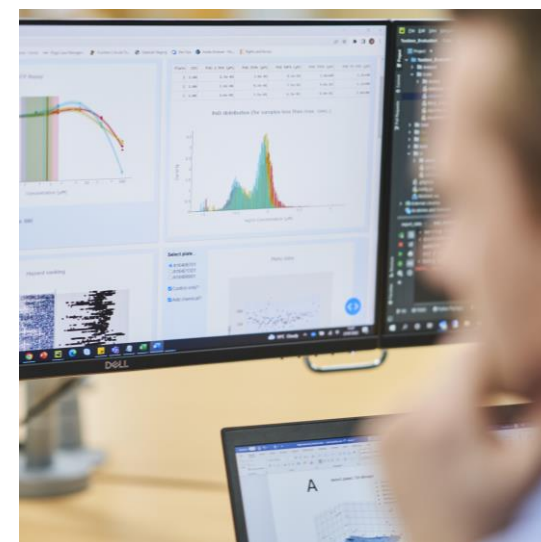


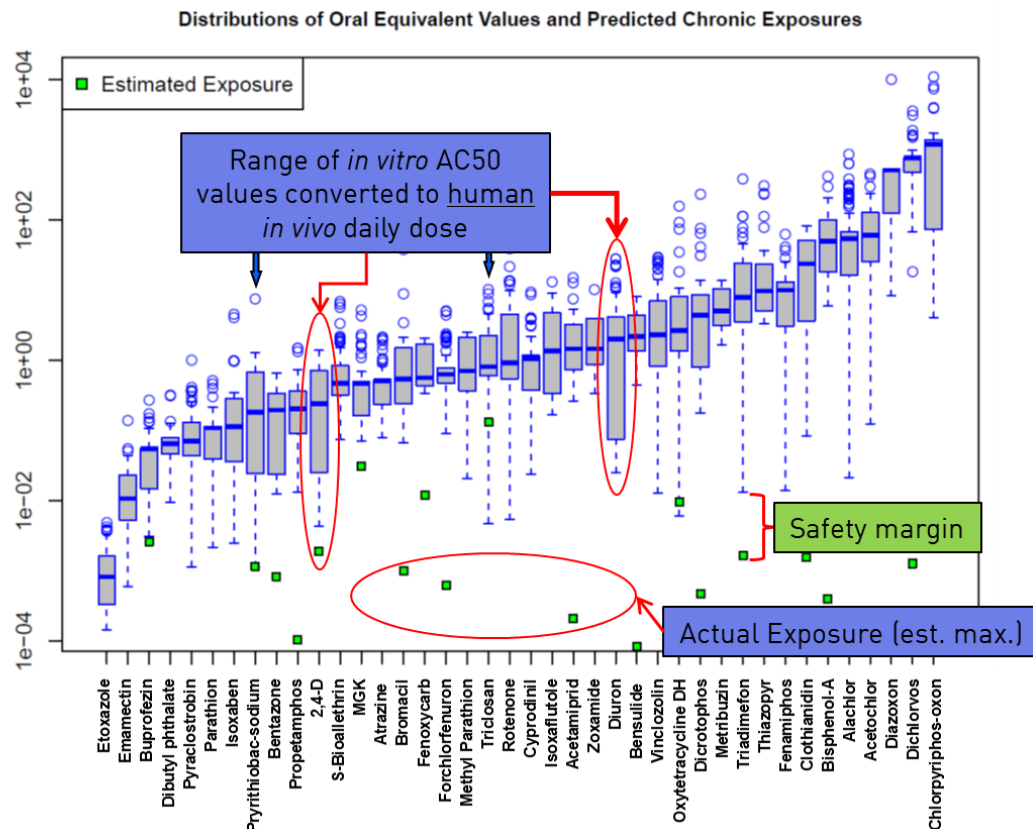
Practical Application of New Approach Methods in Developmental and Reproductive Toxicity (DART) Testing

Alistair Middleton on behalf of Iris Muller

**12th World Congress on Alternatives and
Animal Use in the Life Sciences**



Safety without animal testing - Next Generation Risk Assessment (NGRA)



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.

Rotroff, et al. Tox.Sci 2010, 117, 348-358

Slide from Dr Rusty Thomas, EPA, with thanks



REACH information requirements for DART often lead to significant use of experimental animals

CLP Regulation (EC) No. 1272/2008

**The 19th FRAME Annual Lecture,
November 2022: Safer Chemicals and
Sustainable Innovation Will Be Achieved by
Regulatory Use of Modern Safety Science,
Not by More Animal Testing**

Julia H. Fentem

Alternatives to Laboratory Animals
2023, Vol. 51(2) 90-101
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DOI: 10.1177/02611929231158236
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Study	Annex VII	Annex VIII	Annex IX	Annex X
Screening test for reproductive /developmental toxicity (OECD TG 421 or 422)		Required	Strongly recommended if no higher tier fertility study (such as OECD 443) is/will be available	
Prenatal developmental toxicity study (EU B.31, OECD TG 414)		May be proposed in case of (serious) concern ¹ for prenatal developmental toxicity. However, it is	Required in one species; second species may be triggered ²	Required in two species

Article 54 Council Directive 2001/83/EC

Regulation (EC) No 1907/2006

Regulation (EC) No 528/2012

**OECD TG for prenatal developmental toxicity (OECD 414)
two generation reproductive toxicity (OECD 416)
reproduction/developmental screening test (OECD 422/421)
one generation reproductive toxicity (OECD 415/443)**

**Upholding the EU's Commitment to
'Animal Testing as a Last Resort' Under
REACH Requires a Paradigm Shift in How
We Assess Chemical Safety to Close the
Gap Between Regulatory Testing and
Modern Safety Science**

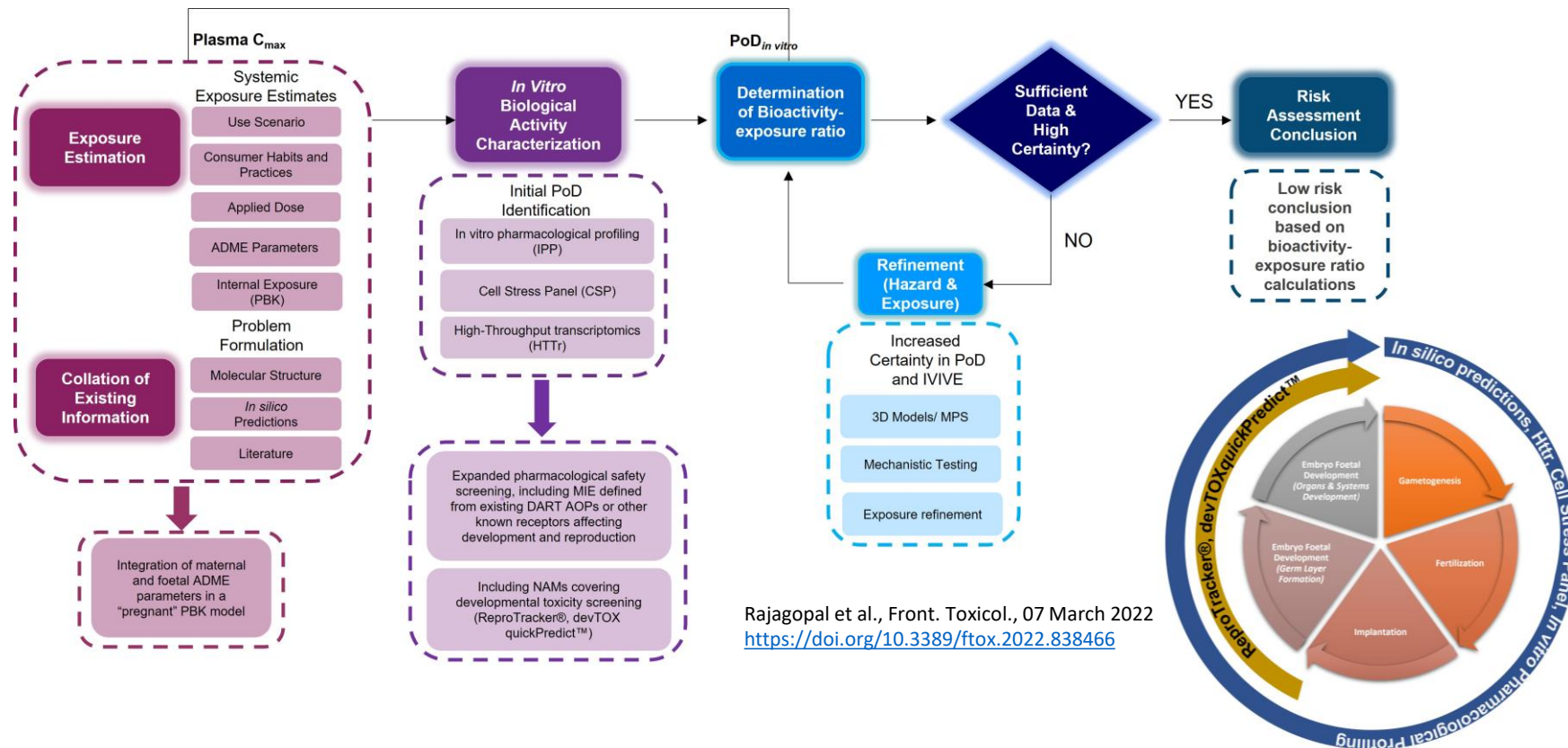
Julia Fentem, Ian Malcomber, Gavin Maxwell and Carl Westmoreland

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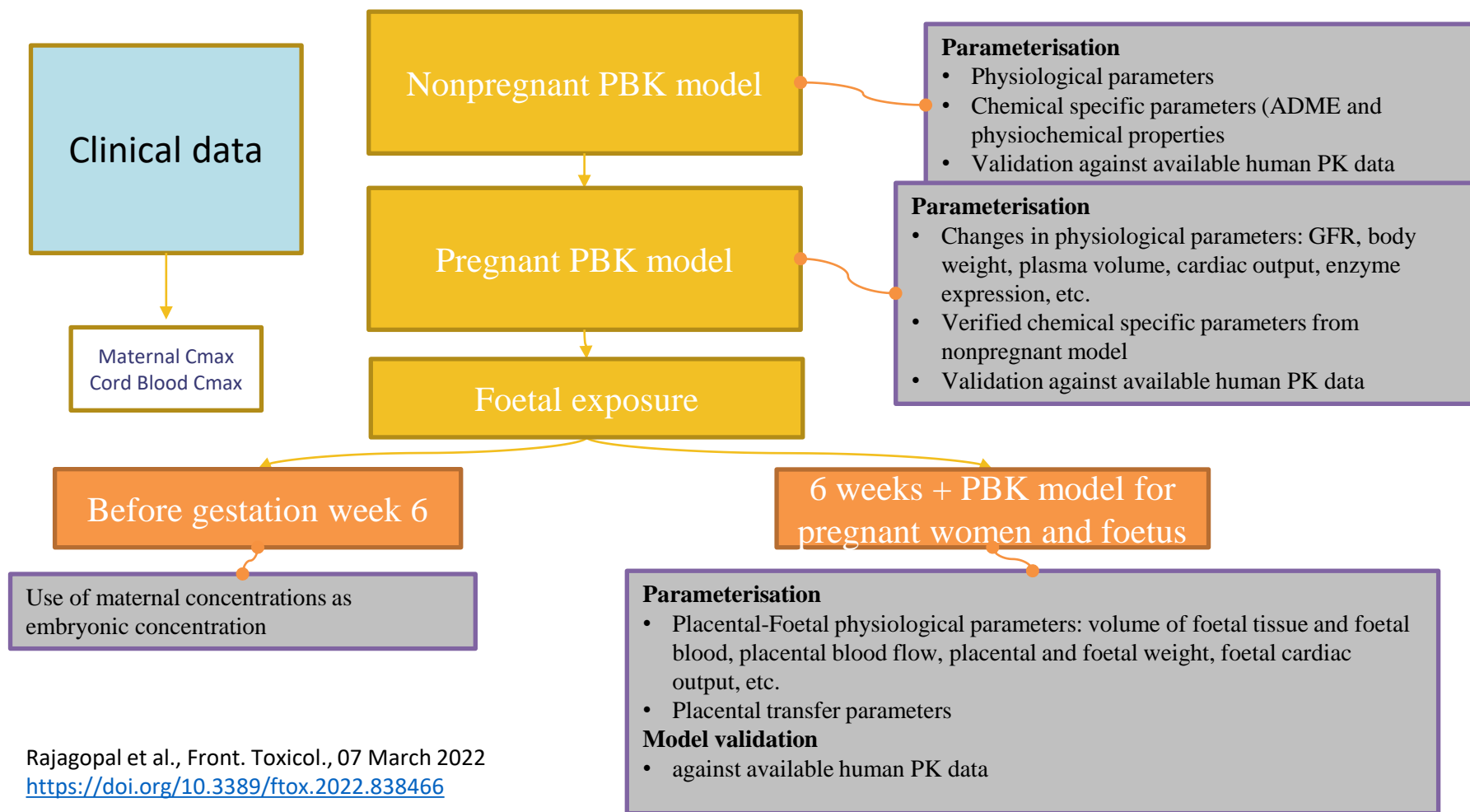


Our DART NGRA framework- a tiered and iterative approach



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

Our DART NGRA framework – the exposure module



Our DART NGRA framework – the bioactivity module

› 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 Middleton¹, Joe Reynolds¹, Sophie Cable¹, Maria Teresa Baltazar¹, Hequn Li¹, Samantha Bevan², Paul L Carmichael¹, Matthew Philip Dent¹, Sarah Hatherell¹, Jade Houghton¹, Predrag Kucic¹, Mark Liddell¹, Sophie Malcomber¹, Beate Nicol¹, Benjamin Park², Hiral Patel³, Sharon Scott¹, Chris Sparham¹, Paul Walker², Andrew White¹

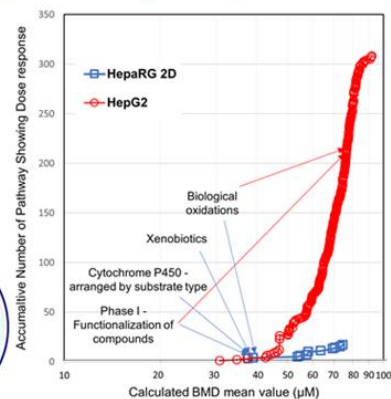
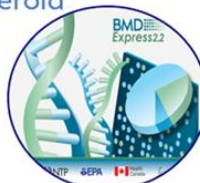
PP)



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)

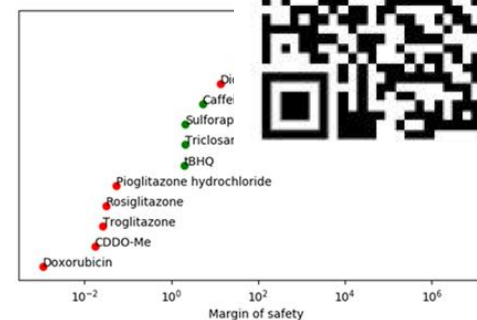
13 chemicals, 36 Biomarker Stress Pathways

Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective):

- Nicotinamide (food, cosmetic)
- Caffeine (beverages, cosmetic)
- Phenylethanol (cosmetics)
- Sulfasalazine (food)
- IBHQ (antioxidant)
- Triclosan (antimicrobial)

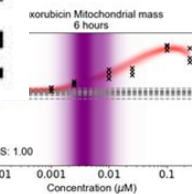
Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective):

- CDDO-Me (drug)
- DDM (industrial chemical)
- Doxorubicin (drug)
- Diclofenac (drug)
- Troglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (drug)



Measurements; ~ 10

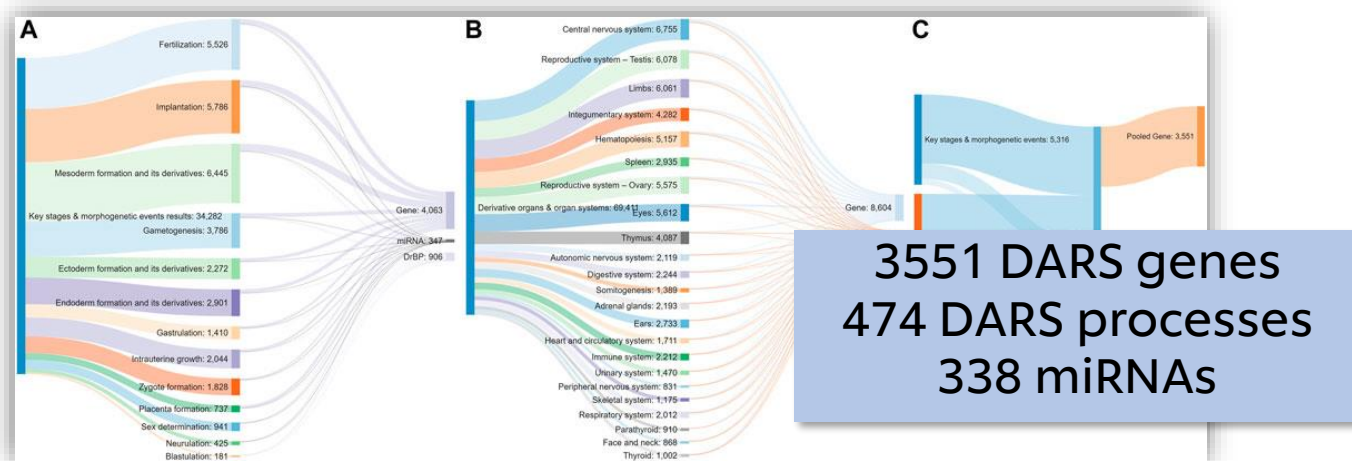
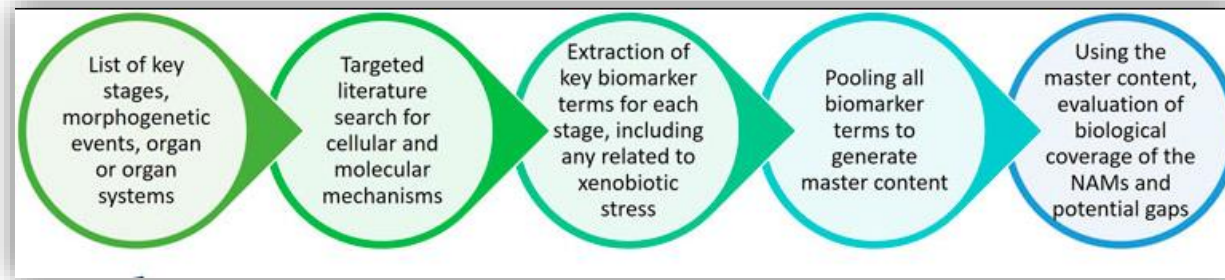
byproteX
AN EVOTEC COMPANY



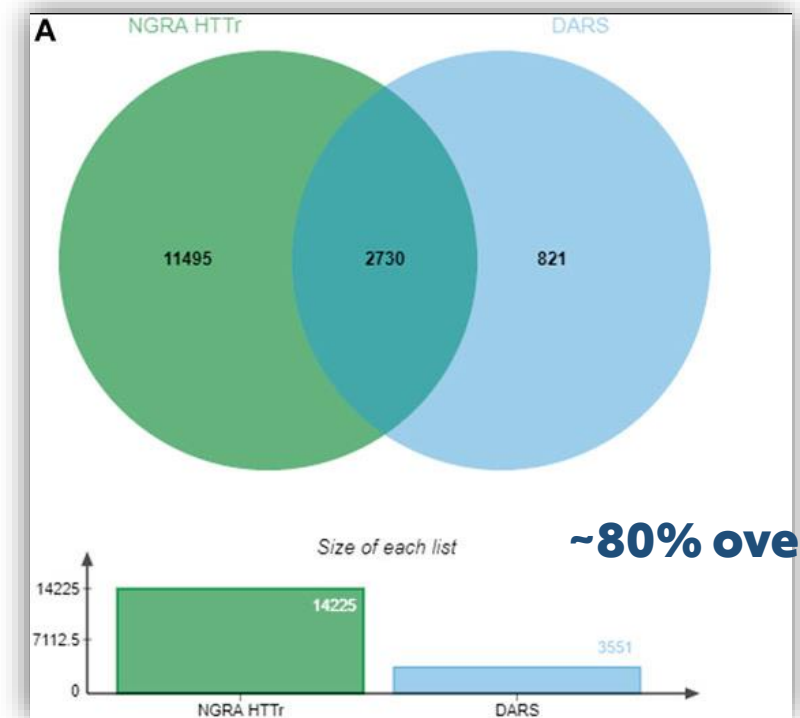
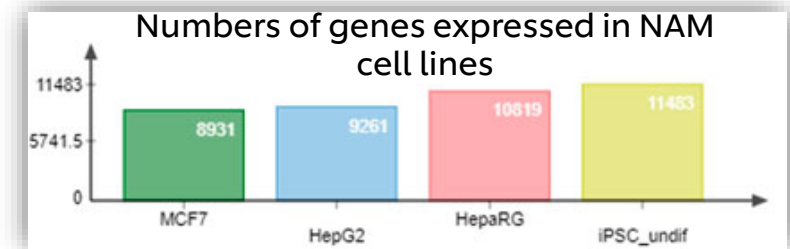
Toxicol Sci (2020), 176, 11-33

Biological coverages of NAMs for DART hazard endpoints

Strategy overview:



Automated literature extraction -> Developmental and reproductive signalling (DARS) marker



DART framework evaluation - first results

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



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



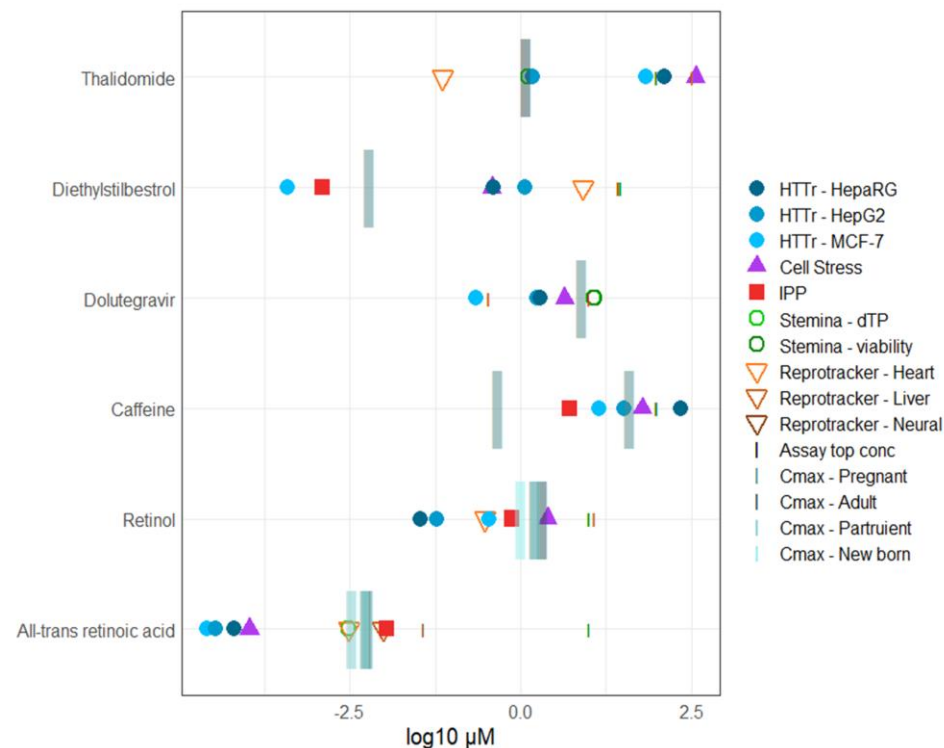
50mg oral application of Dolutegravir, high risk, causing dev. toxicity.



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.



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 newborns³.



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.

Initial results are encouraging, we are protective for some key known high risk exposure scenarios, and we are generating data for more compounds (40 in total) to evaluate the approach further.

ACKNOWLEDGMENTS

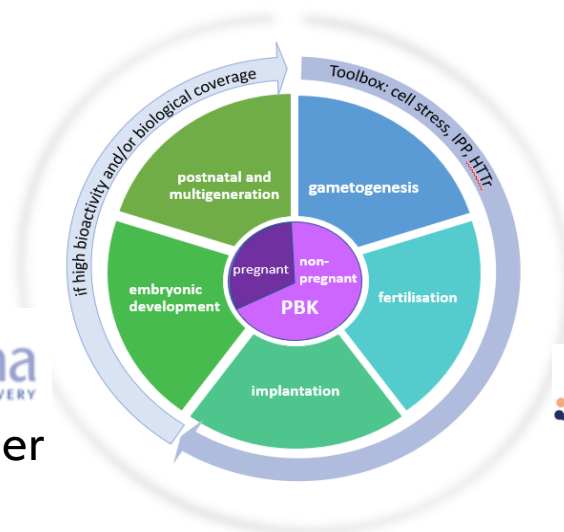
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Jessica Palmer



Bio:Spyder™



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