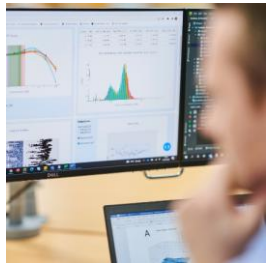
Practical Application of a Next Generation Risk Assessment Approach for Developmental and Reproductive Toxicity

Predrag Kukic, PhD
Unilever SERS

SERS
Safety, Environmental & Regulatory Science



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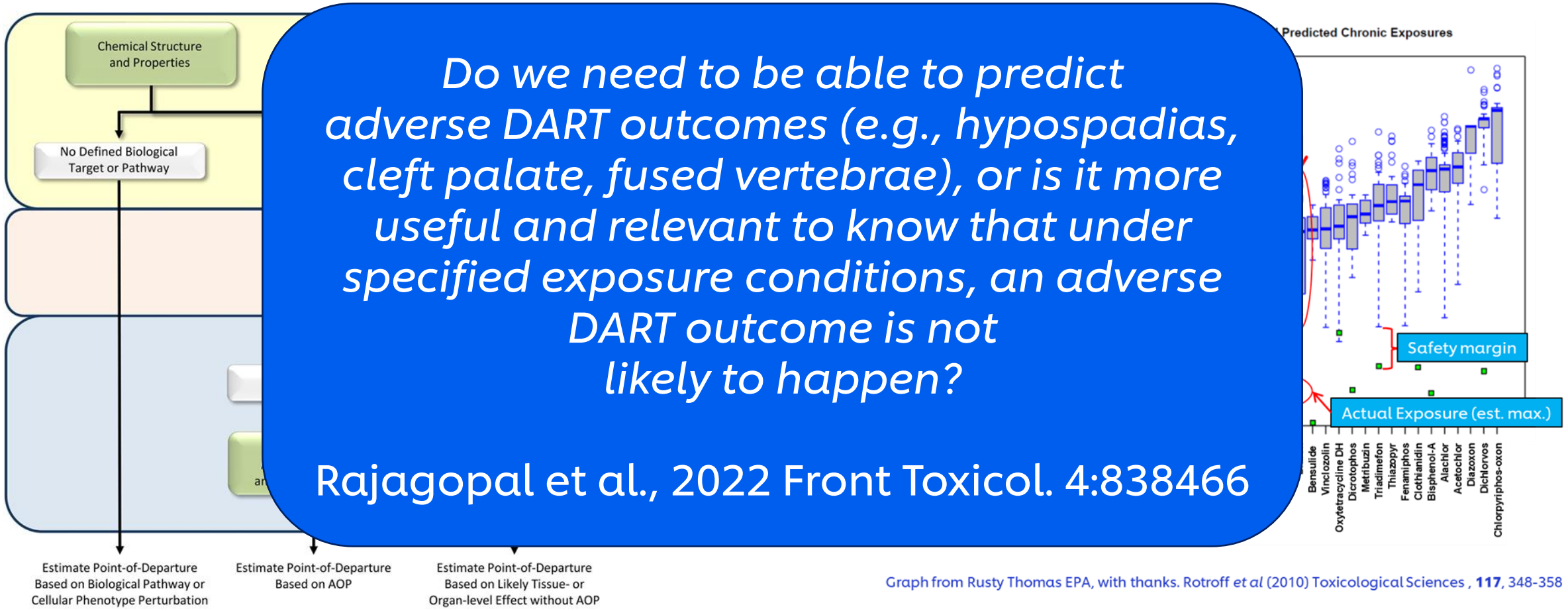
Paradigm shift requires a different approach to systemic and DART toxicity – Focus on protection



Do we need to be able to predict adverse DART outcomes (e.g., hypospadias, cleft palate, fused vertebrae), or is it more useful and relevant to know that under specified exposure conditions, an adverse DART outcome is not likely to happen?

Rajagopal et al., 2022 Front Toxicol. 4:838466

Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

*NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

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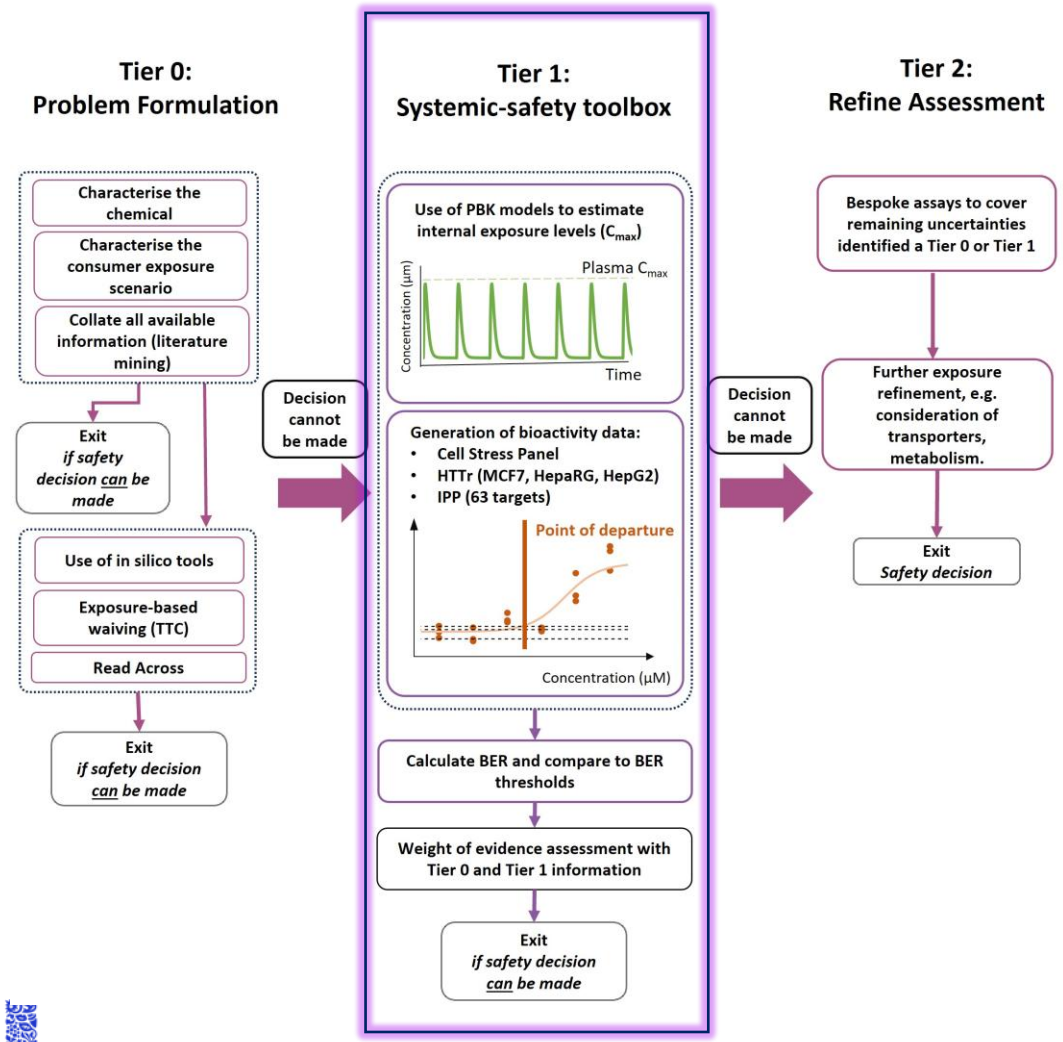
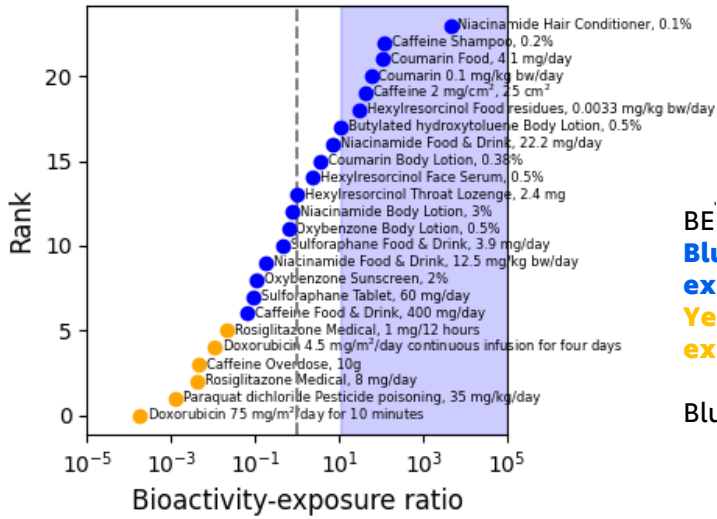
EPA United States Environmental Protection Agency

Our approach for systemic toxicity – A NAM toolbox and workflow



NAM Systemic toolbox provides similar level of protection as traditional approaches for a total of 48 chemicals and 100 chemical exposure scenario



BER=lowest POD/Plasma Cmax
Blue: low risk chemical-exposure scenario
Yellow: high risk chemical-exposure scenario

Blue shaded region BER> 11

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159; Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Systemic toolbox designed to protect against systemic and DART-related toxicities – Context of use

- A systemic toolbox intended to be used as **a Tier 1 within an NGRA/IATA framework for systemic toxicity including DART** (i.e. quantitative risk assessment of ingredients in consumer goods products).
- A systemic toolbox which provides **protective thresholds (PoDs) for systemic toxicity and DART**.
- A systemic toolbox that provides **better or equivalent levels of protection of human health** and useful for risk assessment by integrating bioactivity and exposure.

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Evaluation strategy

Define the Toolbox components

Choose a set of NAMs covering exposure modelling and **bioactivity which provide wide biological coverage**

Set performance criteria

The performance of the NAM toolbox is **assessed against historical safety decisions**

Select test chemicals

Maximise coverage of different **chemistries and biological effects/toxicities**

Evaluate performance of the Toolbox

Data-driven evaluation based on **Bioactivity Exposure Ratio (BER)**

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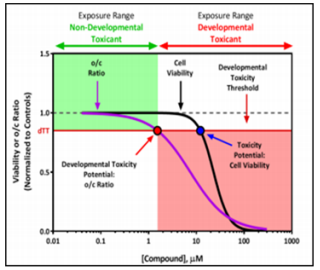
Define the Toolbox components including additional DART-specific NAMs

devTOX quickPredict™

- human iPSC cells
- metabolic perturbation of the biomarker's ornithine and cystine
- predicts concentration at which a test article shows developmental toxicity potential (dTP).

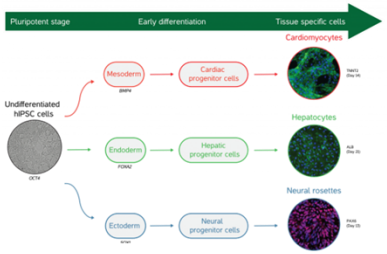
Stemina BIOMARKER DISCOVERY



Toxicology in vitro (2020) Apr 1;174(2):189-209

ReproTracker®

- human iPSC cells
- differentiated into cardio-myocytes, hepatocytes and neuronal rosettes
- Dose depended changes of lineage-specific gene biomarkers are measured to identify potentially teratogenic effects.



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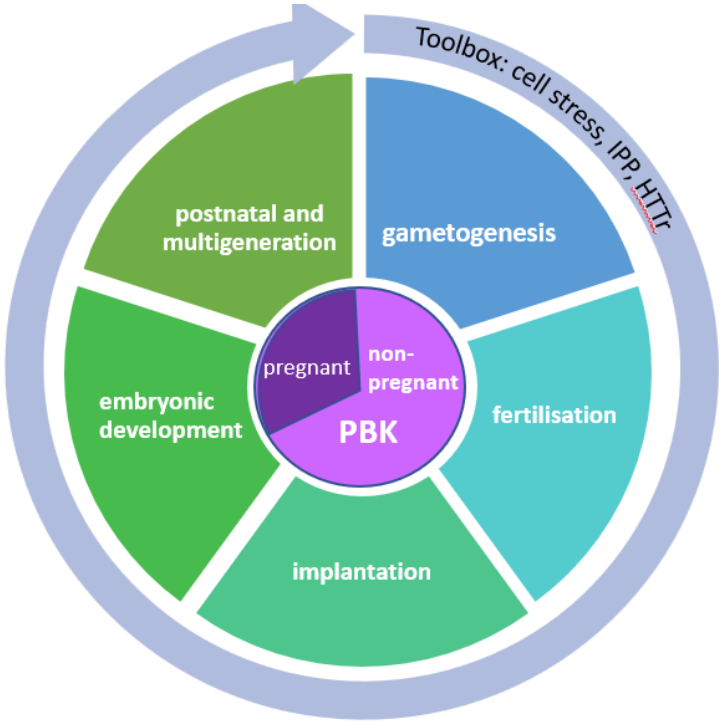
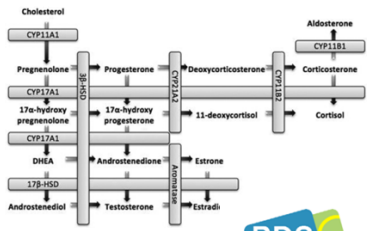
Birth defects Res. (2022) Nov 15;114(19):1210-1228.



H295R steroidogenesis assay

- human adenocarcinoma cell line NCI-H295R and U2-OS
- *in vitro* effect-based responses of compounds using the H295R steroidogenesis assay coupled to two CALUX® bioassays as a read-out: the ERα and AR CALUX®
- OECD Test No. 456



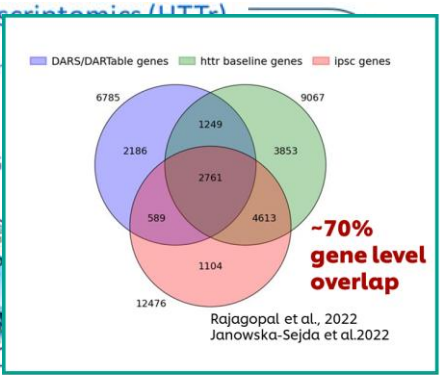
BDS

Toxicological Sciences, 2023, 194(2), 191–208

High-throughput Transcriptomics (HTTr)

- Use of full human gene par ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG MCF7
- 3D HepaRG spheroid

BMDExpress 2



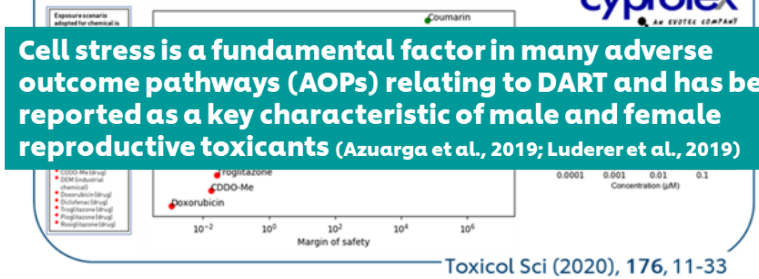
Rajagopal et al., 2022
Janowska-Sejda et al.2022

Cell Stress Panel (CSP)

13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

cyprotex

Cell stress is a fundamental factor in many adverse outcome pathways (AOPs) relating to DART and has been reported as a key characteristic of male and female reproductive toxicants (Azuarga et al., 2019; Luderer et al., 2019)



Toxicol Sci (2020), **176**, 11-33

In vitro Pharmacological Profiling (IPP)

51 out of the 75 targets are DART relevant. This includes 20 nuclear hormone receptors, 6 DNT targets, aromatase ...

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Set performance criteria

What we are trying to test: Are the decisions made with the Tier 1 toolbox equivalent or better than the decisions we have been making with animal data?

What we are not trying to test: Is the Tier 1 Toolbox predictive of all possible adverse effects for a given chemical?

Select test chemicals with known human exposure and associated risk assessments

Selection of 37 chemicals

Maximised **different chemical properties**, and from **different sectors** (pharmaceutical, cosmetic, plant protection, and food).

Assignment of exposure scenario and risk classification and

High or low risk for DART (based on existing data in humans or animal toxicology studies from different regulatory authorities).

Outcome:

- **27 low risk**
- **17 high risk**
- **5 uncertain risk**

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 foetus (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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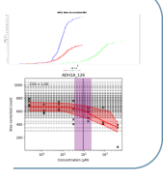
Evaluate performance of the Toolbox: Differentiate high and low risk chemical exposure scenarios using BER

Point of Departure determination from Bioactivity assays

Non-specific effects

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

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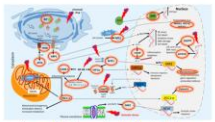

Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Specific effects

In vitro pharmacological profiling

PERSPECTIVES

Reducing safety-related drug attrition: the use of in vitro pharmacological profiling

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DART related assays

devTOX quickPredict™

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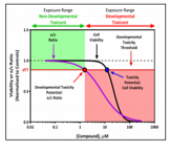

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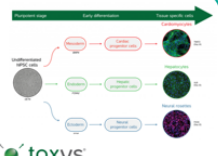

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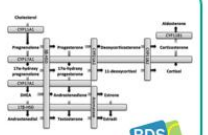
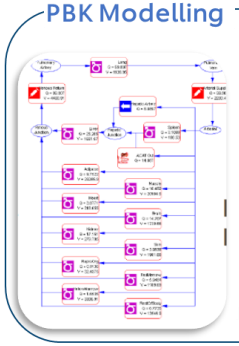


BDS

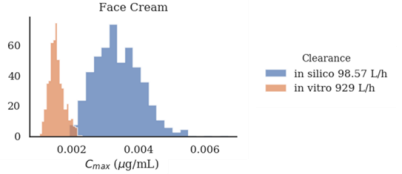
Toxicological Sciences, 2023, 194(2), 191–208

Bioactivity Exposure Ratio (BER)

PBK Modelling



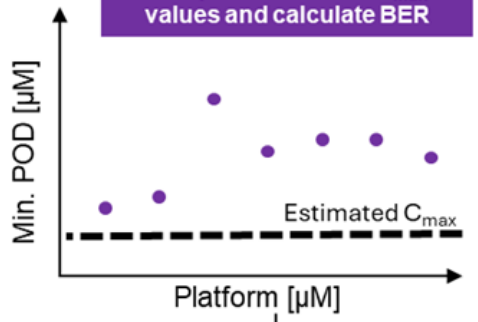
Face Cream



Toxicology in Vitro (2020), **63**, 104746

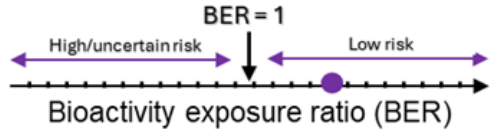
Plasma C_{max} estimate

Compare PODs with C_{max} values and calculate BER



$$BER = \frac{Minimum\ POD}{Plasma\ C_{max} estimate}$$

Conceptually define risk for DART using BERs



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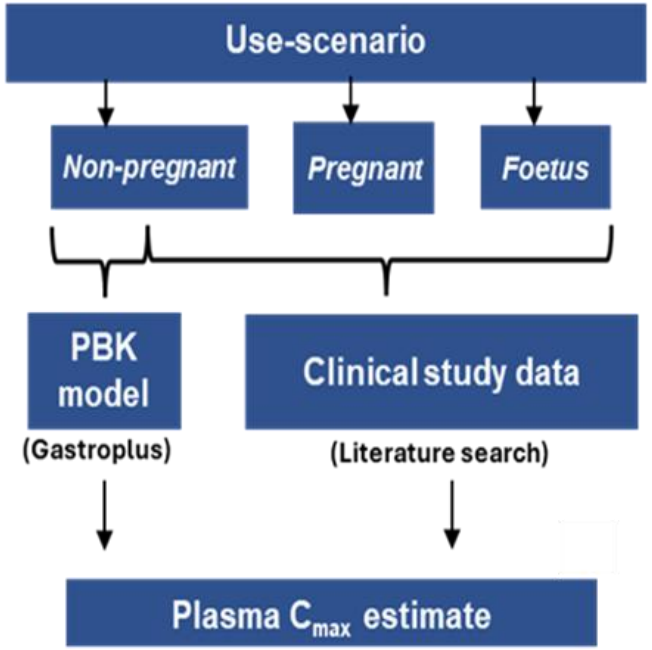
DART exposure strategy for NGRA - Modelling of DART relevant exposures

Human Chemical Exposure



Data curation

- Physico-chemical properties (in silico or measured)
- ADME properties (in silico or measured)
- Non-pregnant adult pharmacokinetic studies (IV, Oral & dermal)
- Pregnant PK studies (IV, Oral)
- In vitro/ex vivo placental transfer studies
- Generic or pregnancy PBPK models

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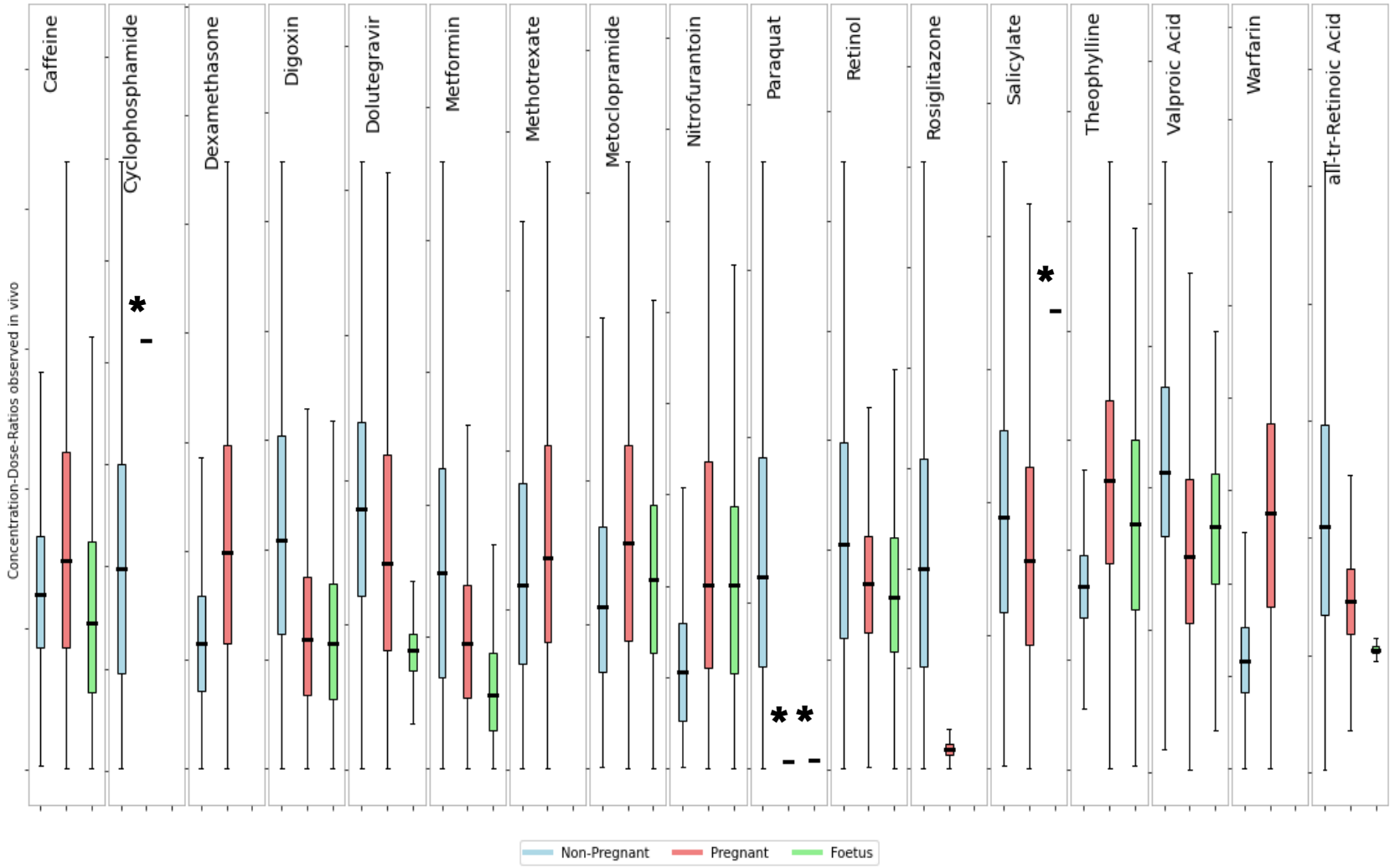
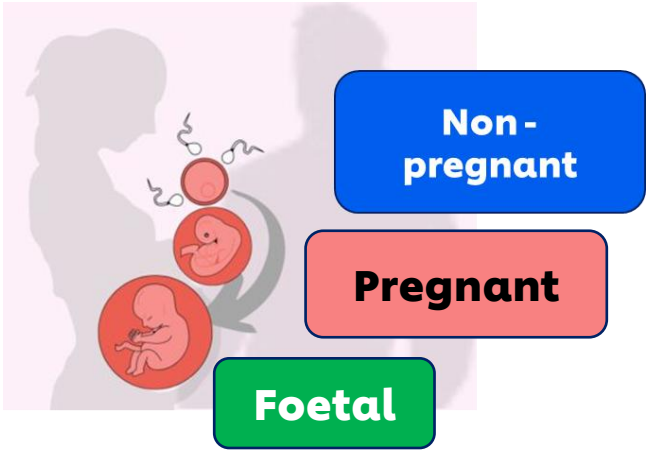
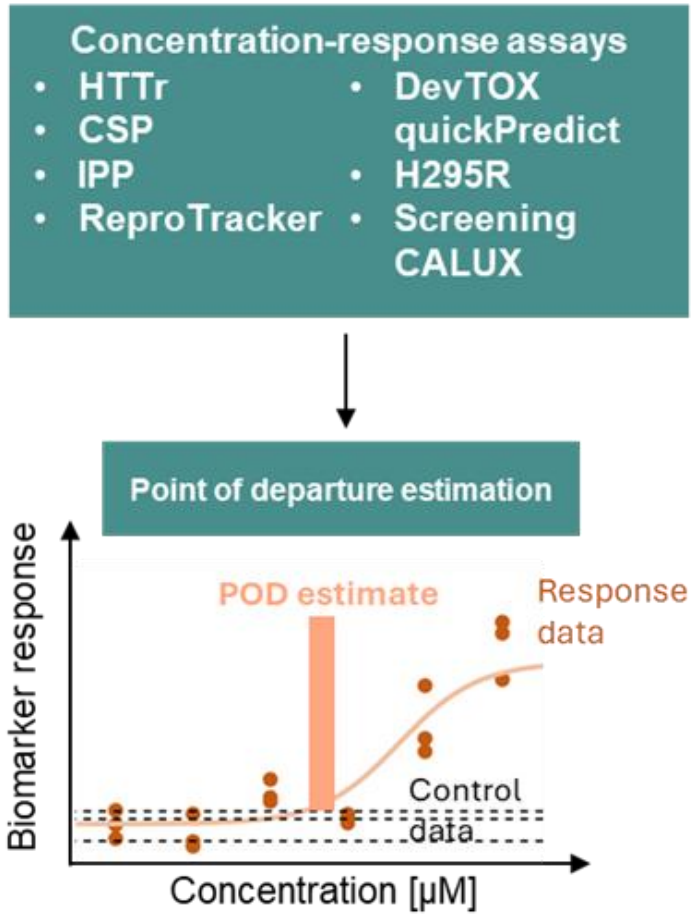
For most chemicals, internal exposure estimates for a general population cover the exposures in the pregnant and foetal sub-group



- Clinical data for pregnant and foetal exposure is scarce
- Most exposures for the 3 different populations are within a factor of 2



Determining the lowest Points of Departure across the 7 bioactivity NAMs



HTTr

- Bifrost global POD (gene level) (for each cell line tested)
- BMDExpress2 Pathway level BMDL

CSP

- Bifrost global POD

IPP

- Bayesian modelled lowest IC50

ReproTracker

- Minimum POD from cytotoxicity or gene biomarker dose response (Lowest BMDL (down regulated, BMR=10%)

DevTox quick predict

- Minimum PoD frm devTox quickPredict cytotoxicity or development toxicity potential (dTP) dose response

H295R stereoidogenesis assay

- Mininum LOEC

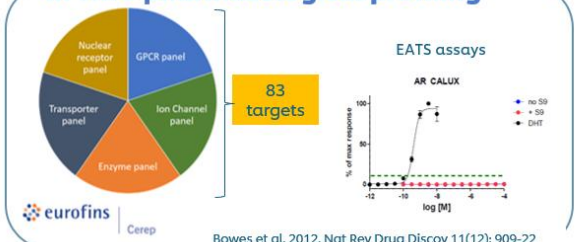
Screening CALUX assay (U2-OS ERα and AR)

- Mininum LOEC

Bioactivity exposure ratios

BIOACTIVITY

In vitro pharmacological profiling



Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22
Sonneveld et al. 2005. Toxciol Sci 83(1): 136-48

Developmental Assays

Induced pluripotent stem cell assays

ReproTracker

devTOX qP quickPREDICT

HTTr

IPP

Rajagopal et al. 2022. Front Toxicol. 4:838466

High-Throughput transcriptomics

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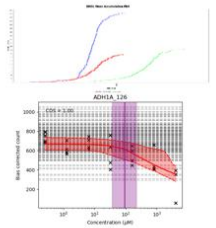

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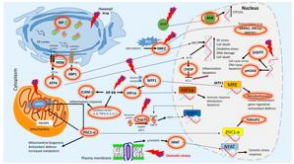

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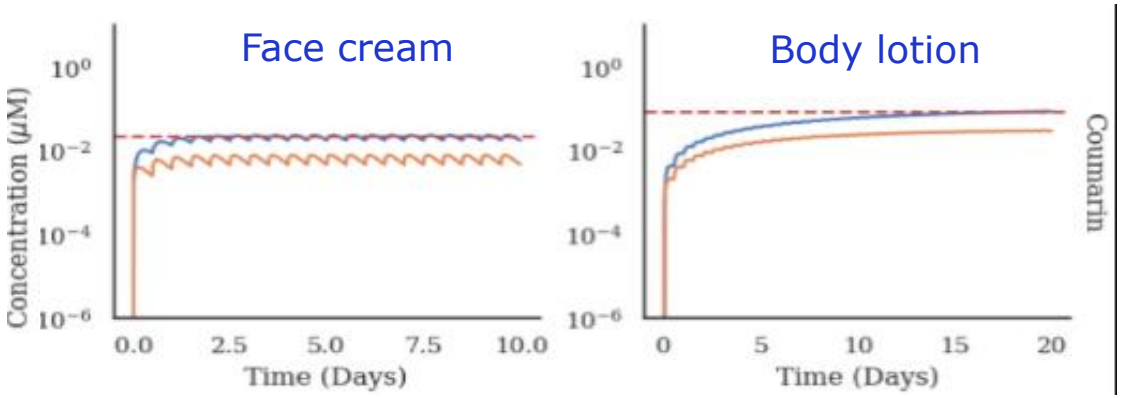
EXPOSURE



Identify lowest (most sensitive) point of departure, expressed in µM

Identify realistic worst-case plasma exposure (C_{max}) expressed as µM

$$\text{BIOACTIVITY EXPOSURE RATIO} = \frac{\text{BIOACTIVITY}}{\text{EXPOSURE}}$$

The larger the BER, the greater the confidence that bioactivity will not occur in exposed population

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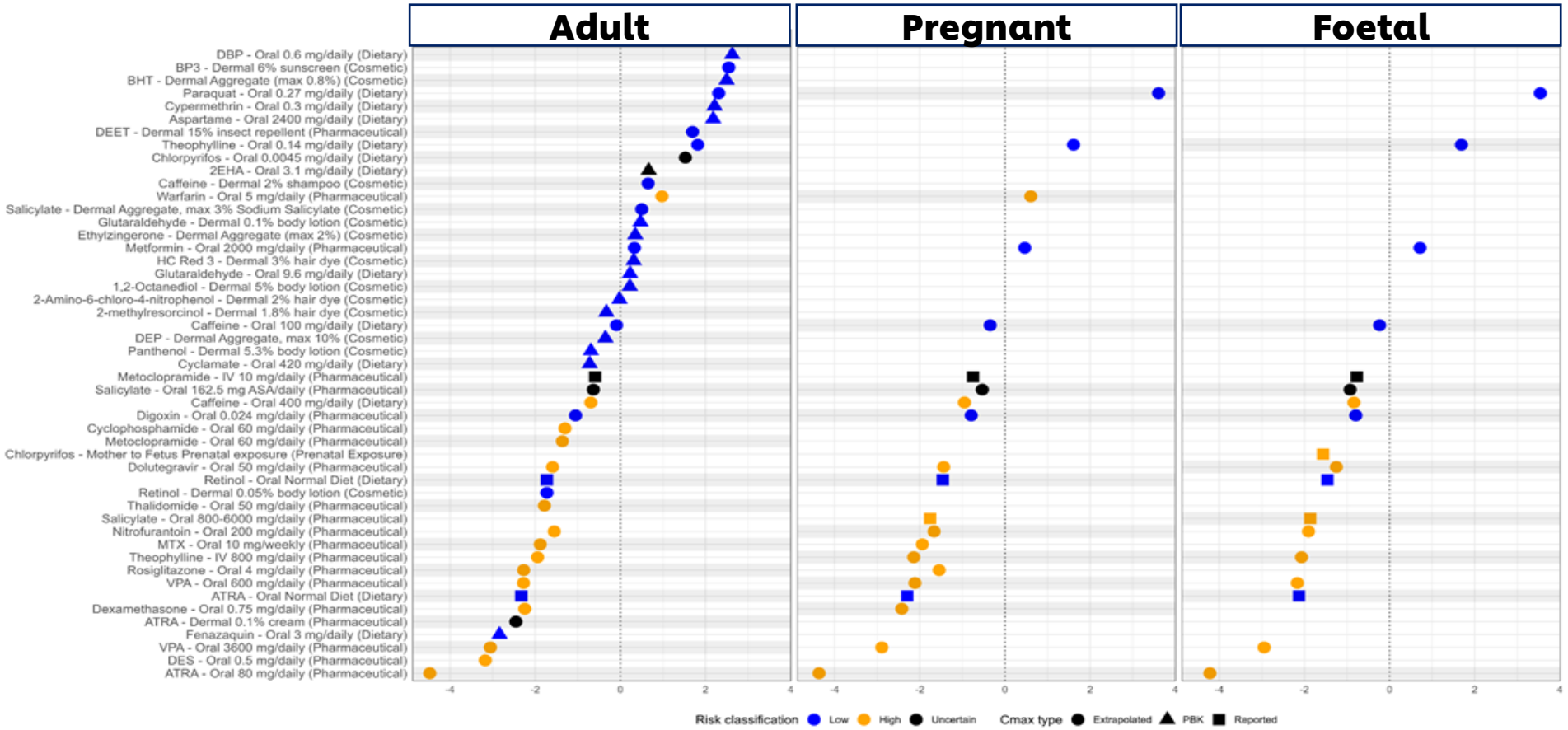
The DART framework is protective for most high-risk scenarios when using a BER threshold of 1



- 16 of the 17 high risk exposure scenarios, as determined by traditional risk assessment methods, are identified as uncertain risk in our NGRA approach (yellow, BER<1)
- 17 of the 27 low risk exposure scenarios are identified as well in the NGRA framework as low risk using our framework (blue, BER >1).

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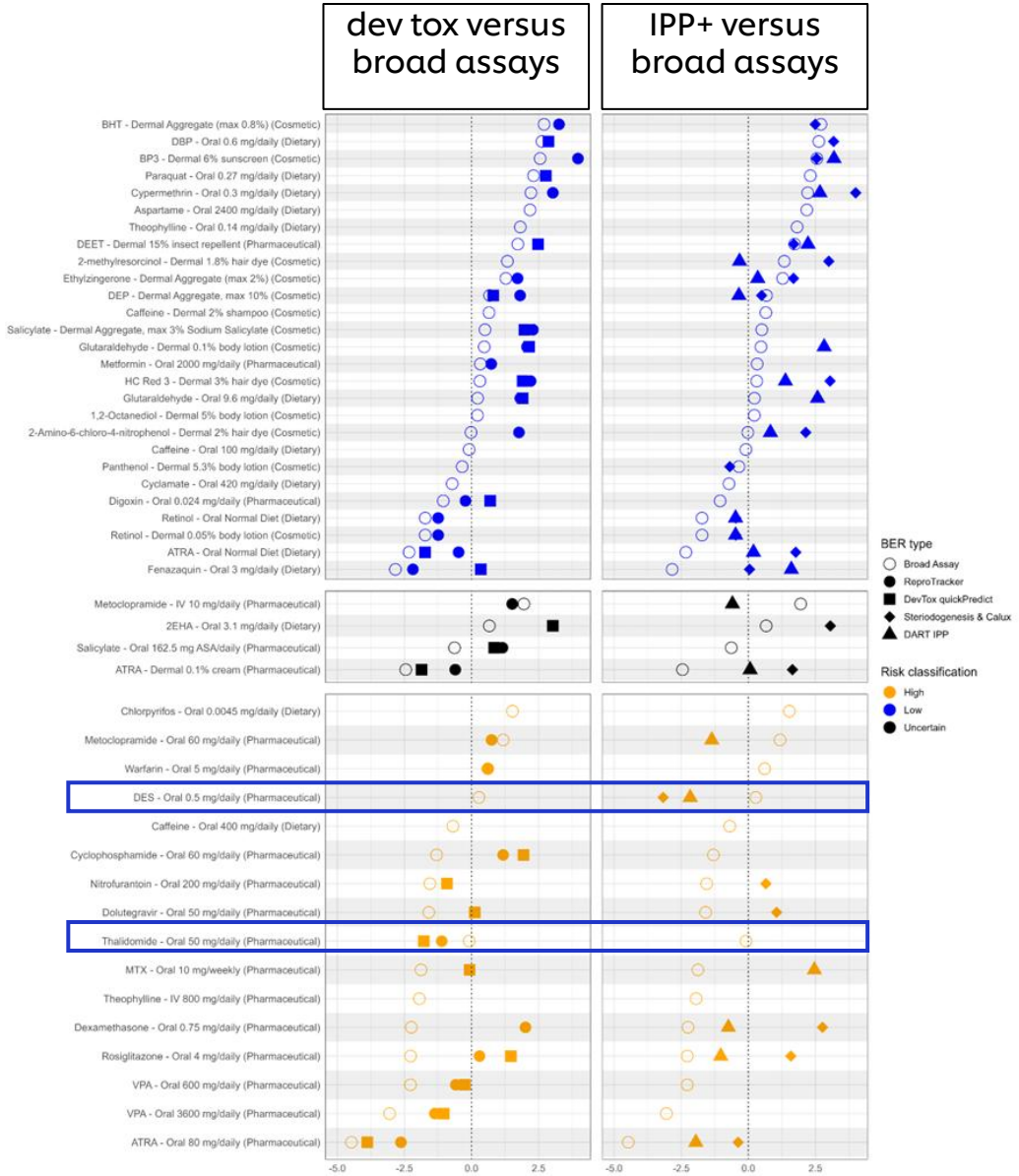
A combination of broad screening and DART targeted NAMs are needed to achieve protectiveness for DART.



- Most often broad screening tools (mainly HTTr) show lower PoDs/BERs.
- Only for thalidomide (dev tox) , DES (ER) and Metoclopramide (dopamine receptor D2) the relevant DART target shows lowest PoD.
- Most high-risk exposure scenarios show DART targeted NAMs with a BER<1
- PoDs from DART target NAMs can also be found for low-risk exposure scenarios with a BER>1

Conclusions

- We do not need to replicate animal studies to make decisions on systemic and DART safety without animals, if:
    - We use a tiered, exposure-led framework
    - We accept that our goal is to be protective rather than to predict pathologies
- This DART framework correctly identified 16/17 high DART risk exposure scenarios.
- BER is based on bioactivity – higher tier tools are required to characterise adversity.
- Protectiveness was achieved with a combination of broad and specific NAMs for DART.

Mueller et al., (2025).
https://www.frontiersin.org/journals/toxicology/articles/10.3389/ftox.2025.1602065/full

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Next steps

❑ Assay refinement/validation

- **ReproTracker®:** extended evaluation, include Osteoblast differentiation, Transferability/reproducibility study
- **HTTr reproducibility** pilot study in HepaRG cell model
- **devTOXqP** has an accepted letter of intent with the FDA's CDER Biomarker Qualification Program (BQP) to qualify the assay as a safety biomarker for detecting human developmental toxicity potential in vitro at the nonclinical stage

❑ Defining a BER threshold

- what 'bioactivity exposure ratio' is sufficient between the *in vitro* point of departure and the predicted or measured plasma exposure level to assure human safety for DART?

❑ Expanding the chemical dataset

- Test with chemicals with different modes of action is needed to build scientific confidence and to fill existing gaps

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ACKNOWLEDGMENTS

toxys

Giel Hendriks
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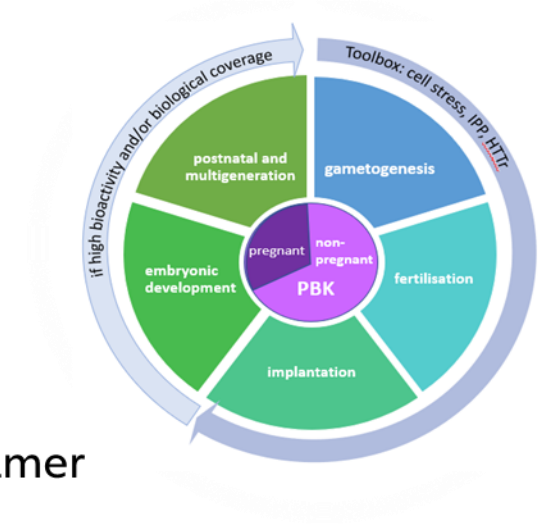
Luke Flatt
Marleen Feliksik



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Bio Spyder

Stemina BIOMARKER DISCOVERY

Jessica Palmer

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Iris Muller, Paul Carmichael, Leonardo Contreas, Renato Ivan de Ávila, Matt Dent, Jade Houghton, Predrag Kukic, Alberto Locca, Sophie Malcomber, Alistair Middleton, Beate Nicol, Magdalena Sawicka, Sandrine Spriggs, Gopal Pawar, Claire Peart, Katarzyna Pryzbylak, Andy White, Katy Wilson, Kathryn Wolton

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