**A. INTRODUCTION**

EPA’s ToxCast program has generated data on a battery of 821 in vitro endpoints for 1066 compounds including pharmacological, natural products, pesticidal active ingredients, consumer use chemicals and industrial ingredients [1].

To increase the diversity of in vitro assays used to assess developmental toxicity, the ToxCast library was evaluated in the Stemina devToxQuickPredict [qP] platform [2]. This assay measures two small molecules (ornithine, cystine) in medium conditioned by human embryonic stem (hES) cells yielding an ornithine:cystine ratio (o/c) indicative of an imbalance in metabolism predictive for teratogenicity in a human system.

Here, we provide a preliminary evaluation of the results focusing on metrics of assay quality, performance, and predictivity.

**B. METHODS**

**Platform:** Metabolomic analysis of the hES cell secretome for predictive developmental toxicity (devTox platform) was reported in 2010 [3]. A 2011 pilot study conducted with 11 ToxCast chemicals predicted developmental toxicity in concordance with animal data with 83% accuracy [4]. In 2013, the Stemina devTox-qP platform was developed as a high throughput screening (HTS) assay for developmental toxicity testing [2]. The model was trained with 23 pharmaceuticals (96% accurate). An independent 13 pharmaceutical test set with known (human) teratogenicity was 77% accurate.

**Dosing:** H9 cells (WA09 line, WiCell Research Institute) were cultured in 96-well plates. Each experimental plate included methyltrexate (MTX) reference concentrations as calibration standards for negative (0 nM) and positive (40 uM) response as well as media blanks on 0.1% DMSO vehicle. Undifferentiated cells were exposed for 72h to test compound (blinded or in triplicates) with media and test compound replacement every 24h; maximum test concentration (MTC) for single concentration screen and/or 8-plate conc. series set at 1, 10, or 100 uM based on ToxCast cytotoxicity burst (TC-Cytoburst) [1] or compound availability.

**Evaluation:** Cell-conditioned media from the final 48h treatment period was analyzed by LC-MS to determine ornithine/cystine (o/c) ratio. Concurrent cell viability was assessed with the CellTiter-Fluo TM assay (Promega). The cytotoxicity Relative Fluence Unit (RFU) was background corrected and normalized to RFU of the neutral control (0.1% DMSO).

**C. METRICS OF ASSAY QUALITY**

**Quality Standards.** Methotrexate (MTX) in the ToxCast library (blinded) gave ornithine/cystine (o/c) ratio and cell viability (o/c) measures identical to the calibration standards.

**D. METRICS OF ASSAY PERFORMANCE AND PREDICTIVITY**

**Replicate Samples.** Concentration (8-point) response for 13 REPs (n=2) with test strategy setting maximum test concentration (MTC) below ToxCast cytotoxicity burst (TC-Cytoburst).

**E. SUMMARY AND TRANSLATION**

- To date, we tested 1079 samples (1066 chemicals = 13 repeats).
- Setting the MTC based on ~18 cytotoxicity assays in ToxCAST® [1] the initial screen showed 3-16% active and 84% predictive accuracy (consistent with previous studies [2-4]).
- 8-point conc. series on an a priori selection of 127 chemicals and 13 reps completed; as concentration increases, positives move into a track where o/c-ratio is linked to cell viability.
- Testing conc. series of a non-a priori subset of 144 samples is currently underway. This will enable the model to be trained with ToxCast (in vitro) and ToxRefDB (in vivo) data.

**Mouse ES (mES) versus human (hES) cell platforms.** Comparison at an LEC for 1054 ToxCast chemicals tested both ways. Results from the o/c-ratio (3-day undifferentiated hES) cells were conditioned on the mES cell response in adherent cultures [6] for Gooseoid (GSC) protein expression - a biomarker for gastrulation (4-days of culture).

**References:**