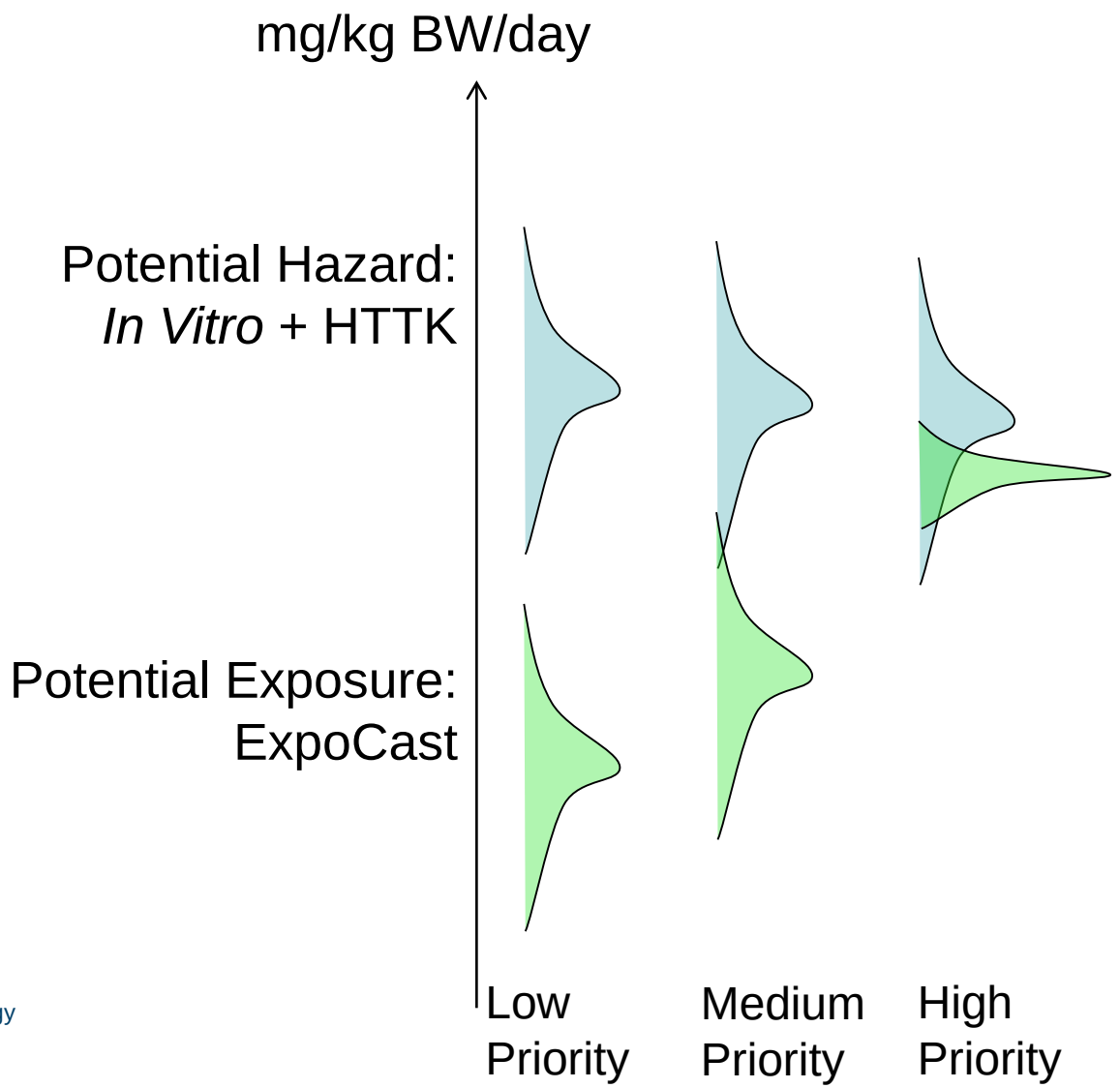
- I have no financial conflicts. All work presented here was funded by the US EPA

Overview

- Goal: assure that food-use chemicals are safe
- How would we construct a cost-effective testing scheme to meet this goal?
- How would we validate this approach?
- What are (remaining) uncertainties?

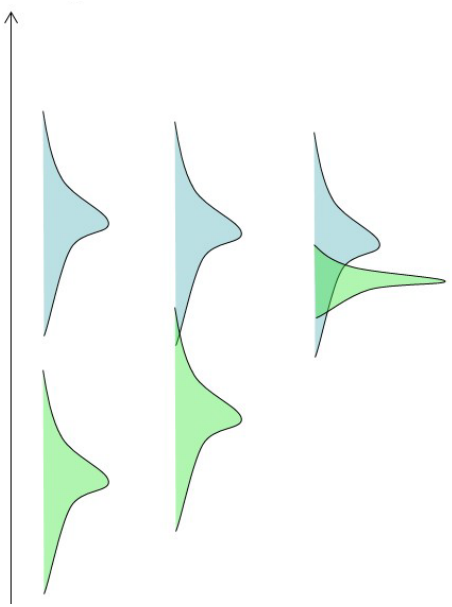
Risk-based Approach
Hazard + Exposure

Semi-quantitative *In Vitro* to *In Vivo* Approach



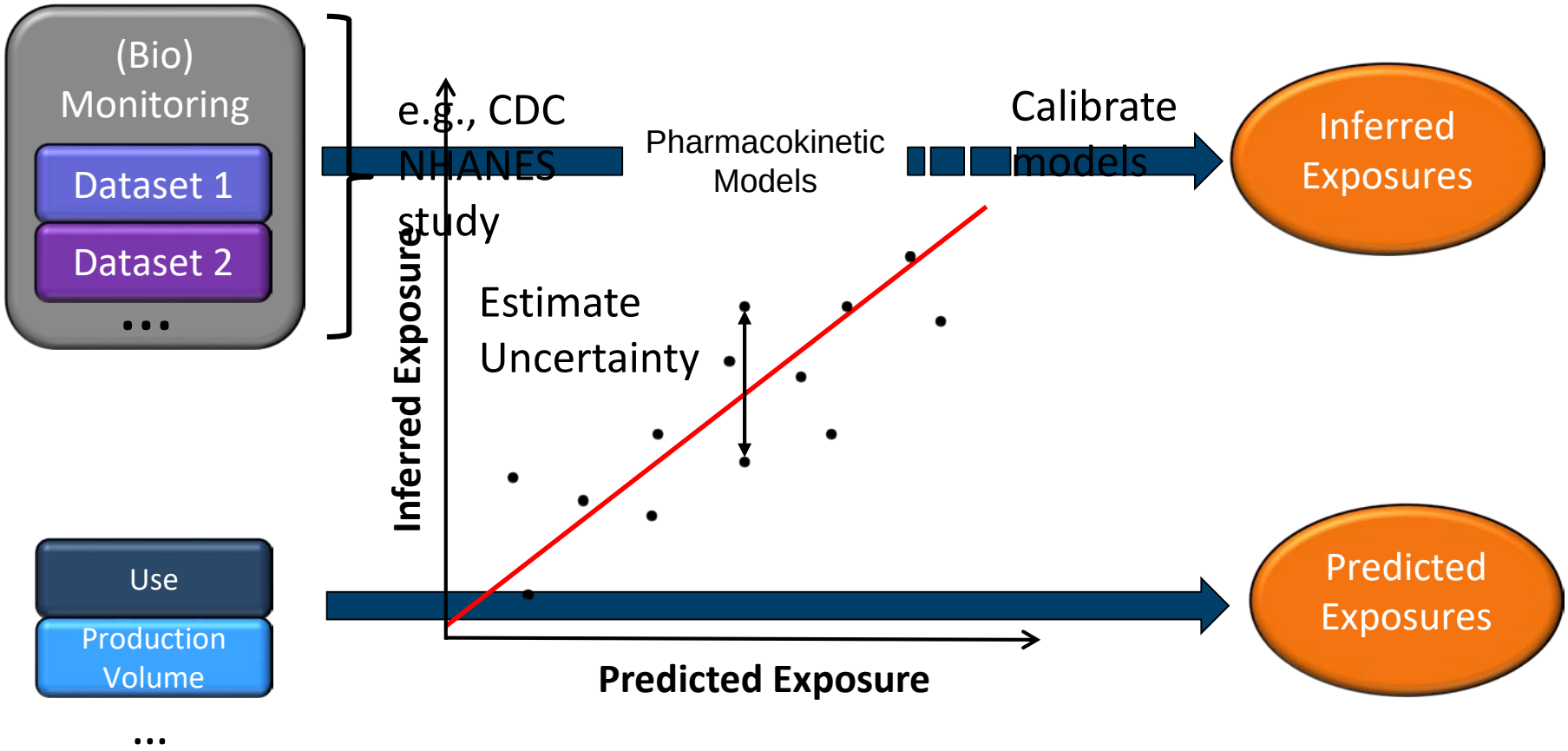
Tools / Models / Data needed

- Exposure information or model
  - Quantify in mg/kg/day
  - Include uncertainties
- Hazard information or model
  - Start in vitro
  - Quantify in uM required to trigger bioactivity
  - Include uncertainties
- Toxicokinetics
  - Use to convert between external dose and internal concentration
  - Include uncertainties



Population and Exposure Modeling

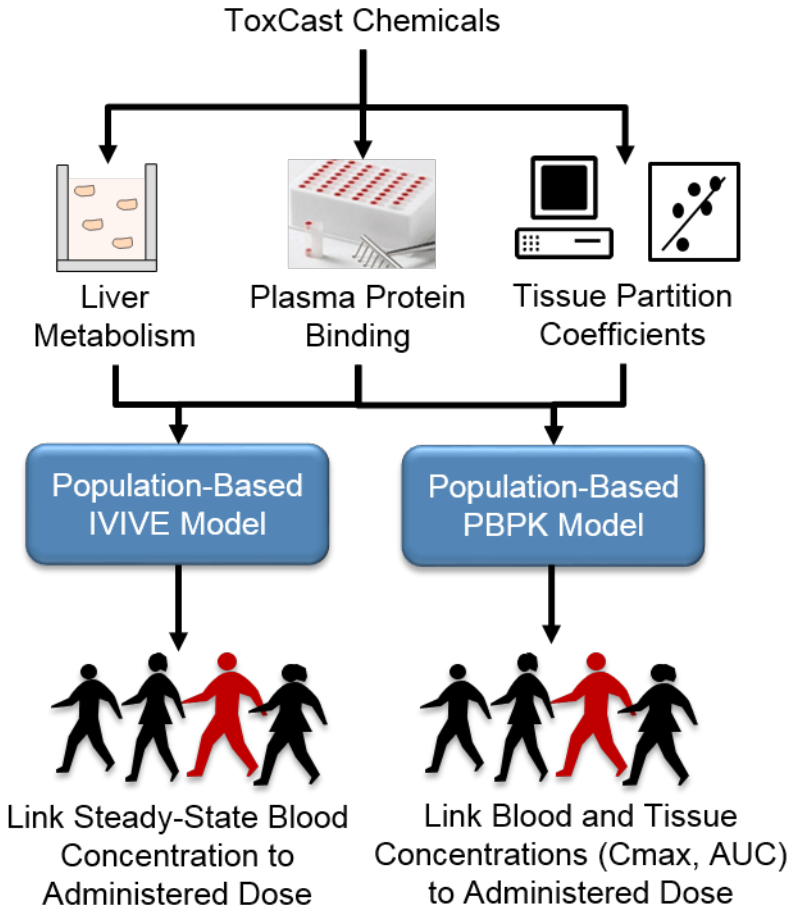
Estimating Exposure and Associated Uncertainty with Limited Data

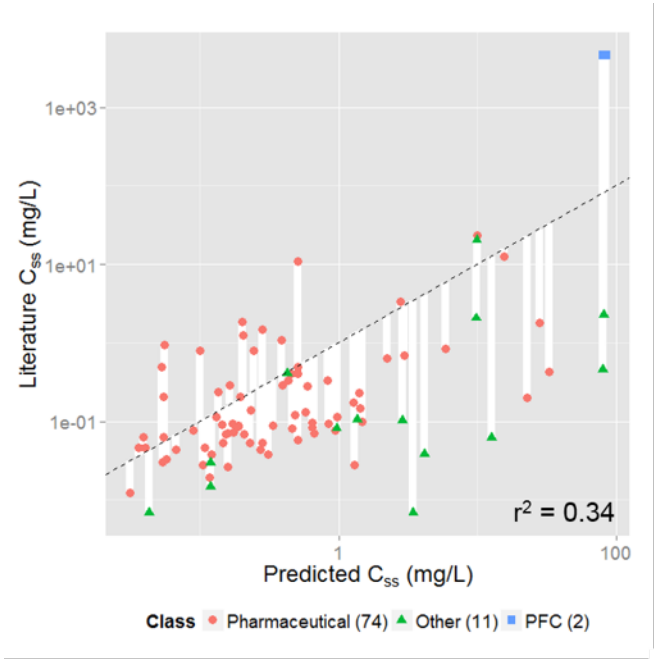


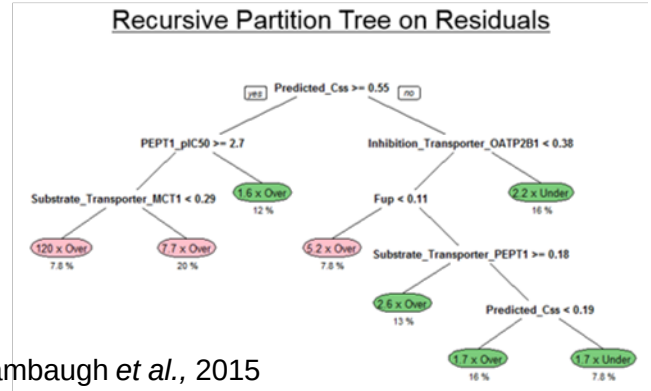
Wambaugh *et al.*, 2014

Toxicokinetics Modeling

Incorporating Dosimetry and Uncertainty into In Vitro Screening



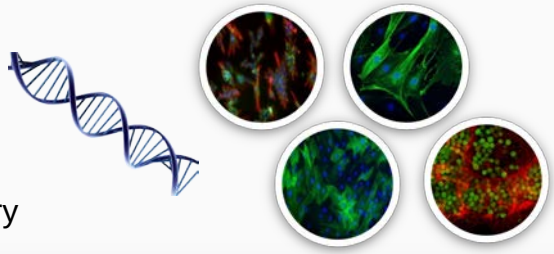




Wambaugh *et al.*, 2015

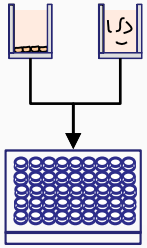
The “Minimal Hazard Battery”

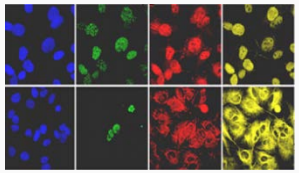
Comprehensive transcriptomic screening + cell-stress / cytotoxicity battery



Multiple Human Cell Types

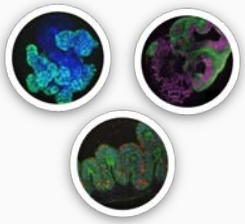
Focused in vitro assays



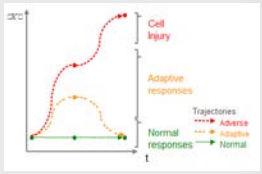


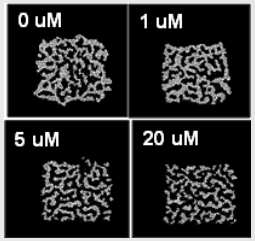
Time Course High Content Assays

Organs-on-a-Chip



Organotypic and Organoid Models





Computational and Statistical Modeling

Comprehensive Characterization

Verification of Affected Processes/ Pathways and Temporal Evaluation

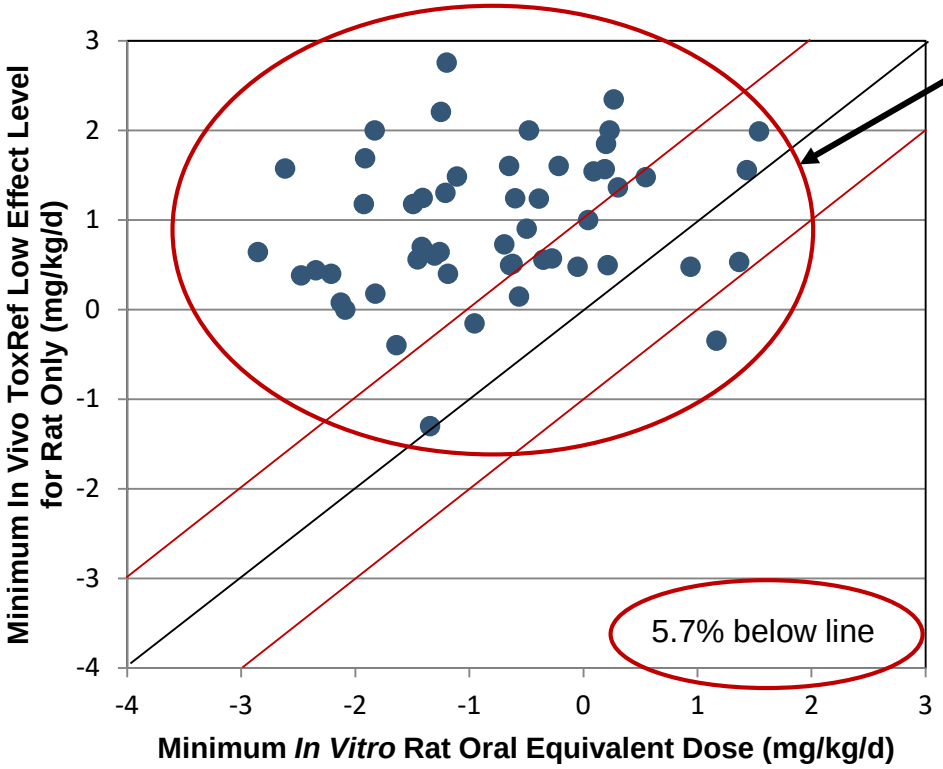
Interpretation of Affected Process/ Pathways and Population Variability

The “Minimal Hazard Battery”

- Still in exploratory stage
- Tier 1 provides
  - *in vitro* LOAEC / NOAEC
  - Survey of perturbed pathways
  - Concentrations where cell stress may interfere with assays giving false positive signals
  - If expected doses overlap with cell-stress concentrations, then the chemical is probably dangerous
- Tier 2
  - Confirmation of pathways perturbed
- Tier 3
  - More in vivo-like context around findings

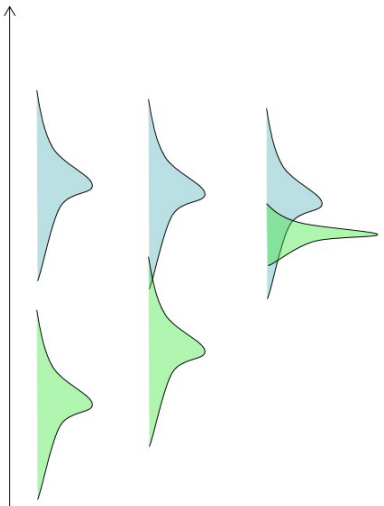
First test:
Can the battery predict *in vivo* POD?

Spanned 38 *In Vivo* Endpoints across Multiple Tissues, Organ Systems, and Study Types (Repro, Chronic, and Dev)

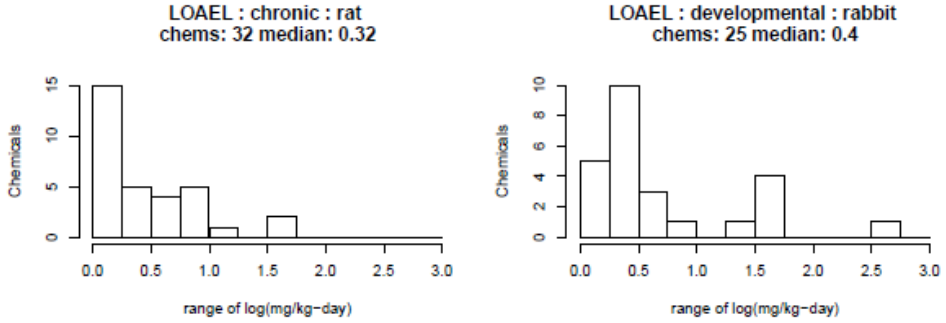


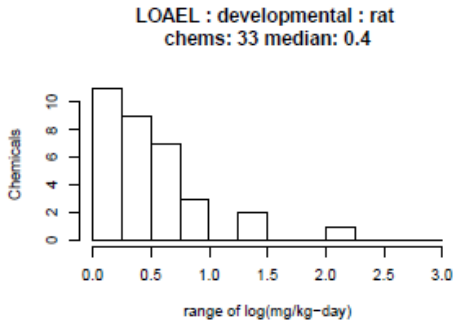
- Start with battery of in vitro assays
- Convert to dose with HT toxicokinetics
- 94% of chemicals have a health-protective prediction of POD

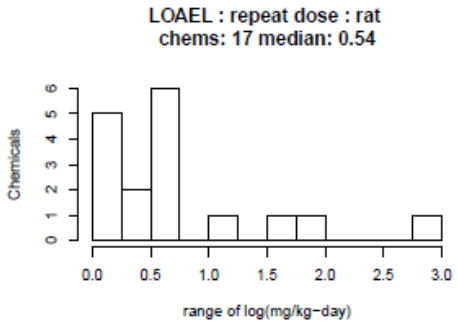
- But: How golden is the gold-standard?

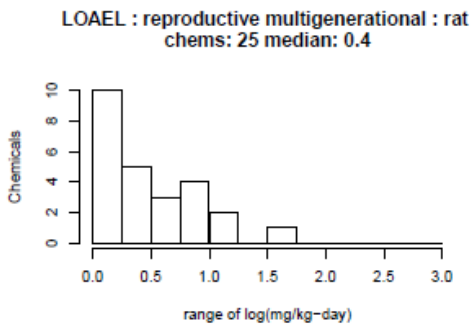


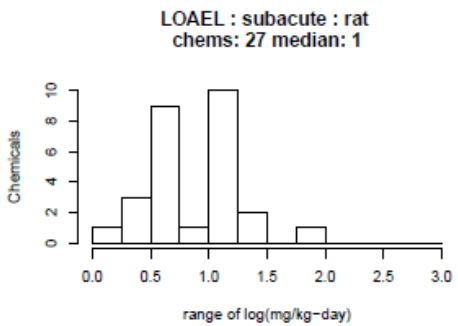
Wetmore *et al., Tox Sci.,* 2013

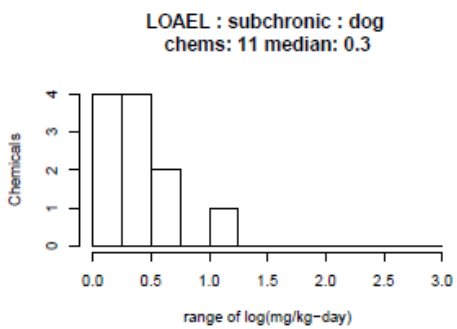


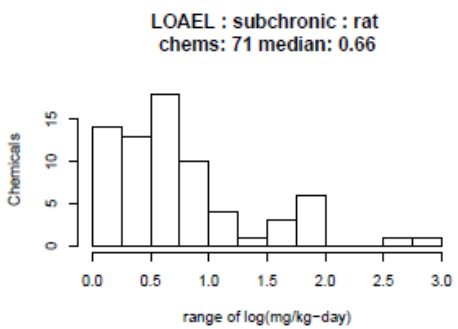












How golden is the goal standard?

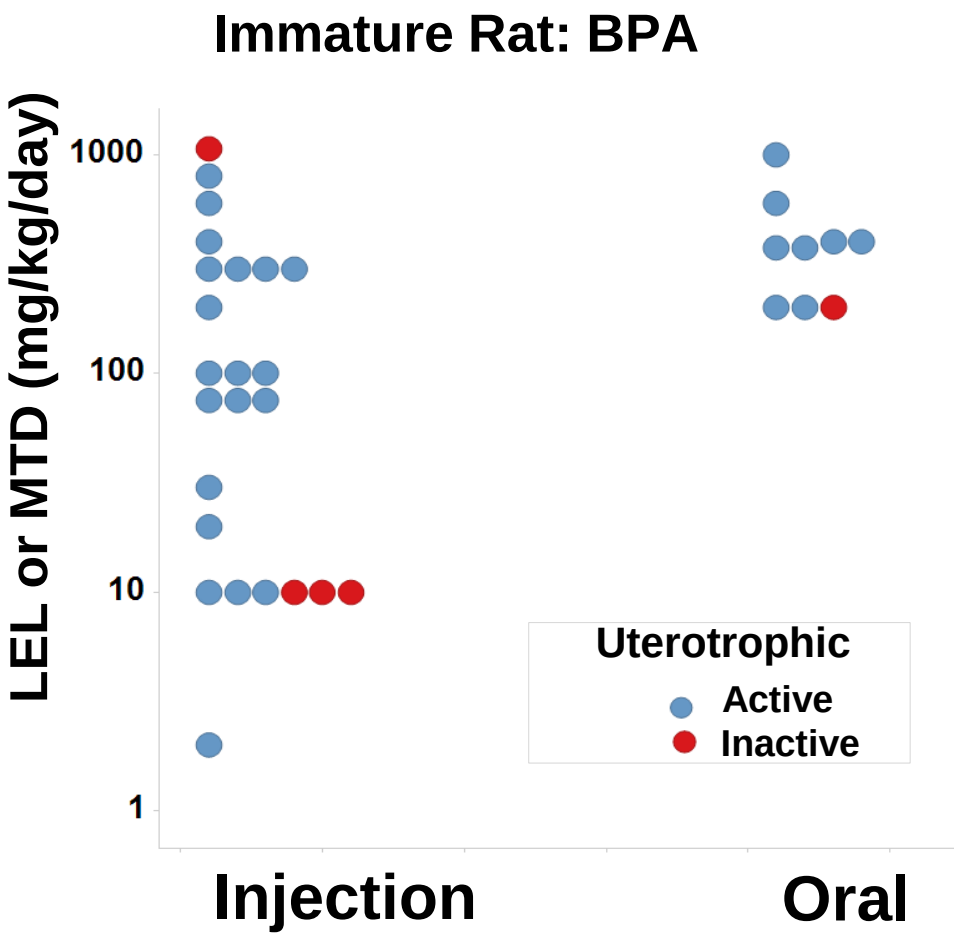
PODs vary from one lab to the next

Median span from lowest LOAEL to highest is 0.3 to 1.0 log units

Data taken from EPA ToxValDB

Uterotrophic guideline study uncertainty

26% of chemicals tested multiple times in the uterotrophic assay gave discrepant results



Kleinstreuer et al: “A Curated Database of Rodent Uterotrophic Bioactivity” EHP (2015)

Anemia concordance results

Species / study 1	Species / study 2	Concordant	Not Concordant	Fraction Concordant
rat SUB	rat CHR	18	2	0.90
rat CHR	dog CHR	13	2	0.87
rat CHR	rat SUB	18	4	0.82
rat SUB	**rat SUB**	**16**	**4**	**0.80**
rat SUB	dog CHR	11	4	0.73
mouse CHR	rat CHR	11	4	0.73
mouse CHR	rat SUB	13	7	0.65
dog CHR	rat SUB	11	6	0.65
dog CHR	rat CHR	13	8	0.62
rat CHR	mouse CHR	11	11	0.50
mouse CHR	dog CHR	6	6	0.50
rat SUB	**mouse CHR**	**13**	**14**	**0.48**
dog CHR	mouse CHR	6	8	0.43
mouse CHR	**mouse CHR**	**2**	**3**	**0.40**

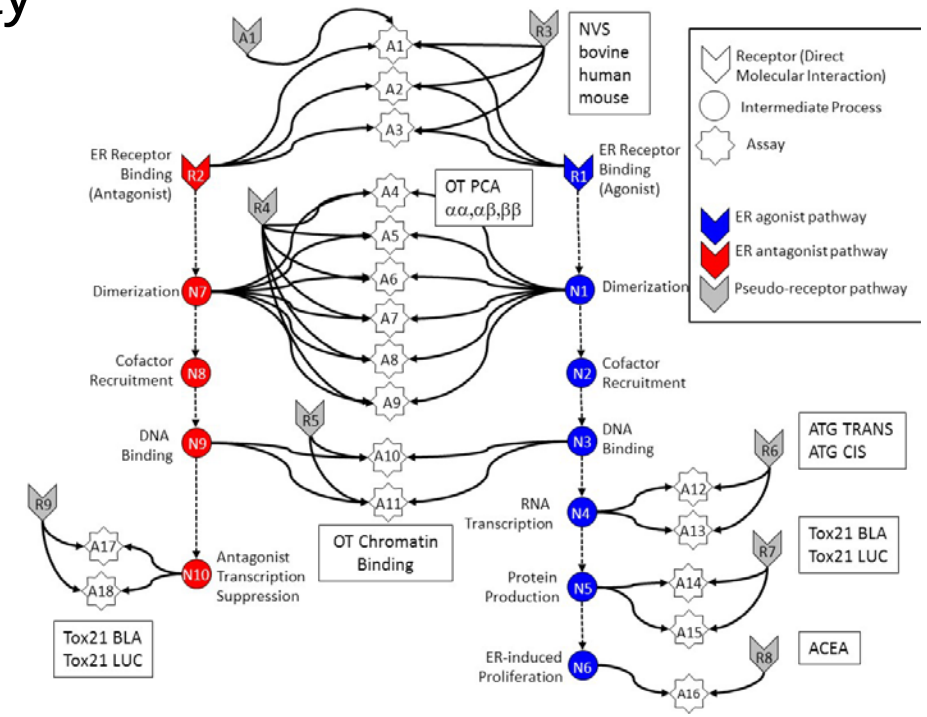
Judson et al. Reg. Tox. Pharm (2017) “Retrospective Mining of Toxicology Data to Discover Multispecies and Chemical Class Effects: Anemia as a Case Study”.

Sources of Variability

- Experimental variability
  - Species, strain, dose range, dose spacing
- Statistical power issues
  - Too few animals to see weak or rare effect
- Reporting bias
  - Was an effect negative or not looked for?
- Observer bias
  - Less sever phenotypes not reported when more severe ones are present
- Diagnostic terminology drift
- Data assimilation and analysis
  - Typos, incomplete transcription

In Vitro Estrogen Receptor Model

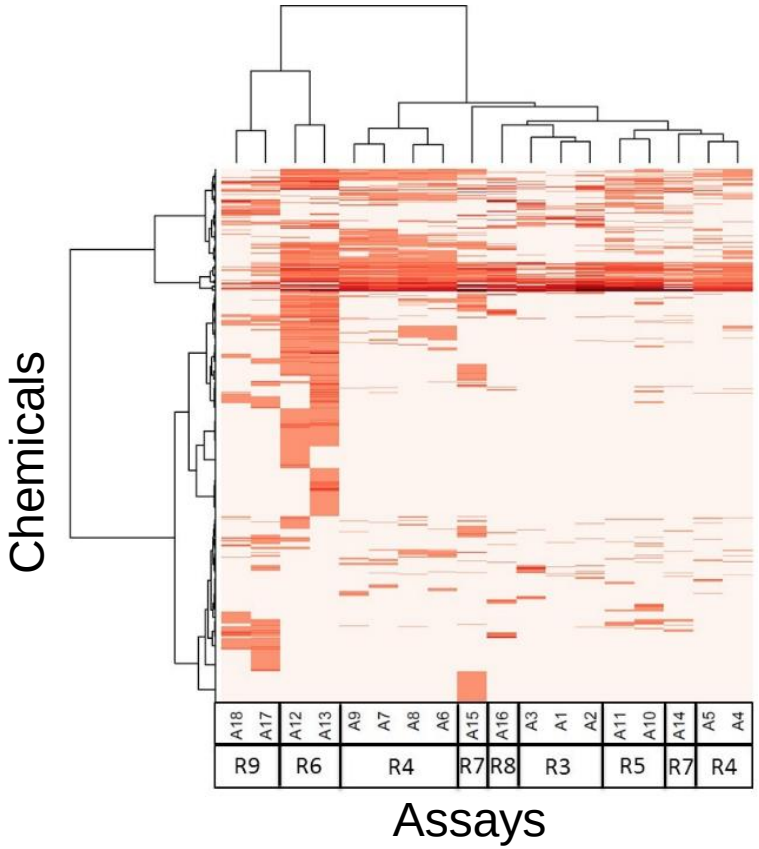
- Use multiple assays per pathway
  - Different technologies
  - Different points in pathway
- No assay is perfect
  - Assay Interference
  - Noise
- Use model to integrate assays
- Evaluate model against reference chemicals
- Methodology being applied to other pathways



Judson et al: "Integrated Model of Chemical Perturbations of a Biological Pathway Using 18 In Vitro High Throughput Screening Assays for the Estrogen Receptor" (EHP 2015)

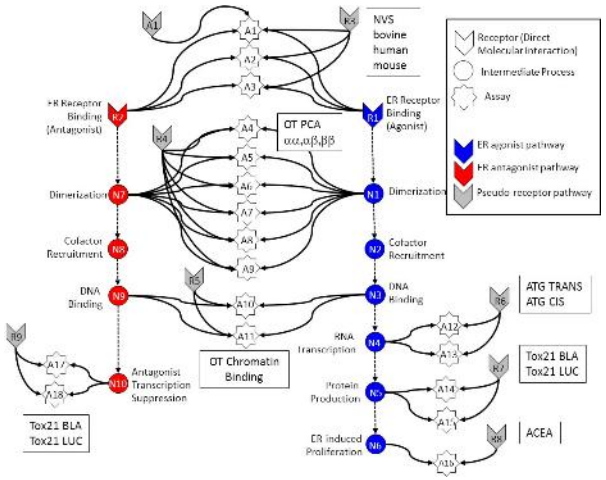
In vitro assays also have false positives and negatives

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



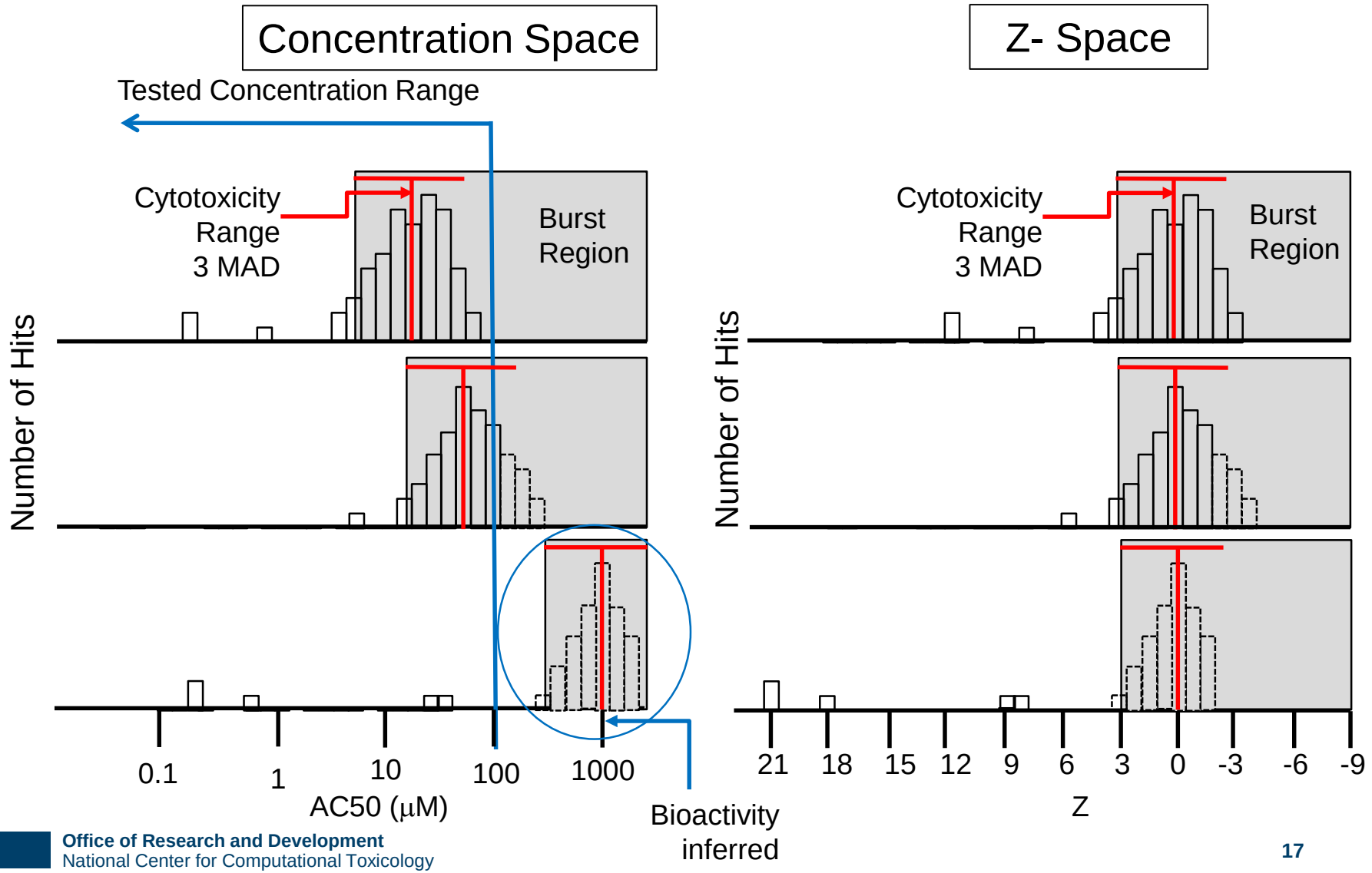
Much of this “noise” is reproducible
- “assay interference”
- Result of interaction of chemical with complex biology in the assay

Chemical universe is structurally diverse
-Solvents
-Surfactants
-Intentionally cytotoxic compounds
-Metals
-Inorganics
-Pesticides
-Drugs



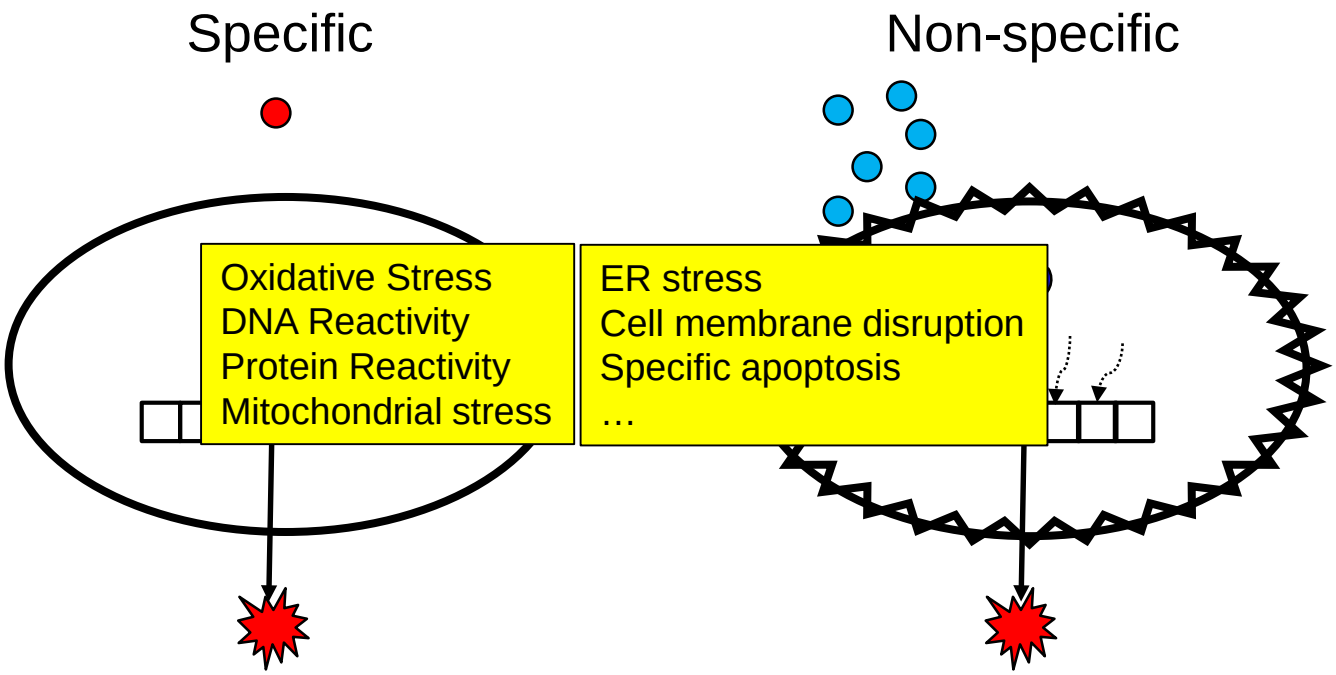
Judson et al: ToxSci (2015)

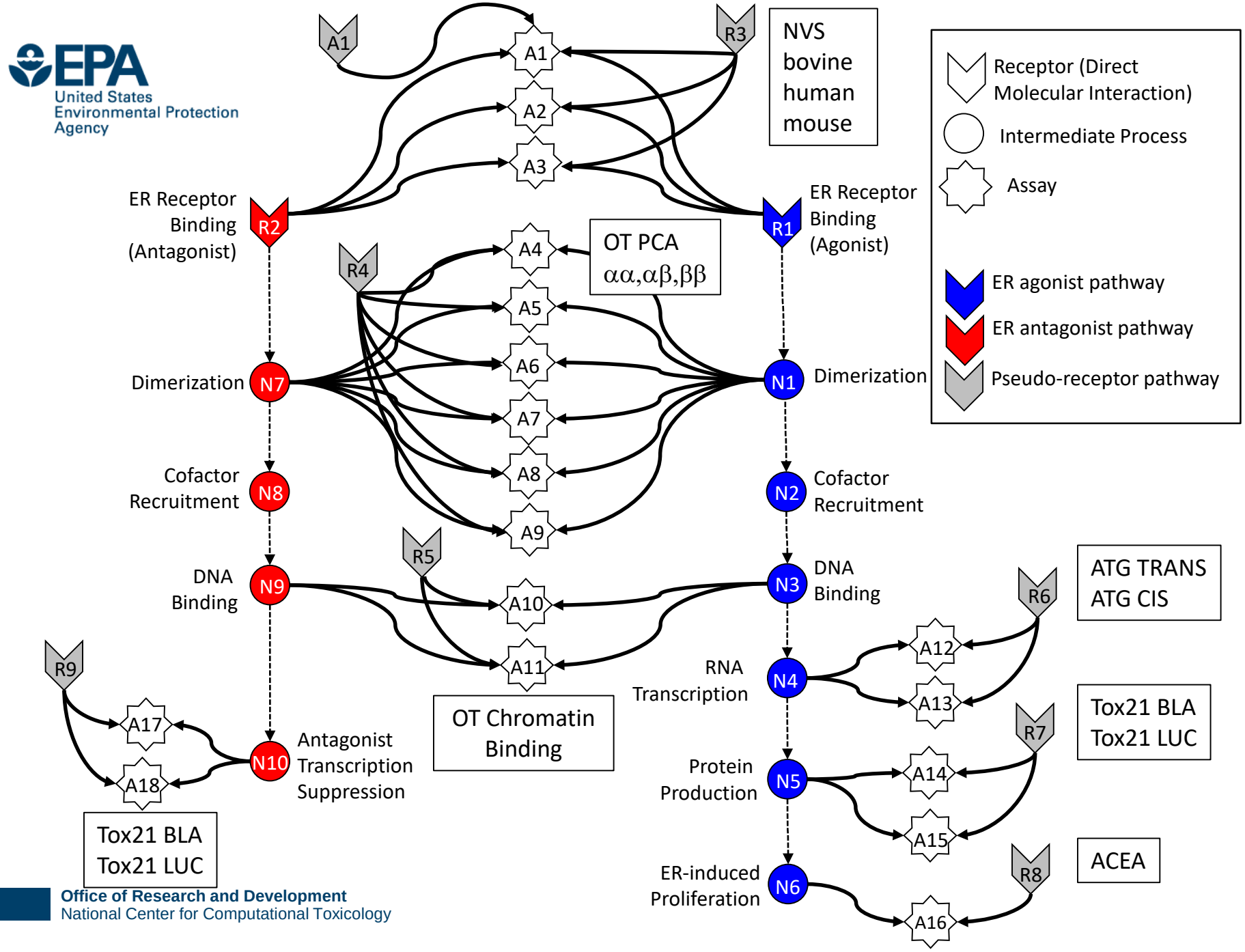
Most chemicals display a “burst” of potentially non-selective bioactivity near cytotoxity concentration



Judson et al. Tox.Sci. (2016)

Schematic explanation of the burst




United States Environmental Protection Agency
NVS
bovine
human
mouse
Receptor (Direct Molecular Interaction)
Intermediate Process
Assay
ER agonist pathway
ER antagonist pathway
Pseudo-receptor pathway
A1
A2
A3
R3
R2
ER Receptor Binding (Antagonist)
R1
ER Receptor Binding (Agonist)
OT PCA
αα,αβ,ββ
R4
A4
A5
A6
A7
A8
A9
N7
Dimerization
N1
Dimerization
N8
Cofactor Recruitment
N2
Cofactor Recruitment
R5
N9
DNA Binding
N3
DNA Binding
A10
A11
OT Chromatin Binding
ATG TRANS
ATG CIS
R6
A12
A13
N4
RNA Transcription
R9
A17
A18
N10
Antagonist Transcription Suppression
Tox21 BLA
Tox21 LUC
R7
Tox21 BLA
Tox21 LUC
N5
Protein Production
A14
A15
N6
ER-induced Proliferation
R8
ACEA
A16
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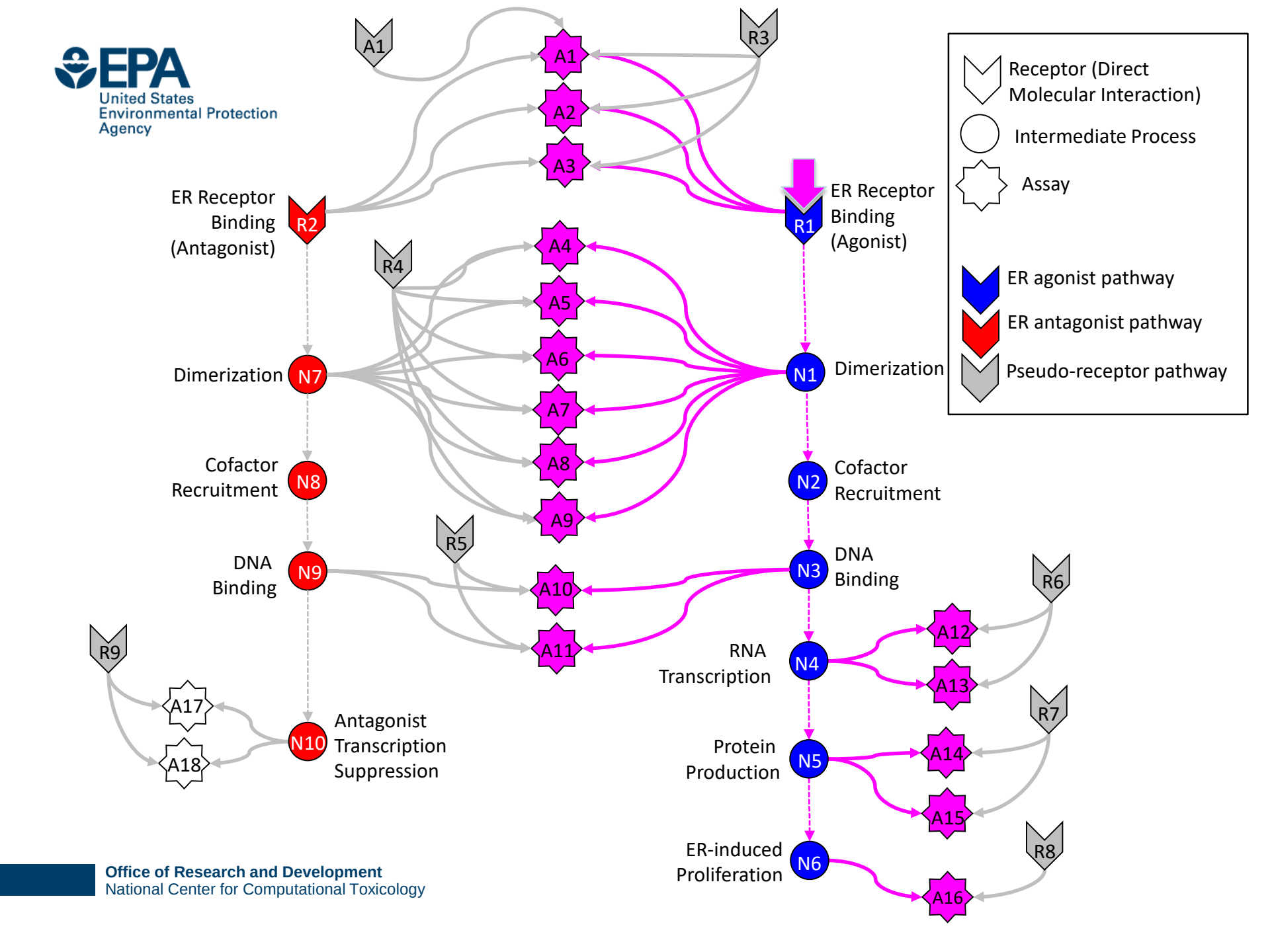
EPA
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DNA
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DNA
Binding
RNA
Transcription
Protein
Production
ER-induced
Proliferation
Antagonist
Transcription
Suppression
R1
R2
R3
R4
R5
R6
R7
R8
R9
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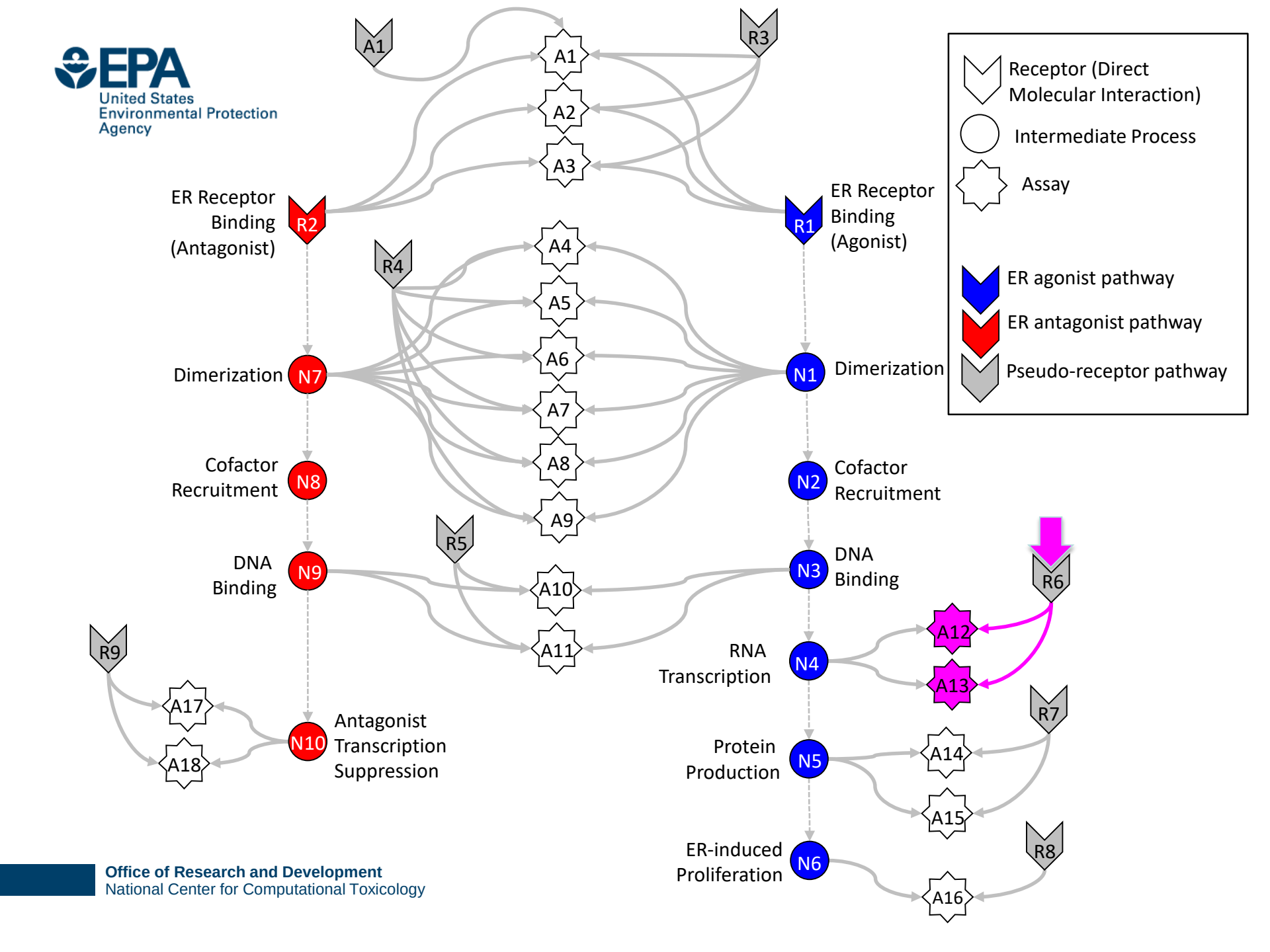
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Receptor (Direct Molecular Interaction)
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R1
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R4
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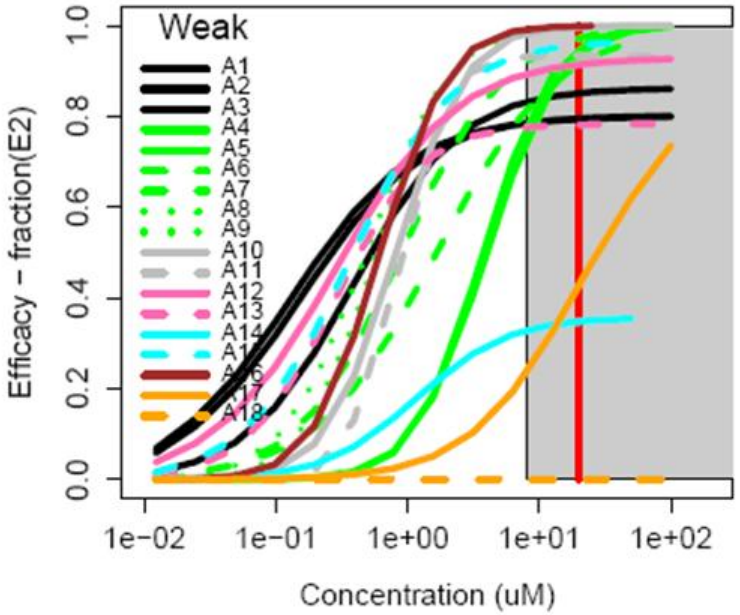
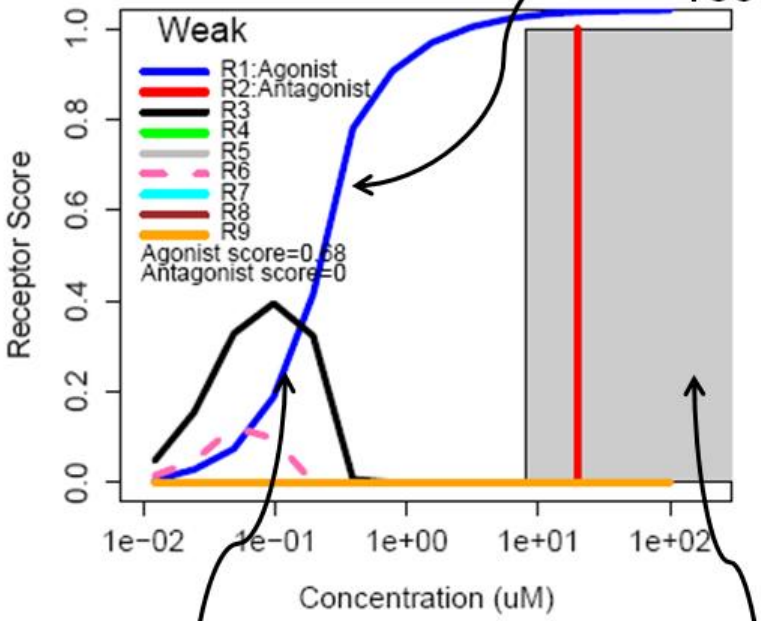
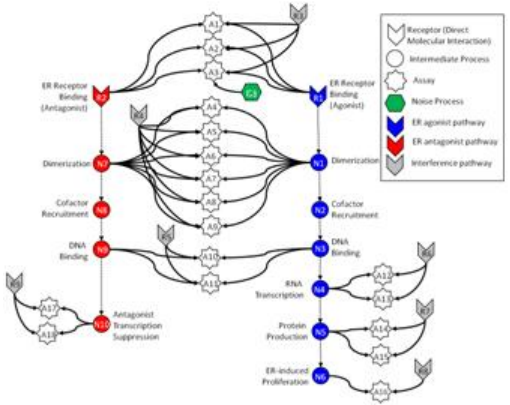
Example 1 – BPA: true agonist (AUC=0.66)





Blue: agonist "receptor"



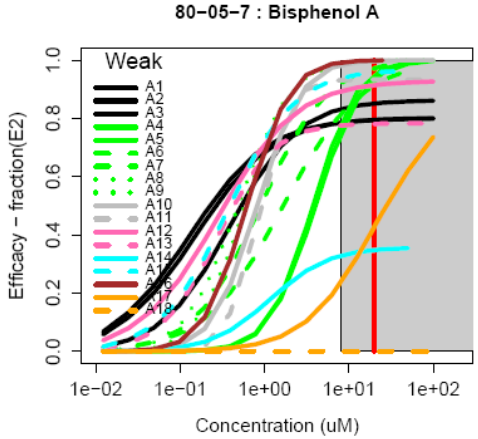
Binding assays active at lowest concentration

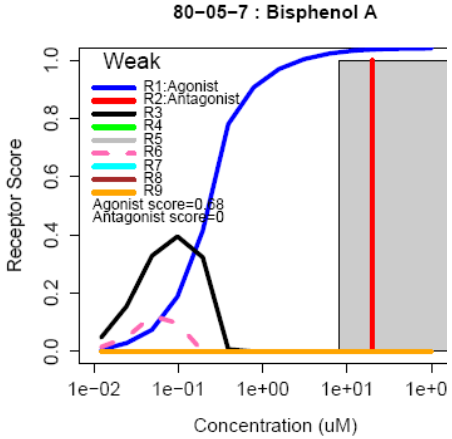
AUC "sign" feature will discount this

Cytotoxicity Region: red line is median cytotox AC50

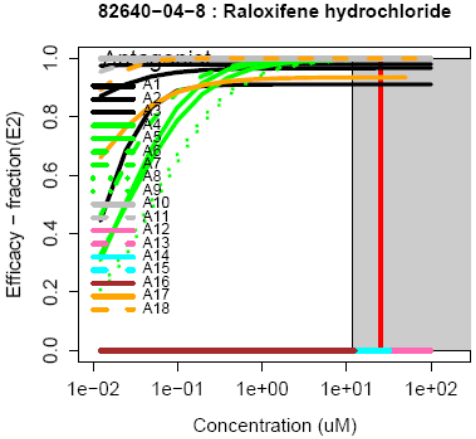
Example curves

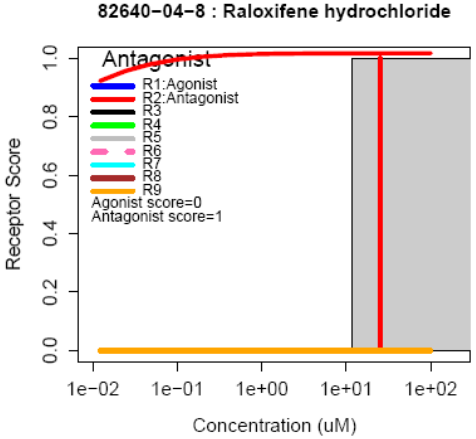
True Agonist



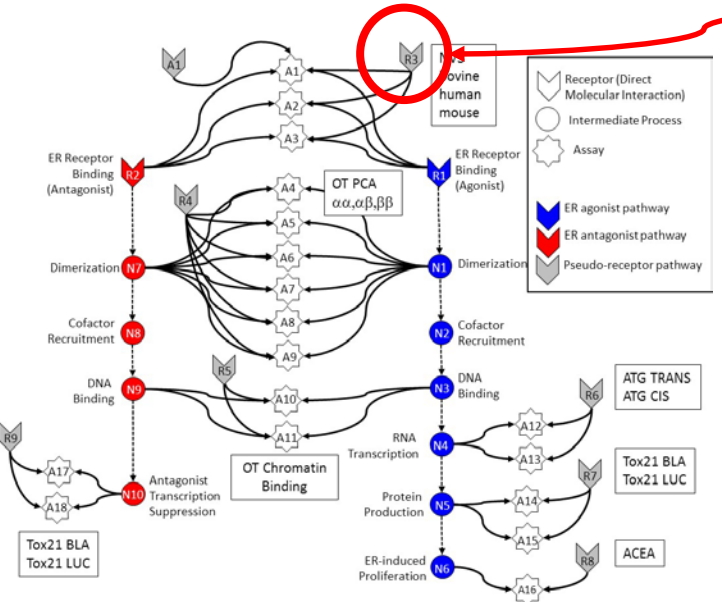


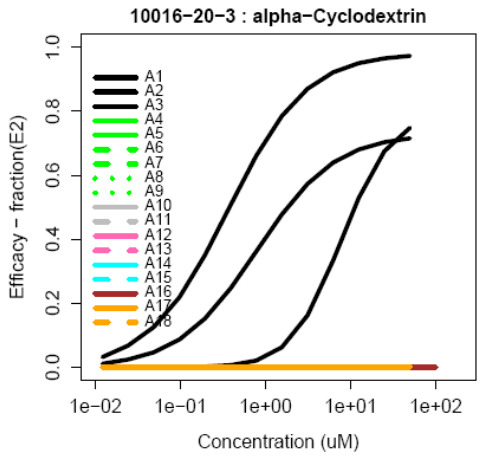
True Antagonist

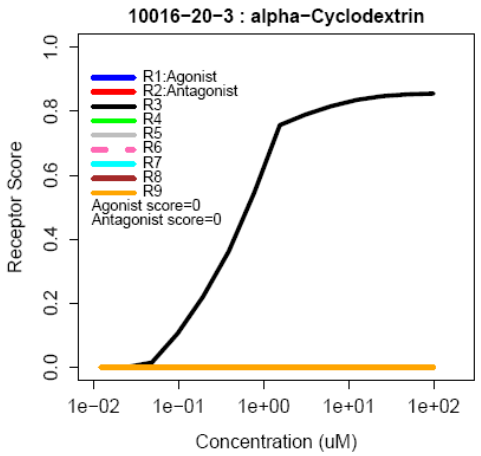




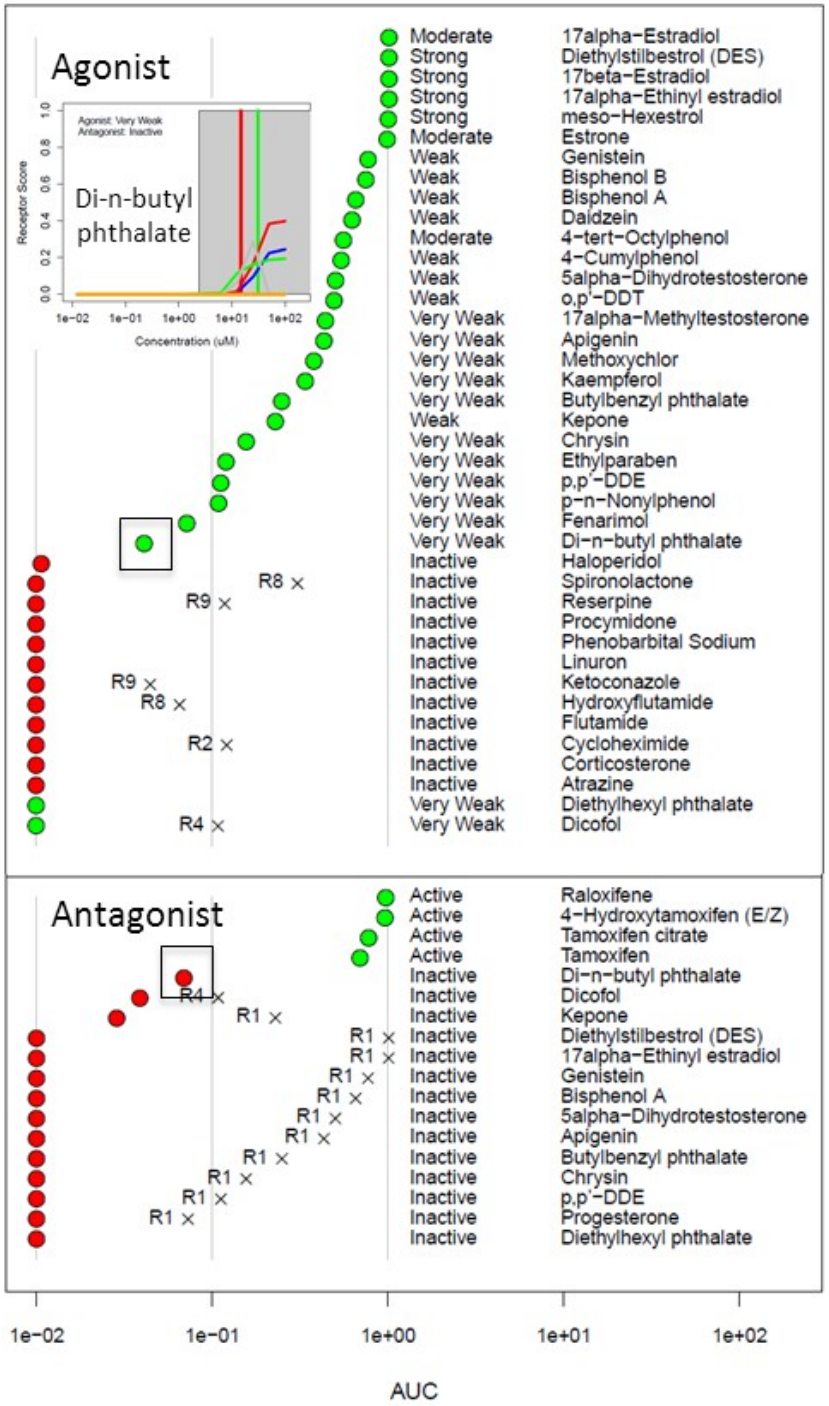
Assay Interference Example “R3”



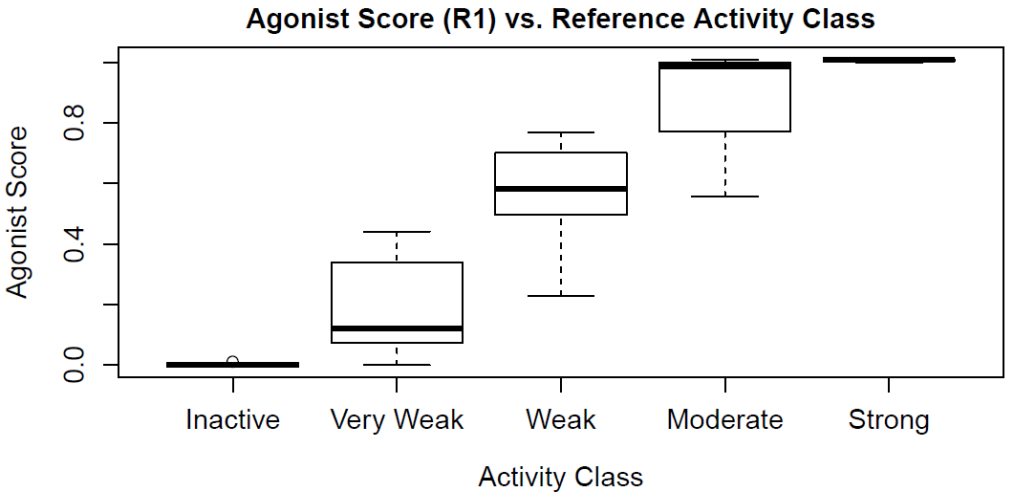




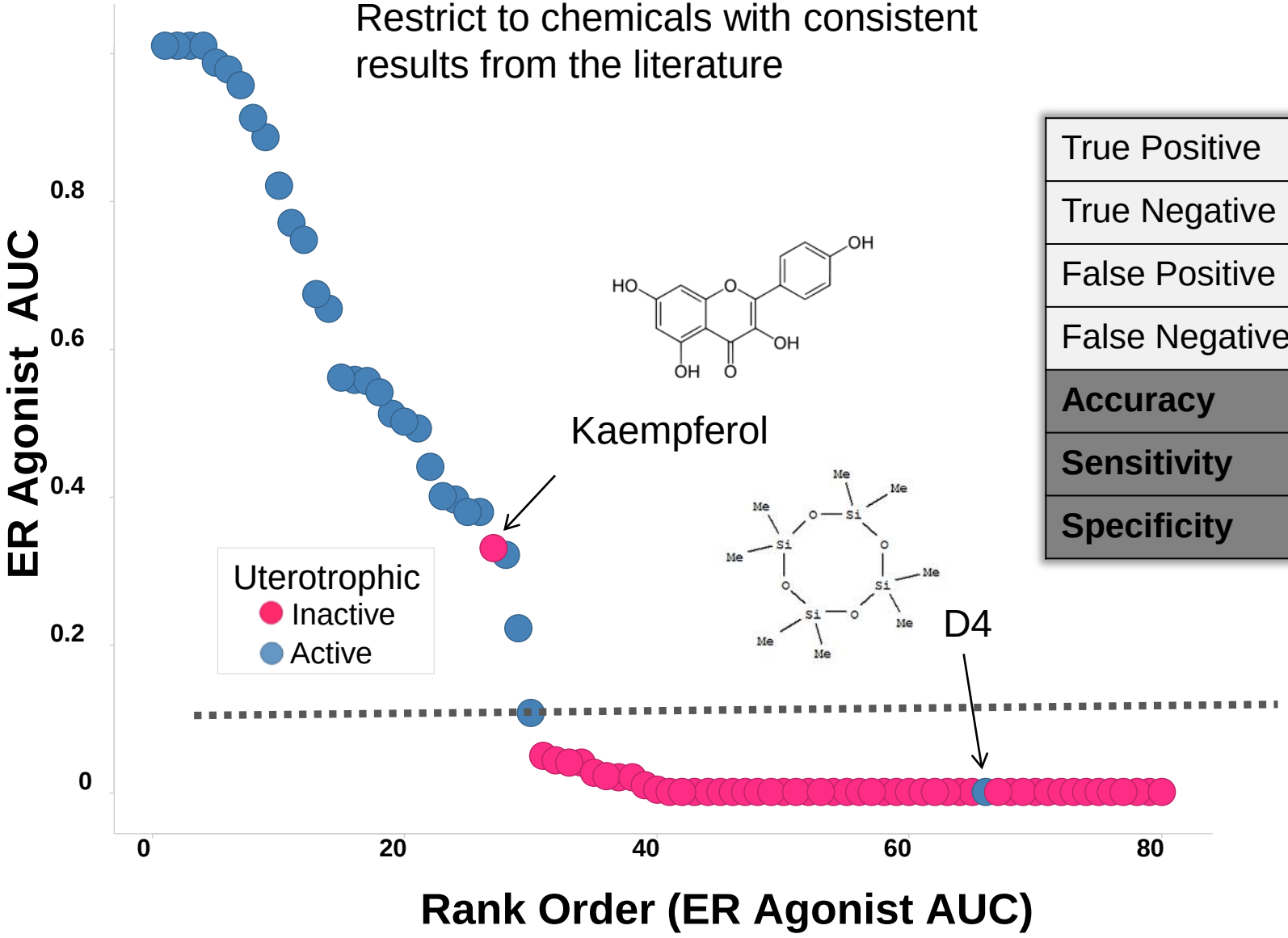
In Vitro Reference Chemical Performance



By using battery of assays and model of noise, we can accurately predict activity



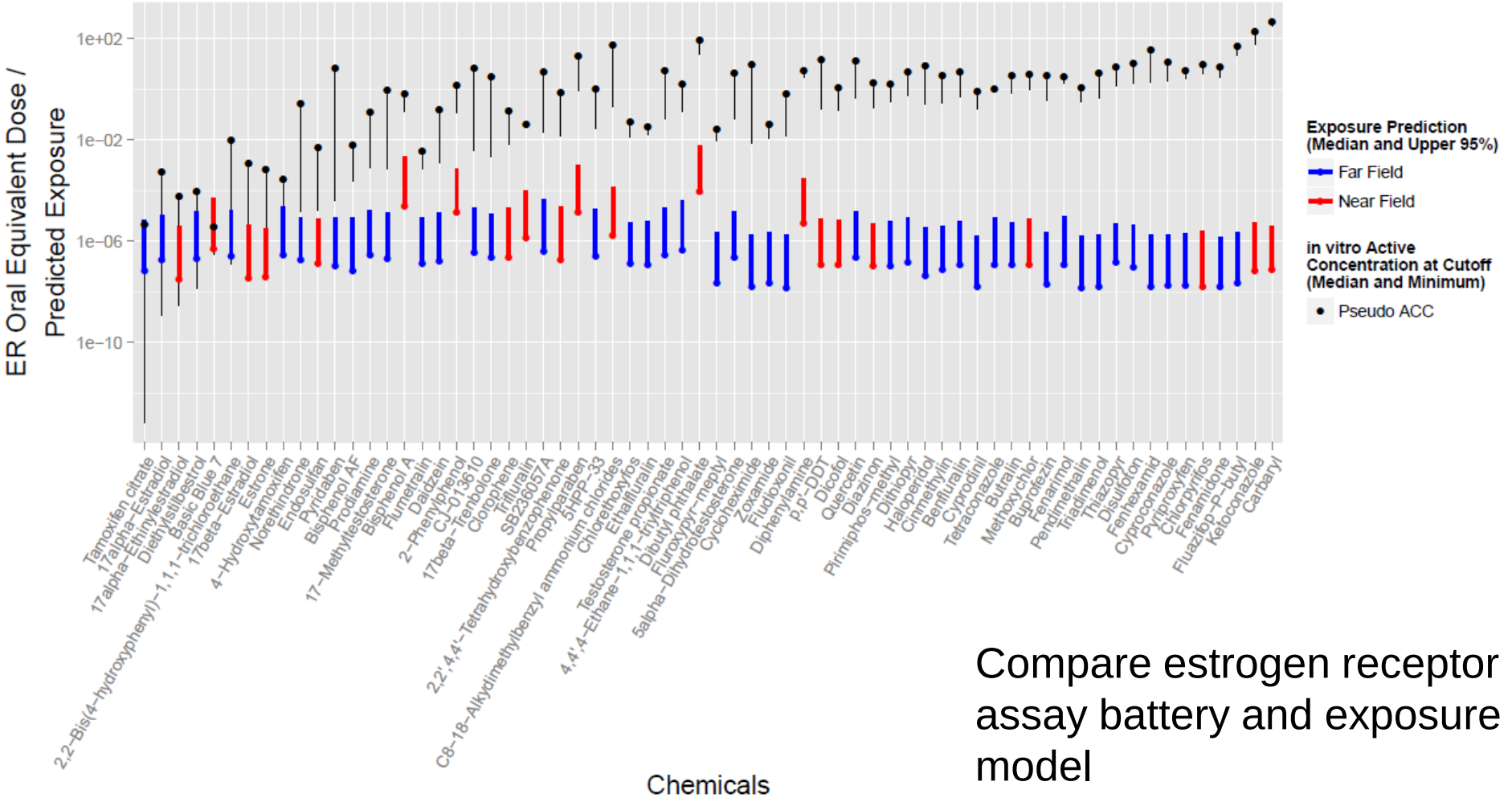
Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic



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

Browne et al. ES&T (2015)

Prioritization (Replacement) Example
Compare predicted exposure and hazard POD



Compare estrogen receptor assay battery and exposure model

Moving Towards Regulatory Acceptance From FIFRA SAP, December 2014

- Can the ER Model be used for prioritization?
  - "… the ER AUC appears to be an **appropriate tool for chemical prioritization** for … the EDSP universe compounds."

- Can the ER model substitute for the Tier 1 ER in vitro and uterotrophic assays?
  - "… **replacement of the Tier 1 *in vitro* ER endpoints …with the ER AUC model will likely be a more effective and sensitive measure for the occurrence of estrogenic activity** …"
  - "… the Panel **did not recommend that the uterotrophic assay be substituted** by the AUC model at this time. The Panel suggested that the EPA considers: 1) conducting limited uterotrophic and other Tier 1 in vivo assay testing, using the original Tier 1 Guidelines (and/or through literature curation)"

- Based on follow-up presented here (FR notice, June 18 2015) …
  - **"EPA concludes that ER Model data are sufficient to satisfy the Tier 1 ER binding, ERTA and uterotrophic assay requirements."**

Summary

- We are developing a minimal hazard battery
- In combination with in vitro TK it can provide
  - in vitro LOAEC/NOAEC
  - In vivo POD estimate
  - Information on pathways perturbed above POD
- Initial example is validated, based on:
  - Comparison with reference chemicals
  - Accounting for uncertainty in both in vitro and in vivo data
  - Uncertainty in both can be quantitative (POD value)
  - Uncertainty in both can be qualitative (active / inactive)