**A. INTRODUCTION**

EA's ToxCast program has generated a data set on 821 in vitro endpoints for 1066 compounds including pharmaceuticals, 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 deCODEquickPREDICT® [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 ratio) 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 predictive utility.

**B. METHODS**

**Platform:**

Metabolomic analysis of the hES cell secretome for predictive developmental toxicity (deCODEqP 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 deCODEqP® 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 methotrexate (MTX) reference controls as calibration standards for negative (0 µM) and positive (100 µM) response as well as media blanks and on 0.1% DMSO vehicle. Undifferentiated cells were exposed for 72h to test compound (blinded and in triplicate) with media and test compound replacement every 24h; maximum test concentration (MTC) for single concentration screen and 8-point conc. series was set at 1, 10, 100 µM on test compound cytotoxicity burst (TC-CytoBurst) [1] or compound available.

**Evaluation:**

Conditioned media from the final 24h treatment period was analyzed by LC-MS to determine ornithine/cystine (o/c) ratio. Concurrent cell viability was assessed by the CellFluir-CellTM assay (Promega). The cytotoxicity Relative Fluorescent Unit (RFU) was background corrected and normalized to RFU of the neutral control (0.1% DMSO). Teratogen Index [3] using the default threshold values 0.88 and concurrent cell viability (RFU values for test compound relative to DMSO control).

**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.

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

**E. SUMMARY and TRANSLATION**

**A blinded study under EPA contract EP-D-13-055 is evaluating the ToxCast Phase II-ll library http://www.epa.gov/ncct/toxcast/chemicals.html in the Stemina deCODEqP® platform [2].**

**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 15%-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 repts 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 Gooseneck (GSGD) protein expression - a biomarker for gastrulation [4-days of culture].

**References**