Robust Phenotypic Profiling Assays for Predictive Toxicity on Human Hepatic Cells

Wei Chen[1], Eric Sherer[1], Zachary Sutake[1], Clinton Willis[2], Eduardo P. Costa[1], Enrica Bianchi[1], Joshua A. Harrill[2], Jessica LaRocca[1]

[1]Corteva Agriscience, Crop Protection Research and Development, Indianapolis, IN, US

[2]US Environmental Protection Agency, Center for Computational Toxicology & Exposure, Office of Research and Development, Research Triangle Park, NC

Background

- Cell Painting, a high content imaging-based phenotypic profiling assay that multiplexes six fluorescent dyes, is currently being leveraged for high-throughput toxicology screening across diverse human-derived cell lines (Bray, 2016).
- We sought to develop the cell painting technology in the metabolically competent human HepaRG cell line.

Fully differentiated HepaRG cells are metabolically competent

- HepaRG cells were purchased from Biopredic International.
- Propagation and differentiation were completed at Corteva Agriscience.
- Functional analysis confirmed that HepaRG cells are metabolically competent.

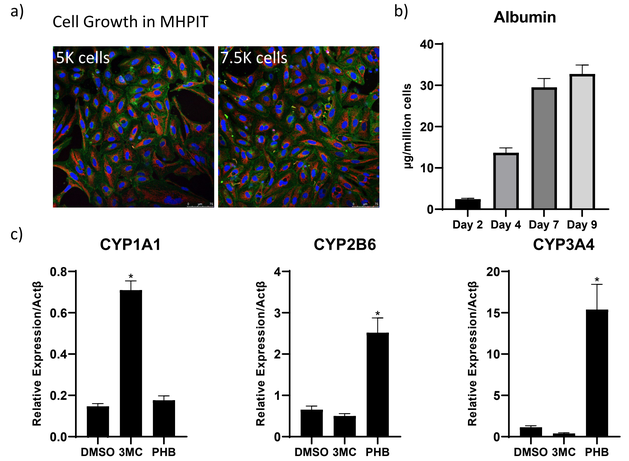


Figure 1: Characterization of HepaRG cells. a) Cells seeded at 5K and 7.5K cells/well in 384-well plate after 7 days growth in HepaRG™ Preinduction and Toxicity Medium (MHPIT) maintained healthy morphology (Blue: Hoechst; Red: MitoTracker® Deep Red FM; Green: Wheat germ agglutinin. b) Albumin production maintained at acceptable levels measured by ELISA (Bethyl Laboratories). c) qRT-PCR results illustrate the CYP induction from 1 µM 3-Methylcholanthrene (3MC) and 500 µM Phenobarbital (PHB).

Workflow for Image-based Phenotypic Profiling (Cell Painting)

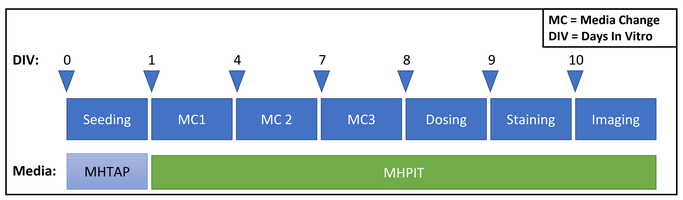
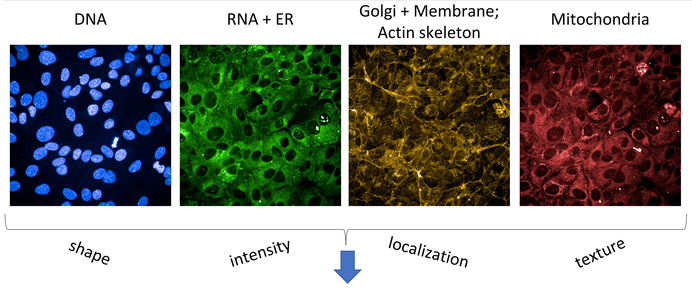


Figure 2: Cell Culture Timeline: HepaRG cells were thawed and plated in 384-well PerkinElmer PhenoPlates with HepaRG™ Thawing and Plating Medium (MHTAP) and maintained in HepaRG™ Preinduction and Toxicity Medium (MHPIT) for a minimum of 7 days to obtain hepatocyte-like metabolic functionality and establish a monolayer at ~90% confluency for chemical testing. Cells were stained with PhenoVue™ Cell Painting Kit following manufacture's recommendations and imaged with Opera Phenix confocal microscopy.

Representative images for Cell Painting



Optimization of data analysis for accurate point of departure (PoD) estimation

The effects of 22 chemicals on HepaRG cells were evaluated at EPA using Cell Painting. PoDs were then calculated at Corteva using the approach of Nyffeler et al. 2020 ("well-averaged" in results below).

- Each compound was tested at 8 concentrations with half $\log_{10}$ spacing
- 2 technical replicates for each concentration
- 12 biological replicates with a total of 12 384-well plates

Subsets of 12 plates were then used to estimate the PoDs and compared to the results from all 12-plates combined.

- With 4 plates (well-averaged), 100% of PoDs were within 1 $\log_{10}$, and 92% were within ½ $\log_{10}$ of the 12-plate (well-averaged) PoDs

The field of view (4 FOV per well) can be treated as separate observations to reduce the number of replicates required for accurate PoD determination and increase throughput.

- With 2 plates (field-averaged), 100% of PoDs were within 1 $\log_{10}$ and 94% were within ½ $\log_{10}$ of the 12-plate (well-averaged) PoDs

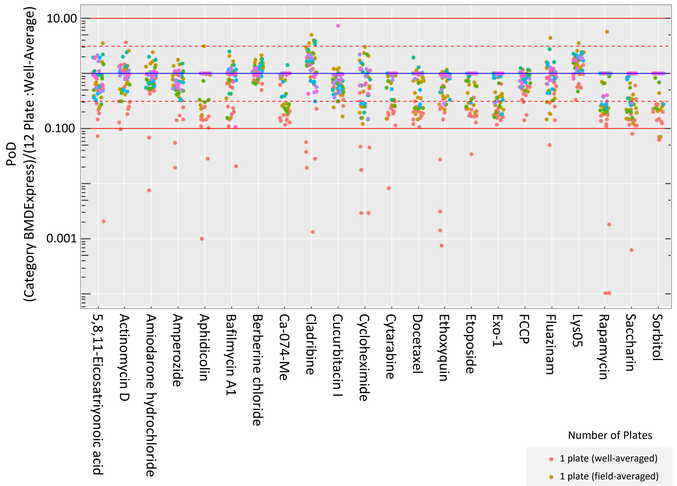


Figure 3: PoDs using well-averaged and field-averaged method with # of plate combinations relative to the 12-plate PoD (well-averaged). The solid blue line indicates unity; the dashed line indicates half $\log_{10}$ difference, and the solid red line indicates a 10-fold difference.

Inter-laboratory comparison demonstrated high concordance

Corteva followed EPA's protocol and repeated the same experiments with the 22 compounds using 4 biological replicates from independent cultures (i.e., plate), each plate with 2 technical replicate wells and imaged with 12 FOV per well. The PoDs were compared to those obtained at the EPA.

- The average PoDs were within 1 $\log_{10}$ for all 22 compounds (100%) and within ½ $\log_{10}$ for 18 of the 22 compounds (82%)
- High accuracy across Corteva and EPA datasets was achieved using either wells or FOVs as the sampling unit

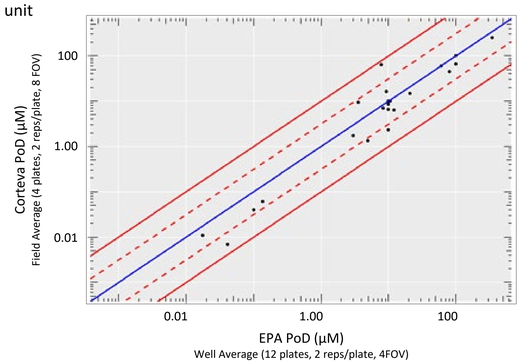


Figure 4: Scatter plot of PoD indicating the potencies of active chemicals with EPA PoD as the x-axis and Corteva PoD as the y-axis.

Comparing Cell Painting PoD to Apical PoD

The concordance between Cell Painting PoD and apical PoD values was assessed for 63 compounds from the TG-GATEs database. The most sensitive apical endpoints PoD were generated using the EPA BMDS software version 3.2.

- All molecules were tested with 8 concentrations for Cell Painting on HepaRG: 158, 50, 15.8, 4.97, 1.56, 0.47, 0.16, 0.05 µM
- 30 compounds had no phenotypic alteration concentration (PAC) within the tested range. The remaining 33 compounds active in HepaRG had Cell Painting POD positively correlated with the most sensitive apical POD (Figure 5).
- *In vitro* to *in vivo* extrapolation (IVIVE) was conducted using ADMET Predictor version 10.4. PK simulation for dose estimation (Cavg: mg/kg/day) was conducted for 12 compounds with rat liver microsome (RLM) clearance and plasma binding (PPB) data measured in Corteva analytical lab.
- 75% of the IVIVE results estimated from Cell Painting PoD were within 1 $\log_{10}$ of the most sensitive apical PoD when RLM and PPB data are available (Figure 6). Low concordance was observed when there was no RLM and PPB measurements for IVIVE (data not shown).

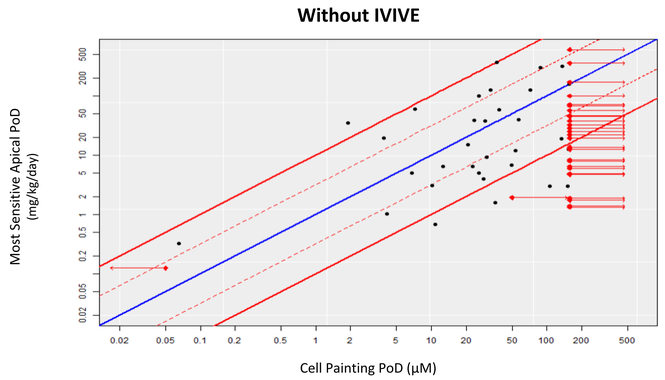


Figure 5: Scatter plot comparing Cell Painting PoD (x-axis) with the most sensitive Apical PoD (y-axis). Compounds in red indicates PoDs were outside the testing range for Cell Painting. The highest concentrations assessed were reported as the lower limit.

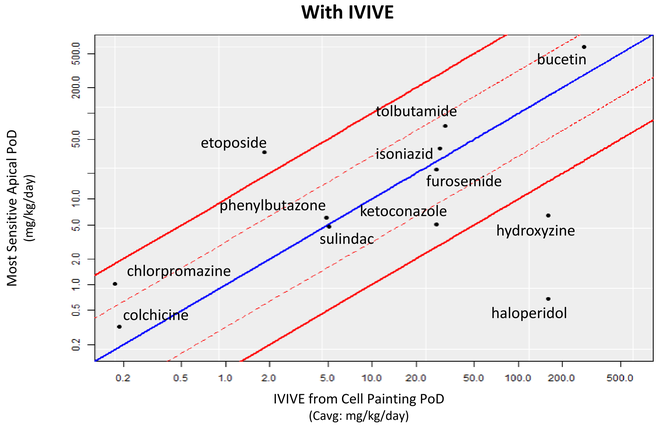


Figure 6: Scatter plot comparing estimated IVIVE data (Cavg: mg/kg/day using ADMET Predictor) from Cell Painting PoD (x-axis) with the most sensitive Apical PoD (Y-axis).

Table 1: Molecules outside the 1 $\log_{10}$ difference between the IVIVE from Cell Painting PoD and the most sensitive apical PoD

Molecule	Compartment With the Most Sensitive Apical POD	Treatment Related Effect in Liver
etoposide	Body Weight	No
haloperidol	Clinical Observations (Decrease in locomotor activity)	No
hydroxyzine	Liver	Yes

Conclusions and future studies

- We have developed a robust Cell Painting assay with human hepatic cell line, HepaRG, for predictive liver toxicity screening.
- Preliminary data indicates high concordance of IVIVE results estimated from Cell Painting PoD to apical endpoint.
- RLM and PPB measurements significantly enhance the accuracy of IVIVE. More studies and IVIVE evaluations are ongoing.

1. Bray, M. A., et al. (2016). Nat Protoc 11(9): 1757-1774
2. Nyffeler J et al. Toxicology and Applied Pharmacology. 2020;389:114876
3. Igarashi, Y., et al. Nucleic Acids Res 2015 43 (Database issue): D921-927

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