Evaluating the ReproTracker Assay as a New Approach Methodology (NAM) for Developmental and Reproductive Testing

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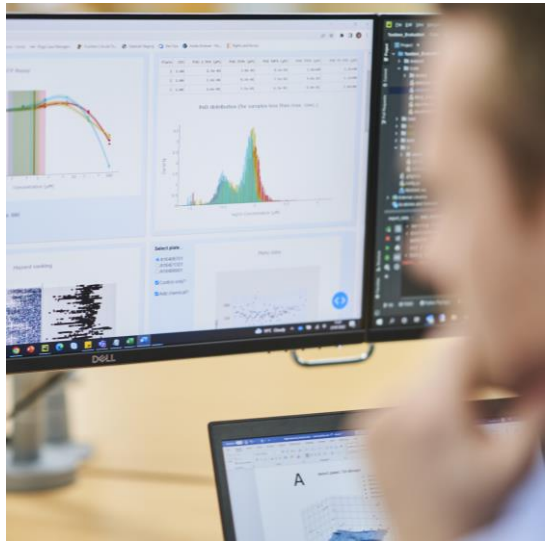
ESTIV 2024



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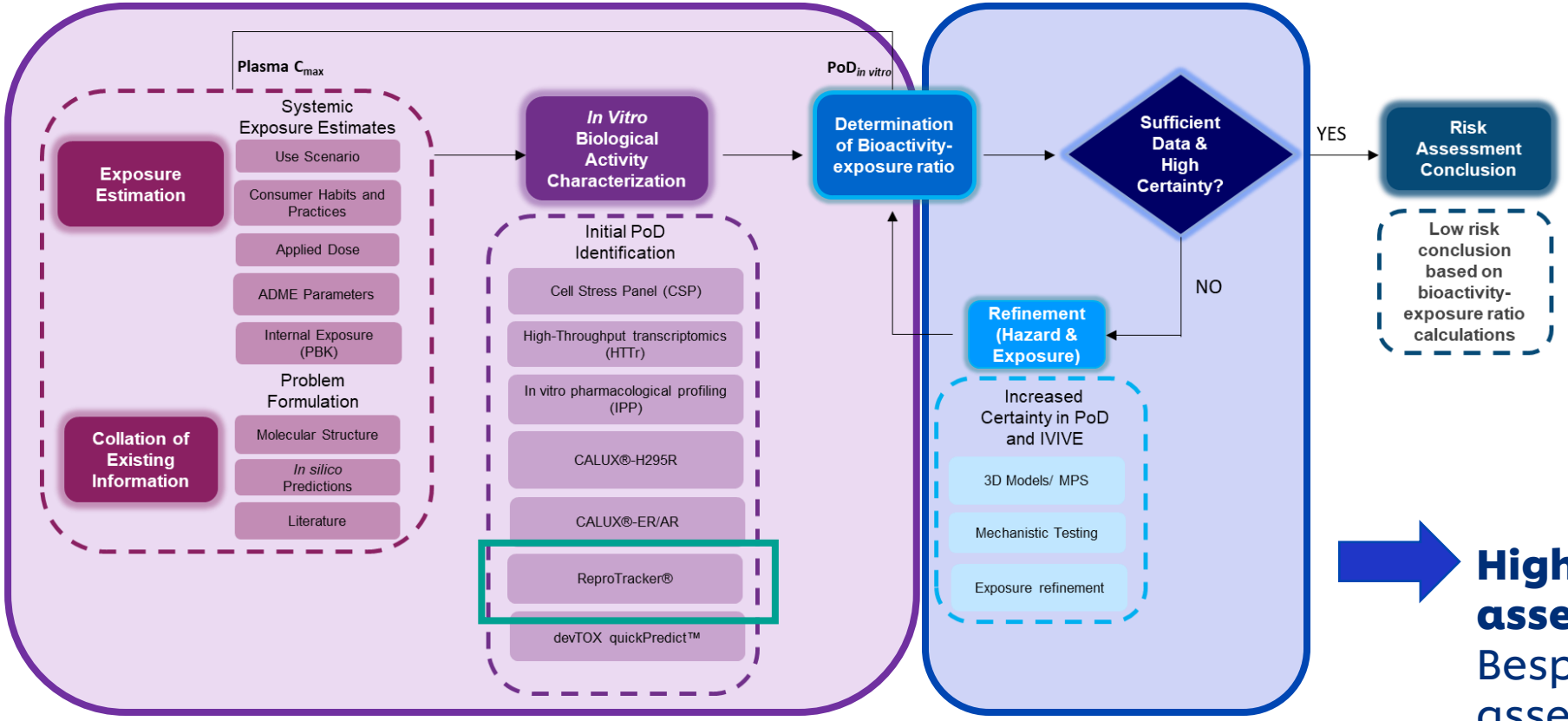
An integrated testing approach for DART – combining broad screening tools with targeted NAMS



modified after
Rajagopal et al., 2022
Mar7; 4:838466

Tier 0/1: Building a "fit for purpose" toolbox ensuring efficient protectiveness of DART/ED for consumer products

Higher Tier assessment : Bespoke assessment, depending on previous results

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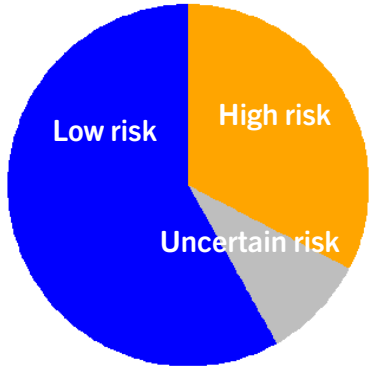
Evaluation of ReproTracker within our NGRA DART approach

33 compounds

43 exposure scenarios

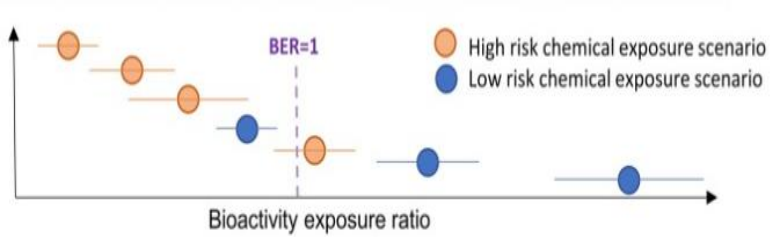
*Risk classifications based on what would be **traditionality considered high or low risk** based on **animal or human toxicology studies***



Chemical	Exposure Scenario	Risk Classification	Reason
Theophylline	Black Tea 0.14 mg	Low	Estimated daily intake USA (NIH)
Theophylline	Pharmaceutical 800 mg	High	Only use during pregnancy if the potential benefit justifies the potential risk to the fetus (FDA, EMA)
Thalidomide	Pharmaceutical 50 mg	High	Contraindicated in pregnancy (FDA, EMA)
Methotrexate	Pharmaceutical 10 mg	High	Contraindiacted in pregnancy (FDA, EMA)
Paraquat	Dietary Residues 0.27 mg	Low	ADI (EFSA)
2-methylresorcinol	Hair Colourant 1.5 mg	Low	Favourable MoS (SCCS)

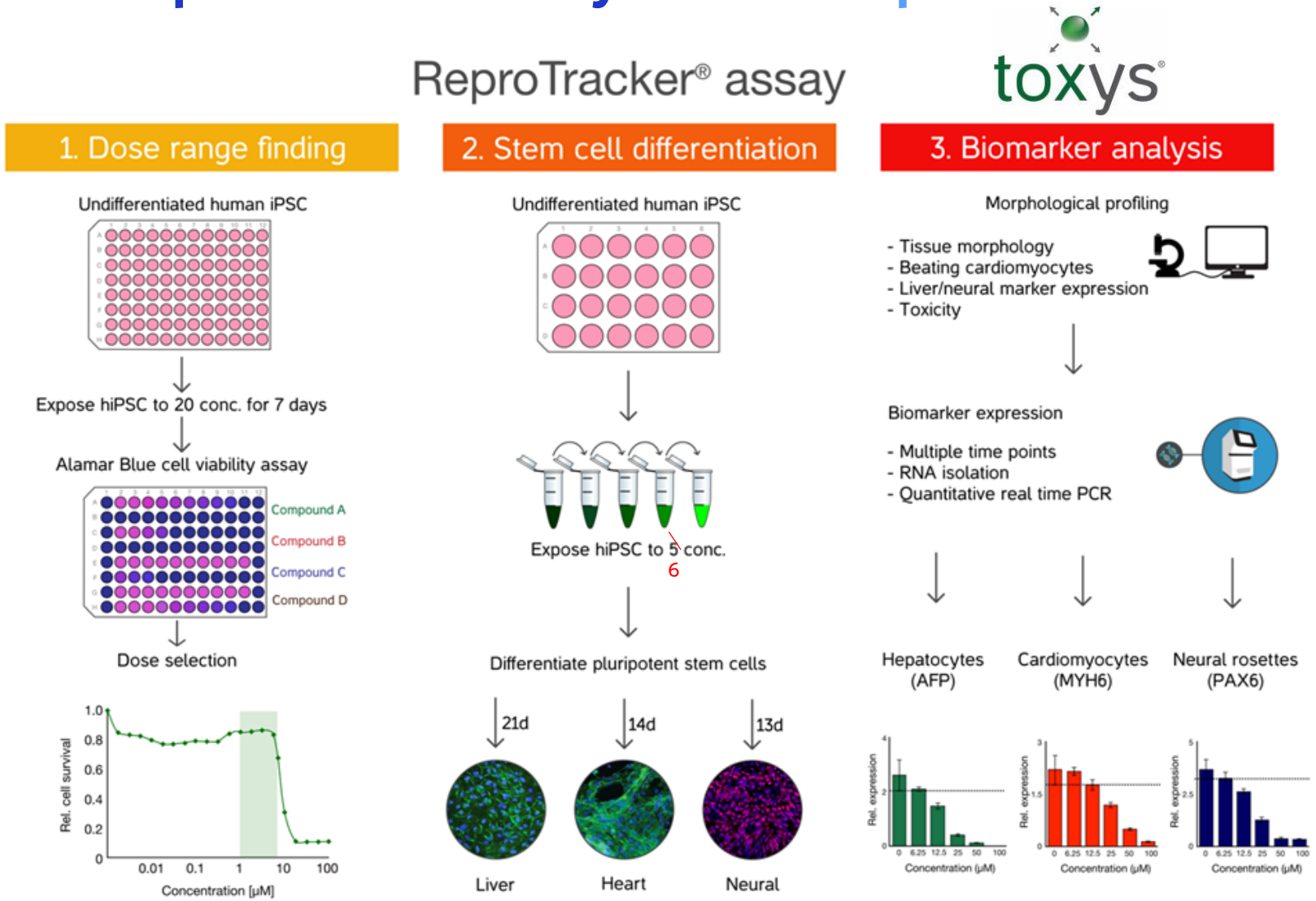
Examples of high and low risk exposure scenarios

$$BER = \frac{Lowest\ bioactivity\ POD}{Internal\ in\ vivo\ exposure\ (Cmax)}$$



Inform safety decision

The ReproTracker Assay – and adaptations for an NGRA approach



To **optimise the POD modelling**, adaptations to the ReproTracker experimental design have been made from the standard assay (left).

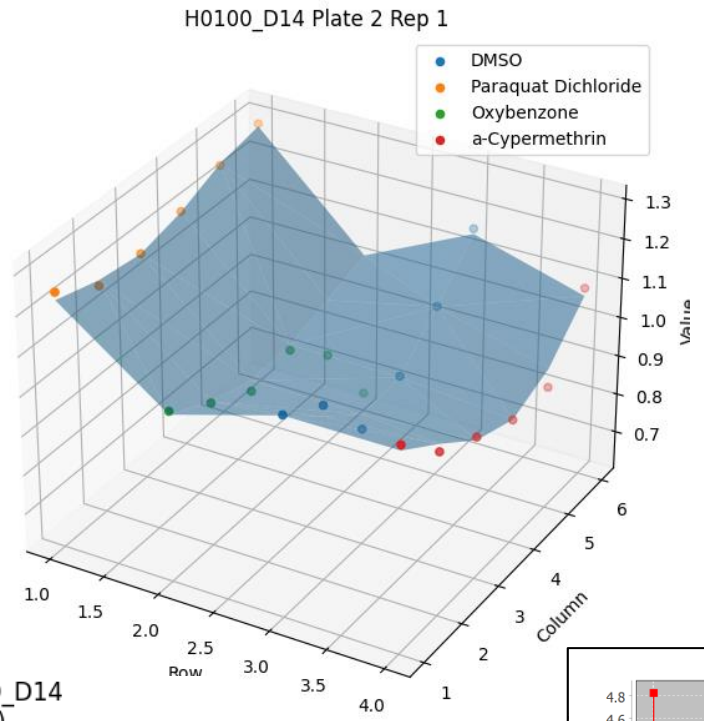
- **Increasing throughput of dose range finding**
  - By reducing number of concentrations tested per chemical
- **Increasing dose response modelling suitability**
  - By increasing number of concentrations tested in main stem cell differentiation testing and by increasing concentration dilution steps.
- **Improving baseline estimation:**
  - By increasing number of controls for better baseline estimations; reducing experimental variability between treated samples compared to previous design where controls were on separate treatment plates.
- **Increasing protectiveness for risk assessment**
  - By including AlamarBlue as an additional endpoint on differentiating cells and calculating cytotoxicity POD.

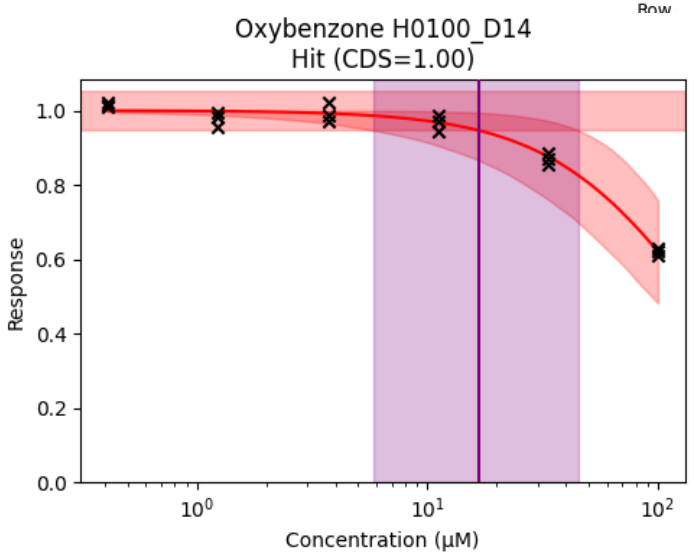
Jamalpoor, A., Hartvelt, S., Dimopoulou, M., Zwetsloot, T., Brandsma, I., Racz, P. I., Osterlund, T., & Hendriks, G. (2022). A novel human stem cell-based biomarker assay for in vitro assessment of developmental toxicity. Birth Defects Research, 114(19), 1210–1228. https://doi.org/10.1002/bdr2.2001

Modelling Methods – deriving cytotoxicity and teratogenicity point of departures

Cytotoxicity Modelling

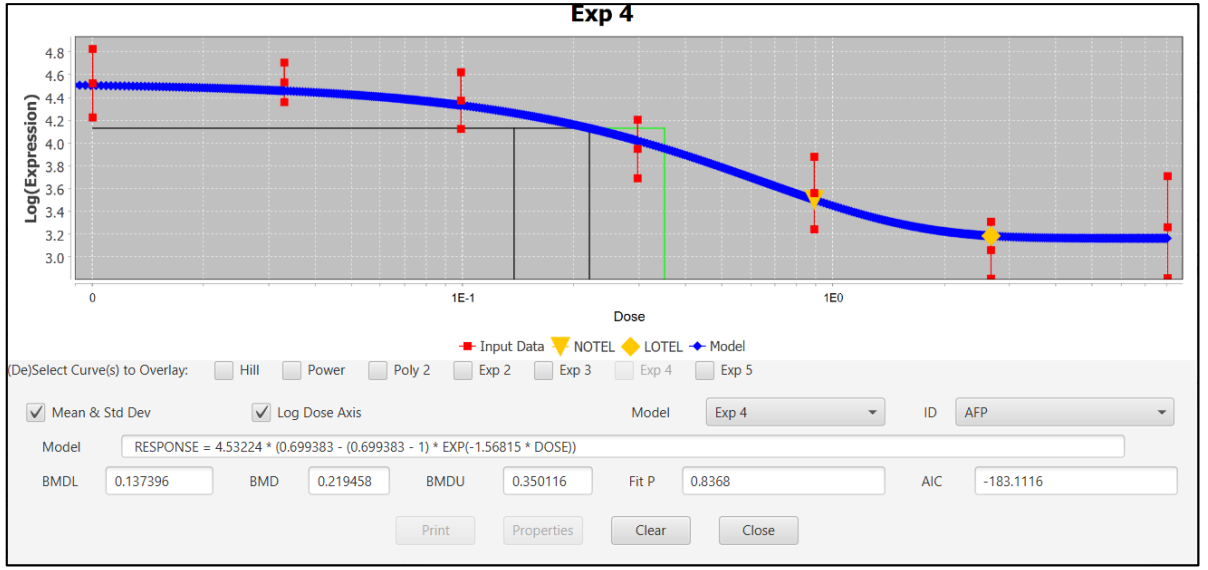
- Effects of chemical concentrations are modelled using **Bayesian methods** to **account for local variations** in fluorescence.
- **PODs are defined as a decrease in viability from the baseline** inferred per row.
- Model assumptions state that baseline **RFU response between rows correlated** but can have different means allowing for row dependent offset.



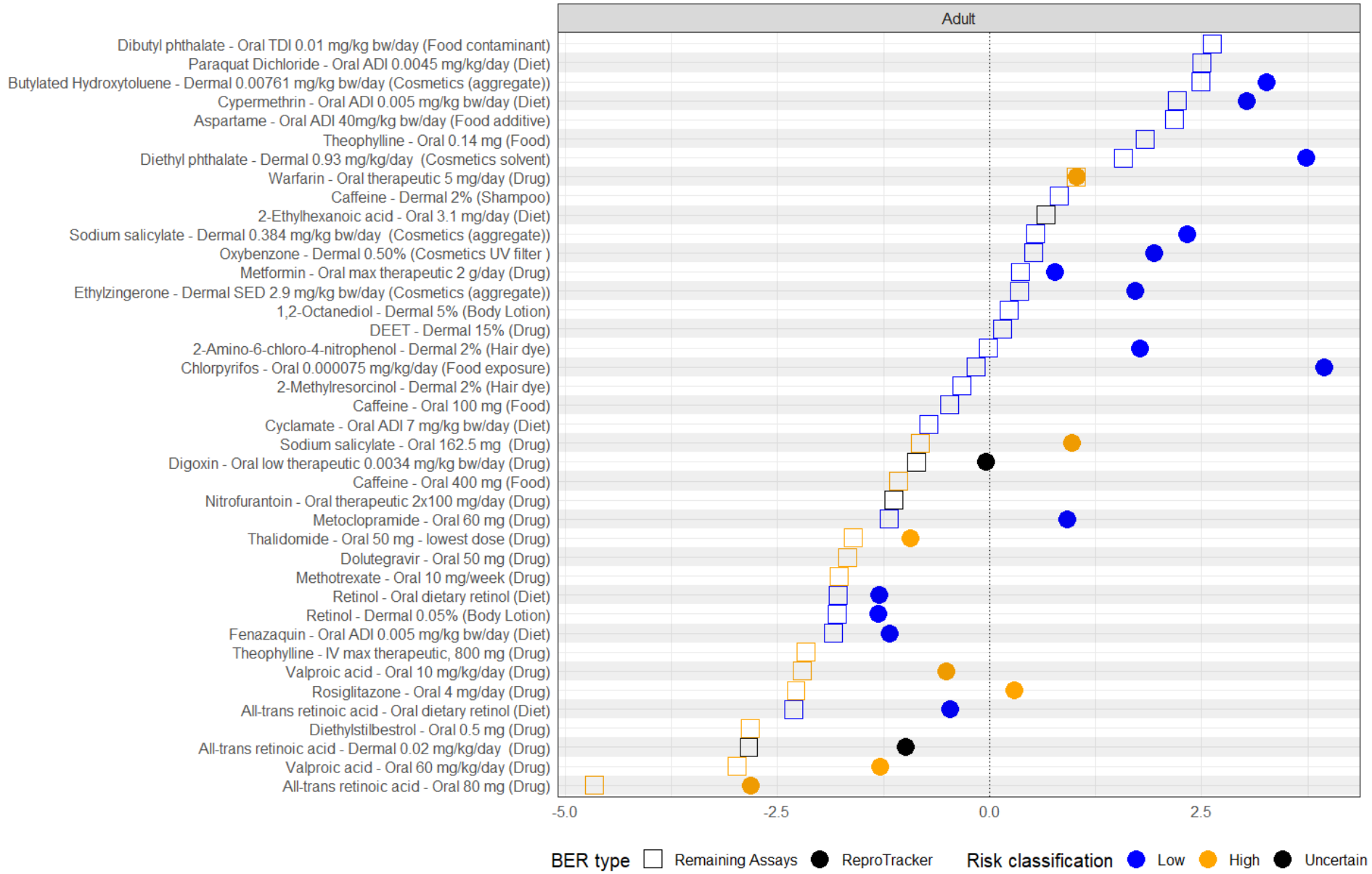


Biomarker BMDExpress2 Modelling

- BMD modelling is a **well-recognised approach** and is used for various dose response data, particularly for transcriptomics. **BMDExpress2** is a **parametric modelling** software and is used to derive PODs from biomarker **response across concentrations**.
- A **benchmark response factor (BMR) of 1.349** is used to calculate BMD as **10% transcriptomic change** from control baseline - A lower bound **(BMDL) is taken as a final POD.**
- Point of departures are only calculated for down regulated responses.



Evaluating ReproTracker –based on a BER of 1



Conclusions

Tailoring experimental design allows ReproTracker data to be used for dose response modelling and to derive point of departures (PODs)

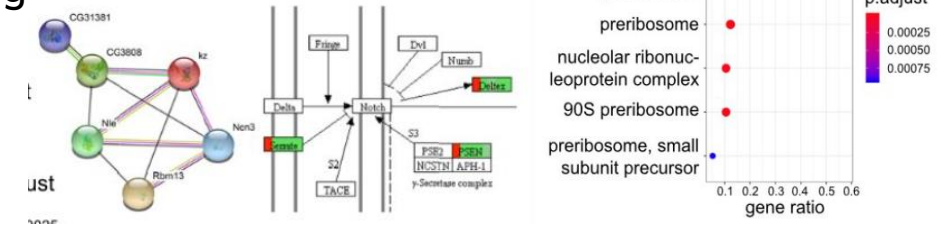
Testing of more compounds is needed to better define biological relevance of ReproTracker and how the data can be used for a weight of evidence approach

A toolbox of broad and DART targeted assays should be used to calculate conservative POD protective overall for DART.

Related Projects and Next Steps

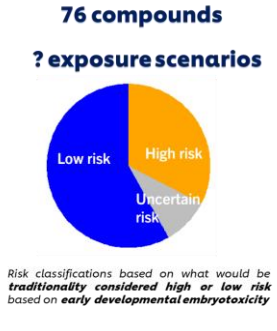
Further understanding of biological relevance using HTTr

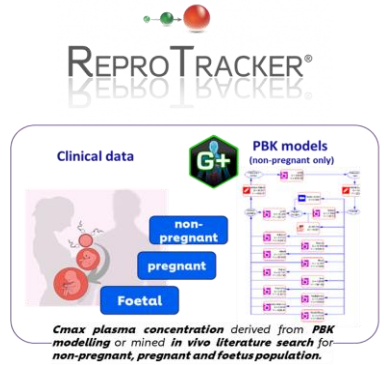
- Run TempO-seq 5-point time course data to 1) define ReproTracker baseline expression profile and 2) explore pathway analysis for informing higher tier testing

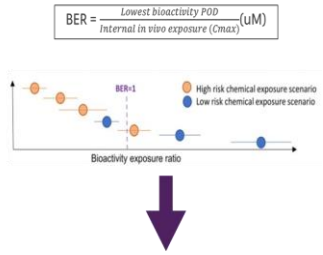


ReproTracker Focused Evaluation

- Evaluate 76 compounds against early embryotoxicity risk exposure scenarios using various concentration response methods

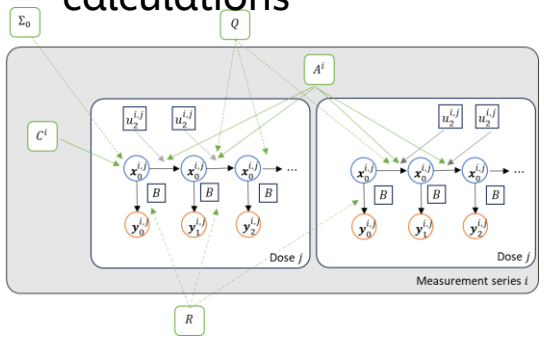


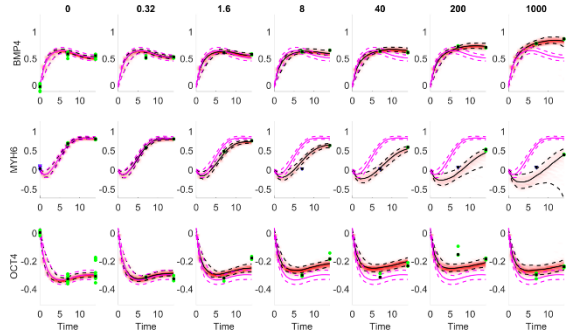




Advancing and improving modelling approaches

- Incorporate time and hierarchical modelling into POD calculations





Transferability study

- To demonstrate inter-laboratory transferability and reproducibility

Testing of 10 blinded compounds in ReproTracker assay in UNILEVER and TOXYS labs

Data analysis conducted by independent team in 2025

Thank you

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