Response to NICEATM Request for Data and Information on Developmental Toxicity Test Methods

Terms and Definitions

Teratogenicity Threshold	A threshold of metabolic perturbation that is associated with the potential for teratogenesis. The threshold was empirically determined to be 0.85 for the targeted biomarker assay using results from the training set. This threshold was applied to all test set and unknown compounds evaluated using the assay to determine the teratogenicity potential.
Ornithine/Cystine Ratio (O/C Ratio)	The fold change of ornithine (Orn) for treatment x relative to the 0.1% DMSO reference treatment divided by the fold change of cystine (Cyss) for treatment x. $$O/C\ Ratio\ =\ \frac{Orn_x\,/Orn_{DMSO}}{Cyss_x\,/Cyss_{DMSO}}$$
Teratogenicity Potential	Interpolated exposure level (concentration) of a test compound where the dose response curve for the o/c ratio or cell viability crosses the teratogenicity threshold. Exposure levels greater than this concentration are associated with teratogenicity.
Accuracy	Number of correct predictions divided by the number test compounds evaluated.
Sensitivity	Detection of teratogens, True Positives divided by the number of False Negatives plus the number of True Positives.
Specificity	Detection of non-teratogens, True Negatives divided by the number of True Negatives plus the number of False Positives.
Training Set	Set of compounds that have well established human developmental toxicity information used to identify biomarkers of developmental toxicity. This set of compounds was tested in both phases of the study and used to set the o/c and viability teratogenicity thresholds.
Test Set	Set of compounds with well-established human developmental toxicity information that were not a part of the training set and were not used to identify the biomarkers. The test was only used to independently evaluate the biomarkers of developmental toxicity capacity to predict teratogens and non-teratogens.

Background/Assay Overview

The devTOX *quick*Predict (devTOX^{qP}) assay is a high-throughput *in vitro* developmental toxicity assay used to signal whether a test compound has the potential to cause developmental toxicity in humans. A significant advantage of the devTOX assays is that it is a human system, eliminating the risk of false-negatives due specifically to inter-species differences in developmental pathways and pharmacokinetics. This is particularly important since human embryos can have a higher sensitivity to chemicals than rodent models. The assay measures changes in the metabolism of ornithine and cystine in human pluripotent stem cells (hPSC, both hES and iPS cell types). The undifferentiated state of the hPS cells allows for broader application in evaluating developmental toxicity potential than specific cell types. The assay has been shown to predict the developmental toxicity potential of test compounds known to affect multiple lineages, including cardiovascular, neural and skeletal. Ornithine and cystine are both involved in metabolic pathways important for normal proliferation and differentiation during development and have been experimentally associated with common mechanisms of developmental toxicity (Palmer et al., 2013). Changes in the metabolites are measured across an 8-point dose response curve to identify the concentration at which a given test compound shows developmental toxicity potential.

Assays such as this help define exposure ranges where a toxic response may be expected, as well as those where a response would not be expected to occur. With wider acceptance, the human endpoint provided by the devTOX *quick*Predict assay, in combination with testing in one species, may support elimination of a second species in certain categories of compounds or as part of read-across or weight of evidence approach for similar chemical structures.

The assay is currently used to assess developmental toxicity risk potential in discovery and preclinical development programs aimed at characterization and prioritization of new investigational compounds. These compounds may be intended for use as pharmaceuticals, agricultural or industrial chemicals, cosmetics or consumer products. Measurement of a compounds' toxicity potential across an exposure range can be put into perspective in terms of the overall risk profile when combined with additional assays conducted during a compound's discovery and development.

Ultimately, the assay could become part of a new paradigm utilizing a panel of human cell-based assays aimed at early decision making. Such an approach is aligned with the objectives of Tox 21 and REACH and the Cosmetics Directives in the EU. The devTOX assay, with its broad applicability and human endpoints is uniquely positioned to become part of regulatory requirements/filings in the future. The assay can be included as part of a panel of assays aimed at defining where potential adverse responses in human populations may exist. This information can help to drive decisions as to whether a test article should progress along its development path.

Methods Overview

Human pluripotent stem cells are exposed to eight concentrations of each test compound for 48 to 72 hours (depending on cell type). Each experimental plate contains reference (0.1% DMSO), positive, and negative controls to ensure the hPSC metabolism meets the assay specifications.

Spent media from the last 24-hour treatment period is collected and cell viability is measured. Proteins are removed and samples are concentrated prior to analysis. Spiked-in internal standards are added to the samples for quality control purposes. Ornithine and cystine are measured using liquid chromatography-high resolution mass spectrometry (LC-HRMS). Relative fold changes for each metabolite are calculated by normalizing the response of each treatment to the reference sample. The reference-normalized value for ornithine is divided by the reference-normalized value for cystine to calculate the o/c ratio. Figure 1 illustrates dose-response curves for the o/c ratio (purple line) and cell viability (black line) fit using a 4 parameter log-logistic non-linear model. The concentration at the point where the non-linear 4-parameter dose response curve of the o/c ratio crosses the developmental toxicity threshold (dTT, red line) indicates the exposure level where a metabolic perturbation is indicative of developmental toxicity potential (dTP, red point). The toxicity potential concentration from cell viability (blue point) is the point where the cell viability dose response curve exceeds the developmental toxicity threshold. The developmental toxicity threshold creates a two-sided toxicity model based on exposure: one where exposure does not perturb metabolism in a manner associated with developmental toxicity (green box) and another where exposure shifts metabolism in manner associated with developmental toxicity (red box).
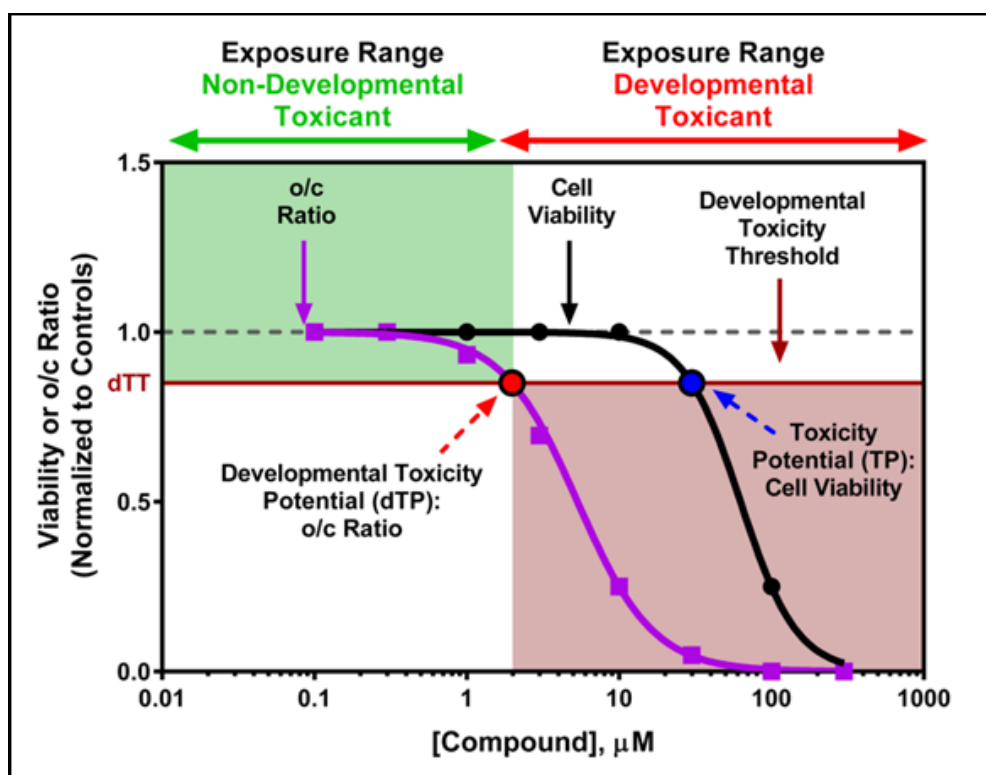

Figure 1. Graphical representation for interpreting the results obtained with the devTOX^{qP} assay.

Results Summary

Assay performance has been evaluated in 80 chemicals known developmental toxicity outcomes *in vivo* (45 positives, 35 negatives), including pharmaceuticals, plasticizers, pesticides, food additives and preservatives, flame retardants, industrial solvents, mycotoxins, isoflavones and stilbenoids, The assay predicted the developmental toxicity potential of these chemicals with 85% accuracy when compared to known *in vivo* toxicity (82% sensitivity, 89% specificity). Within individual chemical classes (i.e., antineoplastic agents or pesticides), assay accuracy ranged from 74% to 100%, demonstrating the broad applicability of the devTOX^{qP} assay.

Test compound predictions were scored against human data when available. However, human data was only available for the compounds in the pharmaceutical compound set. For the non-pharmaceutical compounds, data from rodent in vivo studies was used to classify the compounds as teratogenic or non-teratogenic. Compounds with a lowest effect levels for developmental effects (dLEL) <50 mg/kg were classified as teratogens and dLEL >200 mg/kg were classified as non-teratogens. In addition, developmental toxicity needed to be observed in the absence of maternal toxicity for a compound to be classified as teratogen. Accuracy of predictions was based on these classifications and a concentration threshold of the teratogenicity potential determined using the training set compounds. This data was derived for each compound using the AcTOR and TERIS (Teratogen Information System) databases.

The assay offers a prediction of the likelihood that a test compound has the potential to be a human developmental toxin at concentrations identified as the teratogen potential and above. The Cmax has been used to evaluate the concentration at which the compound is considered a teratogen where Cmax is known. For those where information regarding the physiological concentration is not known or for chemicals which are not intended to be consumed, a 65 µM threshold was used. Application of the assay does not require any pharmacokinetic information (e.g., Cmax) for its prediction, nor is it required that the 65 µM cut-off be applied. Both the Cmax and 65 µM have been used to evaluate performance of the assay using information available for the test compounds. Assay results must be put into context using additional end points from relevant compound development studies such as estimated human exposure levels extrapolated from pre-clinical data.