Screening Chemicals Using High-Throughput Phenotypic Profiling (HTPP) in Two Zebrafish Cell Lines

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HTPP is an *in vitro* New Approach Method (NAM) that aims to characterize chemical bioactivity through measuring changes in the morphology of cells labeled with fluorescent probes.

HTPP has previously been used primarily in human cells.

Expanding to organisms with a wealth of historic *in vivo* data such as zebrafish is beneficial for many open questions in the NAMs research space, including assessment of ecotoxicity hazard.

Sample Preparation

- Immortalized ZFL and ZEM2S cells were ordered from ATCC and expanded to generate passage 8 (P8) cryostocks. Cultures were maintained in media formulations based on manufacturer's recommendations at 28°C and ambient CO_2.

Cell type	Experimental passage	Seeding density cells/well (cells/cm²)*	Base media	Media supplements
ZFL *Liver* (CRL-2643™)	Passage 10	6,000 (56,444)	50% Leibovitz's L-15 Medium 35% High glucose Dulbecco's Modified Eagle's Medium 15% Ham's F12 Nutrient Mix.	0.15 g/L sodium bicarbonate, 15 mM HEPES, 0.01 mg/mL bovine insulin, 50 ng/mL mouse EGF, 5% heat inactivated fetal bovine serum (**).
ZEM2S *Embryo* (CRL-2147™)	Passage 11	15,000 (141,110)		0.18 g/L sodium bicarbonate, 15 mM HEPES, 10% heat inactivated fetal bovine serum.

Table 1. Culture conditions for ZFL and ZEM2S cells used during screening. (*) Cells/cm² were based on a culture well surface area of 0.1063 cm² for 384-well PhenoPlates. (**) 0.5% trout serum as recommended by the manufacturer was omitted from the ZFL cultures.

Targeted Organelle	Stain	Channel
Nucleus	Hoechst 33342	DNA
Nucleoli + RNA	SYTO 14	RNA
Endoplasmic reticulum	Concanavalin A/Alexa Fluor™ 488 conjugate	ER
Actin skeleton	Alexa Fluor™ 568 Phalloidin	AGP
Golgi body + plasma membrane	Wheat Germ Agglutinin/Alexa Fluor™ 555 conjugate	AGP
Mitochondria	MitoTracker™ DeepRed	Mito

Table 2. Organelles targeted by Cell Painting, the corresponding fluorophores, and channel outputs. All fluorophores are applied after fixing cells, except for MitoTracker™ DeepRed, which is applied to live cells prior to fixation.

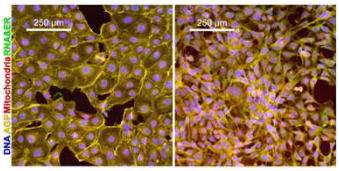

Figure 1. Morphology of ZFL (liver) and ZEM2S (embryo) cells. ZFL cells (left) and ZEM2S cells (right) exposed to 0.5% dimethyl sulfoxide (vehicle control) and "painted".

Data Analysis

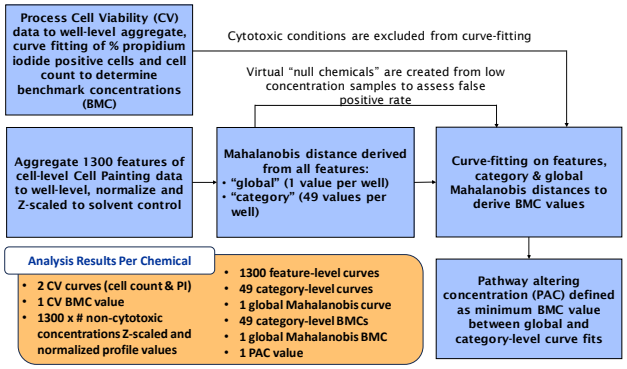

Figure 2. HTPP data analysis pipeline. Cell viability (CV) and Cell Painting feature data are exported from the Revvity Harmony® software. All further analysis and visualization is performed using the R statistical programming language using previously developed internal data pipelining scripts[1]. The ratio of the modeled maximum response and the variability in vehicle controls for each curve is reported as the "top_over_cutoff" value and, referred to as "Effect Size" for the rest of this poster.

Screening Results

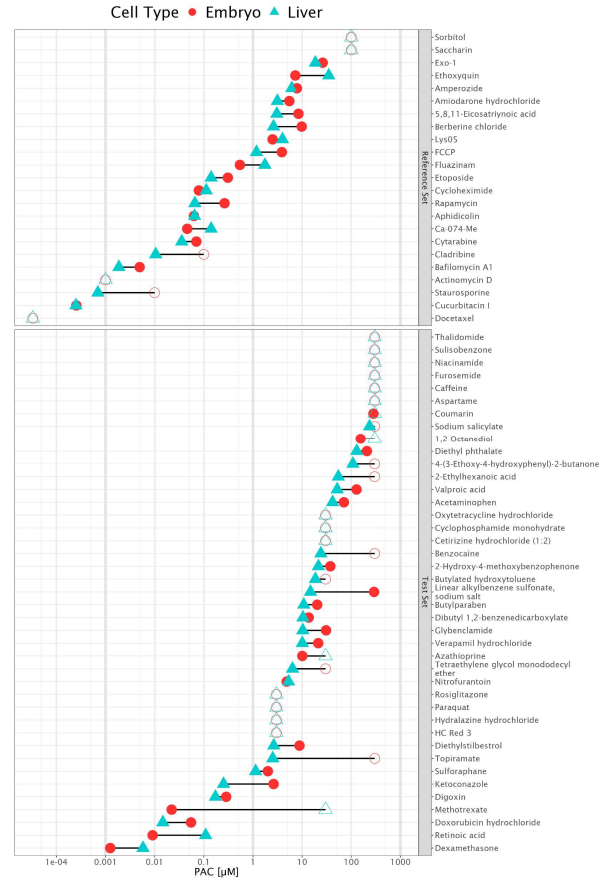

Figure 3. Summary of results for all chemicals tested in ZFL (Liver) and ZEM2S (Embryo) cells. All chemicals tested are displayed and organized into the "reference set" (top) and the "test set" (bottom) of chemicals. Chemicals for which a PAC could not be derived are displayed at the maximum tested non-cytotoxic concentration with an open point.

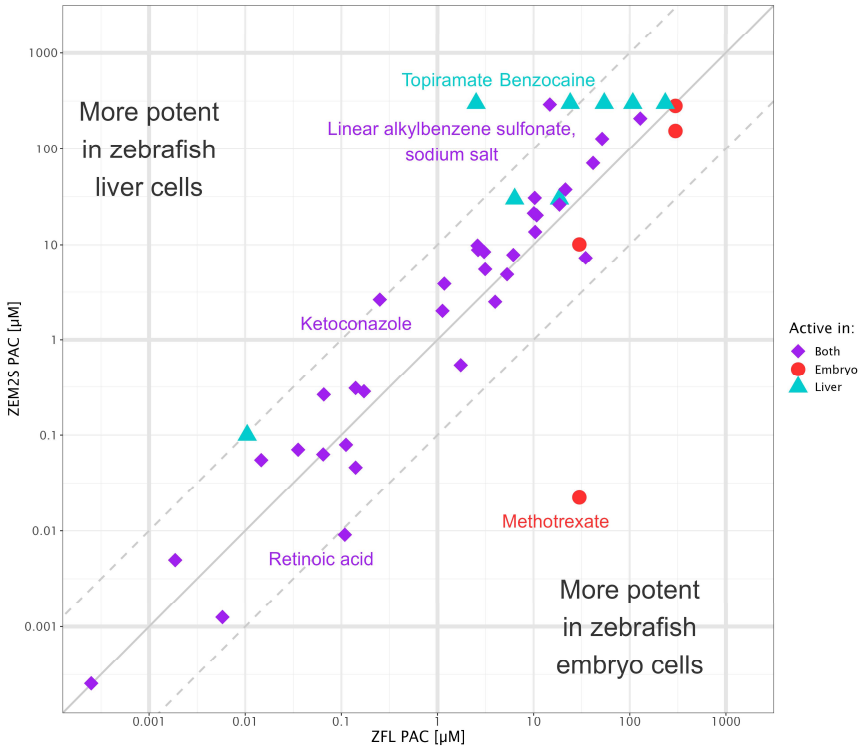

Figure 4. Comparison of ZFL (liver) and ZEM2S (embryo) pathway altering concentrations (PACs) for all chemicals tested. The solid line is the unity line where ZEM2S PAC = ZFL PAC. The dashed lines represent one order of magnitude deviation from unity in either direction. Labeled points fall outside of one order of magnitude difference in either direction. Chemicals for which a PAC could not be derived were assigned the value of the maximum non-cytotoxic concentration tested for that chemical.

References

1. Nyffeler J, Willis C, Harris FR, Foster MJ, Chambers B, Culbreth M, Brockway RE, Davidson-Fritz S, Dawson D, Shah I, et al. 2023. Application of Cell Painting for chemical hazard evaluation in support of screening-level chemical assessments. Toxicol Appl Pharmacol. 468:116513. doi: 10.1016/j.taap.2023.116513.

Sixty-five chemicals were tested using HTPP in two zebrafish cell lines: ZFL (liver) and ZEM2S (embryo).

47 of the 65 chemicals tested were active in at least one cell type (ZFL or ZEM2S)

Of those 47, ~70 % were active in both cell types and most of those 47 had phenotypic altering concentrations (PACs) within one order of magnitude of each other.

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