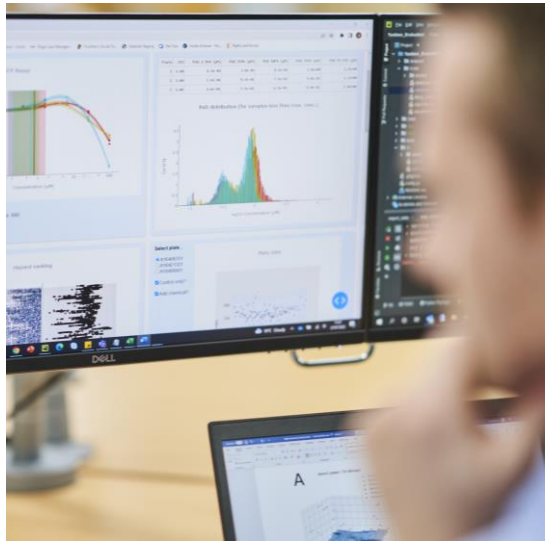
Practical Application of NAMs in DART Testing



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Outline

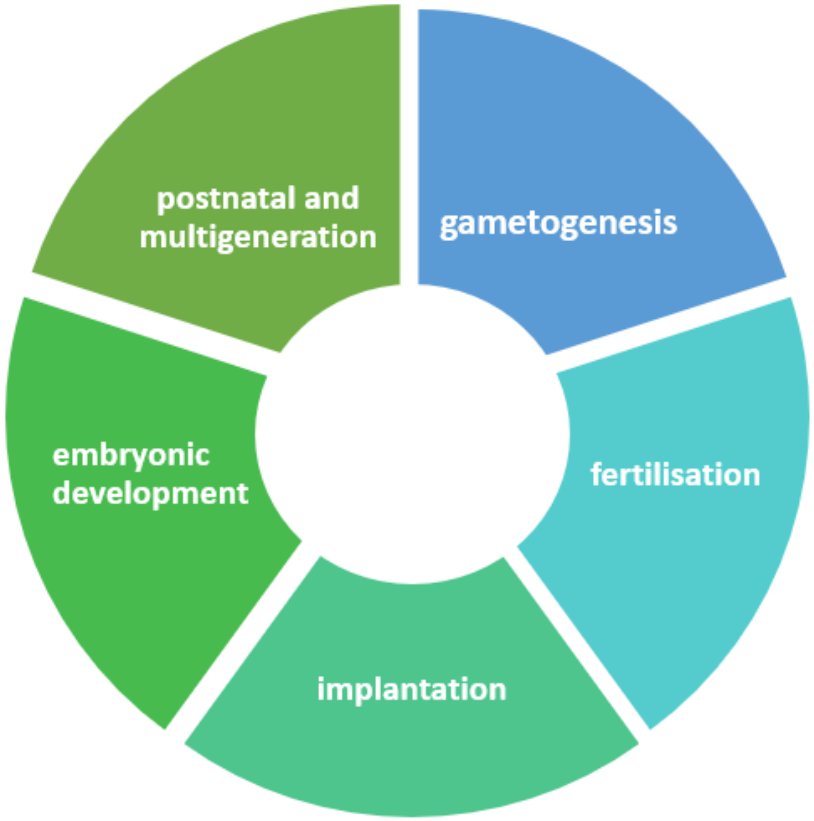
- **Overview of Unilever's NGRA Framework for DART testing**
- **Biological coverage of the NGRA Framework for DART testing**
- **Case studies / fit for purpose validation, next steps**

A paradigm shift is underway as use of non-animal safety science increases & safety assessment frameworks evolve to embed NGRA

Opportunities:

- Human-relevant
- Safe and sustainable chemicals by design
- High throughput

...



DART endpoint

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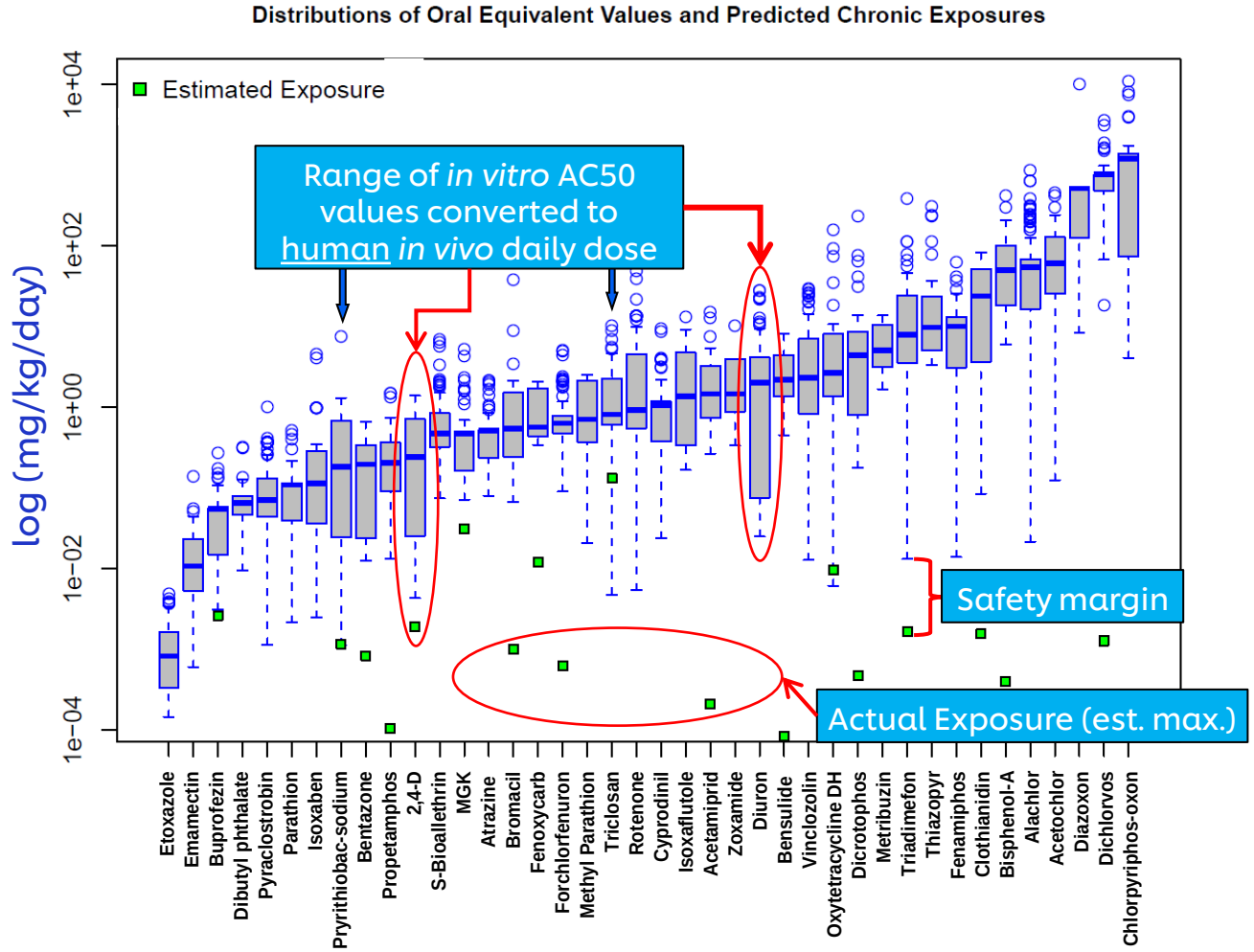
Unilever's approach: use of 21st century science to assure safety



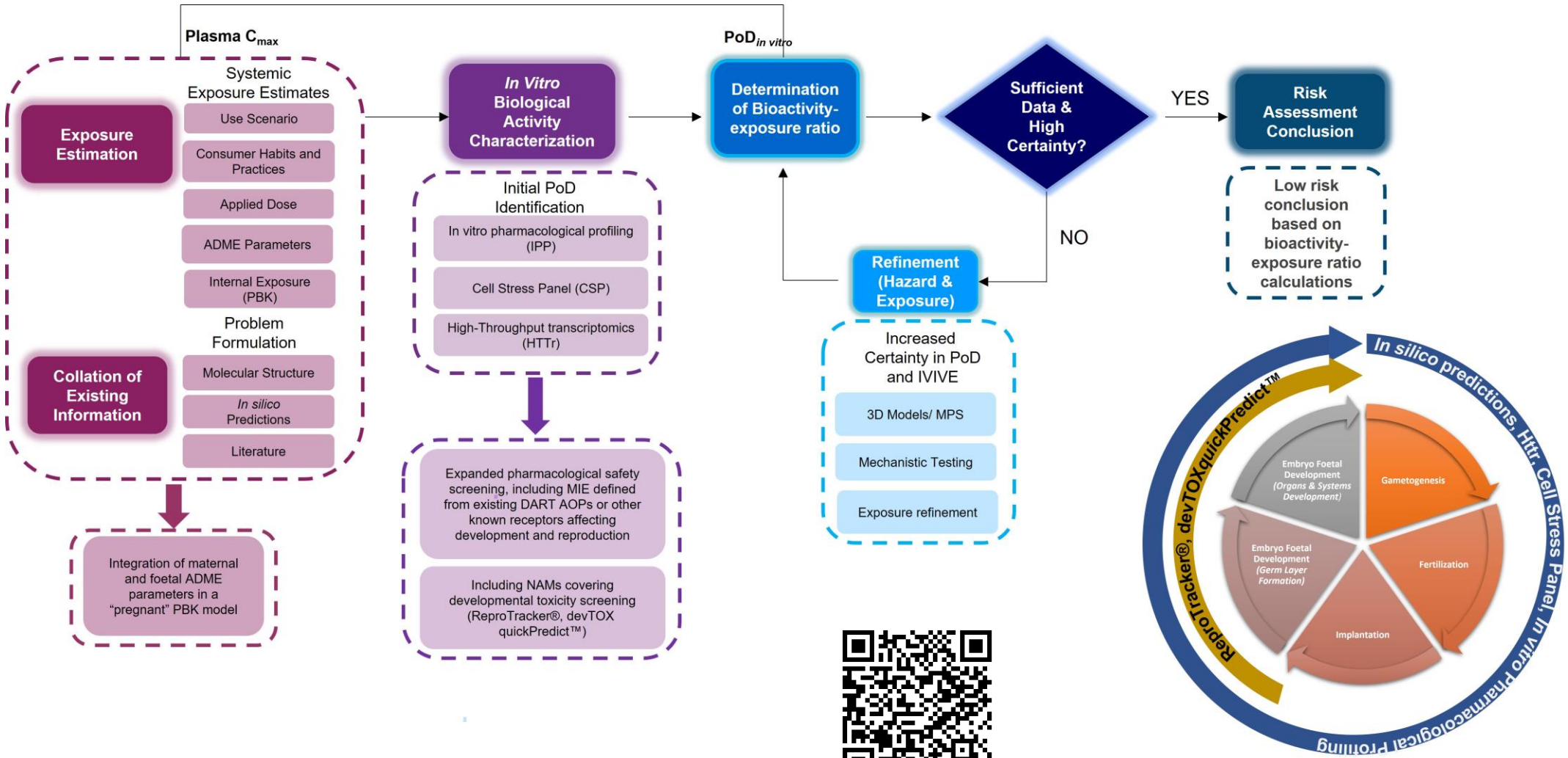
NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing**

The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

At no point does NGRA attempt to predict the results of high dose toxicology studies in animals.

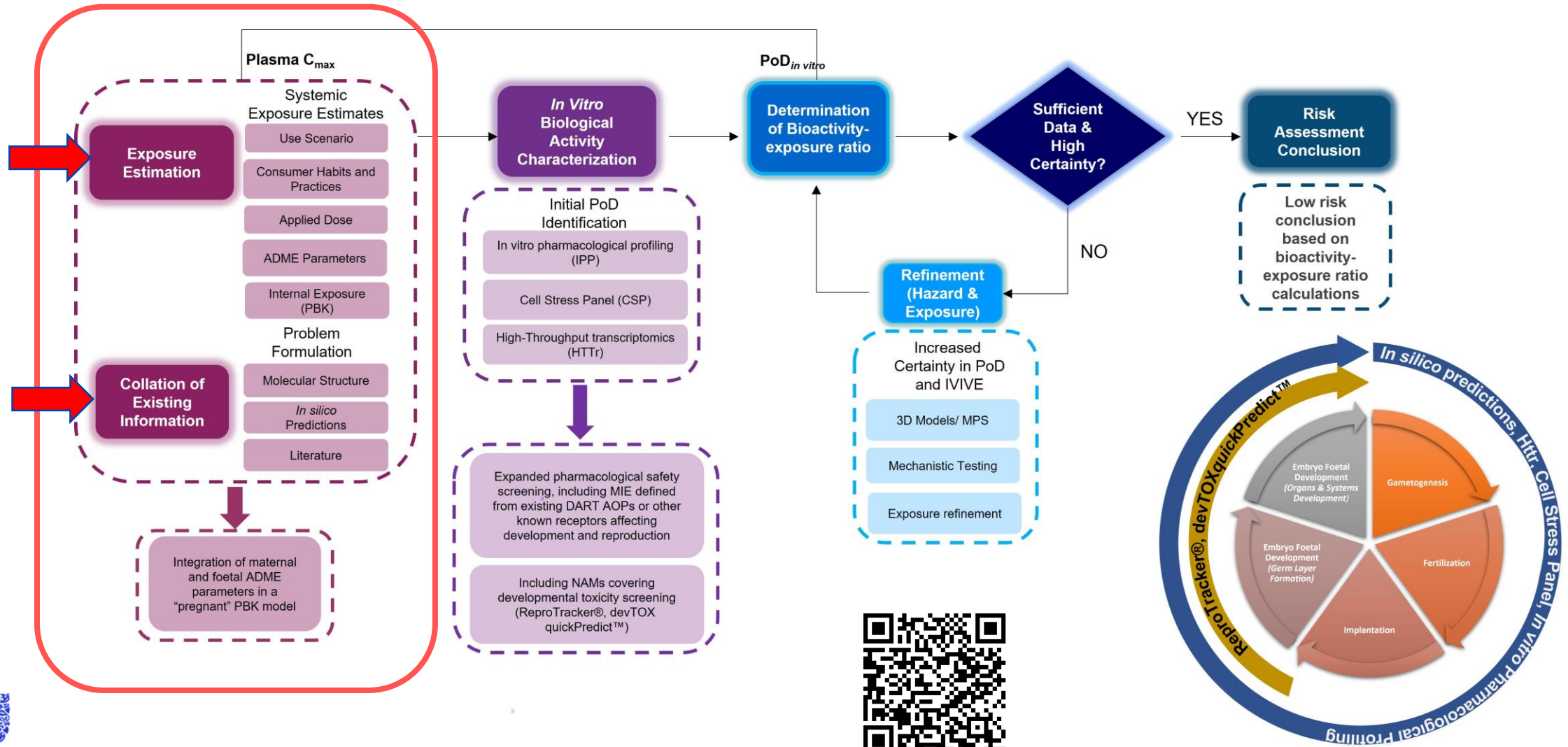
Graph from Rusty Thomas EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

NGRA Framework for DART – tiered approach



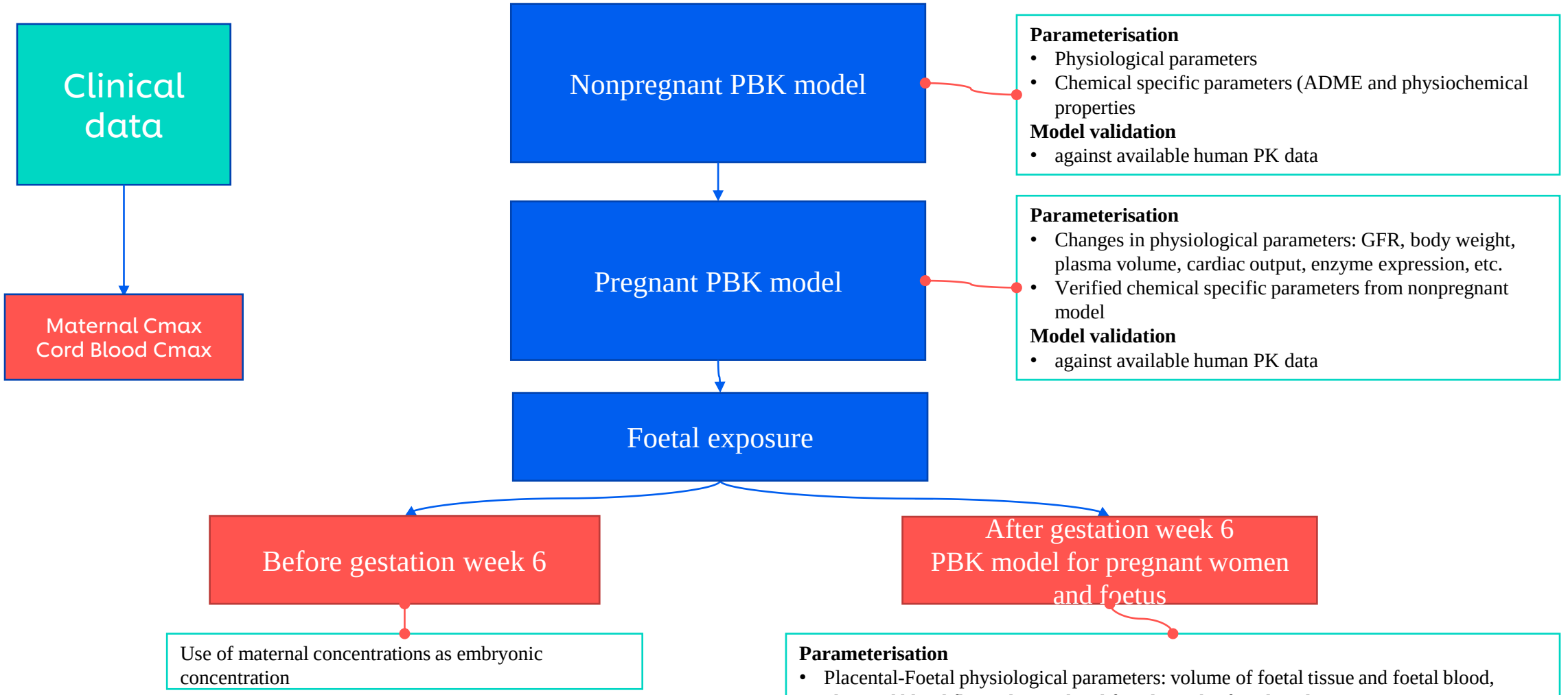
Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – exposure module



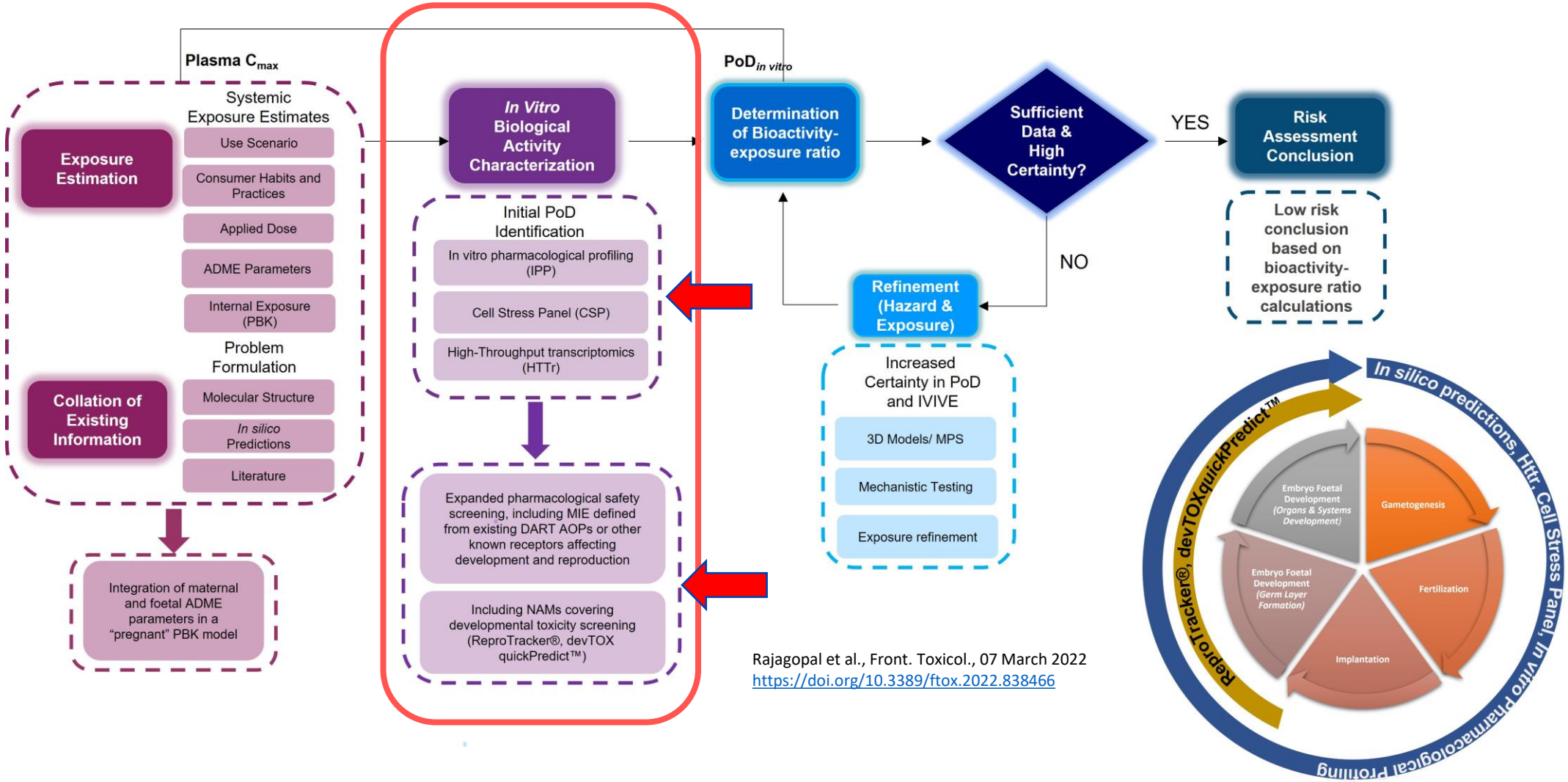
Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – exposure module (see P08-18 – Gopal Pawar)



Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – bioactivity module



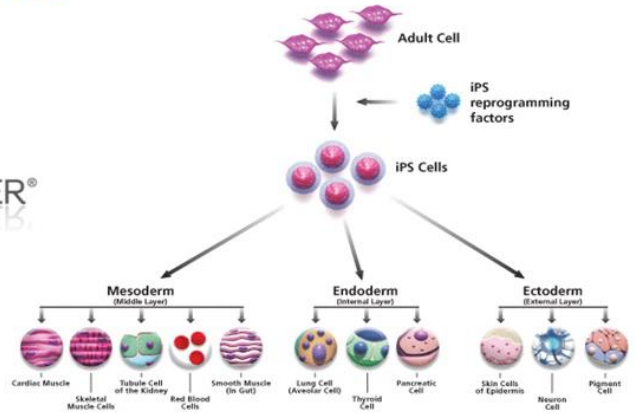
Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

NGRA Framework for DART – bioactivity module

iPSC based tools

devTOX qP quickPREDICT

REPROTRACKER®

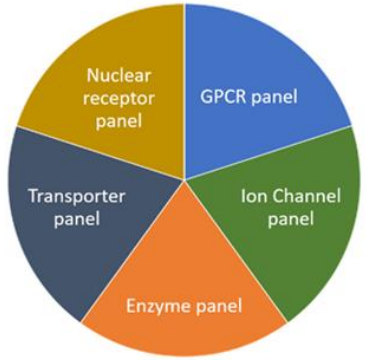


Toxicology in Vitro (2020), **63**, 104746

In vitro Pharmacological Profiling (IPP)


PERSPECTIVES

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

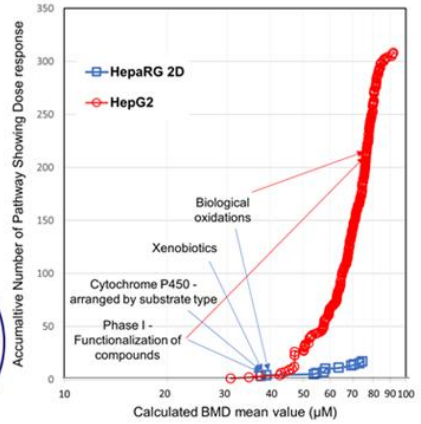


eurofins | Cerep

High-throughput Transcriptomics (HTTr)

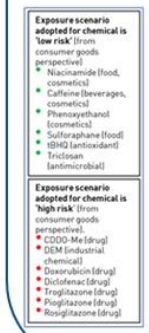
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

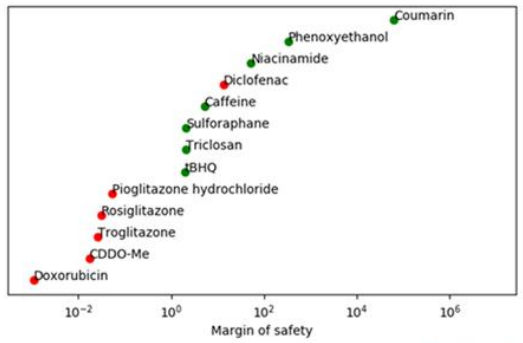
BMDExpress 2



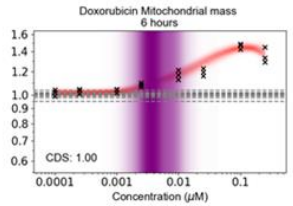
Cell Stress Panel (CSP)

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





cyprotex AN EVOTEC COMPANY



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

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NGRA Framework for DART – Scientific and Technical challenges

- Metabolic capacity of the framework (cell models, MPS, alginate technology, etc.)
- Short duration exposures and extrapolation to chronic effects
- Complex data interpretation and uncertainty analysis
- Spatio-temporal complexity of developmental and reproductive processes
- Coverage of important cellular and intercellular processes
- Chemical domain of applicability / case studies – need for a flexible and fit for purpose validation

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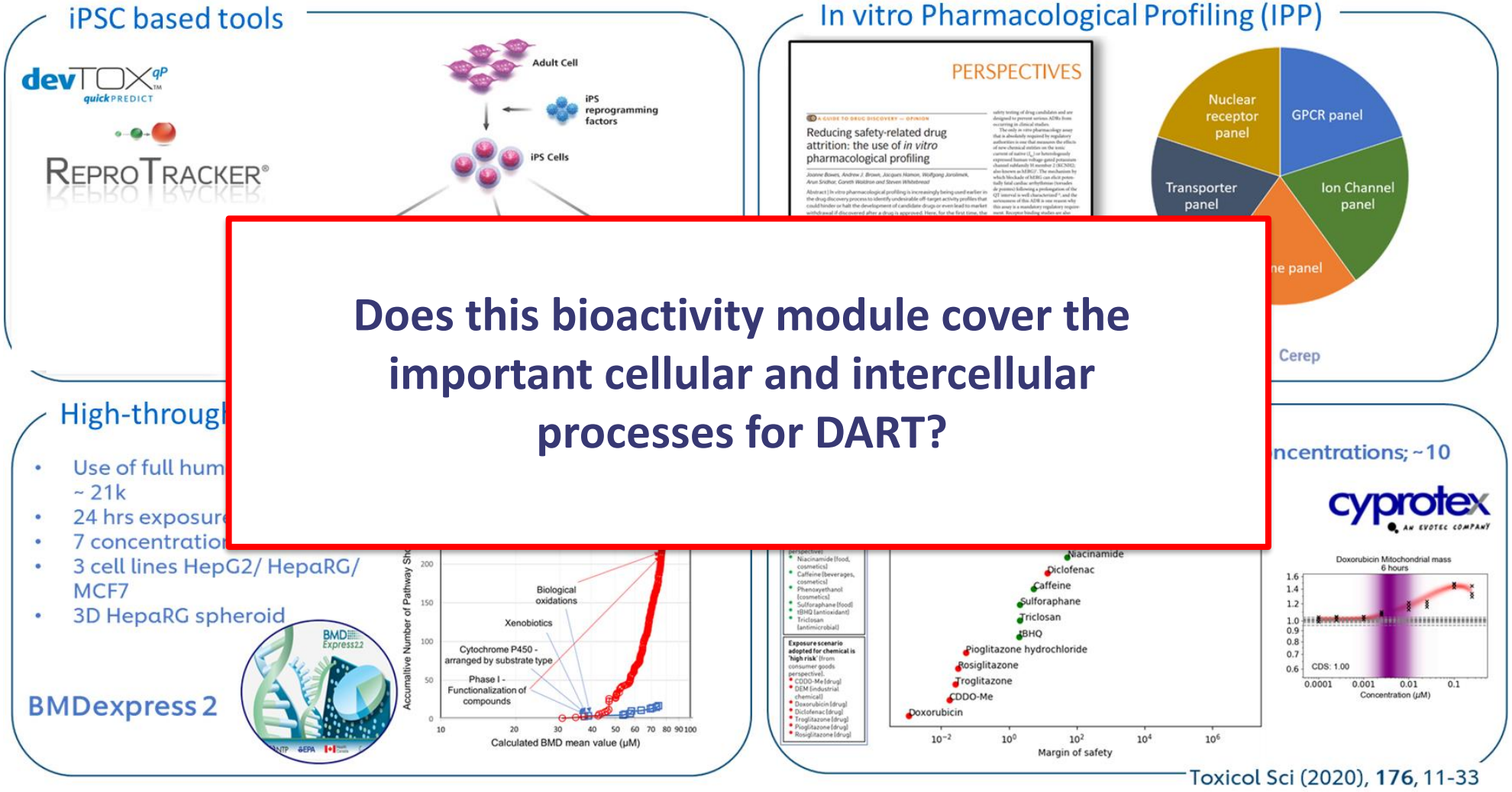
Coverage of important cellular and intercellular processes for DART

Does this bioactivity module cover the important cellular and intercellular processes for DART?



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

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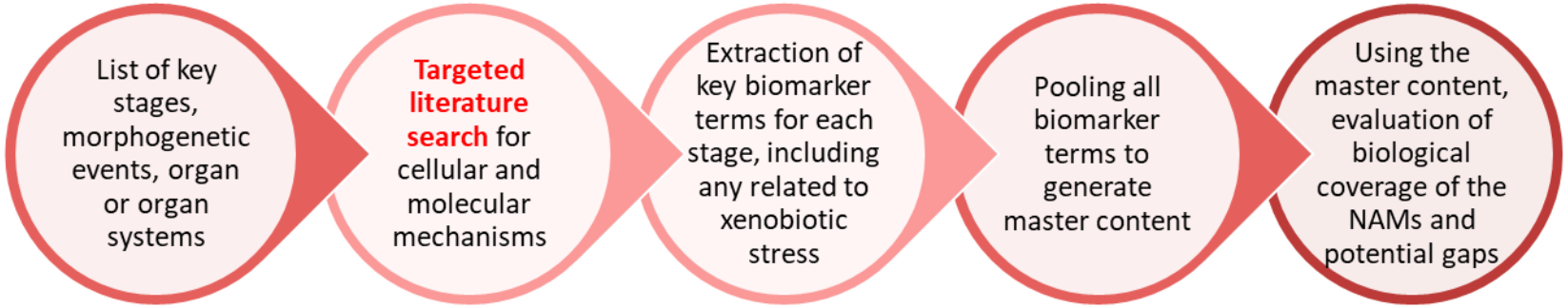
Mining of important DART biomarkers using Literature Search

- Morphological and physiological processes are underpinned by cellular events
- These cellular events in turn are orchestrated by molecular signalling events
- Hypothesis : Gathering the cellular and molecular information pertaining to embryonic development is a useful approach for developing a **master list of biological markers of significance**



Biological markers:

- **3,551 genes**
- **474 biological processes**
- **338 miRNAs**

Query run: ("CNS") AND (embryonic development OR fetal development) AND (cell physiology OR nervous system physiology) OR (signalling OR pathway OR gene OR protein) AND (human OR mammalian) NOT (infections)

34,308 articles on key stages and morphogenetic events

69,299 articles on organs and organ systems development

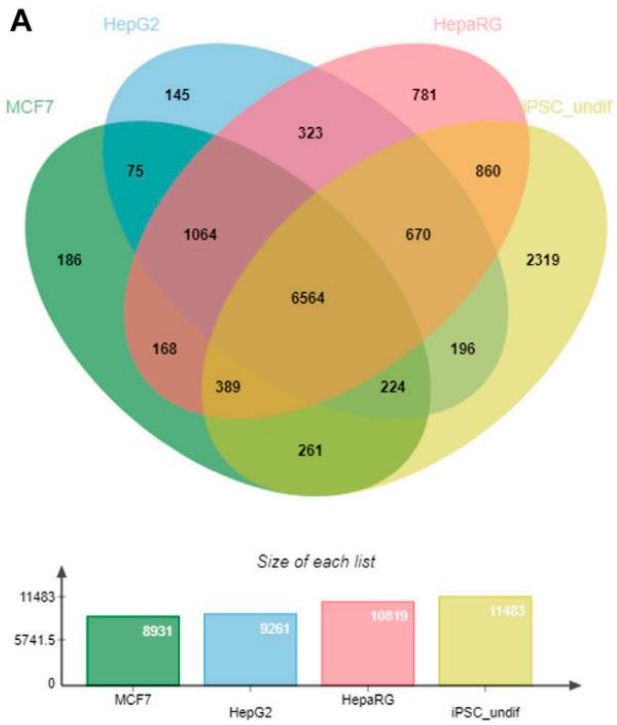
103,607 total articles

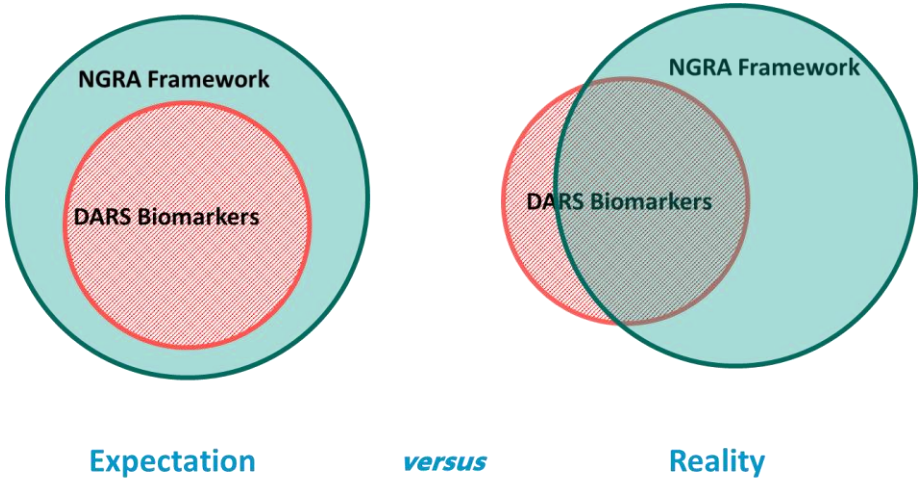
Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

Coverage of important DART biomarkers using Literature Search

- HepG2, MCF-7, HepaRG, hiPSCs

14,225 genes in total





Differentiated hiPSCs not included in this study but in scope for future work

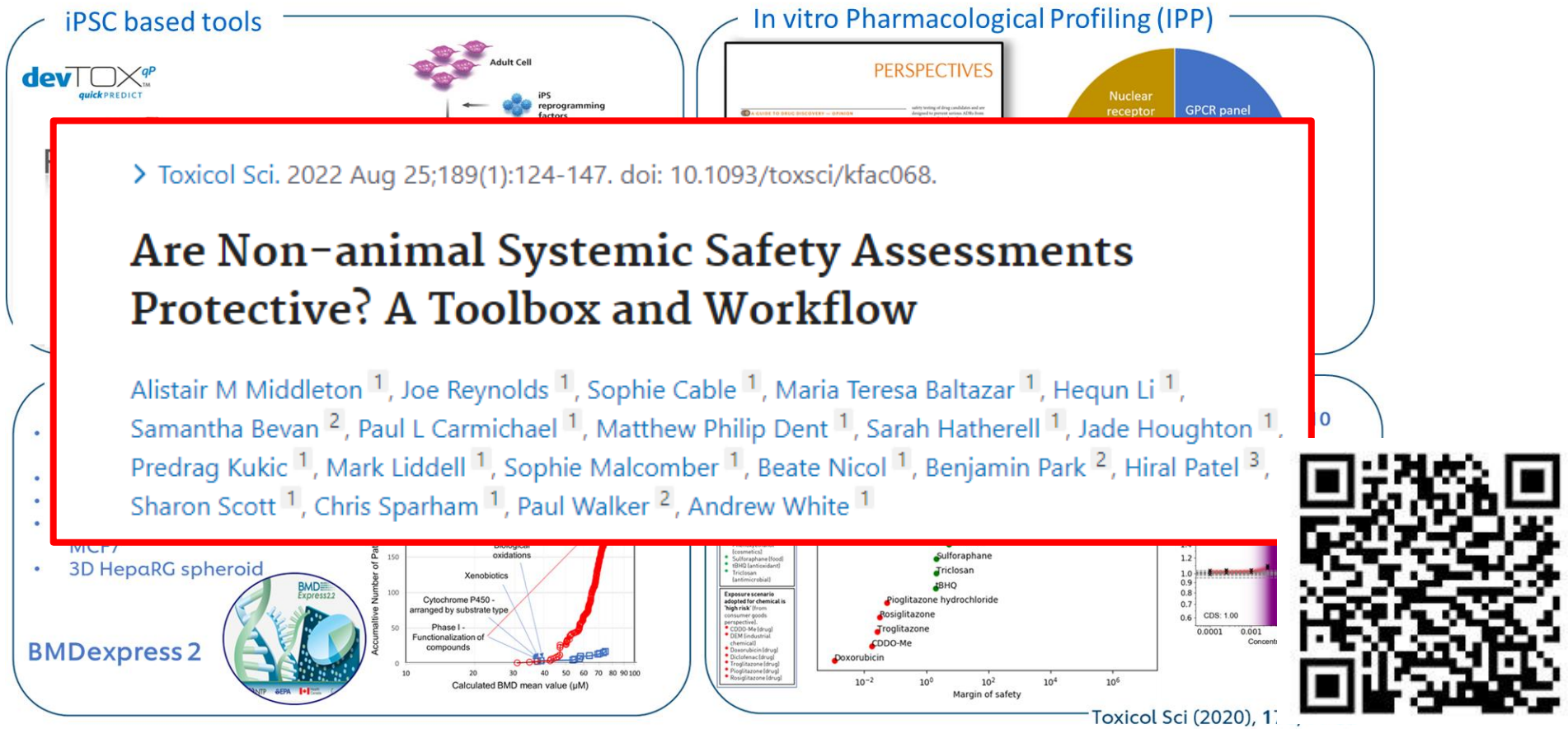
Gaps

- 41 **GPCRs** (6 present in IPP)
- 60 HTH transcription factors (mainly **homeobox transcription factors**)
- **Intercellular** signal molecules (chemokines, cytokines, growth factors, neurotropic factors, peptide hormones)

➢ **Filling the gaps – work in progress: placenta transfer measurements, DNT, DIT, studying epigenetics in germline development, advanced cell models for refinement.**

Is the NGRA Framework protective – fit for purpose validation

- Aim: evaluate protectiveness of the NGRA Framework for DART for a given chemical-exposure scenario
- Each chemical-exposure scenario is classified as “high” or “low” risk for pregnancy
- For each chemical-exposure scenario we generate NAM data using NGRA Framework



> Toxicol Sci. 2022 Aug 25;189(1):124-147. doi: 10.1093/toxsci/kfac068.

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

Alistair M Middleton [1], Joe Reynolds [1], Sophie Cable [1], Maria Teresa Baltazar [1], Hequn Li [1], Samantha Bevan [2], Paul L Carmichael [1], Matthew Philip Dent [1], Sarah Hatherell [1], Jade Houghton [1], Predrag Kukic [1], Mark Liddell [1], Sophie Malcomber [1], Beate Nicol [1], Benjamin Park [2], Hiral Patel [3], Sharon Scott [1], Chris Sparham [1], Paul Walker [2], Andrew White [1]

Is the NGRA Framework protective – fit for purpose validation

Exposure Scenario: Oral 0.5 mg tablet daily during pregnancy = risk for pregnancy

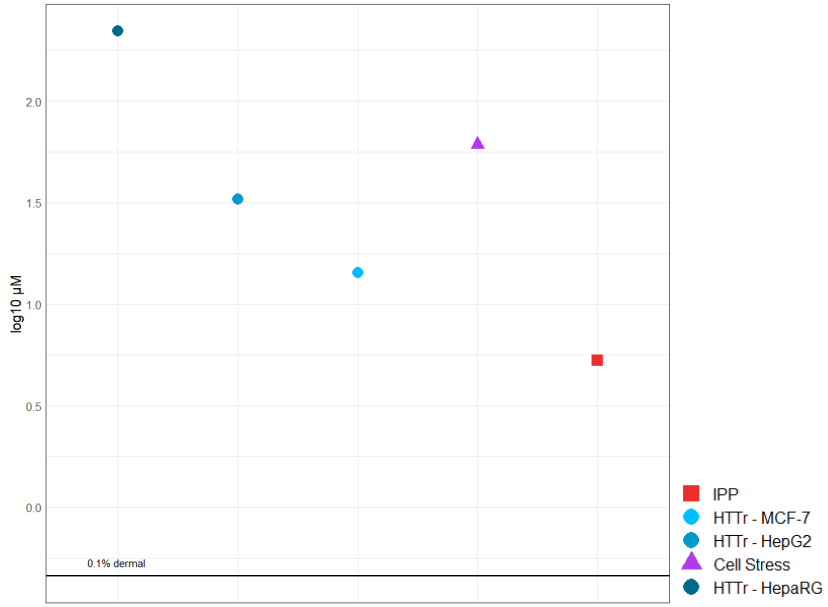
Diethylstilbesterol

Outcome: Bioactivity detected at or below the plasma Cmax = <u>risk for pregnancy</u>

The lowest PoD is coming from HTTR data from MCF7 cells expressing the Estrogen receptor, and from IPP (ER binding)

Exposure Scenario: Daily dermal application of 0.1% caffeine in a body lotion = low risk for pregnancy

Caffeine



Outcome: Bioactivity across the DART toolbox occurring at much higher concentrations than the plasma C_{max} = <u>low risk for pregnancy</u>

The lowest PoD coming from IPP ADORA2A

Is the NGRA Framework protective – fit for purpose validation

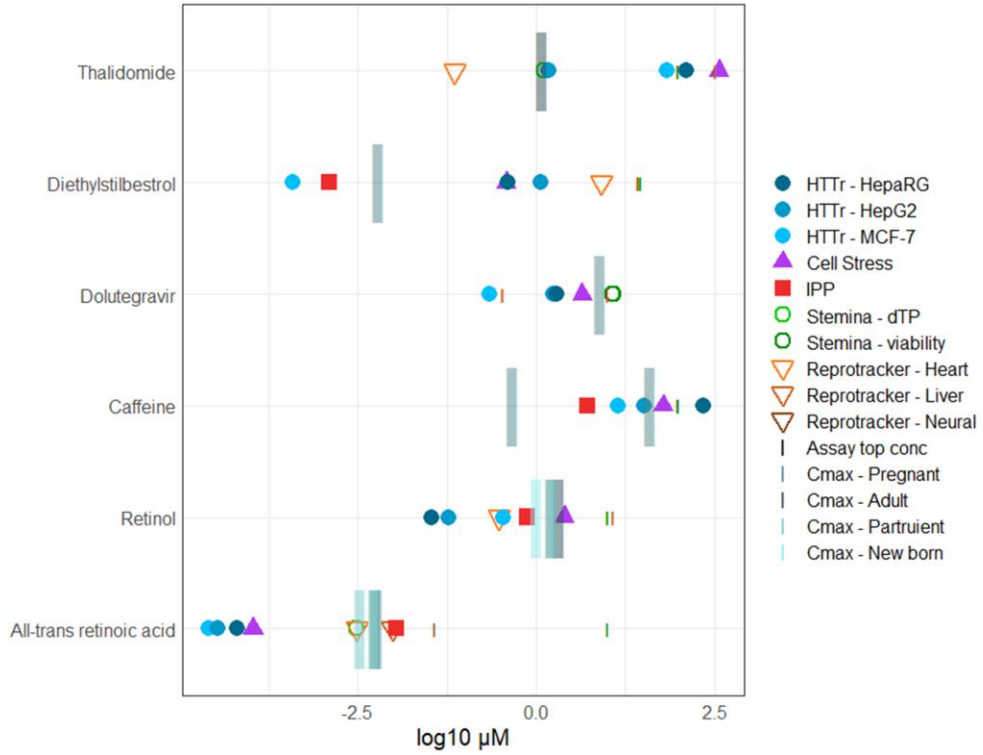
50mg oral application of Thalidomide, high risk, causing dev. toxicity.

5mg oral application of DES, high risk, causing estrogen activity/ED

50mg oral application of Dolutegravir, high risk, causing dev. toxicity

Dermal application of 0.1% caffeine in body lotion (lower Cmax), or oral uptake at recommended TDI of 200mg per days (higher Cmax) of caffeine, both low risk risk.

Uptake of vitamin A/retinol or retinol equivalents in normal diet, low risk. Cmax concentration of retinol and all-trans retinoic acid (metabolite of retinol) were measured in blood of adult, pregnant and parturient woman as well as in newborns[3].



Lowest PoD for Thalidomide is below Cmax value, the toolbox has correctly identified Thalidomide as high risk with lowest PoD coming from ReproTracker® assay.

Lowest PoD for DES is below Cmax value, the toolbox has correctly identified DES as high risk, lowest POD coming from MCF7 HTTr and estrogen receptor binding (IPP).

Lowest PoD for Dolutegravir is below Cmax value of exposure scenario, the toolbox has correctly identified it as high risk. Refinement for hazard classification as dev. Toxicant would be needed, if requested, as there are indications on dev. tox. but above Cmax values. Cell models like gastroloid systems can detect effects at relevant conc.[4].

Cmax for dermal application of caffeine is below lowest PoD, the toolbox has correctly identified it as low risk. For oral uptake of caffeine, the lowest PoD is below Cmax values indicating risk. Refinement for risk assessment would be needed.

Lowest PoD for retinol as well as all-trans retinoic acid is below Cmax values indicating high risk. Further tools would be needed to refine between bioactivity versus adversity of the compound .

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Is the NGRA Framework protective – fit for purpose validation

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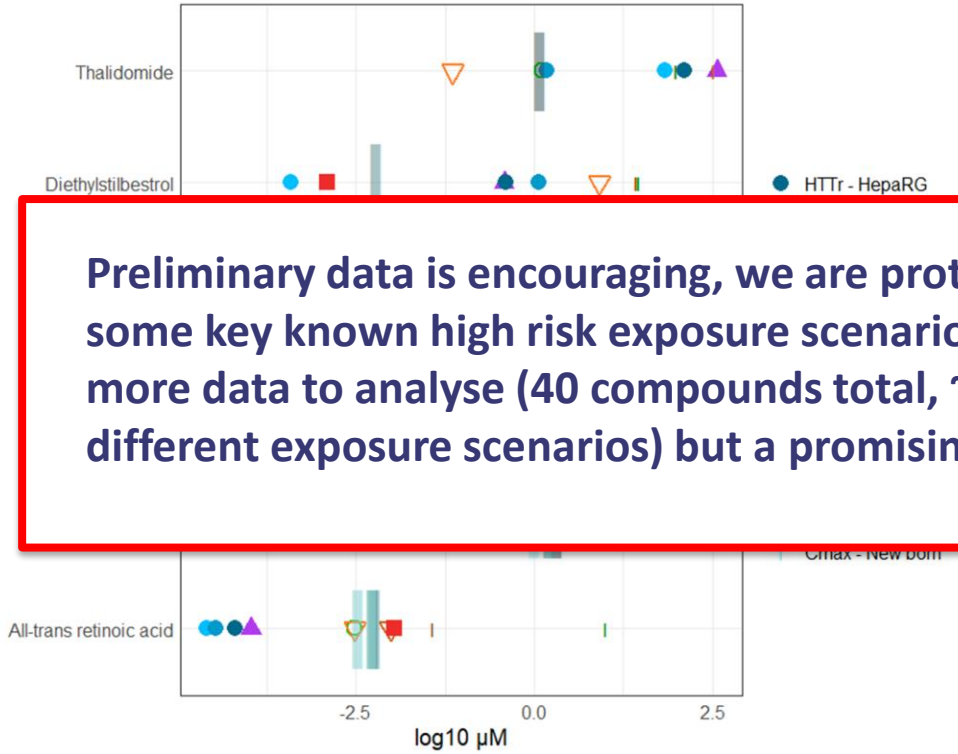

Preliminary data is encouraging, we are protective for some key known high risk exposure scenarios. Lots more data to analyse (40 compounds total, ~60+ different exposure scenarios) but a promising start!

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Lowest PoD for DES is below Cmax value, the toolbox has correctly identified DES as high risk with lowest PoD coming from MCF7 HTTr and estrogen receptor binding (IPP).

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Acknowledgments

DART NGRA Team – Paul Carmichael, Matt Dent, Jade Houghton, Predrag Kukic, Hequn Li, Alistair Middleton, Iris Muller, Beate Nicol, Ramya Rajagopal, Sandrine Spriggs, Gopal Pawar, Katy Wilson, Kathryn Wolton

40+ years of developing non-animal safety science

70+ collaborations

600+ publications

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