EPA United States Environmental Protection Agency

Assay Design, Reproducibility Assessment and Downstream Applications for Imaging-Based High-Throughput Phenotypic Profiling (HTPP) Data

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EPA United States Environmental Protection Agency

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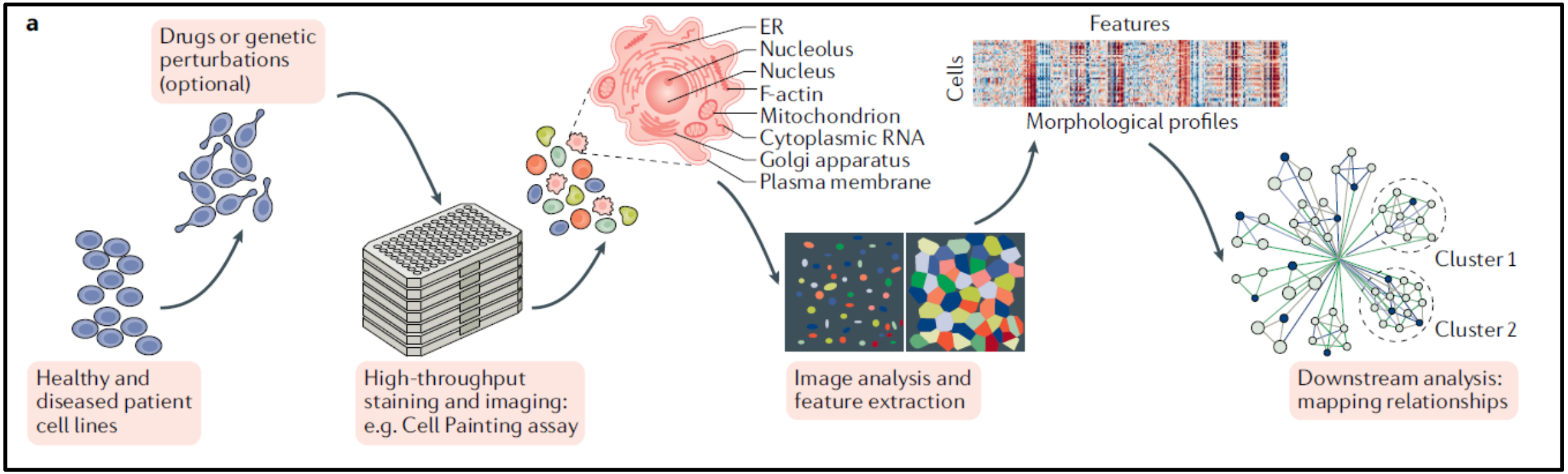
Imaging-Based High-Throughput Phenotypic Profiling (HTPP)



Chandrasekaran et al. Nat Rev Drug Discov. 2020 Dec 22:1–15

- A high-throughput testing strategy where rich information present in biological images is reduced to multidimensional numeric profiles and mined for information characteristic to a chemical's biological activity.
- Originated in the pharmaceutical sector and has been used in drug development to understand disease mechanisms and predict chemical activity, toxicity and/or mechanism-of-action.

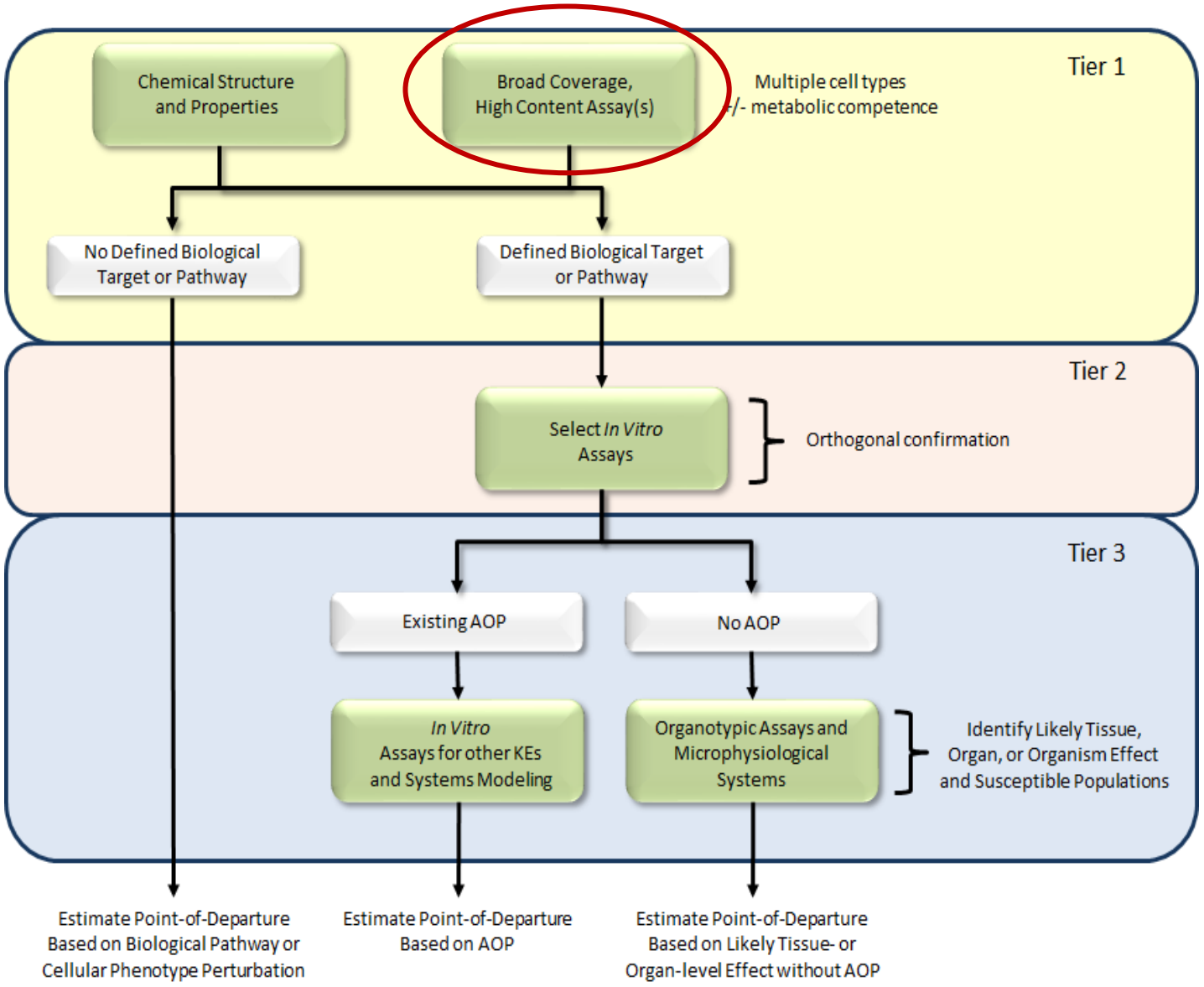
Tiered Hazard Evaluation Approach (1)

- **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

HTPP with the Cell Painting Assay

Cell Painting is a profiling method that measures a large variety of phenotypic features in fluoroprobe labeled cells *in vitro*.

- High-throughput
- Scalable
- Amenable to lab automation
- Deployable across multiple human-derived cell types.
- Reproducible
- Cost-effective (¢ / well)
- Infrastructure investment
- High volume data management

Laboratory & bioinformatics workflows for conduct of this assay have been established at CCTE.

OPEN ACCESS Freely available online

PLOS ONE

Multiplex Cytological Profiling Assay to Measure Diverse Cellular States

Sigrun M. Gustafsdottir, Vebjorn Ljosa, Katherine L. Sokolnicki, J. Anthony Wilson, Deepika Walpita, Melissa M. Kemp, Kathleen Petri Seiler, Hyman A. Carrel, Todd R. Golub, Stuart L. Schreiber, Paul A. Clemons, Anne E. Carpenter, Alykhan F. Shamji

Broad Institute of Harvard and MIT, Cambridge, Massachusetts, United States of America

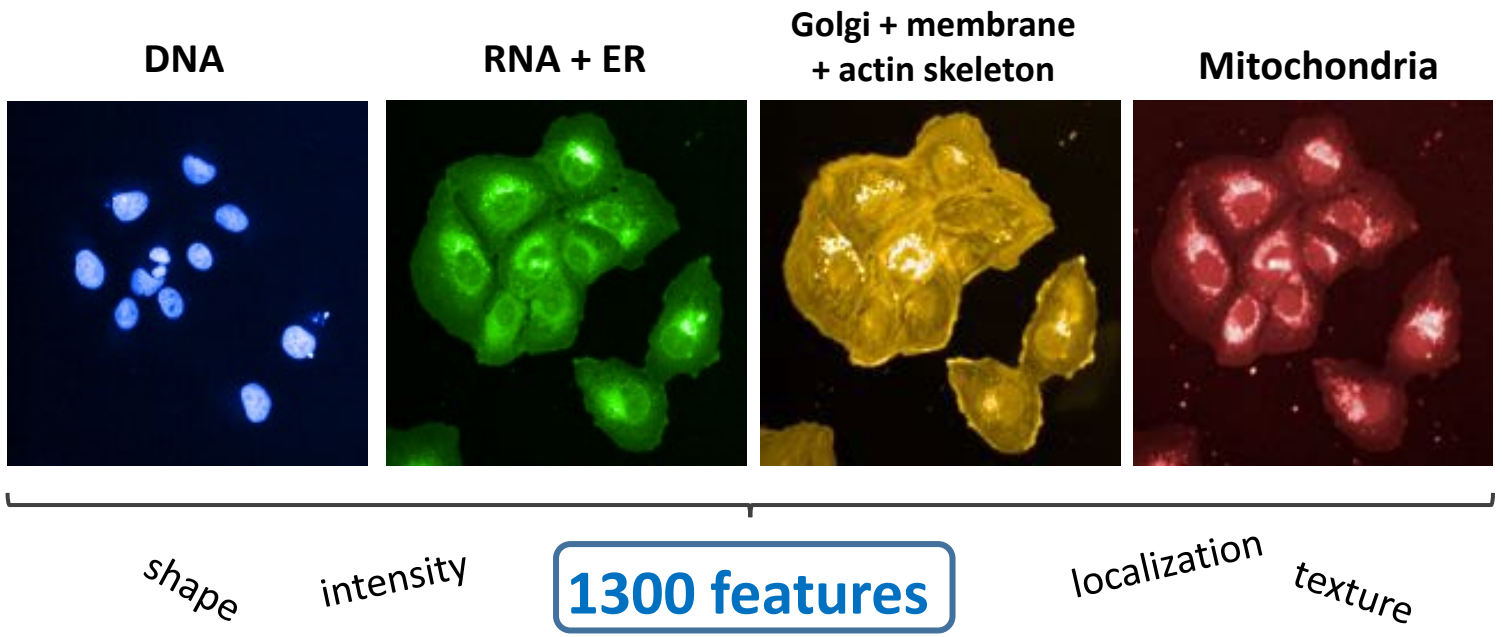
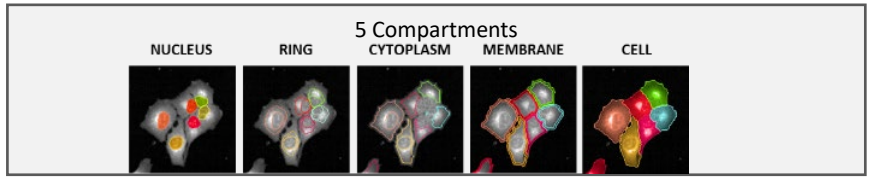
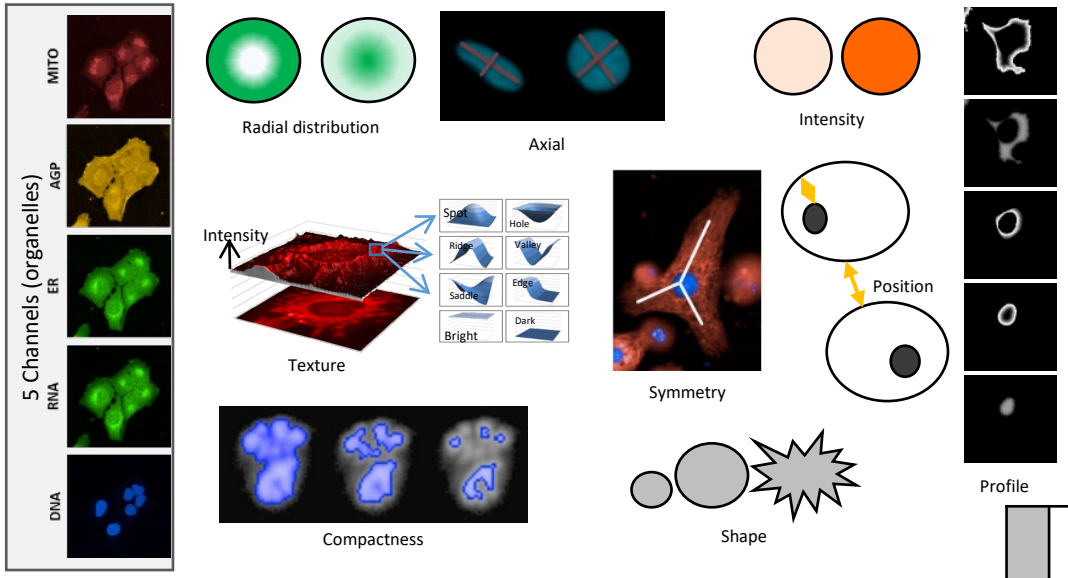


Image Acquisition & Phenotypic Feature Extraction





1300 features / cell

49 Feature Categories
(ex. MITO_Texture_Cytoplasm)

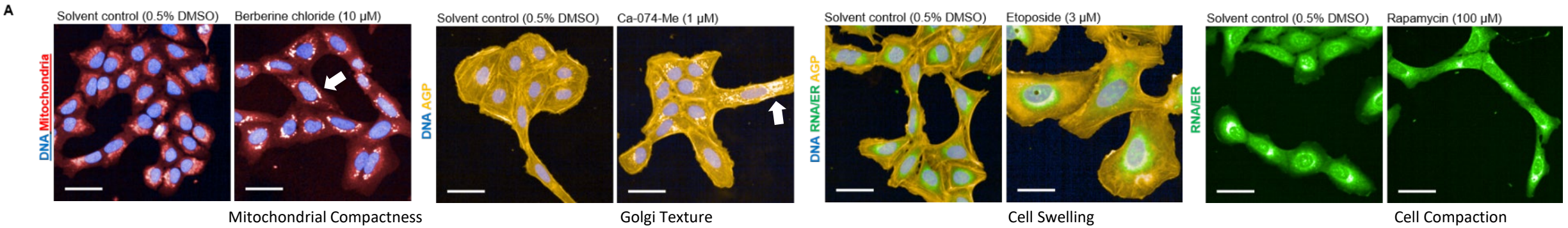
Channel	Module: Position [7]	Module: Basic morph-ology [5]	Module: SCARP morphology: Symmetry [80]	Module: SCARP morphology: Compactness [40]	Module: SCARP morphology: Axial [20]	Module: SCARP morphology: Radial [28]	Module: SCARP morphology: Profile [20-30]	Module: Intensity [9]	Module: Texture [14]
DNA			Nuclei	Nuclei	Nuclei	Nuclei Cell	Nuclei Cytoplasm	Nuclei	Nuclei
RNA			Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei	Nuclei
ER			Cell	Cell	Cell	Cell	Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
AGP			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm Membrane	Ring Cytoplasm Membrane
Mito			Cell	Cell	Cell	Cell	Nuclei Cytoplasm	Ring Cytoplasm	Ring Cytoplasm
Not associated with a channel	Nuclei Cell	Nuclei Cell							

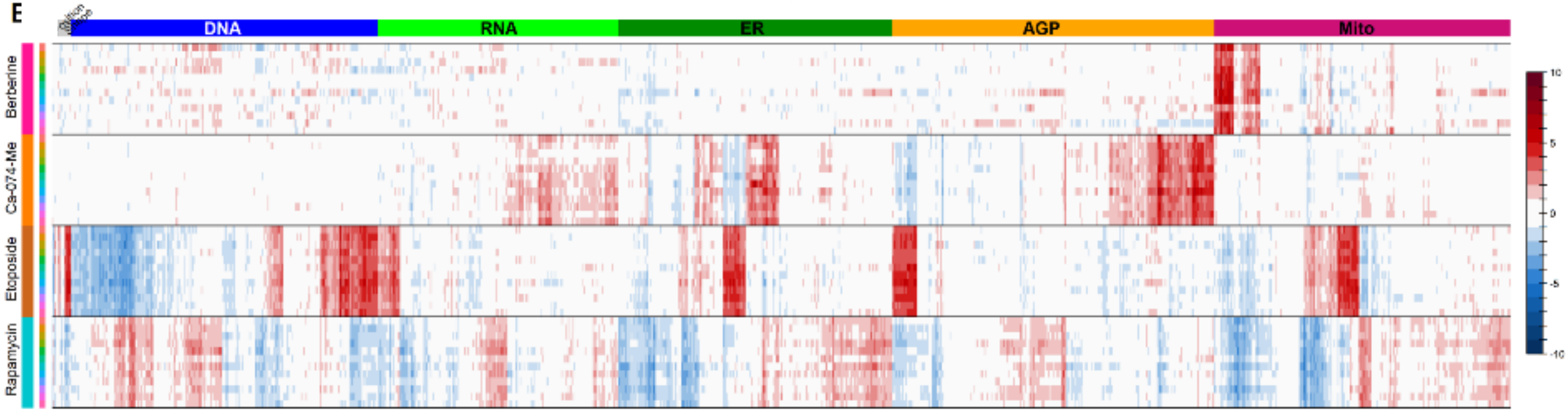
PerkinElmer Opera Phenix

Modality: Confocal (single *z*)
Objective: 20X Water
Plate: CellCarrier-384 Ultra
Fields: 5 or 9

With illustrations from Perkin Elmer

Examples of Chemical Induced Phenotypes



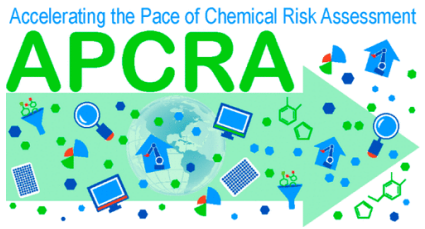


- Strong phenotypes are observed qualitatively and produce distinct profiles when measured quantitatively.

Adapted from Nyffeler et al. Toxicol Appl Pharmacol. 2020 Jan 15;389:114876

U-2 OS ToxCast Screen Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	U-2 OS
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	1,202	TSCA Chemicals of interest to USEPA **Includes 462 APCRA case study chemicals** **Includes 179 chemicals with annotated molecular targets**
Time Points:	1	24 hours
Assay Formats:	2	High Throughput Phenotypic Profiling (Cell Painting) *High Throughput Transcriptomics (TempO-Seq)*
Concentrations:	8	3.5 $\log_{10}$ units; ~half-$\log_{10}$ spacing
Biological Replicates:	4	--



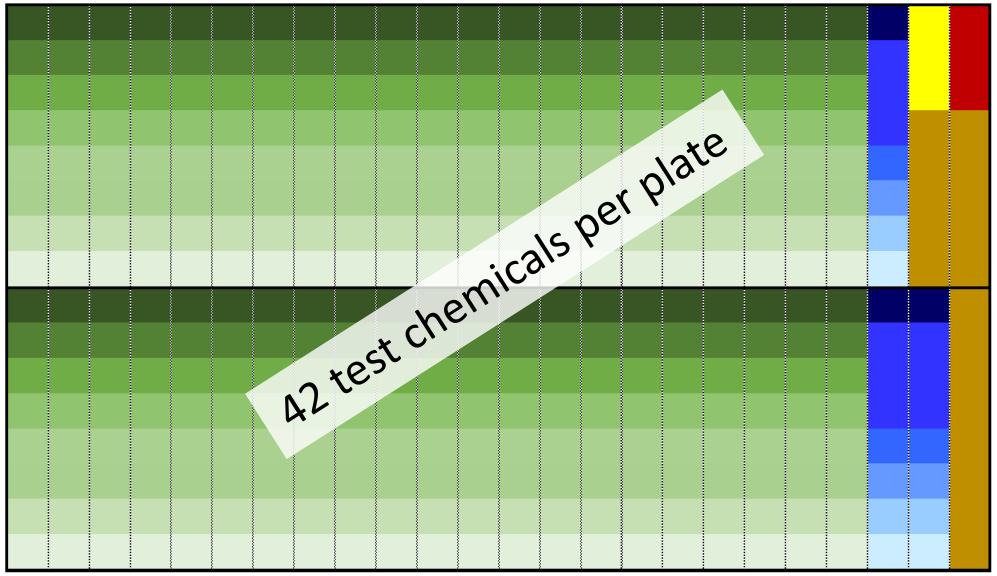
Kavlock et al. (2018)
Chem. Res. Tox; 31(5): 287-290

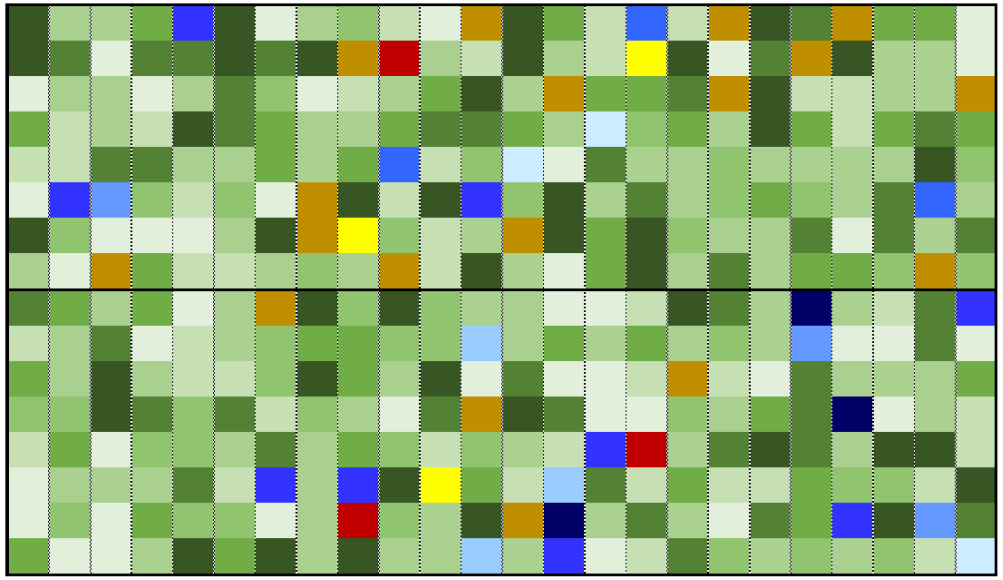
International collaboration of regulatory scientists focused on next generation chemical risk assessment including **deriving quantitative estimates of risk based on NAM-derived potency information and computational exposure estimates.**

APCRA Chemicals → PK parameters necessary for *in vitro* to *in vivo* extrapolation (IVIVE)
in vivo toxicity data

Preliminary results. Do not cite or quote.

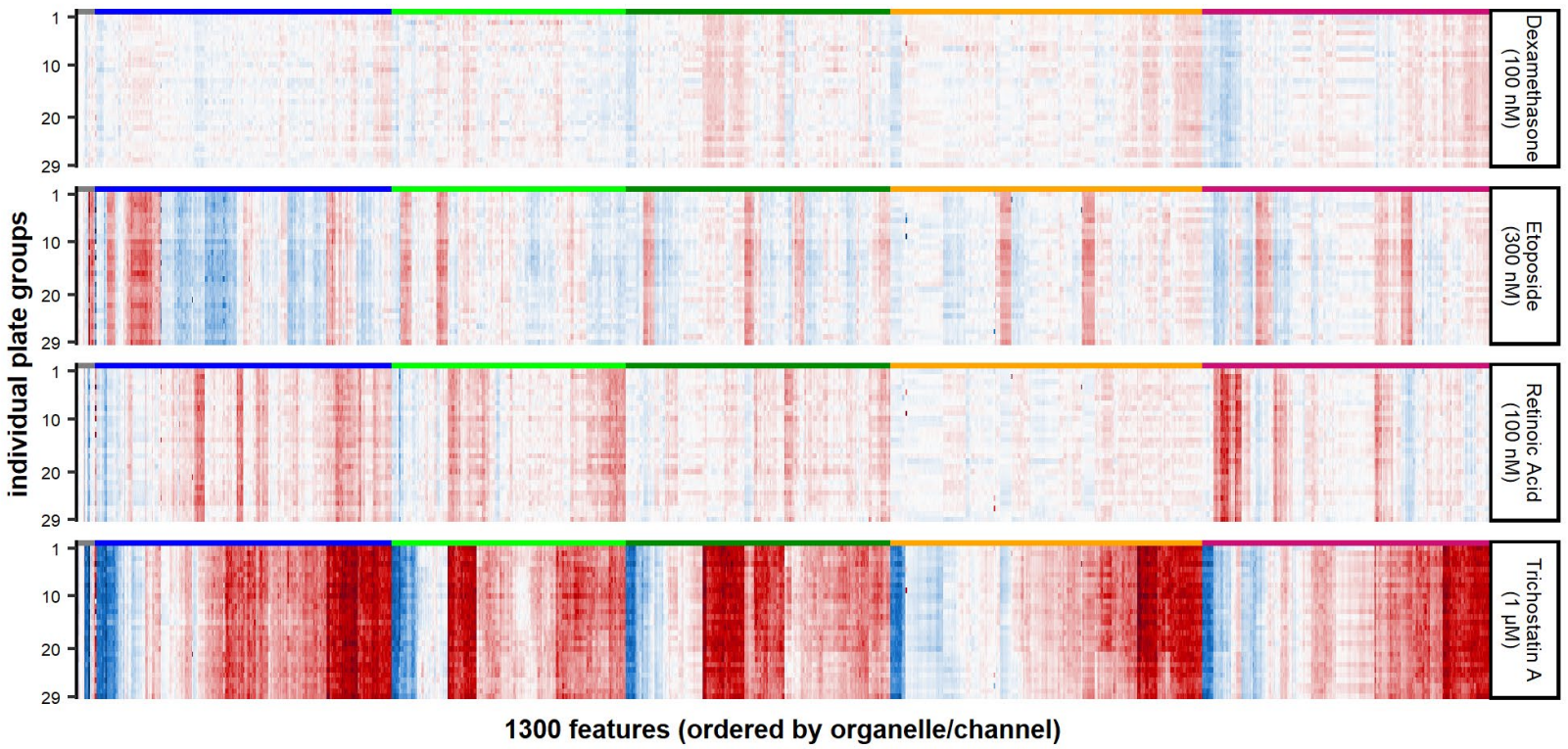
U-2 OS ToxCast Screen Dose Plate Design

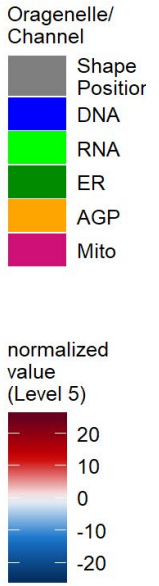


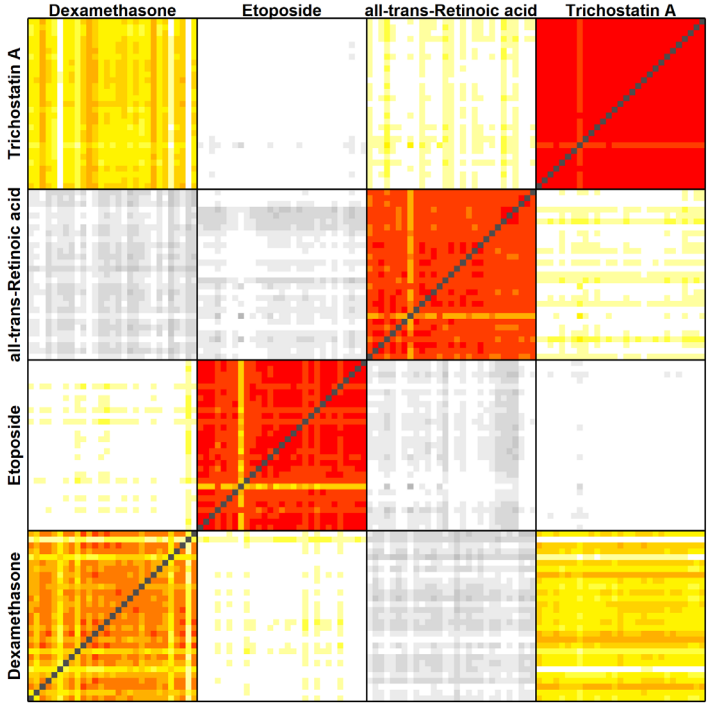


Label	Reference Chemicals:	Molecular Mechanism-of-Action	Test Concentrations
A	Etoposide	DNA topoisomerase inhibitor	0.03 - 10 µM
B	all-trans-Retinoic Acid	Retinoic acid receptor agonist	0.0003 – 1 µM
C	Dexamethasone	Glucocorticoid receptor agonist	0.001 – 3 µM
D	Trichostatin A	Histone deacetylase inhibitor	1 µM
E	Staurosporine	Cytotoxicity control	1 µM
F	DMSO	Vehicle control	0.5 %

Assay Performance / Reproducibility







⇨ **Reference chemicals produce <u>reproducible</u> and <u>distinct</u> profiles.**

HTPP Data Analysis Pipeline

Data reduction

cell-level data

Normalization
MAD normalization

$$\frac{\text{cell value} - \text{median}_{DMSO}}{1.4826\ \text{MAD}_{DMSO}}$$

normalized cell-level data

Aggregation
median

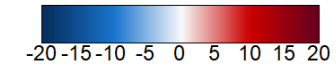
well-level data

Standardization
Z transformation

Cell Count Info
Conc. > 50% cell loss

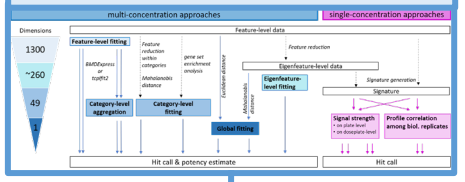
scaled well-level data

clipped well-level data



Concentration Response Modeling

Calculate Response Metrics



See Nyffeler et al. SLAS Discov. 2021 Feb;26(2):292-308.

Fit Multiple Curve Shapes

Best Model Selection

BMC

Berberine chloride
Mito_Cells_Morph_STAR

Preliminary results. Do not cite or quote.

Comparison of Concentration Response Analysis Approaches

Comparison of Approaches for Determining Bioactivity Hits from High-Dimensional Profiling Data

Johanna Nyffeler[1,2], Derik E. Haggard[1,2], Clinton Willis[1,3], R. Woodrow Setzer[1], Richard Judson[1], Katie Paul-Friedman[1], Logan J. Everett[1], and Joshua A. Harrill[1]

SLAS Discovery
2021, Vol. 26(2) 292–308

DOI: 10.1177/2472555220950245
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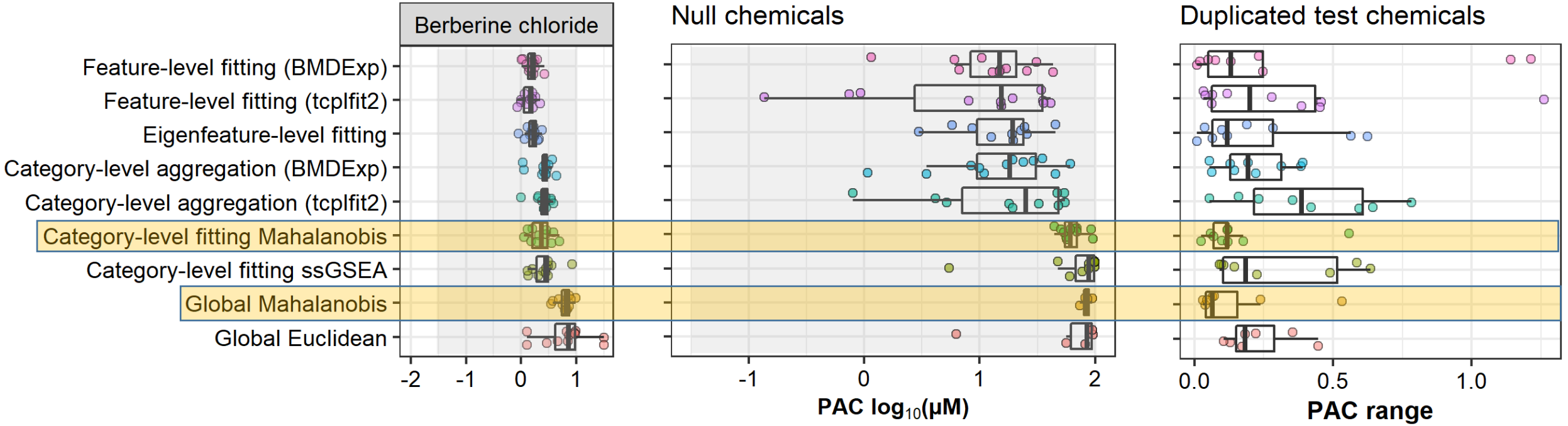
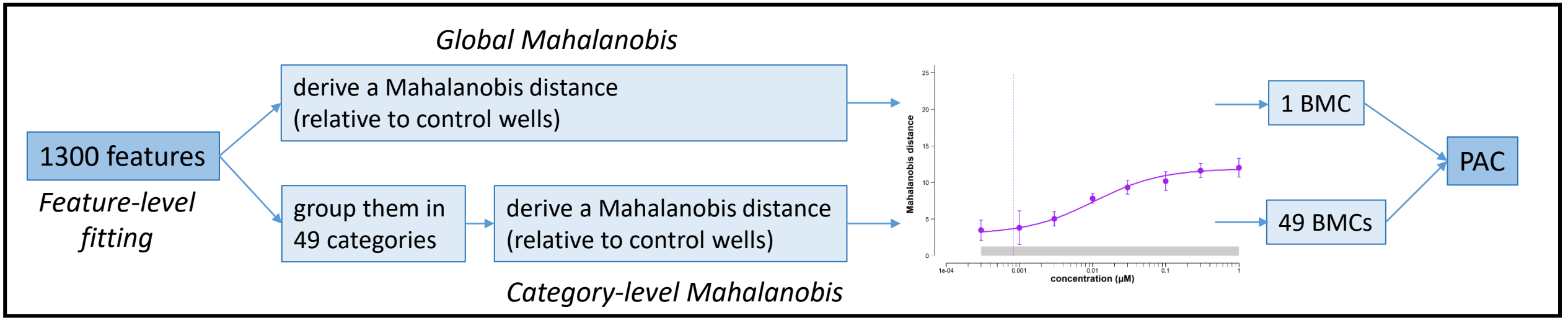


- **Analysis of reference chemicals identified methods that 1) minimized false positives and 2) maximized reproducibility of potency estimates.**

Phenotype Altering Concentrations (PACs)

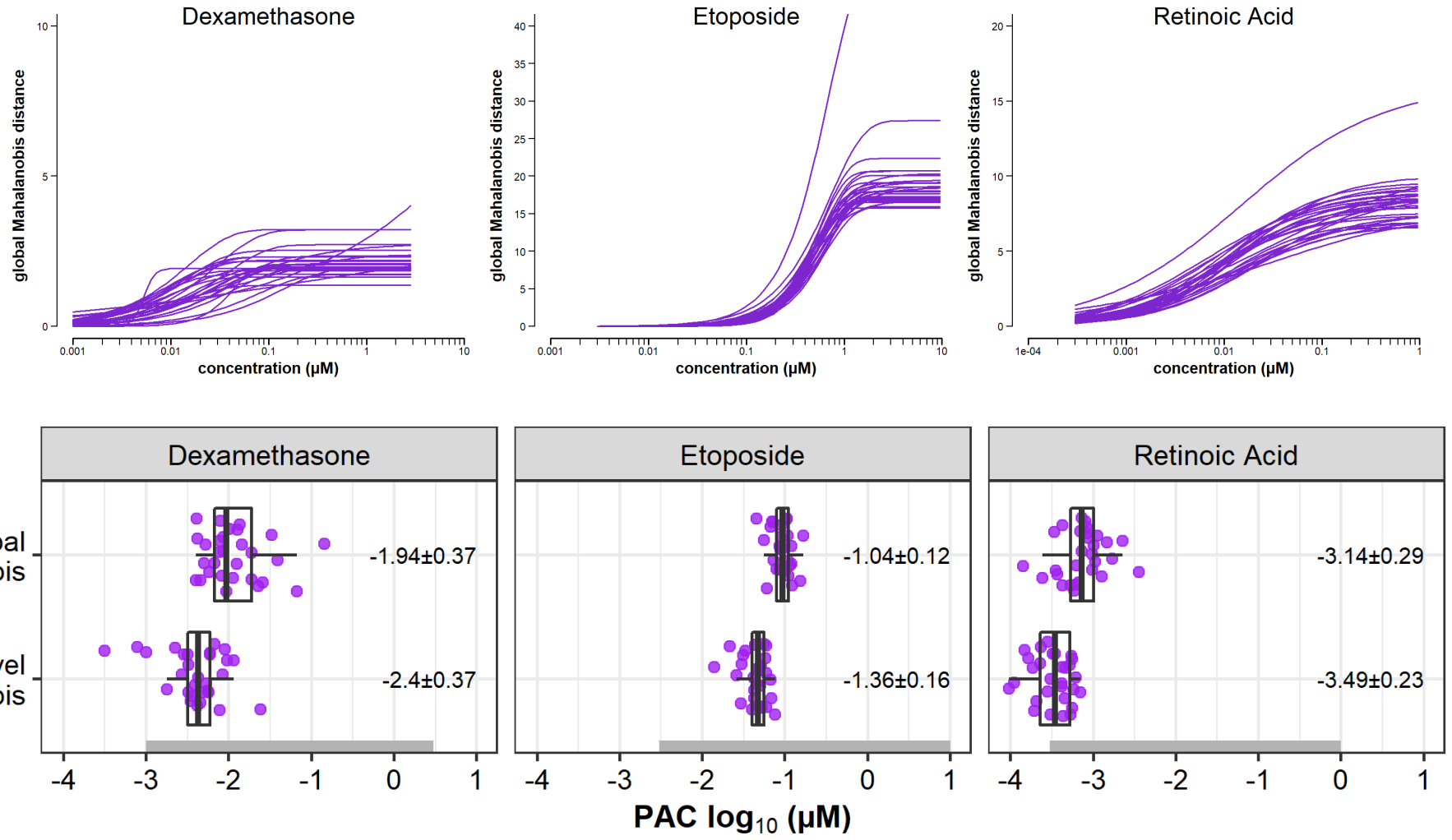
Mahalanobis Distance (D_M):

- A multivariate metric that measures the distance between a treatment and a distribution of controls in feature space.
- Accounts for unpredictable changes in cell states across test concentrations and inherent correlations in profiling data.



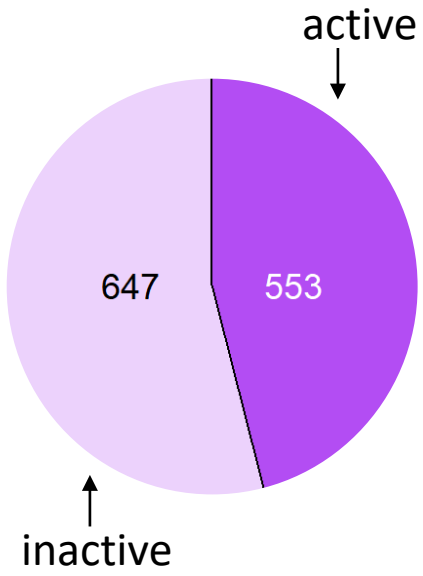
- Chemicals where a BMC can be determined using either the global or category D_M approach are considered active.
- The minimum of the global or most sensitive category BMC is the **Phenotype Altering Concentration (PAC)**

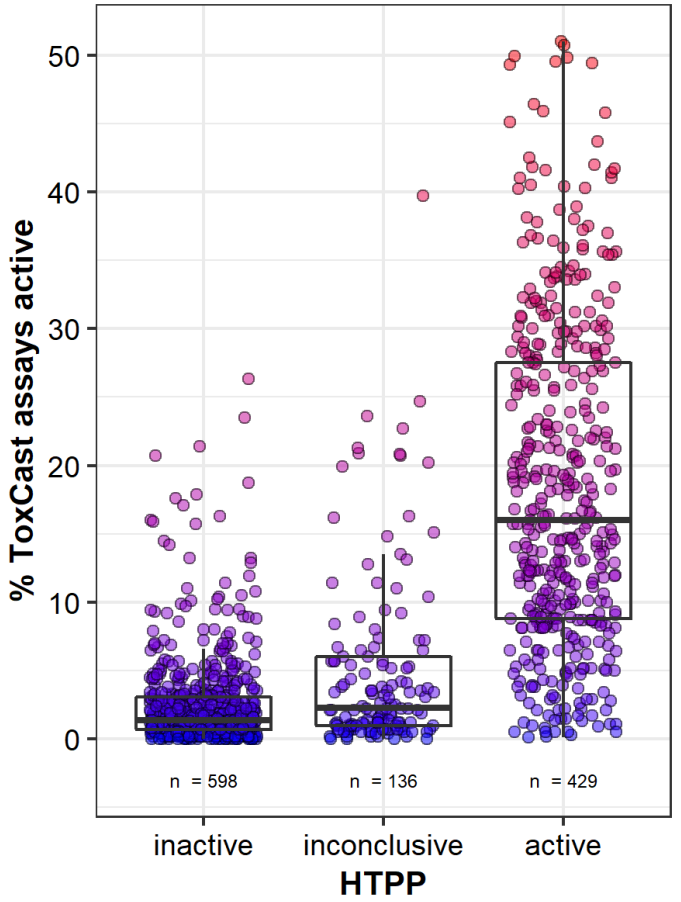
Reproducibility: Potencies

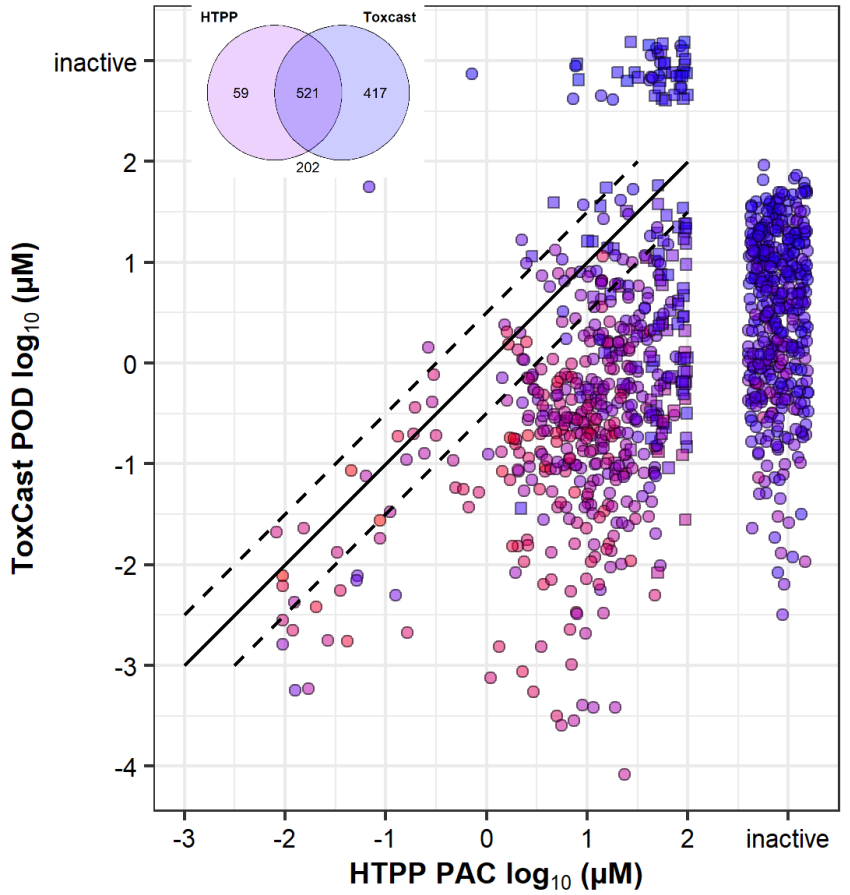


⇨ **Potency estimates vary less than ½ an order of magnitude**

U-2 OS ToxCast Screen Results







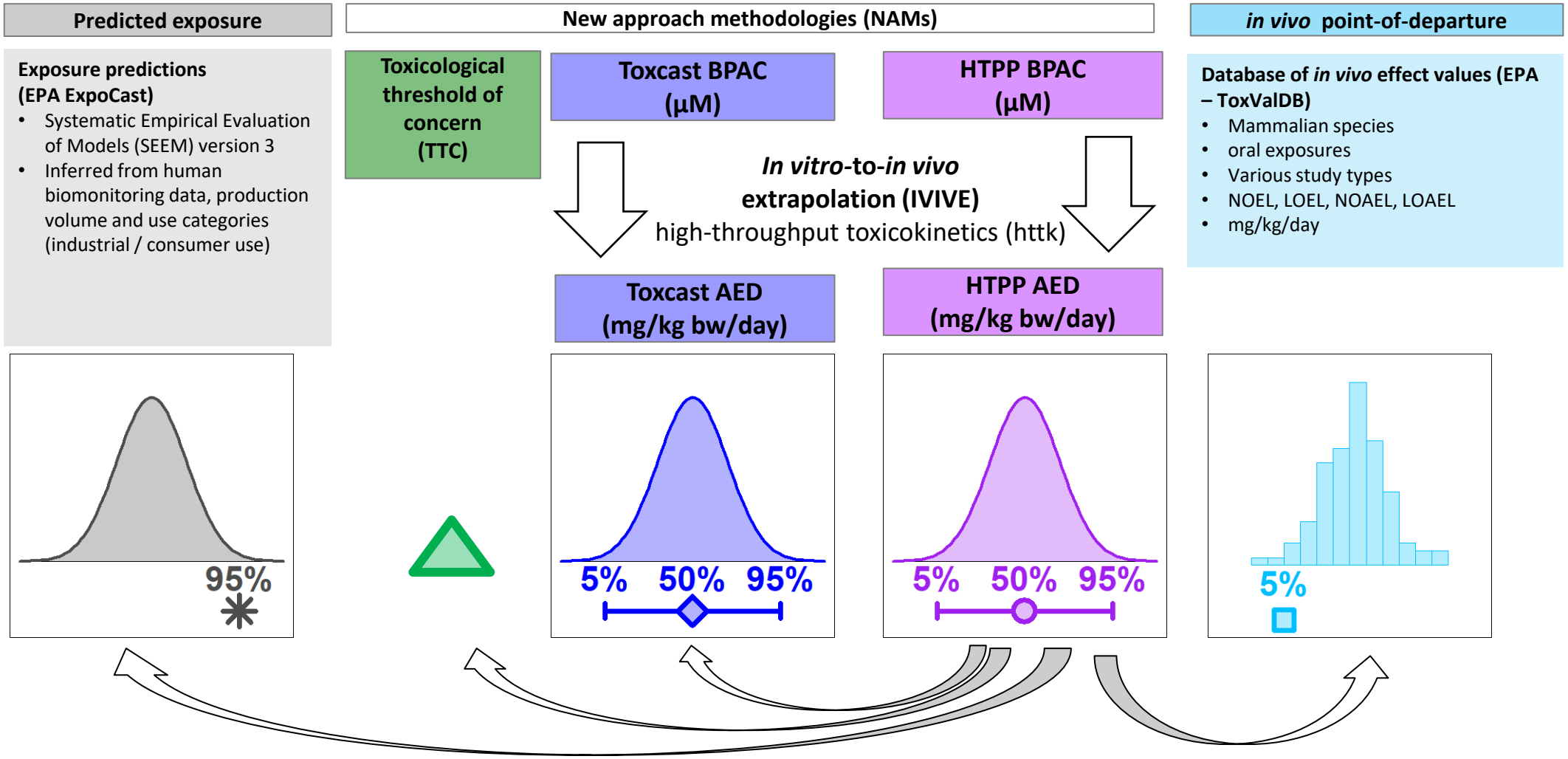
Group
- inactive
- inconclusive
- active

% ToxCast active
50
40
30
20
10
0

⇨ **Potency estimates vary less than ½ an order of magnitude**

Preliminary results. Do not cite or quote.

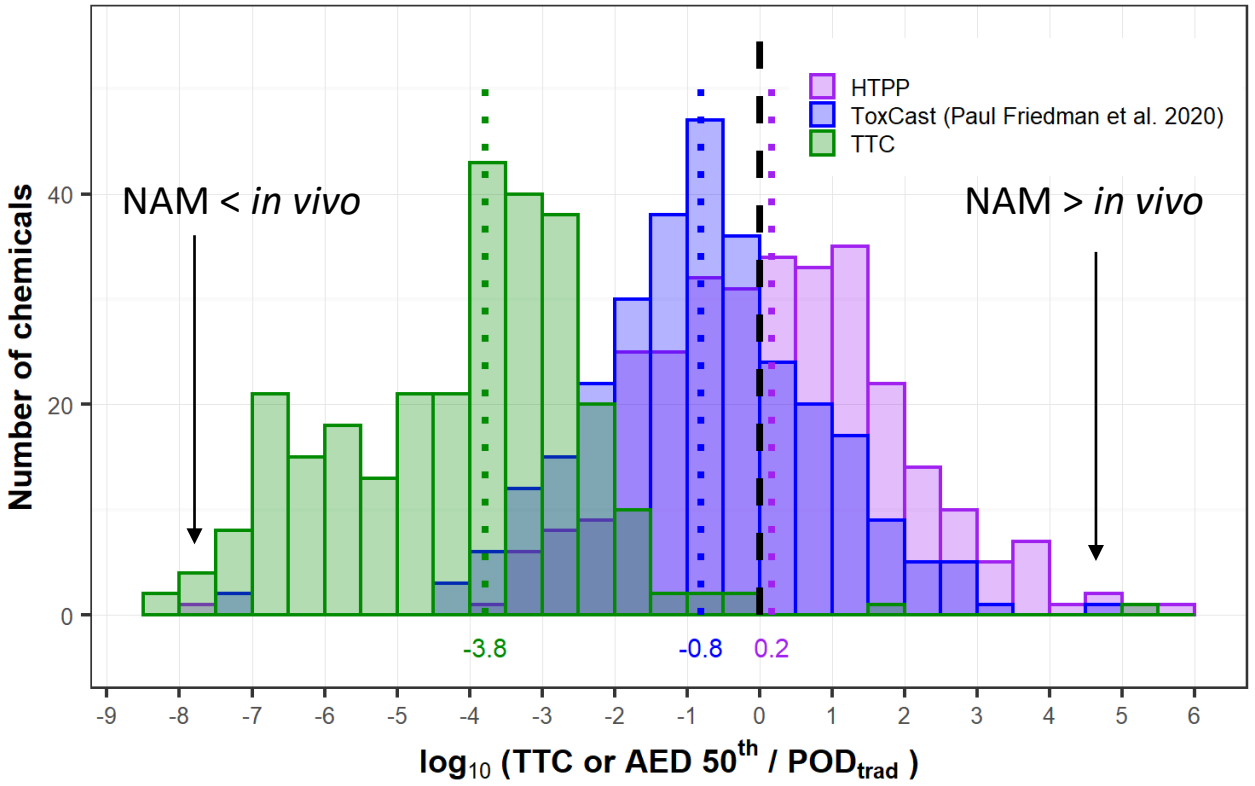
In Vitro to *In Vivo* Extrapolation (IVIVE) & Comparison to *In Vivo* Toxicity Data & Exposure Estimates

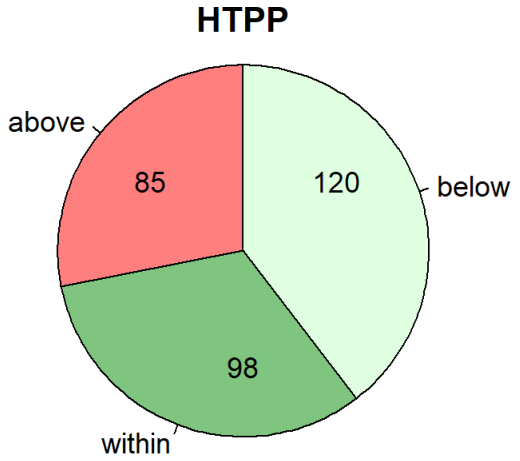


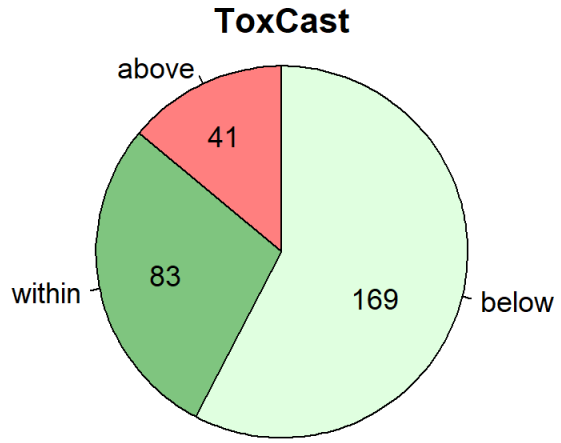
POD: point-of-departure
AED: administered equivalent dose

Comparison to *In Vivo* Effect Values & Other NAMs

- 303 chemicals were active and had pharmacokinetic (PK) information



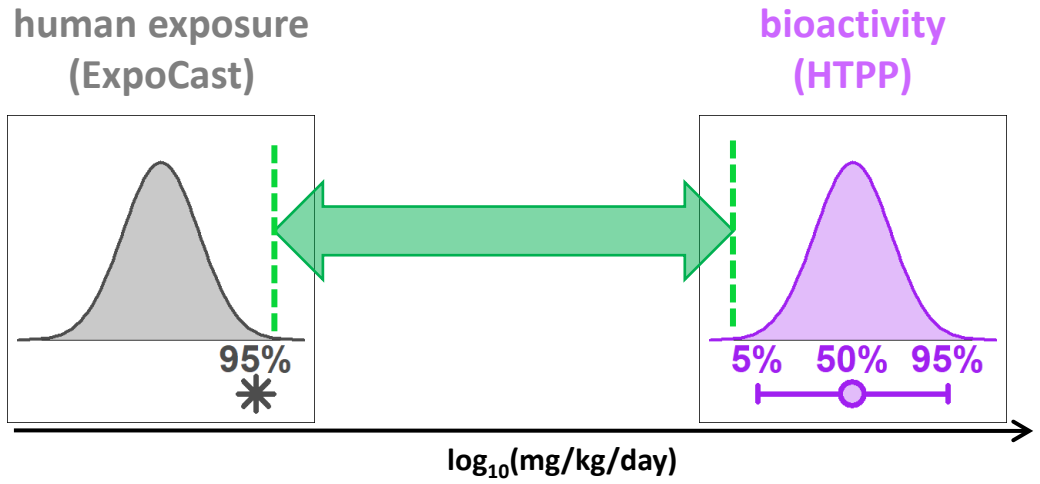


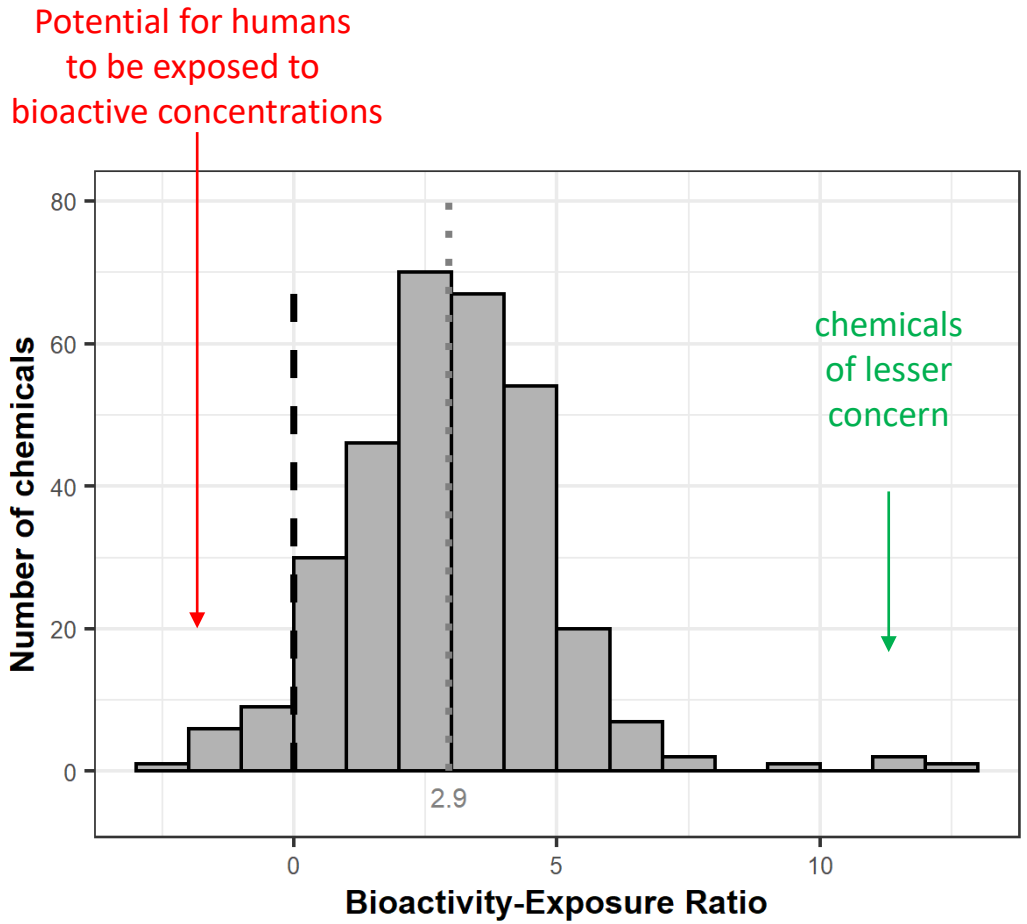


⇨ **HTPP AEDs are higher than ToxCast-derived AEDs and TTC values**

⇨ **78% of HTPP AED are within 2 orders of magnitude of the *in vivo* POD**

Comparison to Exposure Estimates





- for 49% of chemicals, predicted exposure is > 1000x lower than estimated bioactivity
- for a small set of chemicals, the BER was negative, indicating a potential for humans to be exposed to bioactive concentrations of these chemicals

Summary and Conclusions

- **High-Throughput Profiling:** Developed experimental designs and scalable laboratory workflows for high-throughput phenotypic profiling (HTPP) of environmental chemicals that can be used in multiple human-derived cell types.
- **Potency Estimation:** Developed high-throughput concentration-response modeling workflows to identify thresholds for perturbation of cell morphology (e.g. PACs).
- **IVIVE:** Potency estimates can be converted to administered equivalent doses (AEDs) using high-throughput toxicokinetic modeling.
- **Bioactivity to *In Vivo* Effect Value Ratio Analysis:** AEDs derived from the HTPP assay were conservative or equivalent to traditional PODs a majority of the time.
- **Bioactivity to Exposure Ratio (BER) Analysis:** AEDs derived from the HTPP assay were compared to high-throughput exposure predictions. There were very few chemicals where AEDs were within the range of exposure predictions.
- **Comparison to ToxCast:** Applications using HTPP NAMs potencies as input yielded comparable results compared to the use of ToxCast NAMs potencies.

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