

Human Pluripotent Stem Cell-Based Assay Predicts Developmental Toxicity Potential of ToxCast Chemicals

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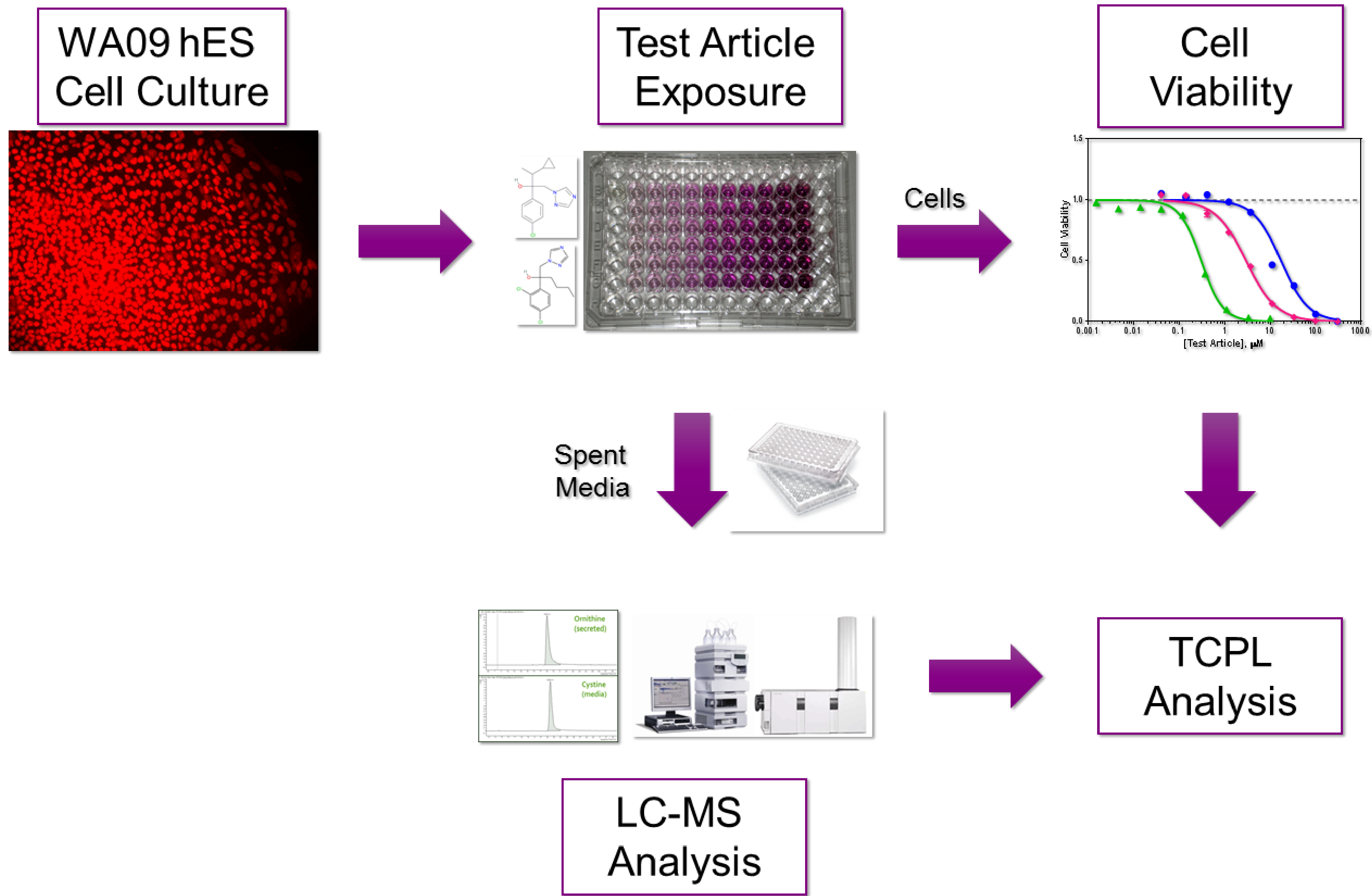


Introduction

- Worldwide initiatives to screen for toxicity potential among the thousands of chemicals currently in use require inexpensive and high-throughput *in vitro* models to meet their goals.
- The devTOX *quickPredict* platform is an *in vitro* human pluripotent stem cell-based assay used to assess a wide range of chemicals for potential developmental toxicity. To increase the diversity of ToxCast assays used to assess developmental toxicity, the United States Environmental Protection Agency (EPA) is using the assay to screen the ToxCast chemical library.
- A two tier testing strategy was employed to screen a total of 1066 chemicals in human embryonic stem (hES) cells, guided by the AC₅₀ (half-maximal activity concentration) across multiple cytotoxicity assays in ToxCast. [1]
- 348 chemicals were tested in an eight concentration dose-response and 731 were tested at a single concentration. Spent media was collected to measure changes in biomarkers of developmental toxicity (ornithine and cystine) together with cell viability measurements.
- Compared with human data, the balanced accuracy, sensitivity and specificity of the model ranged from 87-92%, 80-86%, and 93-100%, respectively, depending on the reference chemical set used.

Methods

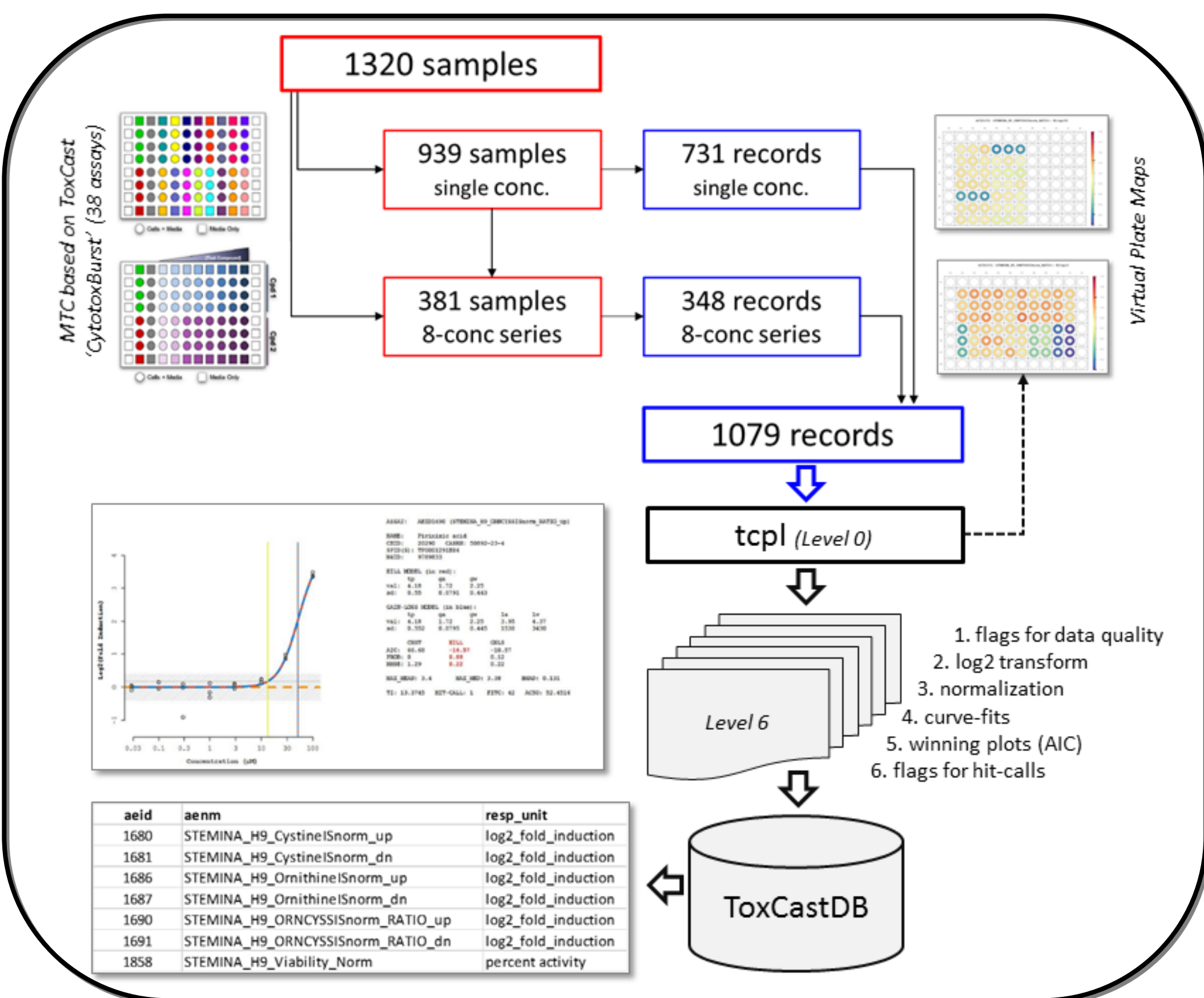
devTOX *quickPredict* Platform



METHODS: WA09 cells (WiCell) were maintained in the undifferentiated state in mTeSR1 (StemCell Technologies) on Matrigel (Corning). Cells were plated in 96-well plates and exposed to test article for 3-days (blinded to the experimenter). Media from the last 24h of exposure was collected and filtered to remove molecules >10 KDa. Samples were analyzed with UPLC-ESI-TOF-MS to determine ornithine (ORN) and cystine (CYSS) levels. Cell viability was determined by CellTiter-Fluor (Promega) assay.

EXPOSURE: Each plate contained controls for vehicle (0.1% DMSO), negative response (5 nM methotrexate (MTX)), and positive response (1 μ M MTX). ToxCast exposures were guided by cytotoxicity determinations [1]. Developmental Toxicity Potential was determined with the ORN/ CYSS ratio (*o/c* < 0.88) and compared to the normalized cell viability response. [2].

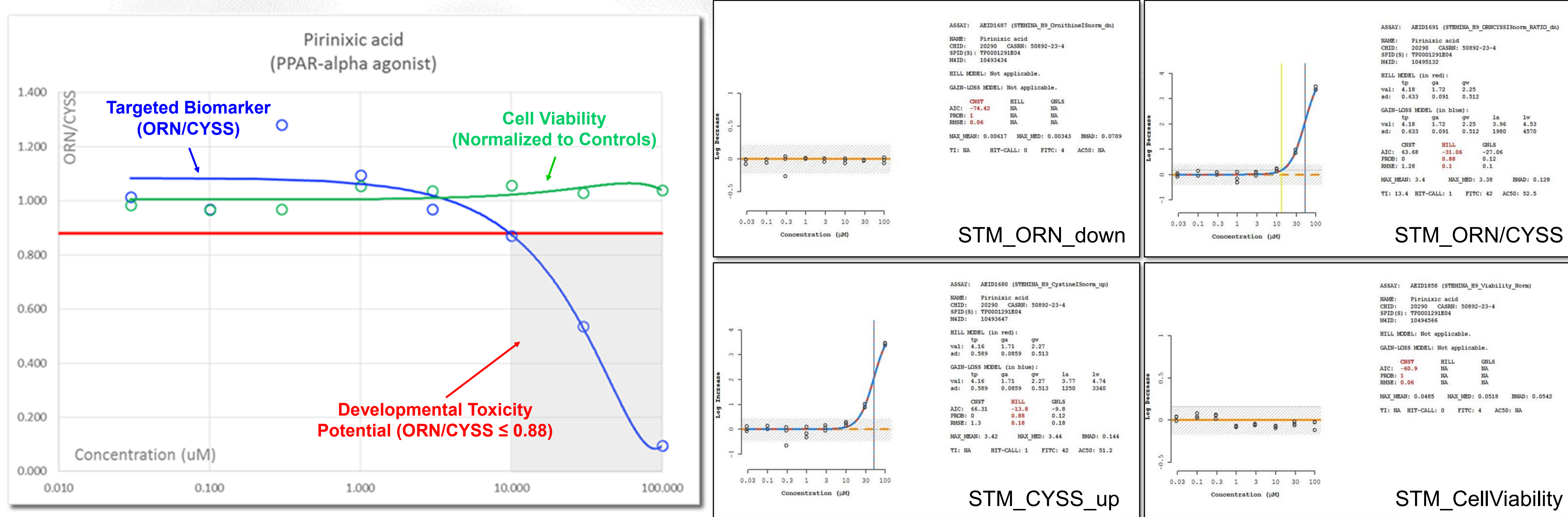
ToxCast Pipeline



Testing Strategy: Maximum Test Concentration (MTC) for exposure was guided by AC₅₀ concentration across multiple cytotoxicity-related assays in ToxCast [1].

Feature \rightarrow Level \downarrow	STM_ORN_down	STM_CYSS_up	STM_ORN/CYSS	STM_CellViability
0	Raw metabolite area normalized to spike-in ¹³ C standard and reference control median		Direct Ratio computed from normalized raw data	Relative Fluorescent Units (RFU) normalized to reference control
1	Removed entries flagged for poor well quality, empty ('0') cells,...			
2	Log2 transformation of raw data (individual measures, n)			
3	Normalization, inverted relevant up/down features to look like ToxCast plots			
4	Calculated parameters for automated curve fitting models (Constant, Hill, Gain-Loss)			
5	Plot winning model based on AIC and output [μ M] for: Hit (0,1); dTP; AC ₅₀ ; fold-change...			
6	Manual flags for curve-fitting issues or data quality concerns			

Example: Pirinixic Acid



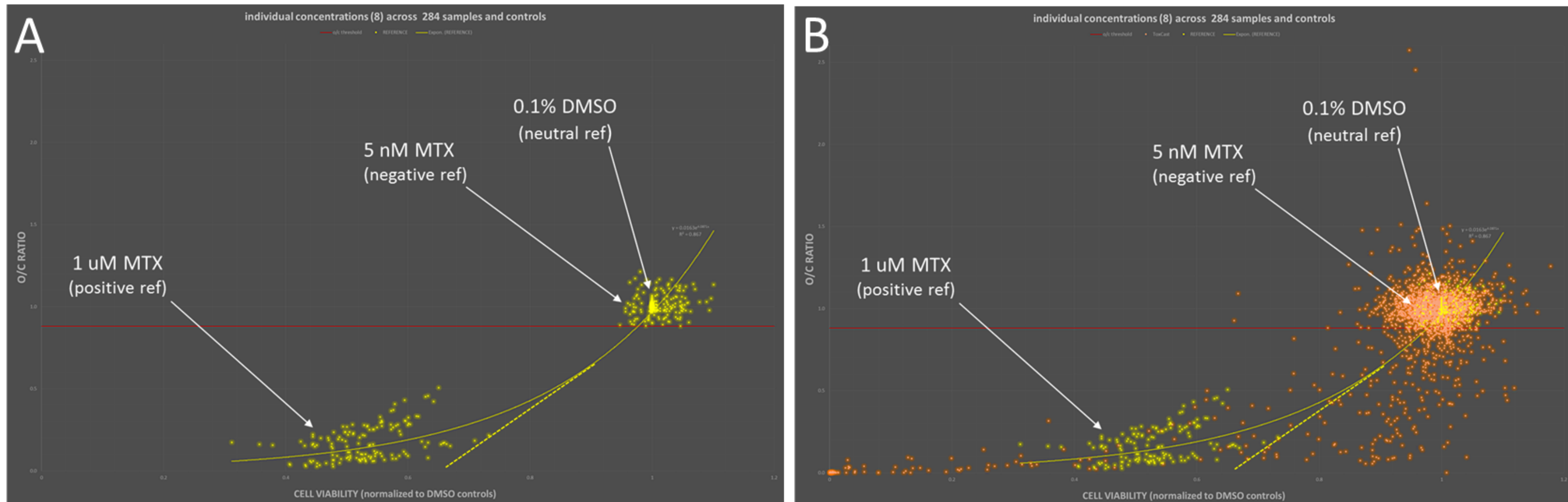
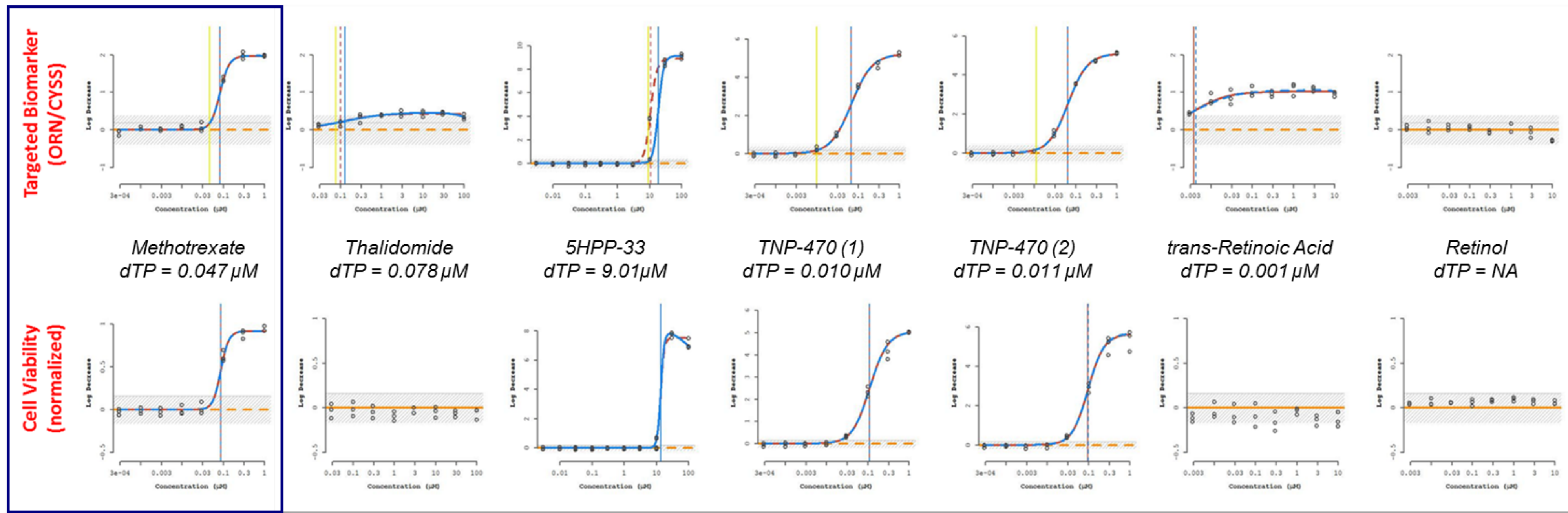
Conventional devTOX^{qP} data representation [2]

TCPL representation [3]

DISCLAIMER: The views expressed are those of the authors and do not necessarily reflect the views of policies of the US EPA. Mention of trade names or commercial products does not constitute endorsement or recommendation for use.

Results

Representative Automated Outputs for ORN/CYSS and Cell Viability



Individual data points graphed for ORN/CYSS as a function of Cell Viability.

- Plate controls, MTX track projects to 65% viability.
- 270 chemicals tested across 8-point conc. response [orange]; MTX projection defines sectors that do and don't co-occur with effects on viability.

Assessing Assay Performance

Number of Chemicals	Sensitivity	Specificity	Accuracy
33	80%	100%	91%
64	86%	97%	92%
71	81%	93%	87%

Preliminary analysis anchored:
1) Conservatively to 33 reference teratogens (ECVAM/FDA labels);
2) Expanded list of 64 by adding 31 chemicals with evidence of developmental toxicity from the literature; and
3) More liberal expansion (71 compounds).

Conclusions

- The devTOX^{qP} dataset for ToxCast was shown to be of high-quality based on replicate samples and reference compounds.
- Overall, 187 samples (~17%) elicited a response in the ORN/CYSS ratio at ≤ 100 μ M. For 348 samples with concentration-response data, 118 actives tracked with decreasing cell viability and 64 samples elicited a response in the ORN/CYSS ratio without a demonstrable loss of cell viability (AC₅₀ >100 μ M).
- Model performance showed a balanced accuracy of 87-92% (sensitivity 80-86%, specificity 93-100%), depending on the chemical set used; the increased specificity over sensitivity was consistent with the MTC testing strategy.
- Some potent actives had a low E_{max} (i.e., retinoic acid, thalidomide) relative to others where ORN/ CYSS paralleled cytotoxicity (i.e., methotrexate, 5HPP-33).

References:

- Judson R, et al. Analysis of the Effects of Cell Stress and Cytotoxicity on *In Vitro* Assay Activity Across a Diverse Chemical and Assay Space. *Toxicol. Sci.* 2016; **152** (2): 323-339.
- Palmer JA, et al. Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. *Birth Defects Res B Dev Reprod Toxicol.* 2013; **98**(4):343-63.
- Knudsen TB, et al. ToxCast Profiling in a Human Stem Cell Assay For Developmental Toxicity. In preparation.