EPA United States Environmental Protection Agency

Bioactivity Screening of Environmental Chemicals Using Cell Painting: Molecular Point of Departure Determination and Mechanistic Prediction Via Profile Similarity

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Disclaimer

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EPA United States Environmental Protection Agency

Outline

- **Background**
  - In vitro hazard assessment
  - High throughput phenotypic profiling (HTPP) using Cell Painting
- **Applications**
  - Chemical bioactivity screening
  - Molecular point of departure estimation
  - Chemical similarity based on profile matching
  - In vitro to in vivo extrapolation (IVIVE)
  - Bioactivity:exposure ratio (BER) analysis
- **Closing thoughts**

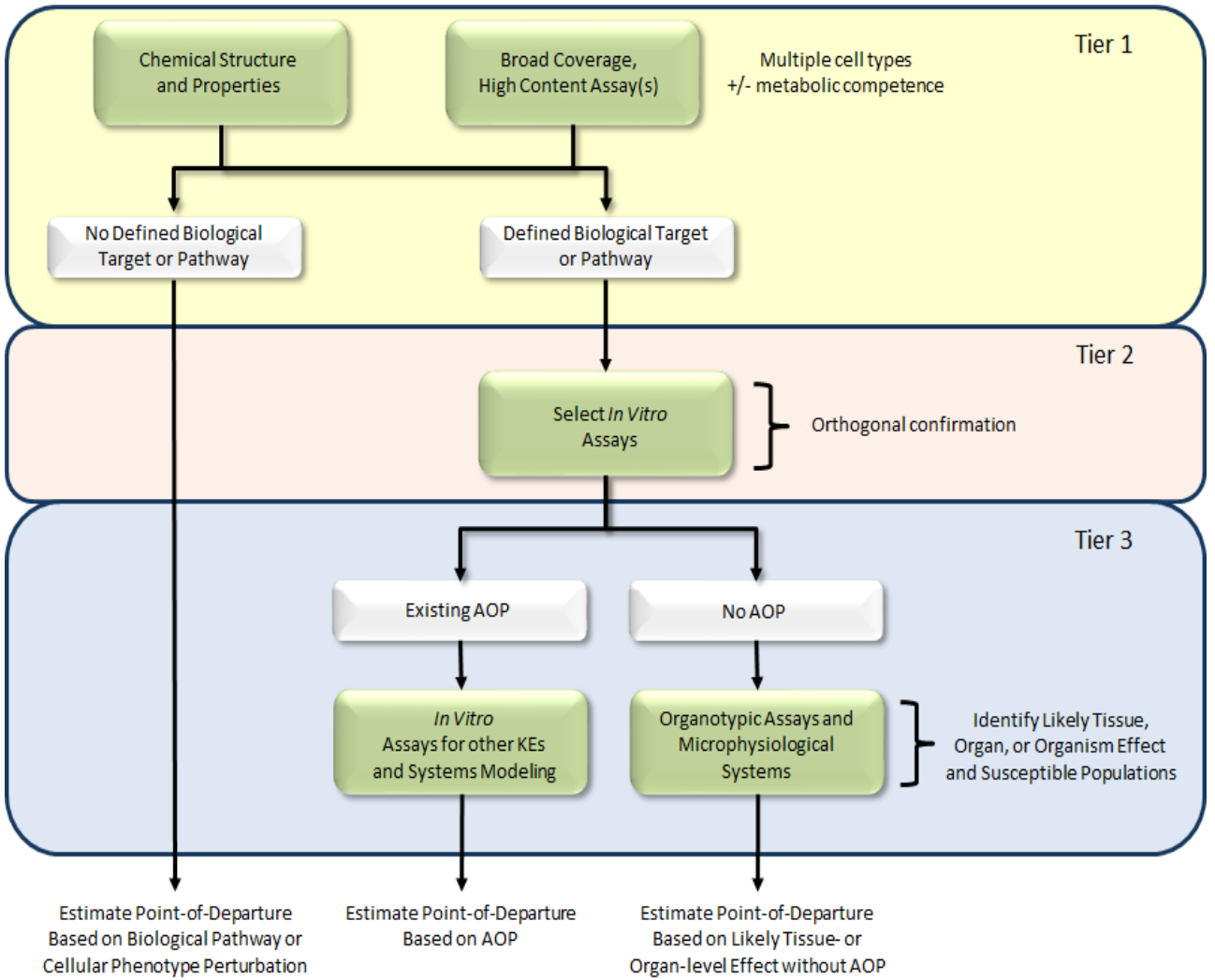
NAMs-Based, Tiered Hazard Evaluation Strategy

- **US EPA** is committed to **reducing, replacing and refining** the use of mammals in toxicity testing.

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

- US EPA CompTox Blueprint proposes a NAMs-based hazard evaluations strategy that advocates the use of **high throughput profiling (HTP) assays** in the initial tier of test.

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



The NexGen Blueprint of CompTox at USEPA, Tox. Sci. 2019; 169(2):317-322

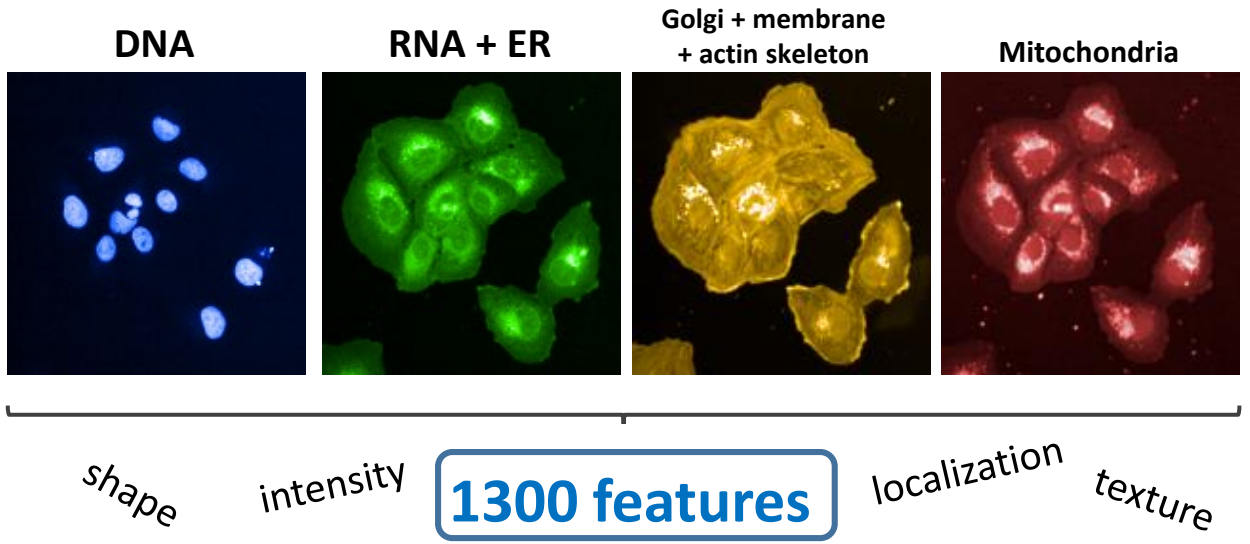
High Throughput Phenotypic Profiling (HTPP) with Cell Painting

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

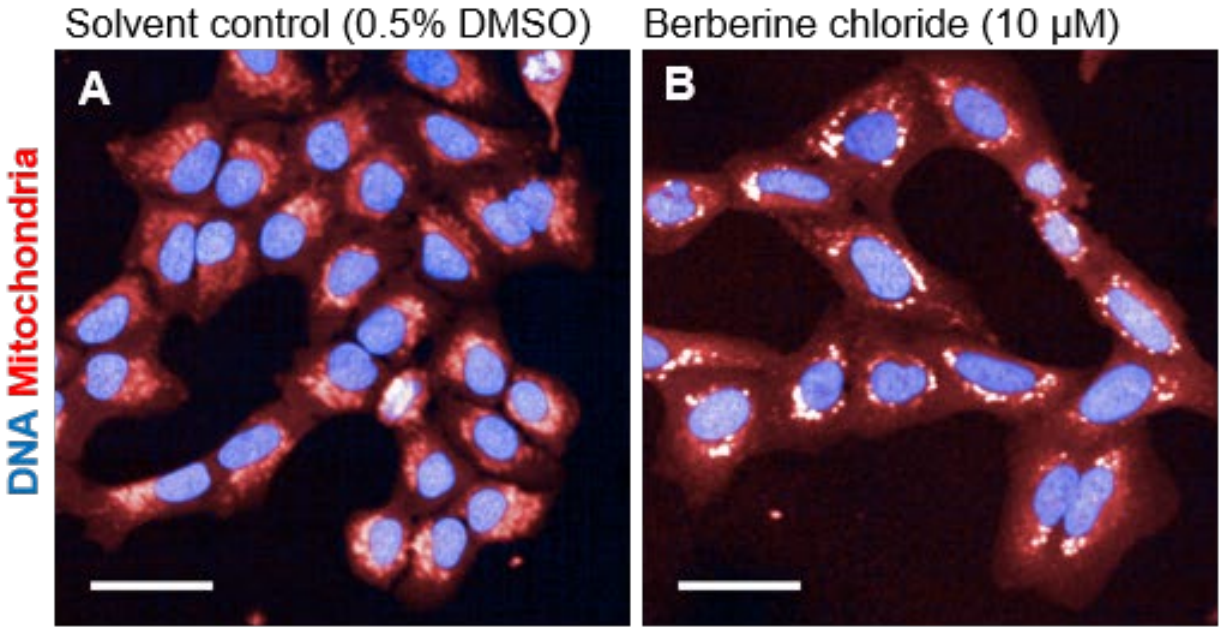
- **Previous Uses:**
  - Functional genomics
  - Drug discovery
  - Compound efficacy and toxicity screening
  - Mechanism-of-action identification
  - Chemical grouping

- **Efficient** and **cost-effective** method for evaluating the bioactivity of environmental chemicals.

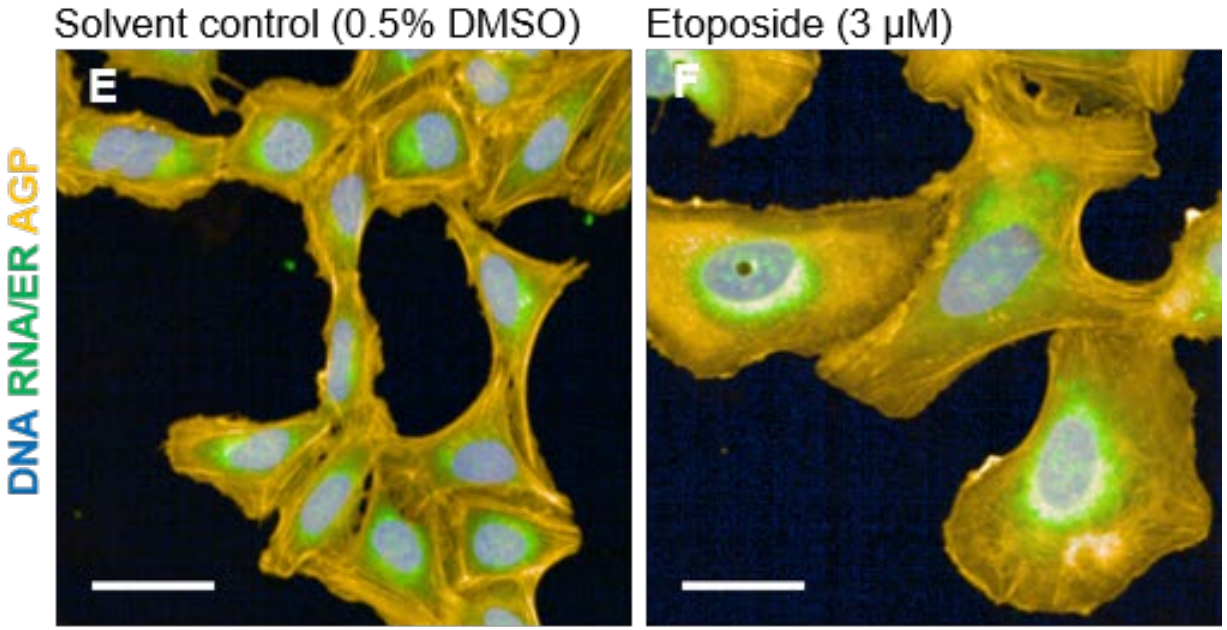
Marker	Cellular Component	Labeling Chemistry	Labeling Phase	Opera Phenix	
				Ex.	Em.
Hoechst 33342	Nucleus	Bisbenzamide probe that binds to dsDNA	Fixed	405	480
Concanavalin A – AlexaFluor 488	Endoplasmic reticulum	Lectin that selectively binds to α-mannopyranosyl and α-glucopyranosyl residues enriched in rough endoplasmic reticulum		435	550
SYTO 14 nucleic acid stain	Nucleoli	Cyanine probe that binds to ssRNA		435	550
Wheat germ agglutinin (WGA) – AlexaFluor 555	Golgi Apparatus and Plasma Membrane	Lectin that selectively binds to sialic acid and N-acetylglucosaminyl residues enriched in the trans-Golgi network and plasma membrane		570	630
Phalloidin –AlexaFluor 568	F-actin (cytoskeleton)	Phallotoxin (bicyclic heptapeptide) that binds filamentous actin			
MitoTracker Deep Red	Mitochondria	Accumulates in active mitochondria	Live	650	760



Examples of Chemical-Induced Phenotypes



→ Mitochondrial compactness/texture



→ Cells are larger

⇨ **Strong phenotypes are observable qualitatively and can be measured quantitatively using Cell Painting**

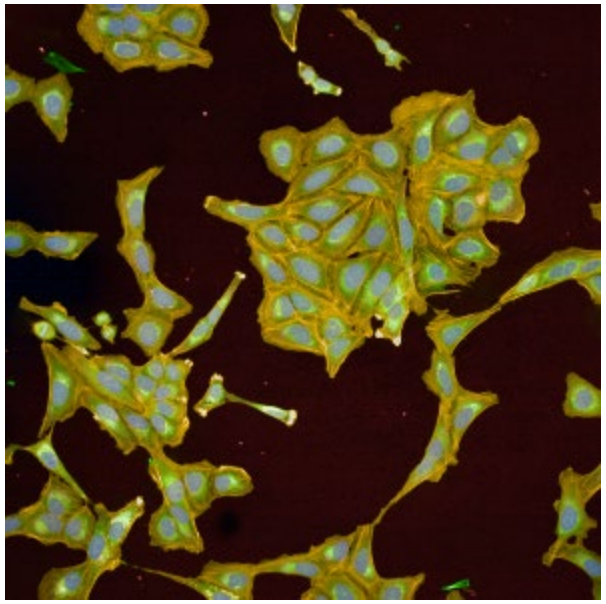
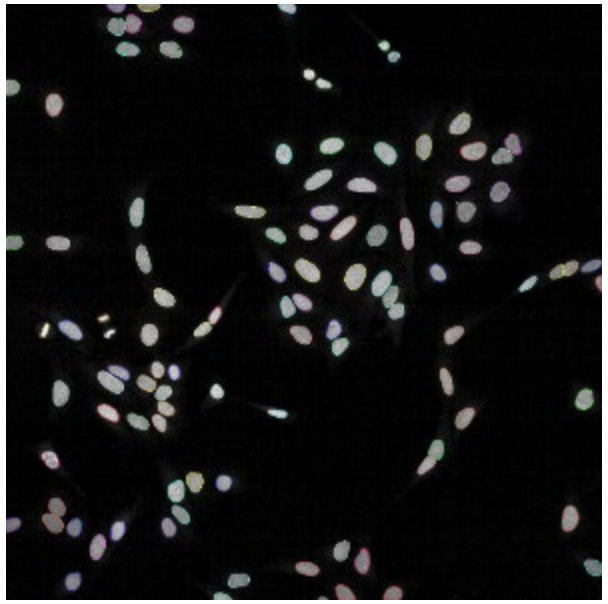
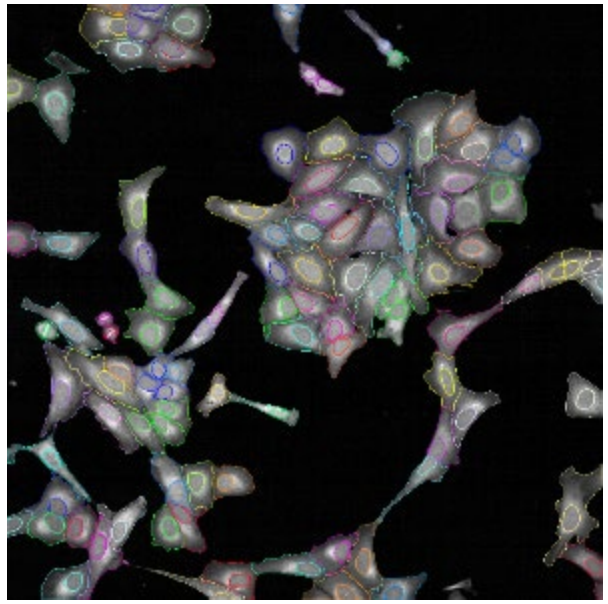
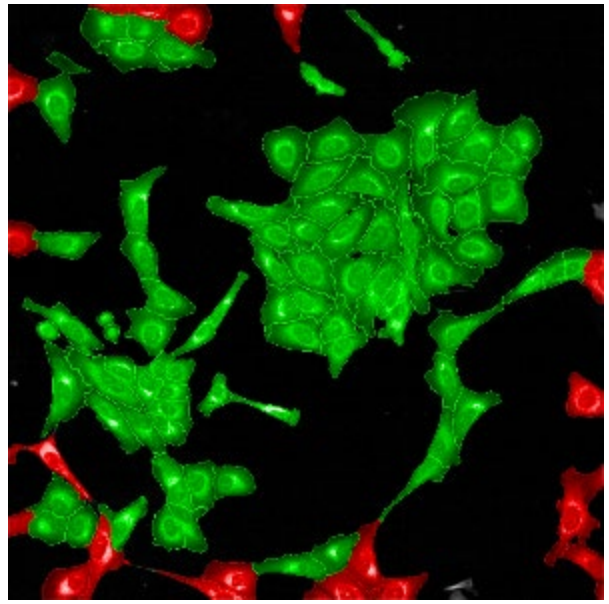
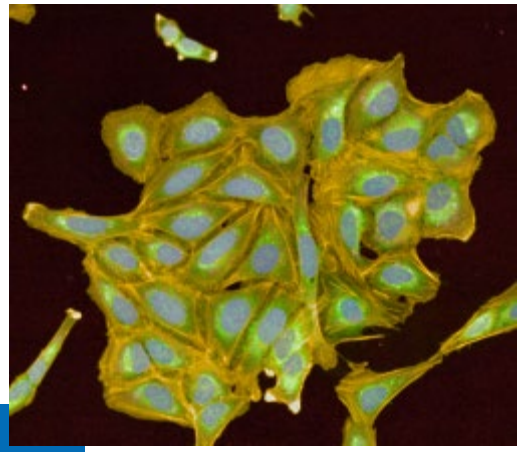
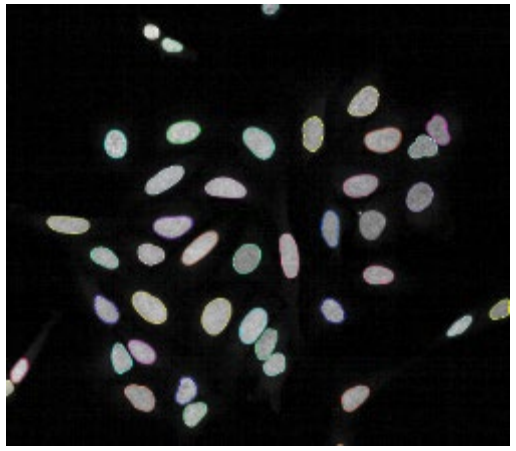
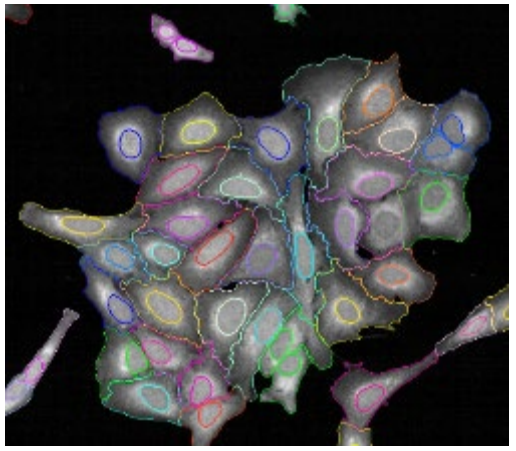
adapted from Nyffeler et al. 2020a

Image Analysis Workflow → Image Segmentation

1. find nuclei

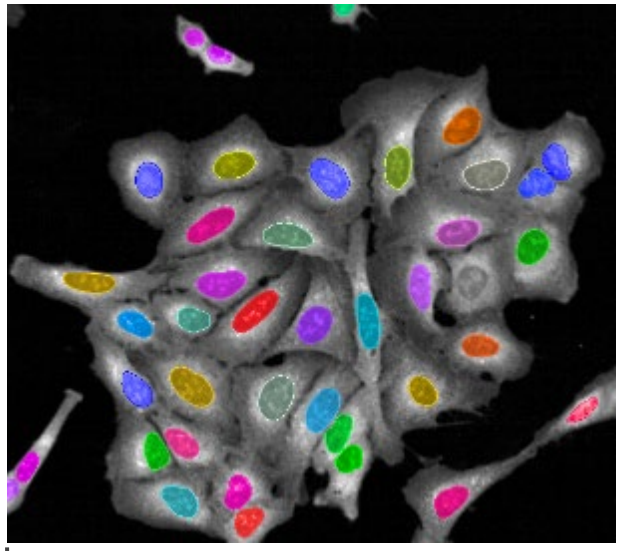
2. find cell outline

3. reject border objects

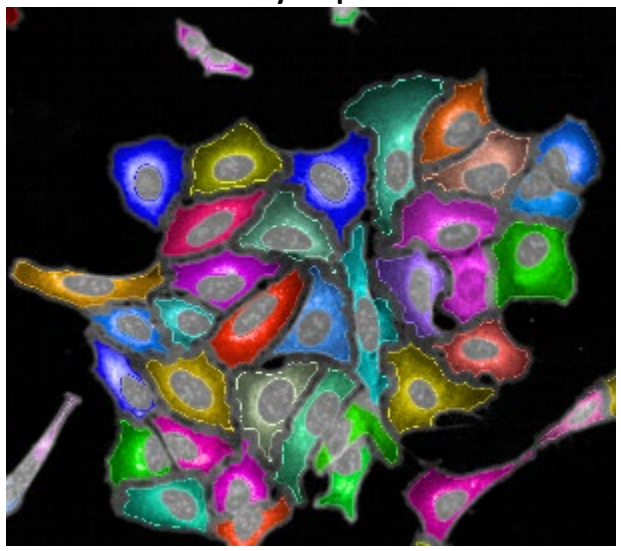








Define Cellular Compartments

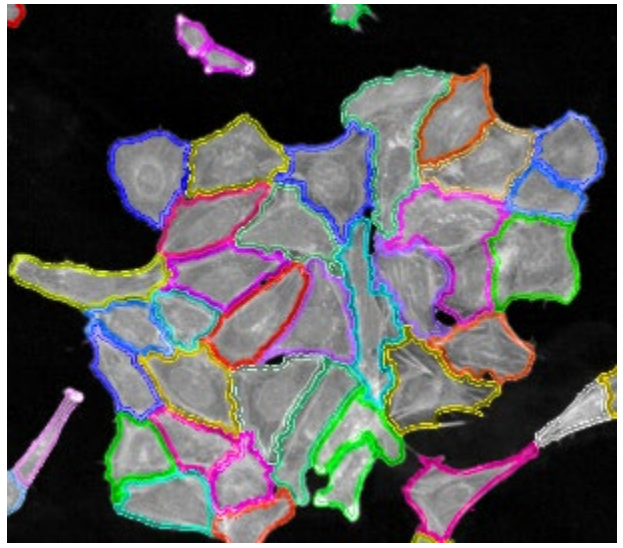
nuclei



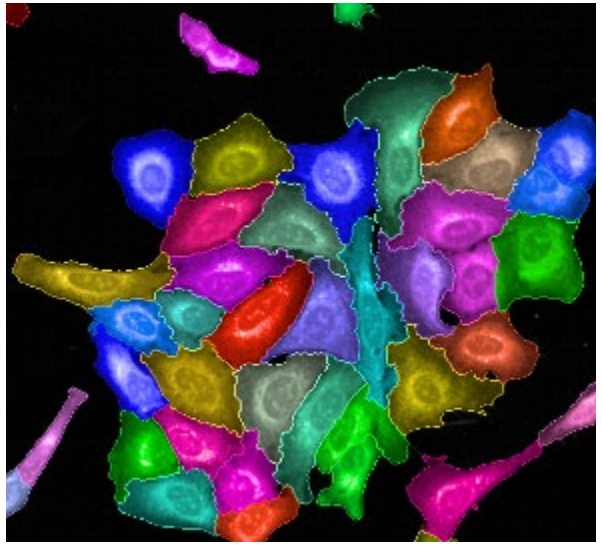
cytoplasm



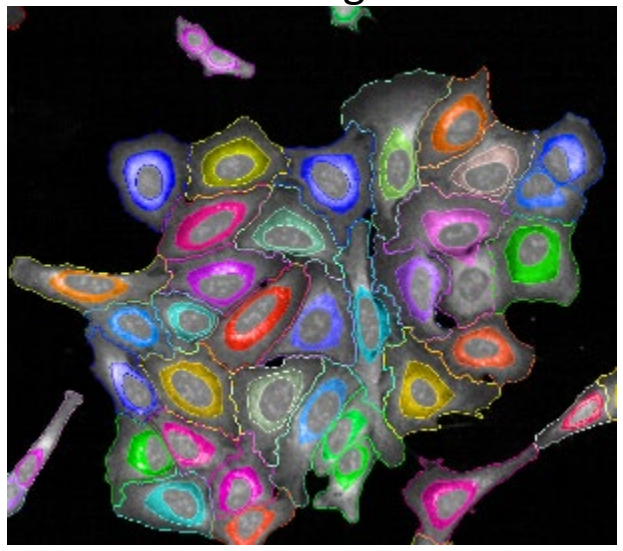
membrane



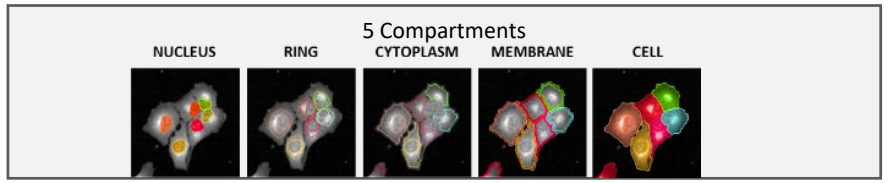
cell

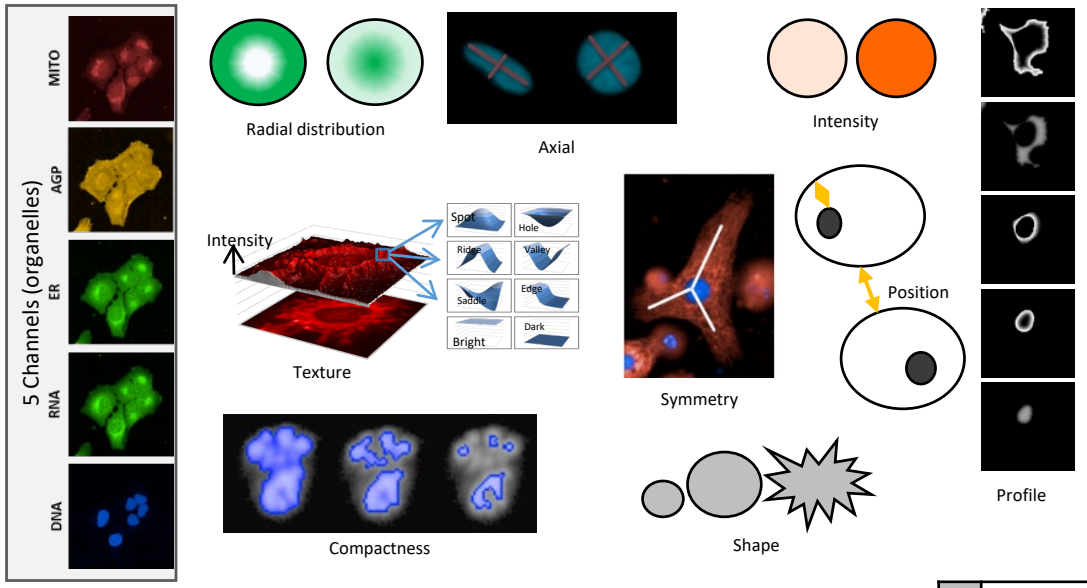


ring



Phenotypic Feature Extraction





49 Feature Categories
(ex. MITO_Texture_Cytoplasm)

1300 features / cell

		Module								
		Position [7]	Basic morph-ology [5]	SCARP morphology					Intensity [9]	Texture [14]
				Symmetry [80]	Compactness [40]	Axial [20]	Radial [28]	Profile [20-30]		
Channel	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

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

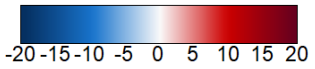
well-level data

Standardization
Z transformation

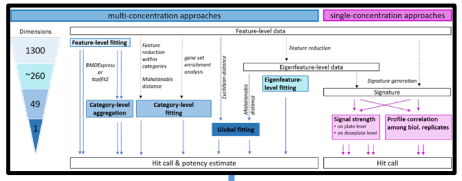
Cell Count Info
Conc. > 50% cell loss

scaled well-level data

clipped well-level data



Concentration Response Modeling

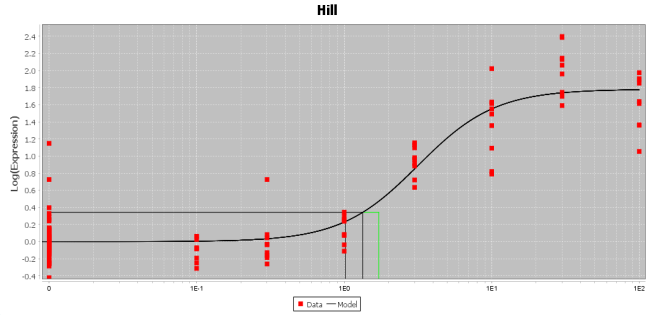


See Nyffeler et al. (2020)
SLAS Discovery
doi: 10.1177/2472555220950245.

Fit Multiple Curve Shapes

Best Model Selection

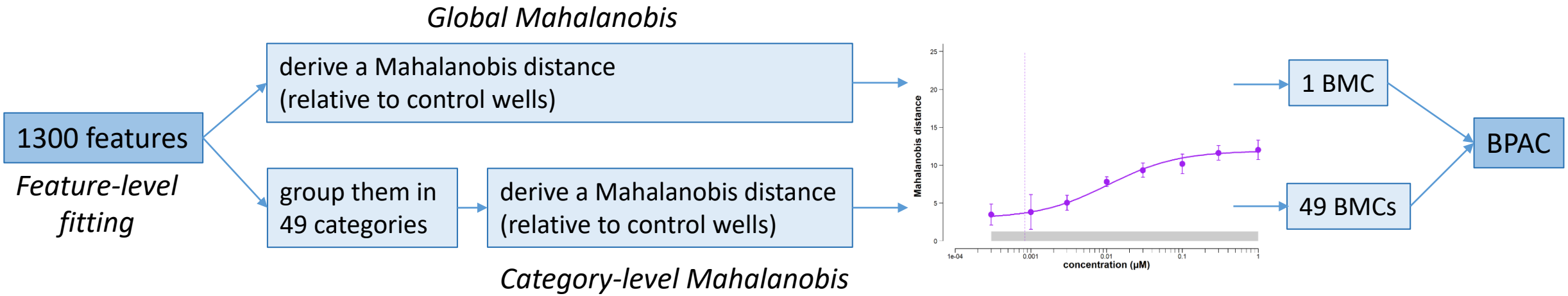
BMC



Berberine chloride
Mito_Cells_Morph_STAR

Mahalanobis Distance

Mahalanobis Distance (D_M): A multivariate distance metric that measures the distance between a point (vector) and a distribution.

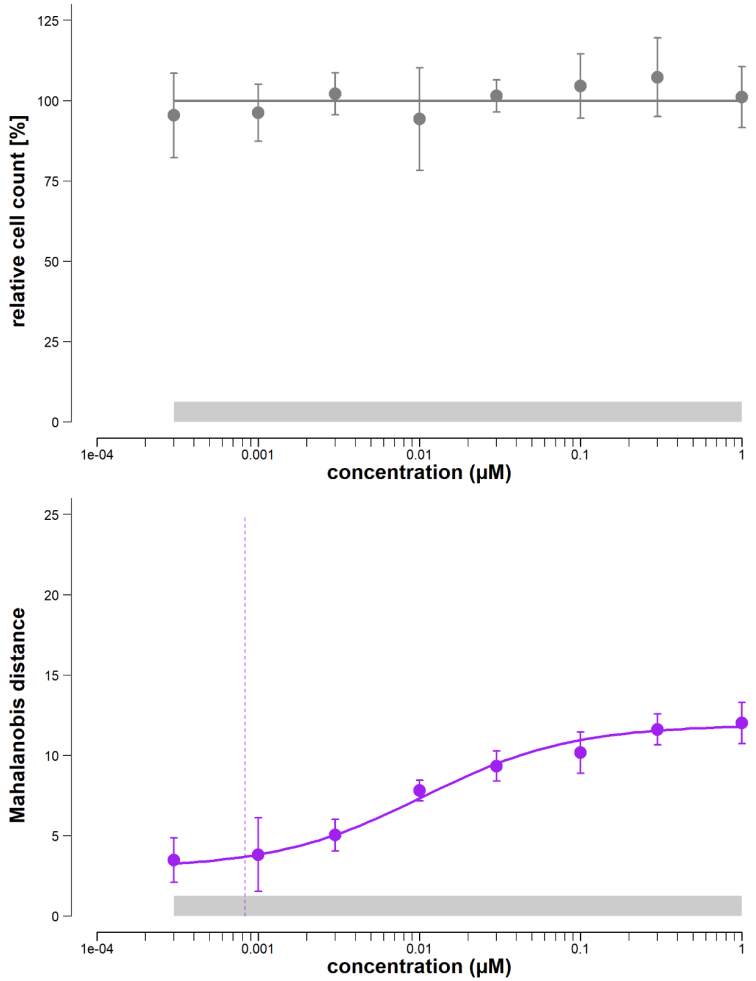


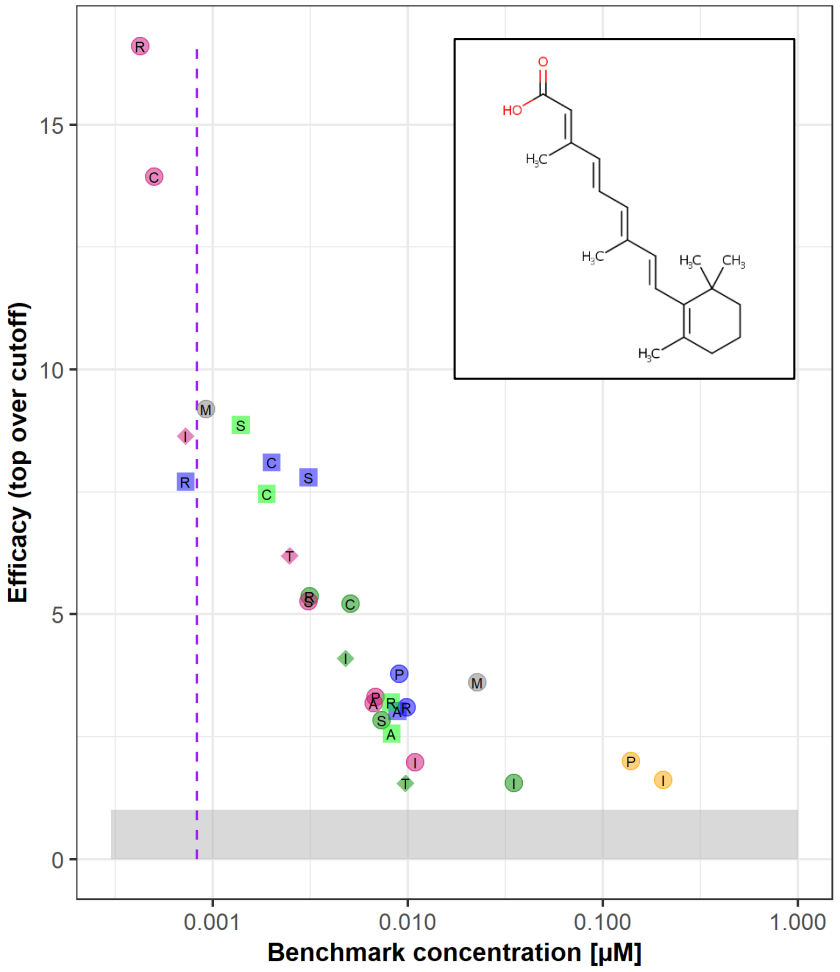
- Chemicals where a BMC can be determined using either the global or category D_M approach are considered active.
- The minimum of the global or most sensitive category BMC is the **Phenotype Altering Concentration (PAC)**

Concentration-Response Modeling Example Chemical

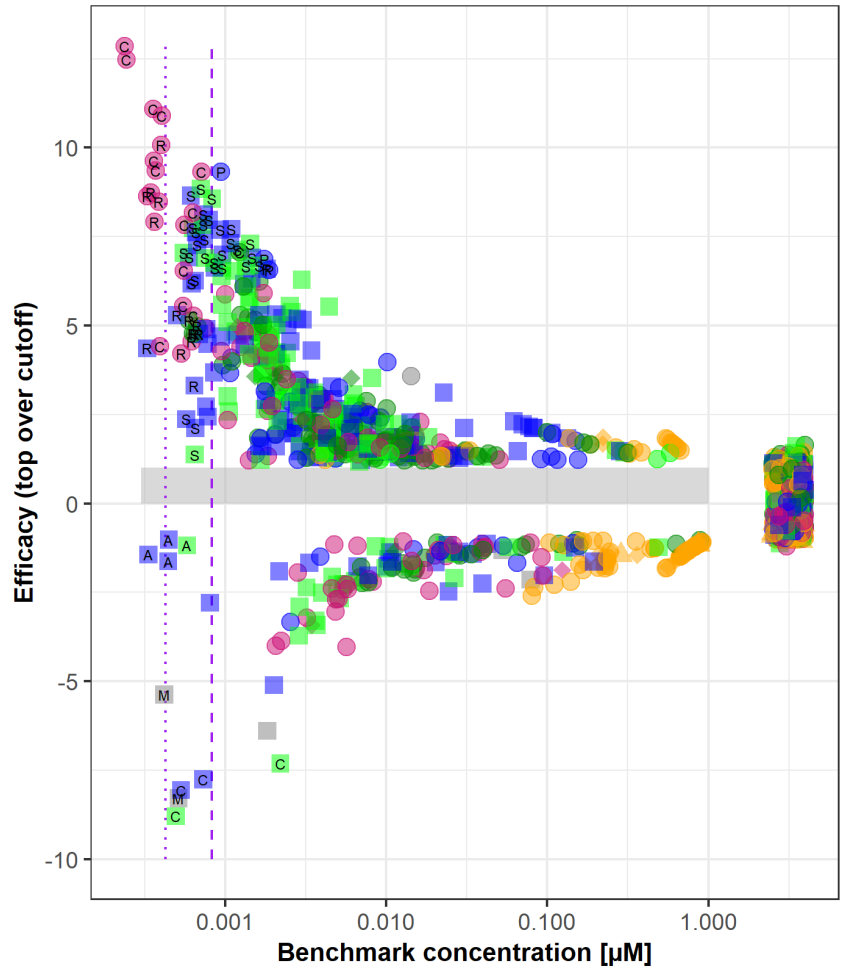
all-trans-Retinoic acid

DTXSID7021239 | 302-79-4 | RA



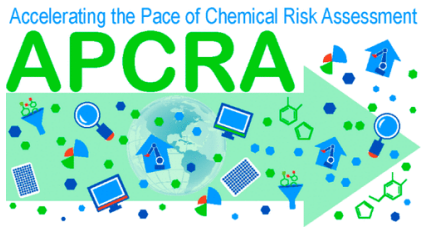


2020-07-27



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	Selected from US EPA ToxCast chemical collection **Includes 179 chemicals with annotated molecular targets** **Includes 462 APCRA case study chemicals**
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	--

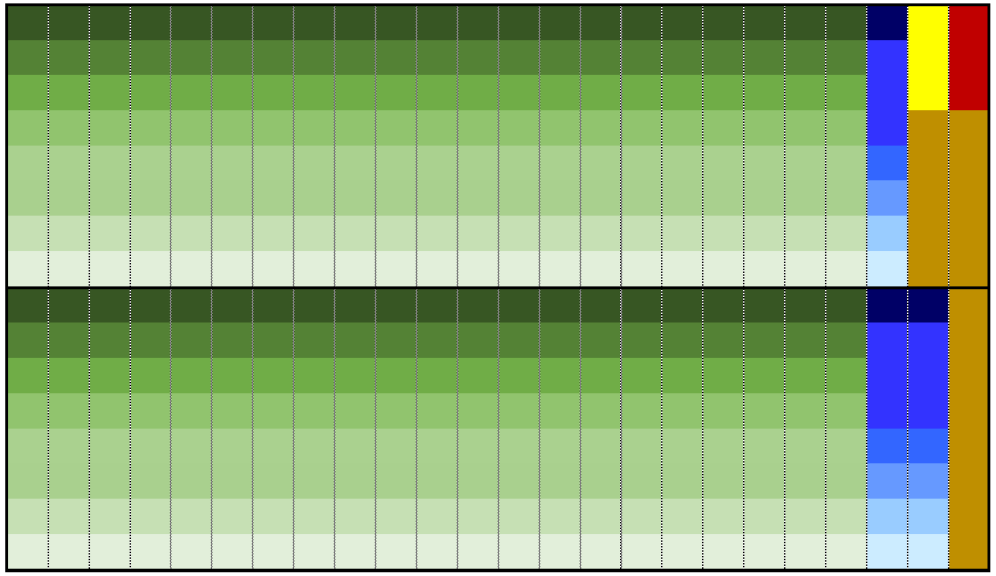


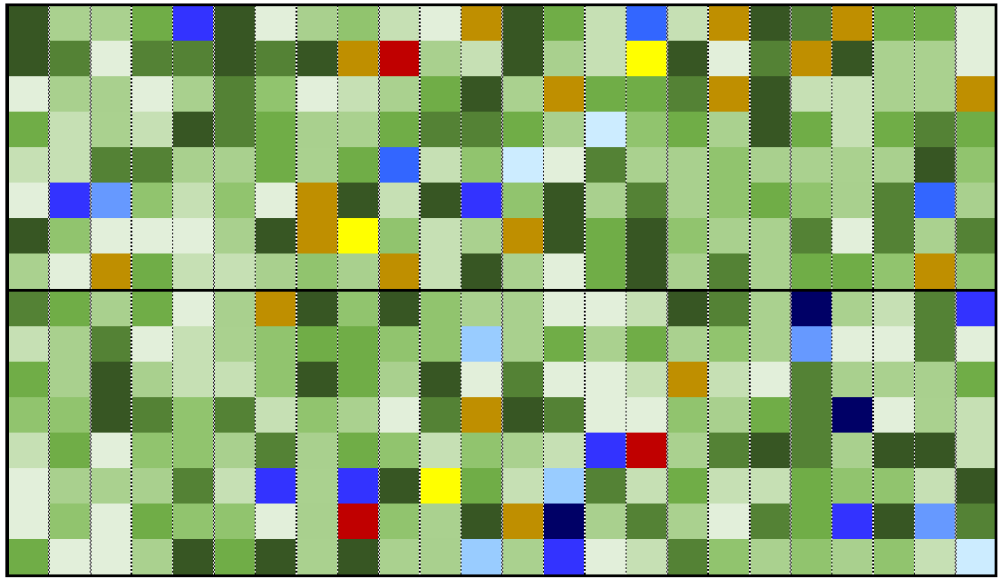
Kavlock et al. (2018)
Chem. Res. Tox; 31(5): 287-290

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

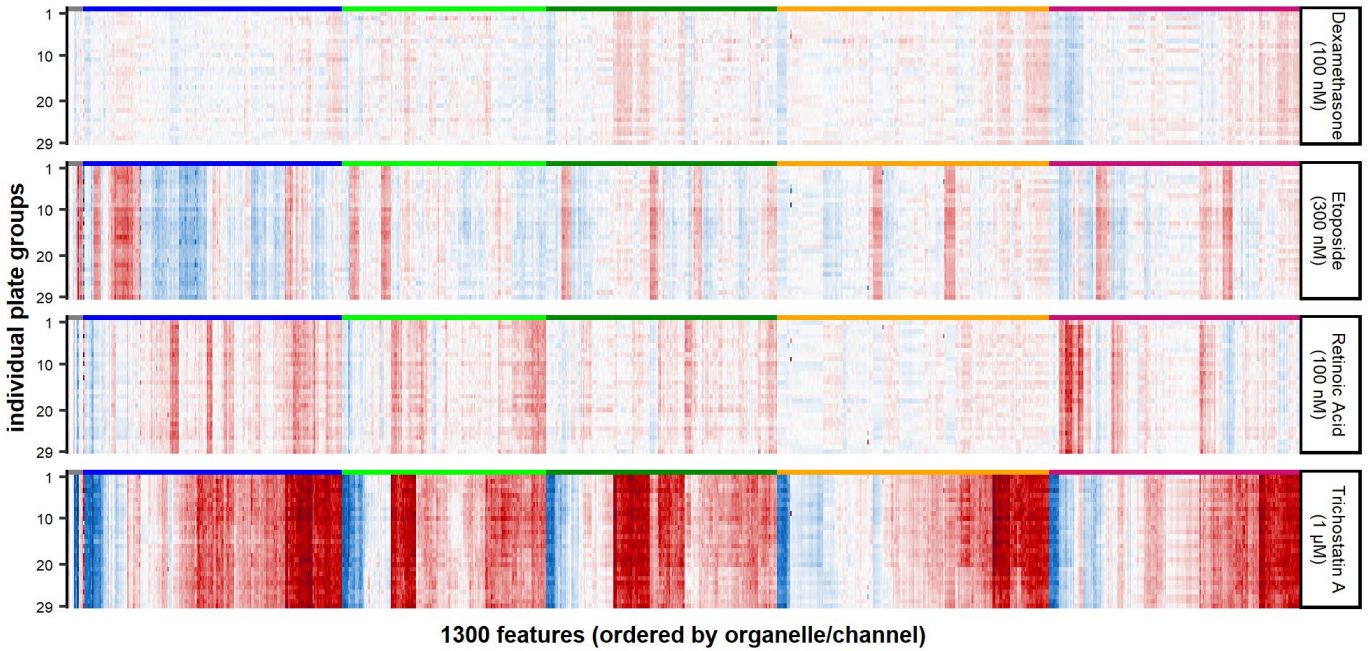
U-2 OS ToxCast Screen Dose Plate Design

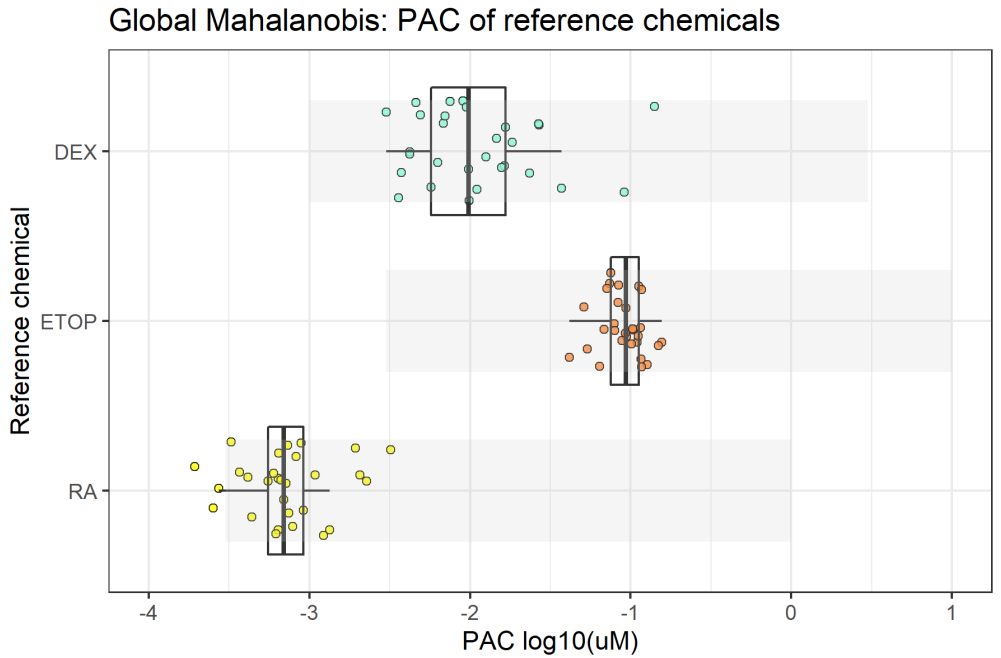




Label	Reference Chemicals:	Molecular Mechanism-of-Action	Test Concentrations
A	Etoposide	DNA topoisomerase inhibitor	0.03 - 10 µM
B	all-trans-Retinoic Acid	Retinoic acid receptor agonist	0.0003 – 1 µM
C	Dexamethasone	Glucocorticoid receptor agonist	0.001 – 3 µM
D	Trichostatin A	Histone deacetylase inhibitor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

Assay Performance / Reproducibility

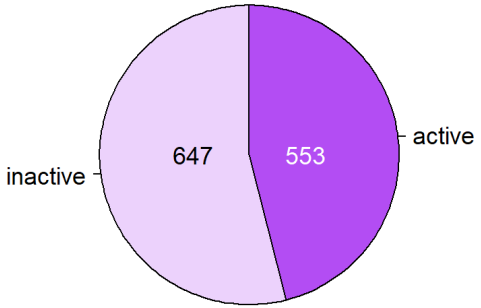


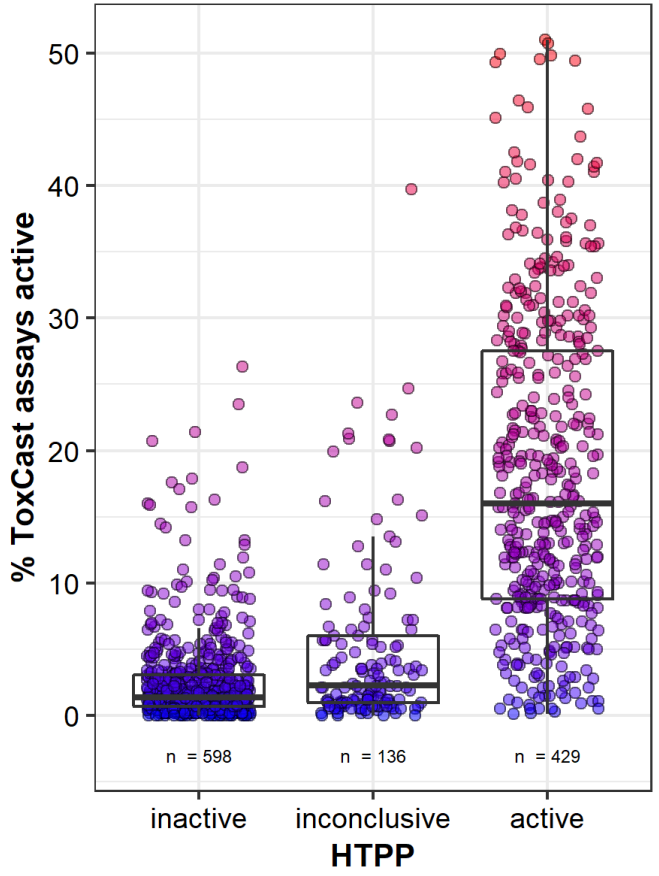


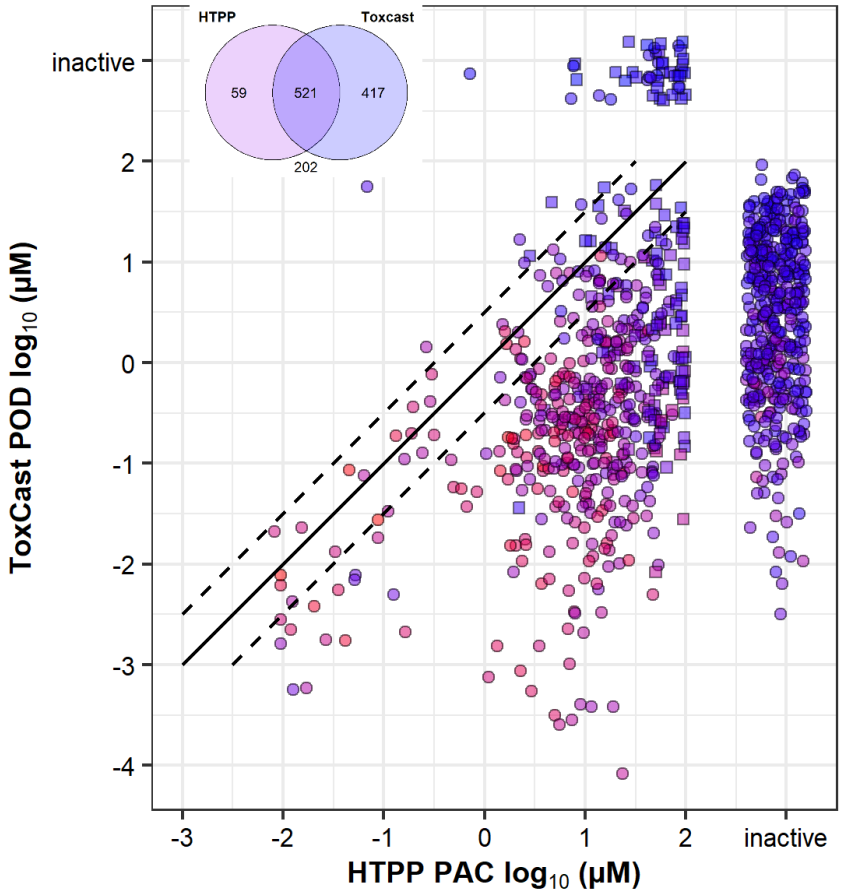
- ⇨ **Reference chemicals produce reproducible and distinct profiles.**
- ⇨ **Reference chemicals produce reproducible potency estimates (PACs).**

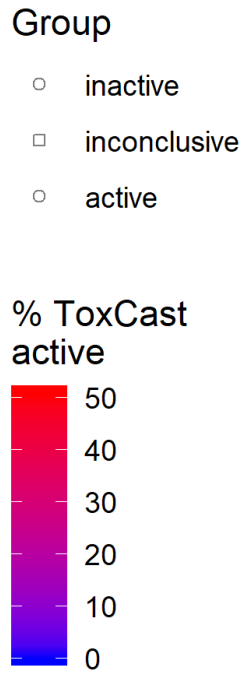
Preliminary results. Do not cite or quote.

HTPP Screening Results









⇨ **Chemicals active in HTPP are more often 'promiscuous' in ToxCast.**

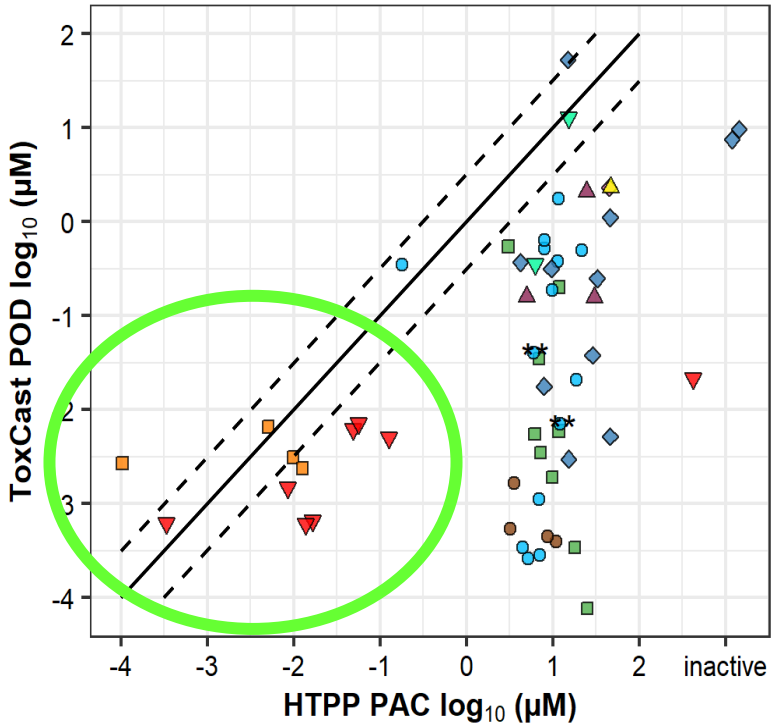
⇨ **Chemicals active in HTPP produce less potency PACs compared to ToxCast.**

Preliminary results. Do not cite or quote.

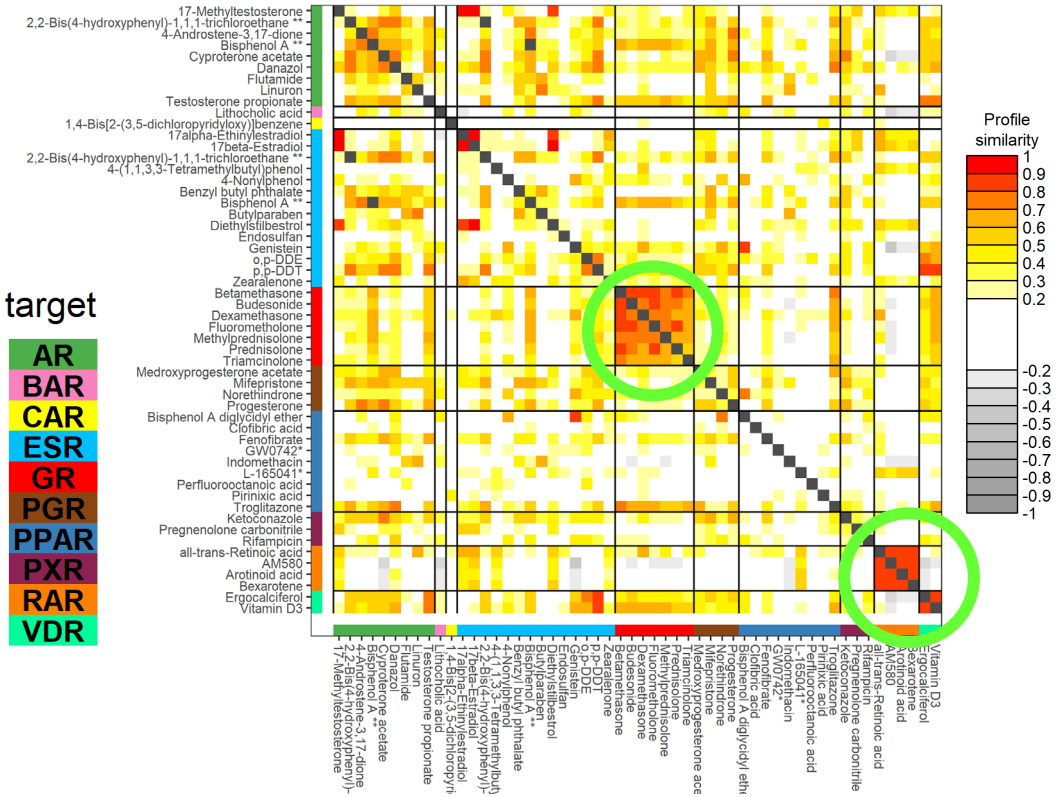
Responses of Nuclear Receptor Modulators

- 52 chemicals were annotated as targeting a nuclear receptor

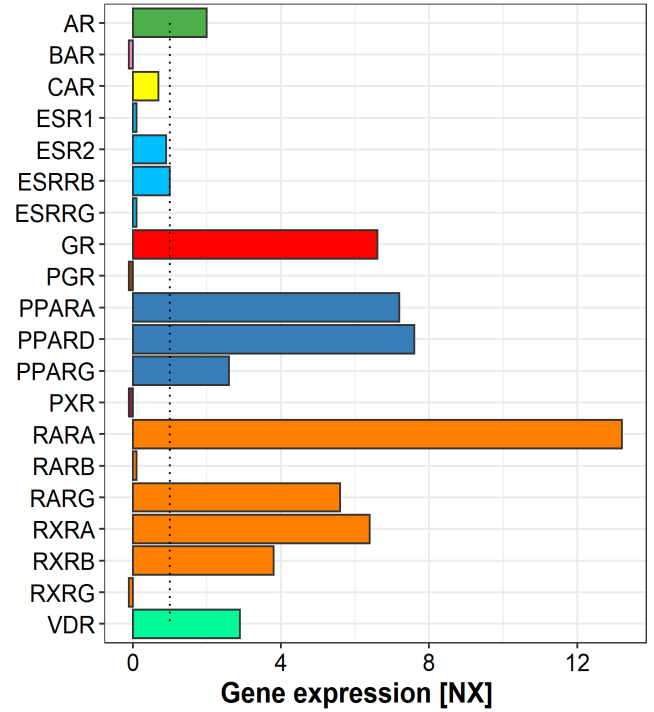
Comparison to ToxCast potencies



Profile Similarity



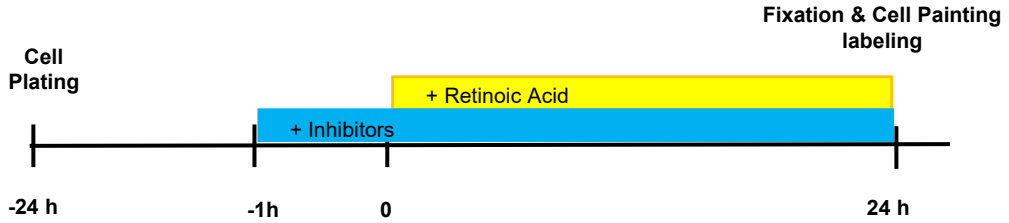
Gene expression in U-2 OS

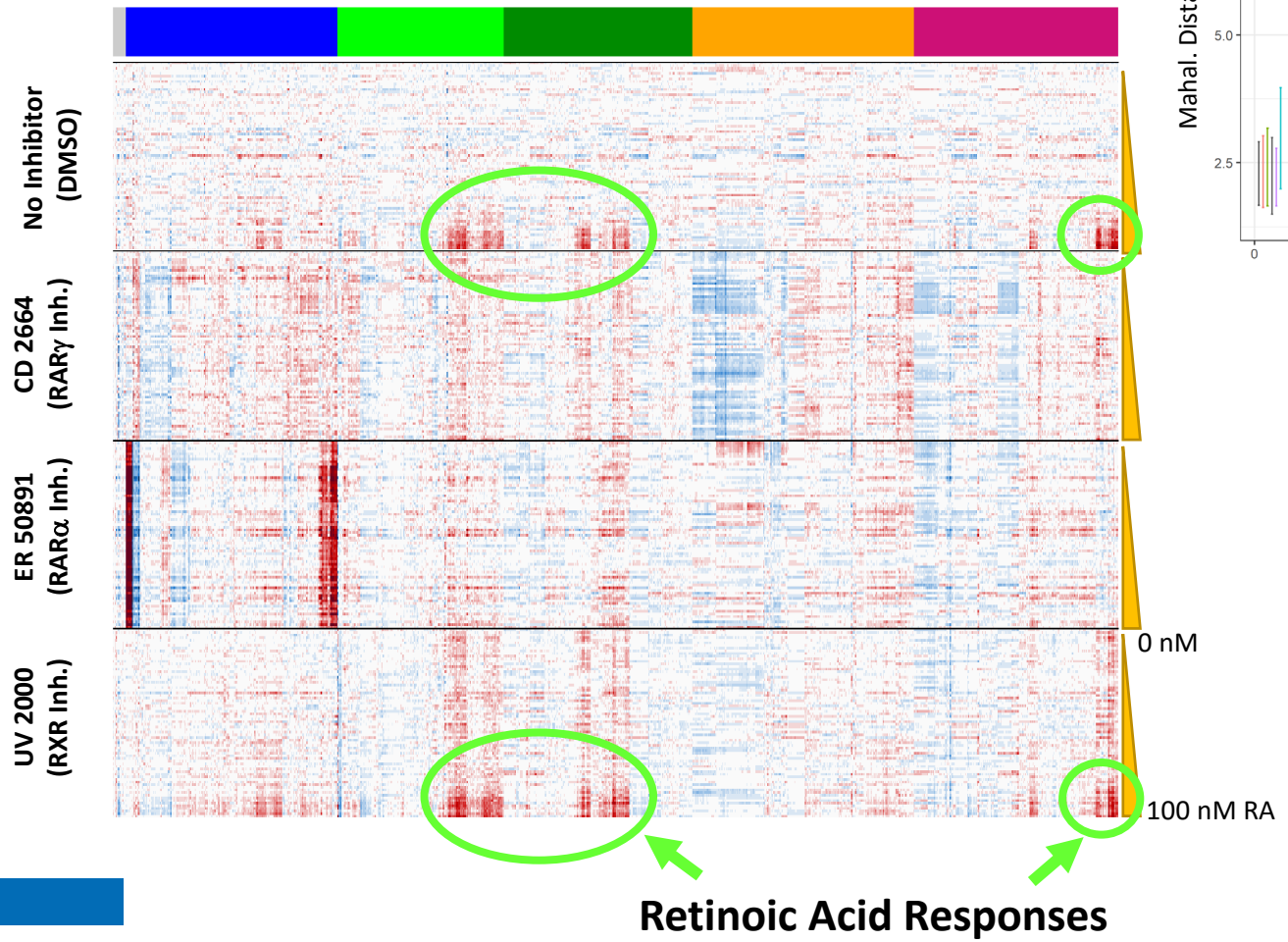


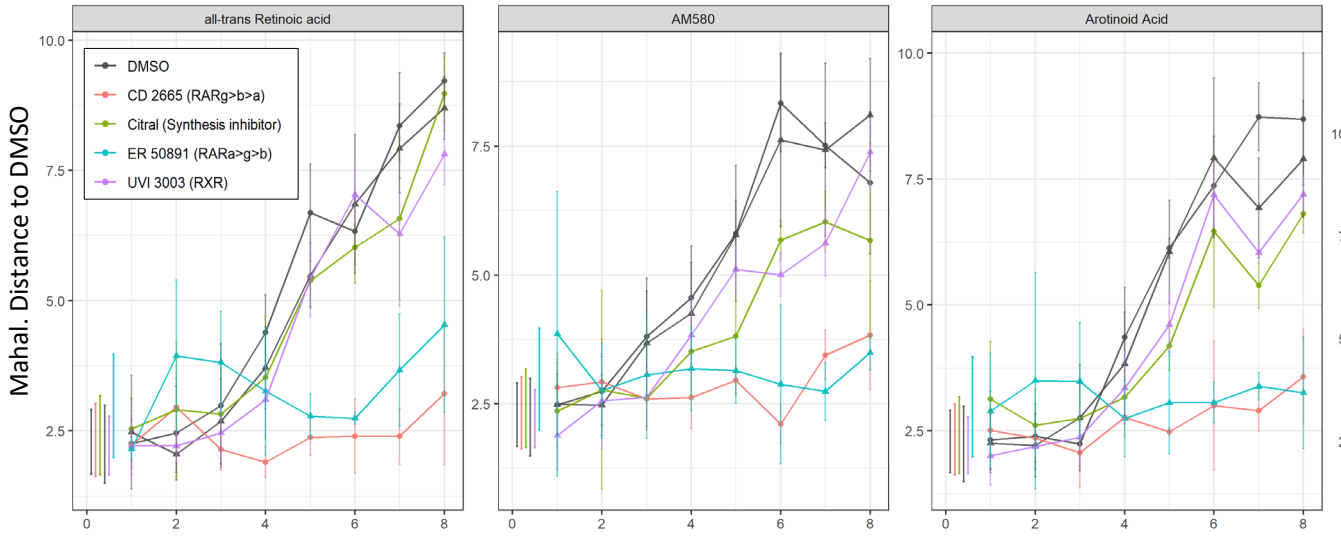
- ⇨ **For two receptor systems that are expressed (GR, RAR/RXR) potencies were comparable with ToxCast**
- ⇨ **Phenotypic profiles for chemicals that affect these receptor systems are similar.**

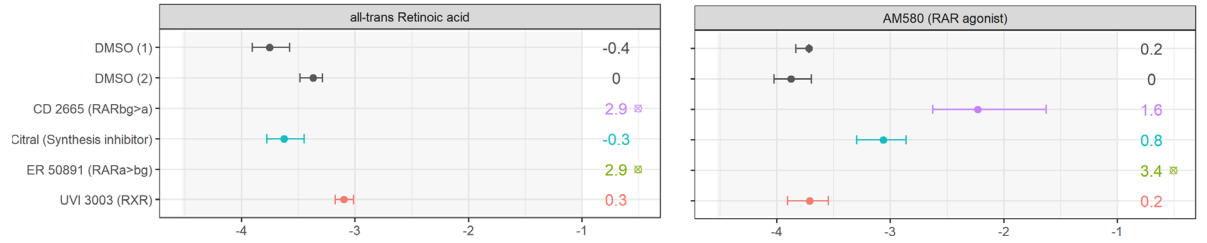
Preliminary results. Do not cite or quote.

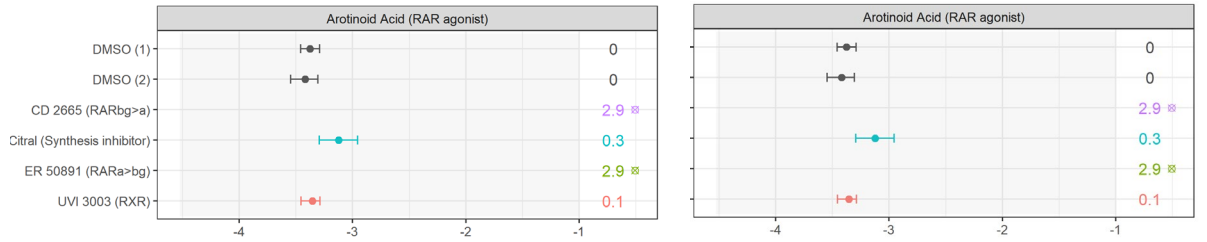
Pharmacological Blockade of Phenotypic Effects







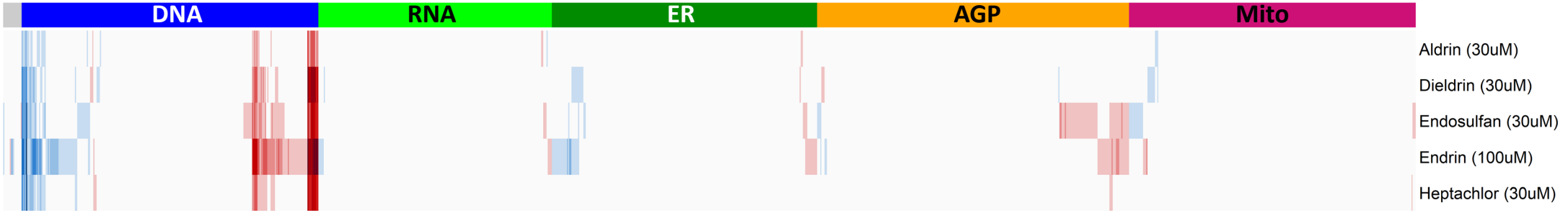




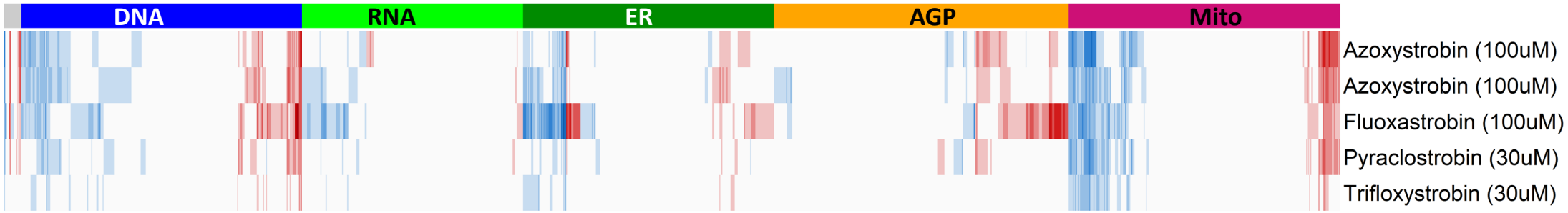
Retinoic Acid Responses

Profile Similarities for Non-Pharmaceutical Chemicals

Organochlorines:



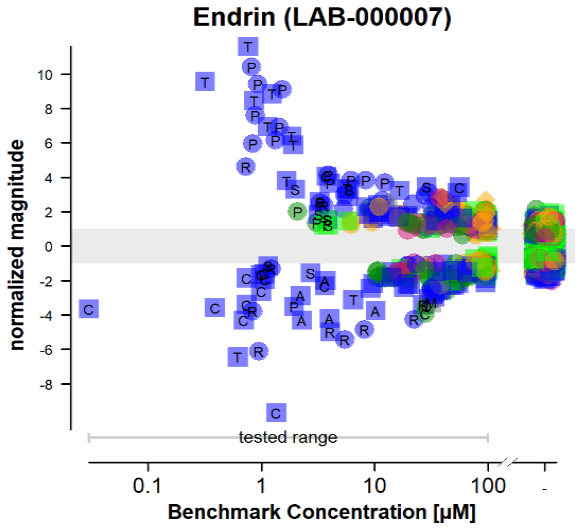
Strobilurins:

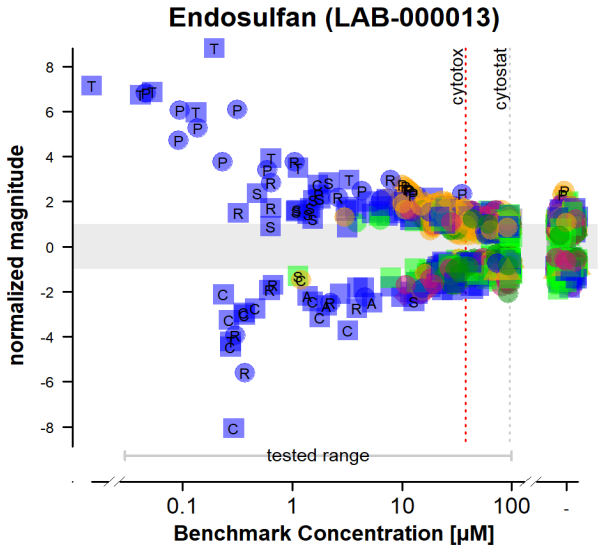


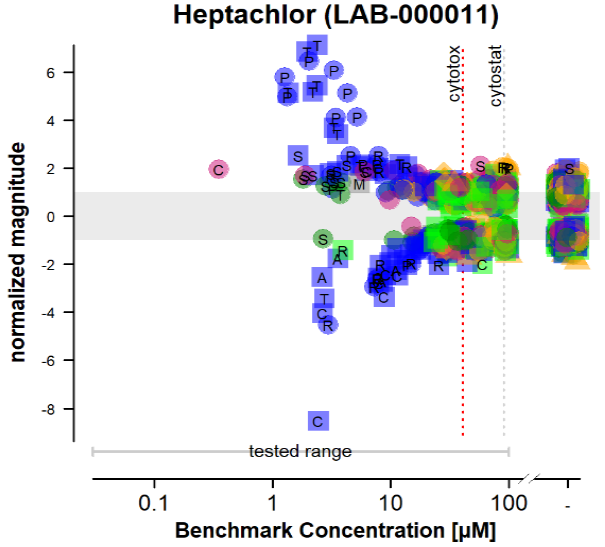
⇨ **Certain groups of environmental chemicals display characteristic profiles**

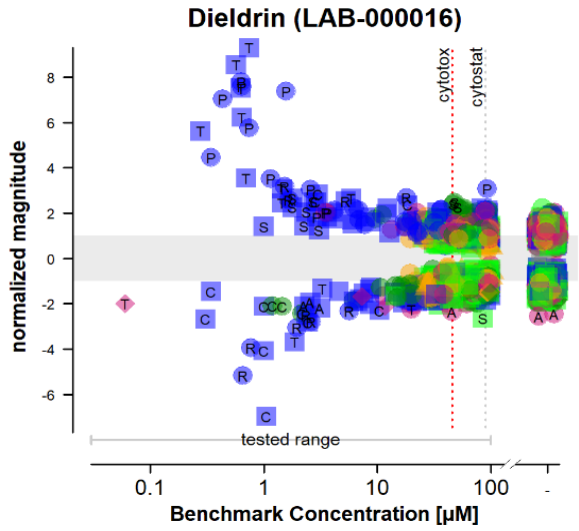
Preliminary results. Do not cite or quote.

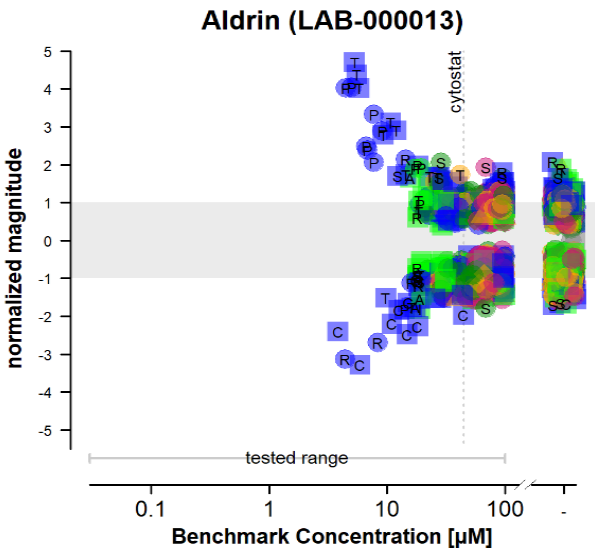
Organochlorine Phenotype

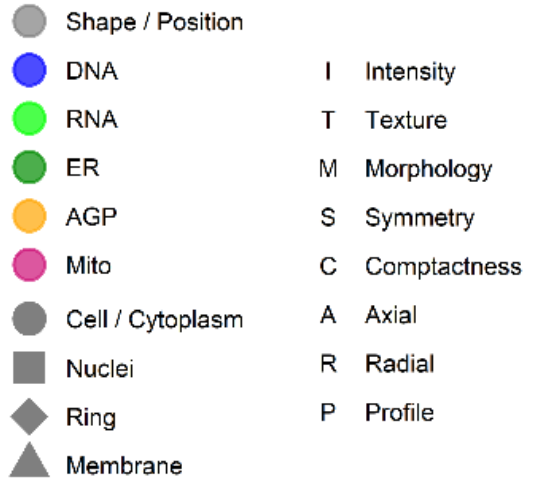


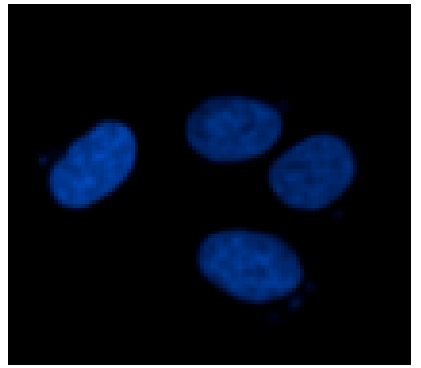


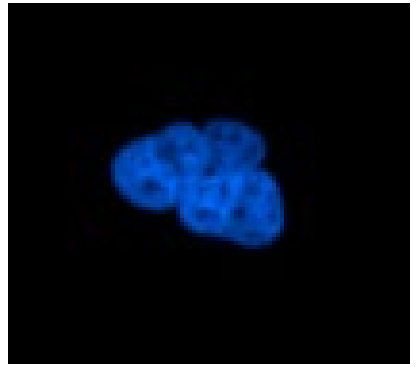


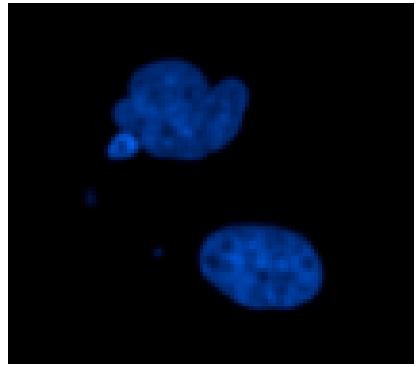






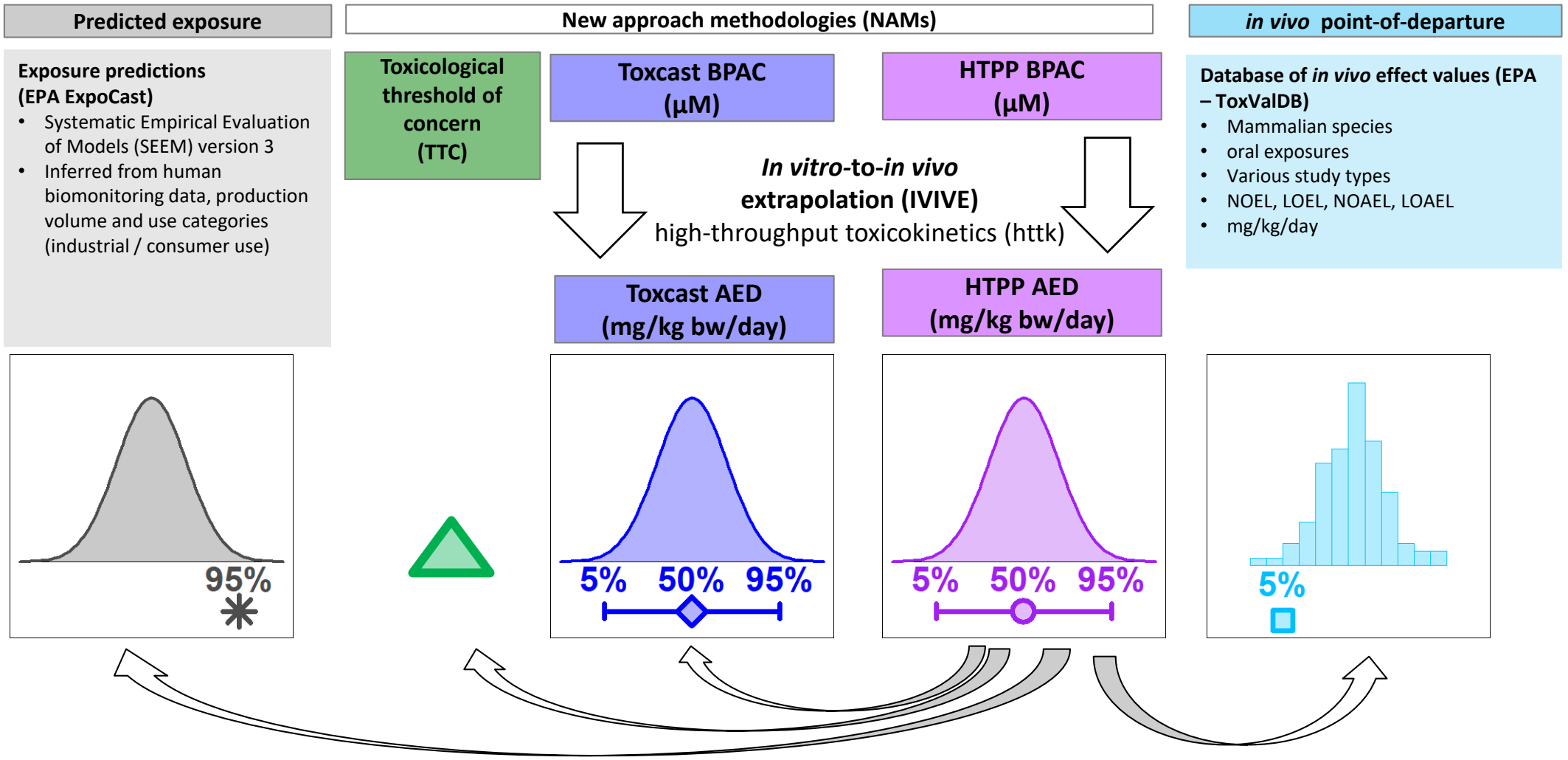






Preliminary results. Do not cite or quote.

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

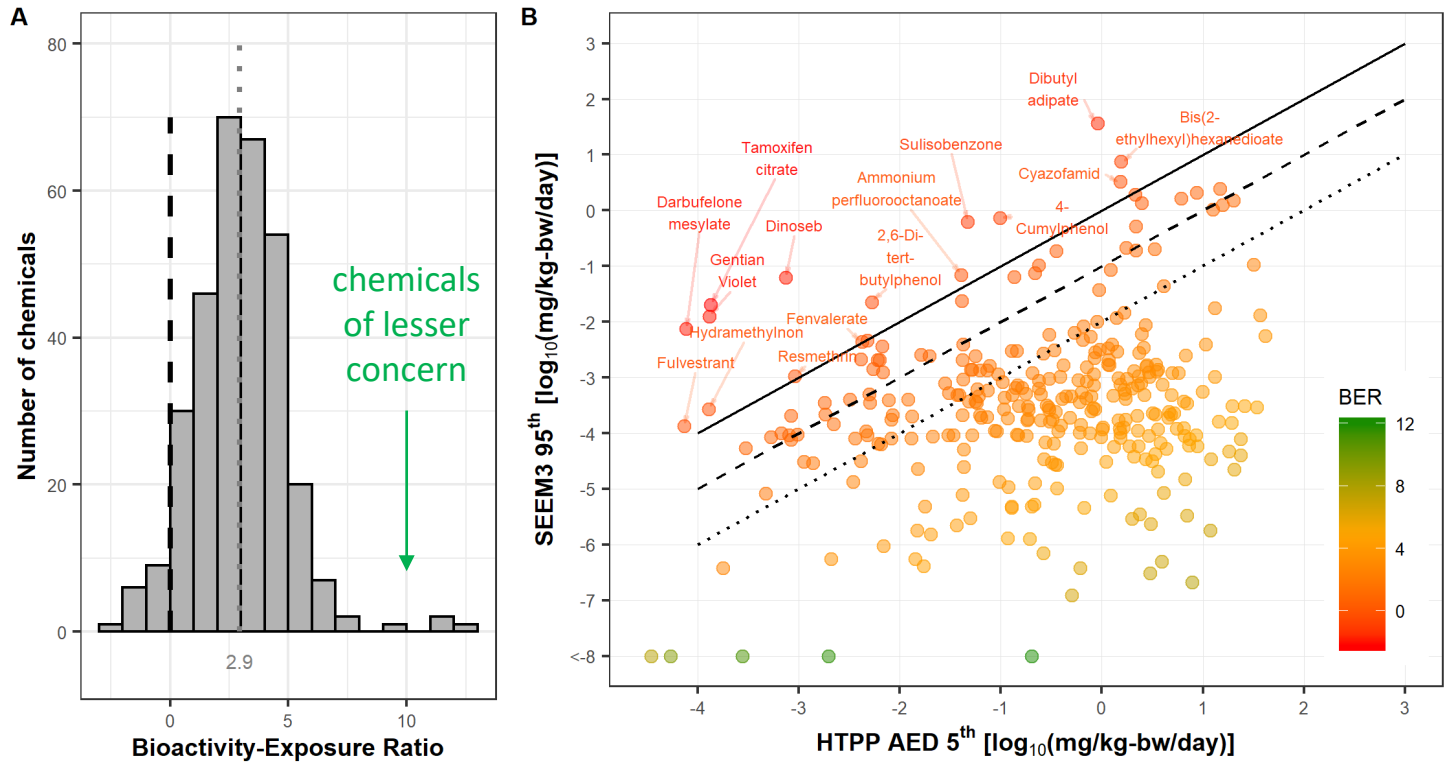


POD: point-of-departure
AED: administered equivalent dose

Bioactivity to Exposure Ratio Analysis

HTPP AEDs were compared to exposure predictions and the bioactivity exposure ratio was calculated as follows:

$$\text{Bioactivity exposure ratio (BER)} = \frac{\text{lower bound of HTPP bioactivity}}{\text{upper bound of exposure estimate}} = \log_{10}\left(\frac{\text{HTPP AED } 5^{\text{th}}}{\text{SEEM3 } 95^{\text{th}}}\right)$$



preliminary

- ⇨ **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

- **Assay Reproducibility:** Demonstrated high assay reproducibility through the use of phenotypic reference chemicals and developed experimental designs that allow for evaluation of assay performance throughout large-scale screening campaigns.
- **Potency Estimation:** Developed a concentration-response modeling workflow to identify concentration thresholds for perturbation of cell morphology (e.g. phenotypic altering concentration, PAC).
- **Mechanistic Prediction:** Chemicals with strong and specific target mode associations can produce similar phenotypic profiles in U-2 OS cells. Strength of similarity varies according to baseline target expression.
- **Chemical Similarity:** Chemicals with similar chemical structures can also produce similar phenotypic profiles in U-2 OS cells.
- **Bioactivity to Exposure Ratio:** Phenotype altering concentrations (PACs) can be converted to administered equivalent doses (AEDs) and compared to human exposure predictions for chemical ranking and prioritization.

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THANK YOU!

QUESTIONS?