Biological profiling and dose-response modeling tools, characterizing uncertainty

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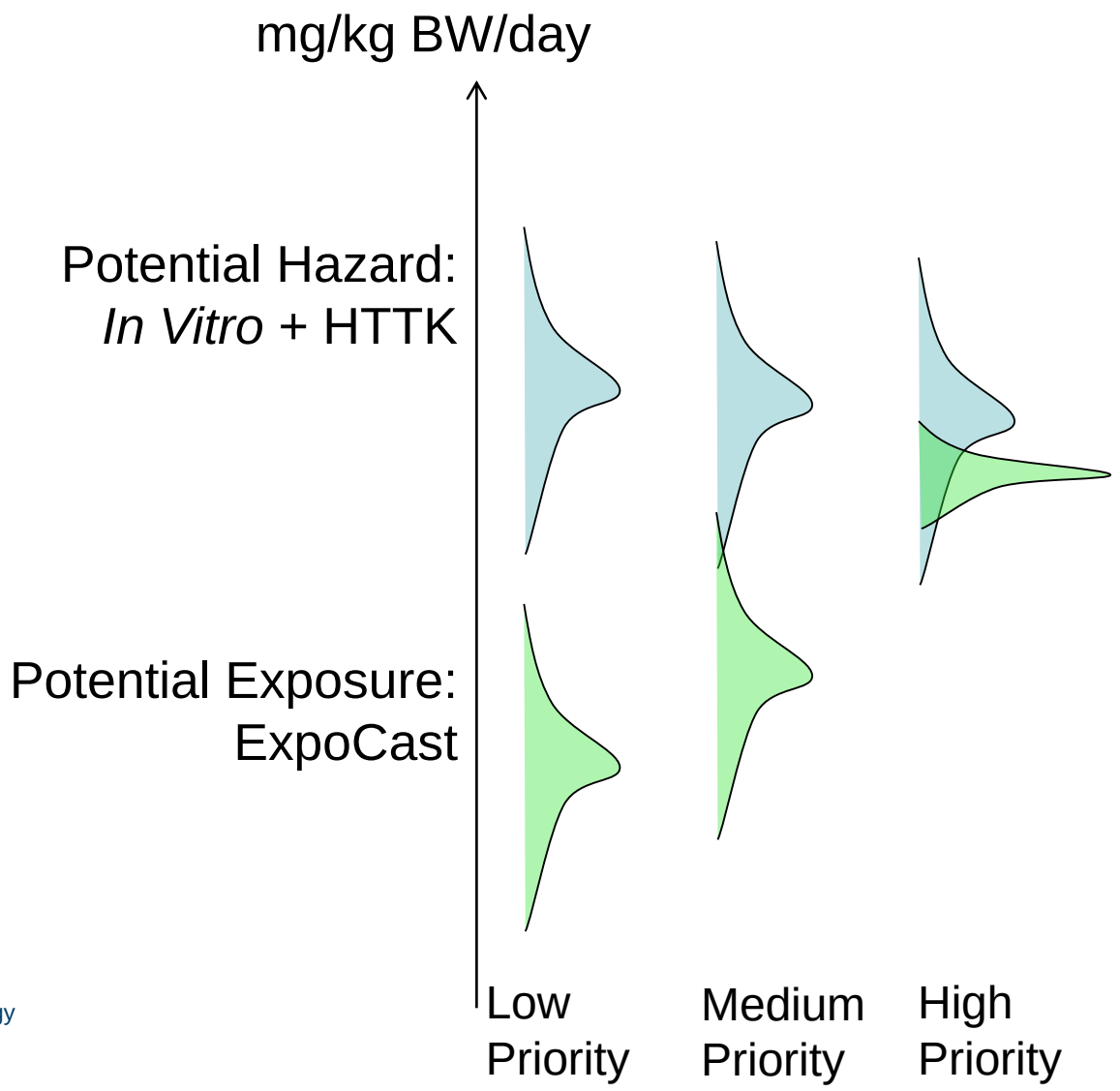
- *In Vitro* assays: Bioactivity Concentration
- Need Bioactivity Dose to compare with exposure
- Convert using High Throughput Toxicokinetics (HTTK)

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

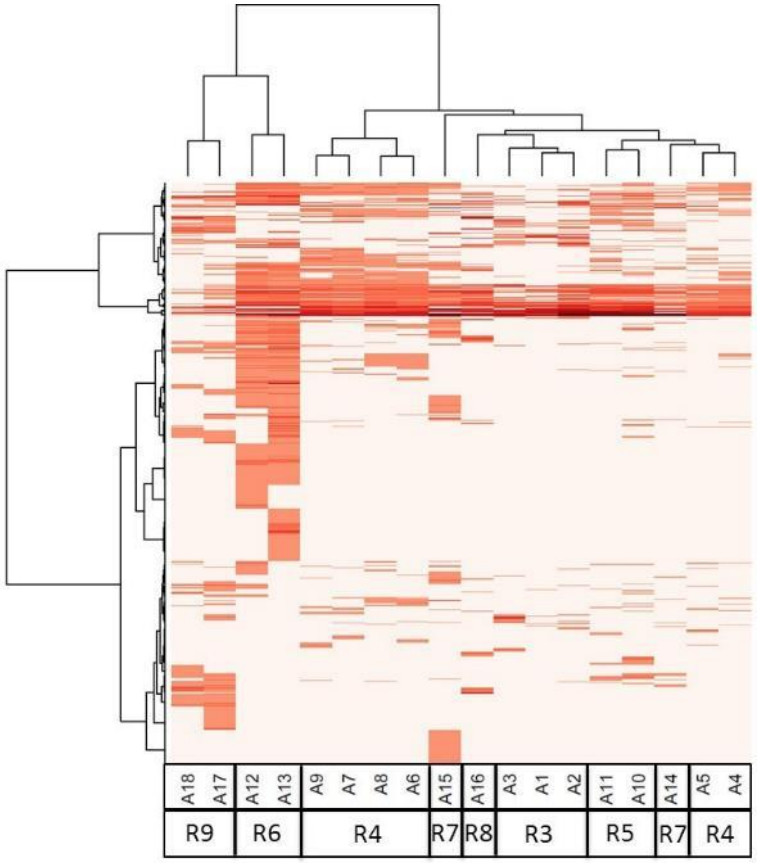


Uncertainty Issues

- How good is the data from a given assay / model?
  - Reference chemicals
  - Domain of applicability
- How certain are we that a chemical perturbs a pathway?
  - Active vs. inactive
  - False positives and false negatives
- If a chemical is active, what is its potency?
  - Quantitative uncertainty in parameter estimates

Major theme – all assays have false positives and negative

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

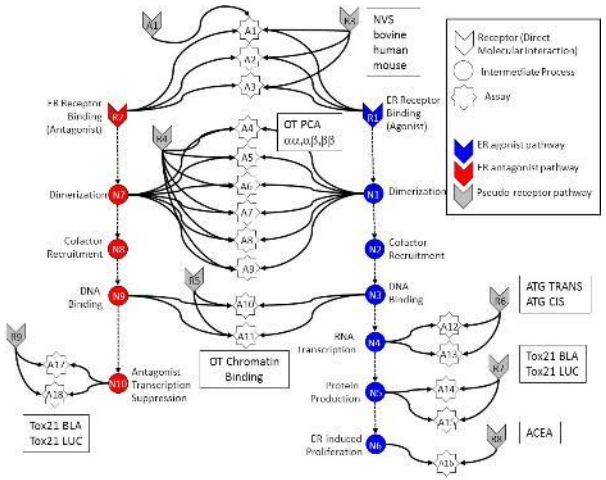


Much of this “noise” is reproducible

- “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



Focus on *in vitro* assay data

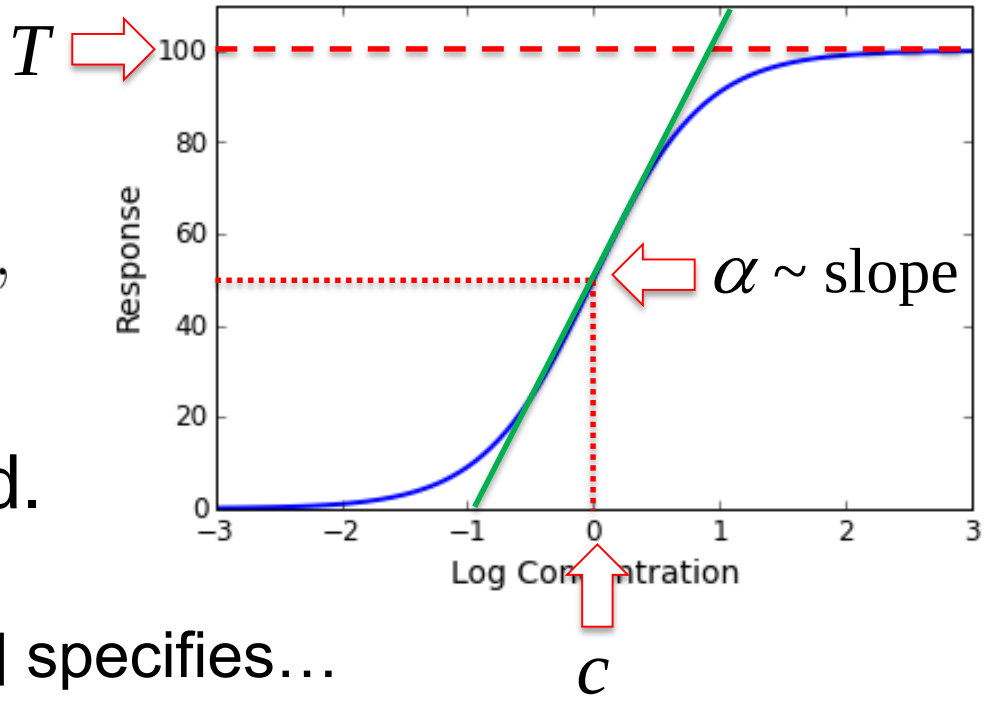
- Fit data to a model (e.g. a Hill curve)
- Is the chemical active or not?
- Estimate parameters
  - Potency plus others
- Estimate uncertainties

Hill Model Formulation

Response is given by

$$y = f(x; q) = \frac{T}{1 + 10^{\alpha(c-x)}},$$

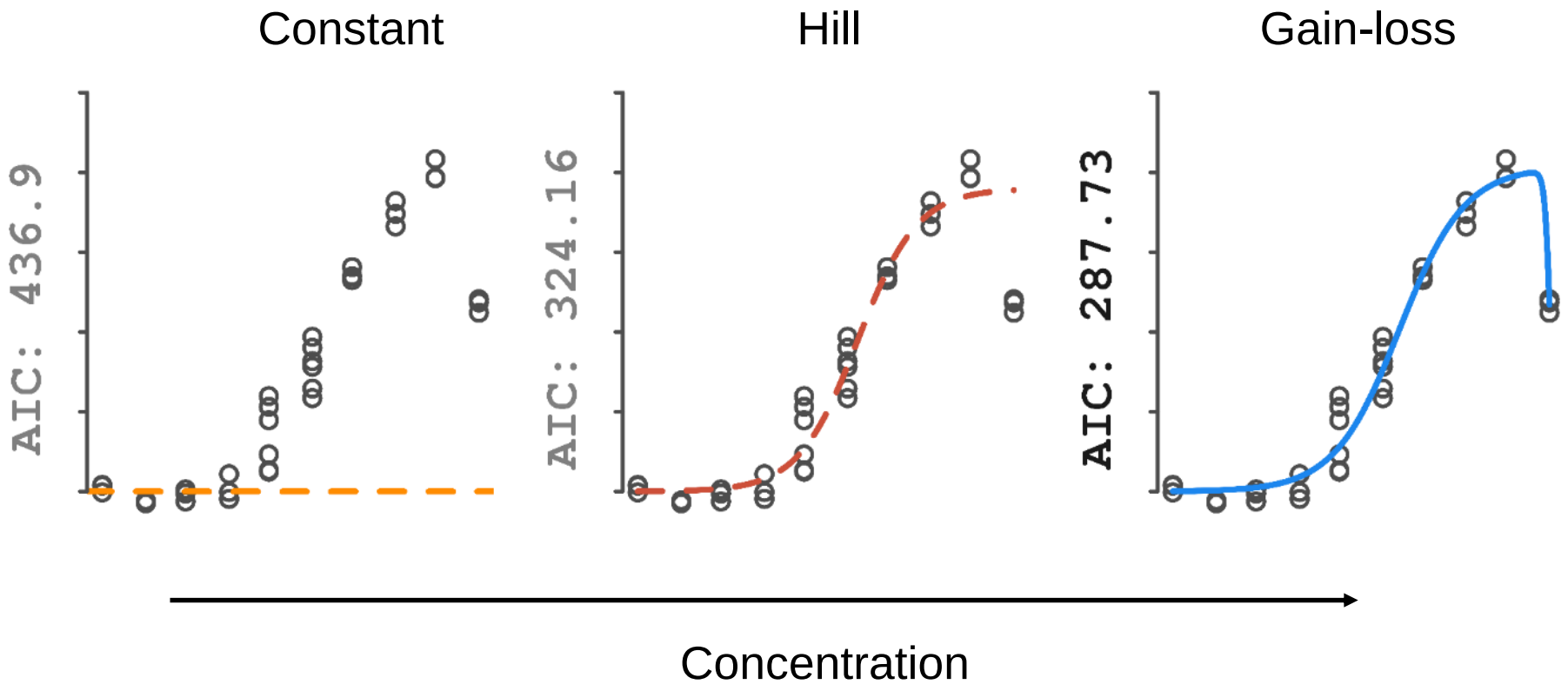
where x is the log of the concentration considered.



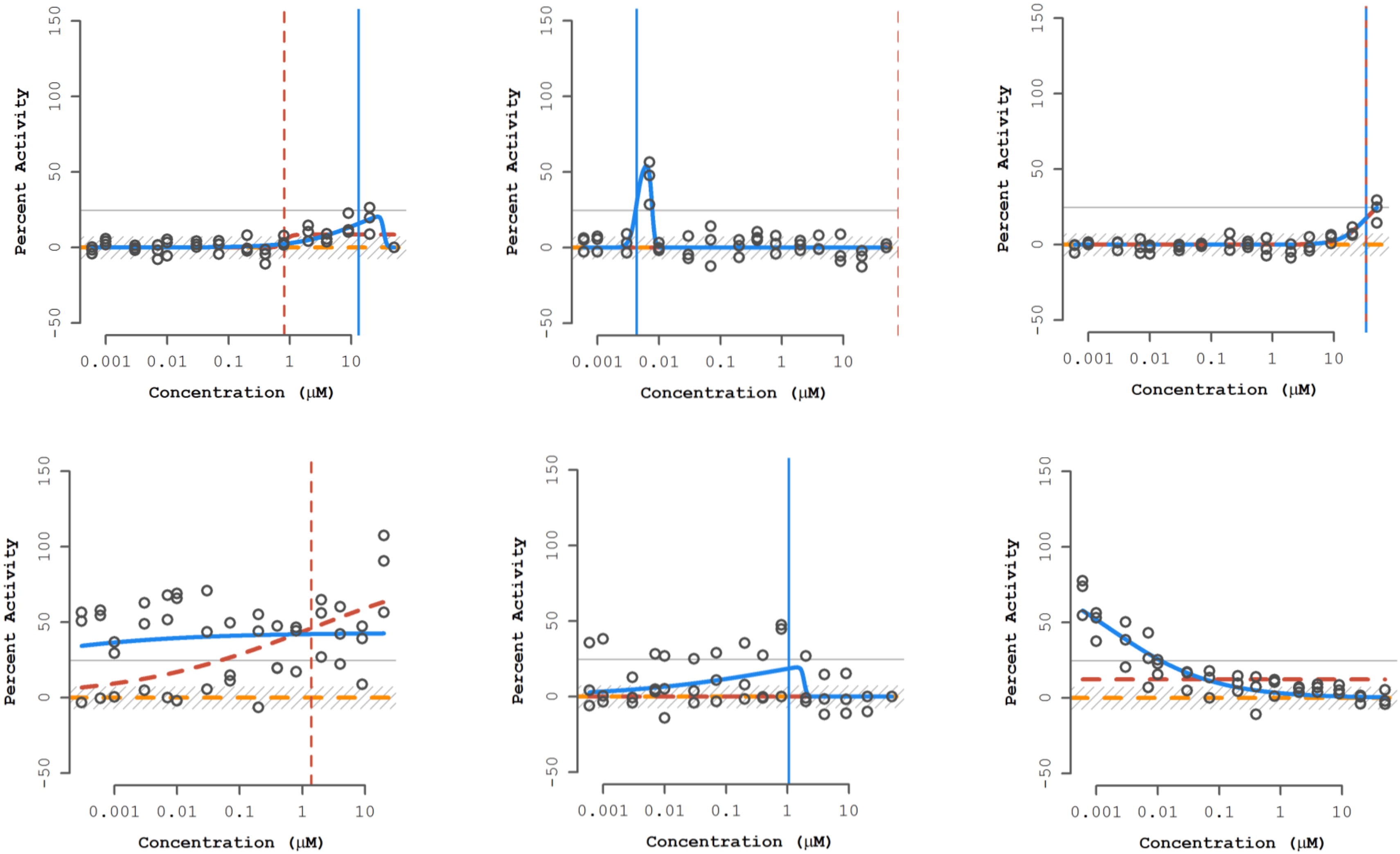
Parameter vector $q = [T, c, \alpha]$ specifies…

- maximal response (T)
- half-maximal activity concentration (c)
- Hill slope (α)

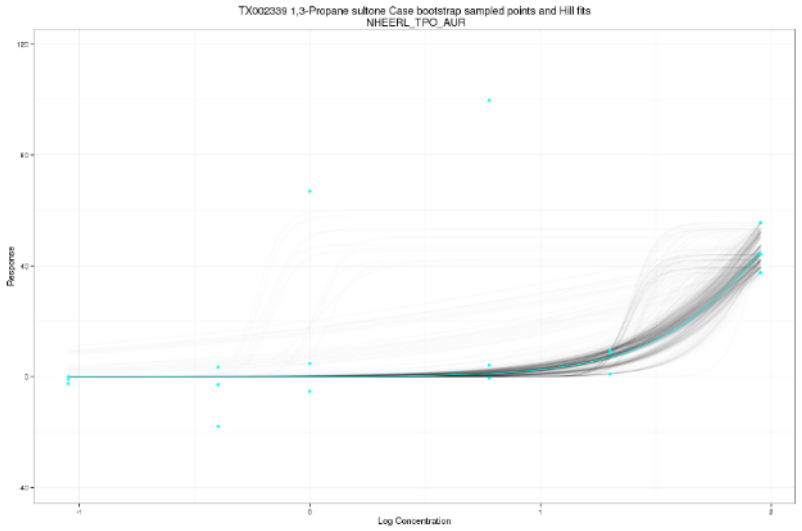
Select the winning model (lowest AIC):

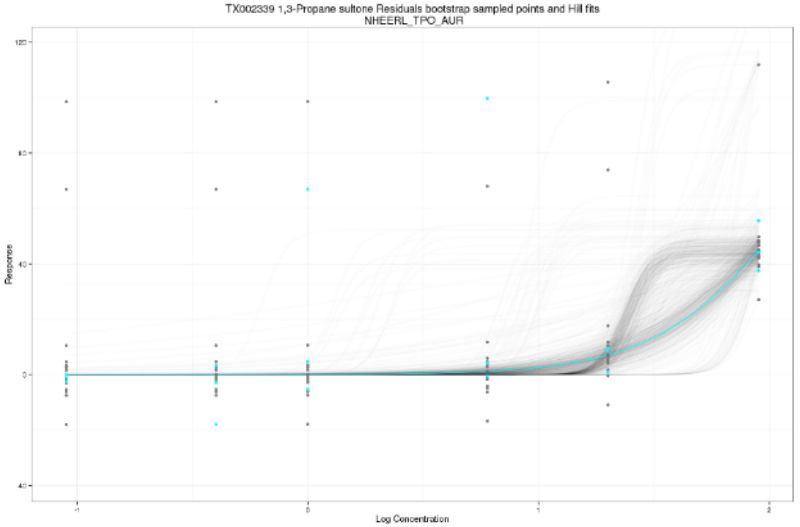


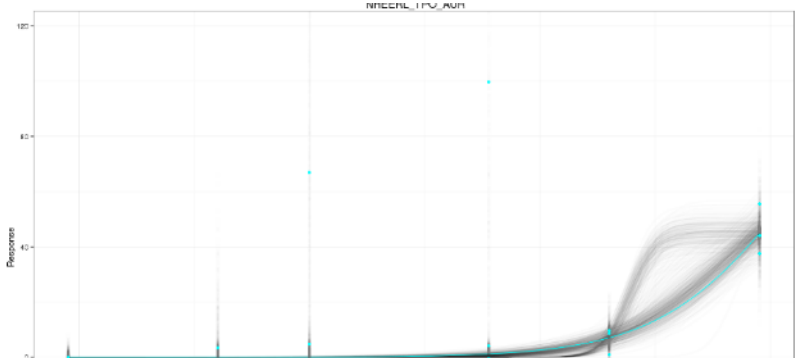
Assay pathologies – active or not?

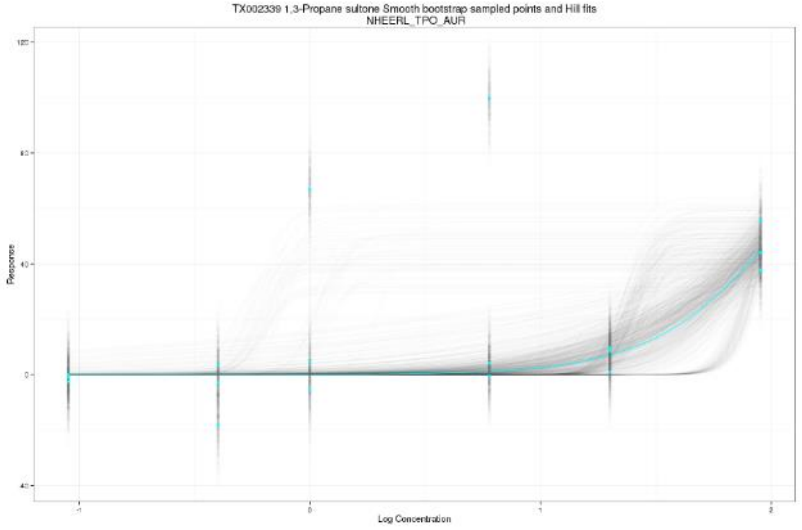


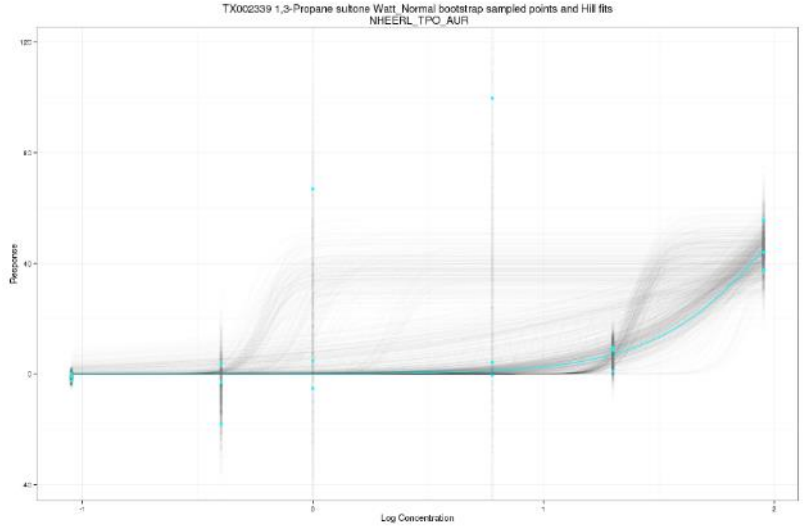
Method used for uncertainty estimate can change the answer



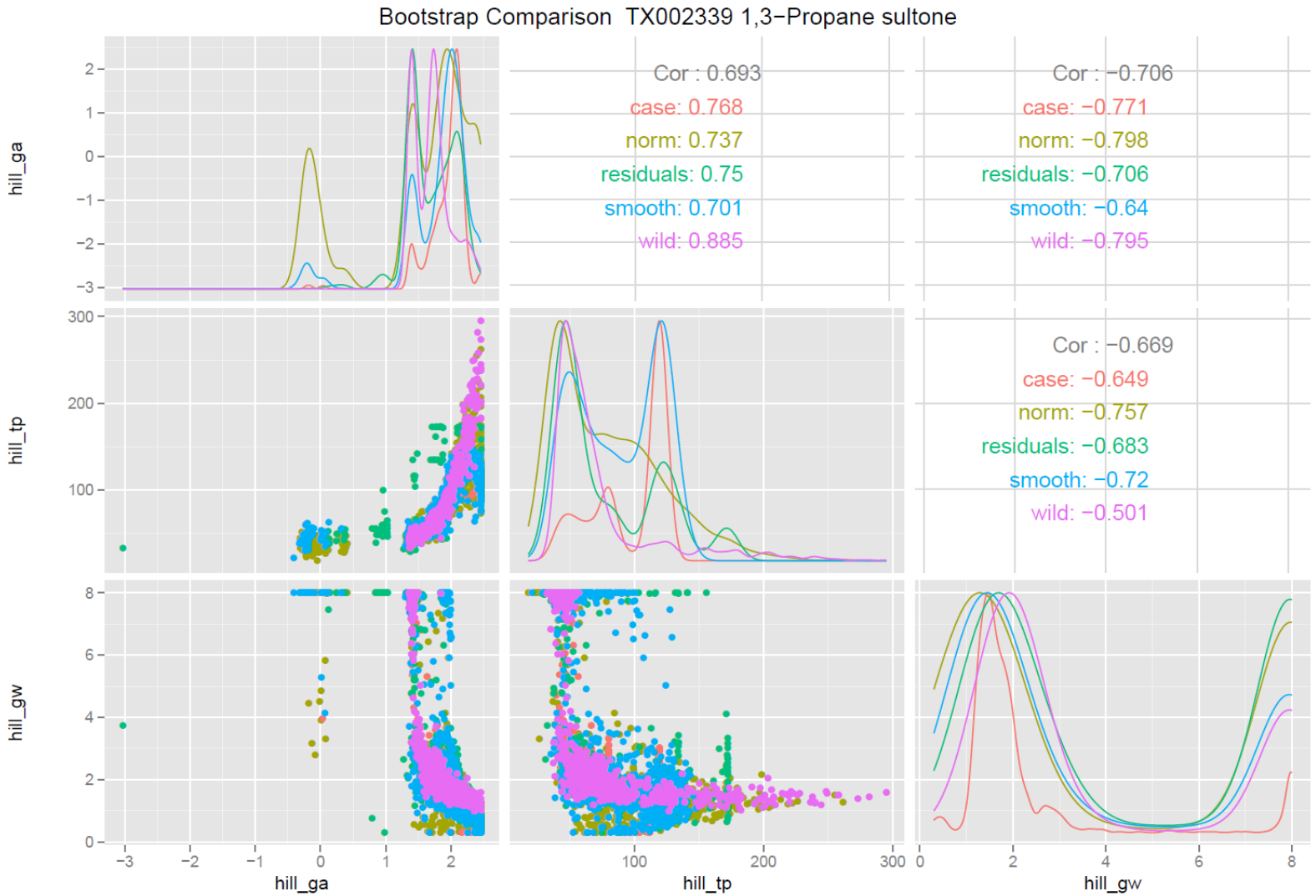




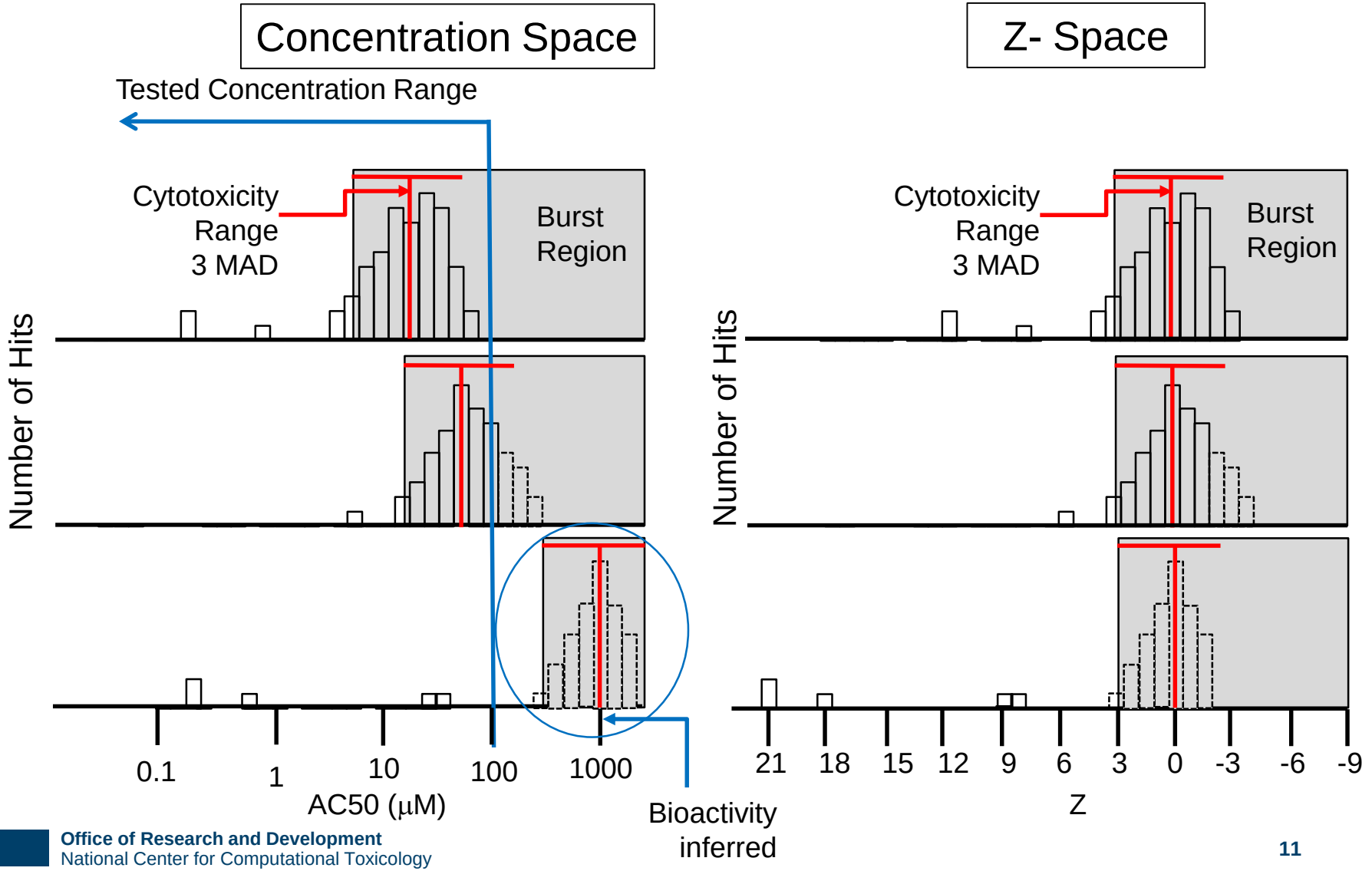




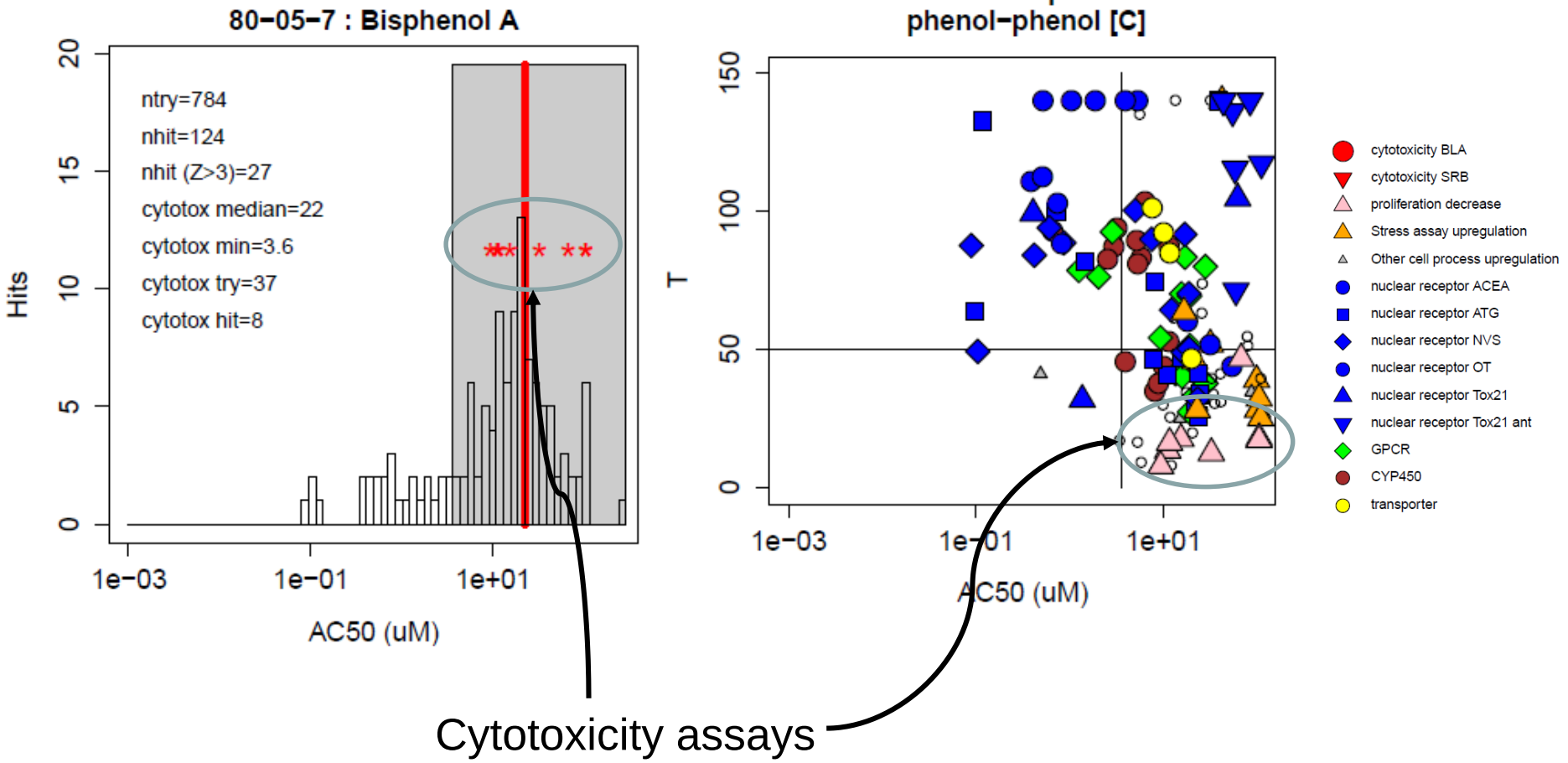
Bootstrap Resampling Comparison



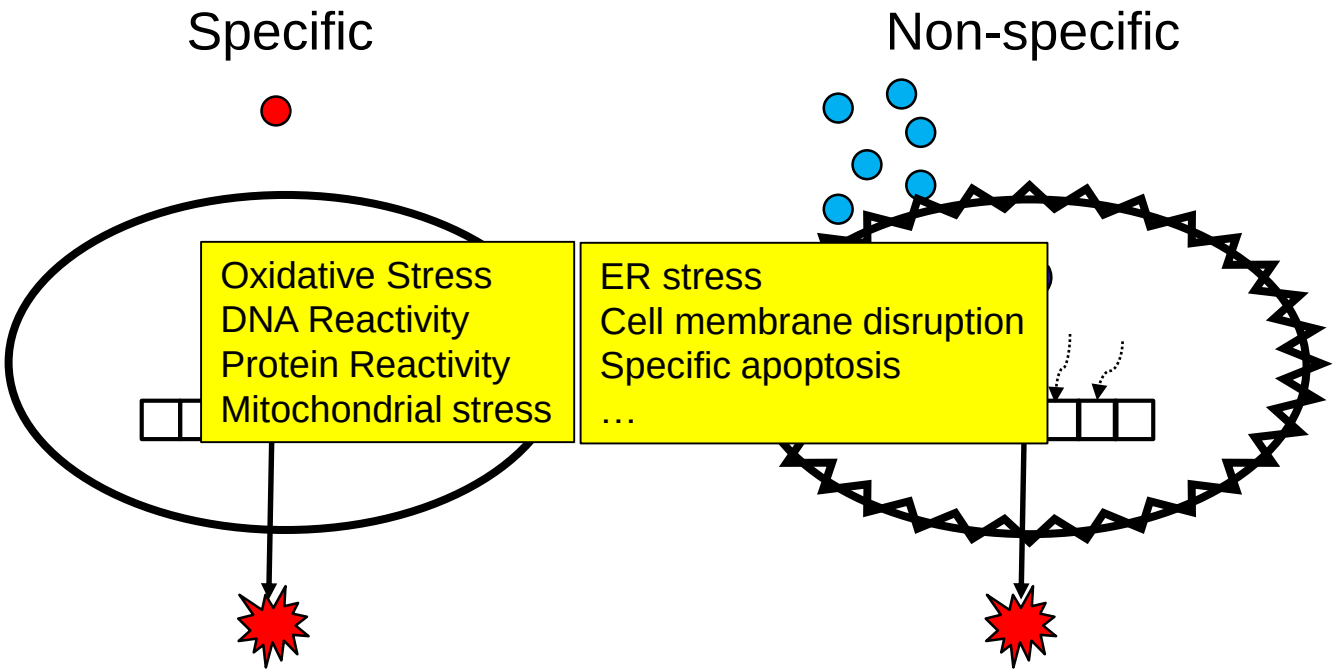
Most chemicals display a “burst” of potentially non-selective bioactivity: Caused by cell-stress / cytotoxity



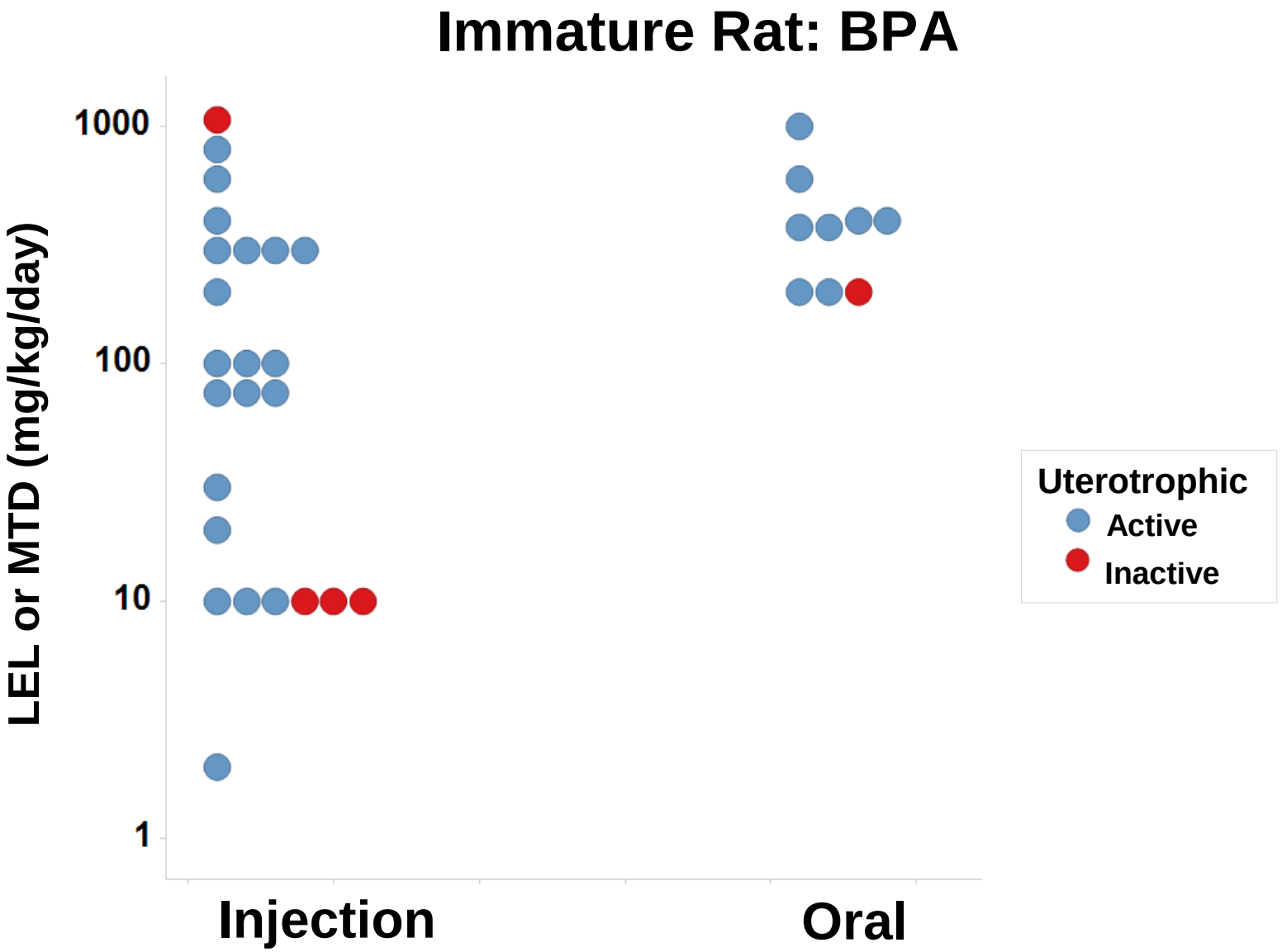
Example of burst bioactivity by chemical



Schematic explanation of the burst

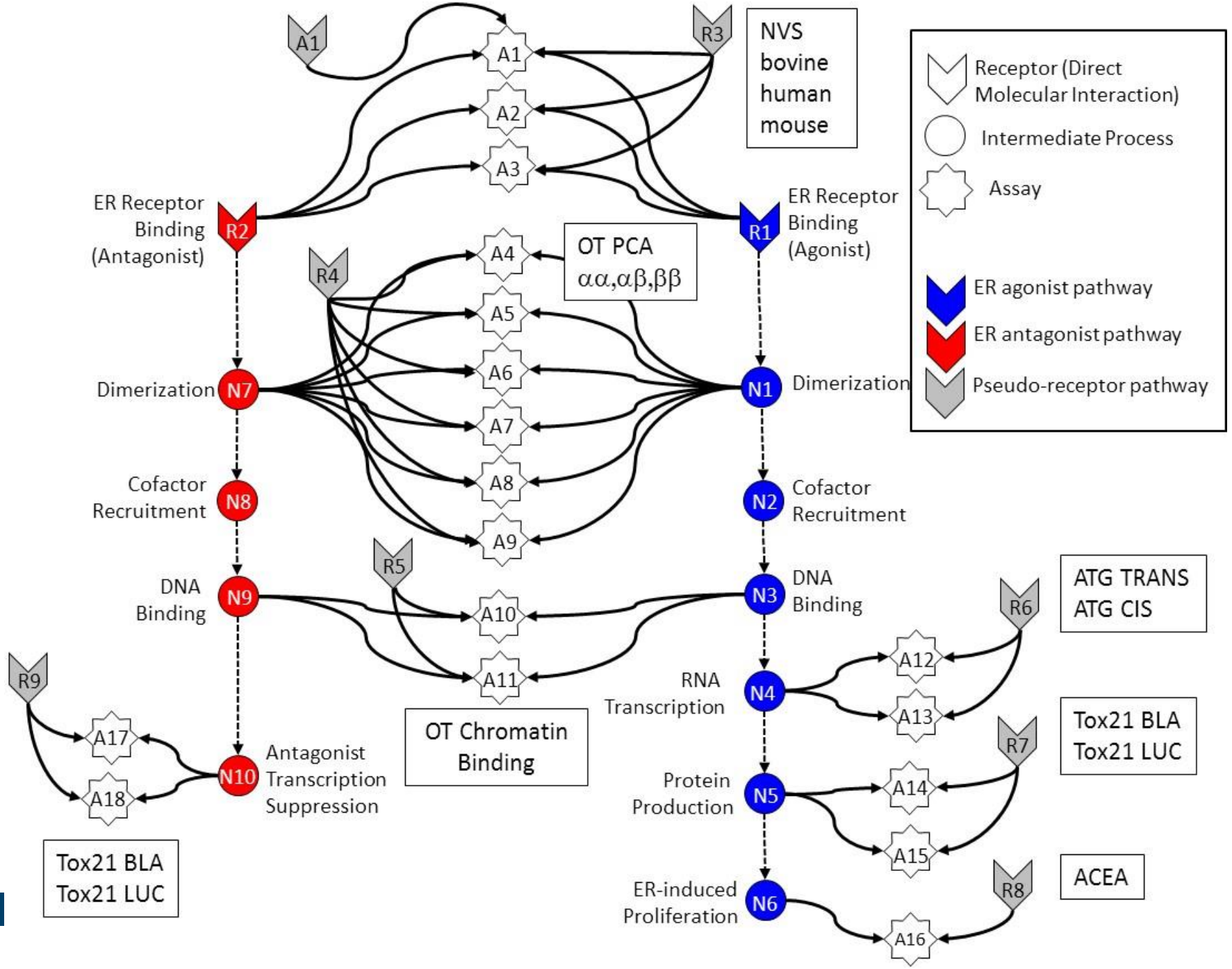


In vivo guideline studies have the same types of uncertainty



Weight-of-Evidence (WOE) Approach

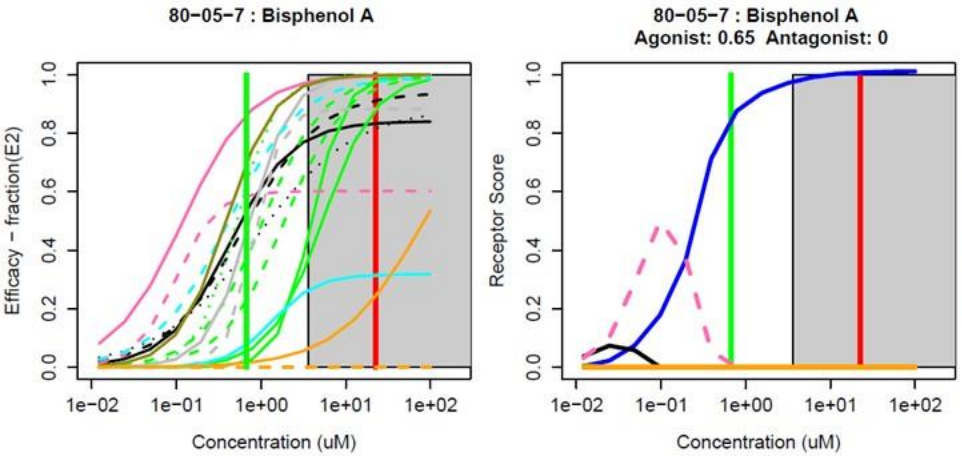
- All data is noisy
- All assays have false positives / negatives
- Using multiple assays can solve the positive / negative quandary
  - Qualitative uncertainty decreases
  - Quantitative (potency) uncertainty may increase


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

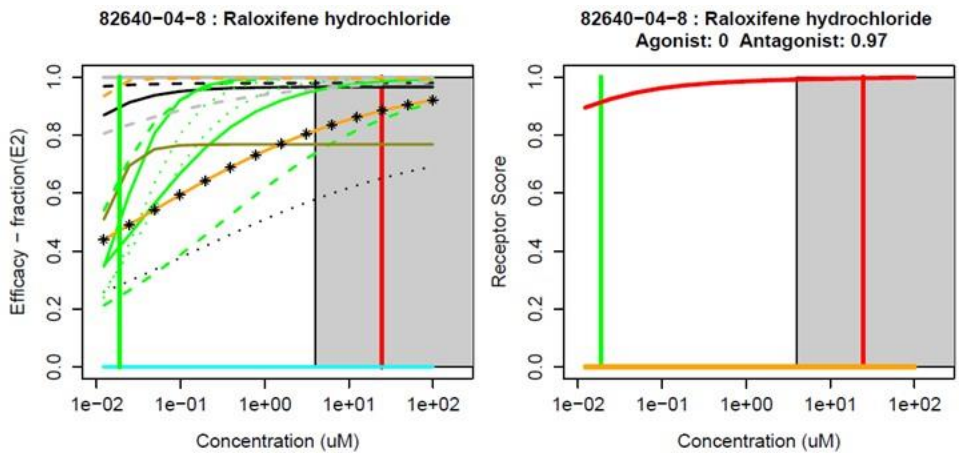
Example curves

United States Environmental Protection Agency

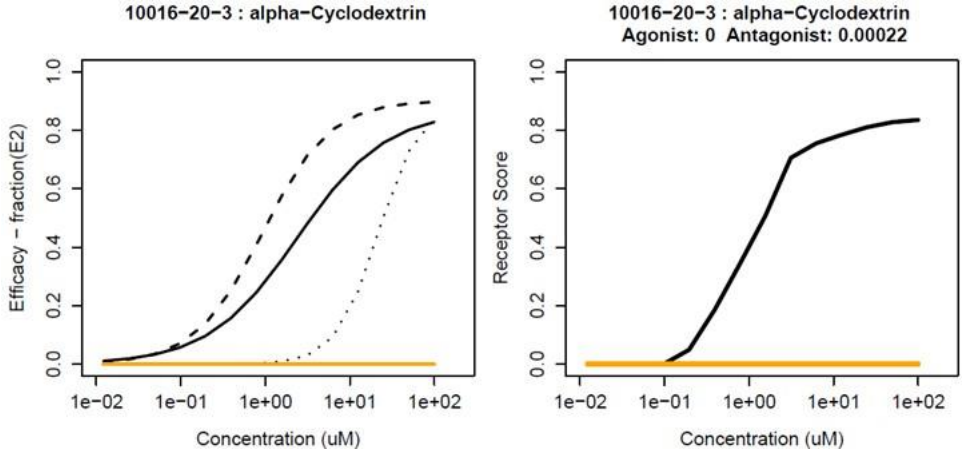
True Agonist



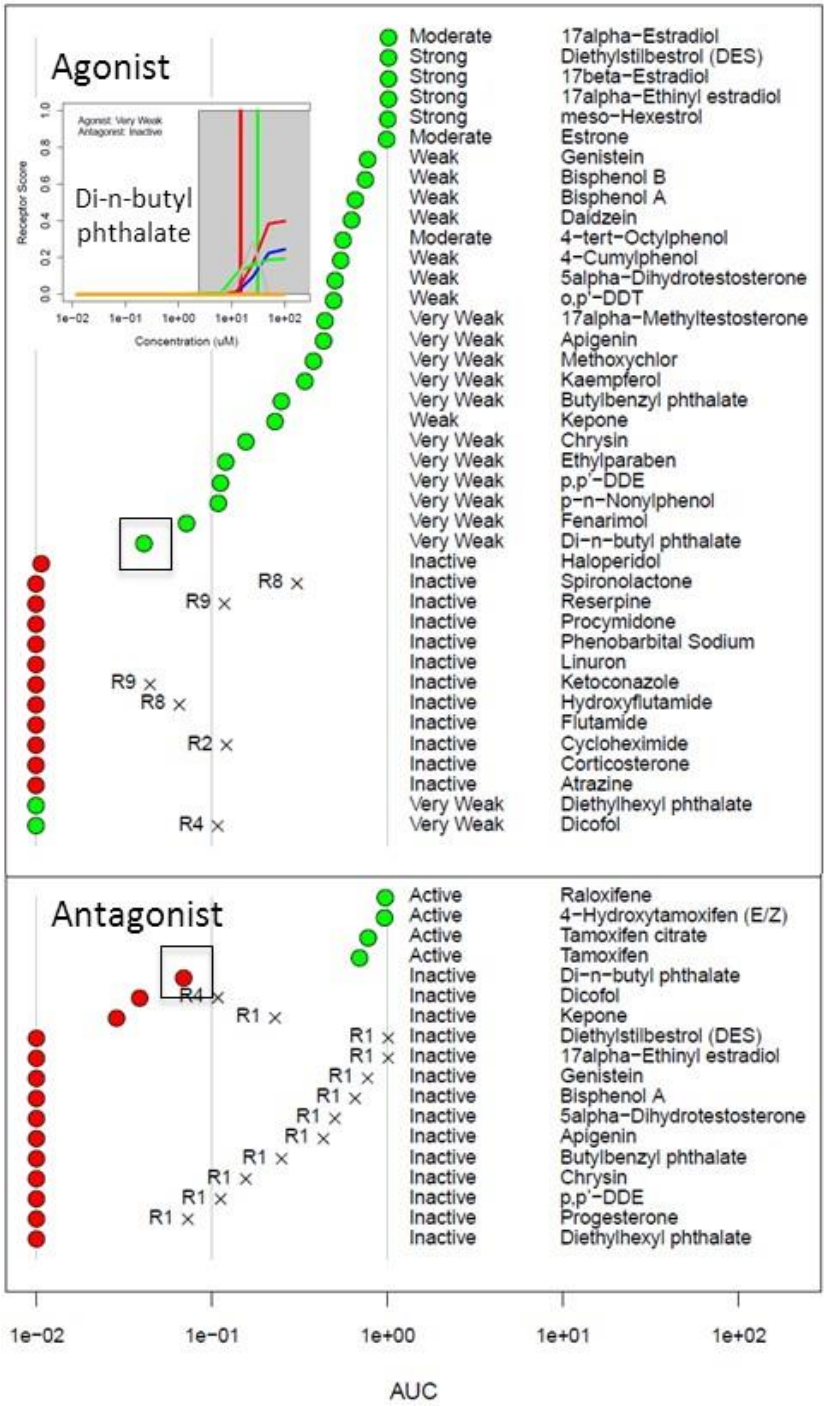
True Antagonist

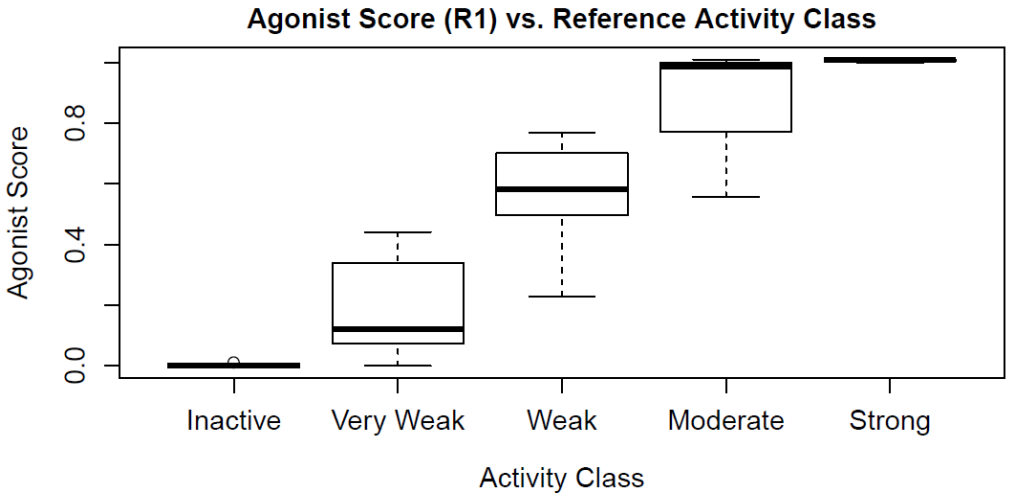


Negative-Narrow Assay Interference

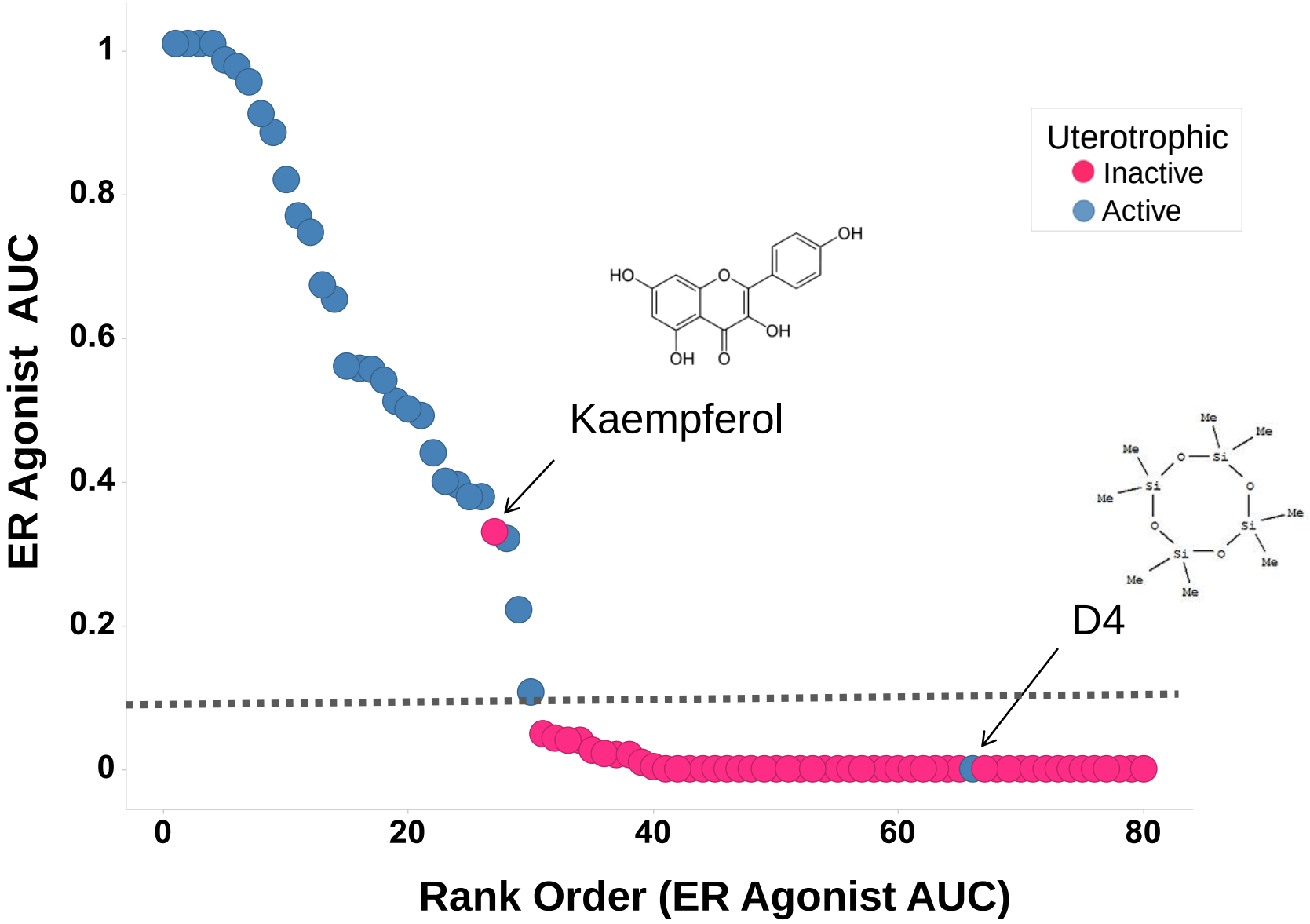


Reference Chemical Performance

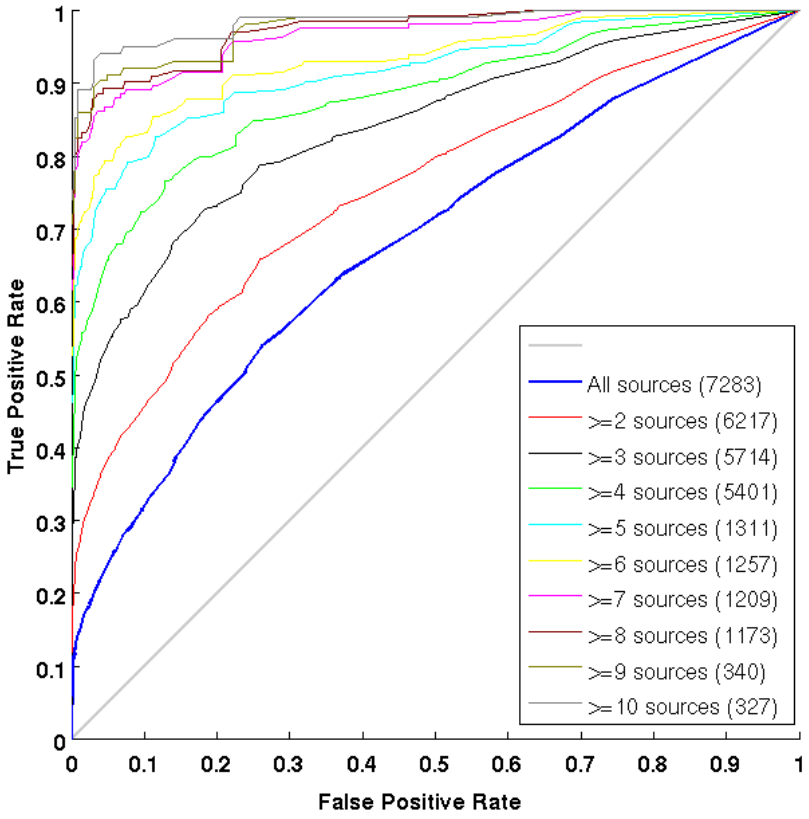




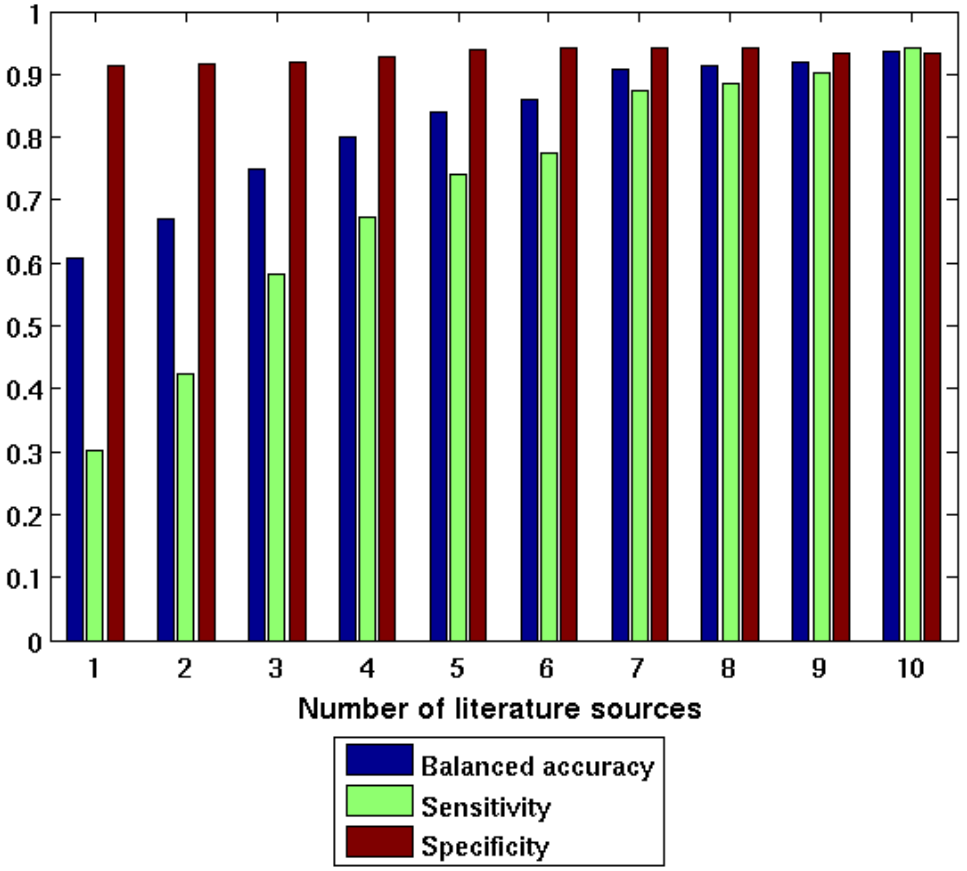
ER Agonist AUC vs Uterotrophic Outcomes



Consensus of models and data helps QSAR accuracy also



Key point: As greater consistency is required from literature sources, QSAR consensus model performance improves



Total Database

Binders: 3961

Agonists: 2494

Antagonists: 2793

Summary

- All assays are noisy (qualitative and quantitative)
  - New in vitro assays are no better or worse than current guideline methods
- Methods to estimate uncertainty exist
  - Topic of considerable development
  - No perfect method
- Using consensus of assays and models can be useful
  - Usually helps decide hit / no hit
  - Uncovers larger issues with quantitative uncertainty

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