The use of *in vitro* stem cell-based assays to assess reproductive and developmental toxicity

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Introduction

Reproductive and developmental toxicity is an important aspect in risk assessment. To address this, currently the primary method for such assessments is to use animal models, which are costly and time consuming and often require more than one OECD Test Guideline to be used. More recently, novel approach methodologies (NAMS) using human stem cells are emerging that have the potential to predict some aspects of *in vitro* developmental toxicity (Fig 1).

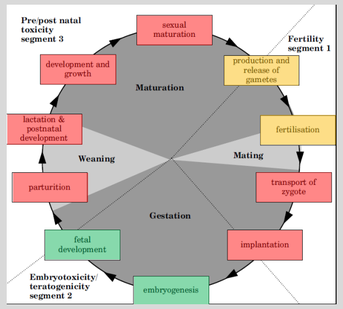

Figure 1. A schematic to represent reproductive and developmental toxicity complexity[1]. Green boxes indicate areas where *in vitro* alternatives exist; amber boxes indicate where *in vitro* is feasible, but would require further investigation and red, where *in vitro* assays are not possible.

Methodology

In vitro stem cell assays

Toxys ReproTracker

- A human induced pluripotent stem cell-based assay that assesses morphological/functional changes and reductions in biomarker expression in cardiomyocytes, hepatocytes and neural rosettes following exposure to potential developmental toxicants (Fig. 2), as a teratogenicity surrogate
- Cells are continuously exposed to the test article at a range of concentrations for up to 21 days
- A compound was considered to have developmental toxicity potential if it caused a dose-related decrease in biomarkers or cell functionality

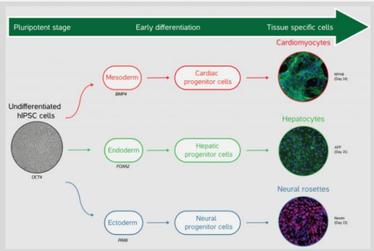

Figure 2. Representation of the ReproTracker assay and morphological/functional changes and biomarkers measured in the assay[2].

Stemina DevTOX^{qp}

- A human metabolomics biomarker-based stem cell assay that measures perturbations in two amino acids, ornithine and cystine, involved in normal cell differentiation and fetal development and provides an indication of teratogenicity
- Cells are dosed for up to 24 hours at a range of test article concentrations
- A compound was considered to have developmental toxicity potential if a decrease in the ratio between ornithine and cystine (o/c) was seen below a defined threshold

Approach to testing

- A phased approach was taken to explore the assays (Fig. 3)

Phase 1 Compounds with known developmental toxicity status in vivo

Phase 2 Complex mixtures, e.g. botanicals and spiked samples

Phase 3 Provisional product assessment

Figure 3. The phased approach to testing

- The compounds tested in Phase 1 consisted of those shown in Table 1, based on a literature search of compounds with known reproductive and developmental toxicity

Table 1. The compounds tested in Phase 1 of this study

Compound	Expected Endpoint
Quassin[3]	Non-teratogen
Piperazine[4]	Teratogen
Dicyclohexyl phthalate[5]	Teratogen
Saccharin[6]	Non-teratogen
Tomatidine[7]	Teratogen
Catechin[8]	Non-teratogen
Coconut oil[9]	Non-teratogen

Results

DevTox^{qp}

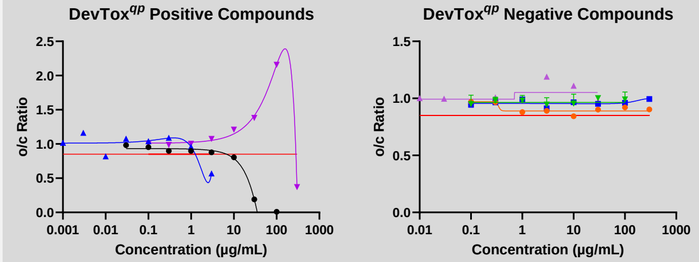

Figure 4. Responses of selected chemicals with known DART properties in the DevTox^{qp} assay.
Dicyclohexyl phthalate; Catechin; Quassin; Saccharin; Coconut oil; Piperazine; Tomatidine; Threshold for a positive response.

- The DevTox^{qp} assay predicted that dicyclohexyl phthalate and tomatidine would have developmental toxicity potential as shown by a decrease in the ornithine/cysteine (o/c) ratio to below the threshold of 0.85 (Fig. 4)
- Catechin was considered equivocal because of its biphasic response (Fig. 4)
- Quassin, Saccharin, coconut oil and piperazine were considered negative (Fig. 4)

ReproTracker assay

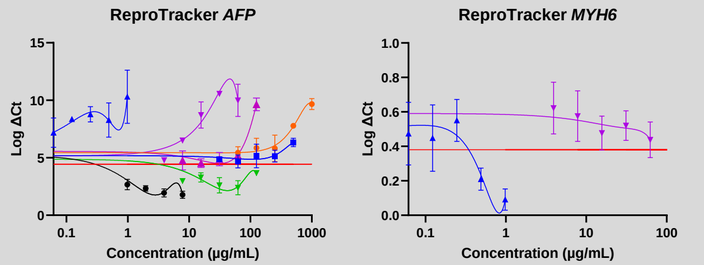

Figure 5. Responses of selected chemicals with known DART properties in the ReproTracker assay.
Dicyclohexyl phthalate; Catechin; Quassin; Saccharin; Coconut oil; Piperazine; Tomatidine; Threshold for a positive response.

- The ReproTracker assay predicted that Dibutyl phthalate (*AFP* (hepatocyte) disruption and Tomatidine (*MYH6* (cardiomyocyte) disruption) would be teratogens (Fig. 5)
- Although catechin did not decrease *MYH6* expression below the threshold, there was a consistent dose related decrease and as such, was considered equivocal (Fig. 5)
- Conversely, although Quassin did decrease *AFP* expression below the threshold, this was not consistently dose-responsive and as such, was not considered to be a developmental toxicant (Fig. 5)
- Saccharin, Coconut oil and Piperazine were also considered negative in the ReproTracker assay

Conclusions

The DevTox^{qp} and ReproTracker assay responses demonstrate concordance with the literature for the studied compounds, showing these assays have potential value as NAMs for the endpoint of developmental and reproductive toxicity

Piperazine was not detected as a reprotoxicant in either assay, however the literature shows that the developmental toxicity of piperazine is a secondary effect of maternal exposure and these assays would not necessarily detect this effect

Because of the complexity of developmental and reproductive toxicity, a battery of *in vitro* assays will be required to address key events in addition to those assessed through the DevToxqp and ReproTracker assays

Acknowledgements

The authors are grateful to Stemina Biomarker Discovery for conducting the DevTox^{qp} assay and to Toxys for conducting the ReproTracker assay

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