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

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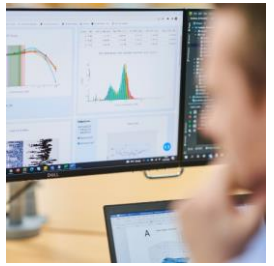
SOT 2025, Orlando, Florida

SERS
Safety, Environmental & Regulatory Science

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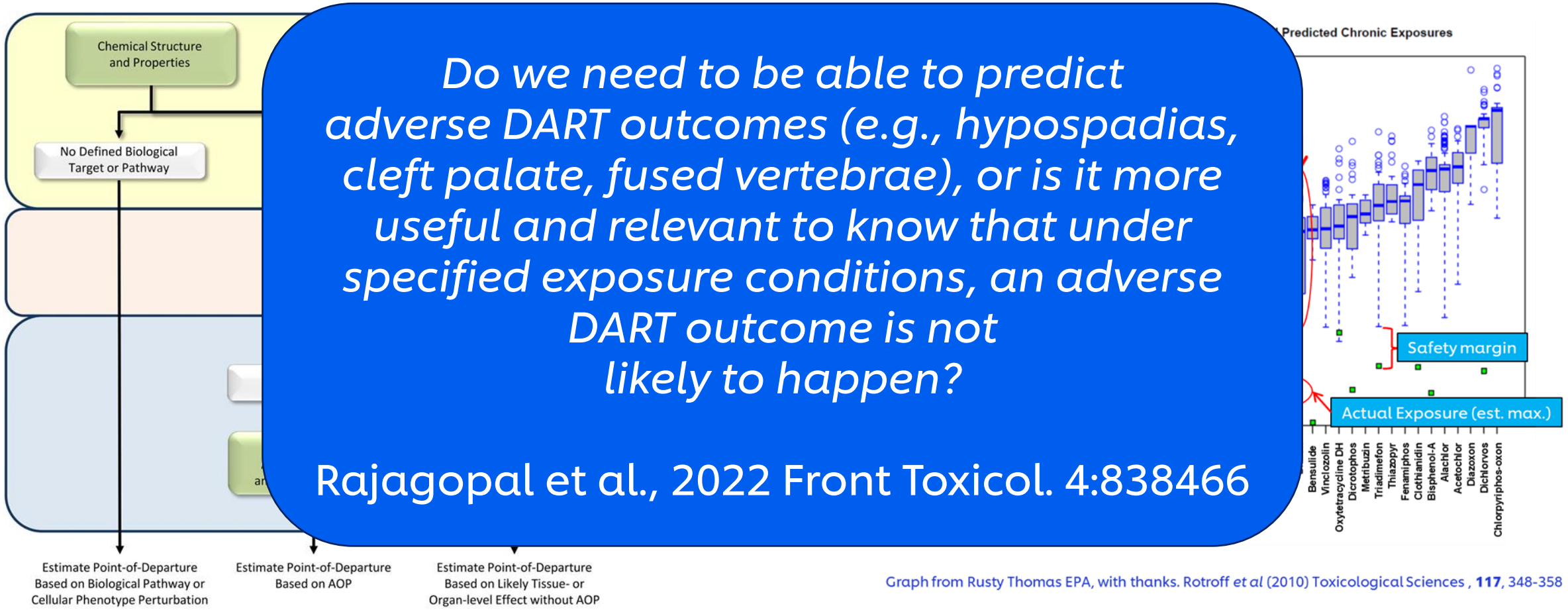
Conflict of Interest Statement

- Dr Maria Baltazar is an employee of Unilever (www.unilever.com)

Paradigm shift requires a different way of approach 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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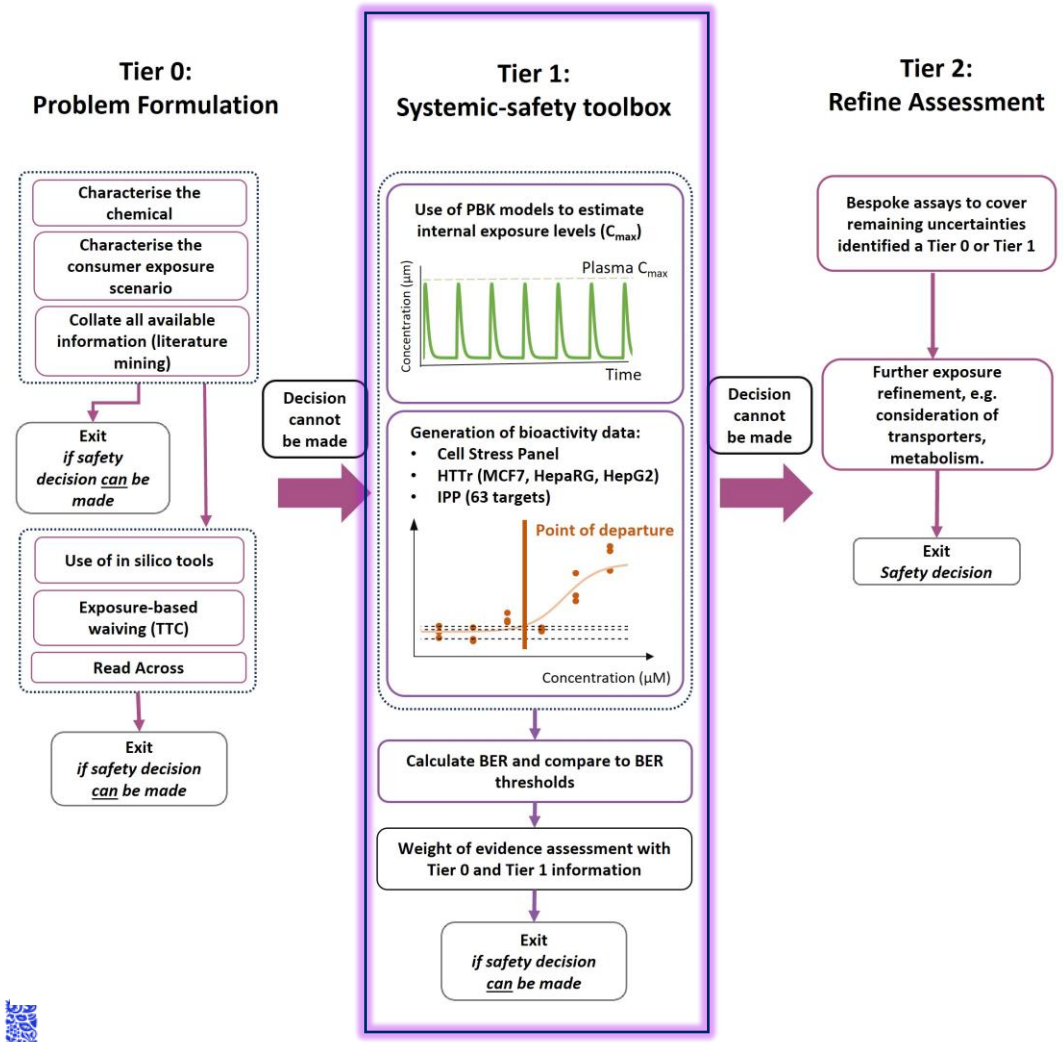
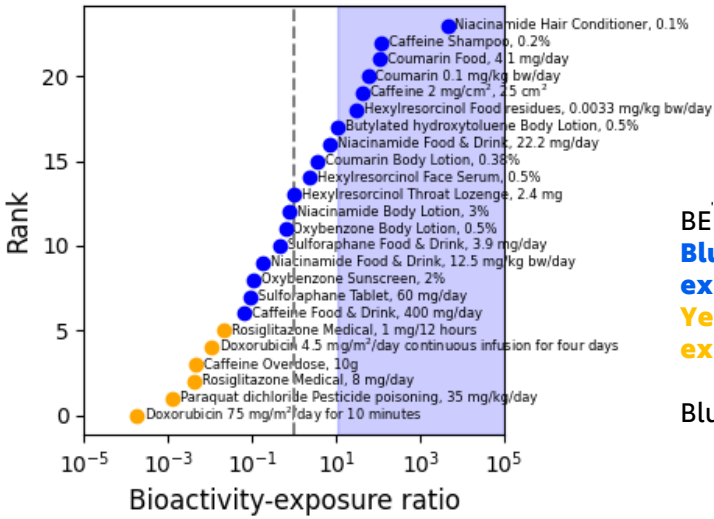
EPA United States Environmental Protection Agency

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

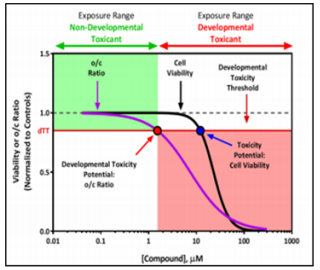
Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159; Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Systemic toolbox biological coverage identified needs for 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).

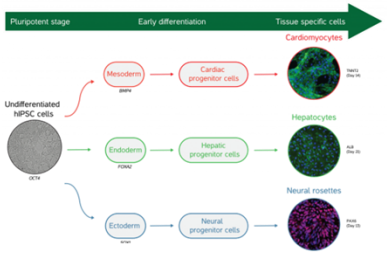
Stemina BIOMARKER DISCOVERY



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

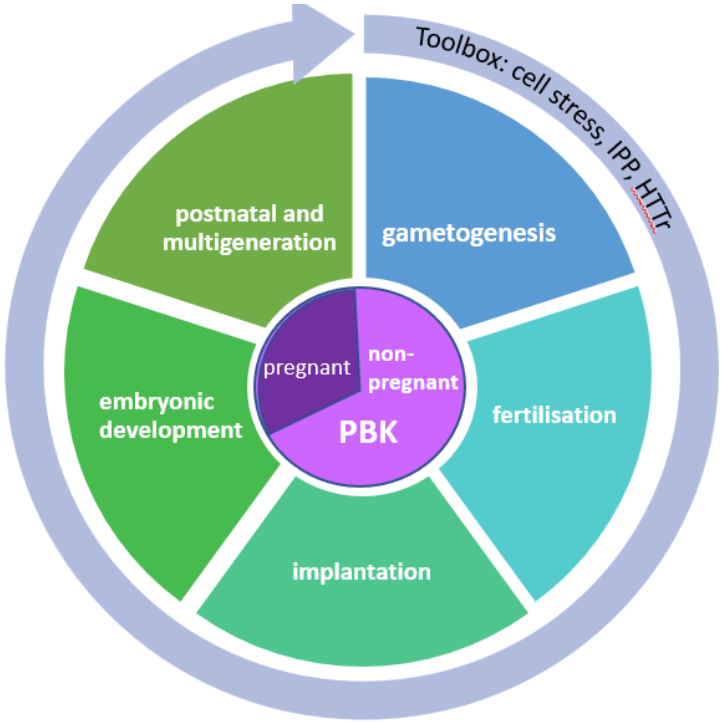
ReproTracker®

- 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.



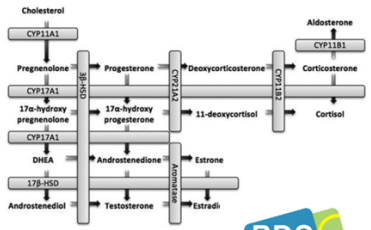
toxys

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



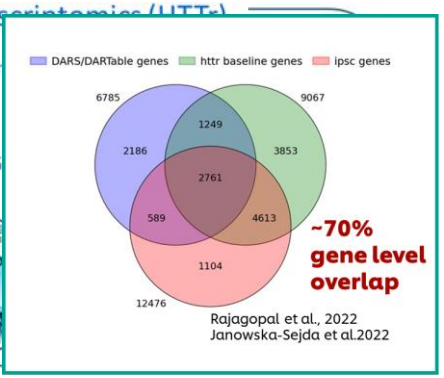
BDS

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

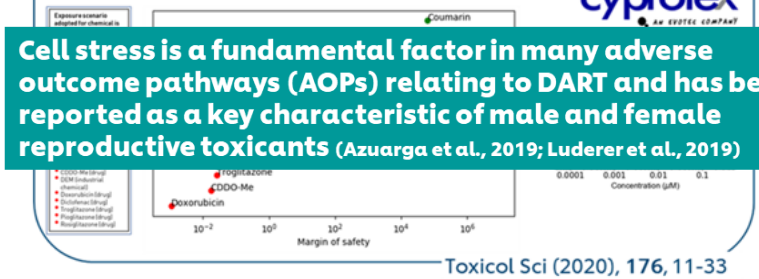


Rajagopal et al., 2022
Janowska-Sejda et al.2022

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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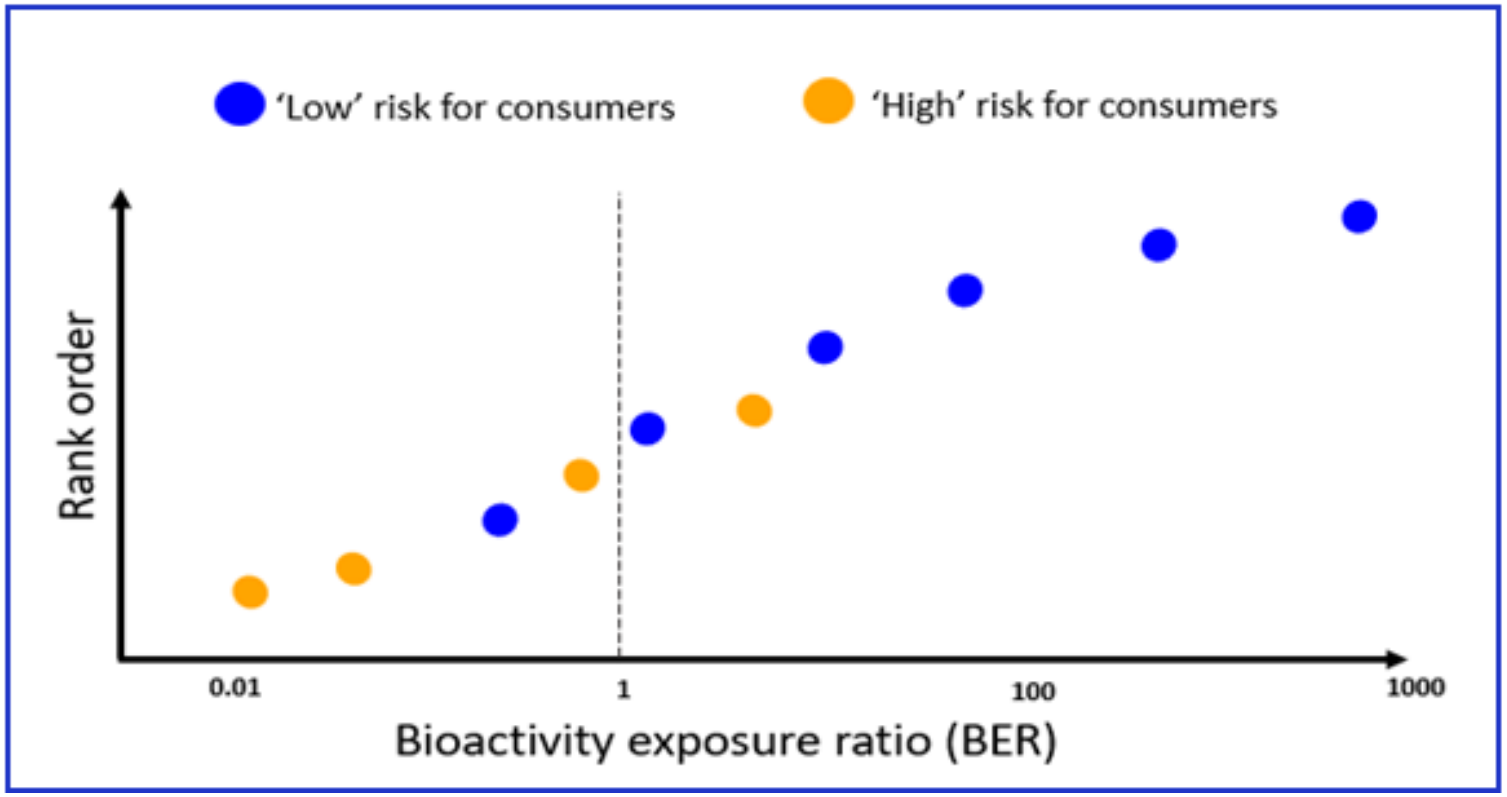
Differentiating High and Low Risk Chemical Exposure Scenarios Using broad and specific DART NAMs and Internal Exposure

Bioactivity exposure ratio (BER):

the ratio between the *in vitro* PoD and predicted human exposure



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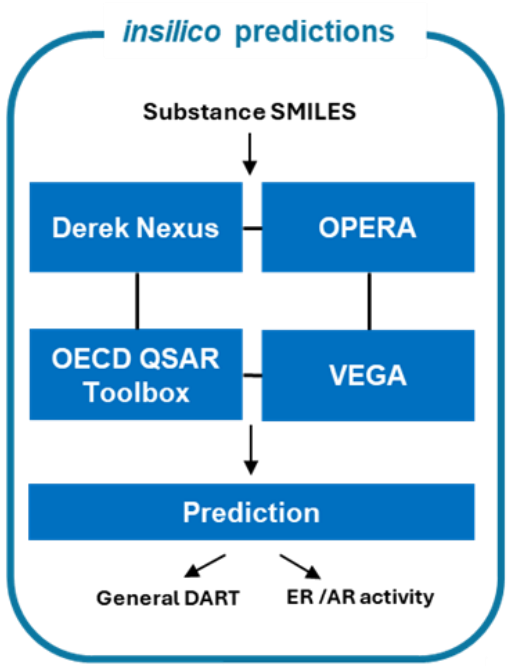
Development and evaluation of a Tier 1 toolbox for DART

Chemical structure



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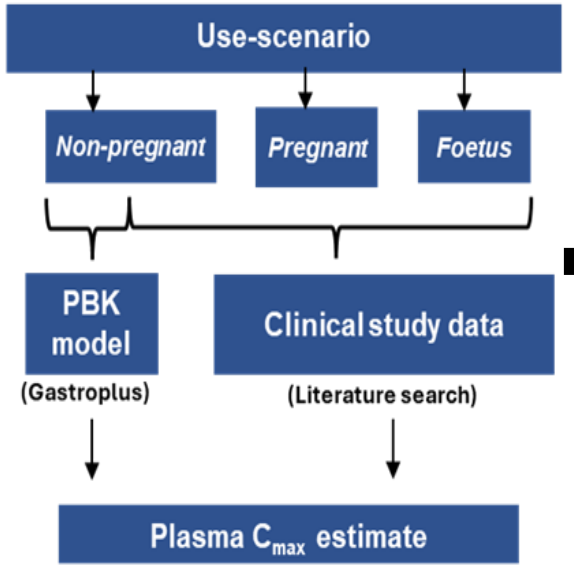
Human Chemical Exposure



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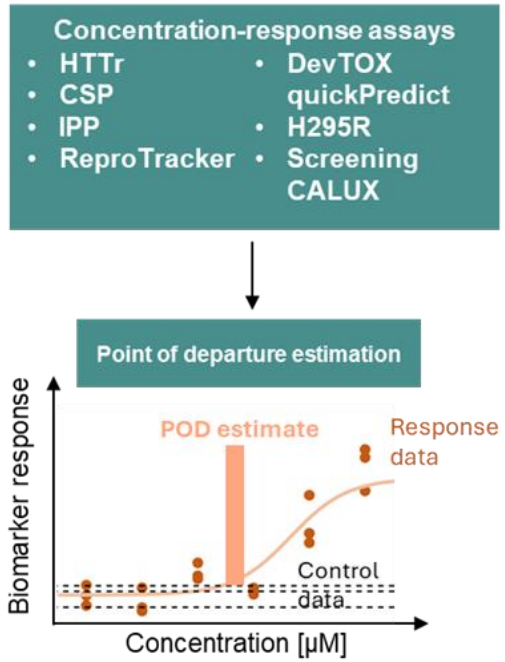
Chemical Bioactivity



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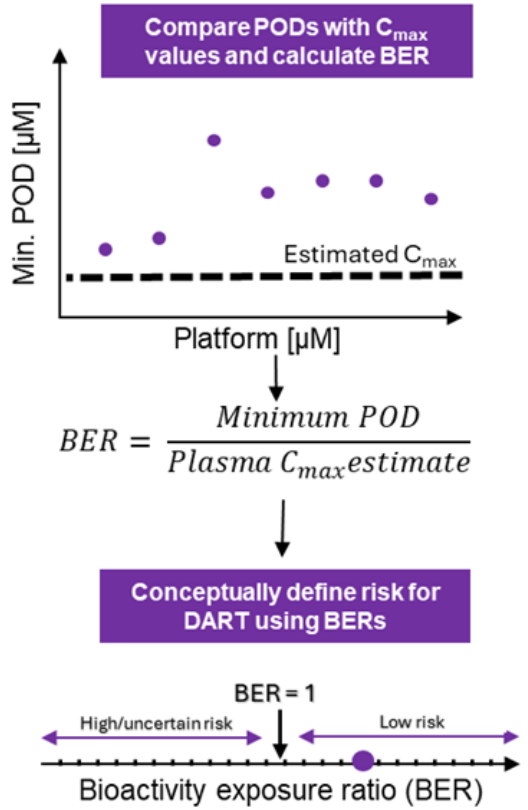
Bioactivity Exposure Ratio (BER)



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Evaluation of the performance of the DART framework: 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**

Example:

- **Thalidomide oral exposure 50 mg/day- High risk**
- **Panthenol- 5.3% in body lotion – Low risk**

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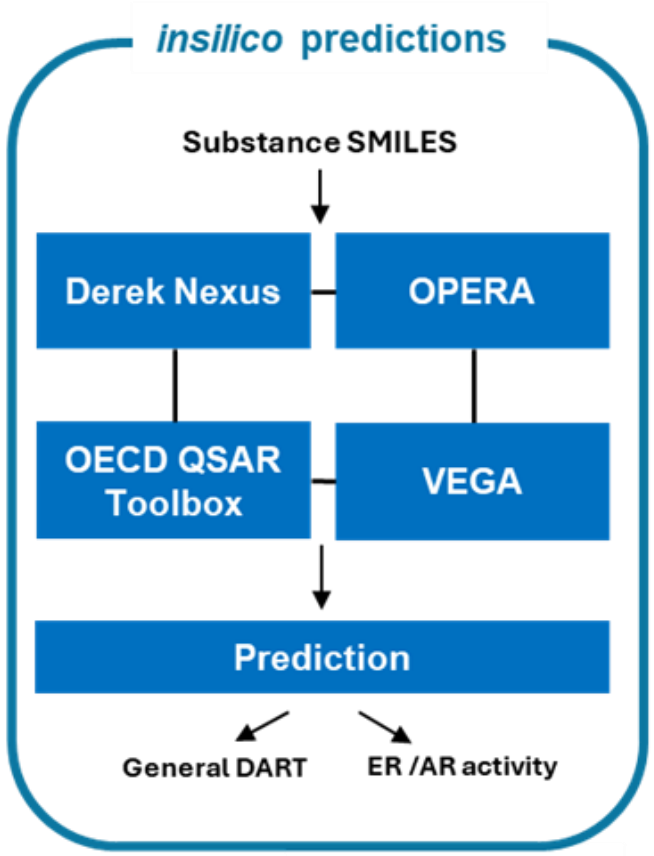
Selection of in silico models for DART Framework



1017 *in vivo* Dev
1376 *in vivo* Repro
1036 *in vitro* Dev

Evaluation

In silico models in DART Framework

1. **Derek Nexus**
   - I. 17 endpoints relevant to DART
   - II. 34 endpoints relevant to DART & systemic tox
2. **OPERA:**
   - I. OPERA_CoMPARA_Androgen_Receptor
   - II. OPERA CERAPP Estrogen_Receptor
3. **OECD QSAR Toolbox**
   - I. DART scheme (P&G decision tree)
4. **VEGA**
   - I. VEGA_ ANDROGEN_COMPARA
   - II. VEGA_ESTROGEN_CERAPP
   - III. VEGA_DEVTOX_PG

general DART in silico models
MoA DART in silico models

In silico results from DART general toxicity models

20 tox & 13 non-tox	TP	FN	TN	FP	SE (%)	SPE (%)
Derek Nexus (34 endpoint)	19	1	4	9	95.00	30.77
Derek Nexus (17 endpoints)	18	2	10	3	90.00	76.92
OECD Toolbox DART scheme	13	5	10	3	72.22	76.92
VEGA DevTox	15	5	9	4	75.00	69.23

	True call	*In silico* prediction			
Chemical	DART Hazard	DEREK Nexus Endpoints (34)	DEREK Nexus Endpoints (17)	VEGA_DEVTOX_PG	OECD Toolbox DART scheme
1,2-Octanediol					
2-Amino-6-chloro-4-nitrophenol					
2EHA					
2-methylresorcinol					
ATRA					
BHT					
BP3					
Caffeine					
Chlorpyrifos					
Cyclophosphamide monohydrate					
Cypermethrin					
DBP					
DEP					
DES					
Dexamethasone					
Digoxin					
Dolutegravir					
Ethylzingerone					
Fenazaquin					
Glutaraldehyde					
HC Red 3					
Metformin					
Metoclopramide					
MTX					
Panthenol					
Paraquat					
Retinol					
Rosiglitazone					
Sodium salicylate					
Thalidomide					
Theophylline					
VPA					
Warfarin					

In vitro & in silico results from MoA (ER &AR) models

	True call				*In silico* prediction							
Chemical	ER Agonist	ER Antag	AR Agonist	AR Antag	VEGA ER Binding	VEGA AR Binding	OPERA ER Agonist	OPERA ER Antag	OPERA ER Binding	OPERA AR Agonist	OPERA AR Antag	OPERA AR Binding
2EHA												
DEP												
Theophylline												
DES												
ATRA												
Retinol												
DBP												
MTX												
Caffeine												
Thalidomide												
VPA												
Cyclophosphamide												
Glutaraldehyde												
Warfarin												
BP3												
Cypermethrin												
Chlorpyrifos												
DEET												
Nitrofurantoin												
BHT												
Aspartame												
Digoxin												

extracted from [CompTox Chemicals Dashboard (epa.gov)]

In silico models are a conservative tool for detecting ER and AR activity. There are more positive results from the predictions when comparing to the output of the ER and AR pathway models (Judson et al., 2017 and Judson et al., 2020) which provide a consensus on activity based on multiple in vitro data points.

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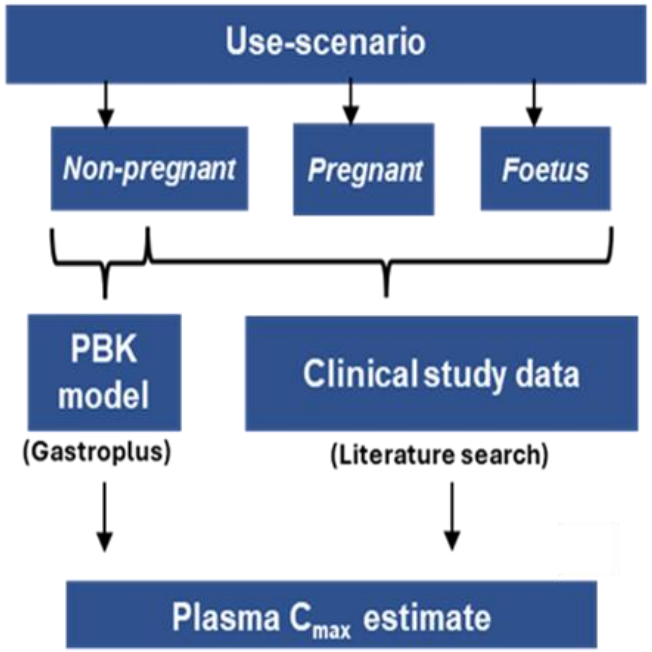
DART exposure strategy for NGRA - Modelling of DART relevant exposures

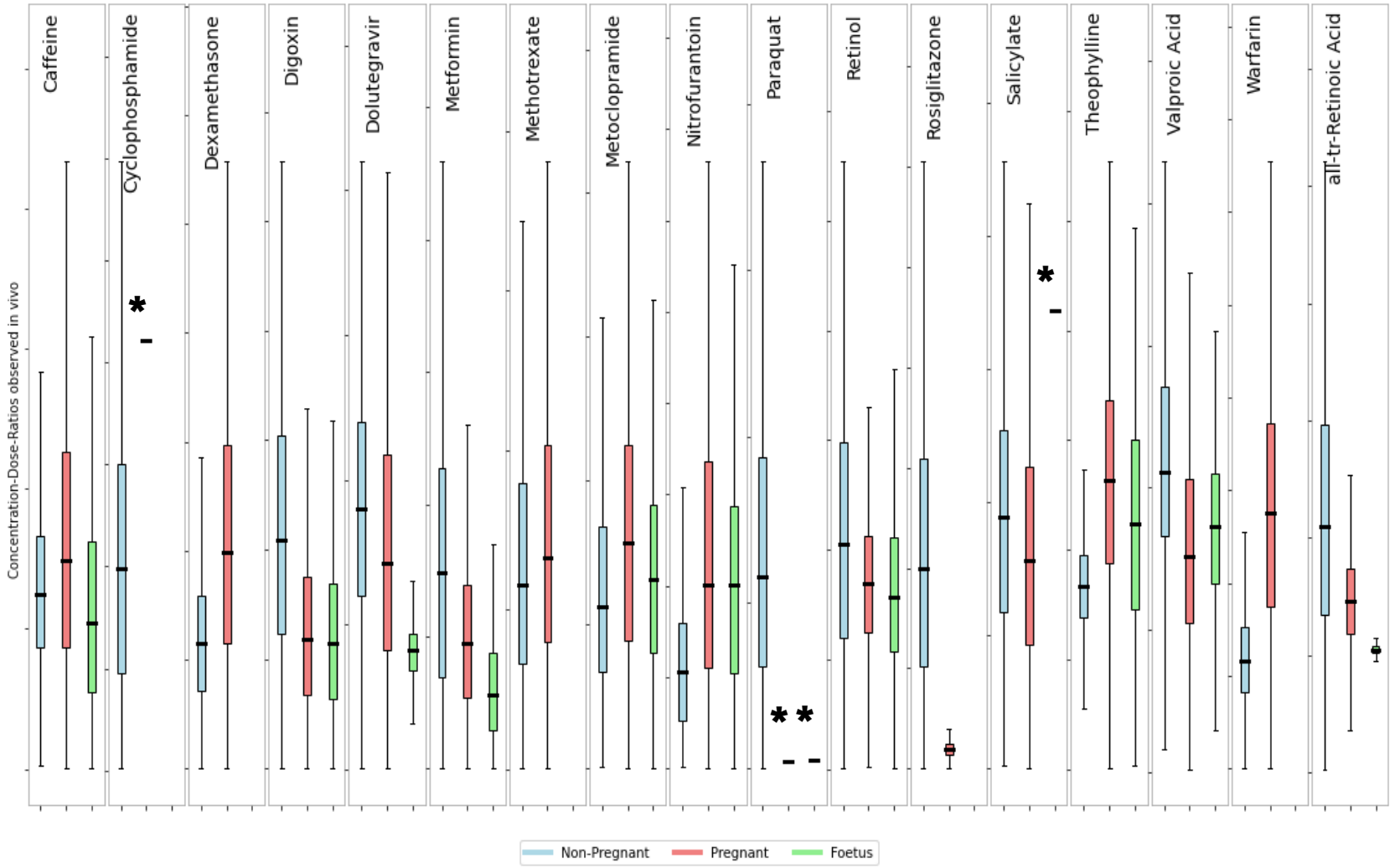
Human Chemical Exposure



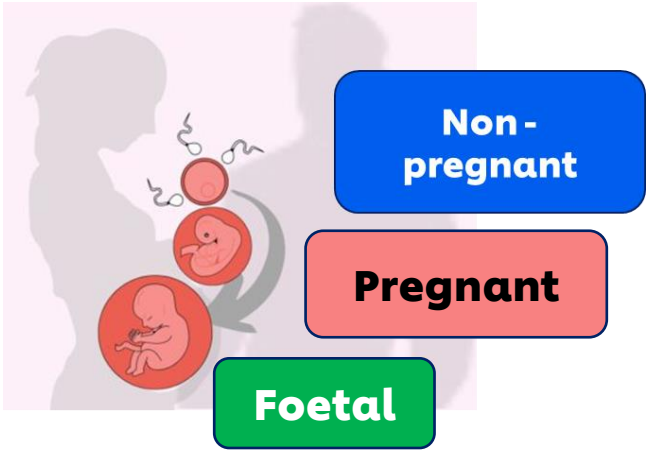
Data curation

- 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)
- Invitro/ex vivo placental transfer studies
- Generic or pregnancy PBPK models

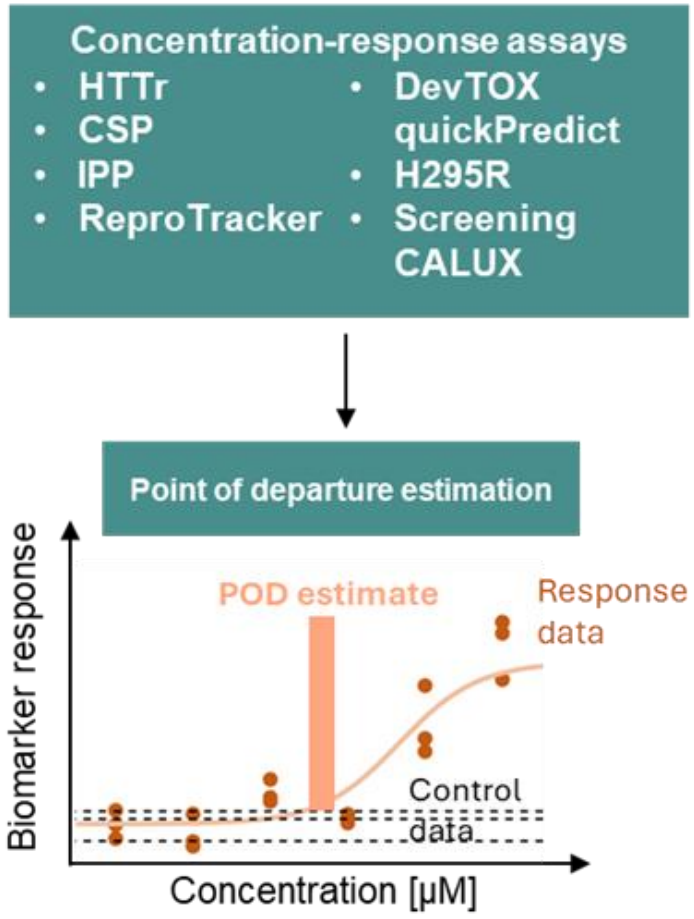
For most chemicals, internal exposure estimates for a general population cover the exposures in the pregnant and foetal sub-group



- 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

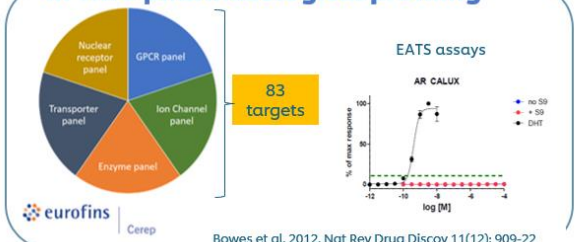
H295R stereoidogenesis assay
- Mininum LOEC

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

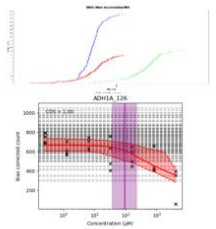
HTTr

IPP

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

High-Throughput transcriptomics

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- HepG2, MCF7, HepaRG cells
- 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

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

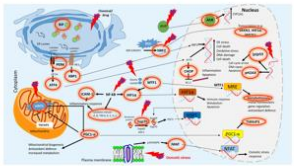
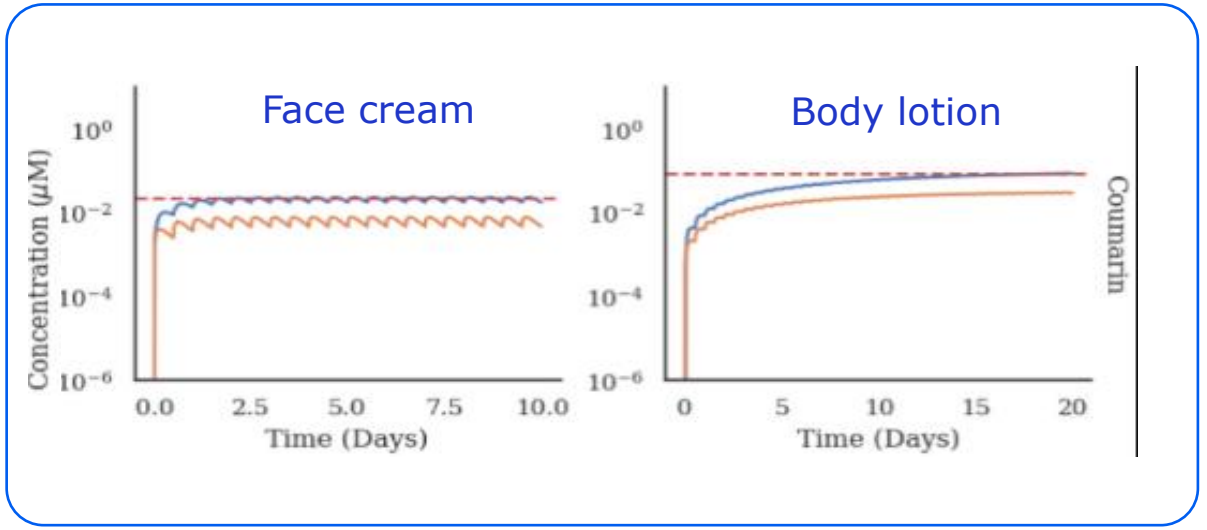


Image kindly provided by Paul Walker (Cyprotex)

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

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

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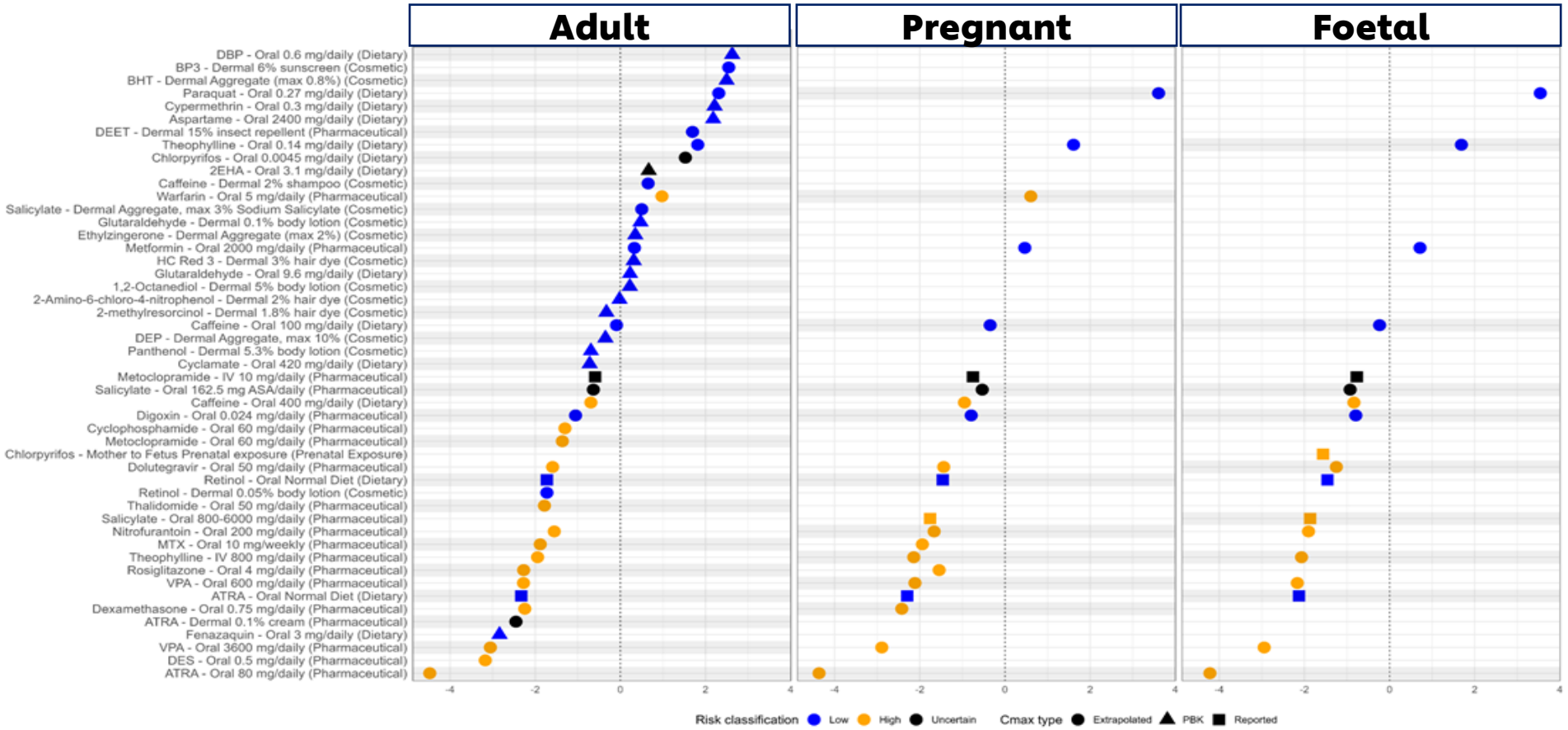
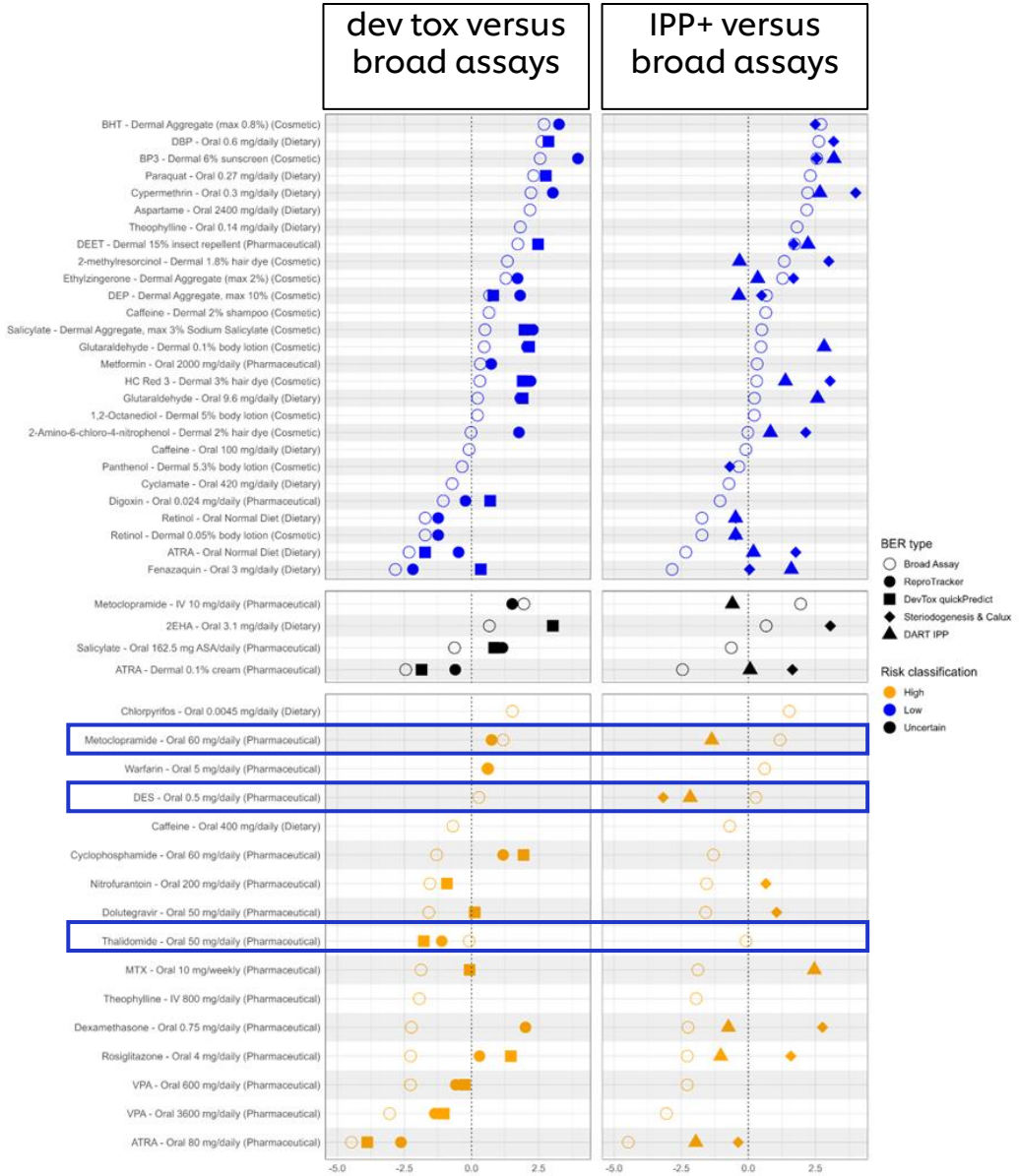
The DART framework is protective for most high-risk scenarios when using a BER threshold of 1



- 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).

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
    - We use our human biology knowledge to develop smart battery of assays
- 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.
- In silico tools can flag potential DART related concerns (~ 70% accuracy) and be used to direct the testing strategy.

Next steps

❑ Assay refinement/validation

- **ReproTracker®:** extended evaluation, include Osteoblast differentiation, testing 80 compounds, and conducting a Transferability and reproducibility study between Unilever and Toxys
- **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

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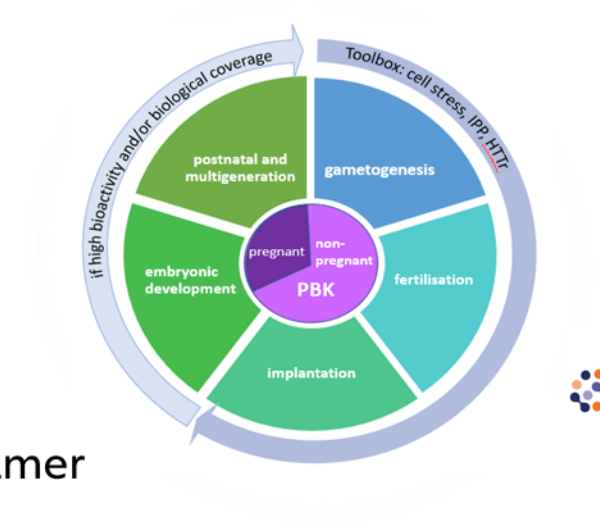

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Bio Spyder

Stemina BIOMARKER DISCOVERY
Jessica Palmer

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