An Integrated Approach for Testing Developmental and Reproductive Toxicity (DART) Endpoints for Next-Generation Risk Assessment (NGRA)

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Introduction

Next Generation Risk Assessment (NGRA) is an exposure-led, hypothesis driven approach that integrates one or more New Approach Methodologies (NAMs), which can be applied to ensure the safety of consumer products without the need for animal testing. The continued development and application of NAMs in a decision-making context will play an increasing role in fulfilling the ambition to assure the safety of novel ingredients without the need for animal testing. Developmental and reproductive toxicity is one area where additional NAMs may be needed to ensure that a NGRA approach is protective. Building on our systemic framework [1] we developed an integrated approach to account for DART endpoints (see Fig. 1).

ReproTracker®

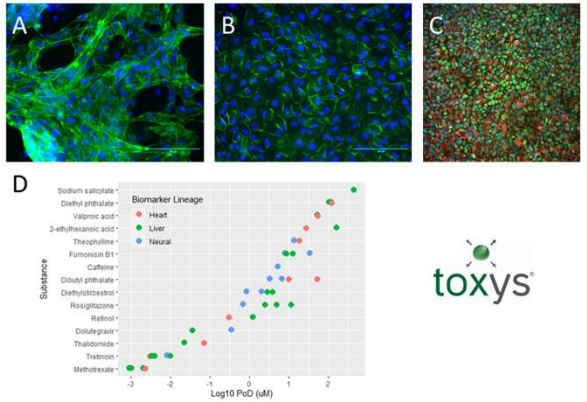

Fig. 2: For the ReproTracker assay hiPSCs are differentiated towards cardiomyocytes (Hoechst blue, TNNT2 green) (A), hepatocytes (Hoechst blue, ALB green) (B) and neural rosettes (Hoechst blue, PAX6 green, Nestin red). Changes in the expression patterns of germ cell layer (BMP4, FOXA2 and SOX1) as well organ specific biomarkers (MYH6, AFP and PAX6) can be used as a read out for teratogenicity of a compound [2]. Dose response analysis were performed on normalised readout using BMDExpress2.3 to model PoD. First results are shown with very light significance filtering on the BMD outputs and further optimisation is needed.

DART Framework

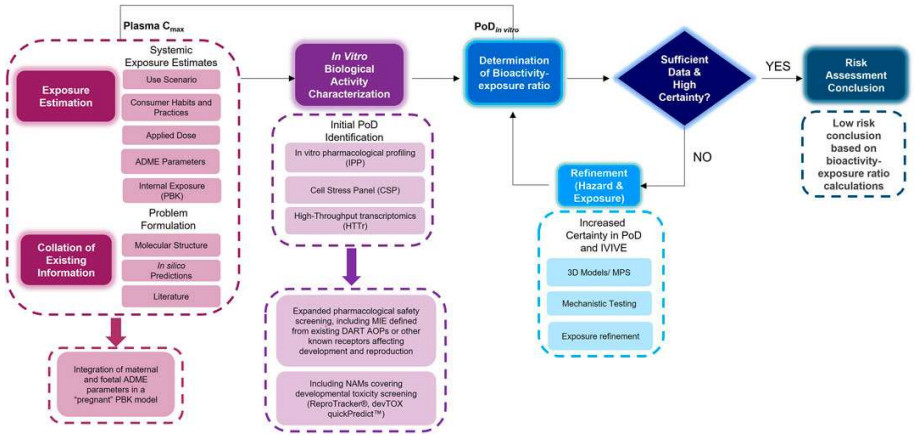

Fig. 1: A NGRA framework outlining the consideration of any existing information with exposure estimation including maternal and foetal ADME parameters with *in vitro* biological activity characterisation including additional NAMs relevant for DART endpoints to determine the bioactivity exposure ratio and further refinements to arrive at a risk assessment conclusion. [3]
Dose response modelling needs to be adapted for the additional NAMs, like the ReproTracker® assay, to determine points of departure (PoD) (see Fig.2).

Caffeine case study: PBK modelling

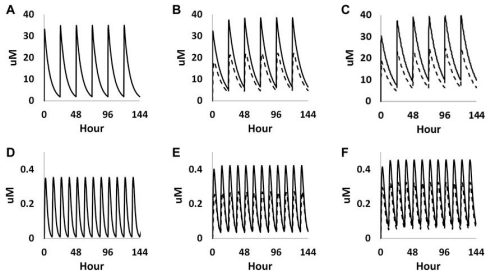

Fig. 3: PBK simulations on plasma concentration time profiles of caffeine in both mother (solid curves) and foetus (dashed curves) through different gestational ages using GastroPlus 9.8 (Simulation Plus, Lancaster, CA, United States). (A–C) represent prediction on week 6, week 20 and week 30 from oral exposure, respectively. (D–F) represent predictions on week 6, week 20 and week 30 from dermal exposure, respectively. [3]

To exemplify the use of the framework caffeine was chosen as a case study. Two distinct exposure scenarios were selected, a dermal exposure to 0.1% caffeine in a hypothetical body lotion and an oral consumption of 200 mg/day of caffeine at different gestational stages of pregnancy (6, 20 and 30 weeks) (Fig. 3). Highest predicted maternal (39.72µM oral and 0.46µM dermal) and foetal (25.27µM oral and 0.32µM dermal) C_{max} values were compared to *in vitro* biological activity data, including IPP, CSP, HTTr, ReproTracker® and devTOXquickPredict™ to predict Bioactivity Exposure Ratio (BER) (see Fig.4).

Caffeine case study: Bioactivity exposure ratios

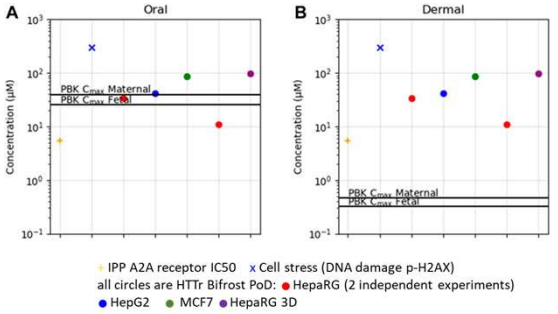

Fig. 4: BER of Caffeine for the oral (A) and dermal (B) exposure scenarios, comparing the IPP, CSP and HTTr PoDs with maternal and foetal C_{max} values. No signs of teratogenicity were observed up to a concentrations of 100 µM in ReproTracker® and caffeine did not affect the ornithine/cysteine ratio in the devTOXquickPredict™ assay up to 500 µM [4]. Graph modified after [3]

Conclusion and outlook

- PoD's can be calculated using ReproTracker results, further work is needed to optimise dose response modelling.
- The caffeine case study demonstrates how to integrate PBK modelling for pregnancy and relevant NAMs for DART safety decisions without generating new animal data. Therefore, work is ongoing using up to 35 chemicals (expanding from the 15 tested so far in ReproTracker®) to evaluate the DART framework.
- Oral exposure of caffeine shows that further refinement might be needed for some substances to separate between bioactivity and adverse effects.
- Identify gaps/problems to define which additional NAMs are needed for a refined NGRA approach for DART (see also poster 3221/P356).

References

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[3] *Rajagopal et al.*, Front. Toxicol., 07 March 2022 https://doi.org/10.3389/ftox.2022.838466
[4] *Zurlinden et al.*, (2020) Toxicol Sci. Apr 1;174(2):189-209