DNTS
DEVELOPMENTAL NEUROTOXICOLOGY SOCIETY

Society for Birth Defects Research & Prevention
EST. 1960 AS THE TERATOLOGY SOCIETY

Practical Application of New Approach Methods (NAMs) in Developmental Toxicity Testing

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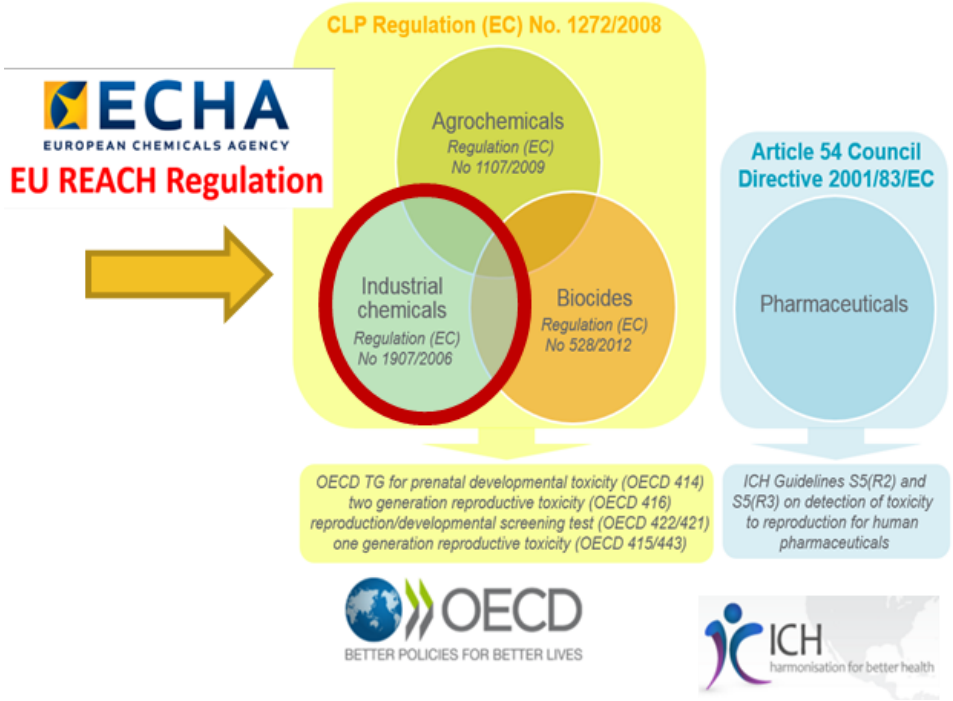
Safety & Environmental Sciences | Unilever

Charleston SOUTH CAROLINA

… animal testing for DART endpoints is “required” under REACH



Study	Annex VII	Annex VIII	Annex IX	Annex X
		~~10-100 tpa~~	~~100-1000 tpa~~	~~1000+ tpa~~
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 strongly recommended to consider conducting a screening study in addition to the prenatal developmental toxicity[2] study	Required in one species; second species may be triggered[2]	Required in two species
Extended one-generation reproductive toxicity study (EU B.56, OECD TG 443)[3]		Recommended instead of the screening study in case of serious concern[1] for fertility	Required if triggered[4]	Required

Draft Guidance document (europa.eu)

… animal testing for DART endpoints is “required” under REACH

Cosmetics
design-europe.com

THE LONG READ: IN CONVERSATION WITH UNILEVER SAFETY & ENVIRONMENTAL ASSURANCE CENTRE (SEAC) EXECUTIVES

The future of animal-free chemical testing? There’s a ‘big frustration’ in the scientific community, say Unilever execs

By Kacey Culliney
20-Oct-2021 - Last updated on 20-Oct-2021 at 09:54 GMT

RELATED TAGS: Animal testing, Animal testing alternatives, cruelty-free, In vivo, Regulation, ECHA, REACH, Animal testing ban, Chemicals

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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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

Alternatives to Laboratory Animals
2021, Vol. 49(4) 122–132
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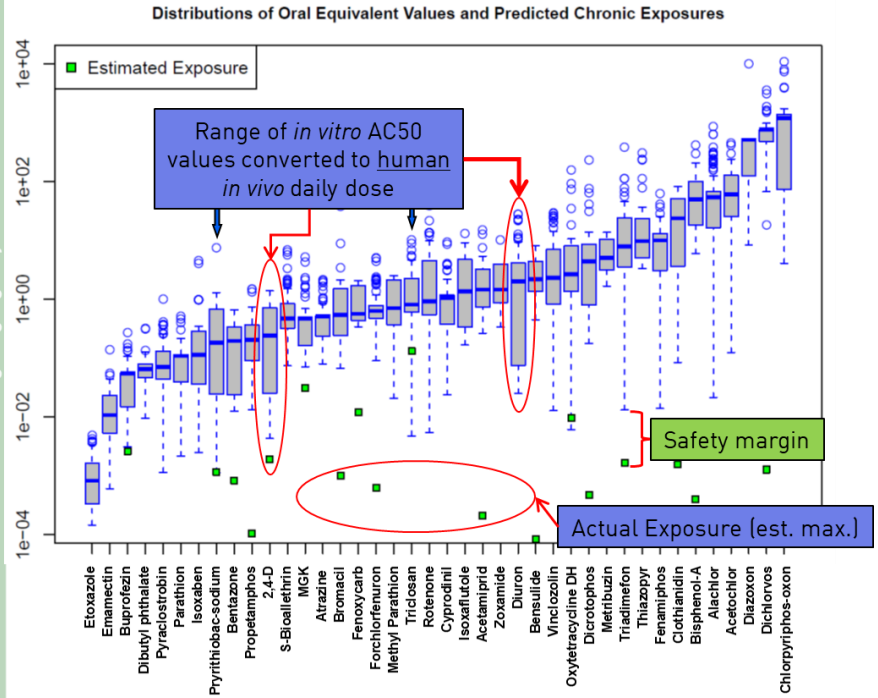
	Required if triggered[4]	Required

Draft Guidance document (europa.eu)

BETTER POLICIES FOR BETTER LIVES

harmonisation for better health

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



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

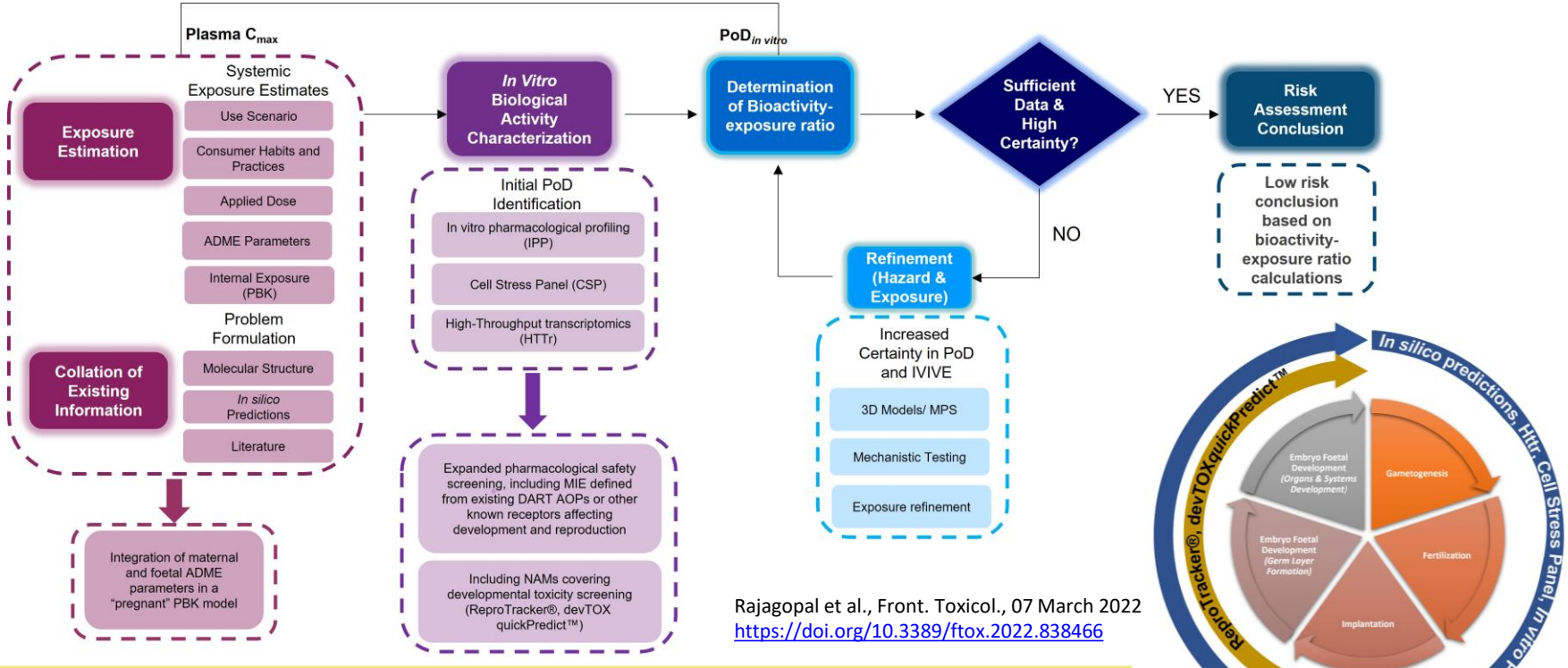
NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing**

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.

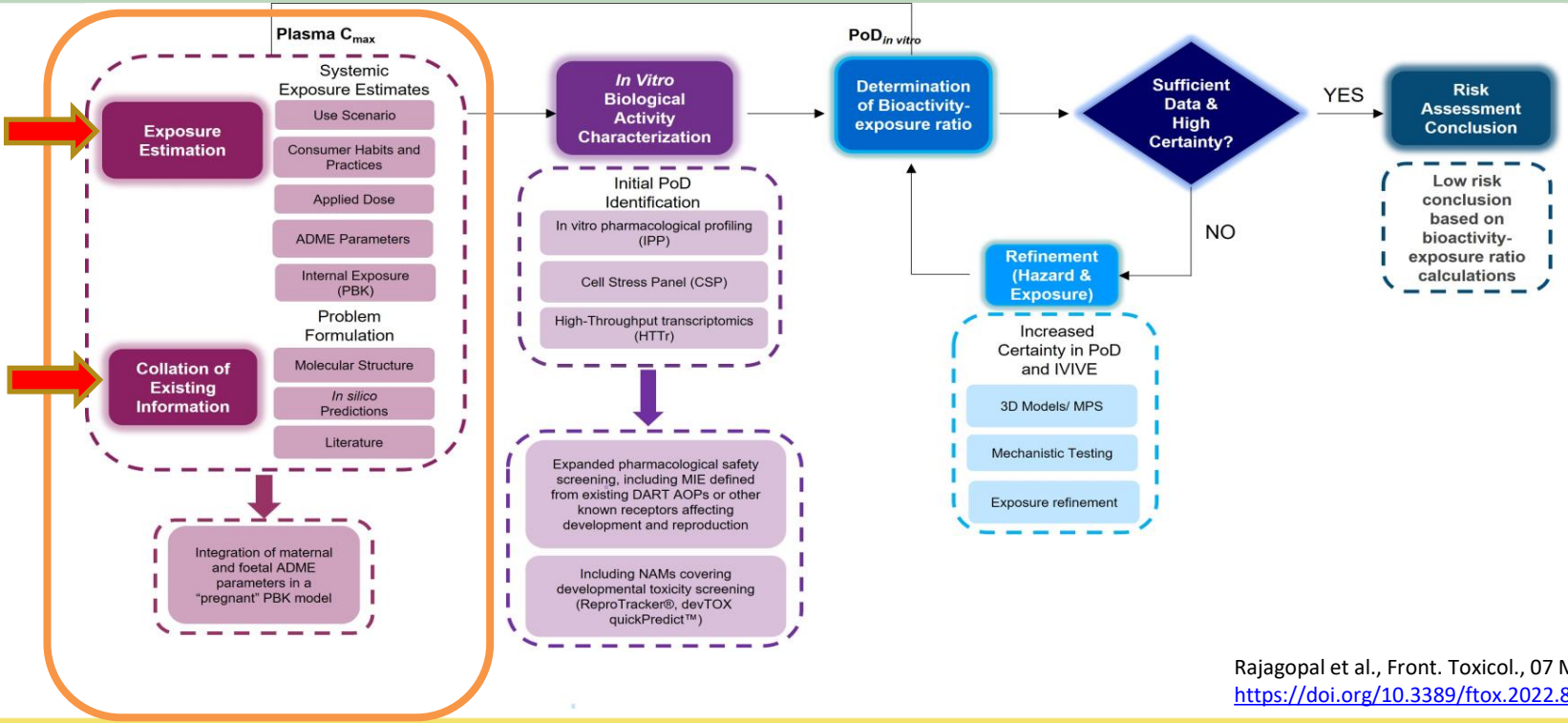
Slide from Dr Rusty Thomas, EPA, with thanks

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



Rajagopal et al., Front. Toxicol., 07 March 2022
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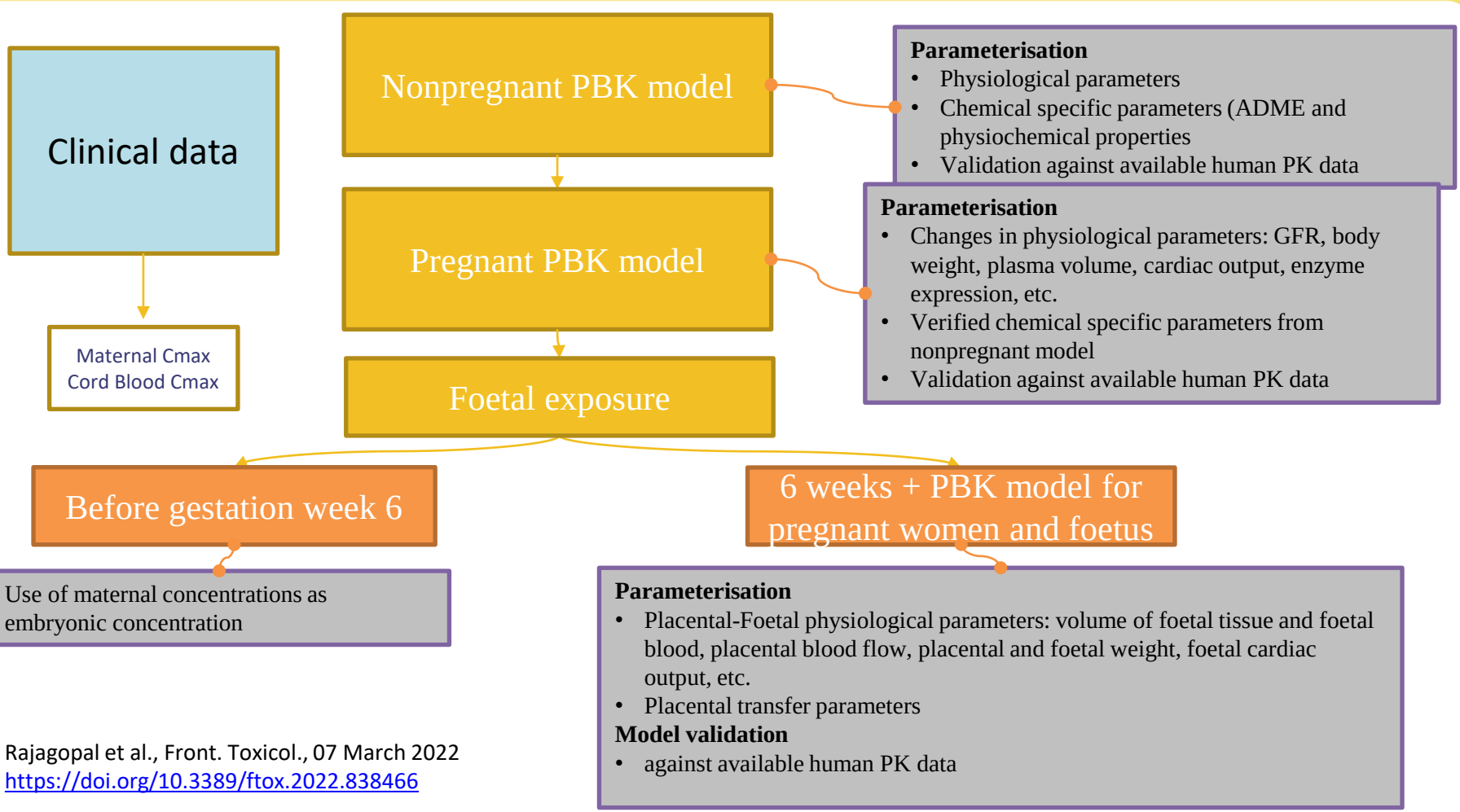
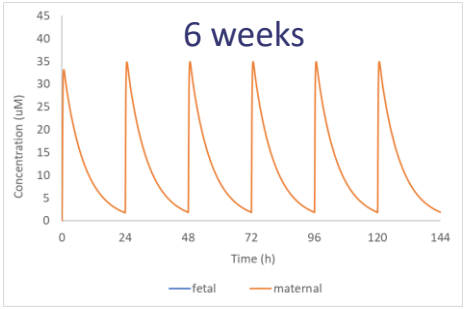
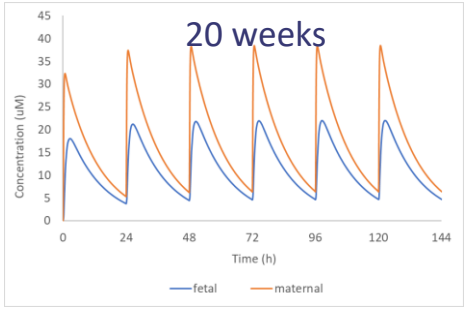


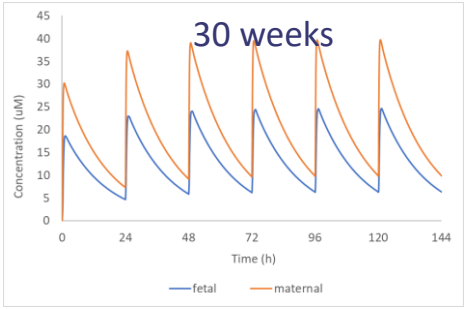
Rajagopal et al., Front. Toxicol., 07 March 2022
https://doi.org/10.3389/ftox.2022.838466

PBPK modelling to derive maternal and fetal plasma Cmax

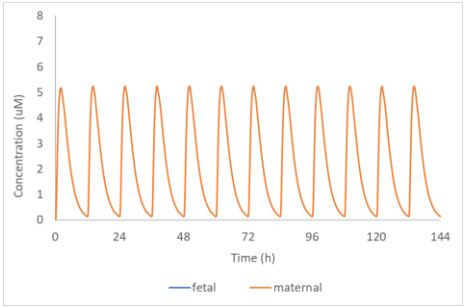
Gestational age

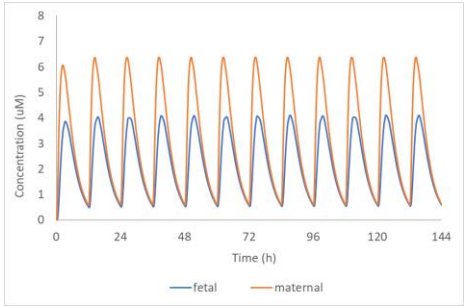


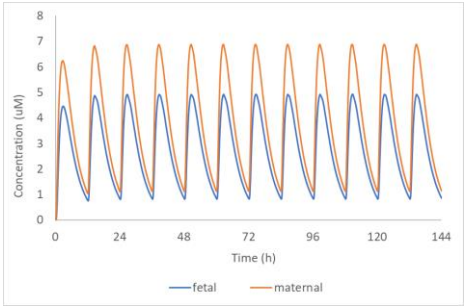




Oral exposure of 200mg caffeine

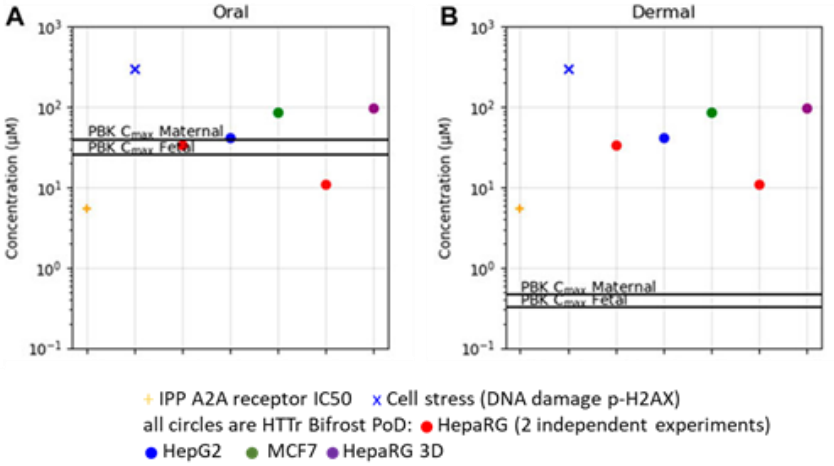






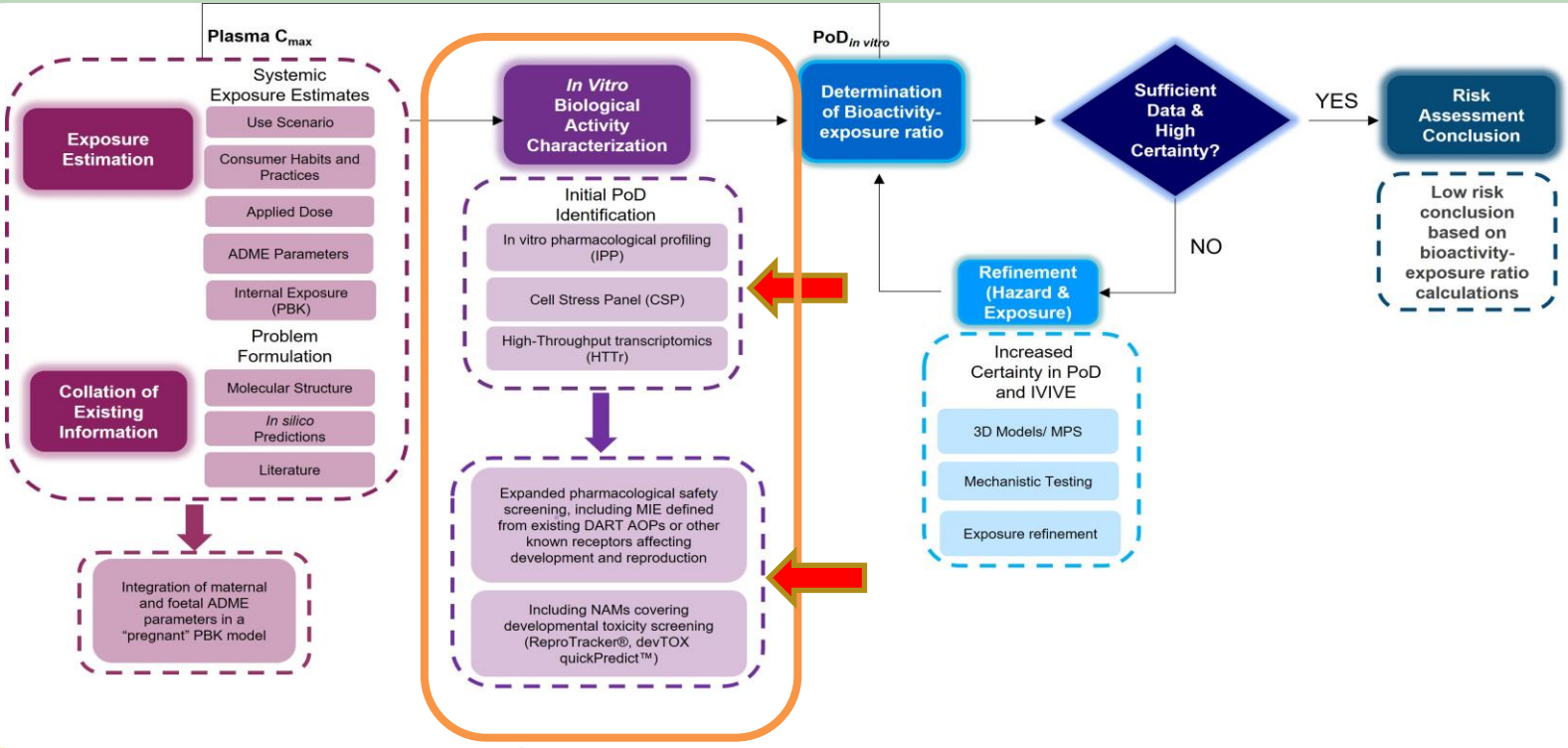
Dermal application of body lotion containing 1.5% caffeine

	Oral: 200 mg/day			Dermal: 0.1% caffeine in body lotion		
	Week 6	Week 20	Week 30	Week 6	Week 20	Week 30
Maternal plasma Cmax (uM)	34.97	38.51	39.72	0.42	0.42	0.46
Foetal plasma Cmax (uM)		22.02	25.27		0.27	0.32



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

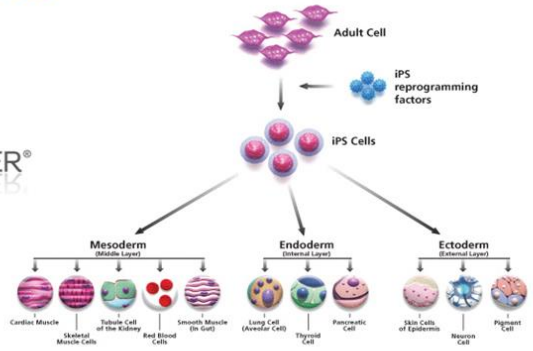
Our DART NGRA framework – the bioactivity module



iPSC based tools

devTOX qP quickPREDICT

ReproTracker®



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

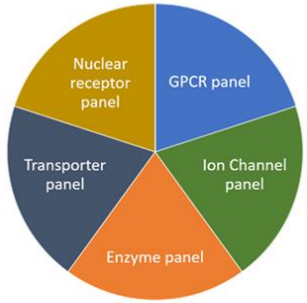
In vitro Pharmacological Profiling (IPP)

PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

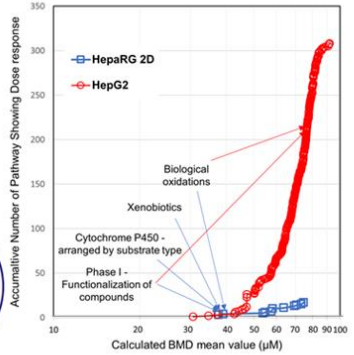


eurofins | Cerep

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

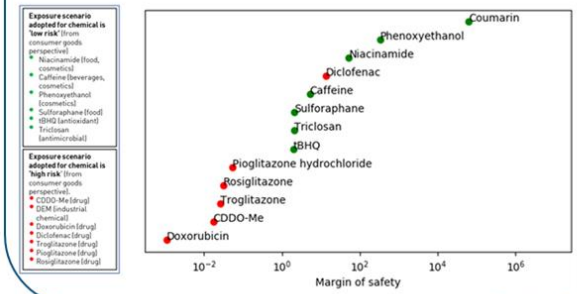
BMDExpress 2

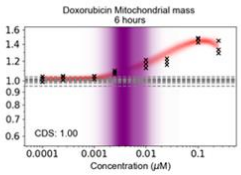


Cell Stress Panel (CSP)

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

cyprotex AN EVOTEC COMPANY





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

iPSC based tools

devTOX qP quickPREDICT

In vitro Pharmacological Profiling (IPP)

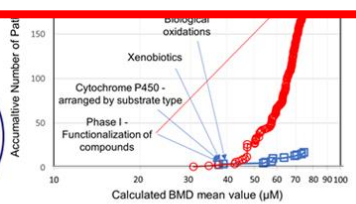
> Toxicol Sci. 2022 Aug 25;189(1):124-147. doi: 10.1093/toxsci/kfac068.

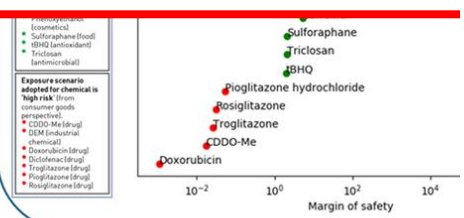
Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

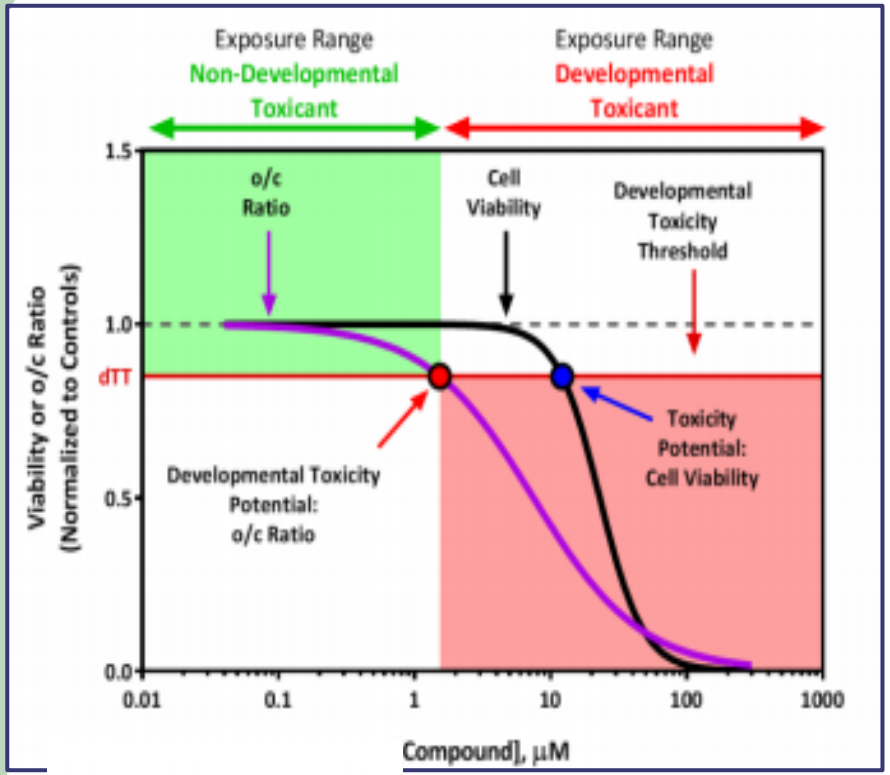
Alistair M Middleton [1], Joe Reynolds [1], Sophie Cable [1], Maria Teresa Baltazar [1], Hequn Li [1], Samantha Bevan [2], Paul L Carmichael [1], Matthew Philip Dent [1], Sarah Hatherell [1], Jade Houghton [1], Predrag Kukic [1], Mark Liddell [1], Sophie Malcomber [1], Beate Nicol [1], Benjamin Park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

- MCF7
- 3D HepaRG spheroid

BMDExpress 2







devTOX qP quickPREDICT

This assay looks at the metabolic perturbation of undifferentiated iPSCs.

Spent media is analysed for the quantity of two metabolites (ornithine and cystine) and a ratio between the two is calculated. Cell viability is also assessed. The dose response curves are used to establish a PoD for developmental toxicity – where the curve drops below the threshold value (dTT) a test article is concluded to have developmental toxicity potential

› Toxicol Sci. 2020 Apr 1;174(2):189-209. doi: 10.1093/toxsci/kfaa014.

Profiling the ToxCast Library With a Pluripotent Human (H9) Stem Cell Line–Based Biomarker Assay for Developmental Toxicity

Todd J Zurlinden [1], Katerine S Saili [1], Nathaniel Rush [1], Parth Kothiya [1], Richard S Judson [1], Keith A Houck [1], E Sidney Hunter [2], Nancy C Baker [3], Jessica A Palmer [4], Russell S Thomas [1], Thomas B Knudsen [1]

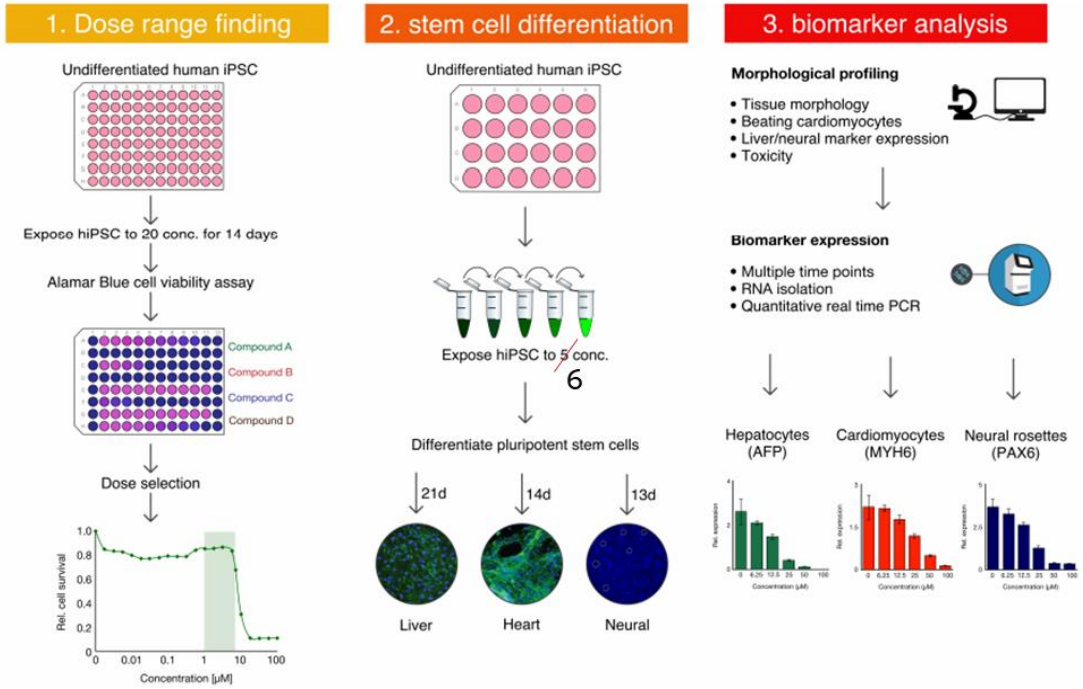


Figure 1: Schematic representation of the experimental setup of ReproTracker.

This assay looks at changes to iPSC differentiation (to cardiac, liver and neural cell lineages)

Biomarker analysis is performed using genes which are specific for all 3 germ layers and genes which are specific to each of the 3 lineages

The assay was originally set up to give a binary response if a substance is teratogenic or not. SEAC have been working with Toxys to further develop the assay, and to calculate a PoD for inclusion within our NGRA toolbox (unpublished work)

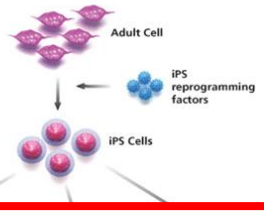
REPROTRACKER®

partly published in Jamalpoor et al 2022 **https://doi.org/10.1002/bdr2.2001**

iPSC based tools

devTOX qP quickPREDICT

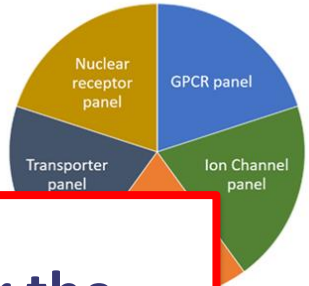
ReproTracker®



In vitro Pharmacological Profiling (IPP)


PERSPECTIVES

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

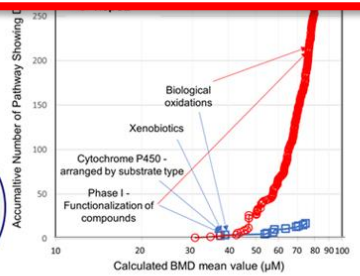


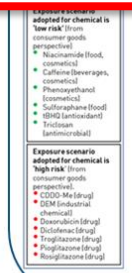
Does this bioactivity module cover the important cellular and intercellular processes for DART?

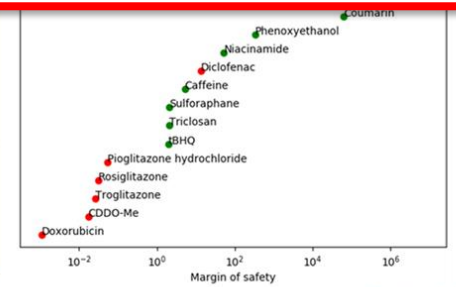
High-throughpu

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

BMDExpress 2

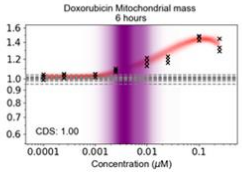






rations; ~10

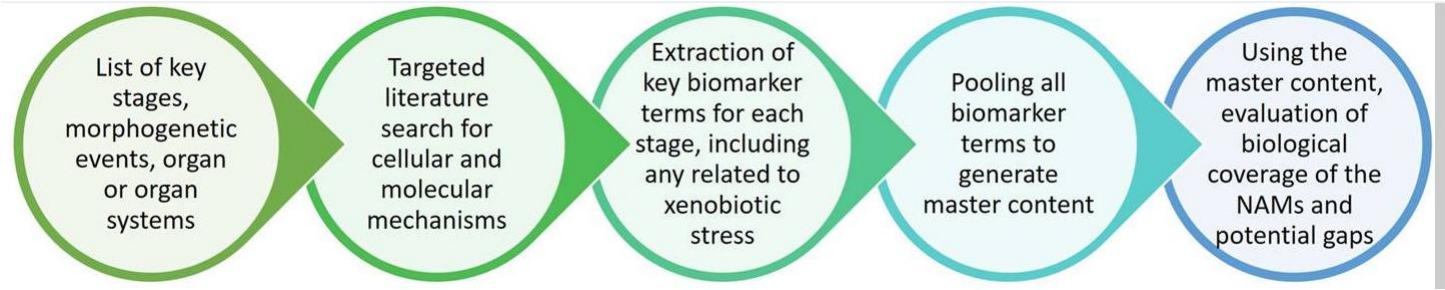
cyprotex
AN EVOTEC COMPANY



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

Biological coverage

- 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



Front Toxicol. 2022; 4: 838466.
Published online 2022 Mar 7. doi: 10.3389/ftox.2022.838466

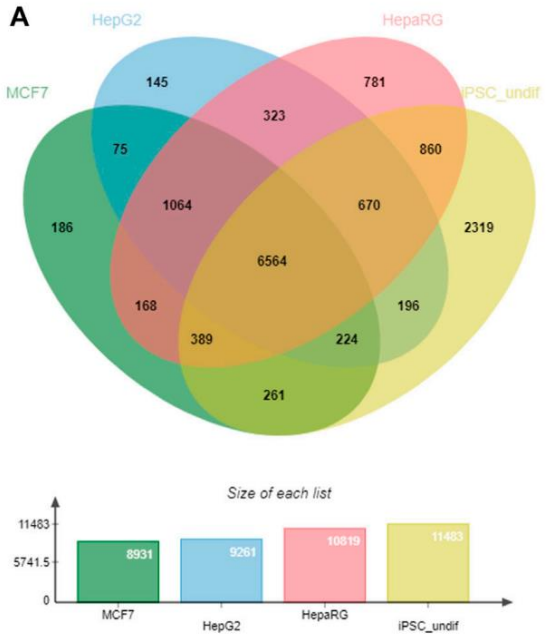
Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing

Rajagopal et al., 2022

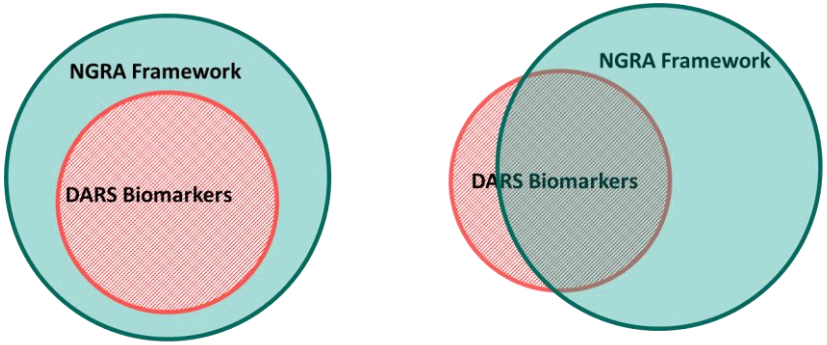
~3500 genes

Baseline gene expression in the DART NGRA framework

- HepG2, MCF-7, HepaRG, hiPSCs



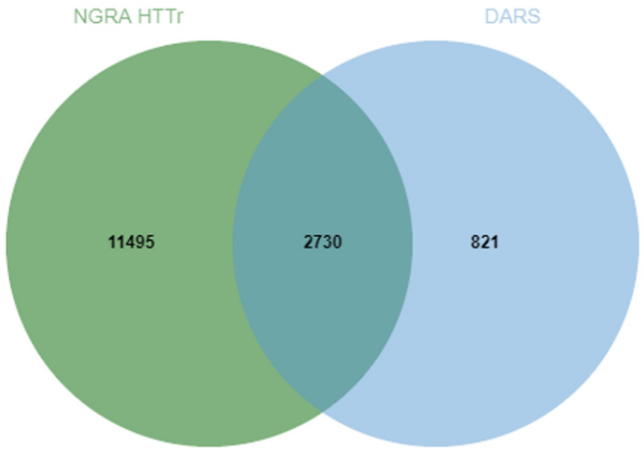
14,225 genes in total

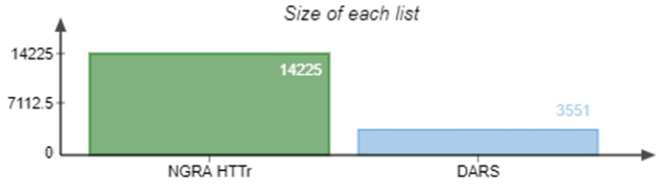


Expectation ***versus*** **Reality**

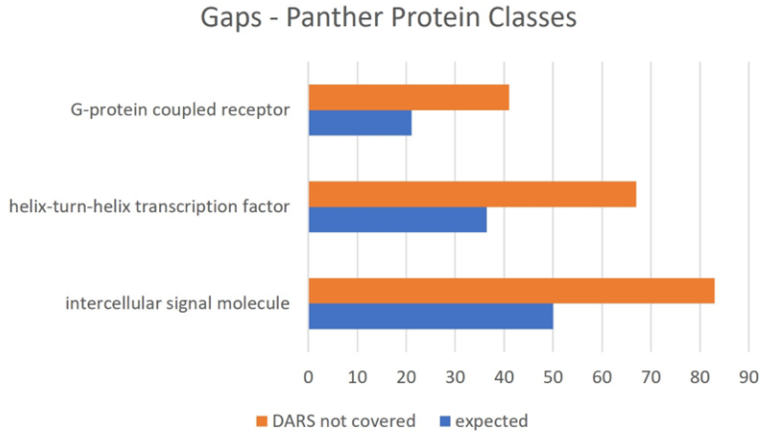
Differentiated hiPSCs not included in this study but in scope for future work

Coverage



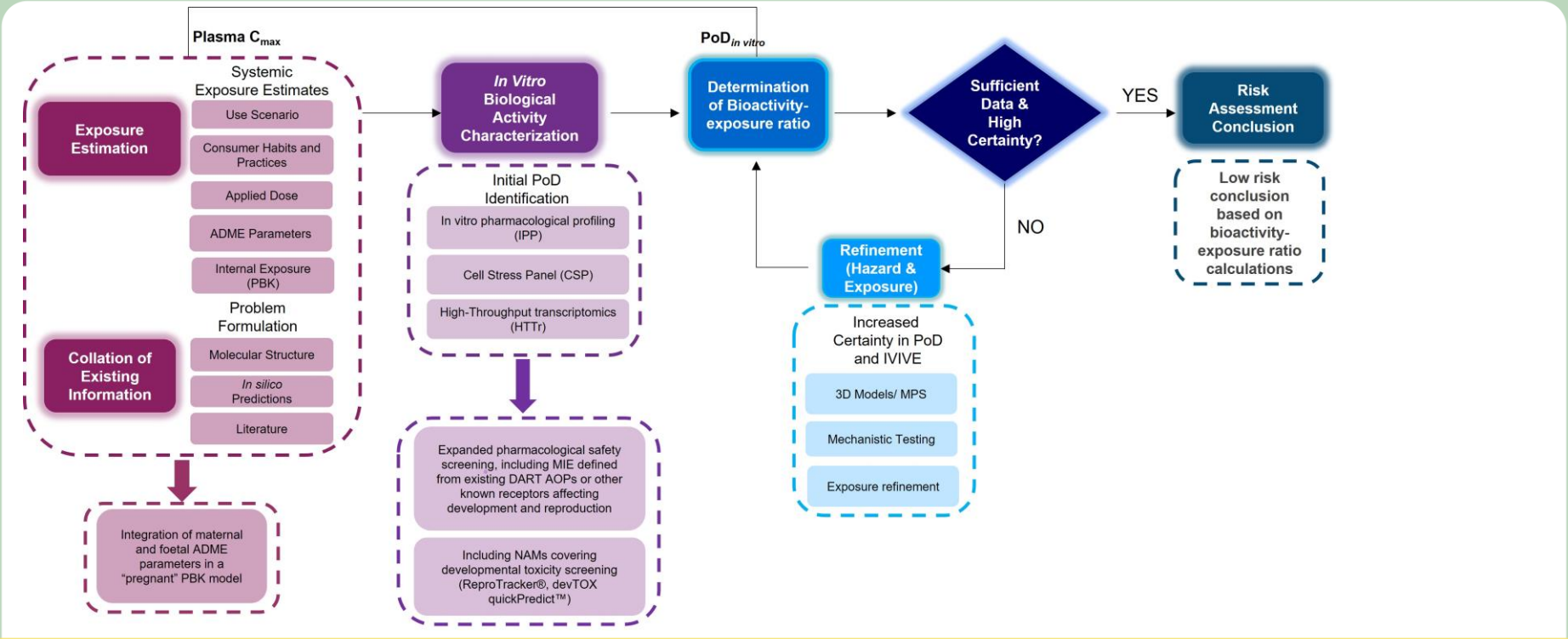


Gaps

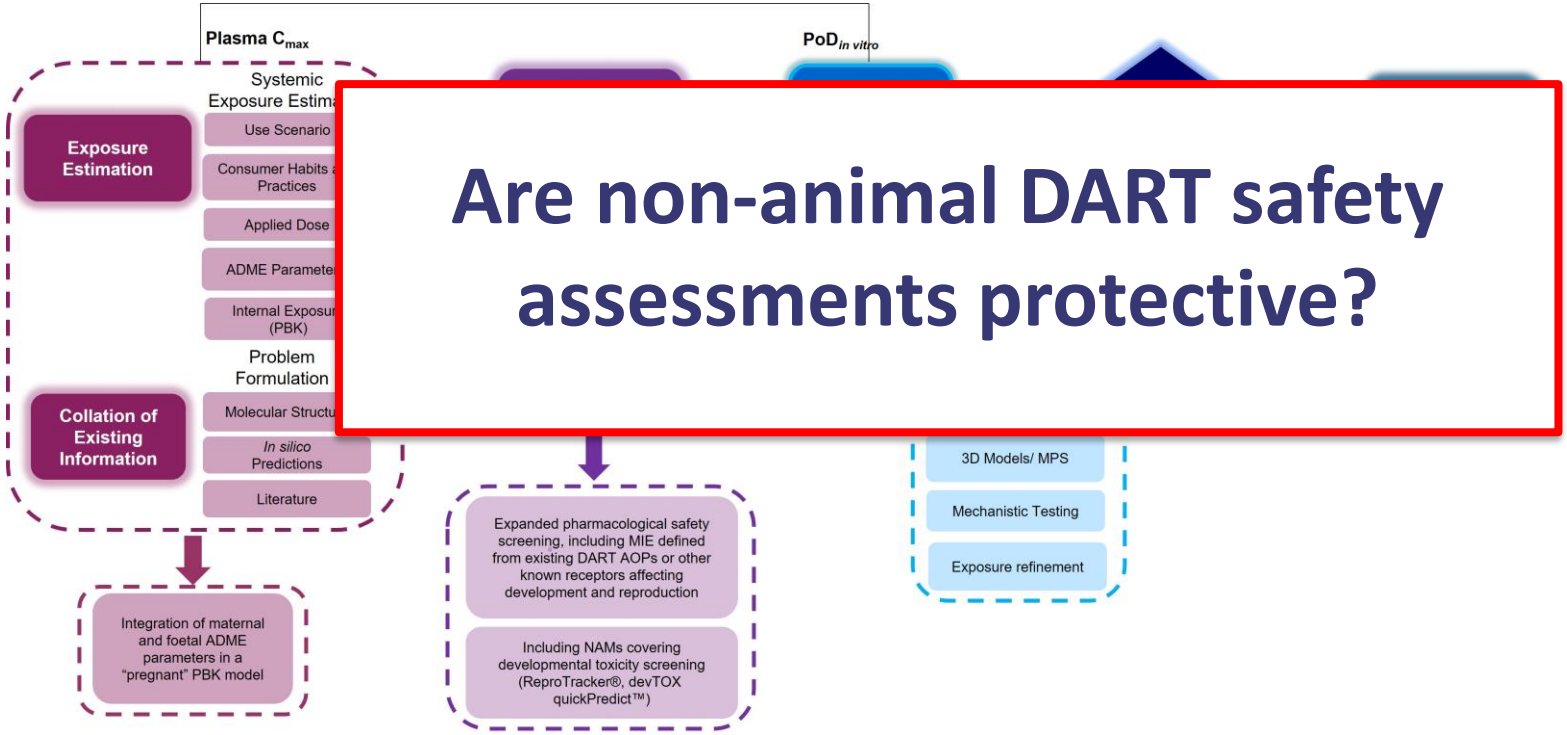


- GPCRs (some present in IPP)
- HTH transcription factors (mainly homeobox transcription factors)
- Intercellular signal molecules (chemokines, cytokines, growth factors, neurotropic factors, peptide hormones)

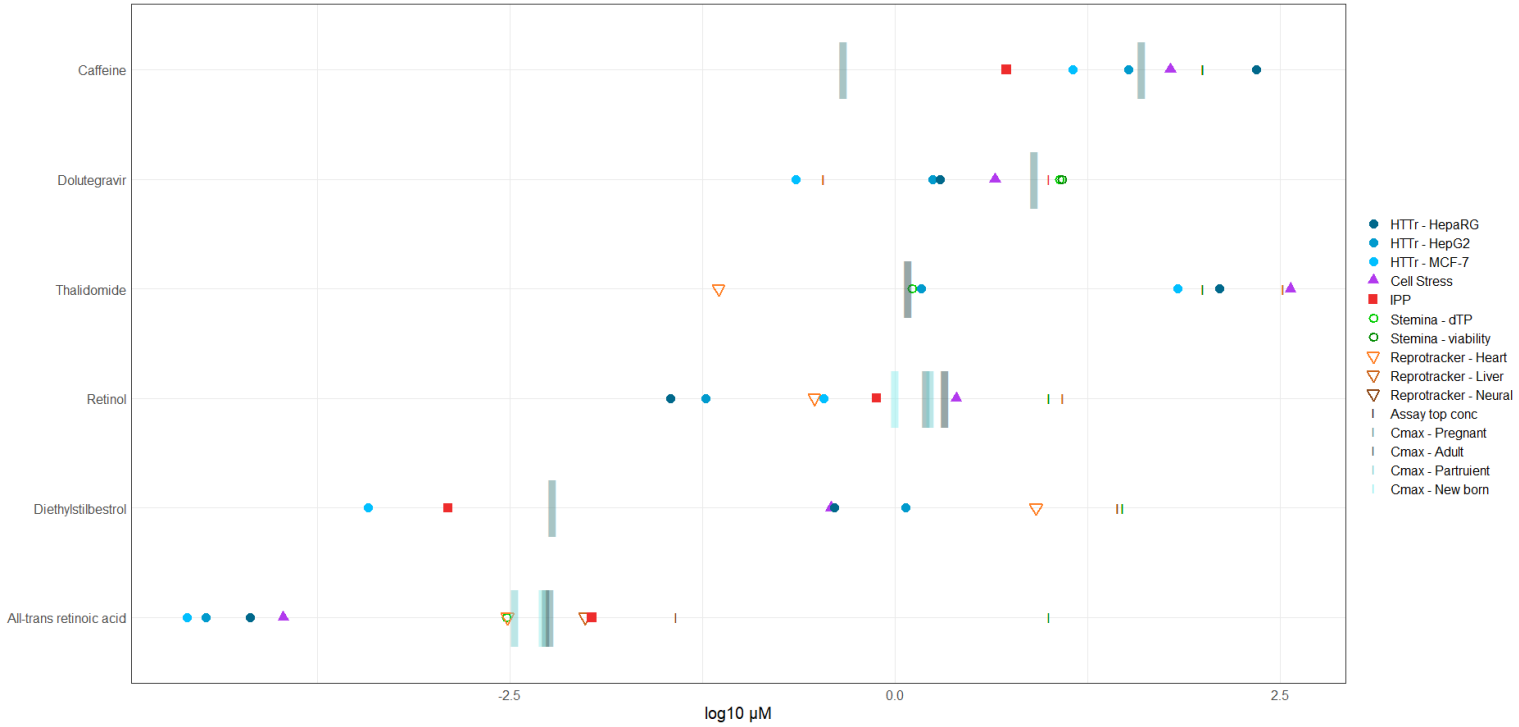
Our DART NGRA framework- a tiered and iterative approach



Our DART NGRA framework- a tiered and iterative approach



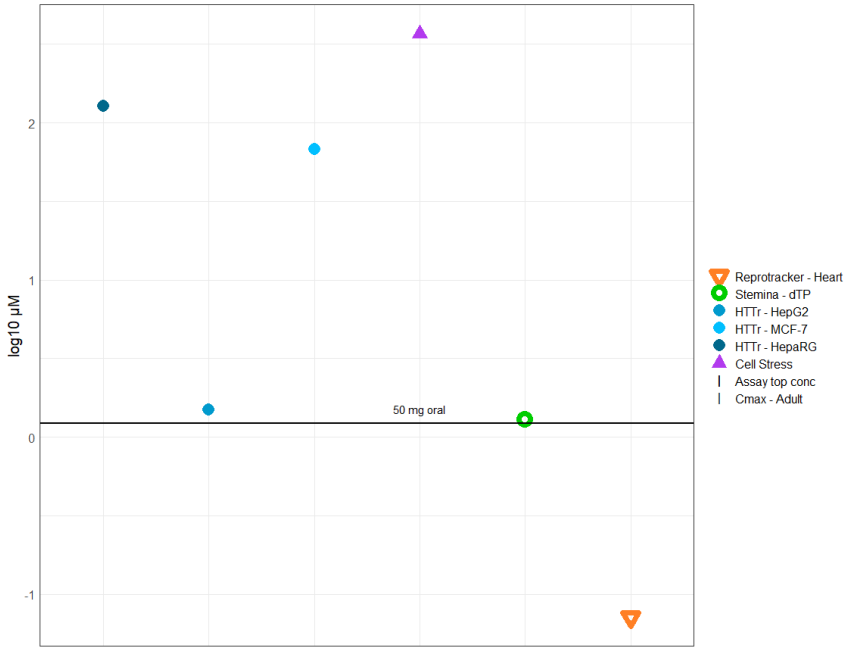
Is our DART framework protective?



Thalidomide

Exposure Scenario

Oral 50 mg tablet daily during pregnancy = risk for pregnancy



Outcome

Bioactivity detected at or below the plasma Cmax = risk for pregnancy

The lowest PoD is coming from the reprotracker cardiac differentiation protocol, with the devTOX qP PoD and HTTR data from HepG2 also providing information on risk

Diethylstilbesterol

Exposure Scenario

Oral 0.5 mg tablet daily during pregnancy = risk for pregnancy

Outcome

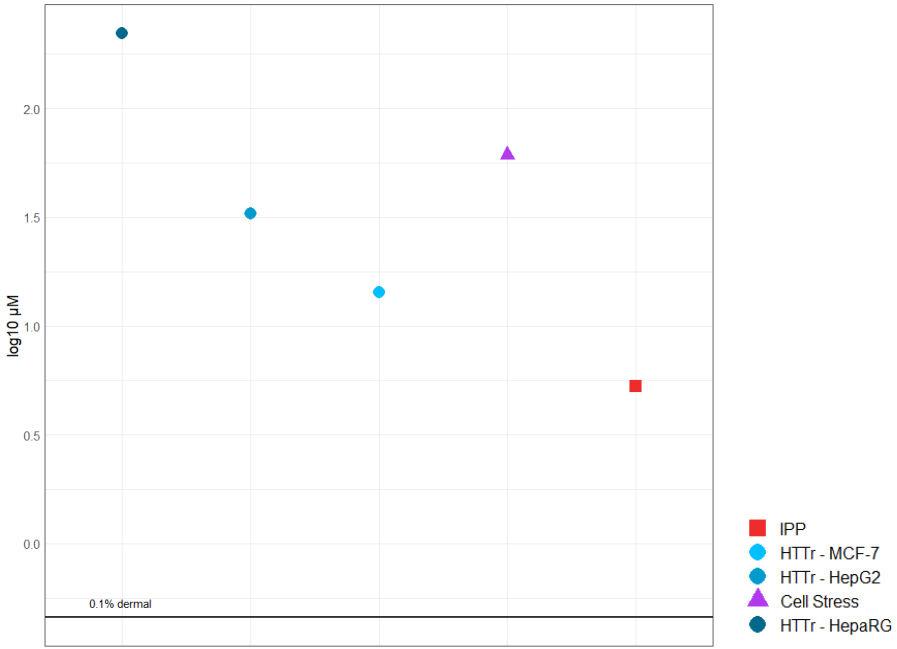
Bioactivity detected at or below the plasma Cmax = risk for pregnancy

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

Caffeine

Exposure Scenario

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



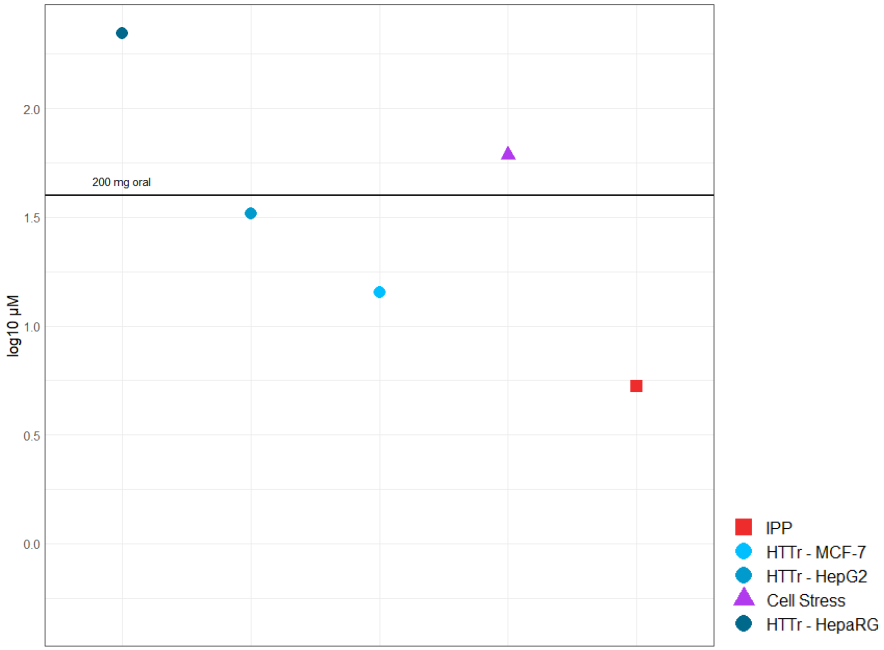
Outcome

Bioactivity across the DART toolbox occurring at much higher concentrations than the plasma C_{max} = low risk for pregnancy

Caffeine

Exposure Scenario

Oral (beverage) consumption of <200 mg caffeine daily during pregnancy = low risk



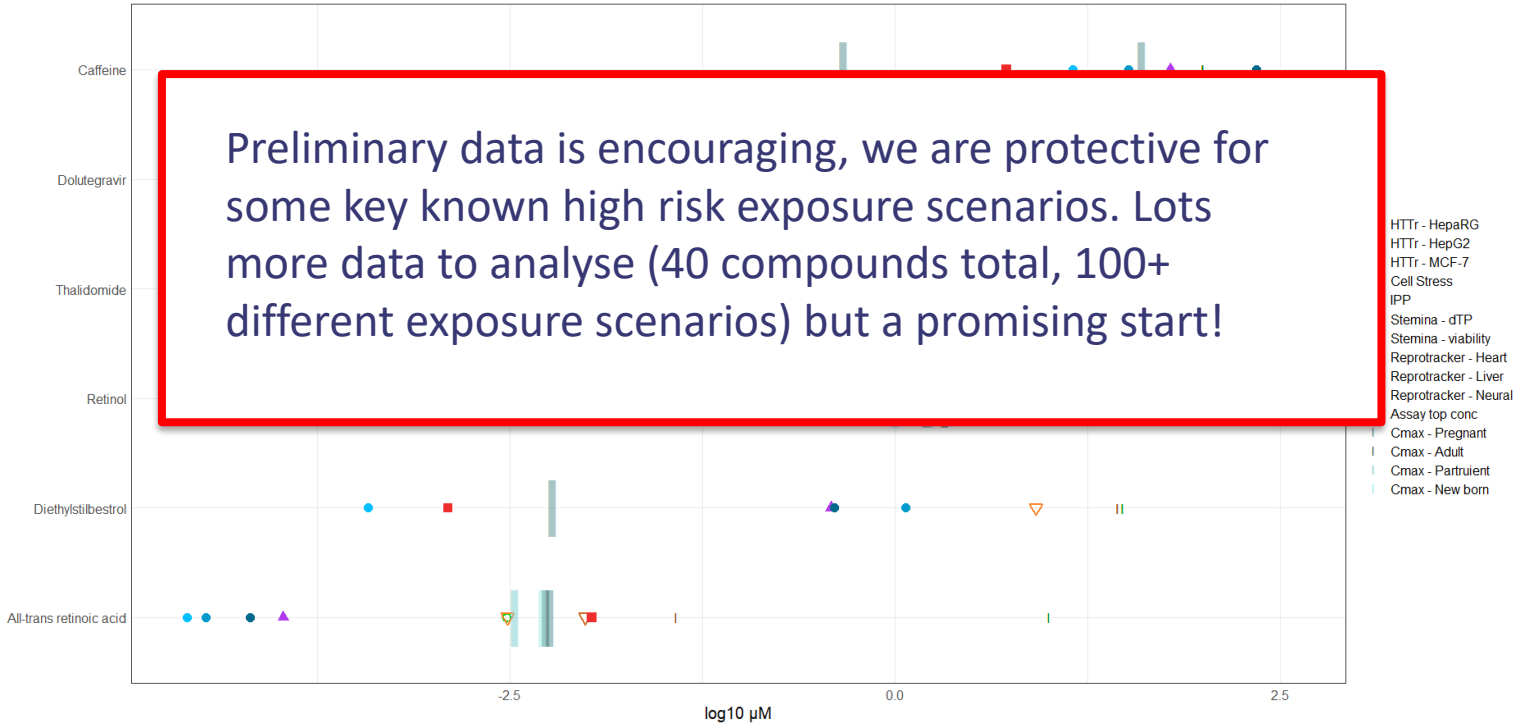
Outcome

Bioactivity detected at or below the plasma Cmax = uncertain risk for pregnancy

The lowest PoD is coming from IPP (5.4µM) which is binding to the adenosine A1 receptor (ADORA1)

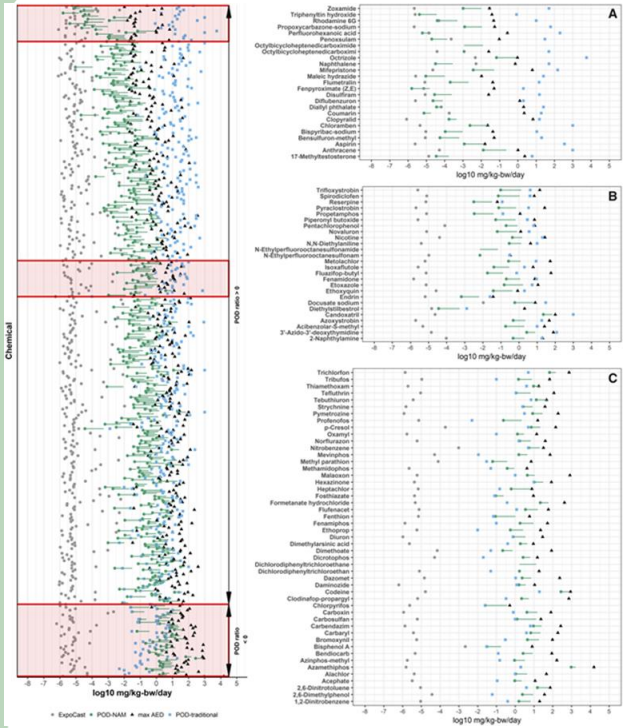
This would trigger additional tiers of testing and further refinement of the risk assessment

Is our DART framework protective?



Preliminary data is encouraging, we are protective for some key known high risk exposure scenarios. Lots more data to analyse (40 compounds total, 100+ different exposure scenarios) but a promising start!

How PODs from NAMs compare to PODs coming from animal studies- including chronic, developmental/reproductive studies



448 chemicals

APCRA
ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

"The primary conclusion of our work is that for 89% of the chemicals in this case study, the HTS approach to derivation of a $POD_{NAM,\ 95}$ for screening and prioritization purposes produced a value less than or equal to the $POD_{traditional}$ from *in vivo* toxicology studies."

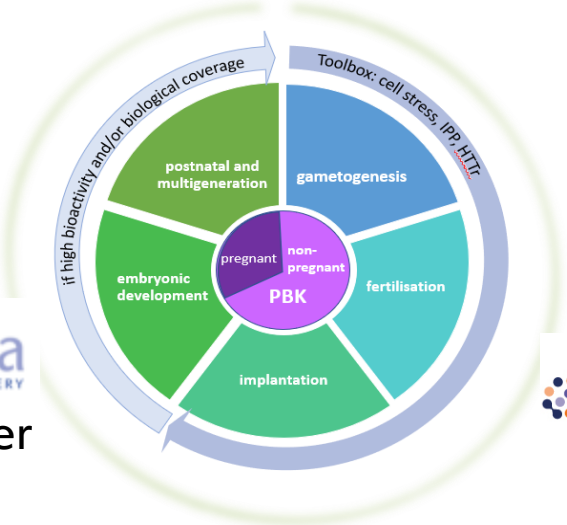
Toxicol Sci, Volume 173, Issue 1, January 2020, Pages 202–225, https://doi.org/10.1093/toxsci/kfz201

#UseScienceNotAnimals

ACKNOWLEDGMENTS

toxys

Giel Hendriks
Amer Jamalpoor
Luke Flatt
Marleen Feliksik



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