Application of an integrated approach using NAMs for DART Next Generation Risk Assessment (NGRA)

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Background and Aims



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

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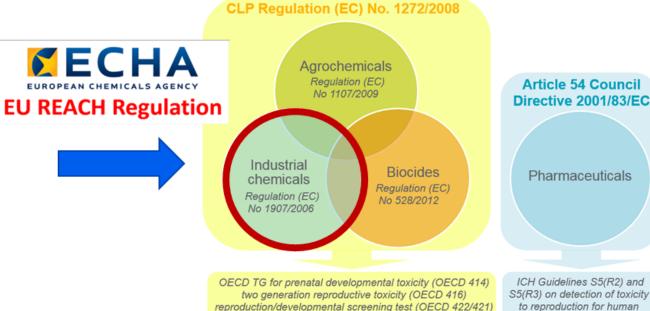
ABSTRACT

An important question in toxicological risk assessment is whether non-animal new approach methodologies (NAMs) can be used to make safety decisions that are protective of human health, without being overly conservative. In this work, we propose a core NAM toolbox and workflow for conducting systemic safety assessments for adult consumers. We also present an approach for evaluating how protective and useful the toolbox and workflow are by benchmarking against historical safety decisions. The toolbox includes physiologically based kinetic (PBK) models to estimate systemic C_{max} levels in humans, and 3 bioactivity platforms, comprising high-throughput transcriptomics, a cell stress panel, and in vitro pharmacological profiling, from which points of departure are estimated. A Bayesian model was developed to quantify the uncertainty in the Cmax estimates depending on how the PBK models were parameterized. The feasibility of the evaluation approach was tested using 24 exposure scenarios from 10 chemicals, some of which would be considered high risk from a consumer goods perspective (eg, drugs that are systemically bioactive) and some low risk (eg, existing food or cosmetic ingredients). Using novel protectiveness and utility metrics, it was shown that up to 69% (9/13) of the low risk scenarios could be identified as such using the toolbox, whilst being protective against all (5/5) the high-risk ones. The results demonstrated how robust safety decisions could be made without using animal data. This work will enable a full evaluation to assess how protective and useful the toolbox and workflow are across a broader range of chemical-exposure scenarios.

Key words: Bayesian modelling; new approach methodologies; point of departure; physiologically based pharmacokinetics probabilistic risk assessment.

The rapid development of new, non-animal approaches for conducting toxicological safety assessments has been driven by several factors. These include ethical considerations, regulatory action (animal test bans for certain types of ingredients), and the need to assure the safety of chemicals using efficient, cost-

et al., 2019). Non-animal approaches also have the potential to improve safety assessments by using more human-relevant tools through coverage of key biological pathways or targets. Next-generation risk assessment (NGRA) provides a way to integrate new approach methodology (NAM) data from various



reproduction/developmental screening test (OECD 422/421) one generation reproductive toxicity (OECD 415/443)

pharmaceuticals

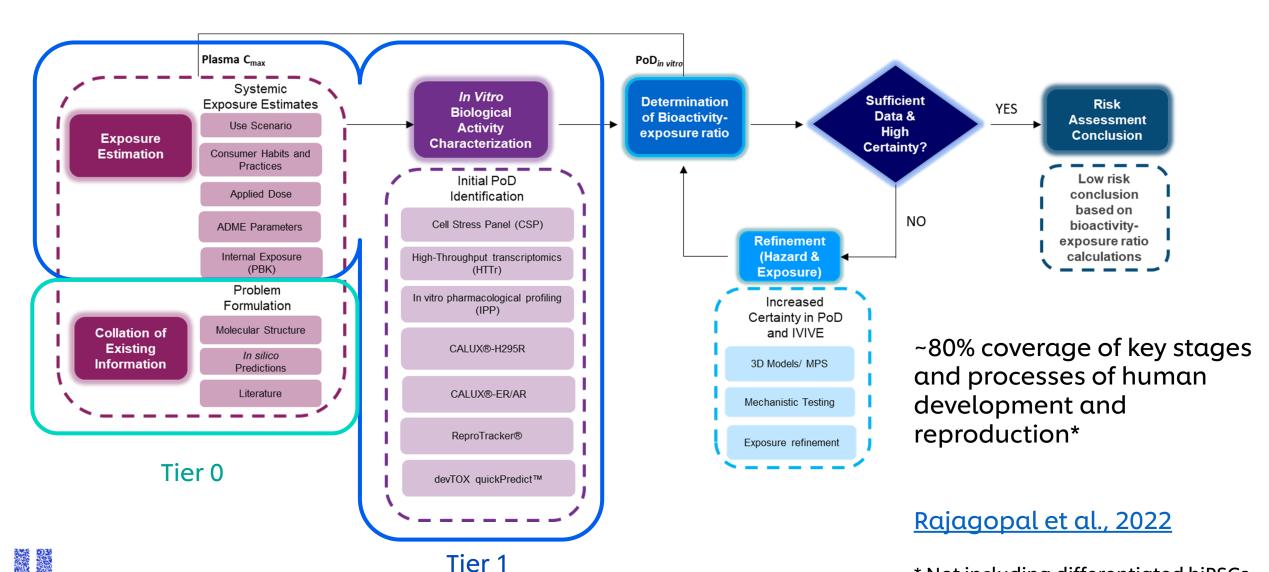
DART being a major endpoint that historically relies heavily on animal testing



Middleton et al. 2022

DART NGRA Framework

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* Not including differentiated hiPSCs and H295R cells

Methods

- 37 compounds
- High Risk, Low Risk and Uncertain Risk- exposure scenarios

Examples:

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 fetus (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)						



Methods

Tier 0- in silico predictions

- Derek Nexus (34 endpoints related to systemic tox and DART)
- OPERA:
 - ✓ OPERA_CoMPARA_Androgen_Receptor
 - ✓ OPERA CERAPP Estrogen_Receptor
- OECD QSAR Toolbox:
 ✓ DART scheme (P&G alerts)
- VEGA:
 ✓VEGA_DEVTOX_PG
 ✓VEGA_ANDROGEN_COMPARA
 ✓VEGA_ESTROGEN_CERAPP

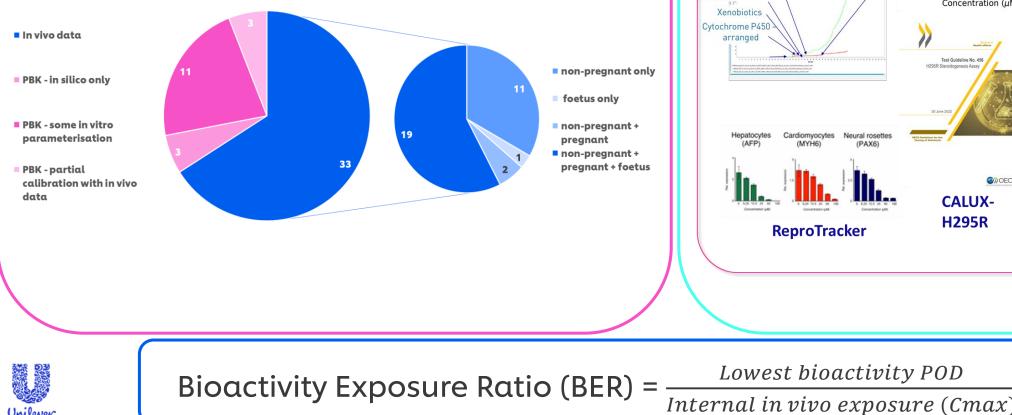


Methods

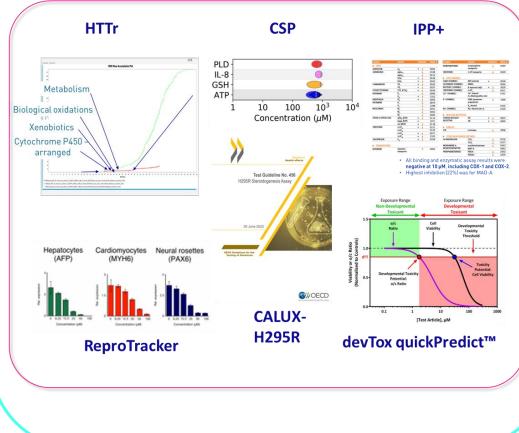
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Tier 1- exposure estimates

Estimation of systemic exposure to a compound through physiologically-based kinetic modelling or clinical data



Tier 1- Bioactivity Characterisation



(uM)

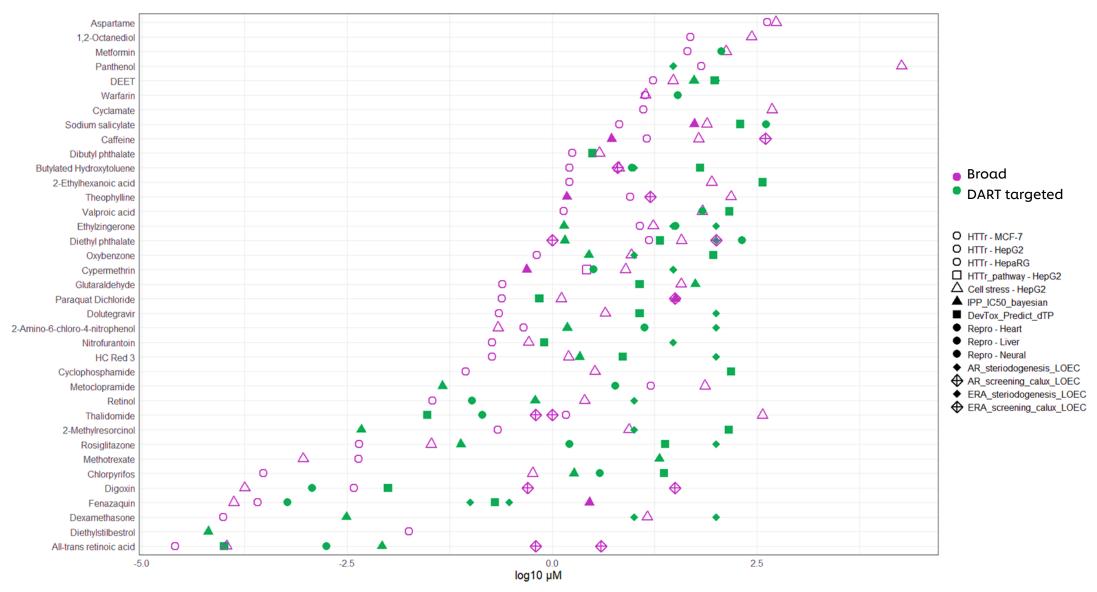
Results-Tier 0

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hloro-4-nitrophenol orcinol	Octanediol													
	nino-6-chloro-4-nitrophenol													
	ethylresorcinol													
	artame													
	aquin													
	nin													
	ngerone													



DART Hazard Flag No DART Hazard Flag

Results-Tier 1





Results-Tier 1

Butylated Hydroxytoluene - Dermal 0.00761 mg/kg bw/day (Cosmetics (aggregate)) Dibutyl phthalate - Oral TDI 0.01 mg/kg bw/day (Food contaminant) Cypermethrin - Oral ADI 0.005 mg/kg bw/day (Diet) Diethyl phthalate - Dermal 0.93 mg/kg/day (Cosmetics solvent)

Warfarin- Oral 5mg/day (Drug)

Oxybenzone - Dermal 0.50% (Cosmetics UV filter) Glutaraldehyde - Dermal 0.10% (Body Lotion) Paraquat Dichloride - Oral ADI (Diet) Metformin - Oral max therapeutic 2 g/day (Drug) Ethylzingerone - Dermal SED 2.9 mg/kg bw/day (Cosmetics (aggregate)) HC Red 3 - Dermal 3% (Hair Colourant) 1,2-Octanediol - Dermal 3% (Hair Colourant) Glutaraldehyde - Oral Chronic Rfd 0.16 mg/kg bw/day (Diet) Caffeine - Dermal 2% (Shampoo) 2-Amino-6-chloro-4-nitrophenol - Dermal 2% (Hair dye) Chlorpyrifos - Oral 0.000075 mg/kg/day (Food exposure)

2-methylresorcinol-Dermal

Cyclamate - Oral ADI 7 mg/kg bw/day (Diet) Metoclopramide - IV 10 mg (Drug) Digoxin - Oral low therapeutic 0.0034 mg/kg bw/day (Drug) Nitrofurantoin - Oral therapeutic 2x100 mg/day (Drug) Metoclopramide - Oral 60 mg (Drug) Caffeine - Oral 100 mg (Food) Chlorpyrifos - Oral Prenatal high exposure () DEET - Dermal 15% (Drug) Sodium salicylate - Oral 162.5 mg (Drug) Methotrexate - Oral 10 mg/week (Drug) Retinol - Oral dietary retinol (Diet) Retinol - Dermal 0.05% (Body Lotion) Dolutegravir - Oral 50 mg (Drug) Diethylstilbestrol - Oral 0.5 mg (Drug) Cyclophosphamide - Oral low therapeutic, 1 mg/kg bw/day (Drug) Fenazaguin - Oral ADI 0.005 mg/kg bw/day (Diet) Caffeine - Oral 400 mg (Food) Rosiglitazone - Oral 4 mg/day (Drug) All-trans retinoic acid - Oral dietary retinol (Diet) Theophylline - IV max therapeutic, 800 mg (Drug)

Thalidomide- Oral 50mg (Drug)

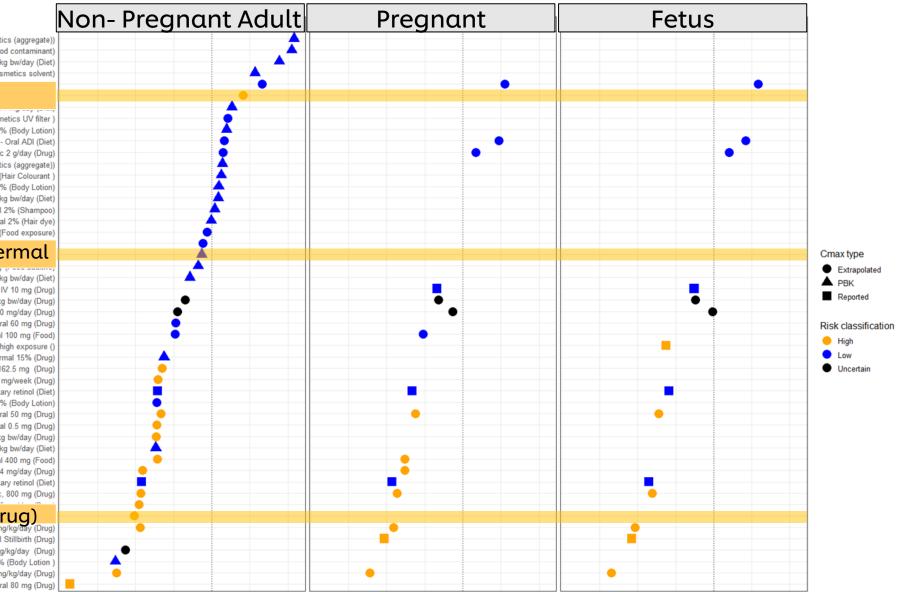
Valproic acid - Oral 10 mg/kg/day (Drug) Sodium salicy/ate - Oral Stillbirth (Drug) All-trans retinoic acid - Dermal 0.02 mg/kg/day (Drug) Panthenol - Dermal 5.30% (Body Lotion) Valproic acid - Oral 60 mg/kg/day (Drug) All-trans retinoic acid - Oral 80 mg (Drug) -5.0

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2.5 -5.0

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Conclusion and Next Steps

- **Conservative** approach and framework is a good starting point for a protective NGRA for DART
- Additional testing of more substances with different MoAs (DNT and thyroid)
- Advanced more physiologically-relevant models are needed for refinement (e.g. placenta transfer).
- Better understanding of pregnant and foetal exposures
- **Expansion of in silico tools** for predicting additional endpoints (e.g. thyroid)
- Uncertainty calculations and models for decision making
- Expansion of **biological coverage** understanding

(11)

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