High-Throughput Transcriptomics (HTTr) Screening as a Component of NAMs-based Tiered Hazard Evaluation

Joshua A. Harrill

USEPA Center for Computational Toxicology and Exposure (CCTE)

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Outline

- **NAMs-Based Tiered Hazard Evaluation Approach**

- **High-Throughput Transcriptomics (HTTr)**
  - Assay Concept
  - Data Analysis Pipeline
  - Concentration Response Modeling of Transcriptomic Signatures

- **Results**
  - Comparison to ToxCast
  - Comparison to ER Model
  - Bioactivity / *In Vivo* Effect Value Ratio Analysis
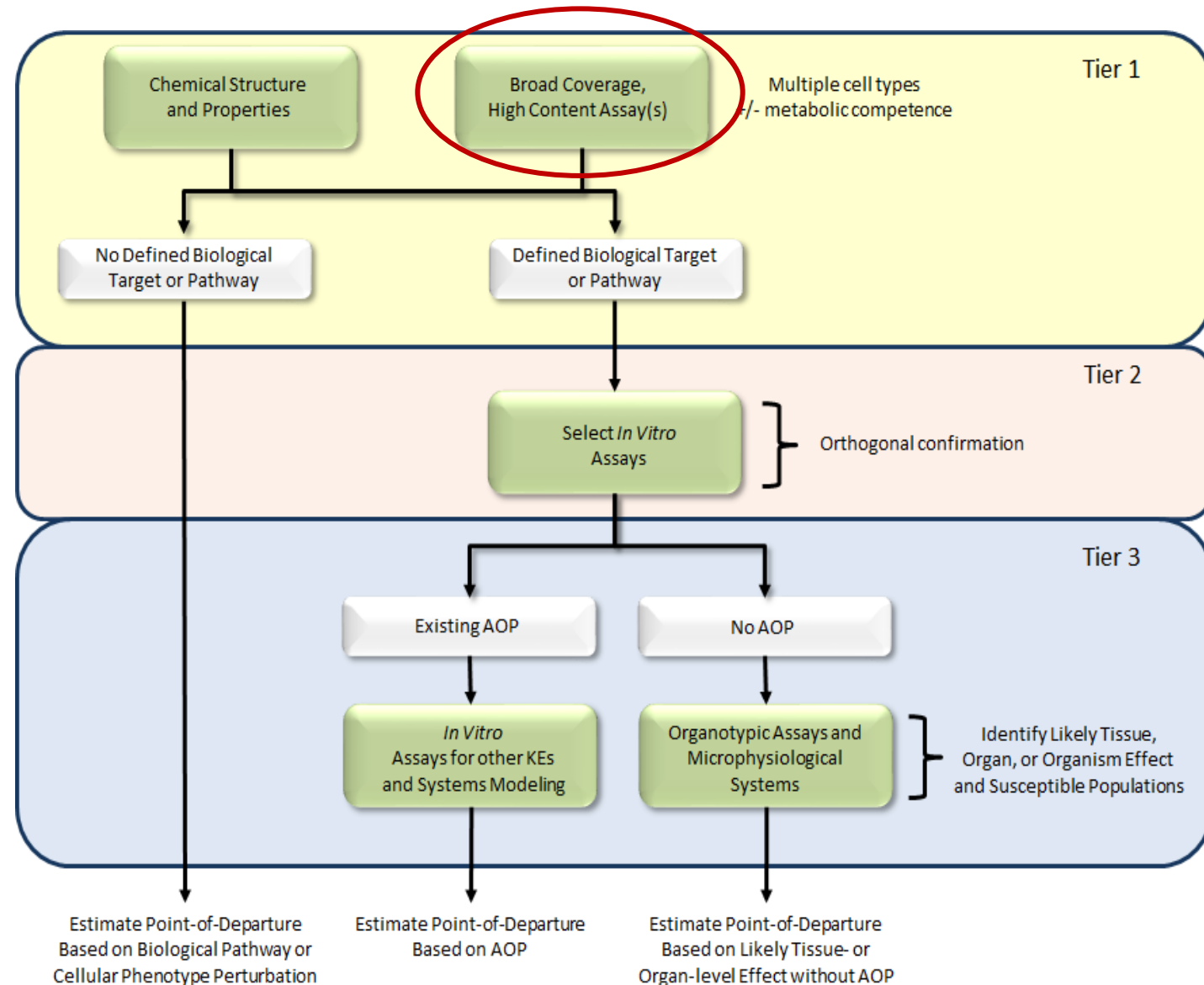
- **Current and Future Directions**

- **New Approach Methodologies (NAMs)** are any technology, methodology, approach or combination thereof that can be used to provide information on chemical hazard and risk that avoids the use of intact animals.

- NAMs are a potential means to **reduce** the use of animals in toxicity testing and **accelerate** the pace of chemical risk assessment.

- US EPA CompTox Blueprint advocates the use of **high throughput profiling (HTP) assays** as the first tier in a NAMs-based hazard evaluation approach.

- **HTP assay criteria:**
  1. Yield bioactivity profiles that can be used for **potency estimation, mechanistic prediction** and evaluation of **chemical similarity**.
  2. Compatible with multiple human-derived culture models.
  3. Concentration-response screening mode.
  4. Cost-effective.

The NexGen Blueprint of CompTox as USEPA Tox. Sci. 2019; 169(2):317-322
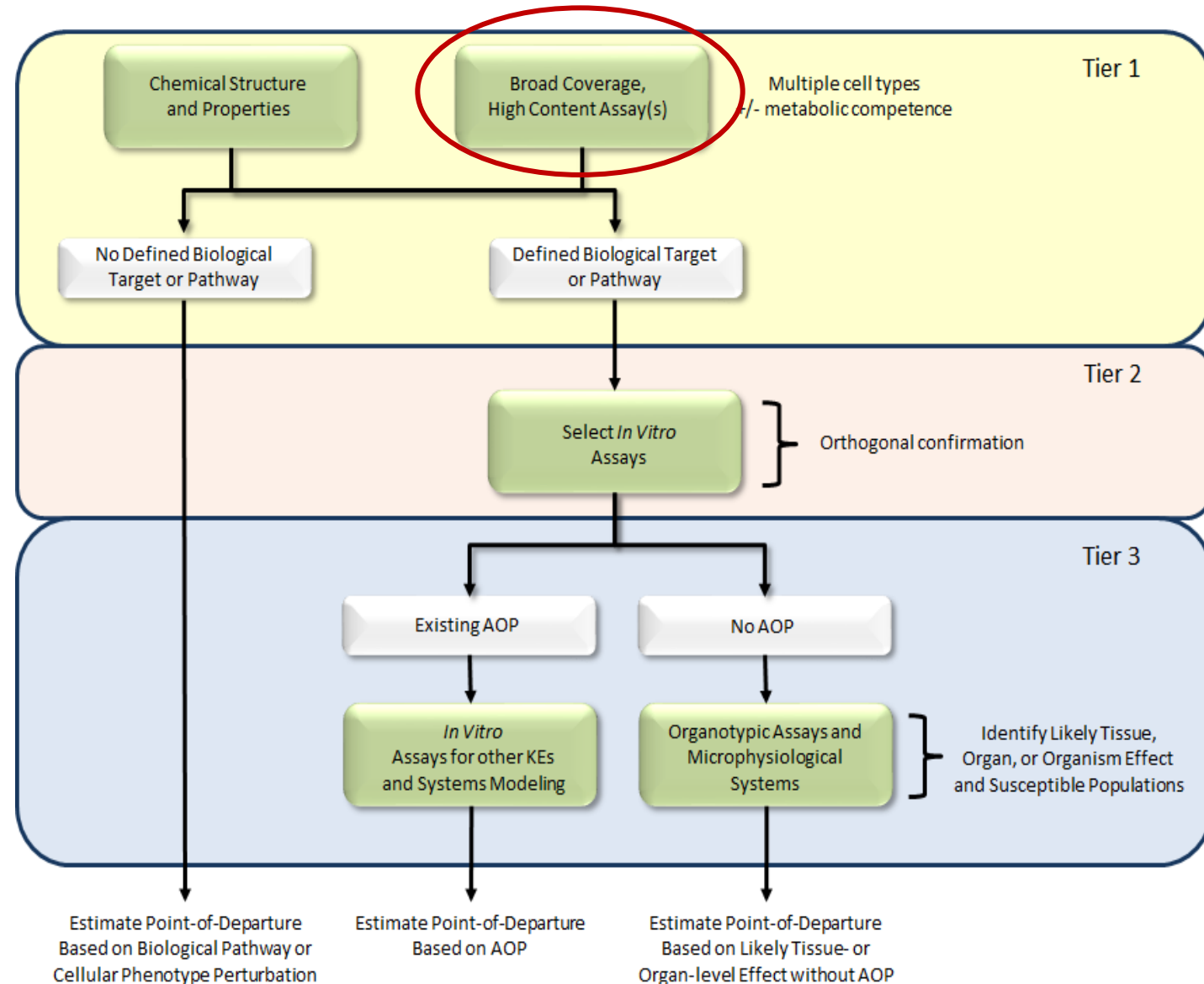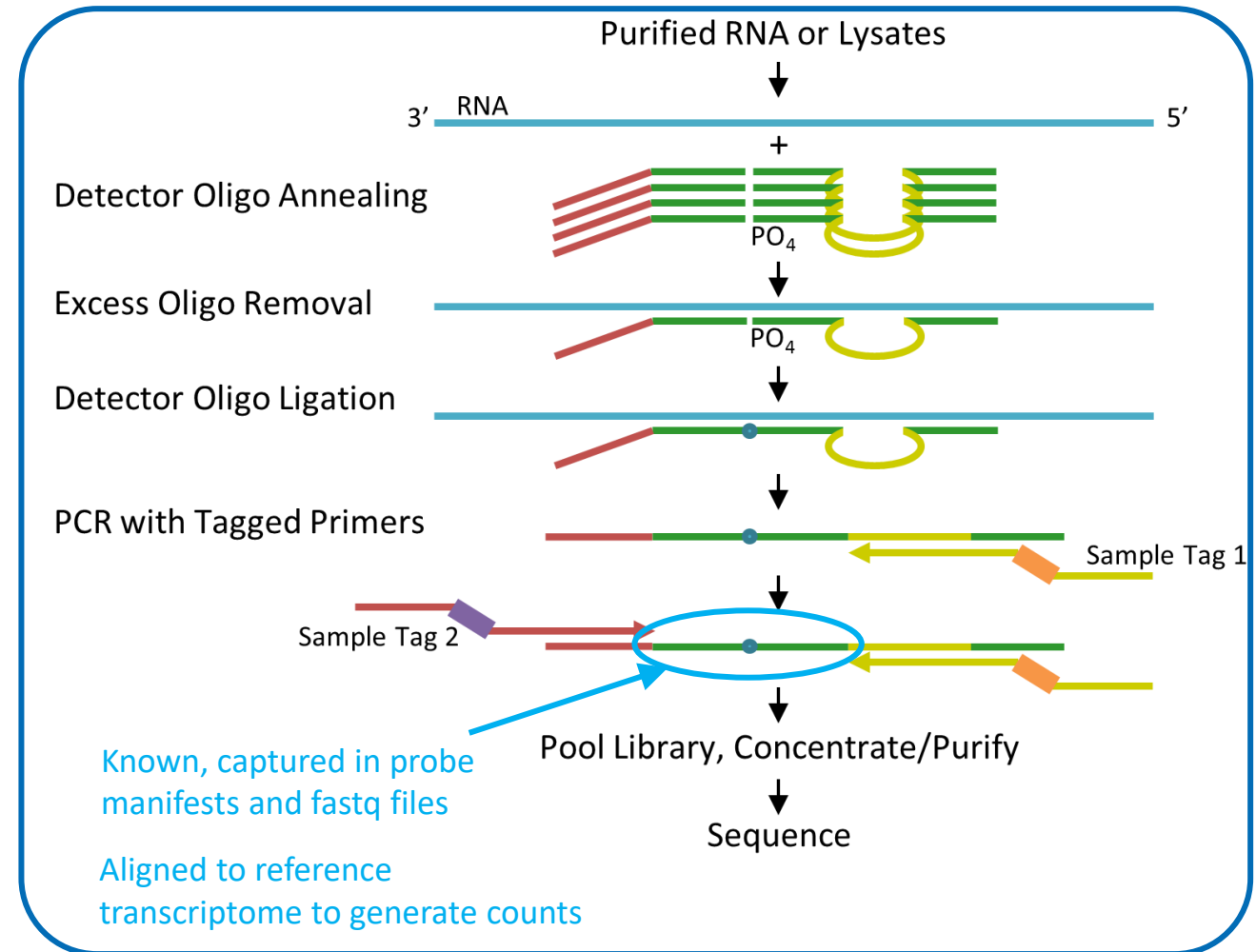
Tiered Hazard Evaluation Approach (2)

- To date, EPA has identified and implemented two HTP assays that meet this criteria.

- **High-Throughput Transcriptomics [HTTr]**

  - Whole Transcriptome TempO-Seq

- **High-Throughput Phenotypic Profiling [HTPP]**

  - Cell Painting

- Both methods are **complementary** to each other and can be used in many different human-derived cell types.

- EPA has established scalable laboratory and bioinformatics workflows for each assay.

Tier 1

Chemical Structure and Properties

Broad Coverage, High Content Assay(s) — Multiple cell types +/- metabolic competence

No Defined Biological Target or Pathway

Defined Biological Target or Pathway

Tier 2

Select *In Vitro* Assays — Orthogonal confirmation

Tier 3

Existing AOP

No AOP

In Vitro Assays for other KEs and Systems Modeling

Organotypic Assays and Microphysiological Systems — Identify Likely Tissue, Organ, or Organism Effect and Susceptible Populations

Estimate Point-of-Departure Based on Biological Pathway or Cellular Phenotype Perturbation

Estimate Point-of-Departure Based on AOP

Estimate Point-of-Departure Based on Likely Tissue- or Organ-level Effect without AOP

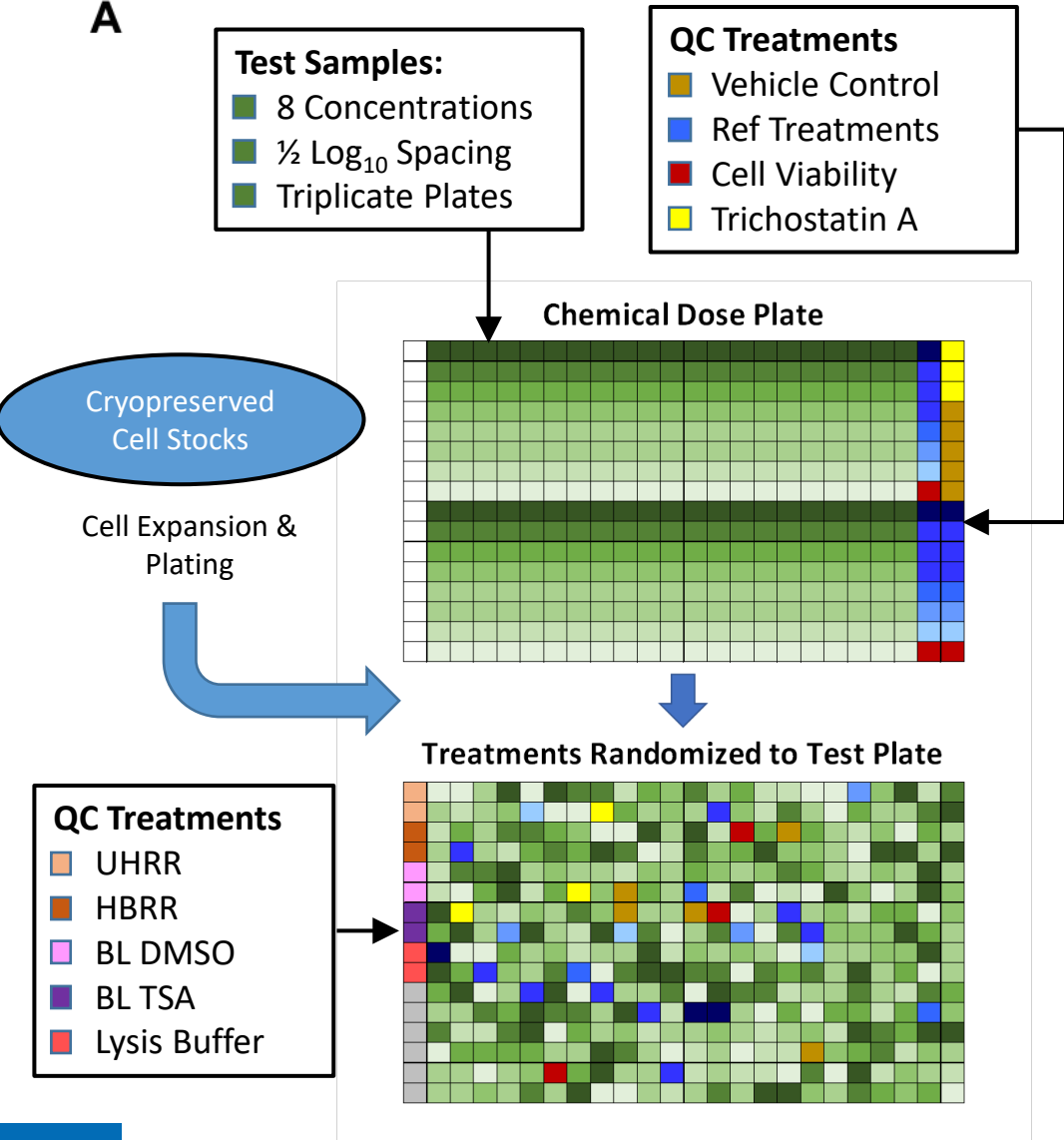# Templated Oligo with Sequencing Readout (TempO-Seq)

- The **TempO-Seq** human whole transcriptome assay measures the expression of greater than 20,000 transcripts.

- Requires only picogram amounts of total RNA per sample.

- Compatible with purified RNA samples or **cell lysates**.

- Lysates are barcoded according to sample identity and combined in a single library for sequencing using industry standard instrumentation.

- Scalable, targeted assay:
  - 1) specifically measures transcripts of interest
  - 2) ~50-bp reads for all genes
  - 3) requires less flow cell capacity than RNA-Seq

TempO-Seq Assay Illustration

Purified RNA or Lysates

3' RNA 5'
+
Detector Oligo Annealing
PO_4

Excess Oligo Removal
PO_4

Detector Oligo Ligation

PCR with Tagged Primers
Sample Tag 1

Sample Tag 2

Known, captured in probe manifests and fastq files

Aligned to reference transcriptome to generate counts

Pool Library, Concentrate/Purify

Sequence
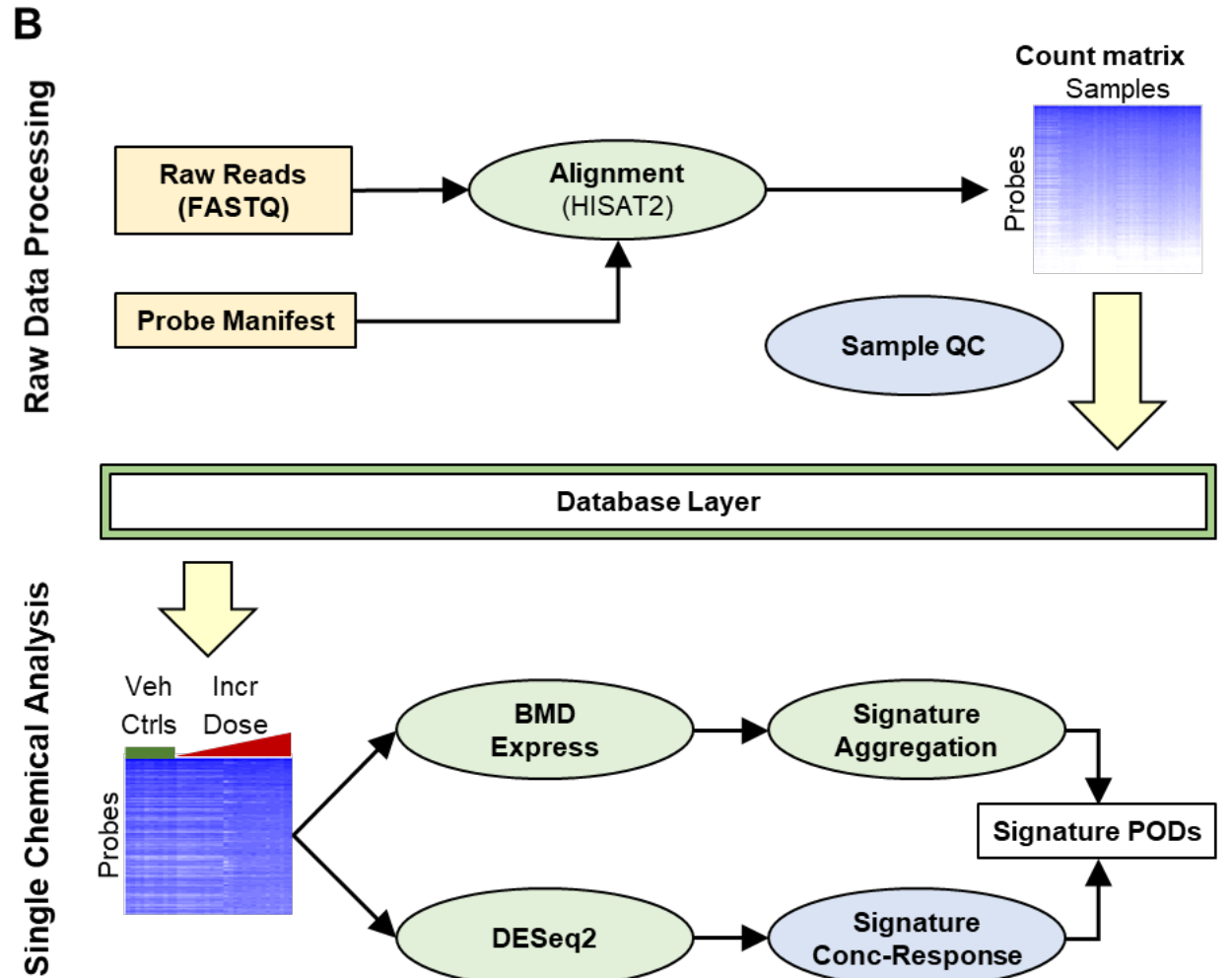
Yeakley et al., PLoS One. 2017 May 25;12(5):e0178302

HTTr Experimental Design and Bioinformatics Workflow

Concentration-Response Modeling of Gene Signatures (1)

- Understanding the results of change in gene expression for **10,000 – 20,000 genes** is difficult.

- Grouping genes into gene signatures aids in **data interpretation**.

- **Examples of signature types**:
  - Genes that are perturbed in diseased tissue vs. healthy tissue
  - Genes perturbed in individuals with congenital diseases vs. those without
  - Genes perturbed by gene knockdowns / knockouts
  - Genes perturbed by drugs or other chemicals with known (or unknown) mechanisms

- **Example use:**
  - If an unknown *chemical X* perturbs genes that are also perturbed by a well-characterized chemical with a specific mechanism of action, then one can infer the *chemical X* may affect the same molecular target(s).

- **CCTE signature collection:**
  - Compiled from many public sources (MSigDB[1], BioPlanet[2], CMAP[3] , DisGeNET[4]) → ~10,000 signatures
  - Each signature has been manually-assigned a "super target" class to aid in interpretation → ~1000 super targets
    - Disease groups (Immune, Cancer, etc.)
    - Biological organization groups (molecule, pathway, cell, tissue, organ, etc.)

[1] Liberzon et al., Bioinformatics. 2011 Jun 15;27(12):1739-40
[2] Huang et al., Front Pharmacol. 2019 Apr 26;10:445
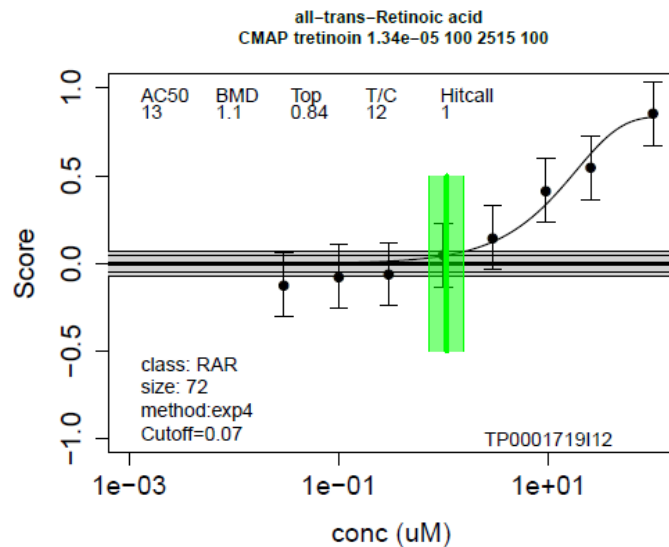[3] Subramanian et al., Science. 2006 Sep 29;313(5795):1929-35.
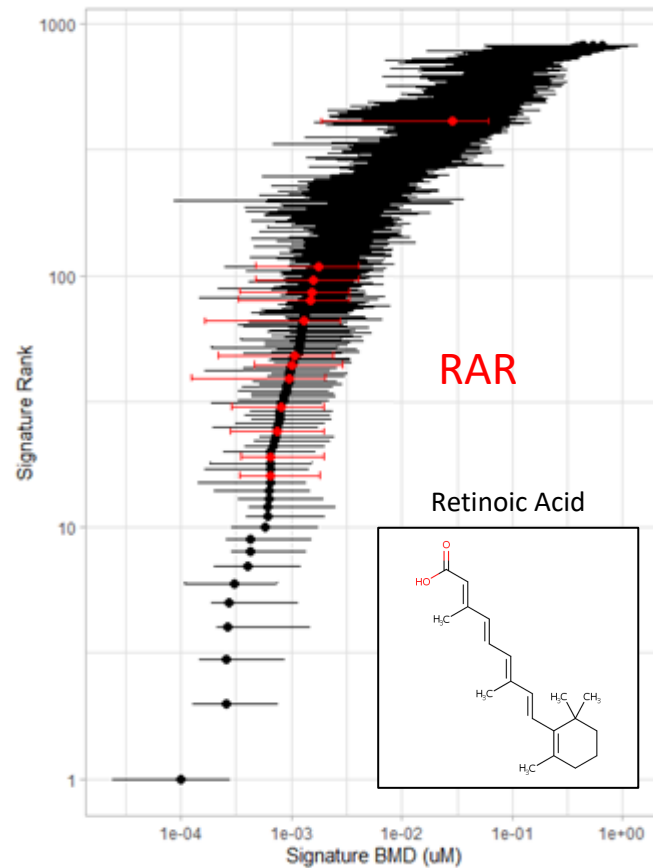[4] Pinero et al., Database (Oxford). 2015 Apr 15;2015:bav028

Concentration-Response Modeling (*tcplfit2*)

Signature-Level:
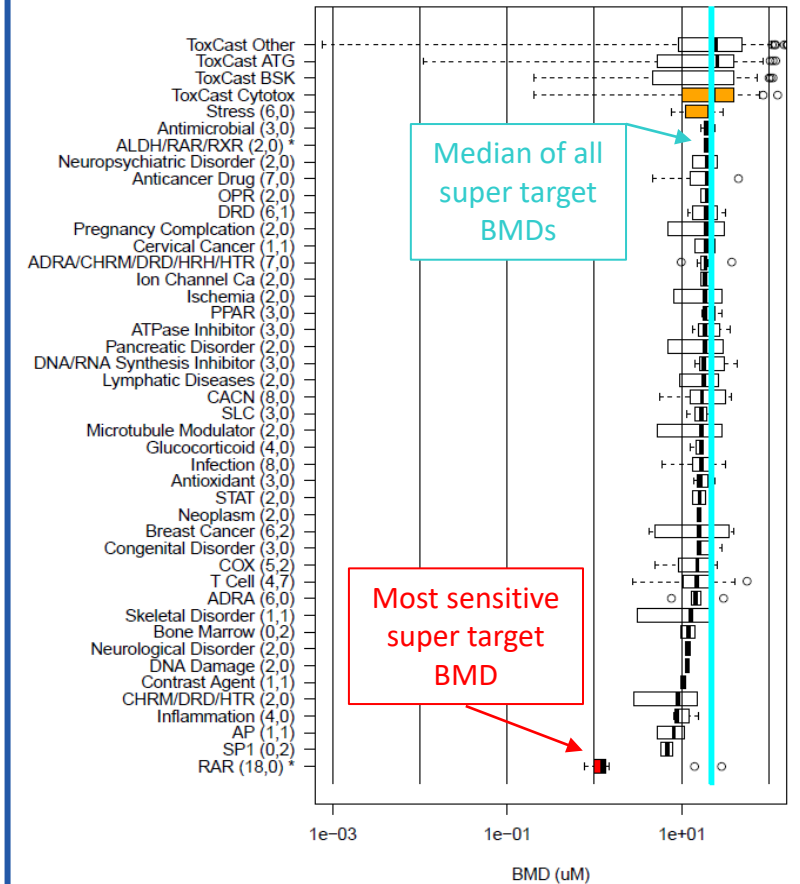- Benchmark Dose (BMD)
- Confidence Interval on BMD
- Hit Call Probability

Ranking of Signatures

RAR

Retinoic Acid

Signature Aggregation

Median of all super target BMDs

Most sensitive super target BMD

- Molecular PODs based on **biological pathway altering concentrations (BPACs)** may be derived in several ways.
- Most sensitive signature **OR** statistic based on distribution of active signatures (5th %ile) **OR** by target class.

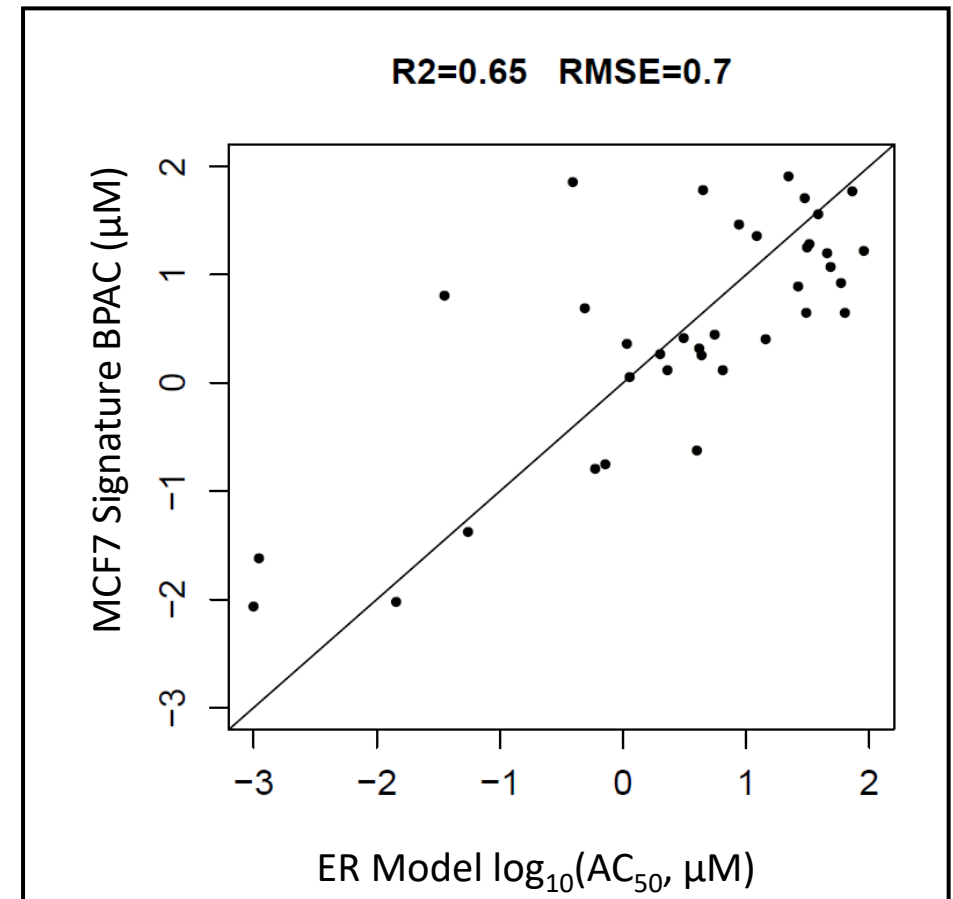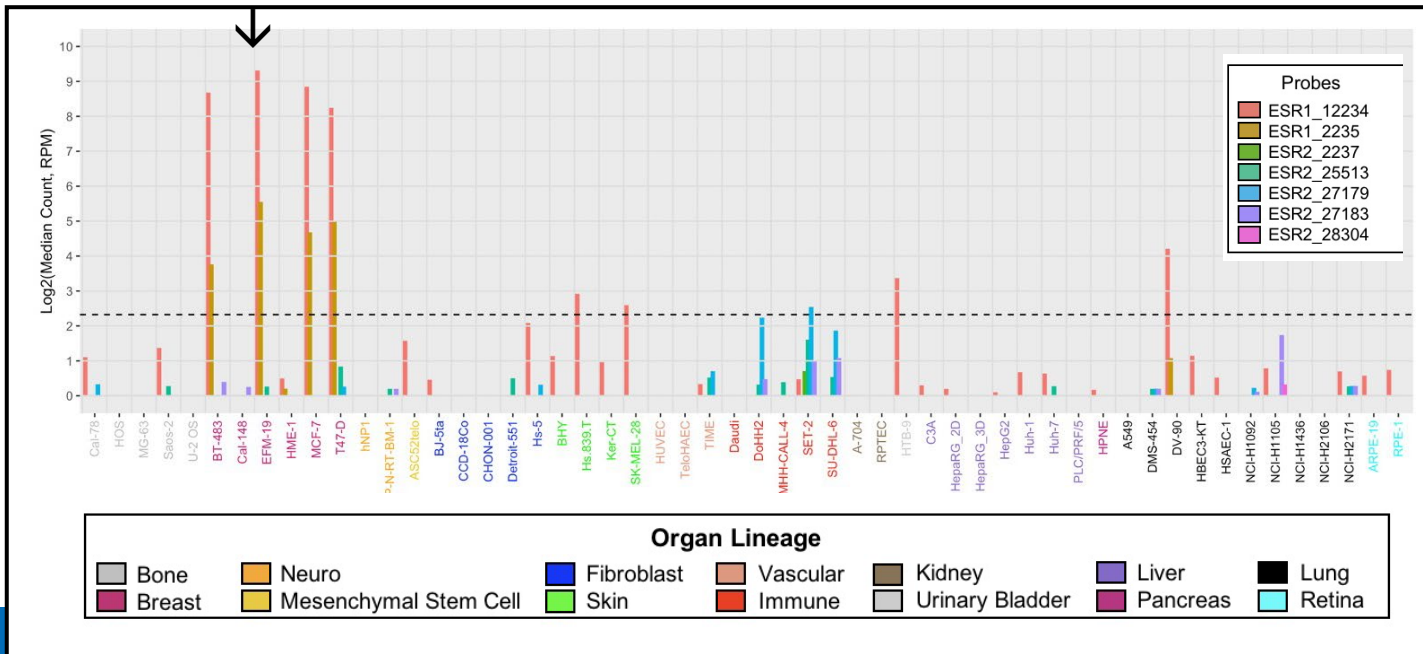# Comparison of Transcriptional BPACs to ToxCast BPACs
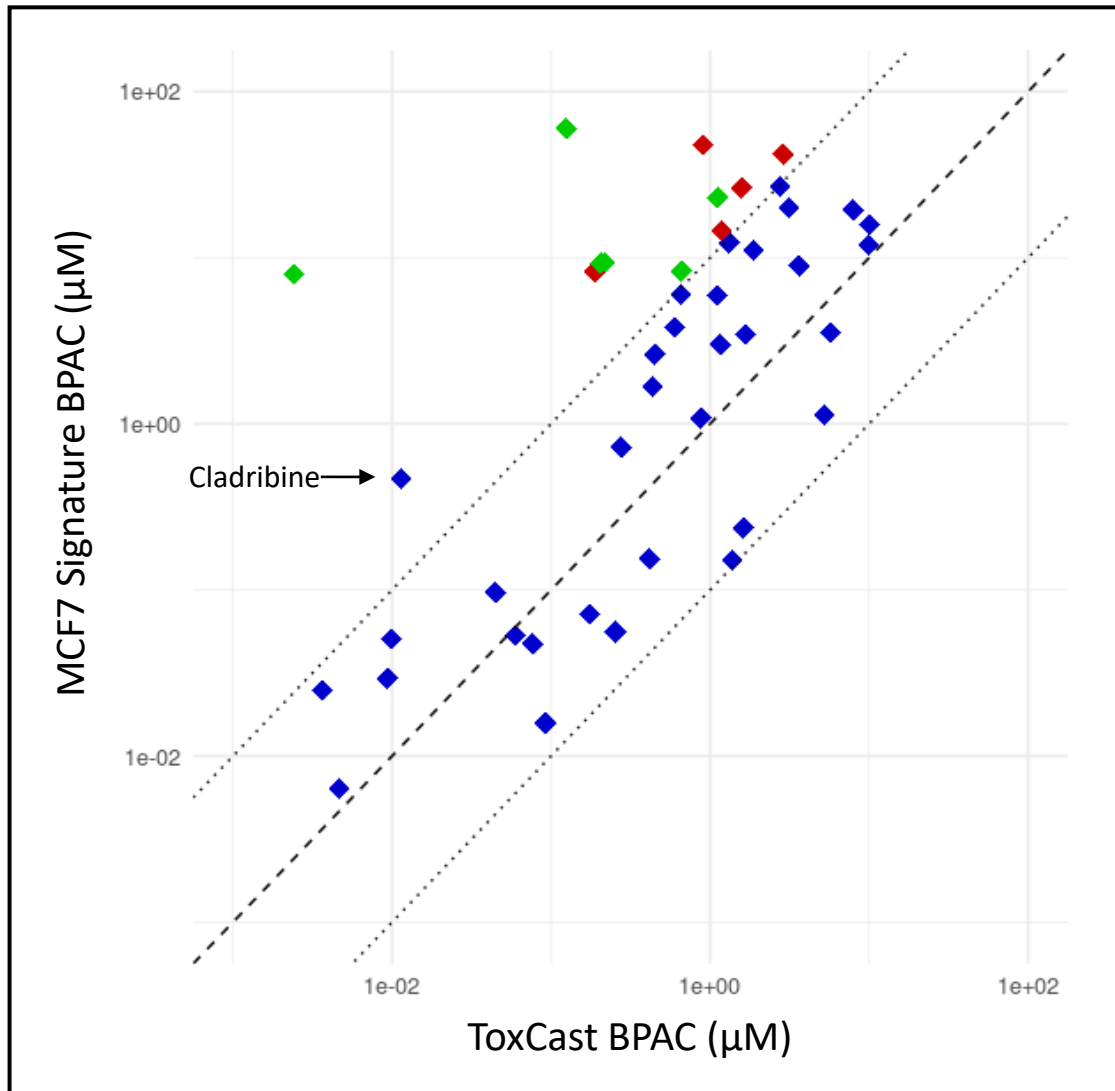
- Pilot study of 44 well-characterized ToxCast chemicals in MCF-7 cells *(Harrill et al., Toxicol Sci. 2021 Apr 27;181(1):68-89)*.

- Compare HTTr-derived PODs from MCF-7 cells to previous ToxCast HTS assay results *(Paul-Friedman et al., Toxicol Sci. 2020 Jan 1;173(1):202-225)*.

- Signature-based BPACs are highly concordant with ToxCast results for a majority of test chemicals in the pilot study.

  - 6 chemicals with targets that low/absent expression in MCF-7 cells.
  - 5 chemicals with off-target hit as most potent assay in ToxCast
  - Cladribine is a non-specific DNA synthesis inhibitor.

Harrill et al., Toxicol Sci. 2021 Apr 27;181(1):68-89.

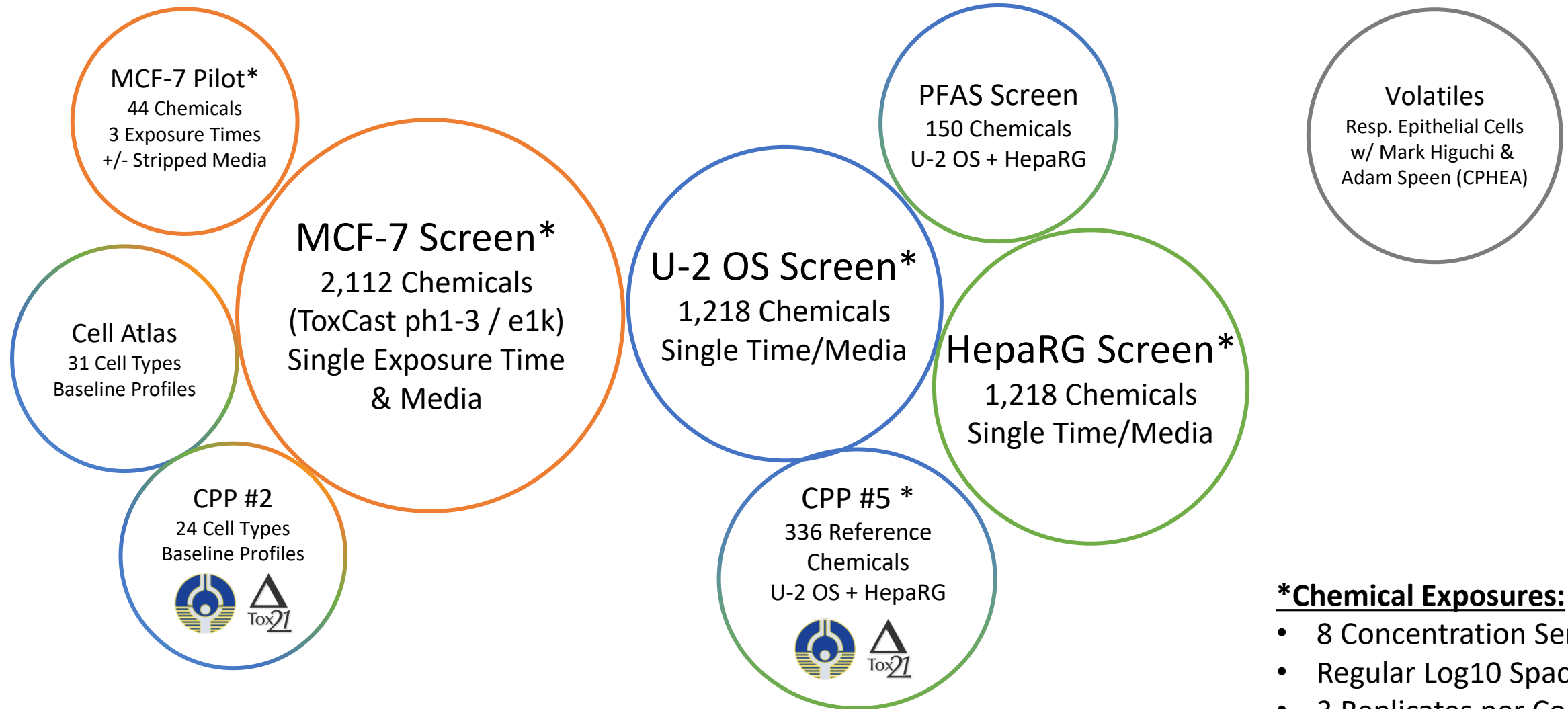# Signature Modeling of Estrogenic Chemicals
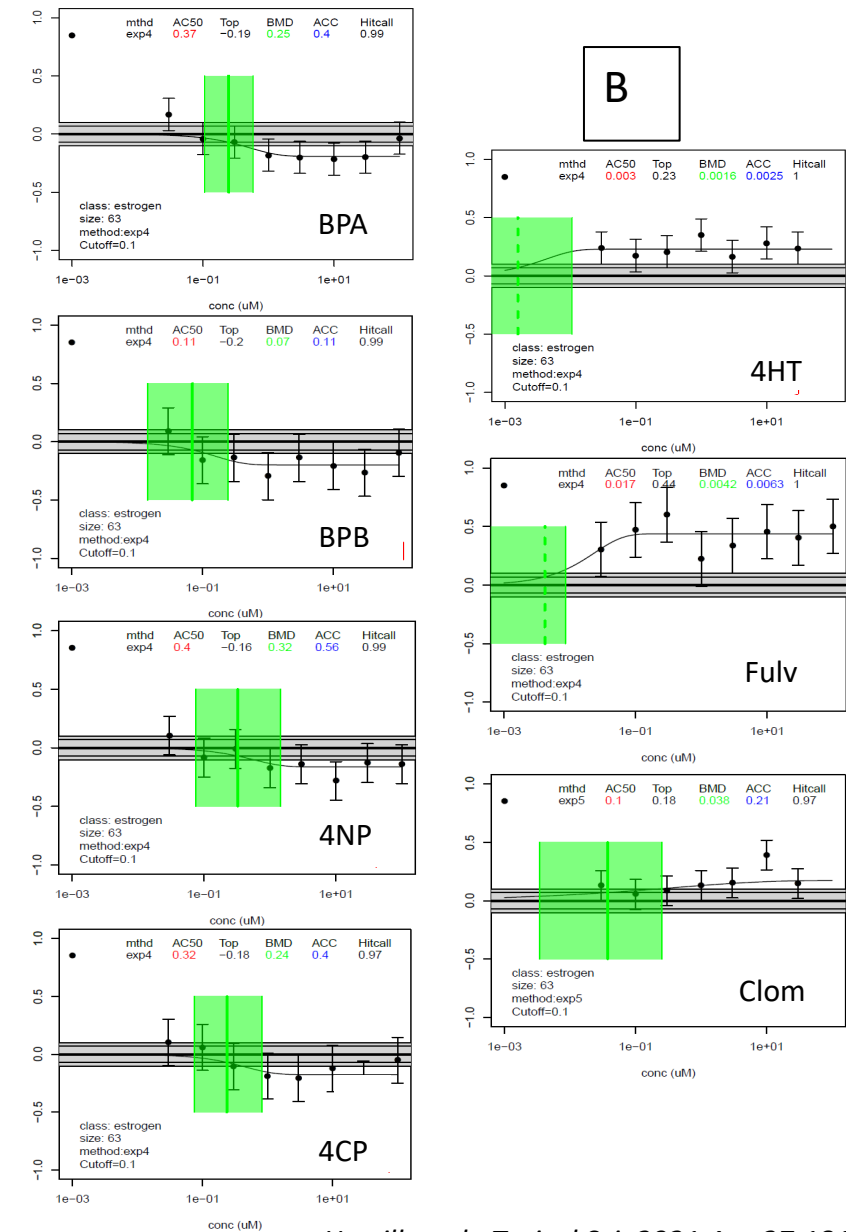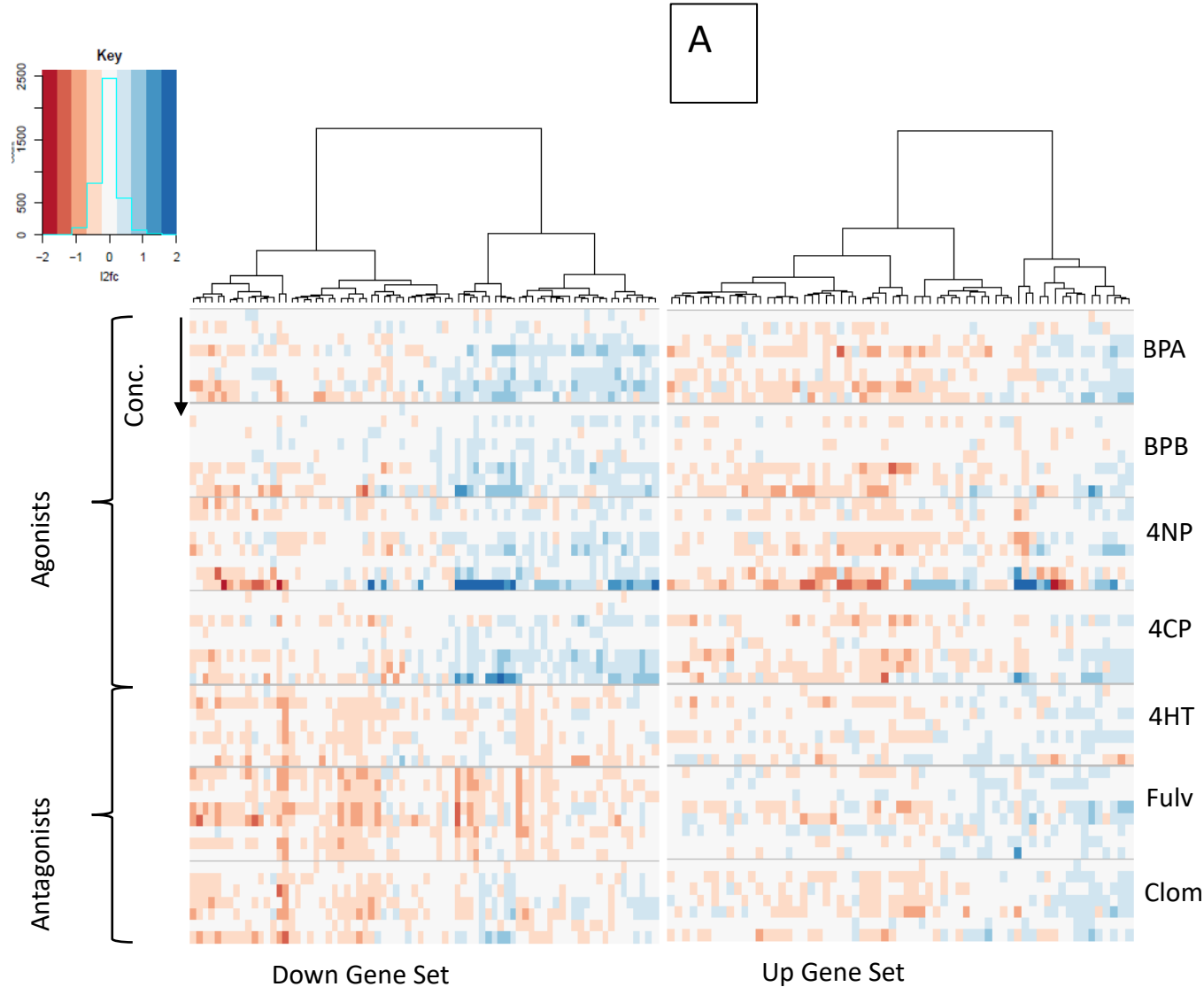
Comparison of Transcriptional BPACs to ER Model

- US EPA has developed a battery of 18 ToxCast assays to predict activity at the estrogen receptor *(Browne et al., Environ Sci Technol. 2015 Jul 21;49(14):8804-14)*

- Log_{10} AC_{50} values from the ToxCast ER model assays were compared to transcriptomic signature BPACs in MCF-7 cells for a collection of 37 estrogenic chemicals.

- Signature-based BPACs are concordant with ER model predictions. →

- Estrogen receptor is also abundantly expressed in MCF-7 cells (and other breast-derived cell lines)
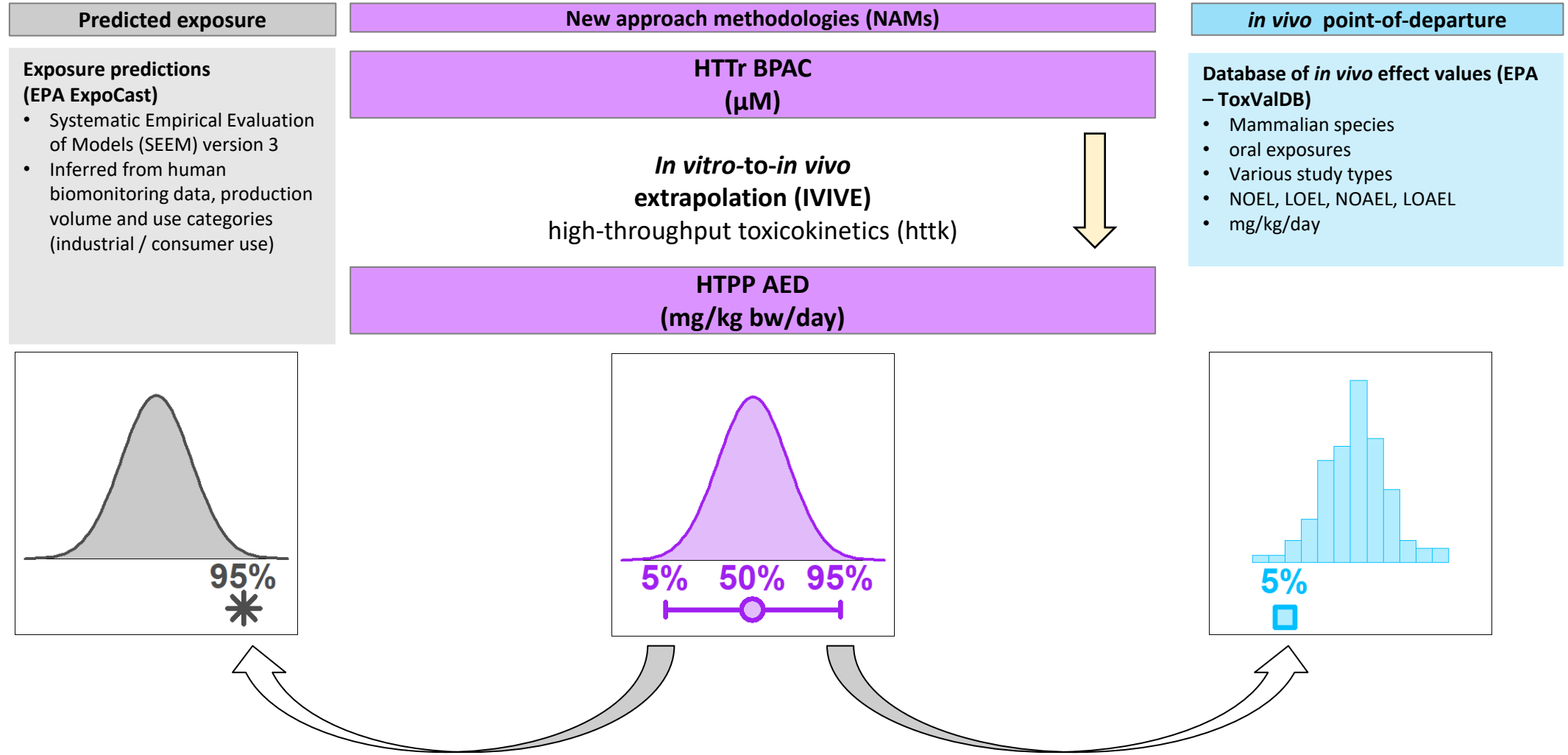

Harrill et al., (unpublished). DO NOT CITE OR QUOTE

High Throughput Transcriptomics (HTTr) Data Landscape

MCF-7 Pilot*
44 Chemicals
3 Exposure Times
+/- Stripped Media

MCF-7 Screen*
2,112 Chemicals
(ToxCast ph1-3 / e1k)
Single Exposure Time
& Media

PFAS Screen
150 Chemicals
U-2 OS + HepaRG

Volatiles
Resp. Epithelial Cells
w/ Mark Higuchi &
Adam Speen (CPHEA)

Cell Atlas
31 Cell Types
Baseline Profiles

U-2 OS Screen*
1,218 Chemicals
Single Time/Media

HepaRG Screen*
1,218 Chemicals
Single Time/Media

CPP #2
24 Cell Types
Baseline Profiles

CPP #5 *
336 Reference
Chemicals
U-2 OS + HepaRG

***Chemical Exposures:**
- 8 Concentration Series
- Regular Log10 Spacing
- 3 Replicates per Conc
- Randomized Plate Layout

Slide courtesy of Logan Everett

In Vitro to *In Vivo* Extrapolation (IVIVE) Using High-Throughput Toxicokinetic (httk) Modeling

BPAC: Biological Pathway Altering Concentration
AED: Administered Equivalent Dose

Bioactivity / *In Vivo* Effect Value Ratio Analysis

APCRA Chemicals

- **Negative ratios** indicate that AEDs derived from HTP NAMs molecular PODs are **conservative** surrogates for traditional *in vivo* PODs.

- When cell lines are considered individually, **~66-68%** of chemicals had negative ratios.

- When considered in combination, the number and percentage of chemicals with negative ratios **increased (82.3 %)**.

- Paul-Friedman et al. (2020):
  - Using ToxCast, **89 %** of APCRA chemicals had negative ratios.

- Positive ratios observed for several organophosphate and carbamate pesticides.

Harrill et al., (unpublished). DO NOT CITE OR QUOTE
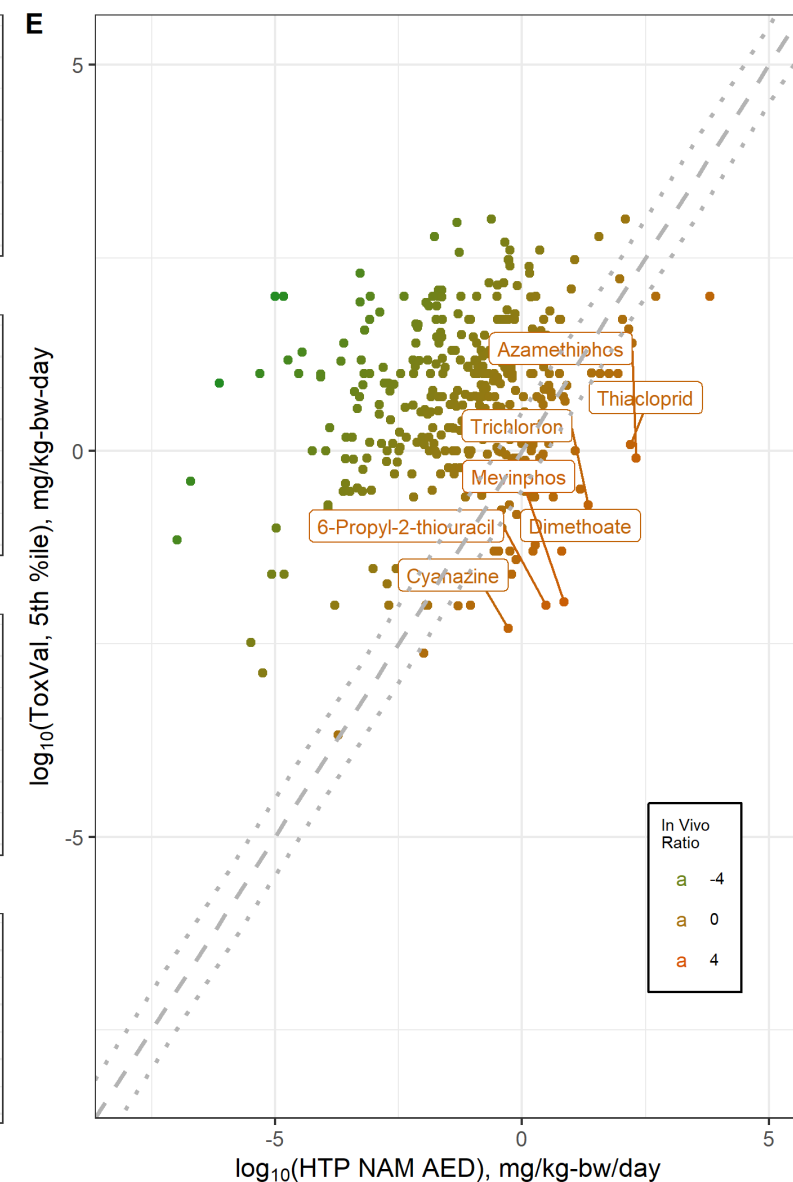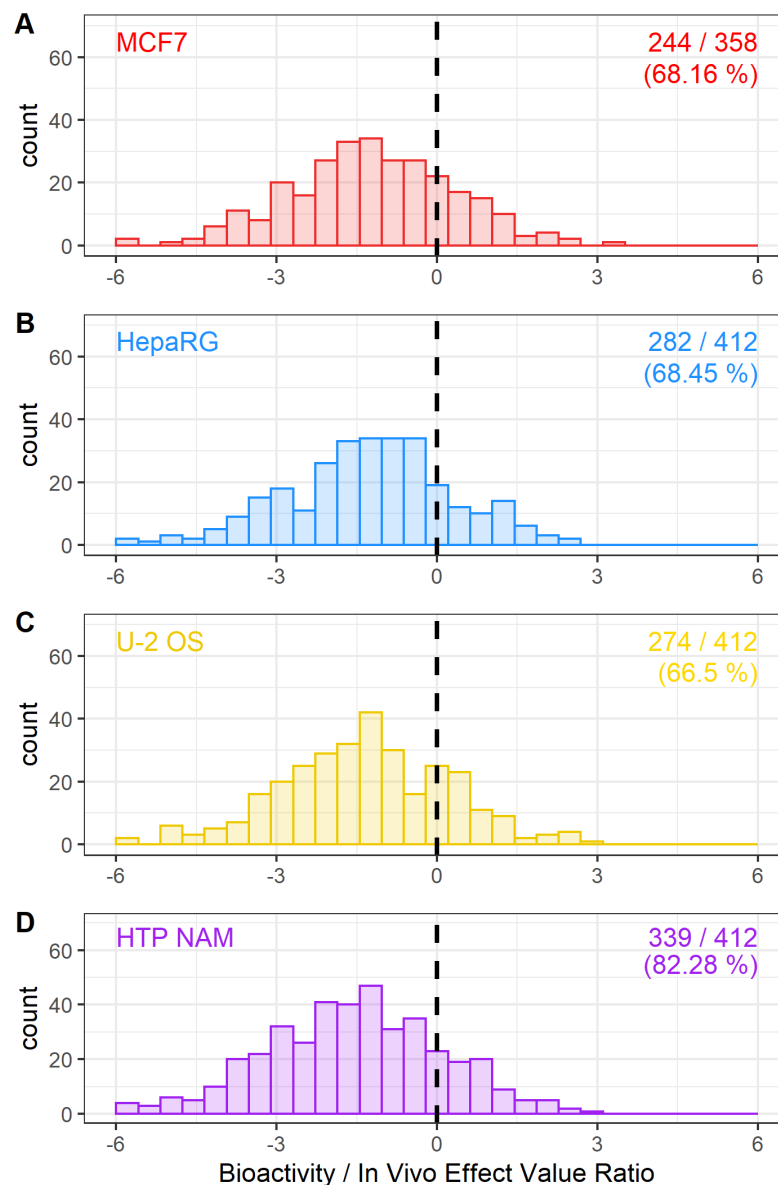
Future Directions

- **Expand chemical space**
  - Screen additional chemicals in accordance with programmatic needs / goals

- **Expand biological space**
  - Continue screening a subset of chemicals through many biologically diverse cell lines

- **Refine signature concentration-response modeling approach**
  - Reduce redundancy in signature collection
  - Continued curation of target annotation

- **Refine methods for putative target prediction & confirmation**
  - Integration with other NAM's data streams
  - Machine learning approaches
  - Bioactivity confirmation within tiered hazard evaluation framework

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