Surveying the Biological Activity of Drugs and Consumer Products Across Ten Biologically Diverse Human-Derived Cell Lines using the Cell Painting Assay

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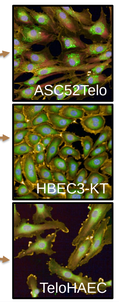
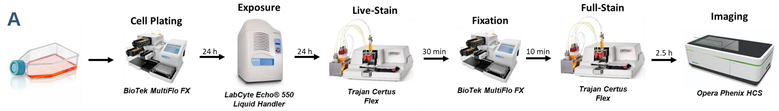
Background

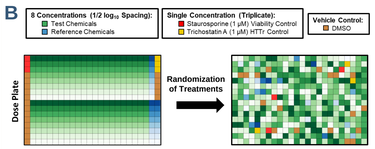
- The Center for Computational Toxicology and Exposure (CCTE) at the U.S. Environmental Protection Agency and the Unilever Safety, Environmental and Regulatory Science (SERS) group are engaged in a Cooperative Research and Development Agreement (CRADA) that aims to assess the utility of a **systemic safety toolbox for next generation risk assessment**.
- The systemic safety toolbox is comprised of **non-animal based new approach methodologies (NAMs)** for *in silico* estimation of human exposures and characterizing the biological activity of chemicals using human-derived *in vitro* models.
- The aim of this project was to investigate the use of **high-throughput phenotypic profiling (HTPP) with the Cell Painting assay** to generate and characterize the variability of *in vitro* potencies for **42 case study chemicals across ten biologically diverse human-derived cell lines**.

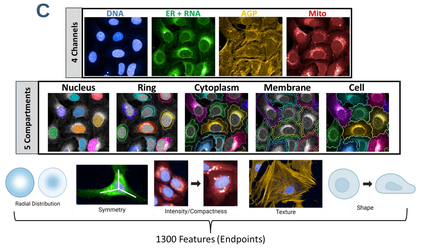
Cell Line	Tissue Origin	Disease State	Morphology
ASC52Telo	Adipose	Normal (hTERT Immortalized)	fibroblast-like
CCD-18Co	Colon	Normal (hTERT Immortalized)	fibroblast-like
CHON-001	Long bone; cartilage	Normal (hTERT Immortalized)	fibroblast-like
HBEC3-KT	Lung; bronchus	Normal (hTERT Immortalized)	epithelial
HepG2	Liver	Hepatocellular Carcinoma	epithelial
hNP1	Brain	Neural Progenitor	epithelial
Ker-CT	Skin; foreskin	Normal (hTERT Immortalized)	epithelial
RPTEC/TERT1	Kidney; proximal tube	Normal (hTERT Immortalized)	epithelial
TeloHAEC	Heart; aorta	Normal (hTERT Immortalized)	endothelial
U-2 OS	Bone	Osteosarcoma	epithelial



Experimental Design







Parameter	Multiplier	Notes
Chemicals:	42	Project chemicals
Concentrations:	8	Dilutions series tailored to each chemical
Cell Types:	10	Human-derived cell lines; various tissues
Assay Formats:	1	HTPP (Cell Painting)
Exposure Duration:	1	24 HR
Biological Replicates:	4	Independent cultures of each cell line

Figure 1: Overall design for the study, including screening details, dose plate layout, and fluorescent channel information. (A) The HTPP assay utilizes a variety of microfluidic instruments that assist with plating, dosing, and staining plates prior to imaging. (B) All dose plates contain 42 test chemicals and 3 reference chemicals in 8-point concentration series, as well as additional vehicle and reference control treatments. (C) Fluoroprobe labeling patterns in four different imaging channels (shown in HBEC3-KT cells). Subsequent cell segmentation and image analysis are performed using PerkinElmer Harmony® software.

Results: High Variability in PACs Observed for Some Chemicals

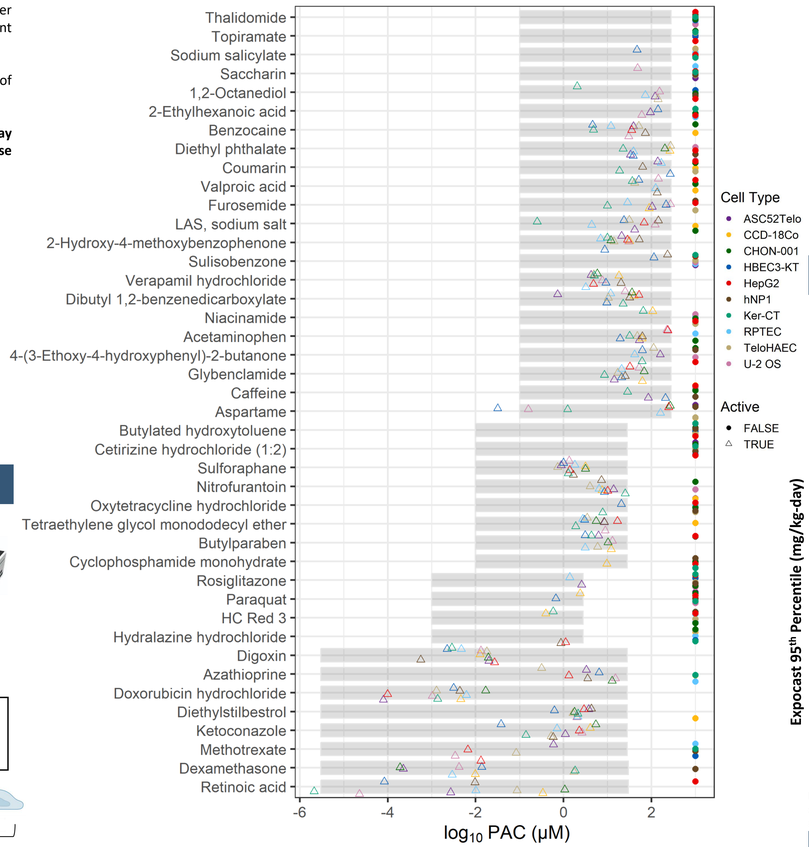


Figure 2: Overall PAC results from 42 chemicals across 10 human-derived cell lines. Phenotype Altering Concentration (PAC) in $\log_{10}$ µM units are plotted along the *x*-axis. Chemical names are listed along the *y*-axis with dose ranges shown in the grey band for each chemical. Chemicals were separated into four distinct dose bands for this study consisting of high (0.1 – 300 µM), medium (0.01 – 30 µM), low (0.01 – 3 µM), and very low ($3x10^{-6}$ – 30 µM). Half-$\log_{10}$ spacing was used for all dose bands except for very low, where a full-$\log_{10}$ dose spacing was utilized. Chemicals in the higher dose bands consist of more consumer products while chemicals in the low to very-low range consisted mostly of drugs. A PAC value is calculated as the minimum of the benchmark concentration for either 49 different "category" Mahalanobis distance calculations or one single "global" Mahalanobis distance calculation.. Most chemicals tested were found to have a PAC for at least one cell type. Active chemical PACs were within 2 orders of magnitude for most chemicals with some of the drugs having higher PAC variability, upwards of 5 orders of magnitude.

Results: Chemical Bioactivity for Most Chemicals is Dependent on Cell Type

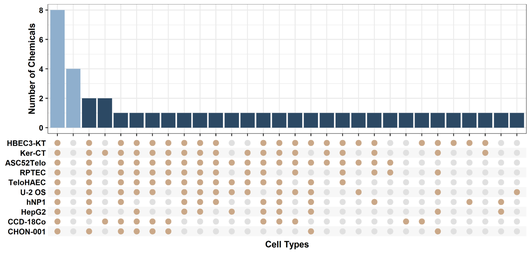


Figure 3: The overlap of active chemicals across cell types. The intersection between cell types is shown across the *x*-axis with a filled circle designating an active chemical hit. The first column on the far left indicates the number of chemicals found active in all 10 cell types tested. The 2nd bar from the left shows the total number of chemicals found inactive across all 10 cell types tested. There are a select number of chemicals that were found to be active in only 1-2 cell types being tested.

- 8 chemicals found active in ALL cell types
- 4 chemicals found inactive across all cell types

Results: Bioactivity to Exposure Ratio (BER) for Chemical Prioritization

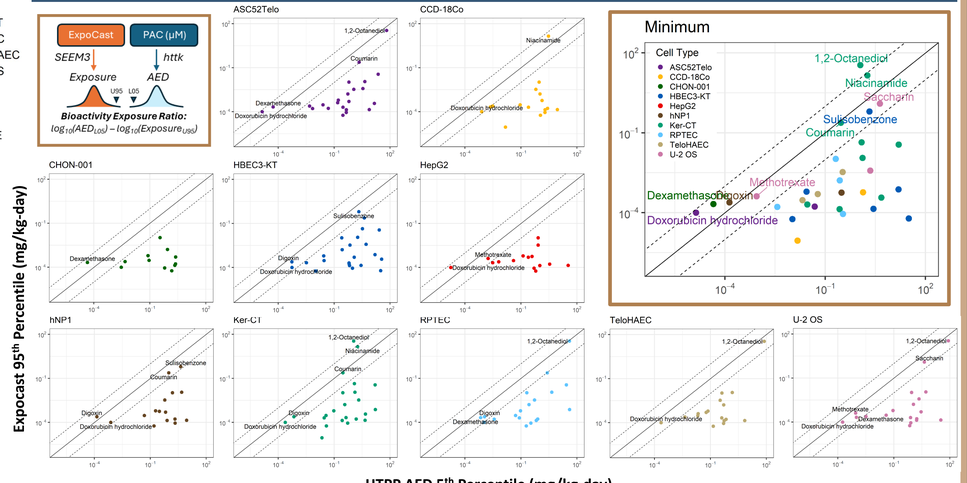


Figure 4: BER results for active chemical hits in each cell line and the minimum BER for each chemical across all cell types. Each plot represents the ratio of the 5th percentile administered equivalent dose (AED) for each chemical, calculated using the HTPP PAC value as input for the *httk* R package, to the 95th percentile of exposure using ExpoCast data. When taking the minimum BER value across all cell types for active chemicals, the results show a higher number of chemicals with a BER of less than one.

Conclusions and Future Directions

- Bioactivity of chemicals is dependent on the cell type being tested and this should be considered when planning future screening studies.
- Gene expression levels corresponding to each individual cell type are being compared to molecular targets of the chemical set tested in this study for potential sources of PAC variability.
- Ongoing analyses also include comparing phenotypic profiles across cell types and conducting *in vitro* to *in vivo* extrapolation to characterize the impact of cell line selection on the biological toxicity effect ratio (BER) results.