Practical Application of New Approach Methods in Developmental and Reproductive Toxicity (DART) Testing

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Evaluation of NGRA Framework for DART safety assessment

- We've built an NGRA framework *(Fig.1)* that uses available knowledge together with NAMs providing broad biological coverage[1] used in exposure-led DART safety assessments.
- For risk assessment, a tiered approach[2] would be followed making use of *in silico* predictions, molecular structure and a literature review at Tier 0 and more detailed comparisons of the exposure calculation and hazard classification at Tier 1. Higher tier testing would only be performed if refinement (exposure and hazard) of results are needed following these early tiers.
- 37 benchmark substances were selected to undergo data generation. Where possible, high and low risk exposure scenarios were identified from DART relevant data (from authoritative sources *e.g.* SCCS, ECHA, EPA, FDA, EMA) for each benchmark substance and evaluation was performed for Tier 0 and Tier 1 using the proposed framework.

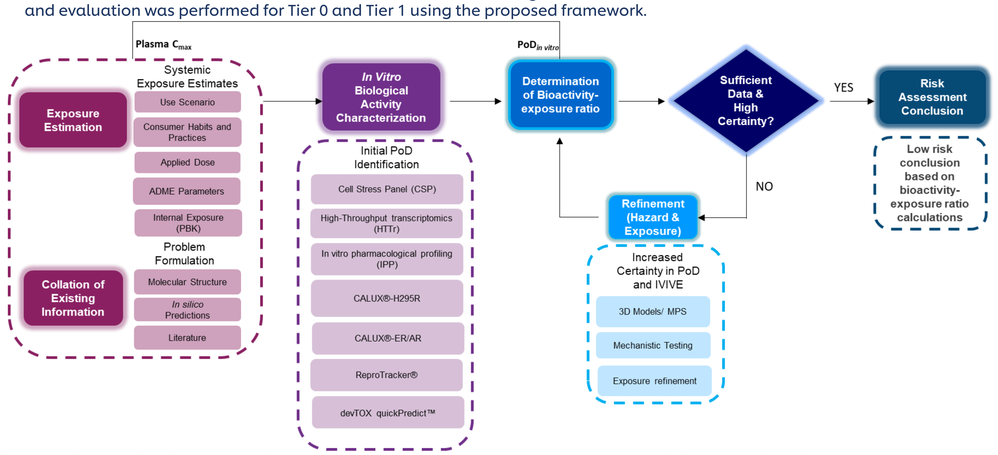

Fig. 1: NGRA framework outlining the consideration of any existing information with exposure estimation including maternal and foetal ADME parameters with *in vitro* biological activity characterisation including broad screening assays together with DART specific NAMs to determine the bioactivity exposure ratio (BER) and further refinements to arrive at a risk assessment conclusion.

Tier 0: *in silico* predictions to flag potential DART risk

- In silico predictions for the 37 benchmark substances were performed using different tools to cover developmental and reproductive toxicity as well as estrogen and androgen activity. The results can be used in risk assessment to identify potential DART related concerns, inform on *in vitro* testing, provide potential mechanistic information and can be used in weight of evidence approach.

Results:

- All benchmark substances with positive hazard characterisation were flagged by the chosen *in silico* tools (see *Fig2.*) as potential DART risk. However, it must be noted that most of the chosen benchmark substances were also part of the training sets used to develop some of these tools and further evaluation is needed.

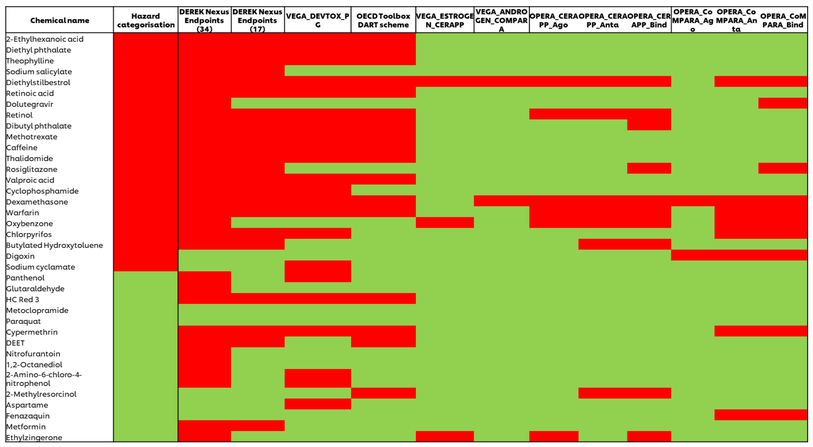

Fig. 2: in silico prediction of DART risk. Classifications from authoritative sources (ECHA, EPA, FDA, EMA) were used to categorize benchmark substances as DART positive (red) or negative (green). *In silico predictions were performed using different tools and the outcome is presented either as* positive (red) or negative (green) flags for DART relevant endpoints (dev tox, *repro tox and estrogen and androgen activity). For Derek Nexus results are divided into systemic tox (34 endpoints, including DART) versus DART relevant endpoints (17 endpoints).*

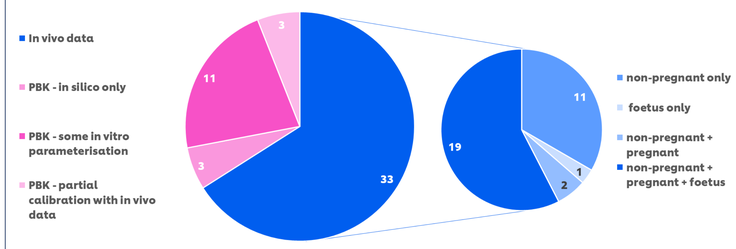

Fig. 3: Overview of the exposure scenarios. The left pie chart shows the derivation of C_{max} values for the 50 exposure scenarios describing high/low/uncertain risk for 37 benchmark substances used for BER calculation (see Fig.5). The right pie chart shows the distribution of available human *in vivo* data for non-pregnant, pregnant and foetal exposure.

Tier 1: Exposure predictions

- To investigate if an adult C_{max} would be protective for foetal and pregnant exposure, a literature review was performed aiming to identify human *in vivo* C_{max} data for benchmark substances for non-pregnant, pregnant and foetal exposures to compare BER values for the 3 different populations.
- Where available, C_{max} data was extrapolated for chosen exposure scenarios from multiple human *in vivo* studies. Where no *in vivo* data or only one clinical study was available, PBK modelling was performed to make predictions of C_{max} values for non-pregnant exposures only.

Results:

- Lack of pharmacokinetic studies in pregnant females (often serum concentrations at the point of delivery) and most data for non-pregnant C_{max} values are from males.
- No pharmacokinetic studies in foetus (no C_{max} data; mostly cord blood concentrations at the point of delivery.
- Only small differences for C_{max} values have been found for pregnant, non-pregnant and foetus populations, not affecting the BER outcome (see *Fig. 5*).

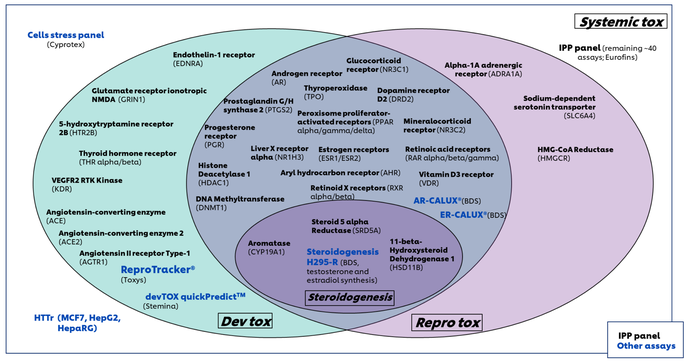

Fig. 4: NAM toolbox for DART. The toolbox has been designed to provide broad biological coverage[1] for DART safety combining broad screening tools (HTTr - high throughput transcriptomics, CSP – cell stress panel and IPP - *in vitro* pharmacological profiling) complemented with NAMs with DART specific endpoints (ReproTracker® from Toxys and the devTOX quickPredict™ assay from Stemina for developmental toxicity, DART specific IPP endpoints, steroidogenesis and CALUX® assays).

Tier 1: Bioactivity measurements and BER calculation

- Data were generated for the 37 benchmark substances for all NAMS of the DART toolbox (see *Fig.4*)
- In vitro points of departure for the 37 benchmark substances were compared to exposure estimates for 50 human exposure scenarios describing high/low/uncertain risk to calculate a BER (*see Fig 5*). Conceptually, a BER>1 indicates low risk (see for example [3]).

Results and future work:

- A first evaluation of the protectiveness of this framework using benchmark substances with known outcomes for DART, at specific human-relevant concentrations, shows that the framework is a good starting point in building a fit-for-purpose and protective NGRA approach for DART risk assessment.
- Pharmaceutical use of warfarin grouped with the low-risk exposures due to a specific mode of action not covered by the toolbox.
- Extended testing with more substances with different modes of action of toxicity is needed to build scientific confidence and to fill existing gaps (*e.g* DNT, and thyroid)
- Advanced more physiologically-relevant models are needed for refinement (*e.g.* placenta transfer).
- Better understanding of pregnant and foetal exposures is needed to build confidence that measured or predicted non-pregnant Cmax values are conservative exposure metrics for DART risk assessment.
- Integration/development of more *in silico* tools for predicting additional endpoints (*e.g.* thyroid)
- Integration of uncertainty calculations and models for decision making (integrating Tier 0 and Tier 1 testing).

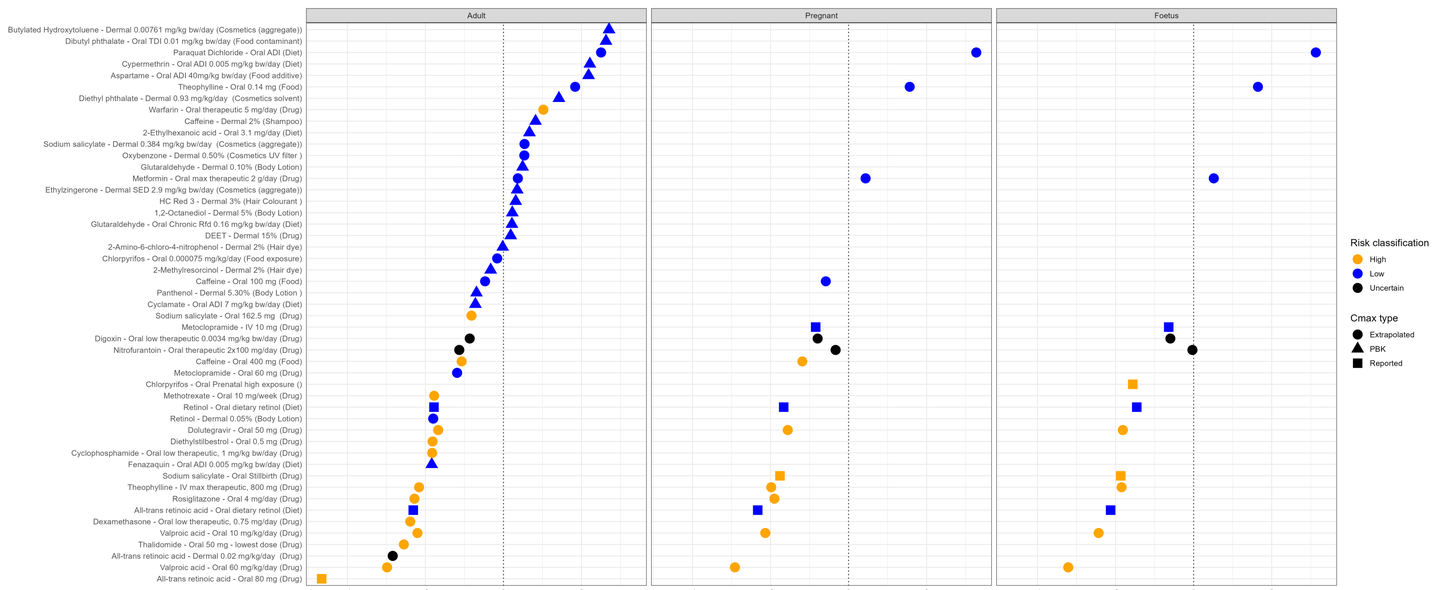

Fig. 5: BER estimates for 37 benchmark substances . BER values for the 50 human relevant exposure scenarios shown for adult (non-pregnant), pregnant and foetal C_{max} values. The lowest measured PoD from the DART toolbox was used for the calculations. The dashed grey line indicates a BER = 1. For the exposure scenarios marked as uncertain, no decision could be made from literature if they represent high or low risk for DART.

Literature

1) Rajagopal et al., 2022 Mar 7;4:838466
2) Thomas et al., 2019 Jun 1;169(2):317-332.
3) Middleton et al., 2022 Aug 25;189(1):124-147

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