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ToxCast experience with (cosmetic relevant) chemicals

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Cosmetics Europe Virtual Workshop October 21-22, 2020

Office of Research and Development
Center for Computational Toxicology and Exposure

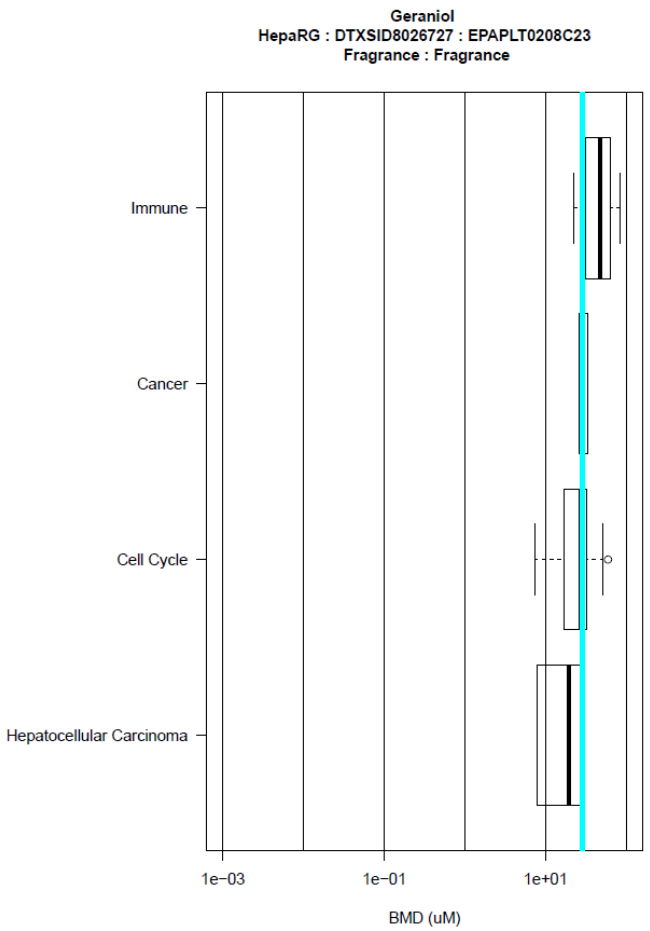
Issue to Address

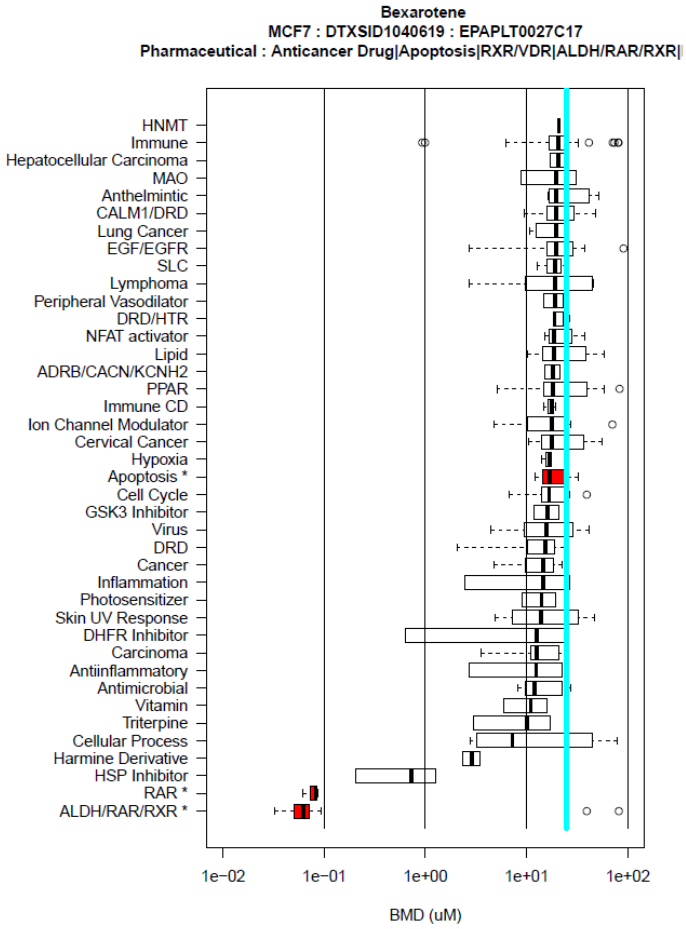
- ToxCast experience with:
  - General activity across different use categories
  - Specific versus generalised activities
  - Cosmetic relevant chemicals

For today "ToxCast" means

- ToxCast HTS in vitro assays (~1000 assays)
  - Many specific targets: NR, GPCR, Enzymes, …
  - Phenotypic assays
  - Cell-base, cell free
- High-throughput transcriptomics (HTTr)
  - Whole genome, 3 cell types
- Zebrafish assays
  - Embryo / Developmental, behavior
- In vitro toxicokinetics
  - Allows IVIVE
- QSAR models
- Other kinds of models

Fragrance vs. Pharmaceutical





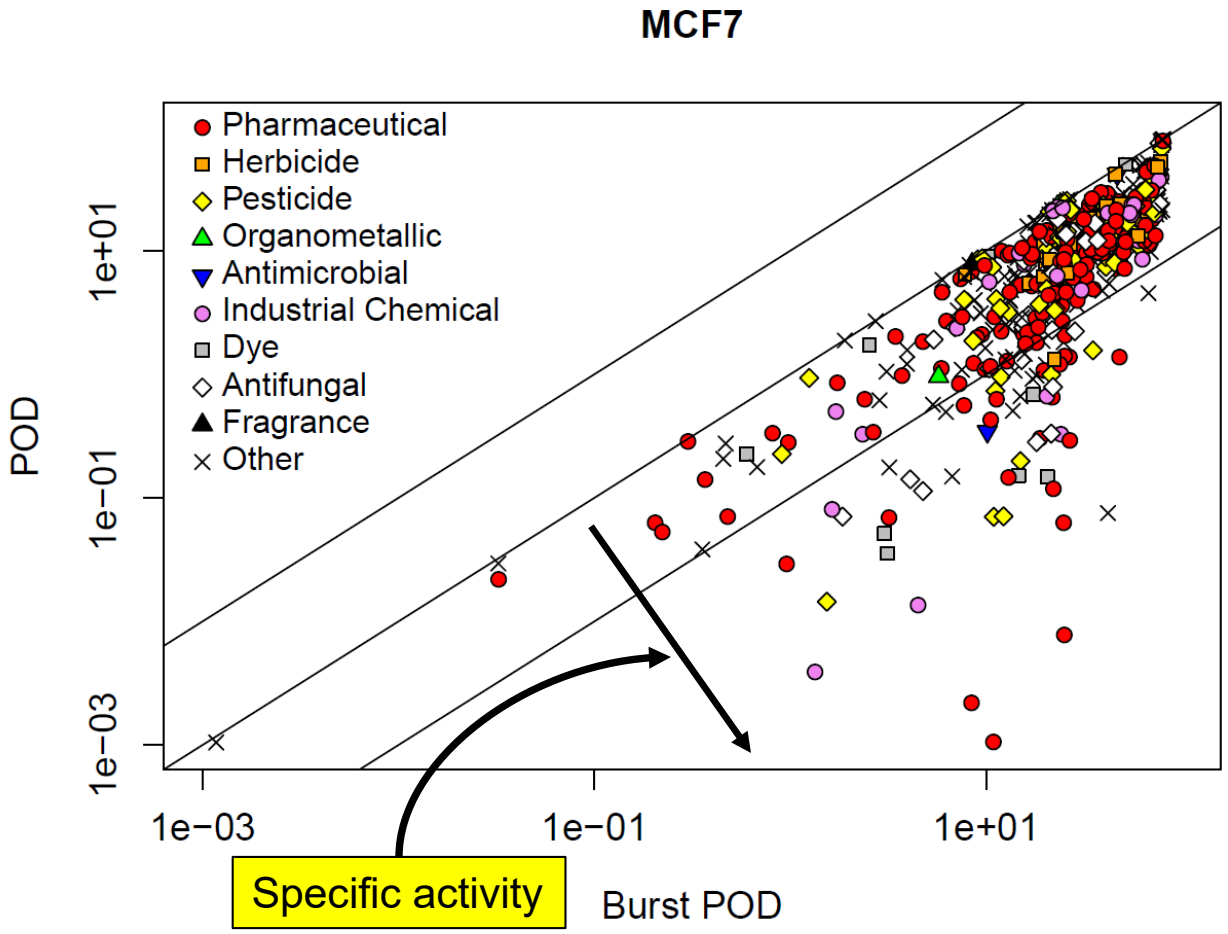
Data is from HTTr
Rows are target pathways (>1 pathway per target class)

Red indicates intended target of the chemical

Only high-conc activity
No specific activity
Few pathways active

Specific (target) pathways active at low conc
Many pathways active at high conc

General Chemical Class Trends

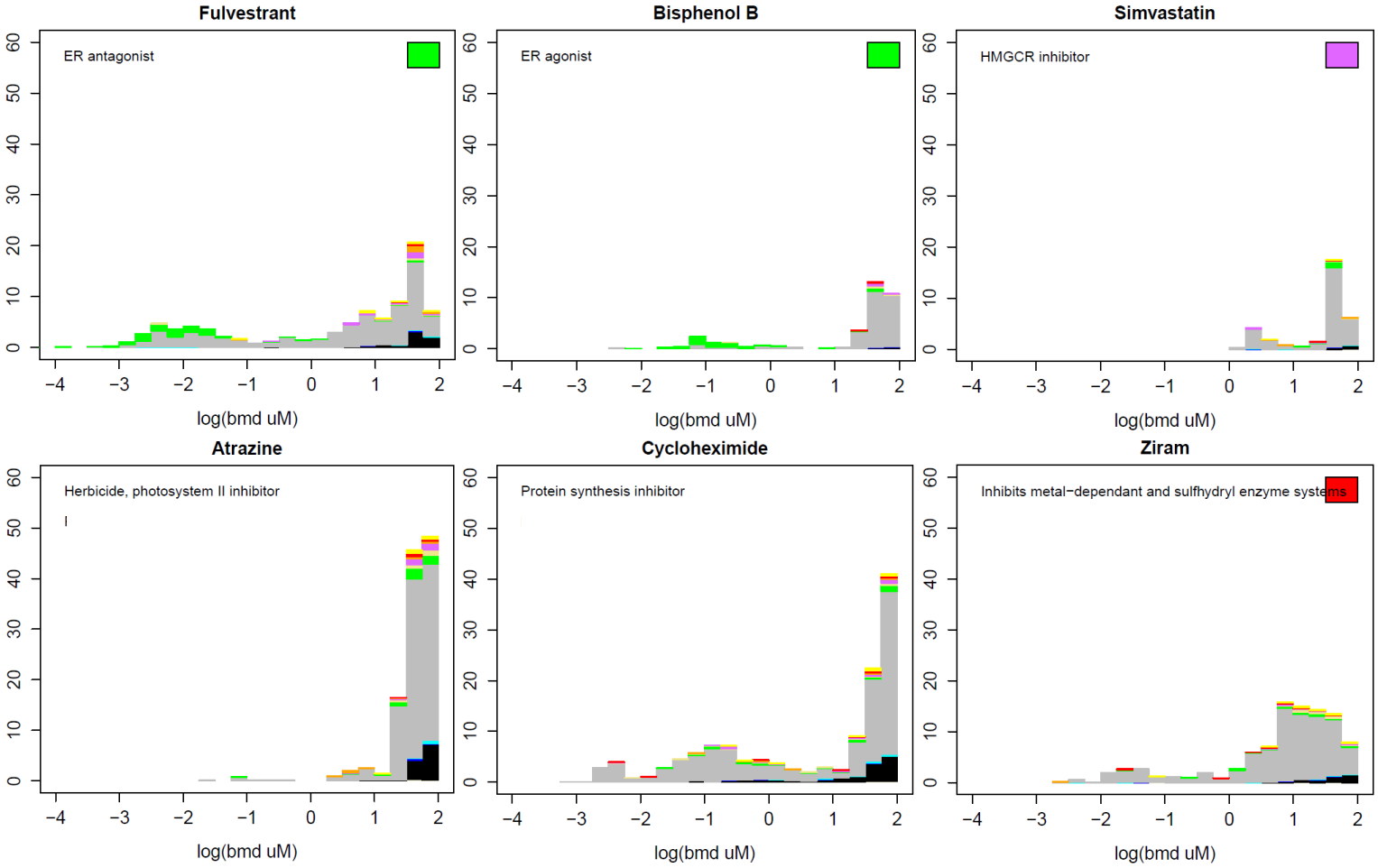


Data from HTTr

Point is the overall chemical POD

Burst POD is the concentration where many pathways are activated (non-specific threshold)

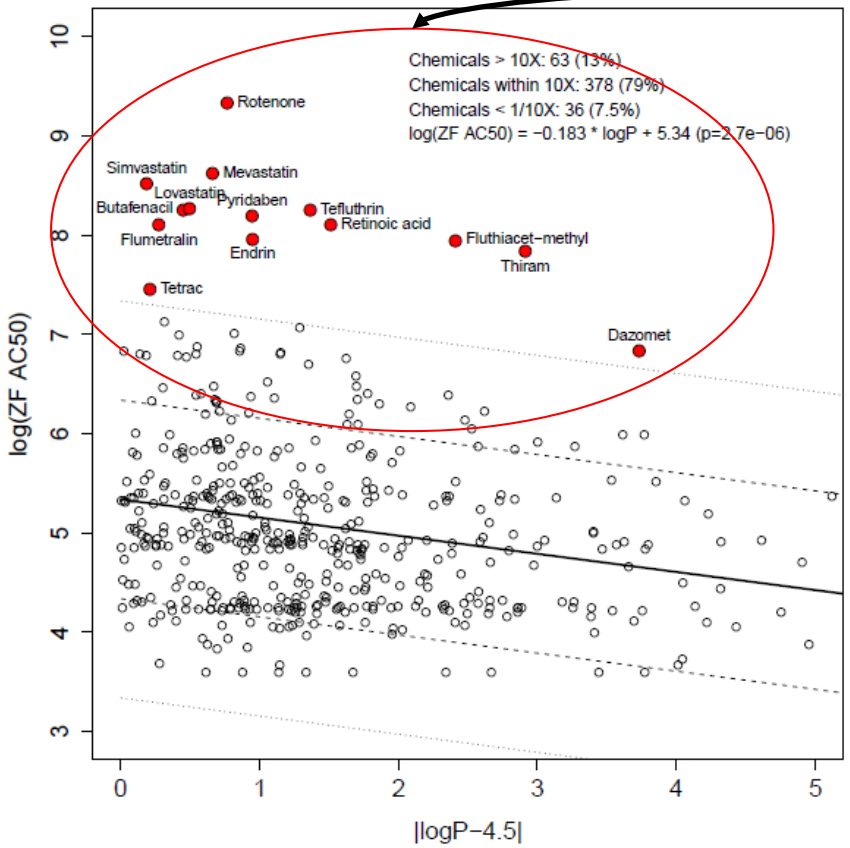
Chemical Level Signature Summary Plots

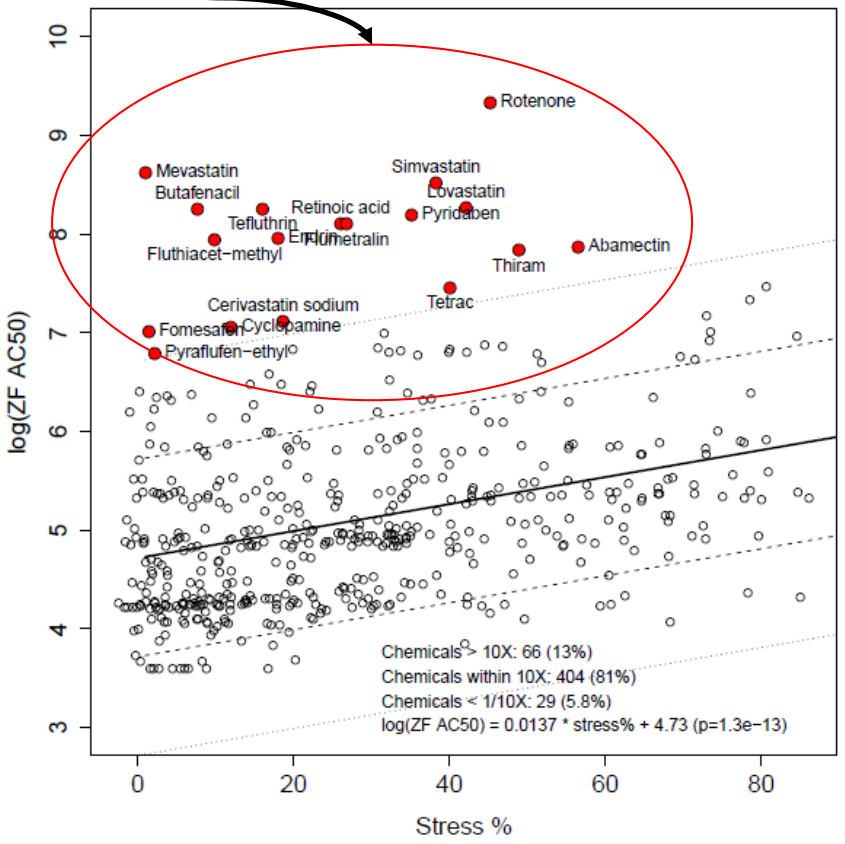


Green=ER
Black=random
Red=cell stress

Zebrafish Data: Subset of chemicals are more potent than expected from stress or logP

Chemical showing “excess” toxicity

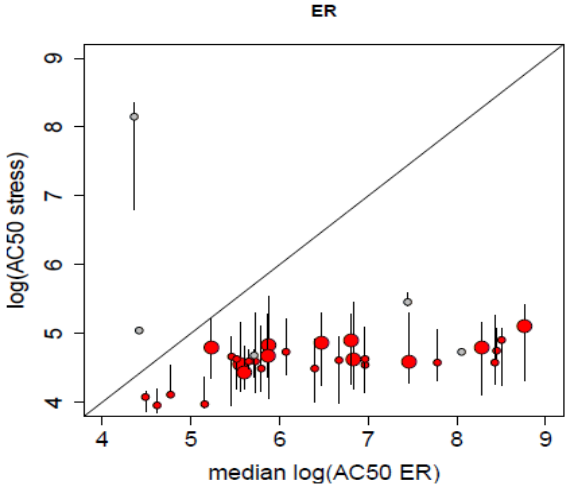


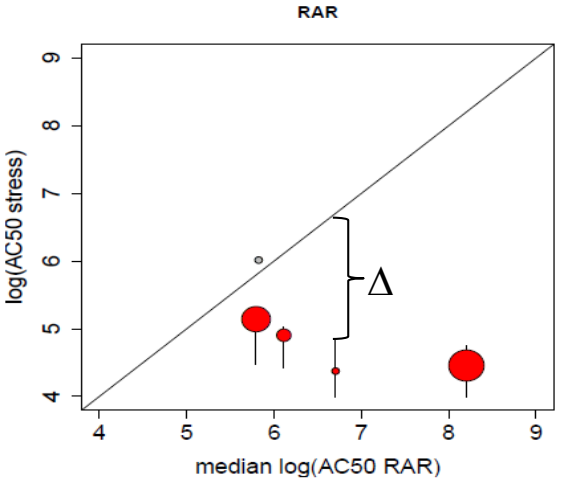


Proposed Mode of Action

- Are target+ chemicals highly likely to be ZF+?
- Does target activity occur below cell stress and cytotoxicity?

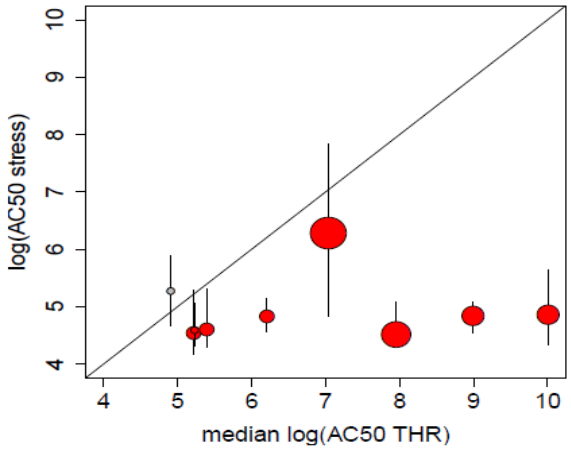
Estrogen Receptor
86% active
(30 / 35)

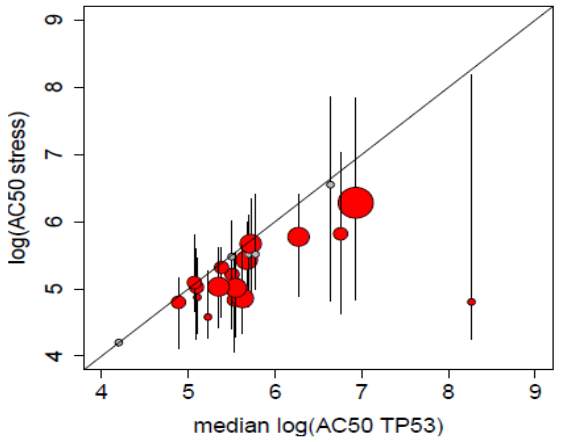




Retinoic Acid Receptor
83% active
(5 / 6)

Thyroid Hormone Receptor
90% active
(9 / 10)





TP53
77% active
(17 / 22)

Relevant Ongoing Work

- Annotate use classes of all chemicals in HTTr (and most in ToxCast)
  - Targets where available
  - Develop some kind of use ontology
  - Relevant to cosmetics: solvents, surfactants, preservatives, fragrances, dyes, (others?)

- Look for trends in activity by use class
  - Potency
  - Specificity (Does the chemical hit some biological pathway at concentrations well below cytotoxicity?)
  - Particular classes of stress

ToxCast experience strategy with reference chemicals, considerations of chemical promiscuity and assay interference

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Issue to Address

- ToxCast experience with:
  - Reference chemicals
  - Considerations of chemical promiscuity and assay interference

Reference Chemicals

- RefChemDB
  - Database of candidate reference chemicals from open source data
- Chemical Annotation Project
  - Dig into specific MOA (not just gene targets) for all HTTr chemicals
- Project specifically on finding reference chemicals for cell stress pathways
  - ER stress, DNA damage, Hypoxia, Oxidative stress, Metal stress, Mitochondrial stress

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RefChemDB

- Database of candidate reference chemicals from open source data
- Goals:
  - Set of reference chemicals for many targets for validating in vitro assays
  - Use in understanding specific vs. non-specific results in HTS and HTTr assays
- Process
  - Mine data from many databases
  - Manually curate a subset to estimate accuracy
  - Annotate each chemical-target pair with a level of “support”

RefChemDB Workflow

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- ChEMBL
- CTD
- DrugBank
- Eurofins
- Iuphar/BPS
- KEGG Drug
- KIDB
- KinaseDB
- LitDB
- NCCT Curation
- Open Targets
- ProDrug
- Repurposing Hub
- ToxCast
- TTD

source_chemical

- source_chemical_id
- name
- casrn
- source
- pubchem_cid
- smiles
- inchi_key
- chemical_id

target

- target_id
- source_chemical_id
- target
- mode
- activity_call
- source
- pmid
- geneid

chemical

- chemical_id
- casrn
- preferred_name
- dsstox_substance_id

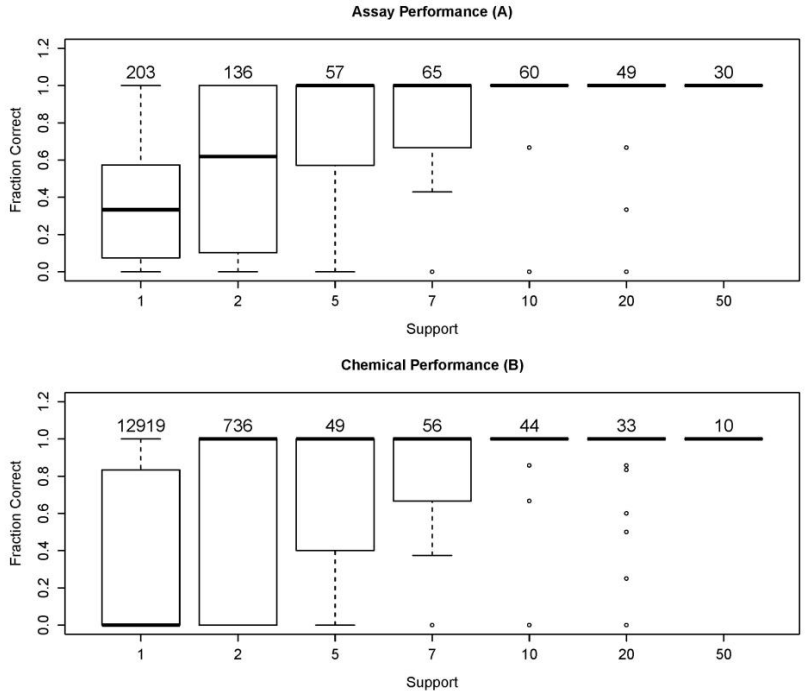
target_summary

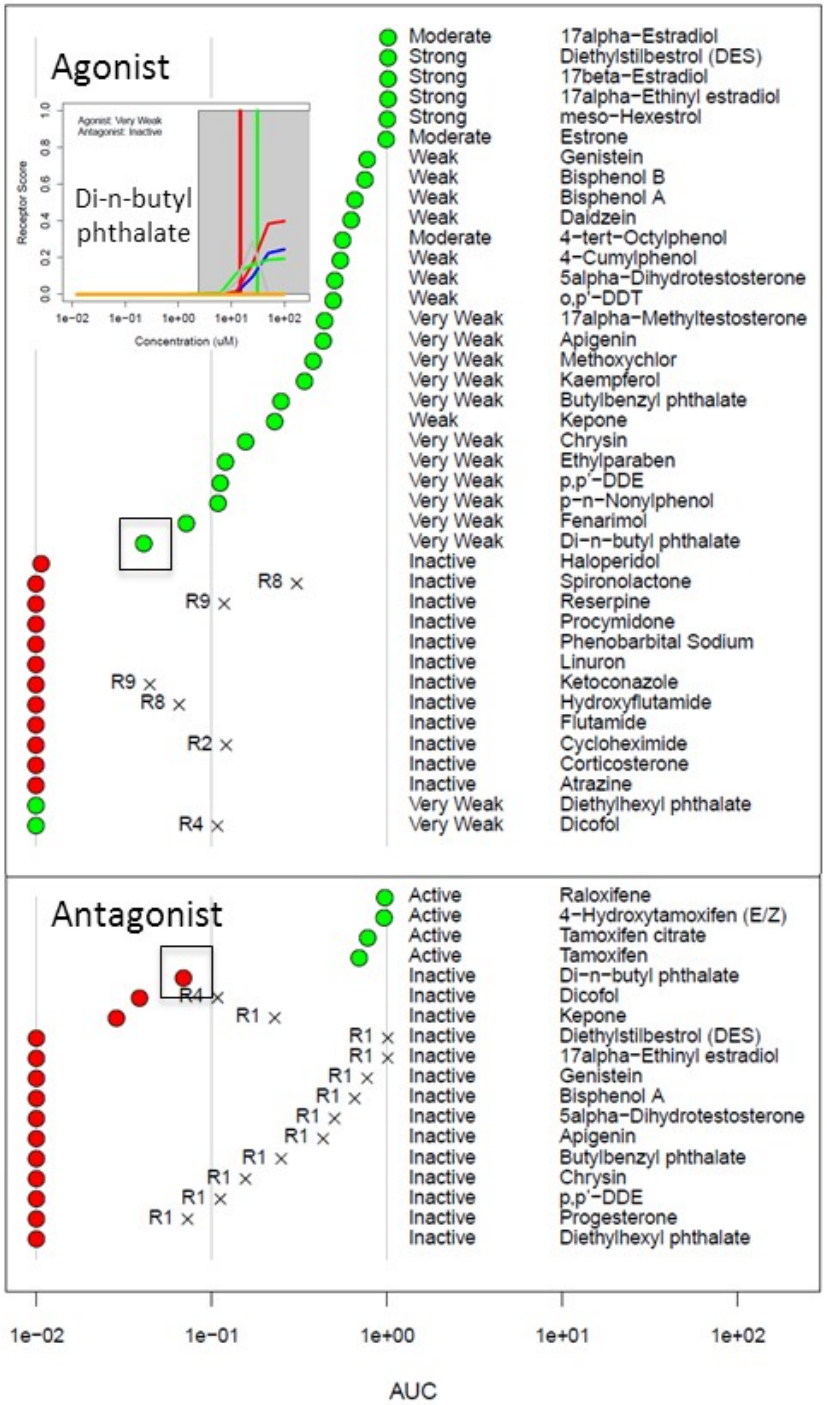
- target_summary_id
- target
- target_type
- mode
- activity_call
- source
- pmid
- geneid
- chemical_id

Field	Description
chemical_id	Database-unique chemical identifier
target	Entrez gene symbol for a gene-related target, or higher-level target such as mitochondrial membranes
name	Target name in target_summary table and chemical name in source_chemical table
geneid	Entrez Gene ID, standardized unique identifiers for genes
target_type	“gene” or “other”
mode	agonist, antagonist, inhibitor, etc.
activity call	active or inactive in the specific source reference
source	Original source of the record
pmid	Links to the PubMed ID (PMID) or other reference information
CASRN	Chemical Abstracts Registry Number

RefChemDB Results

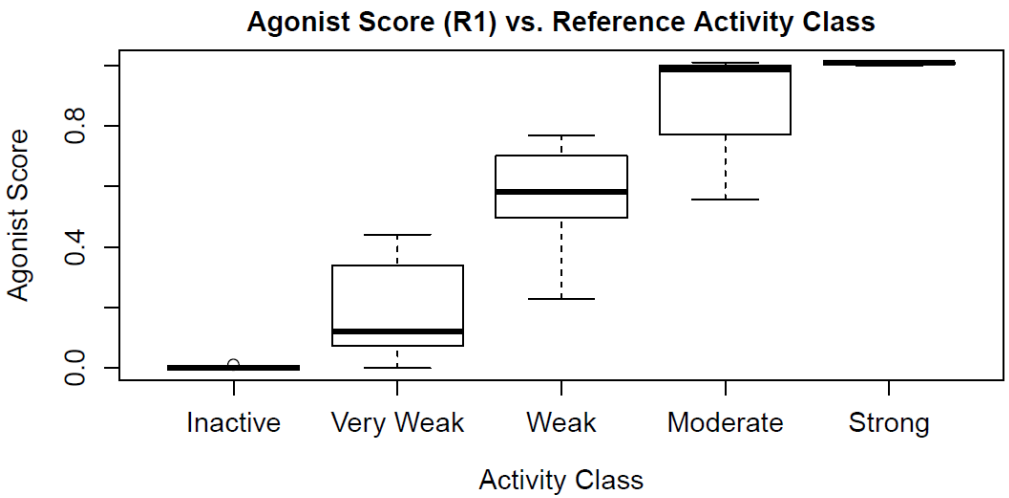
- Total of 2995 targets had at least one chemical in one data source
- The larger the support (number of independent mentions of the chemical-target link) the more likely was the chemical show specificity in the target assay
- Recommend using support ≥ 5



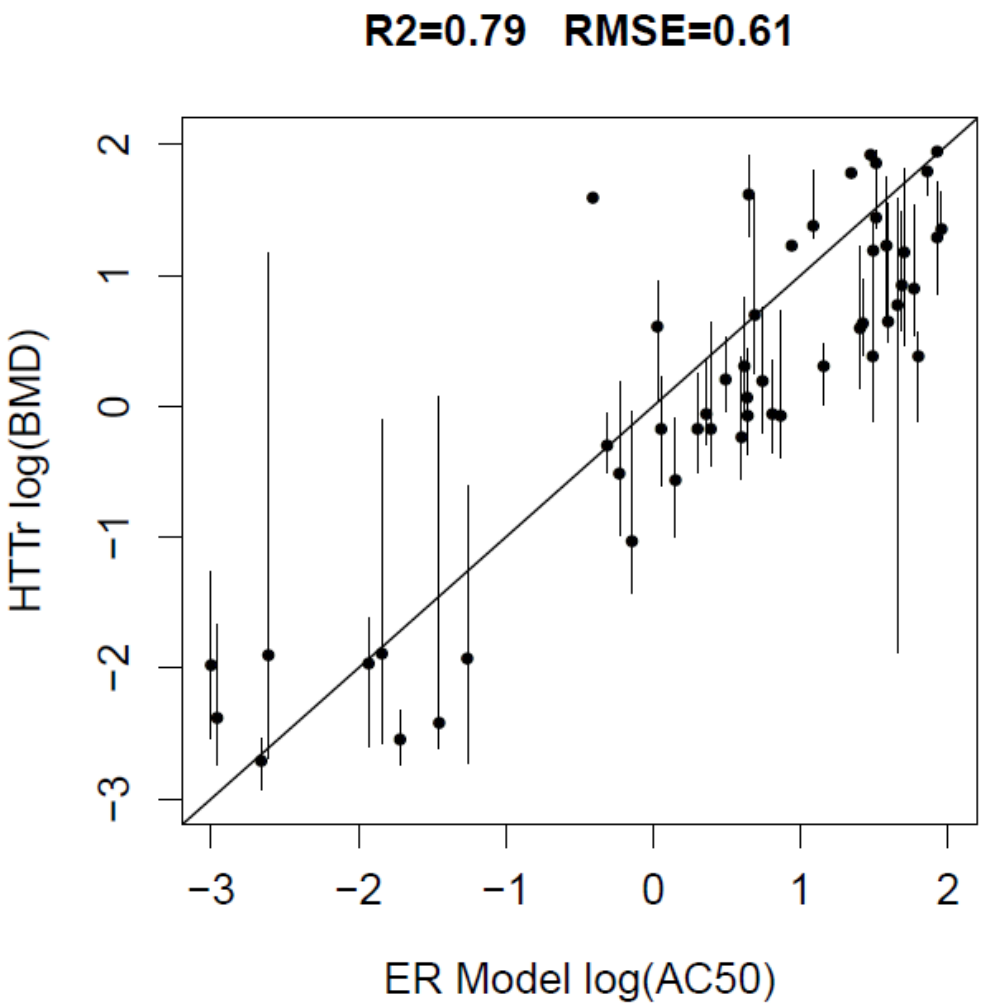


In Vitro Reference Chemical Performance

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



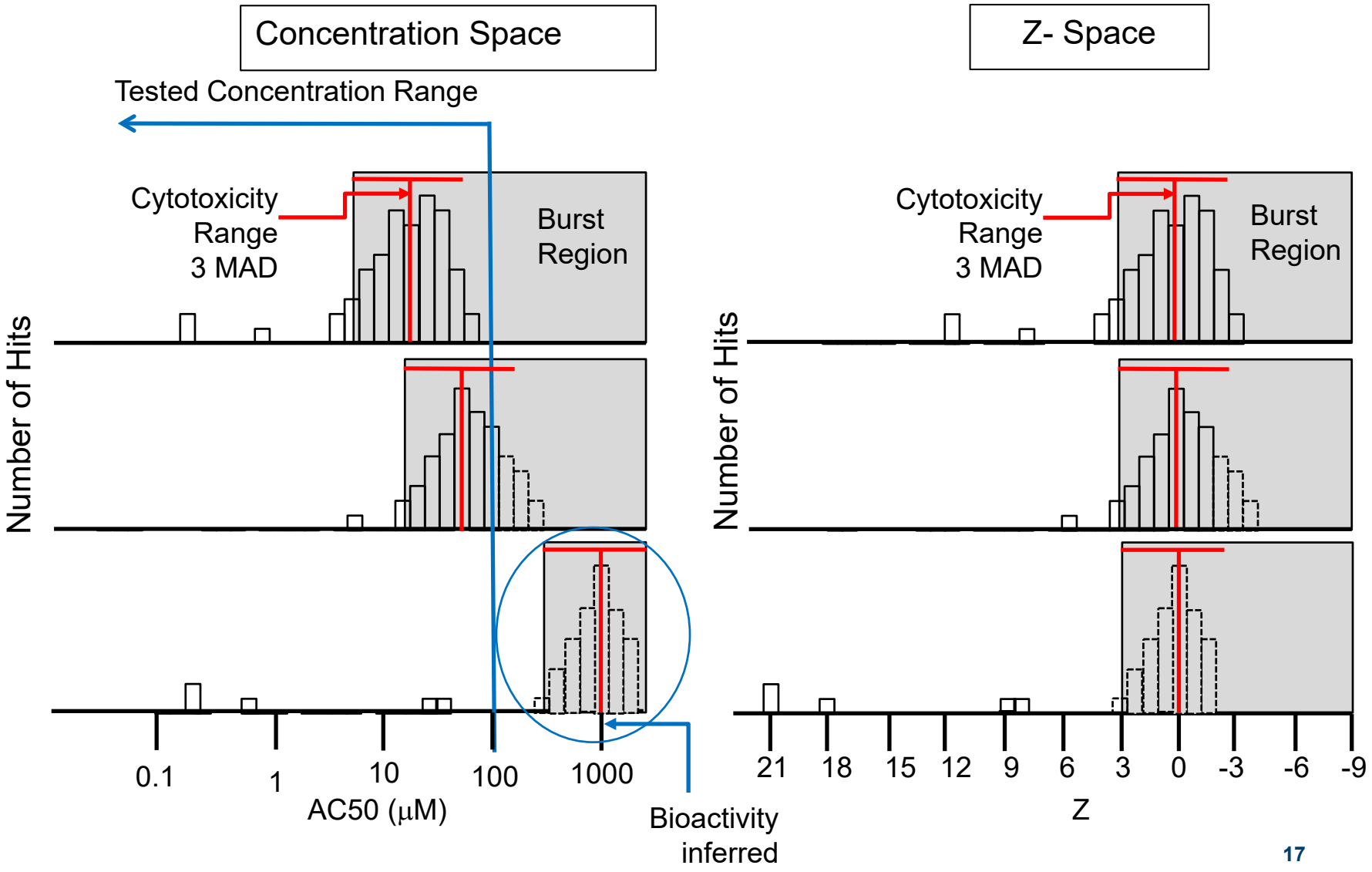
How do HTTr potencies compare with other in vitro assays?



Compare potency with estimates from ToxCast ER model using 18 in vitro agonist and antagonist assays.

HTTr values are BMDs from 10 ER signatures active in the 10 most potent ER reference compounds

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



Judson et al. Tox.Sci.(2016)

Most activity comes at high doses
# Indicates non-specific effects

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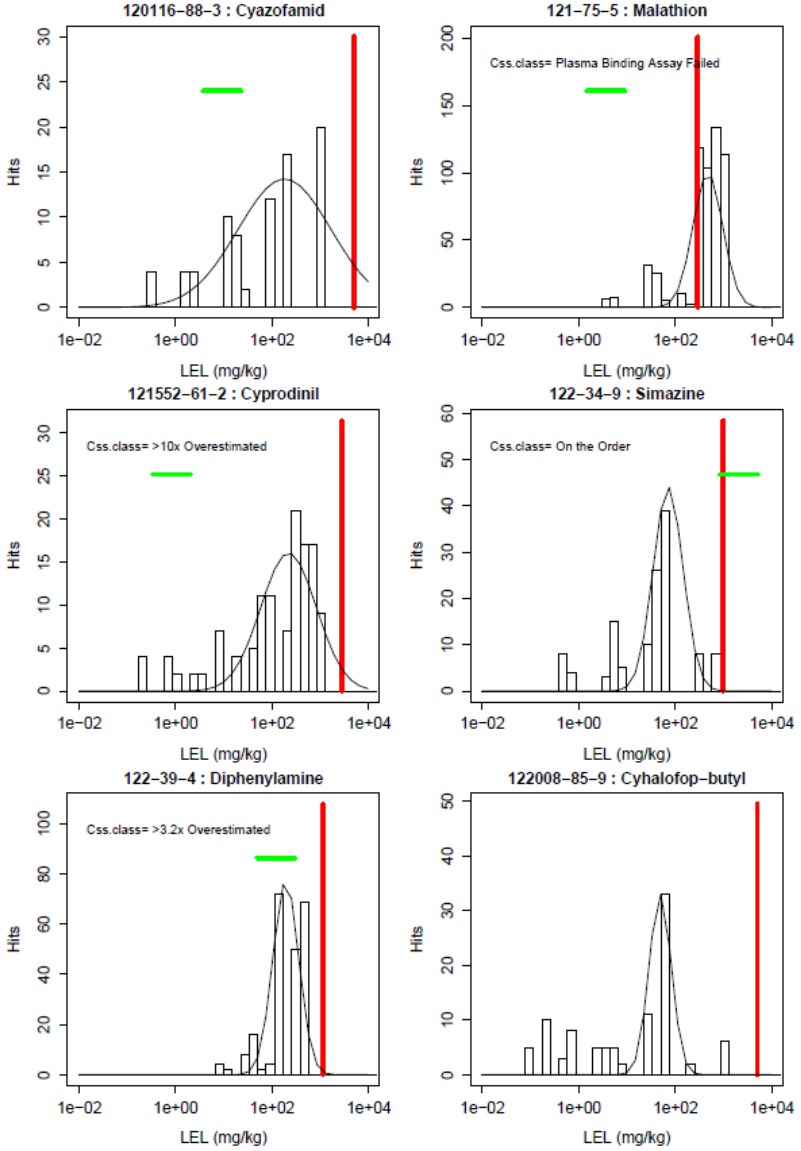
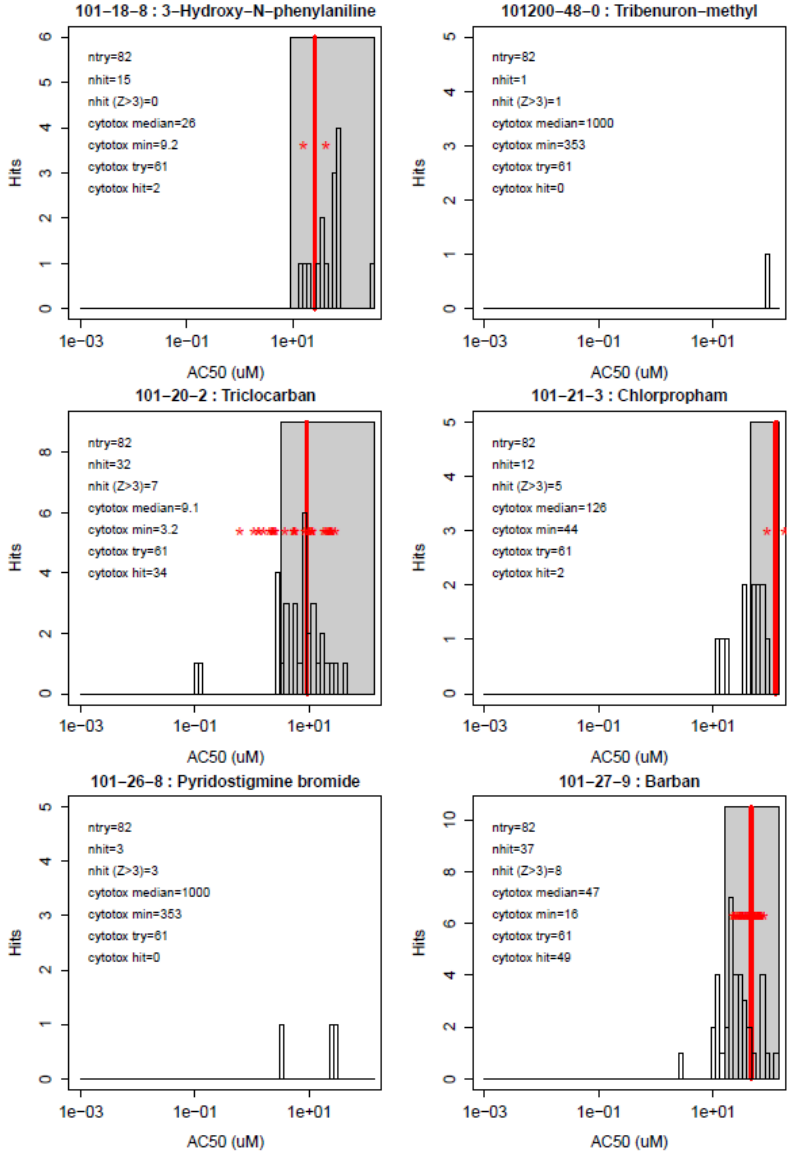
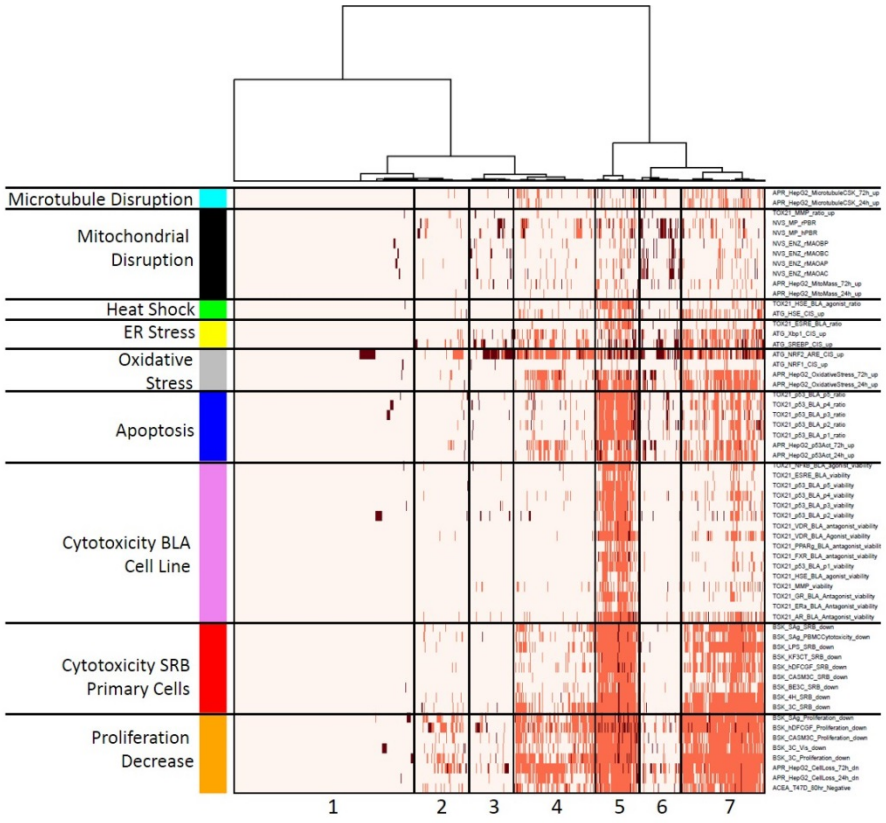
In vivo



In vitro



Stress and Cytotoxicity



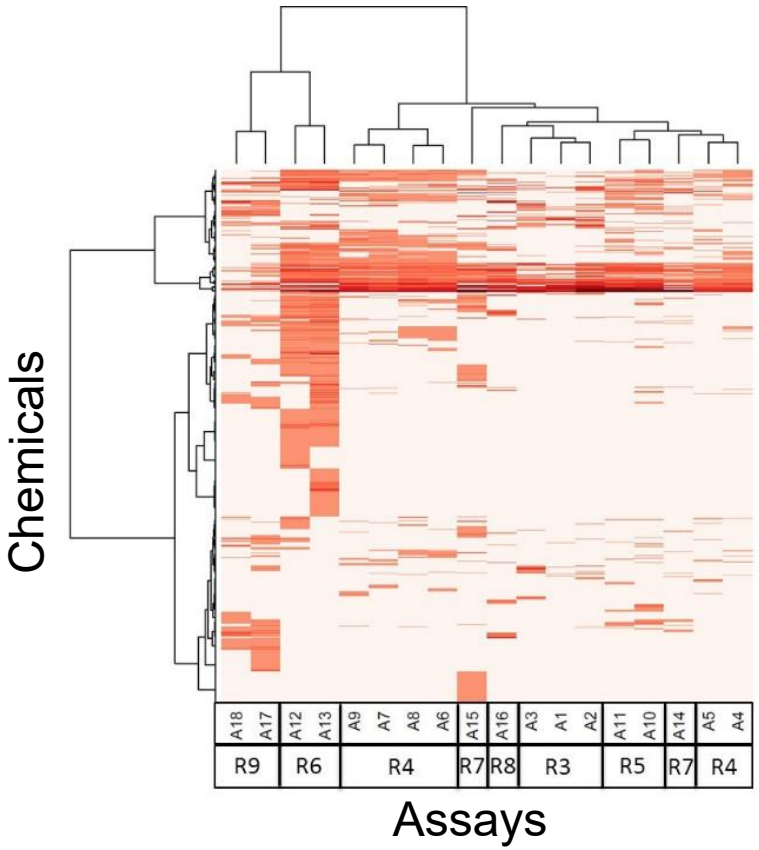
Cell stress and cytotoxicity can manifest themselves differently based on:

- Cell type
  - Cell lines
  - Primary cells
  - Cell origin / tissue
- Readout technology

Heat map shows potency across 1000 chemicals with a collection of cell stress and cytotoxicity assays

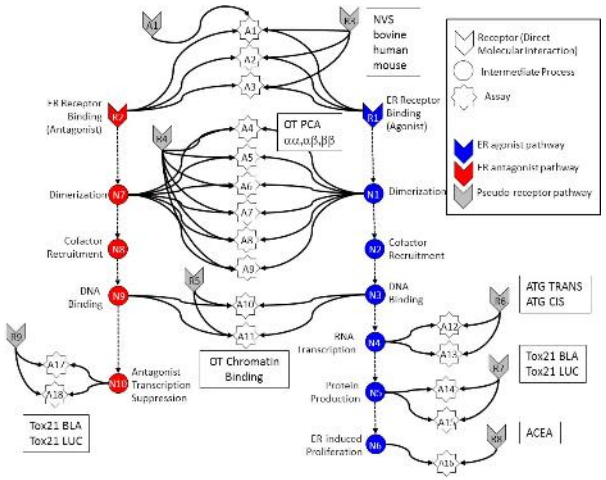
All In vitro assays have false positives and negatives

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)

United States Environmental Protection Agency

Extra Slides

Goals of NAM Hazard Assessment

- Predict a point of departure (POD)
- Predict what pathology would occur at doses>POD
- Understand uncertainties about POD and pathology predictions

Hazard Approach where Animal Data is Lacking

- Goals:
  1. Quantitative point of departure (POD) (e.g. NOAEL)
  2. Estimate of what effects will be seen (e.g. liver hypertrophy)
- Experimental approaches
  - Battery of *in vitro* assays (ToxCast), one per target / pathway
  - High-throughput whole genome transcriptomics
  - Yield POD and MOA / AOP / mechanism information
- Modeling approaches
  - QSAR models
  - Read-across
  - TTC
  - Better at POD estimation than mechanism prediction

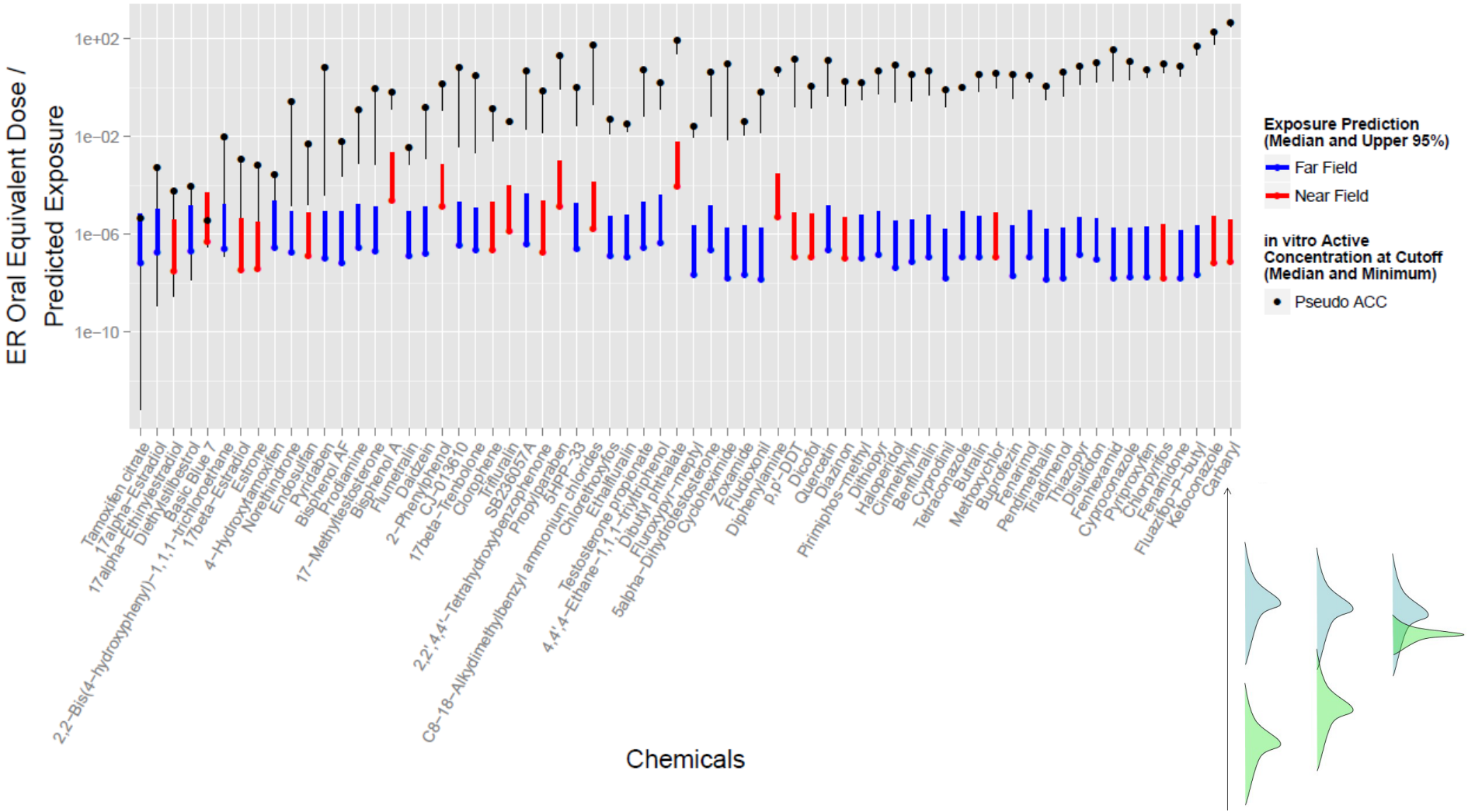
Putting it all together

- *In vitro* assays yield POD in μM
  - Select the minimum "relevant" *in vitro* POD
- TK yields *in vitro* to *in vivo* conversion factor
  - "Concentration at Steady State", C_{ss}
  - Blood concentration for a 1 mg/kg/day steady-state dose
- IVIVE POD ("oral equivalent dose") = *in vitro* POD / C_{ss}
- Exposure model yields estimate of exposure (mg/kg/day)

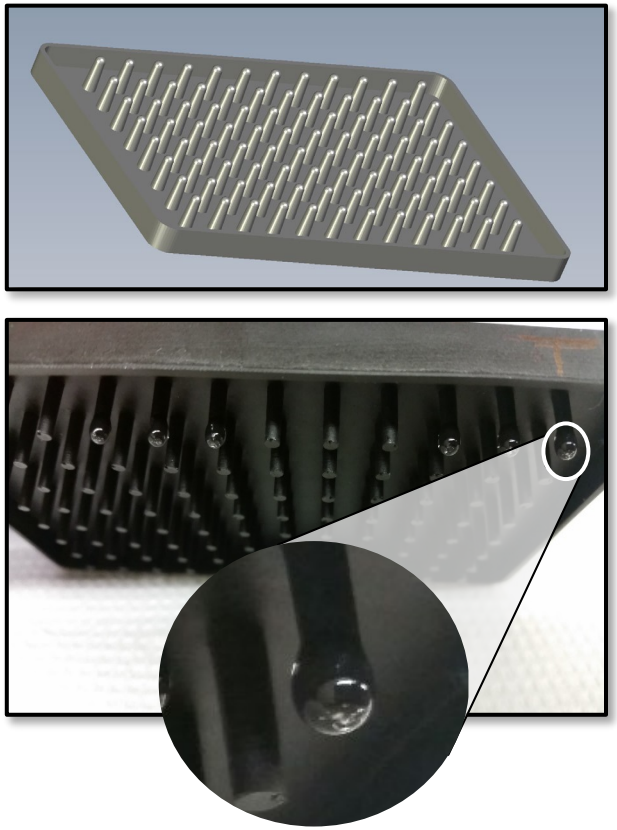
- BER: Bioactivity to Exposure Ratio
  - IVIVE POD / Exposure estimate
  - BER >> 1 implies low concern for risk

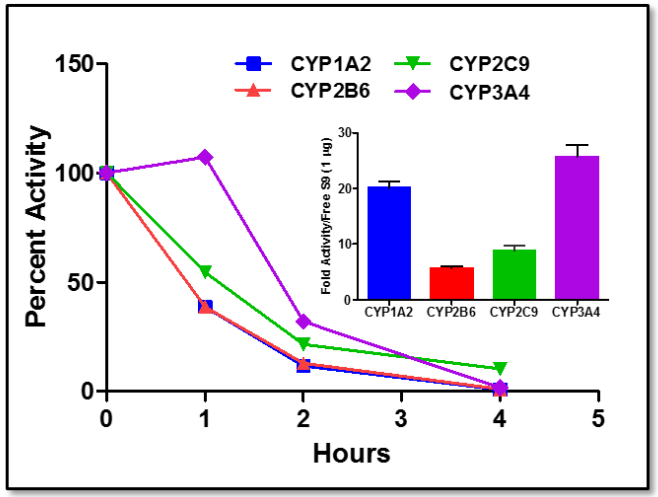
Prioritization (Replacement) Example
Compare predicted exposure and hazard POD

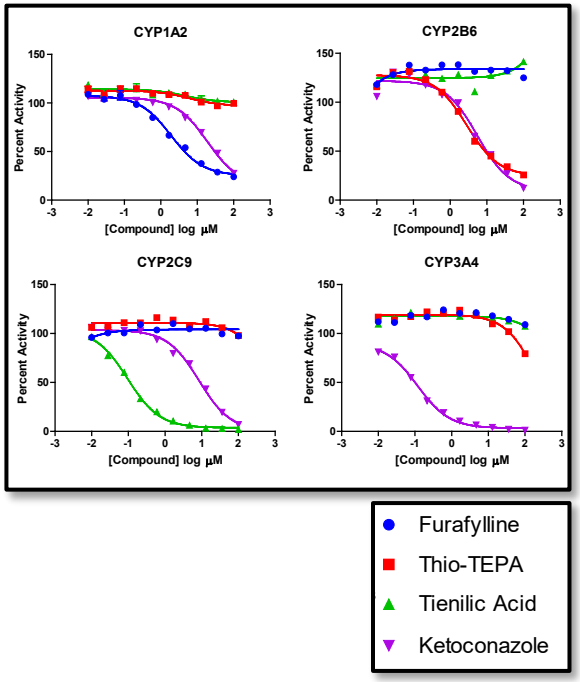
Compare estrogen receptor assay battery and exposure model



Efforts to Address Metabolism Challenge

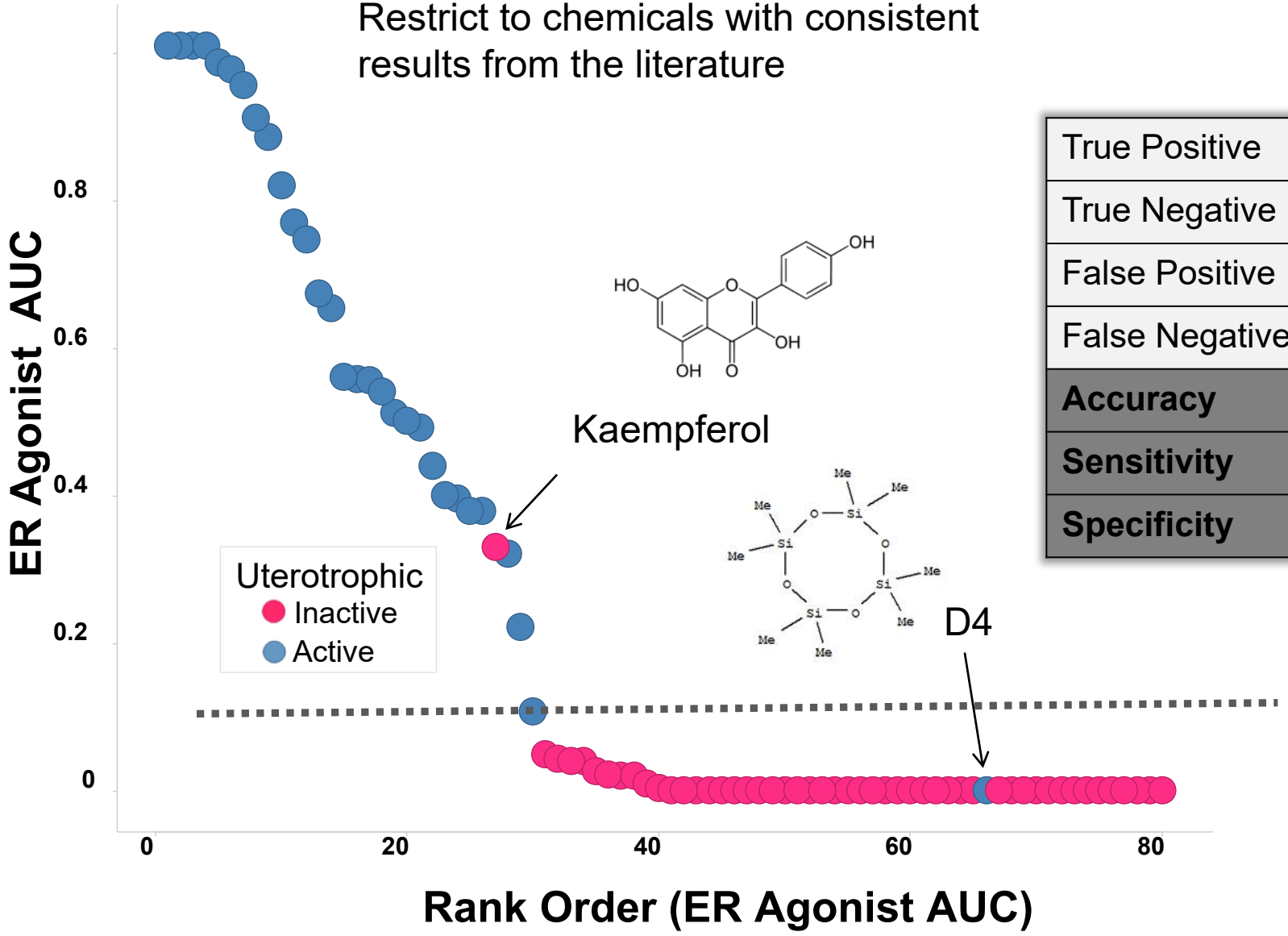






DeGroot and Simmons, Unpublished

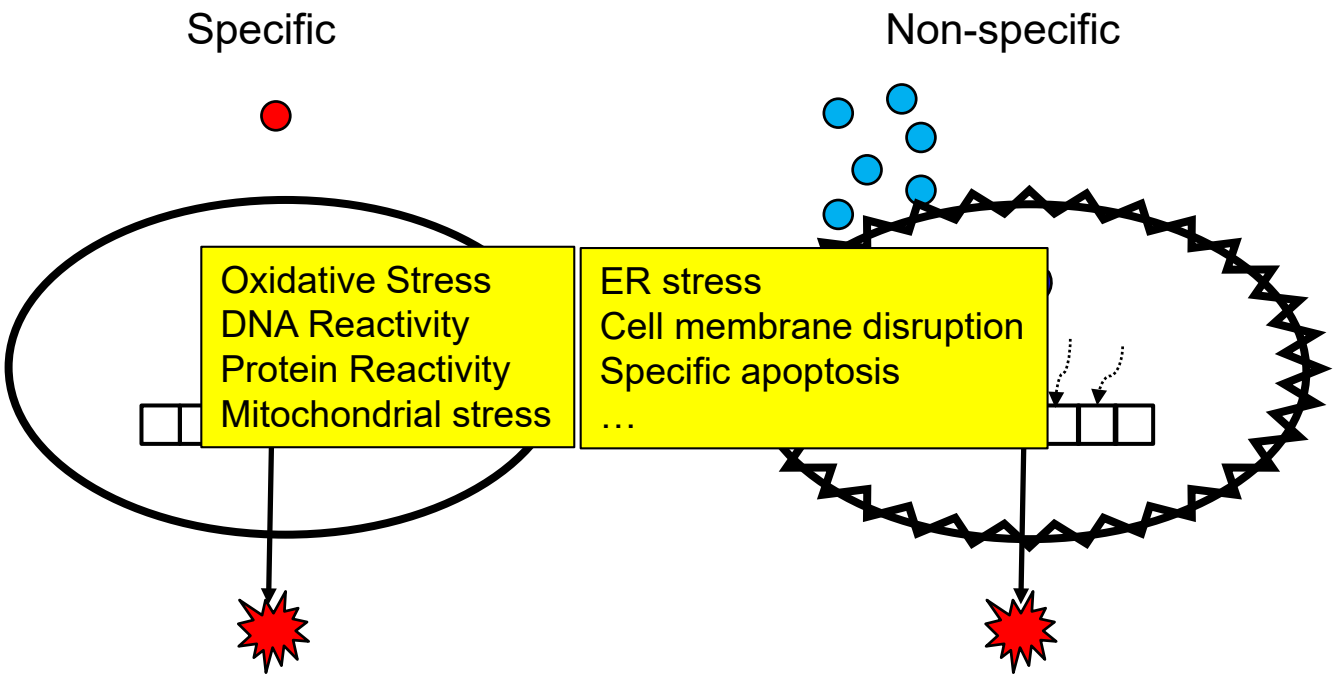
Model predicts *in vivo* uterotrophic assay as well as uterotrophic predicts uterotrophic



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

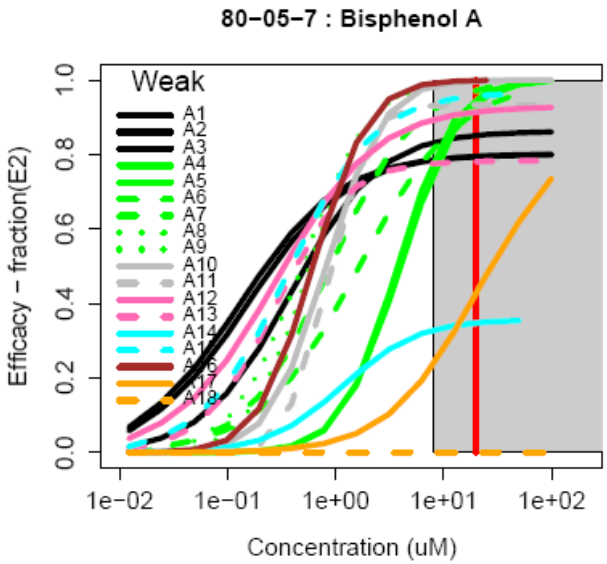
Browne et al. ES&T (2015)

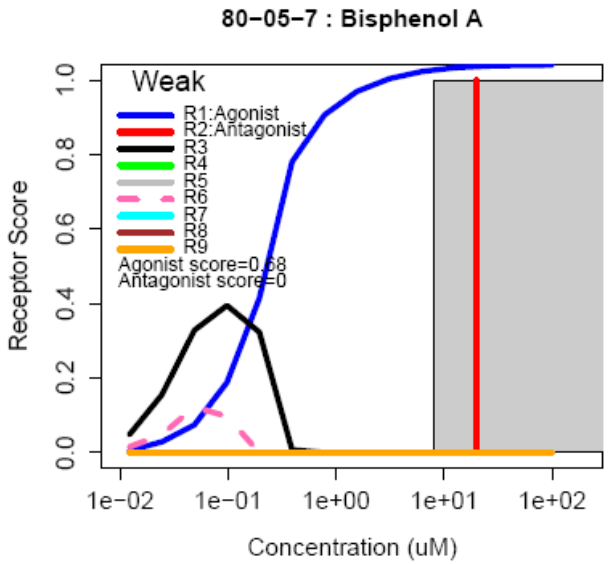
Schematic explanation of non-specific activity



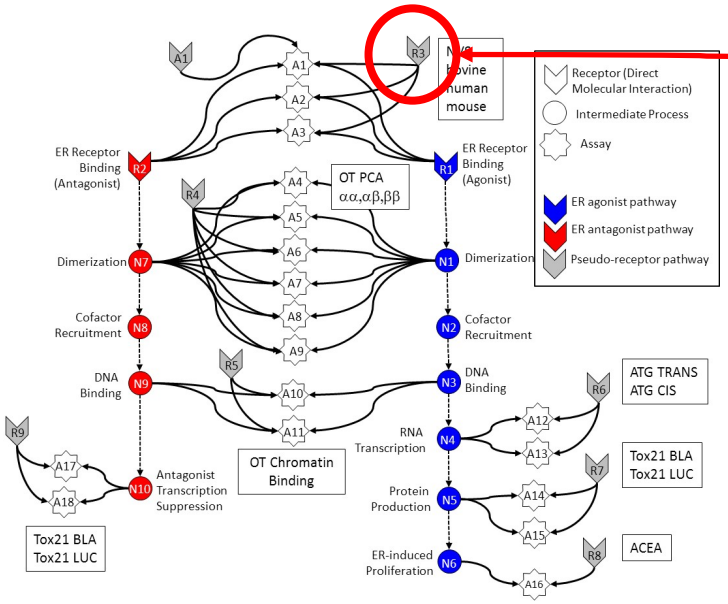
Example chemicals:
Observe quantitative uncertainty

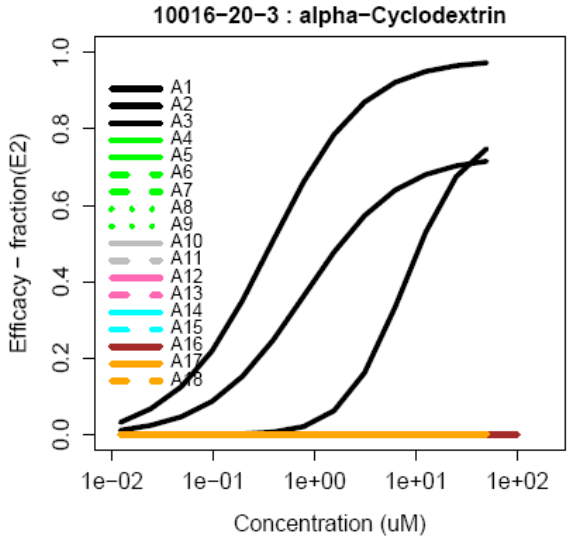
True Agonist

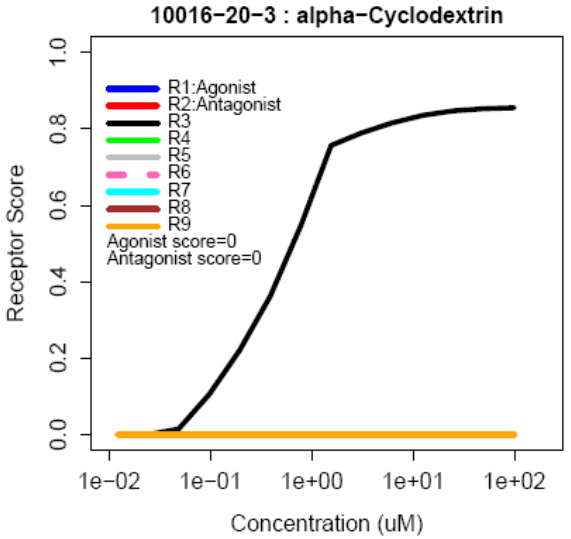




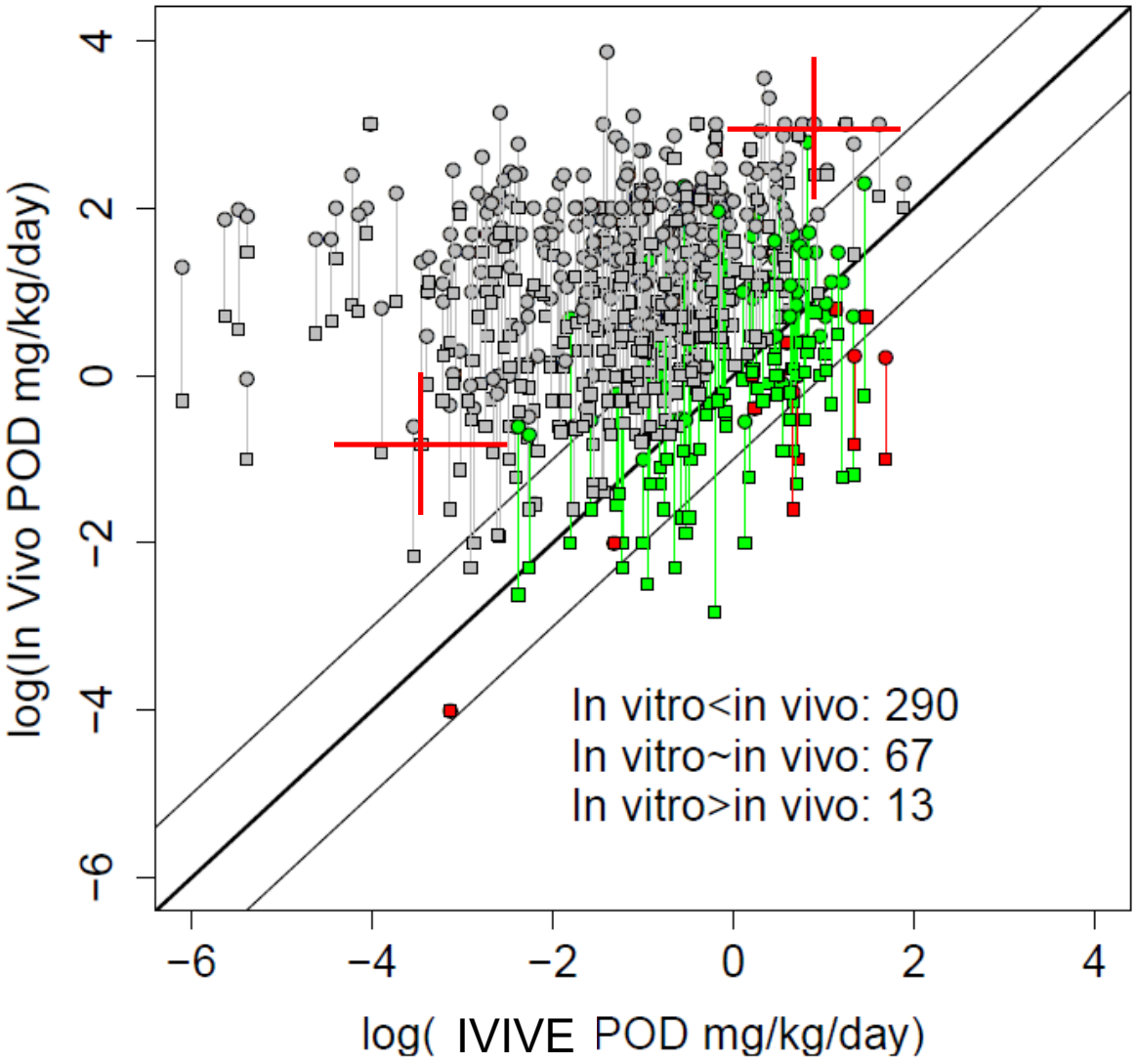
Assay Interference Example “R3”







Simple IVIVE Results …



Conservative: IVIVE POD < POD in most cases

However:

- Correlation is almost zero
- Errors are large: 2-6 orders of magnitude

Mitigated by uncertainty in both *in vitro* and *in vivo*

Tools / Models / Data needed

- Hazard information or model
  - Start with *in vitro* data
  - Quantify concentration (μM) required to trigger bioactivity
- Toxicokinetics
  - Use to convert between external dose and internal concentration
- Exposure information or model
  - Quantify in mg/kg/day
- Include uncertainties everywhere