Application of an integrated approach using NAMs for DART Next Generation Risk Assessment (NGRA)

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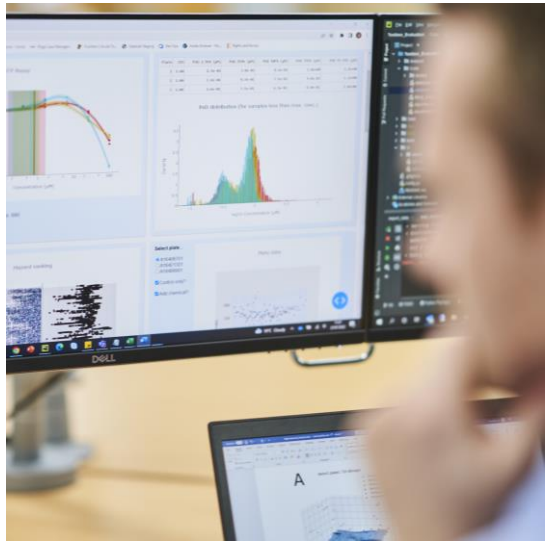

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Background and Aims

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Research article

Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

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ABSTRACT

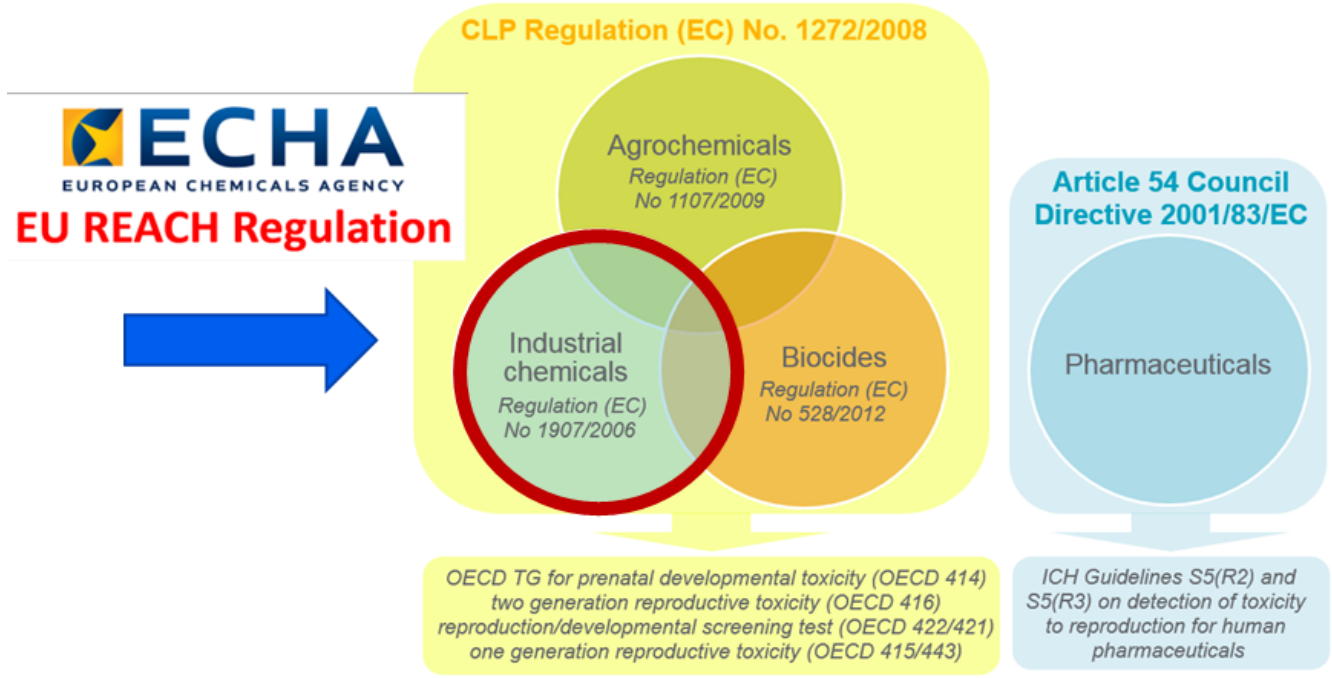
An important question in toxicological risk assessment is whether non-animal new approach methodologies (NAMs) can be used to make safety decisions that are protective of human health, without being overly conservative. In this work, we propose a core NAM toolbox and workflow for conducting systemic safety assessments for adult consumers. We also present an approach for evaluating how protective and useful the toolbox and workflow are by benchmarking against historical safety decisions. The toolbox includes physiologically based kinetic (PBK) models to estimate systemic C_{max} levels in humans, and 3 bioactivity platforms, comprising high-throughput transcriptomics, a cell stress panel, and *in vitro* pharmacological profiling, from which points of departure are estimated. A Bayesian model was developed to quantify the uncertainty in the C_{max} estimates depending on how the PBK models were parameterized. The feasibility of the evaluation approach was tested using 24 exposure scenarios from 10 chemicals, some of which would be considered high risk from a consumer goods perspective (eg, drugs that are systemically bioactive) and some low risk (eg, existing food or cosmetic ingredients). Using novel protectiveness and utility metrics, it was shown that up to 69% (9/13) of the low risk scenarios could be identified as such using the toolbox, whilst being protective against all (5/5) the high-risk ones. The results demonstrated how robust safety decisions could be made without using animal data. This work will enable a full evaluation to assess how protective and useful the toolbox and workflow are across a broader range of chemical-exposure scenarios.

Key words: Bayesian modelling; new approach methodologies; point of departure; physiologically based pharmacokinetics; probabilistic risk assessment.

The rapid development of new, non-animal approaches for conducting toxicological safety assessments has been driven by several factors. These include ethical considerations, regulatory action (animal test bans for certain types of ingredients), and the need to assure the safety of chemicals using efficient, cost-

et al., 2019). Non-animal approaches also have the potential to improve safety assessments by using more human-relevant tools through coverage of key biological pathways or targets. Next-generation risk assessment (NGRA) provides a way to integrate new approach methodology (NAM) data from various

[Middleton et al. 2022]



DART being a major endpoint that historically relies heavily on animal testing

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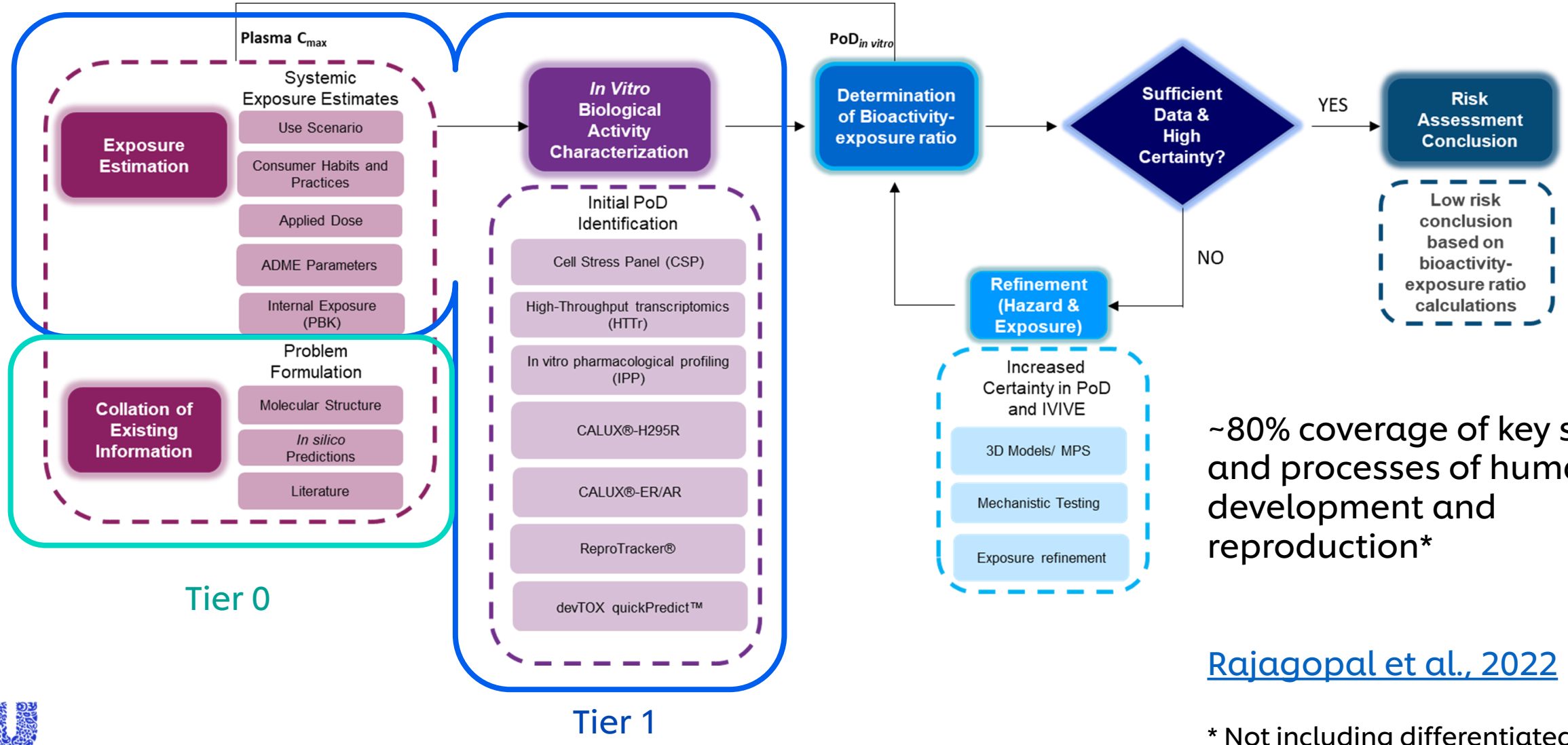
DART NGRA Framework



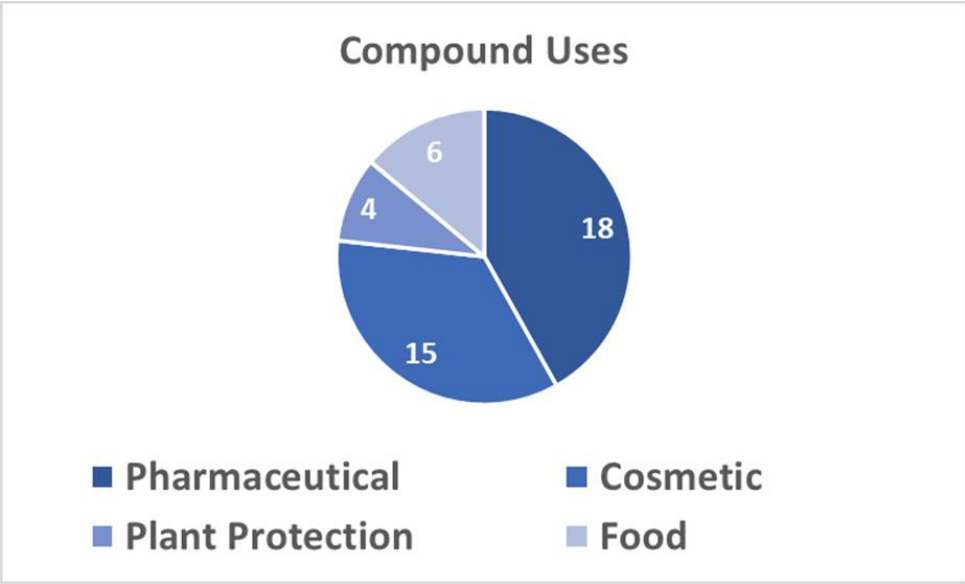
~80% coverage of key stages and processes of human development and reproduction*

[Rajagopal et al., 2022]

* Not including differentiated hiPSCs and H295R cells

Methods

- 37 compounds
- High Risk, Low Risk and Uncertain Risk- exposure scenarios



Examples:

Chemical	Exposure Scenario	Dose	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)

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Methods

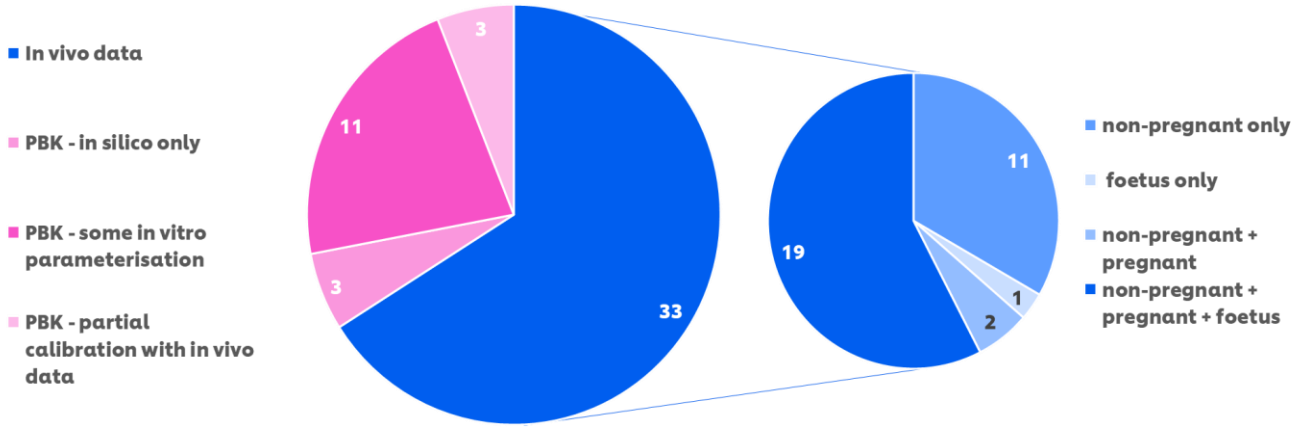
Tier 0- in silico predictions

- Derek Nexus (34 endpoints related to systemic tox and DART)
- OPERA:
  - ✓ OPERA_CoMPARA_Androgen_Receptor
  - ✓ OPERA CERAPP Estrogen_Receptor
- OECD QSAR Toolbox:
  - ✓ DART scheme (P&G alerts)
- VEGA:
  - ✓VEGA_DEVTOX_PG
  - ✓VEGA_ ANDROGEN_COMPARA
  - ✓VEGA_ESTROGEN_CERAPP

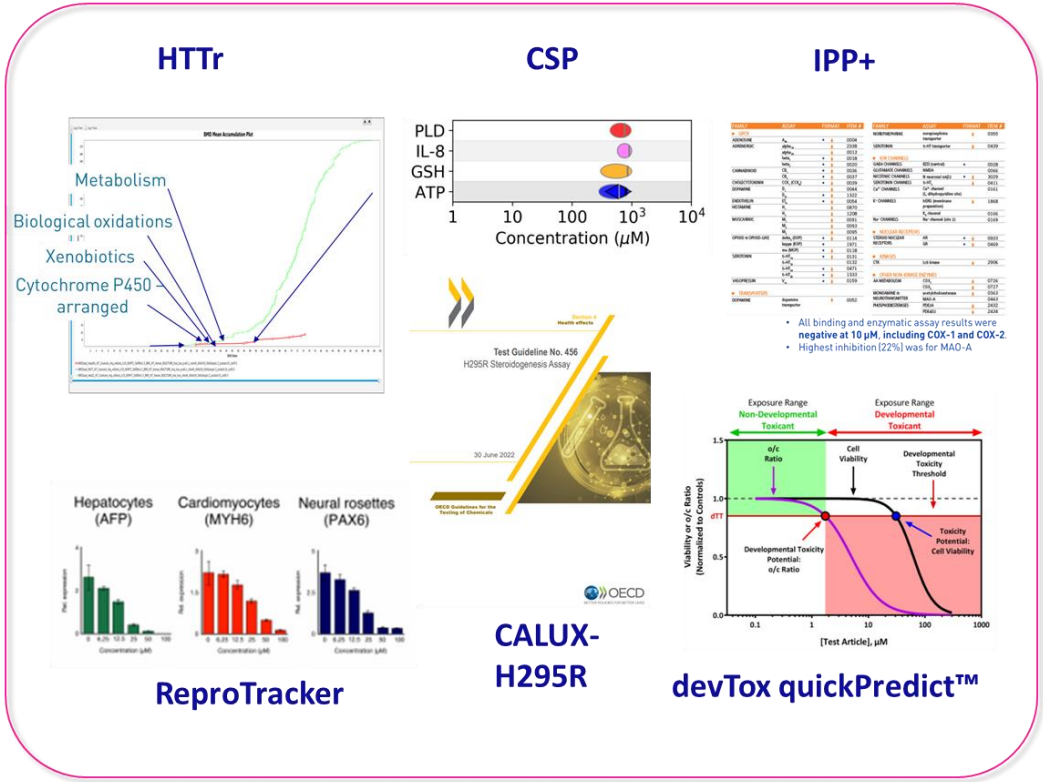
Methods

Tier 1- exposure estimates

Estimation of systemic exposure to a compound through physiologically-based kinetic modelling or clinical data



Tier 1- Bioactivity Characterisation



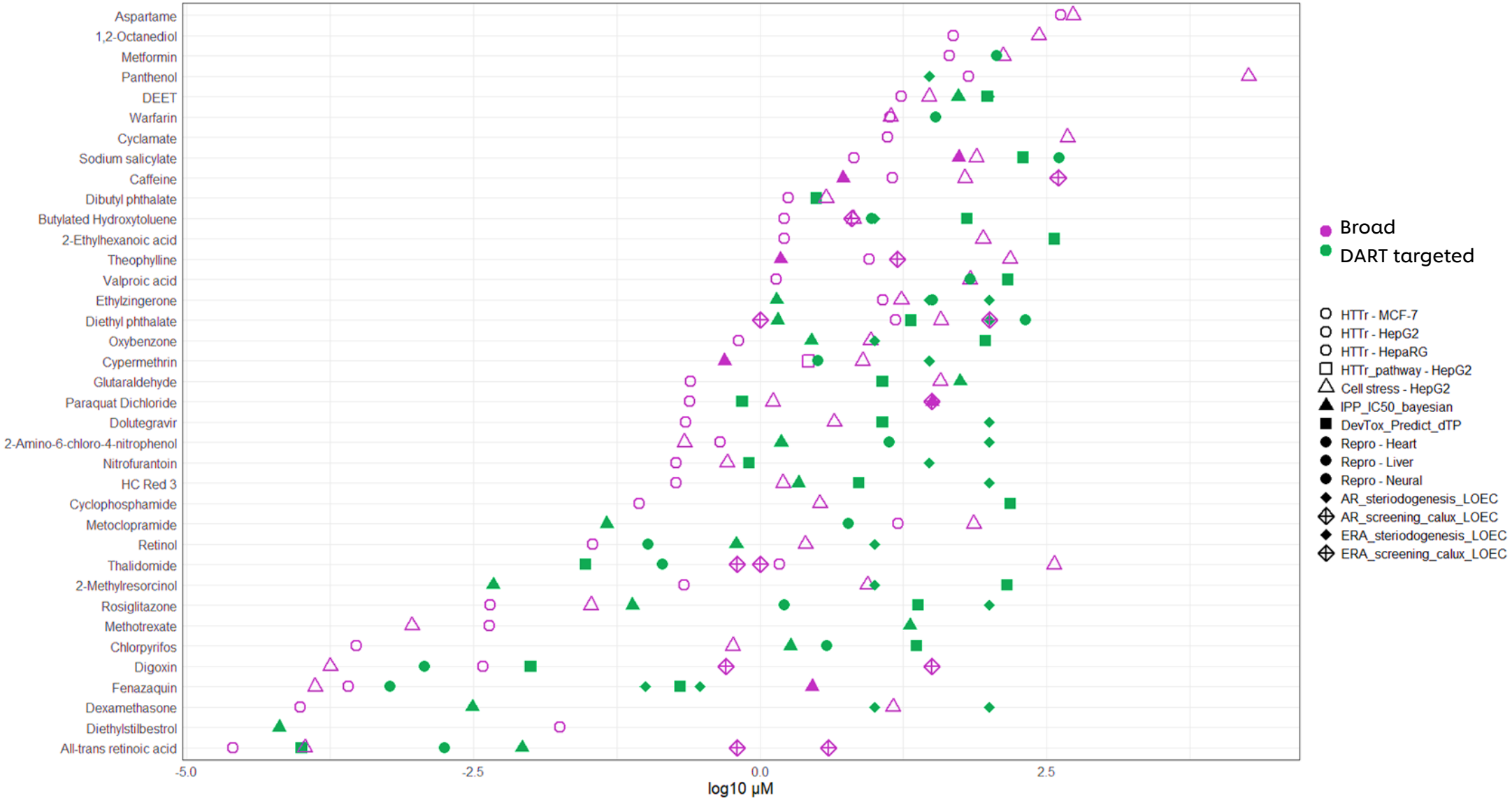
Bioactivity Exposure Ratio (BER) = $\frac{Lowest\ bioactivity\ POD}{Internal\ in\ vivo\ exposure\ (Cmax)}$(uM)

Results- Tier 0

Chemical name	Exp DART Hazard	DEREK Nexus Endpoints (34)	DEREK Nexus Endpoints (17)	VEGA_DEVTOX_PG	OECD Toolbox DART scheme	VEGA_ESTROGEN_CERAPP	VEGA_ANDROGEN_COMPARA	OPERA_CERAPP_Ago	OPERA_CERAPP_Anta	OPERA_CERAPP_Bind	OPERA_CoMPARA_Ago	OPERA_CoMPARA_Anta	OPERA_CoMPARA_Bind
2-Ethylhexanoic acid													
Diethyl phthalate													
Theophylline													
Sodium salicylate													
Diethylstilbestrol													
Retinoic acid													
Dolutegravir													
Retinol													
Dibutyl phthalate													
Methotrexate													
Caffeine													
Thalidomide													
Rosiglitazone													
Valproic acid													
Cyclophosphamide													
Dexamethasone													
Warfarin													
Oxybenzone													
Chlorpyrifos													
Butylated Hydroxytoluene													
Digoxin													
Sodium cyclamate													
Panthenol													
Glutaraldehyde													
HC Red 3													
Metoclopramide													
Paraquat													
Cypermethrin													
DEET													
Nitrofurantoin													
1,2-Octanediol													
2-Amino-6-chloro-4-nitrophenol													
2-Methylresorcinol													
Aspartame													
Fenazaquin													
Metformin													
Ethylzingerone													

DART Hazard Flag | No DART Hazard Flag

Results- Tier 1



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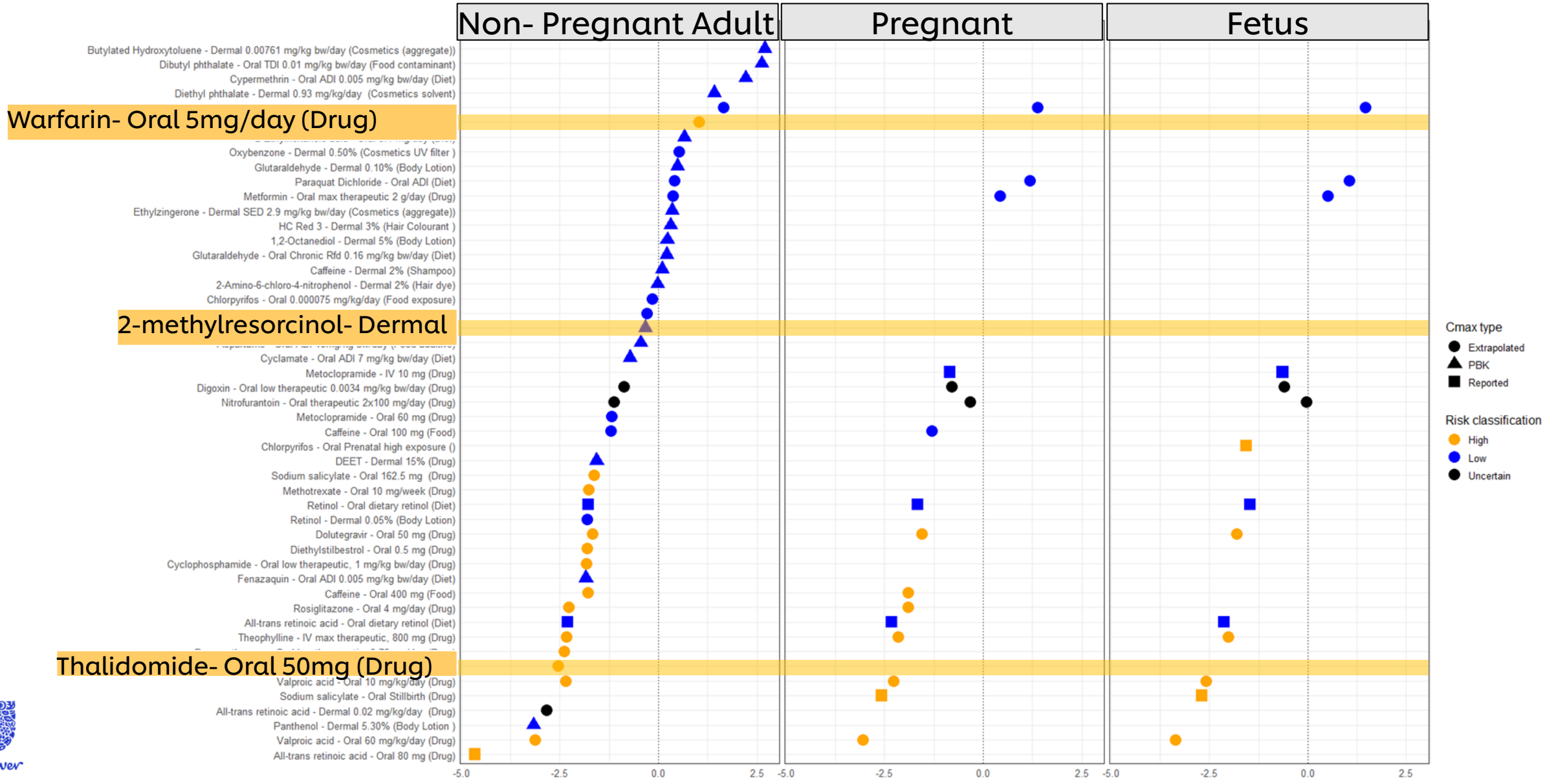
Results- Tier 1



Conclusion and Next Steps

- **Conservative** approach and framework is a good starting point for a protective NGRA for DART
- **Additional testing** of more substances with different MoAs **(DNT and thyroid)**
- Advanced more physiologically-relevant models are needed for refinement (*e.g.* **placenta transfer**).
- Better understanding of **pregnant and foetal exposures**
- **Expansion of in silico tools** for predicting additional endpoints (e.g. thyroid)
- **Uncertainty calculations** and models for decision making
- Expansion of **biological coverage** understanding

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Presentation can be found using this QR code →