EPA
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Environmental Protection
Agency

Development and Use of a High Content Imaging-Based Phenotypic Profiling Assay for Bioactivity Screening of Environmental Chemicals

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USEPA National Center for Computational Toxicology (NCCT)

Session I: HCS-HTS Successes and Learnings
Society for Biomolecular Imaging and Informatics (SBI[2]) Annual Meeting, Boston, MA
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EPA United States Environmental Protection Agency

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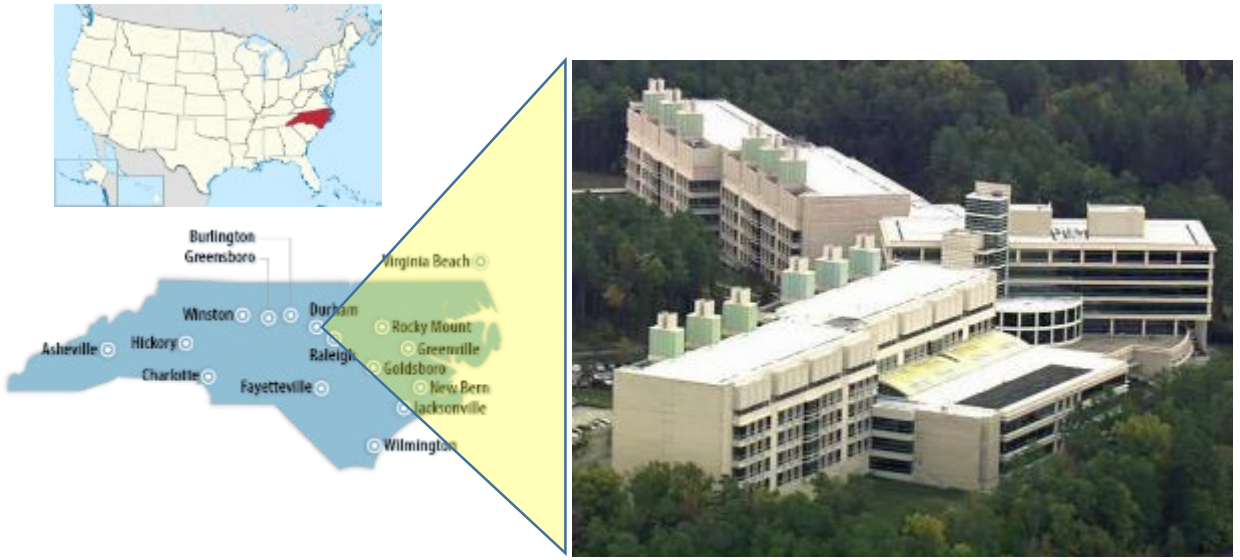
Outline

- **Background**
  - What is NCCT?
  - What does NCCT Do?
  - USEPA Computational Toxicology Blueprint
- **High-Throughput Phenotypic Profiling (HTPP)**
  - Technology Overview → Cell Painting
  - Image Analysis
  - Computational Workflows
- **Potential Applications for Chemical Safety Assessment**
  - Bioactivity to Exposure Ratio (BER) Analysis
  - Profile Similarity for Chemical Read-Across
- **Conclusions**

Who is NCCT?

National Center for Computational Toxicology



Research Triangle Park Campus

Mission Statement:

A research organization tasked with advancing the science of toxicity testing through the **development and/or application of novel experimental and computational approaches** for rapidly characterizing the physiochemical properties, biological activity, exposure potential and potential human health risks associated with chemicals.

Computational Toxicology Research Areas

NCCT research programs focus on developing the **tools, approaches and data** needed to accelerate the pace of chemical risk assessment and foster incorporation of non-traditional toxicity testing data into regulatory decision-making processes.

- **ToxCast:** Use of targeted high-throughput screening (HTS) assays to expose living cells or isolated proteins to chemicals and assess bioactivity and potential toxic effects.

	# of assays	# of chemicals	Types of chemicals
Phase 1 *(2007 – 2009)*	500	300	Mostly pesticides
Phase 2 *(2009 – 2013)*	700	2,000	Industrial, consumer product, food use, "green"

- Mostly targeted assays (*chemical X → protein Y*)
- Incomplete coverage of biological space.

- **New Approach for Hazard Evaluation:** Employ broad-based (i.e. non-targeted) **profiling assays** that cast the broadest net possible for capturing potential hazards associated with chemical treatment and may be used to group chemicals based on similarity in bioactivity profiles.

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

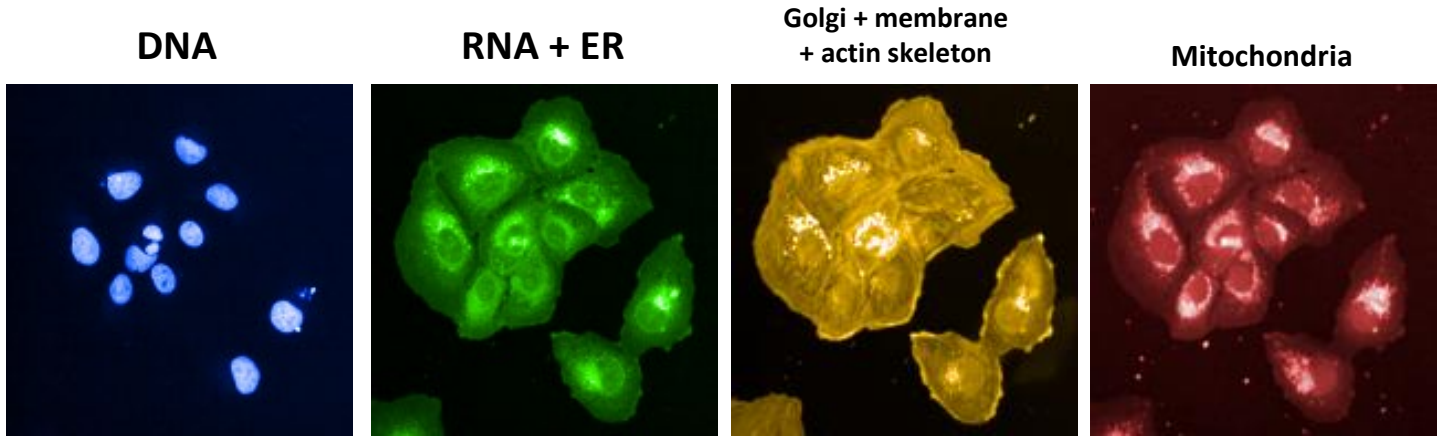
Cell Painting

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

- No requirement for *a priori* knowledge of molecular targets.

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

- **Hypothesis:** **Cell Painting may be an 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

Objectives

1. Adapt the Cell Painting assay (Bray et al. 2016) for use with microfluidics and imaging instruments available at NCCT.

2. Develop computational workflows for phenotypic feature extraction, data normalization, concentration-response modeling and generation of phenotypic response profiles.

3. Evaluate assay performance by replicating published results (Gustafsdottir et al. 2013).

4. Perform concentration-response screening of a set of environmental chemicals selected from USEPA ToxCast collection.

5. Demonstrate data usability in down-stream applications of potential interest to chemical safety assessment practitioners:

    1. *In vitro*-to-*in vivo* (IVIVE) and bioactivity-exposure ratio (BER) analysis

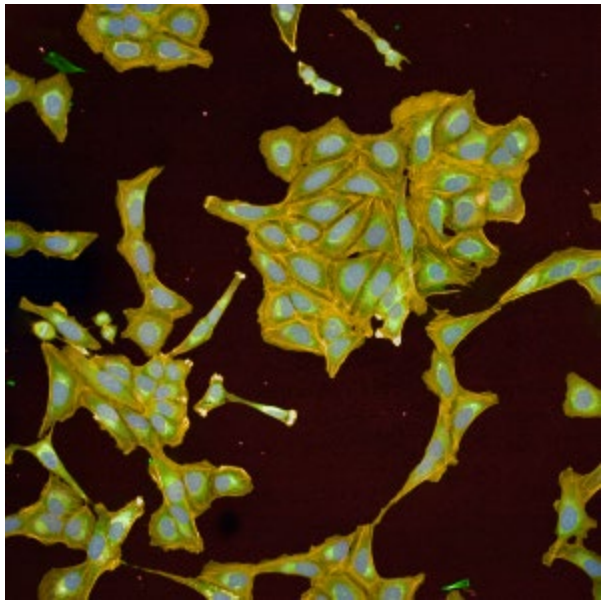
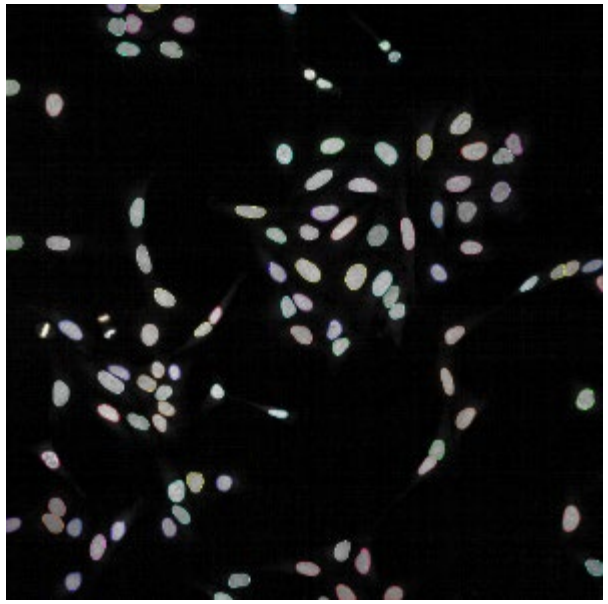
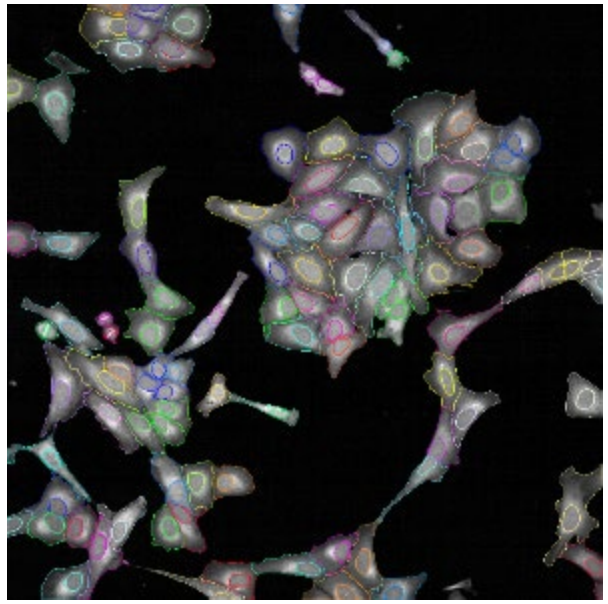
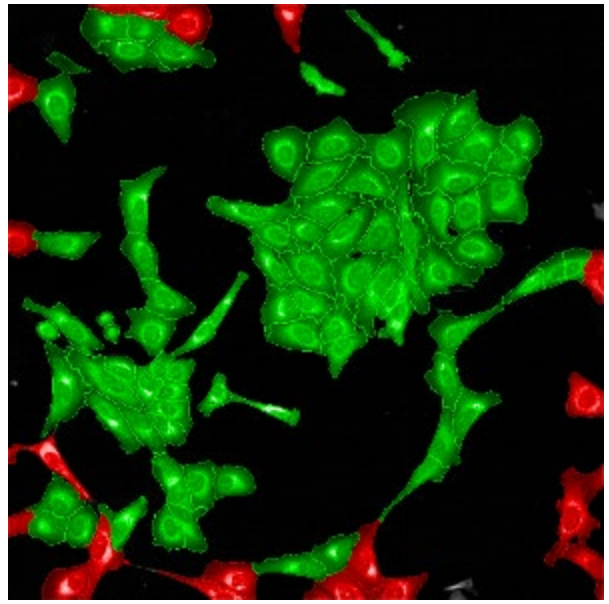
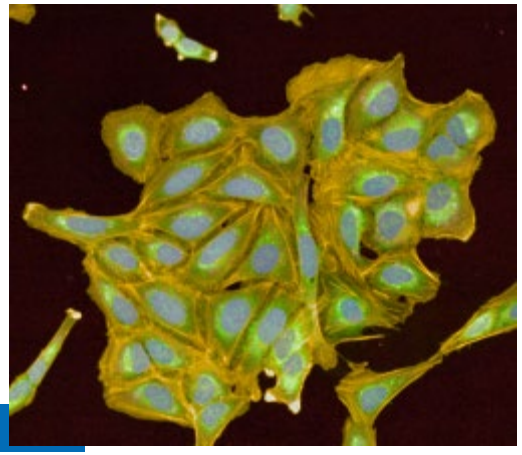
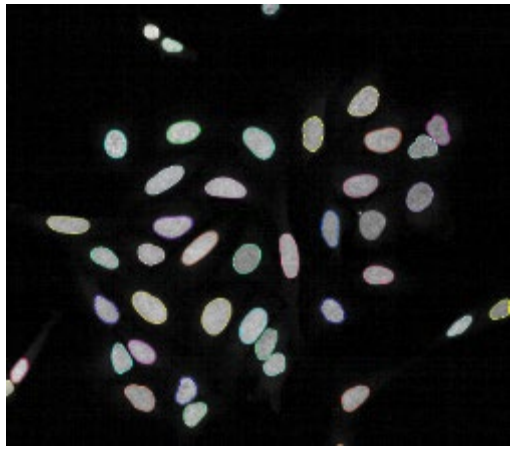
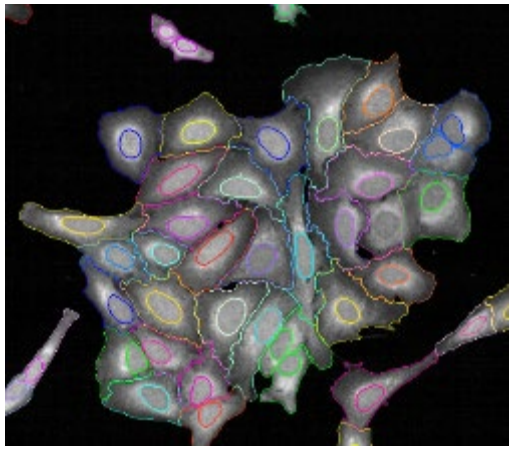
    2. Chemical read across using phenotypic profile similarity

Image Analysis Workflow → Image Segmentation

1. find nuclei

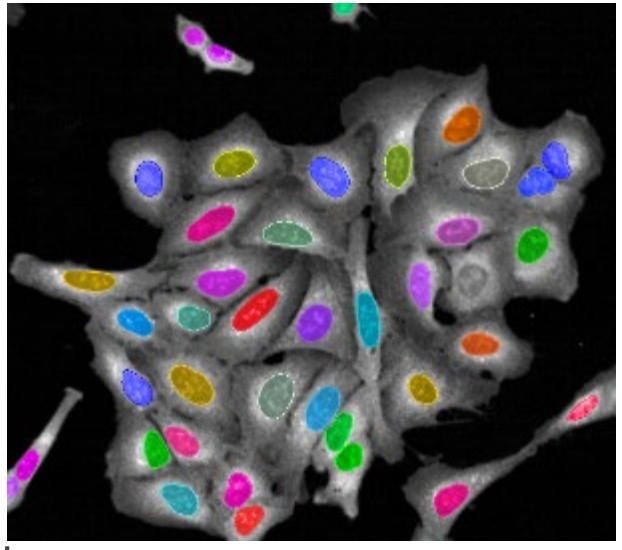
2. find cell outline

3. reject border objects

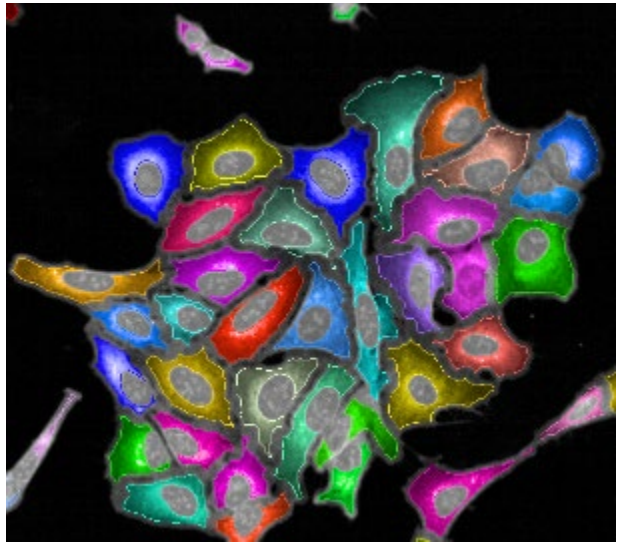








Define Cellular Compartments

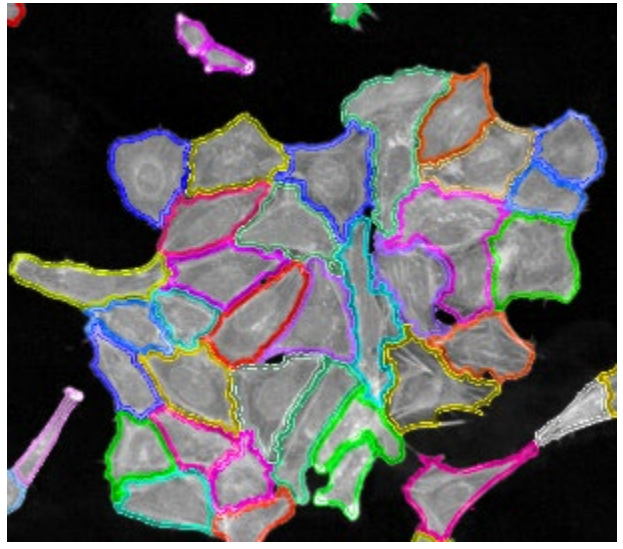
nuclei



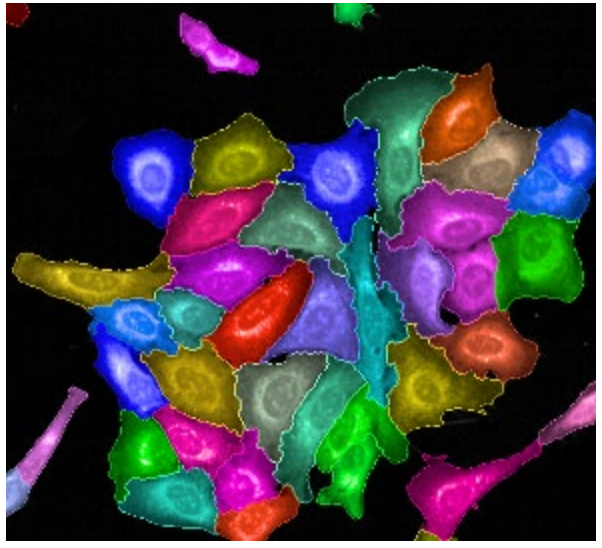
cytoplasm



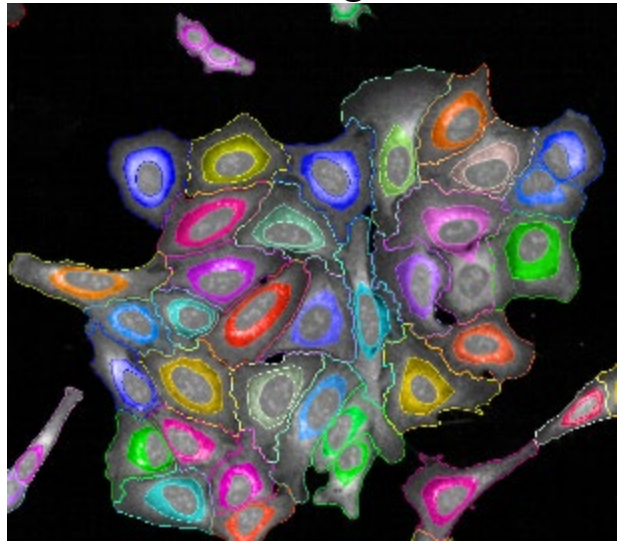
membrane



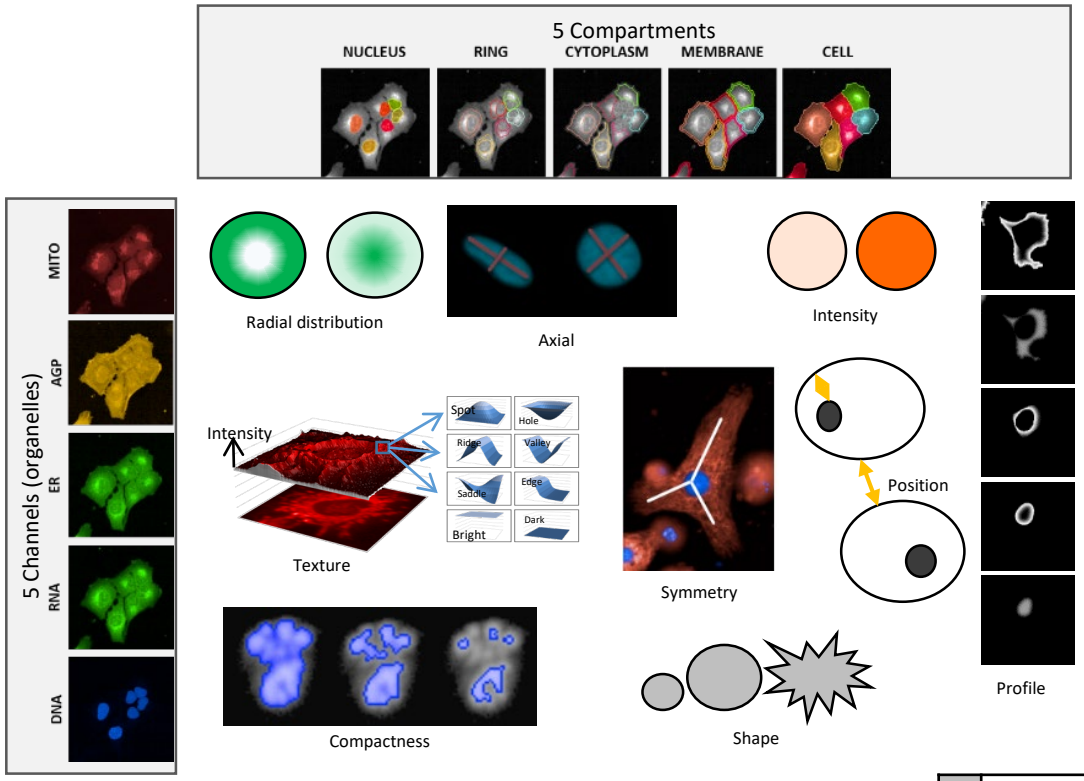
cell



ring



Phenotypic Feature Extraction

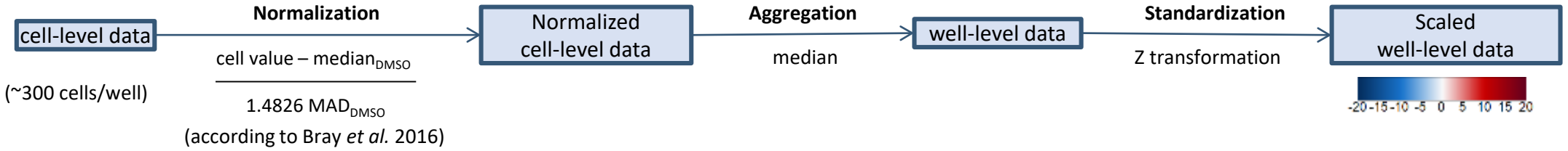


1300 features / cell

		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							

With illustrations from Perkin Elmer

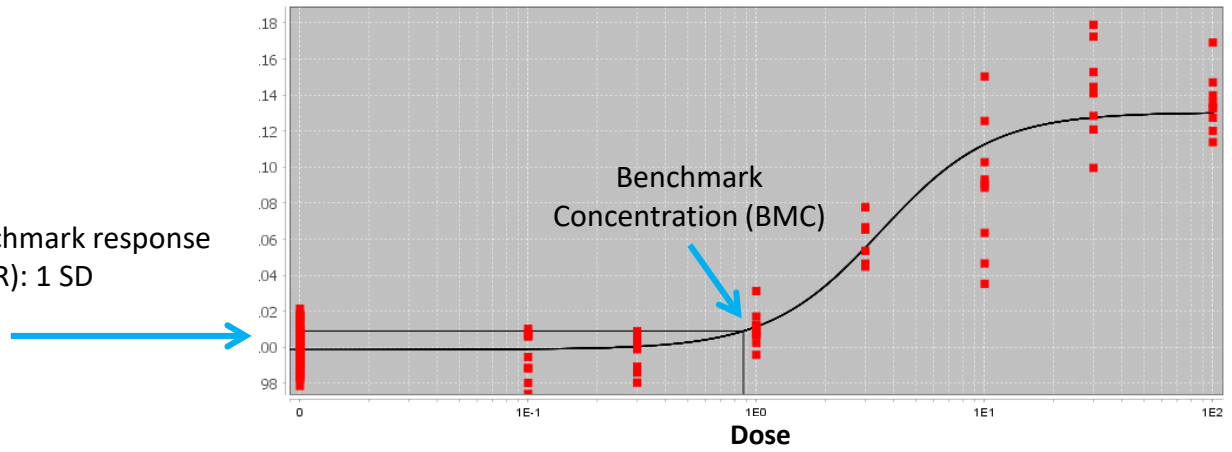
Analysis of phenotypic features



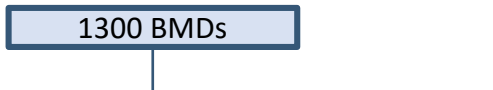
Dose-response modelling

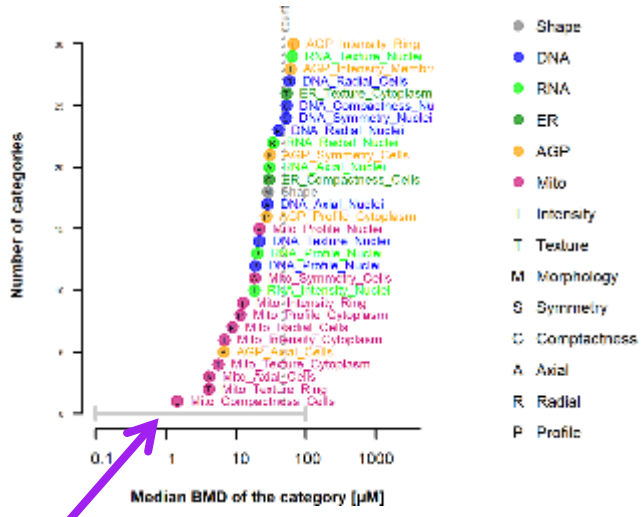
- Scaled well-level data is clipped above the first cytotoxic dose (determined by the CV assay)
- Software: BMDExpress 2.2
- 4 models: Hill, Linear, Poly2, Power
- Best model selection:
  1. nested χ^2 to select the better polynomial (Linear vs Poly2)
  2. best AIC (Hill, Power, Poly)
- BMCs above the tested range are reported as NA
  BMCs below the tested range are assigned $\log_{10}$(min dose)-0.5

Benchmark response (BMR): 1 SD



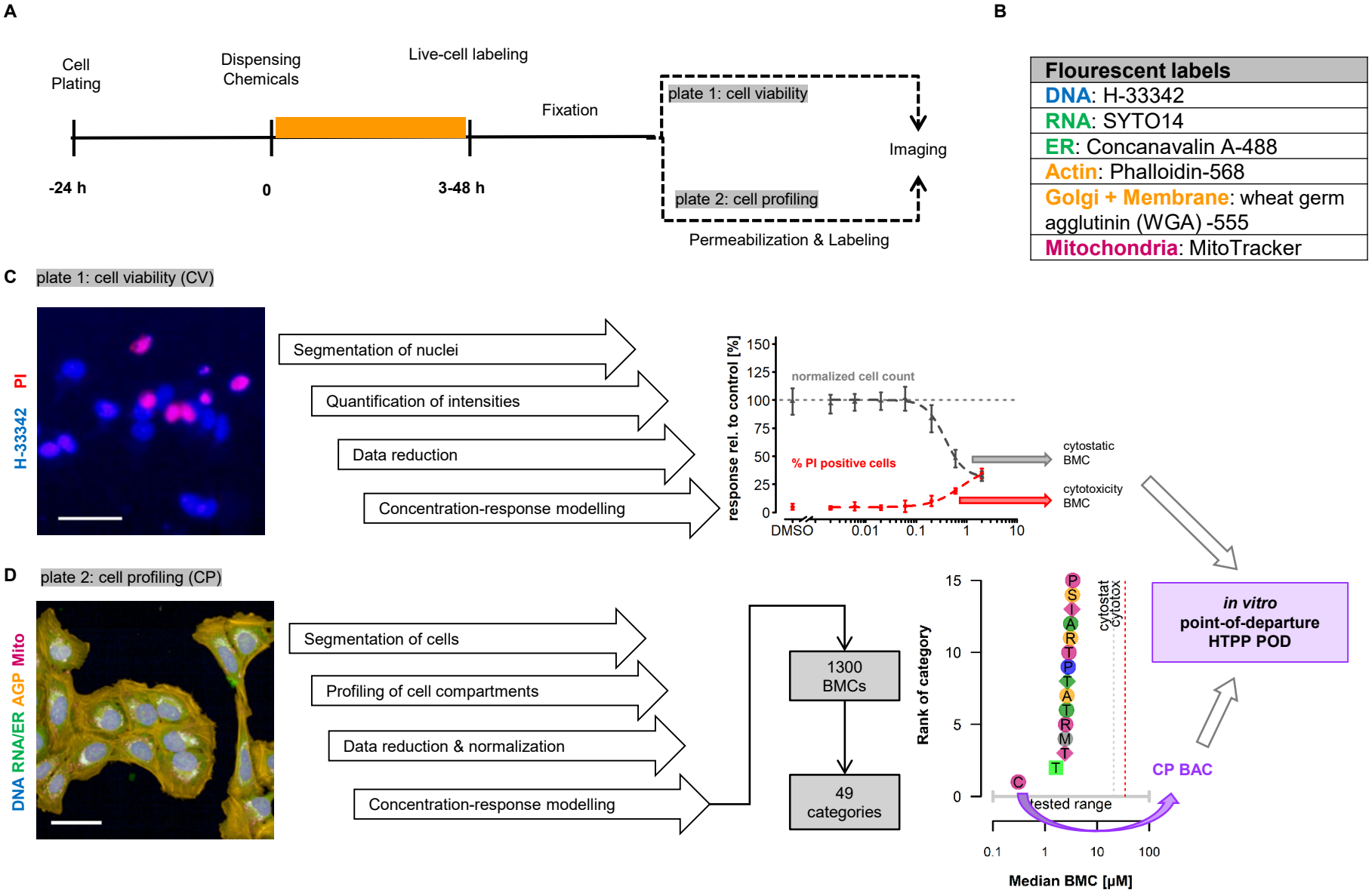
Phenotypic Category Analysis





Bioactivity Altering Concentration (BAC):
Median BMC of the most sensitive category
(where ≥ 30% ontology elements affected)

HTPP Assay Overview



Pilot Study Design

Parameter	Multiplier	Notes
Cell Type(s)	1	**U-2 OS**
Culture Condition	1	DMEM + 10% HI-FBS [a]
Chemicals	16	14 phenotypic reference chemicals, 2 negative controls
Time Points:	1	48 hours
Assay Formats:	2	Cell Painting HCI Cell Viability & Apoptosis
Concentrations:	8	3.5 $\log_{10}$ units; semi $\log_{10}$ spacing
Biological Replicates:	3	Independent cultures

Reference Chemical Set

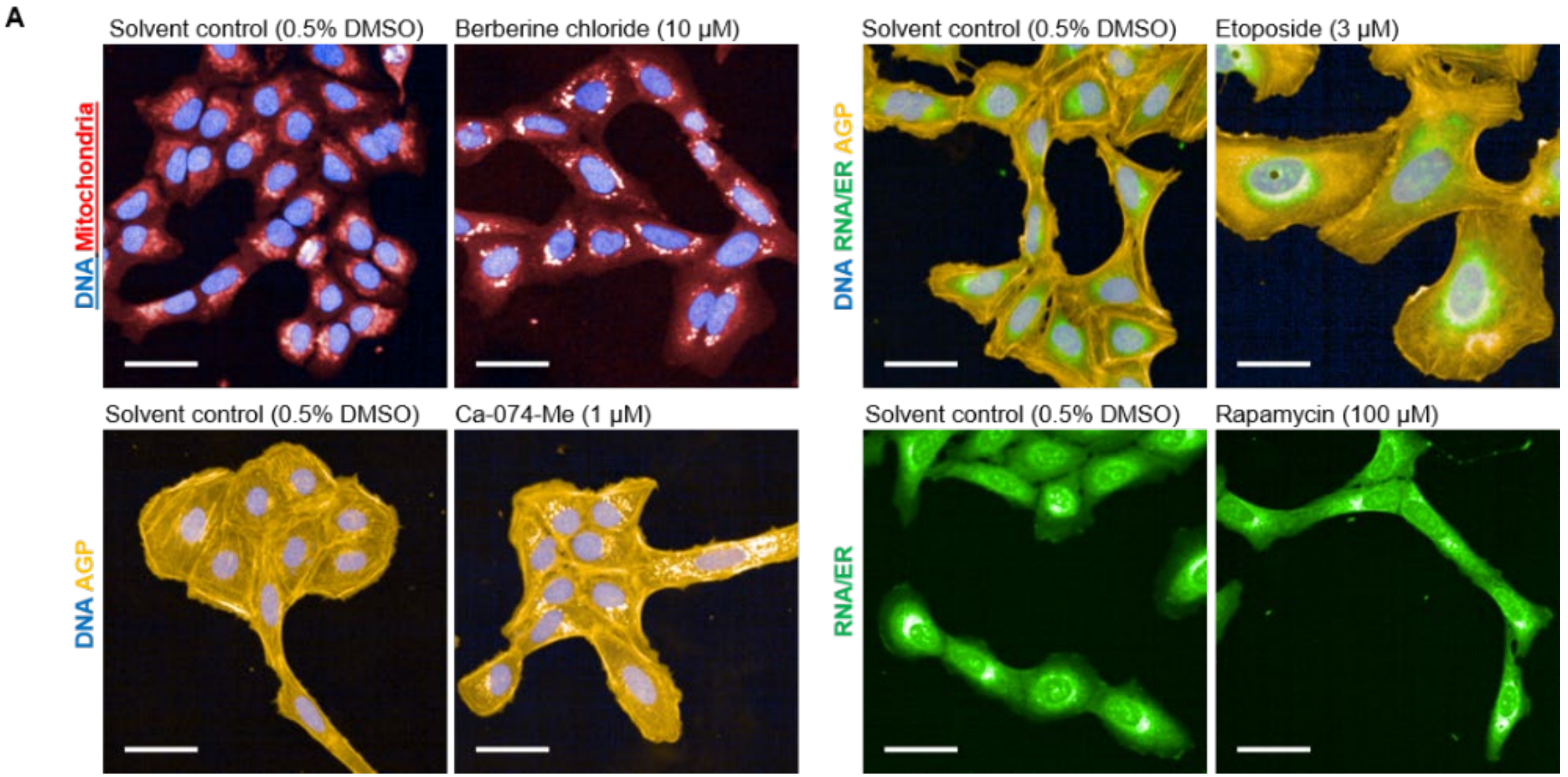
- Reference chemicals (n=14) with narrative descriptions of observed phenotypes were identified from Gustafdottir et al. 2013.
- Candidate negative control chemicals (n=2) with no anticipated affect on cell phenotype were included in the reference set.

Compound Name	Chemical Use	Expected Phenotype
Amperozide	Atypical antipsychotic	Toroid nuclei
Berberine Chloride	Mitochondria complex I inhibitor	Redistribution of mitochondria
Ca-074-Me	Cathepsin B inhibitor	Bright, abundant golgi staining
Etoposide	Chemotherapeutic	Large, flat nucleoli
Fenbendazole	Anthelmintic	Giant, multi-nucleated cells
Fluphenazine	Typical antipsychotic	Enhanced golgi staining and some cells with fused nucleoli
Latrunculin B	Actin cytoskeleton disruptor	Actin breaks
Metoclopramide	D_2 dompaine receptor antagonist	Enhanced golgi staining and some cells with fused nucleoli
NPPD	Chloride channel blocker	Redistribution of ER to one side of the nucleus
Oxibendazole	Anthelmintic	Large, multi-nucleated cells with fused nucleoli
Rapamycin	Macrolide antibiotic / antifungal	Reduced nucleolar size
β-dihydrorotenone [a]	Mitochondria complex I inhibitor	Mitochondrial stressor
Saccharin	Artificial Sweetener	Negative Control
Sorbitol	Artificial Sweetener	Negative Control
Taxol	Microtubule Stabilizer	Large, multi-nucleated cells with fused nucleoli
Tetrandrine	Calcium channel blocker	Abundant ER

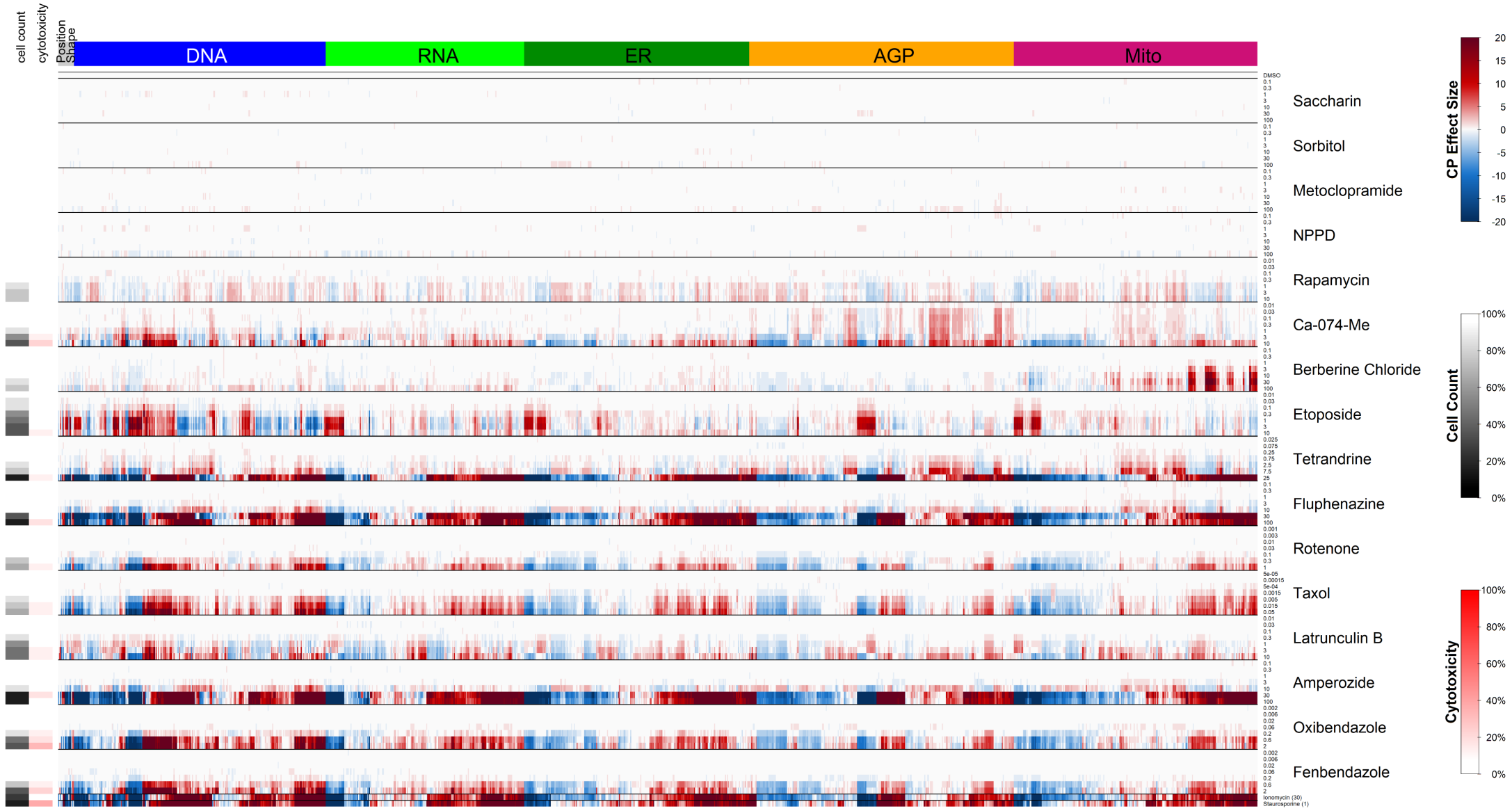
[a] *β-dihydrorotenone not commercially available. Tested rotenone instead*

Gustafsdottir, et al. 2013

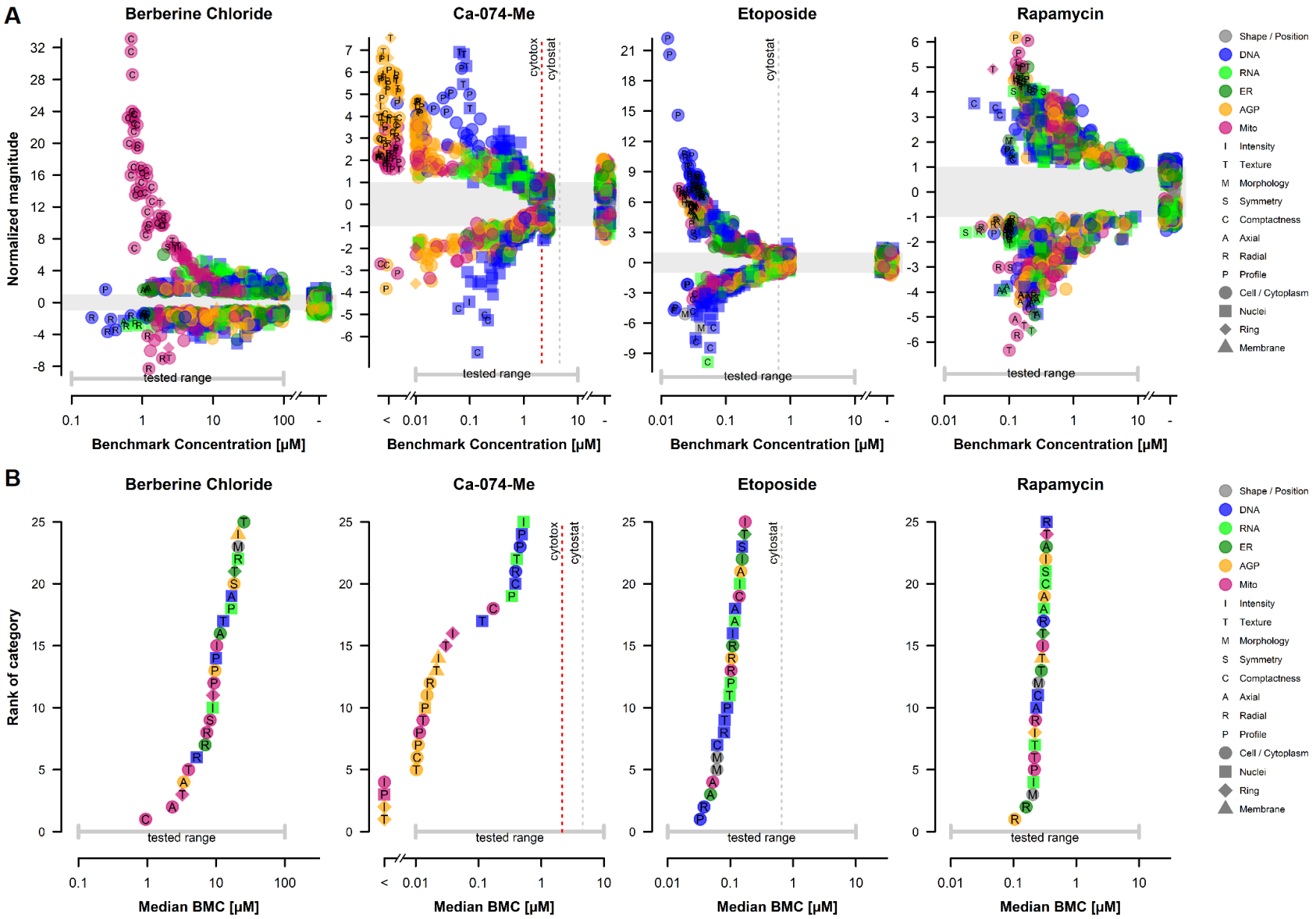
Reference Chemical Phenotypes (1)



Reference Chemical Phenotypes (2)

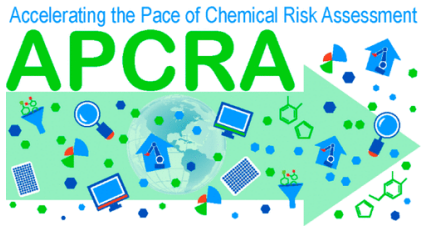


Reference Chemical Phenotypes (3)



U-2 OS APCRA Screen Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	U-2 OS
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	462	APCRA Case Study Chemicals + Duplicates Unilever CRADA Consensus Chemicals HTTr Pilot Chemicals
Time Points:	1	24 hours
Assay Formats:	2	Cell Painting Cell Viability
Concentrations:	8	3.5 $\log_{10}$ units; semi $\log_{10}$ spacing
Biological Replicates:	4	Independent cultures



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

- International collaboration of regulatory scientists focused on developing case studies for evaluating the use of New Approach Methodologies (NAMs) in chemical risk assessment.
- ECHA Workshop (2017) case study focuses on **deriving quantitative estimates of risk based on NAM-derived potency information and computational exposure estimates**

U-2 OS APCRA Screen: Dose Plate Design

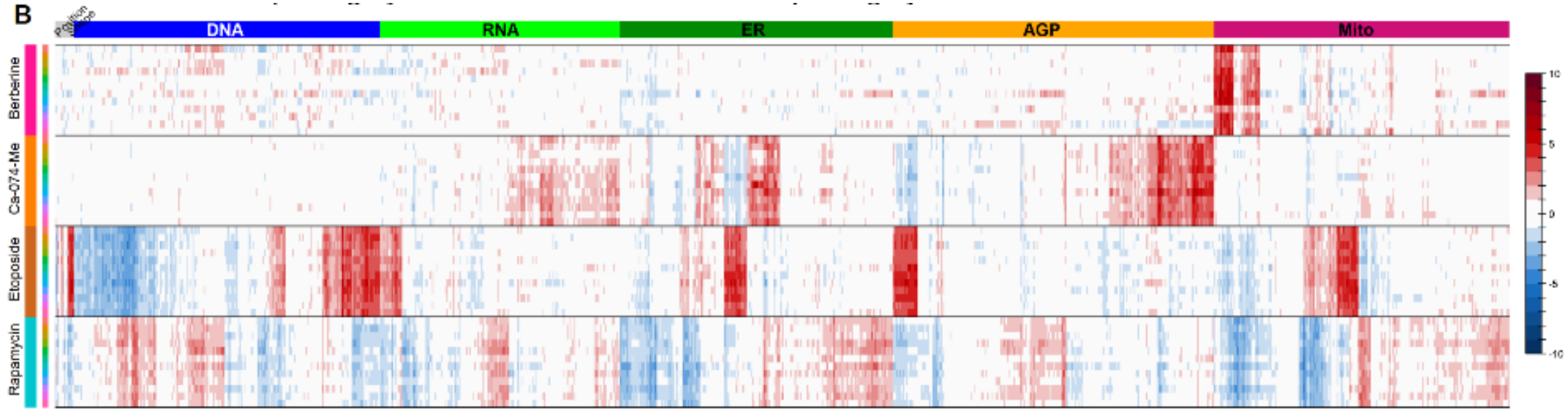
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	A	B	C	DMSO
B	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	A	B	C	DMSO
C	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	A	B	C	DMSO
D	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	A	B	C	DMSO
E	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	A	B	C	DMSO
F	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	A	B	C	DMSO
G	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	A	B	C	DMSO
H	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	A	B	C	DMSO
I	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100	D	STS	DMSO	DMSO
J	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	30	D	STS	DMSO	DMSO
K	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10	D	STS	DMSO	DMSO
L	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3	D	STS	DMSO	DMSO
M	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	D	STS	DMSO	DMSO
N	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	D	STS	DMSO	DMSO
O	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	0.1	D	STS	DMSO	DMSO
P	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	D	STS	DMSO	DMSO

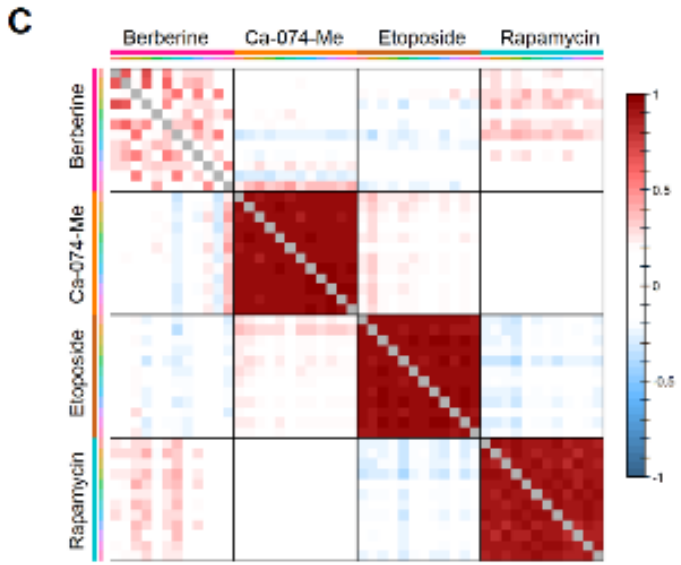
Label	Reference Chemicals:	Phenotypic Observations	Test Concentrations
A	Berberine Chloride	Specific mitochondrial effects	0.03 – 10 uM
B	Etoposide	Cell hypertrophy control that produces effects in every channel / organelle	0.03 - 10 uM
C	Ca-074-Me	Effects on AGP channel at sub-cytotoxic doses	0.03 -10 uM
D	Rapamycin	Effects on RNA and DNA channels	0.03 - 10 uM
STS	Staurosporine	Cytotoxicity Control	0.01 -3 uM

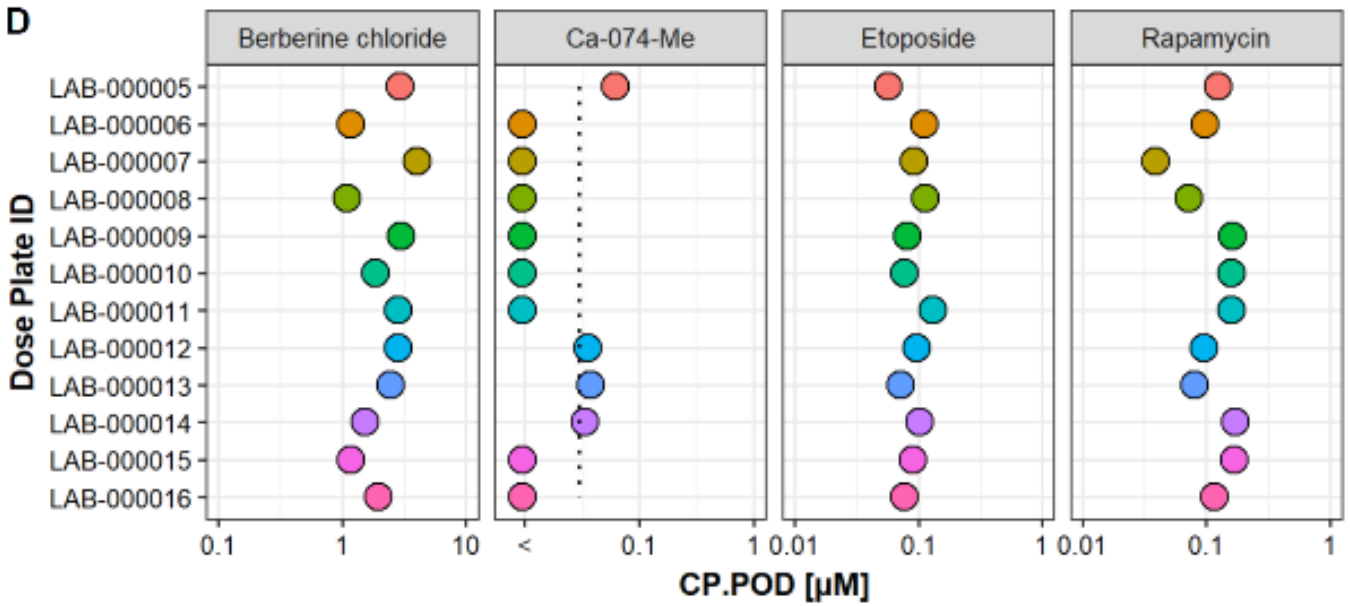
	c2018-10-14		c2018-10-15		c2018-10-16		c2018-10-17		c2018-12-11	
Dose Plate ID	CP	CV	CP	CV	CP	CV	CP	CV	CP	CV
LAB-000005	TC00000189	TC00000201	TC00000213	TC00000225	TC00000237	TC00000249	TC00000261	TC00000273		
LAB-000006	TC00000190	TC00000202	TC00000214	TC00000226	TC00000238	TC00000250	TC00000262	TC00000274		
LAB-000007	TC00000191	TC00000203	TC00000215	TC00000227	TC00000239	TC00000251	TC00000263	TC00000275		
LAB-000008	TC00000192	TC00000204	TC00000216	TC00000228	TC00000240	TC00000252	TC00000264	TC00000276		
LAB-000009	TC00000193	TC00000205	TC00000217	TC00000229	TC00000241	TC00000253	TC00000265	TC00000277		
LAB-000010	TC00000194	TC00000206	TC00000218	TC00000230	TC00000242	TC00000254	TC00000266	TC00000278		
LAB-000011	TC00000195	TC00000207	TC00000219	TC00000231	TC00000243	TC00000255	TC00000267	TC00000279		
LAB-000012	TC00000196	TC00000208	TC00000220	TC00000232	TC00000244	TC00000256	TC00000268	TC00000280		
LAB-000013	TC00000197	TC00000209	TC00000221	TC00000233	TC00000245	TC00000257	TC00000269	TC00000281	TC00000294	TC00000298
LAB-000014	TC00000198	TC00000210	TC00000222	TC00000234	TC00000246	TC00000258	TC00000270	TC00000282	TC00000295	TC00000299
LAB-000015	TC00000199	TC00000211	TC00000223	TC00000235	TC00000247	TC00000259	TC00000271	TC00000283	TC00000296	TC00000300
LAB-000016	TC00000200	TC00000212	TC00000224	TC00000236	TC00000248	TC00000260	TC00000272	TC00000284	TC00000297	TC00000301

= Plates in gray failed QC and had to be re-run

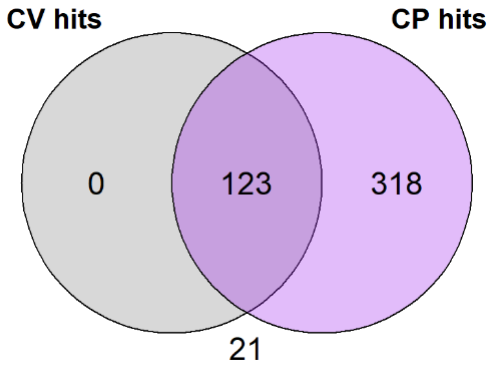
U-2 OS APCRA Screen: Assay Reproducibility

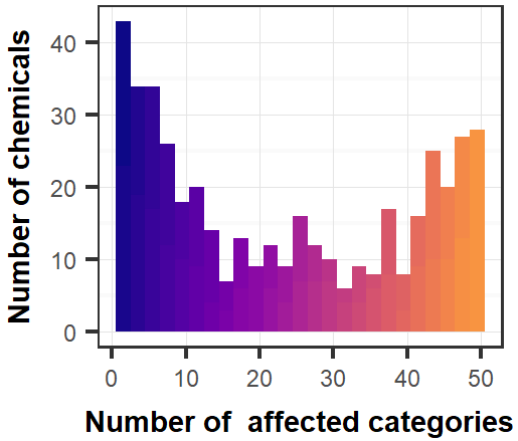


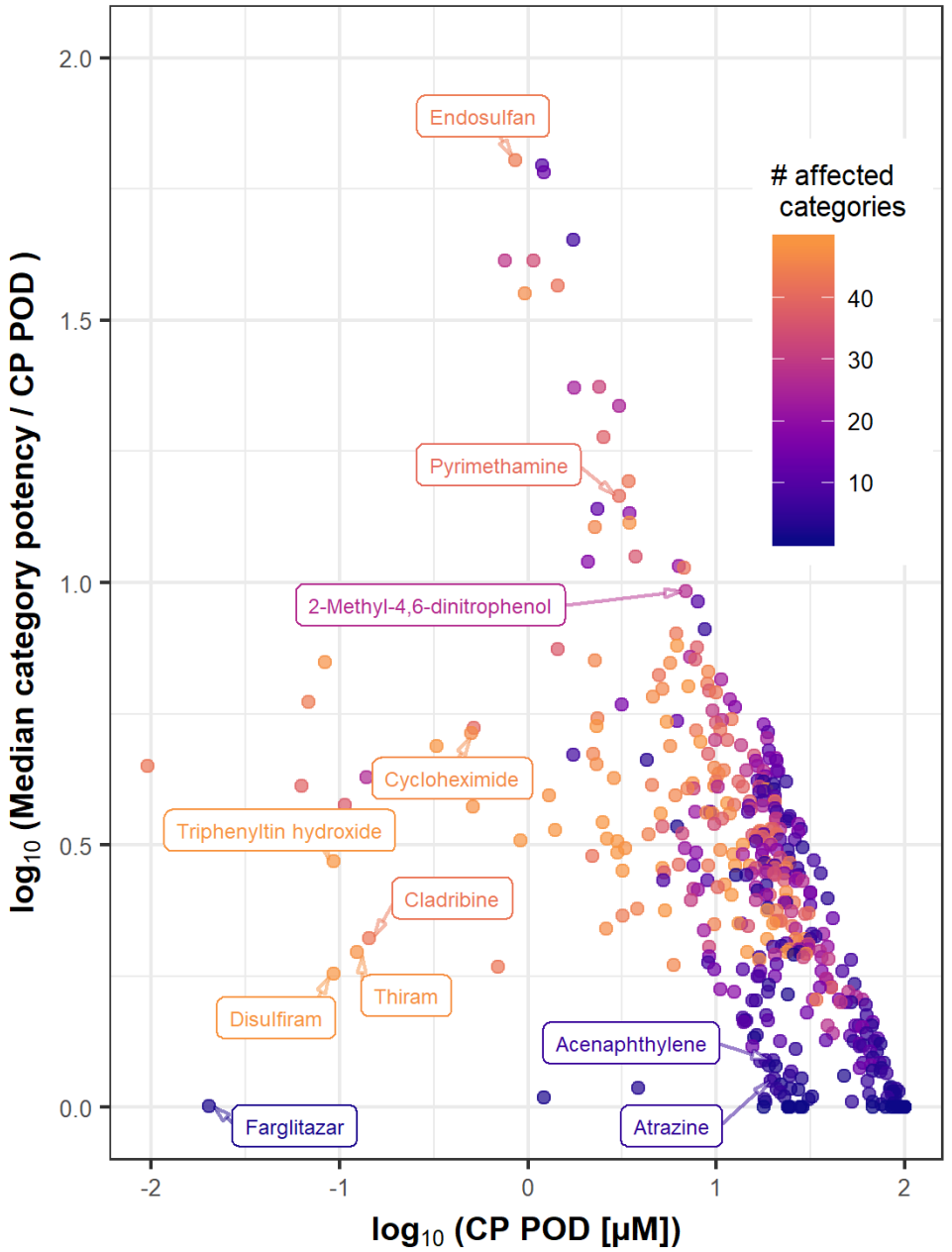


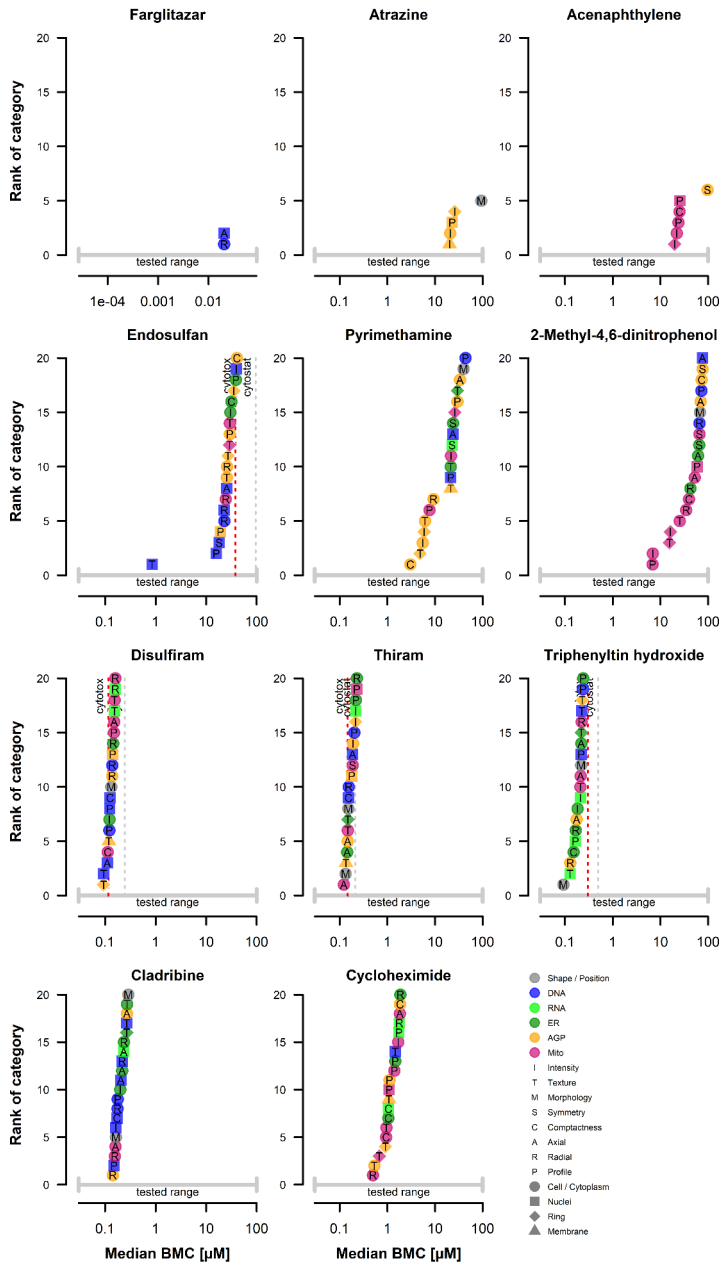


U-2 OS APCRA Screen: Results

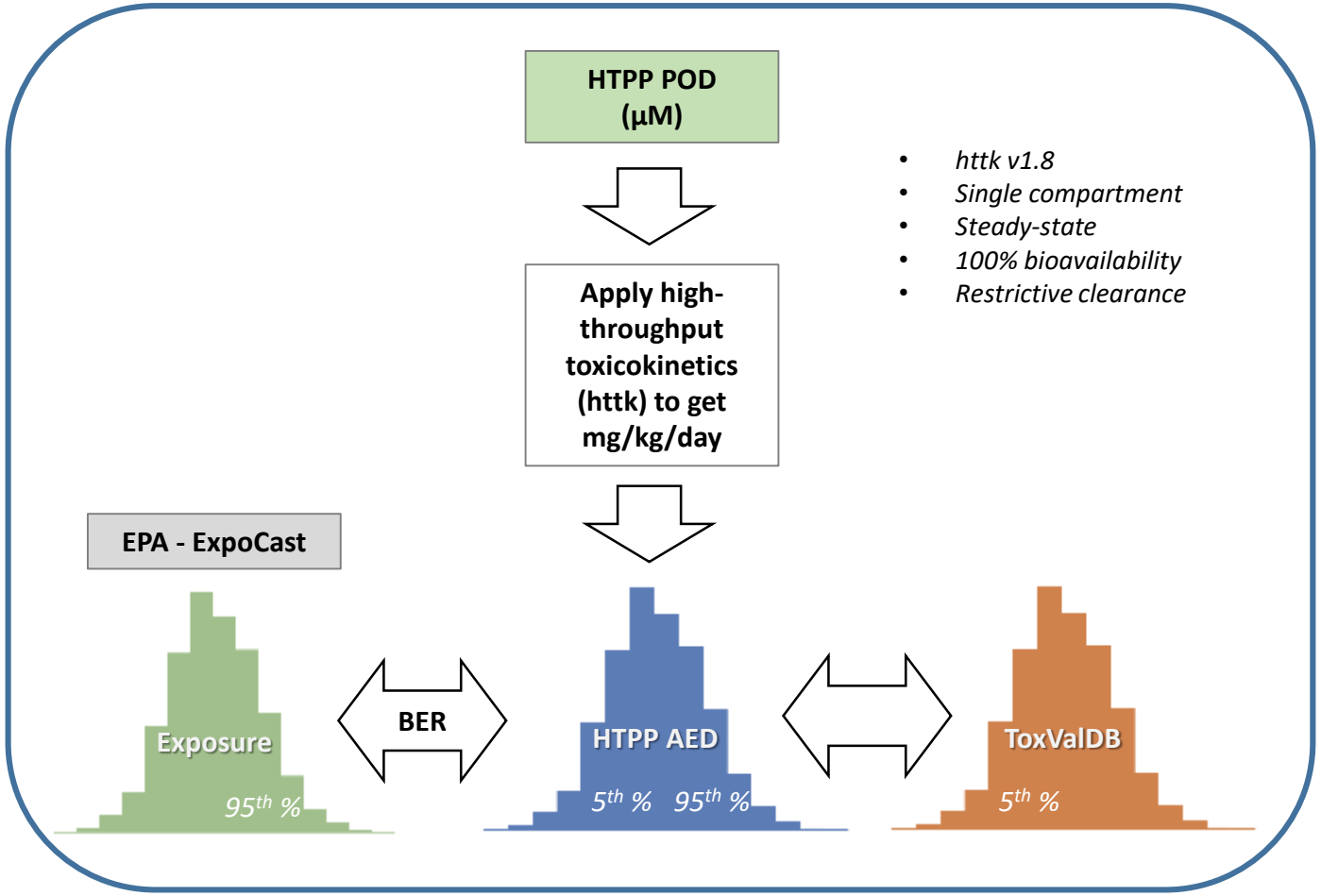


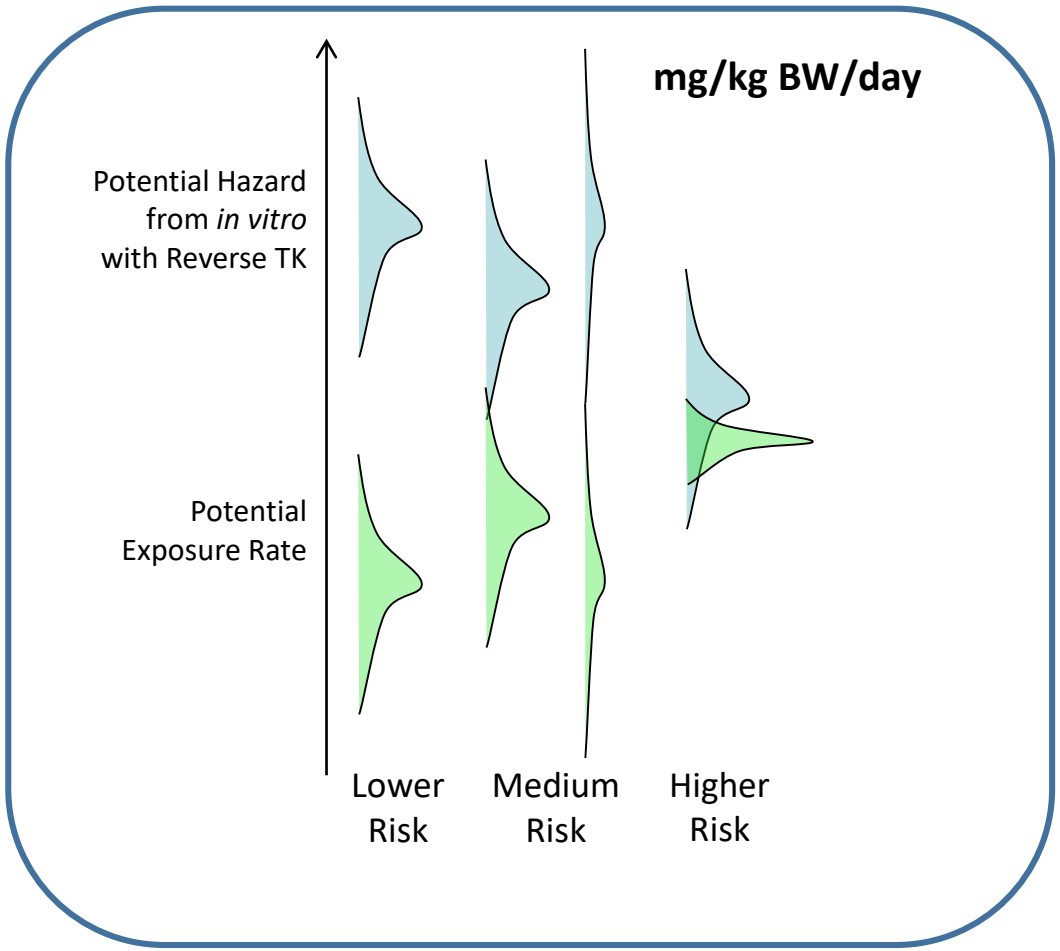






In Vitro-in-*In Vivo* Extrapolation (IVIVE) & Bioactivity Exposure Ratios (BER)



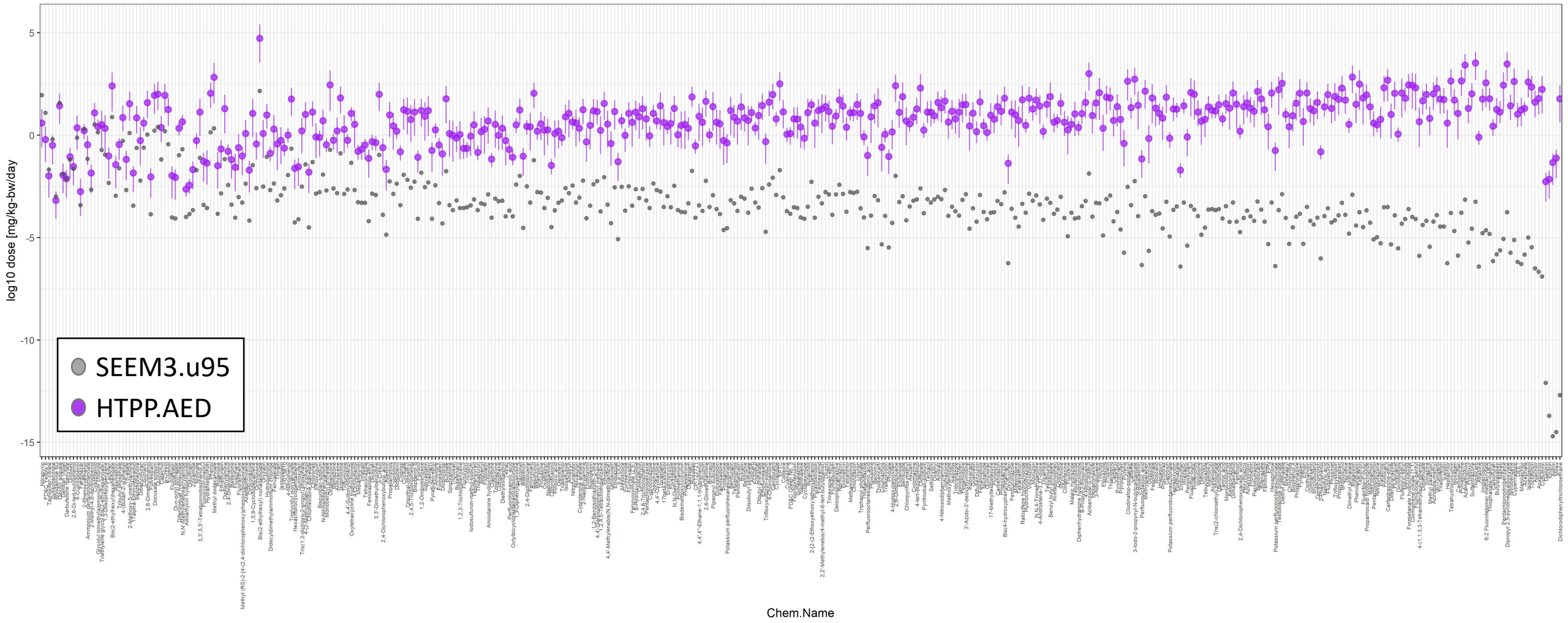


High-throughput toxicokinetic (httk) modeling: Conversion of *in vitro* bioactivity to *in vivo* steady state concentration (C_{ss})

Reverse dosimetry: Conversion of predicted C_{ss} to an administered equivalent dose (AED)

Figure courtesy of Katie Paul-Friedman & John Wambaugh

BER Results



- Chemicals with small BER ratio would be of higher priority that chemicals with a large BER ratio.

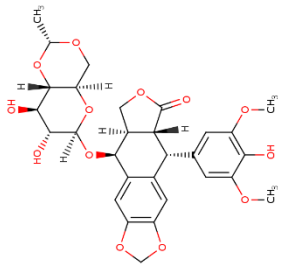
Read Across Pilot, Experimental Design

Parameter	Multiplier	Notes
Cell Type(s)	1	U-2 OS
Culture Condition	1	DMEM + 10% HI-FBS
Chemicals	120	Pharmacological Tool Compounds Model Toxicants Structure Series
Time Points:	1	24 hours
Assay Formats:	2	Cell Painting Cell Viability
Concentrations:	8	3.5 $\log_{10}$ units; semi $\log_{10}$ spacing
Biological Replicates:	4	Independent cultures

Actin cytoskeleton modulators	DNA toxicants: alkylators	Mito. Respiratory complex inhibitor
Actin stabilizers	DNA toxicants: topoisomerase	Autophagy inhibitor
ER modulator	DNA toxicants: antimetabolites	Autophagy activator
Golgi modulator	DNA toxicants: genotoxic	RNA polymerase inhibitor
Mitochondrial fission	Oxidative stress	Benzimidazole structure series
Microtuble modulator	Proteosome inhibitors	Rapamycin analogues
Microtuble stabilizer	Oxidative phosphorylation uncoupler	Ca-074-Me analogues

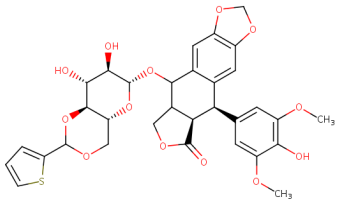
Read Across Pilot, Results (2)

Profile: Feature level response magnitudes
Signature: Response threshold @ 1.5
Similarity Metric: Pearson Correlation
Comparisons: All test chemicals against each other
“Hit” Criteria: Correlation > 0.5

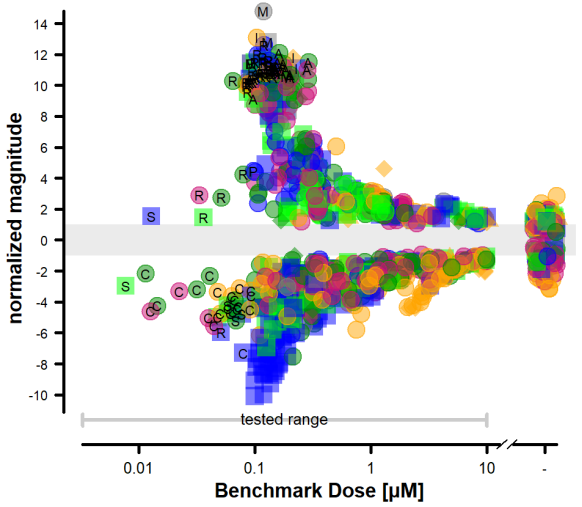


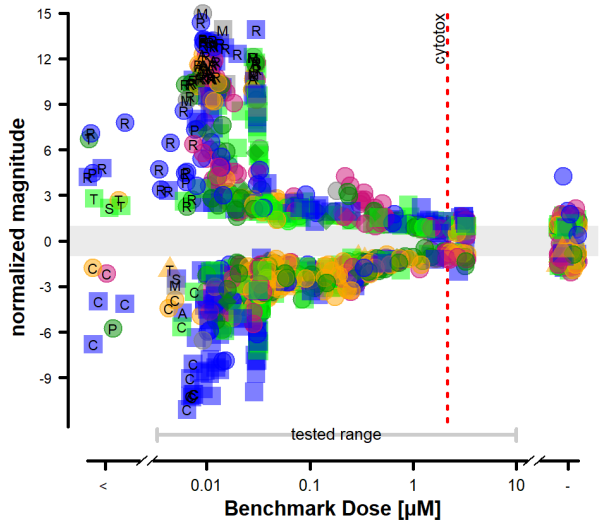
Etoposide | DTXSID5023035

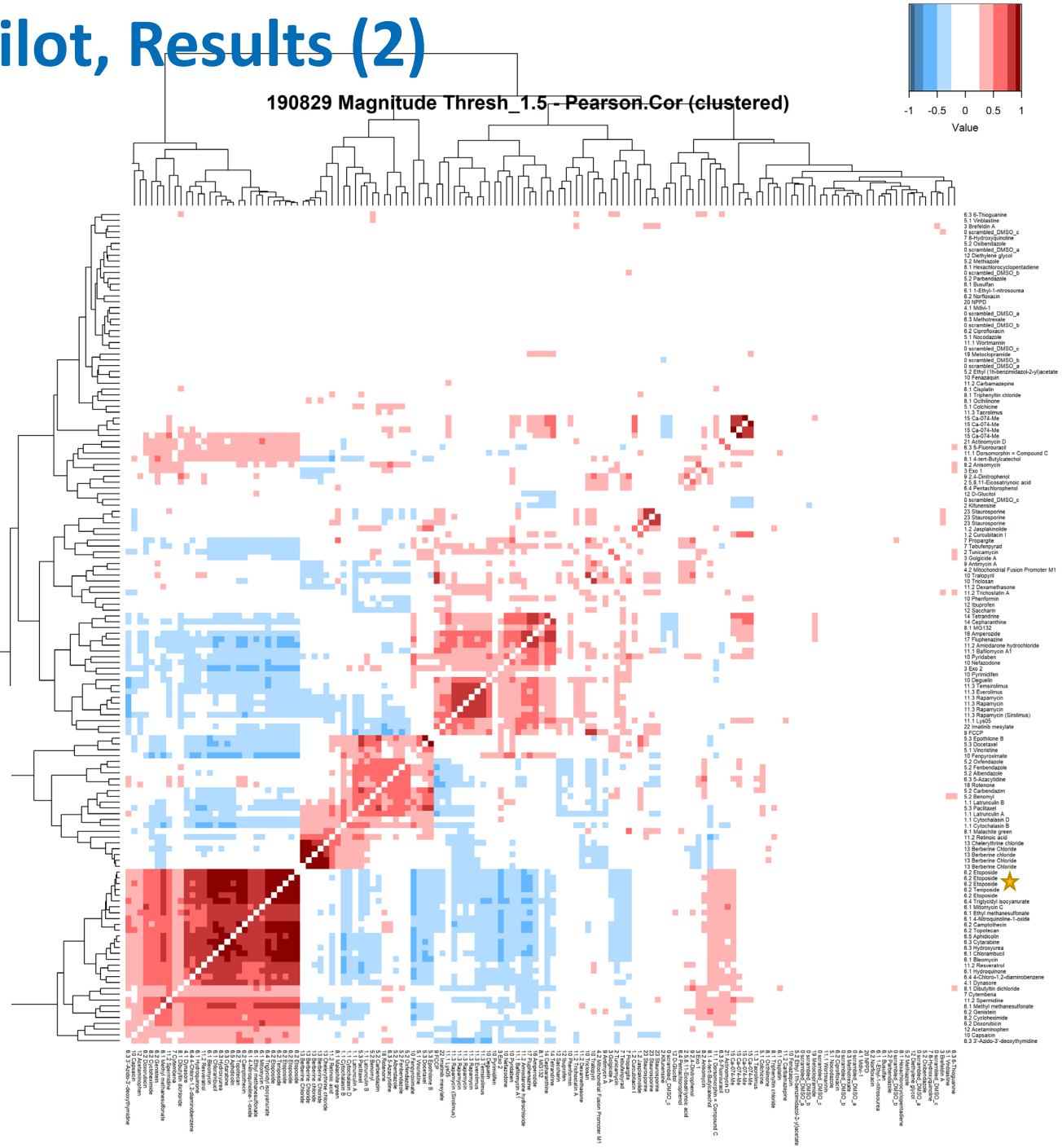
0.966



Teniposide | DTXSID8023638

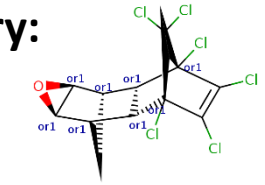






Read Across Example (1)

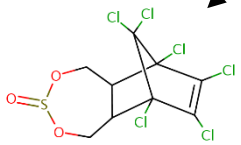
Query:



Endrin
72-20-8 |
DTXSID6020561

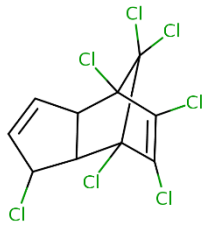
Matches:

0.71



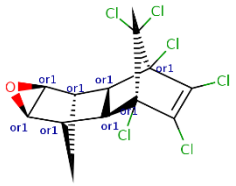
Endosulfan
115-29-7 |
DTXSID1020560

0.70



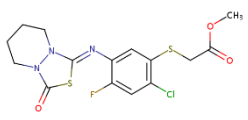
Heptachlor
76-44-8 |
DTXSID3020679

0.61



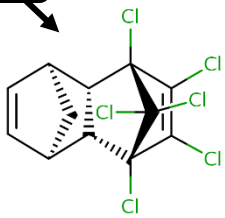
Dieldrin
60-57-1 |
DTXSID9020453

0.59

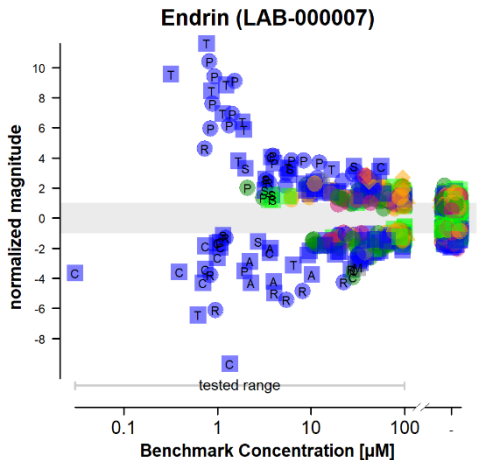


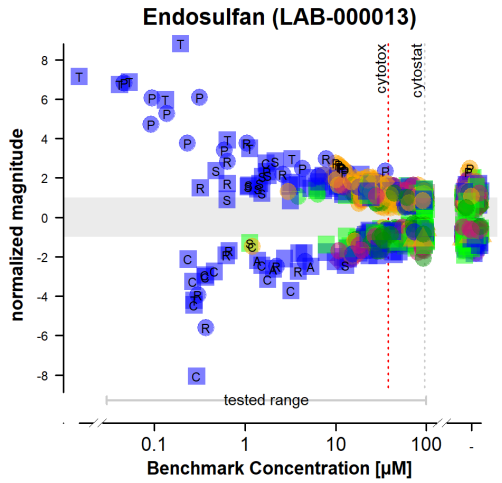
Fluthiacet-methyl
117337-19-6 |
DTXSID2032556

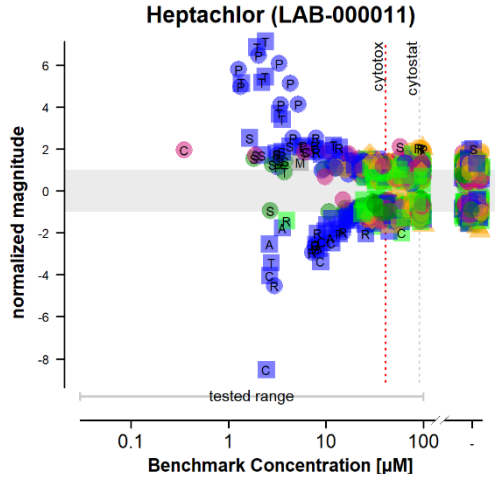
0.59

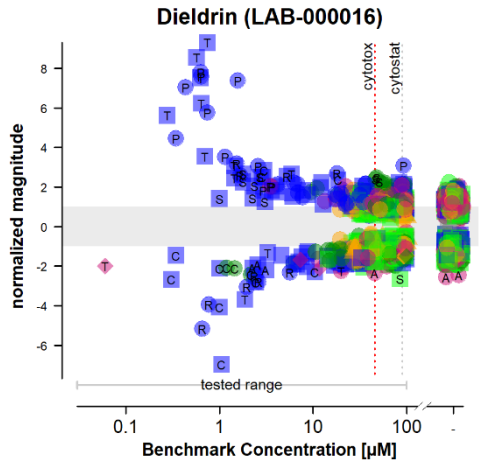


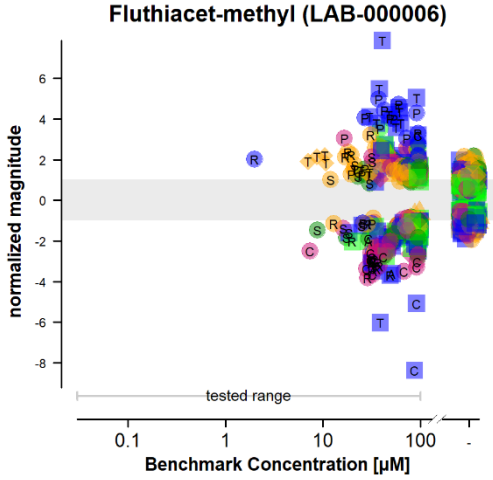
Aldrin
309-00-2 |
DTXSID8020040

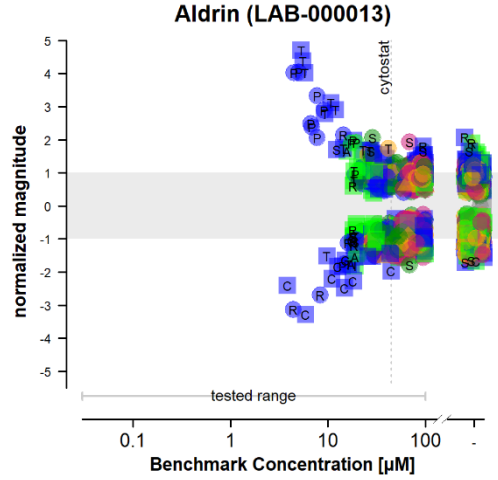






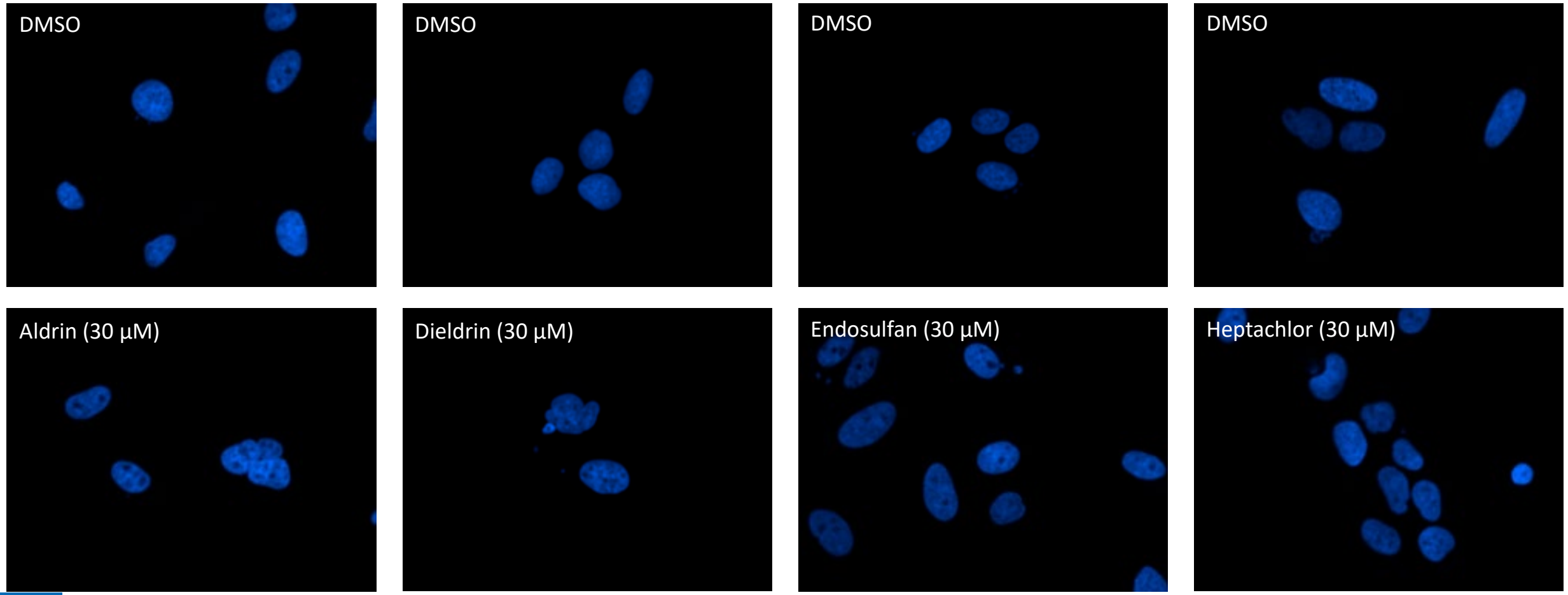






Organochlorine Pesticides

- Changes in nuclear texture manifest as “holes”.



Read Across Example (2)

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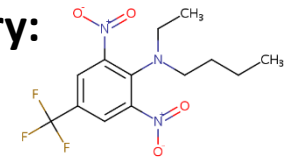
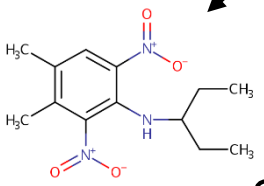


Matches:

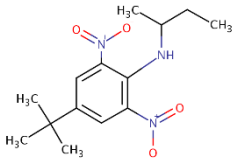
Benfluralin
1861-40-1 |
DTXSID3023899

0.75



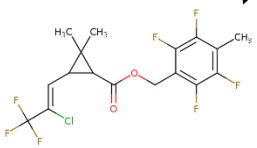
Pendimethalin
40487-42-1 |
DTXSID7024245

0.68



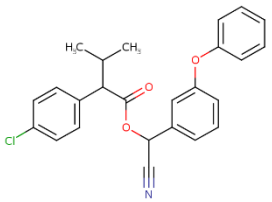
Butralin
33629-47-9 |
DTXSID3032337

0.75



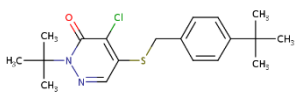
Tefluthrin
79538-32-2 |
DTXSID5032577

0.70

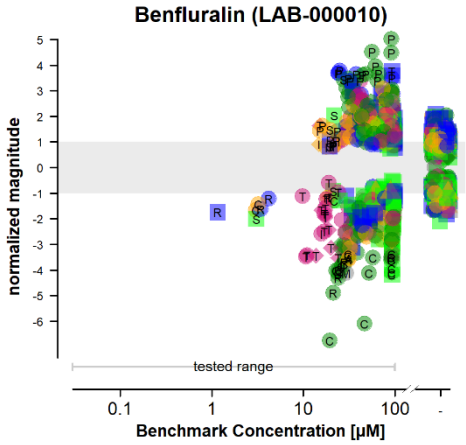


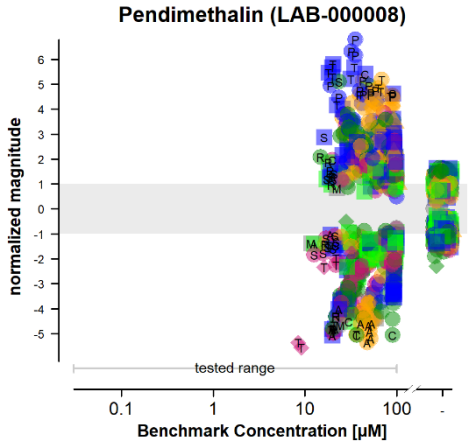
Fenvalerate
51630-58-1 |
DTXSID101017940

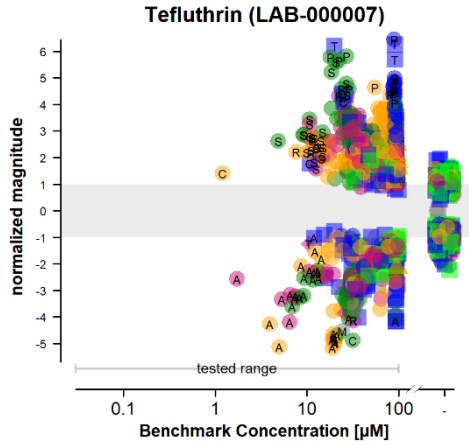
0.69

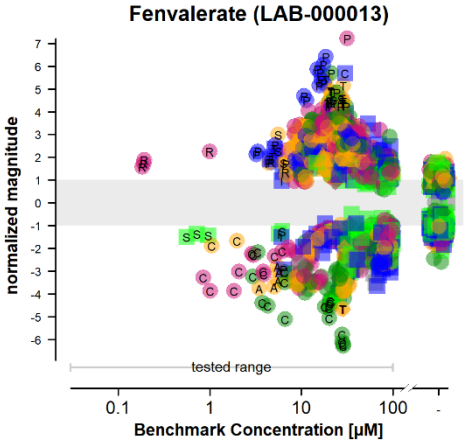


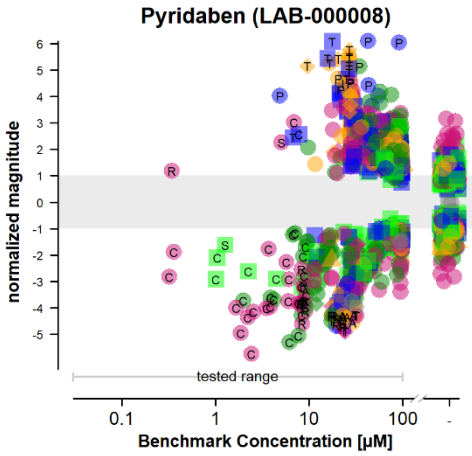
Pyridaben
96489-71-3 |
DTXSID5032573

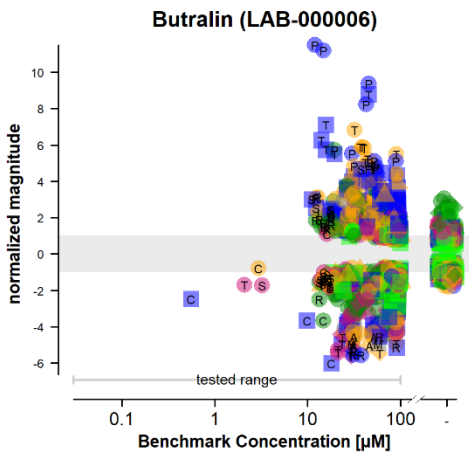












Summary

- **Workflow:** We have established the Cell Painting assay and developed computational workflows for phenotypic feature extraction, data normalization, concentration-response modeling and generation of phenotypic response profiles.
- **Reproducibility:** Demonstrated reproducibility of potency estimates and phenotypic profiles in the Cell Painting assay using phenotypic reference chemicals.
- **Chemical Screening:** Performed concentration-response screening of a set of environmental chemicals (APCRA) and a set of pharmacological chemicals and model toxicants (RefChem120).
- **Bioactivity Exposure Ratio (BER):** HTPP data may be used in combination with IVIVE and ExpoCast estimates to identify chemicals with bioactivity thresholds in human relevant exposure ranges.
- **Chemical Read Across:** Vector-based similarity approaches were able to identify structurally-related chemicals with similar response profiles.

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