Evaluation of a Next Generation Risk Assessment Framework for Developmental and Reproductive Toxicity

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Building Our DART NGRA Framework

A comprehensive chemical safety assessment will always consider the potential for developmental and reproductive toxicity (DART). Traditionally huge numbers of animals have been used to assess numerous defined apical endpoints relating to DART (e.g. pregnancy duration, foetal malformation and sexual maturity) as well as assessing more non-specific or general systemic effects. These guideline animal studies provide a protective approach to ensuring human safety and allow derivation of a point of departure appropriate for human health risk assessment. Encouraged by recent evidence that NGRA approaches may also provide a protective approach for ensuring human safety (Paul-Friedman 2019, Middleton, 2022) we built a fit for purpose NGRA framework to evaluate if NAM-based exposure-led safety assessments can be sufficiently protective for developmental and reproductive toxicity. The framework is designed to be tiered and iterative with Tier 1 evaluated here.

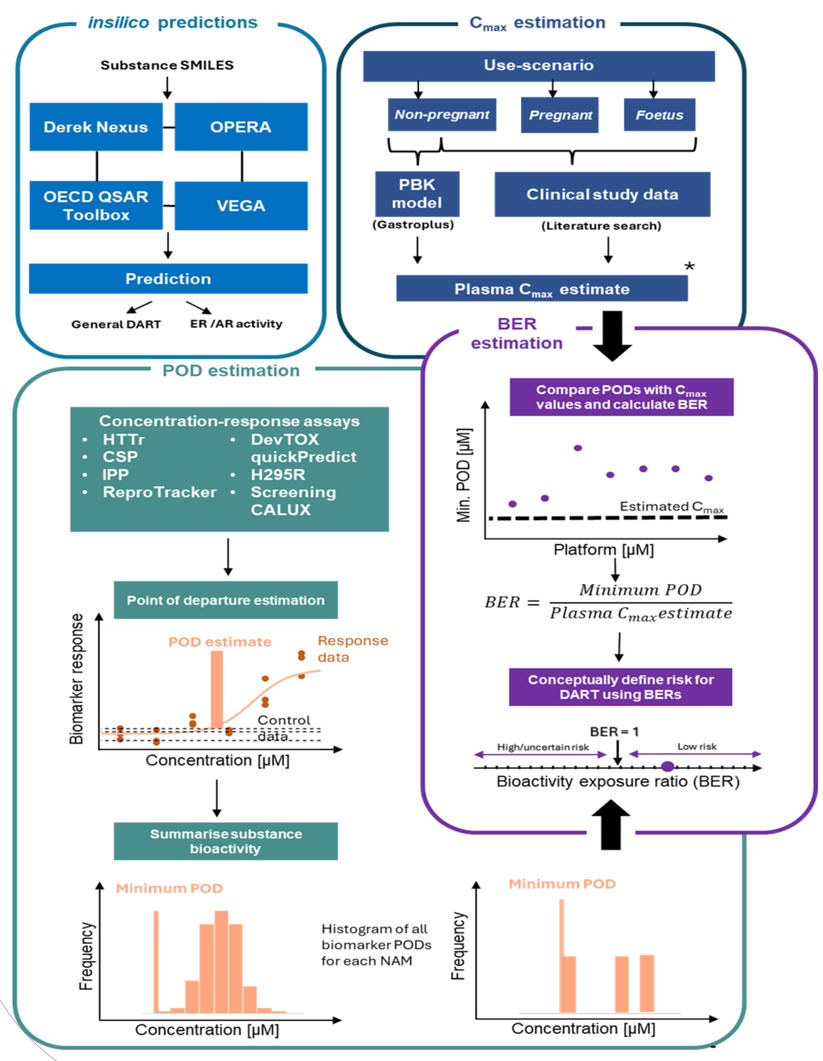

Figure 1: DART NGRA Framework *Combines DART specific in silico predictions and estimation of human exposure at different life stages (via PBK modelling or extrapolation from clinical data) with estimation of chemical bioactivity. Bioactivity is characterised across a panel of biomarkers related to general xenobiotic cell stress pathways (CSP),high-throughput transcriptomics (HTTr) in three cell lines (MCF7, HepG2, HepRG) and through a panel of in vitro pharmacology assays across targets associated with adverse human safety outcomes (~75 targets; GPCRs, enzymes, ion channels and NHRs). To broaden the biological coverage of our NGRA framework with respect to the human reproductive life cycle (as described in Rajagopal 2022) we included additional NAMs into our framework to characterise chemical bioactivity. These include assays measuring iPSC metabolism (DevTox qP) and differentiation (ReproTracker) as well as assays measuring specific endocrine related activity (e.g. H295R steroidogenesis assay, AR/ER CALUX assays). Points of departure (PoD) are calculated from the concentration-response data across all assays and the minimum PoD is compared to the estimated plasma Cmax value for the specific chemical exposure. The Bioactivity exposure ratio (BER) provides an estimation of risk for that chemical exposure, in a similar way to traditional risk assessment (Middleton, 2022).*

Evaluating Our DART NGRA Framework

37 benchmark compounds were selected to evaluate the framework. Compounds were selected to provide at least one human exposure scenario, and to include a variety of different consumer uses (e.g. pharmaceutical, cosmetic, plant protection, food). We assigned each of the chemical-exposure scenarios a risk classification with respect to DART. These risk classifications are considered the 'truth' and form the basis for how we determine if our NGRA framework is sufficiently protective. 7 examples are provided below, in total there are 49 unique exposure scenarios across the 37 chemicals.

Chemical	CAS Number	Exposure Scenario	Exposure Risk
1,2-Octanediol	1117-86-8	Cosmetic, 5% in body lotion	Low Risk
Cyclophosphamide	6055-19-2	Pharmaceutical, 60 mg/daily	High Risk
Panthenol	16485-10-2	Cosmetic, 5.3% in body lotion	Low Risk
Salicylate	69-72-7	Pharmaceutical, 162.5 mg/daily	Uncertain Risk
Thalidomide	50-35-1	Pharmaceutical, 50 mg/daily	High Risk
Theophylline	58-55-9	Pharmaceutical, 800 mg/daily	High Risk
Theophylline	58-55-9	Dietary, 0.14 mg/daily	Low Risk

Chemicals were classified as either high, low, or uncertain risk for DART based on existing risk assessments using traditional methods. This information was gathered from authoritative and regulatory sources. For protection of human health an optimal NGRA framework would identify and flag all high risk exposures (as defined by traditional risk assessment). Conceptually a BER <1 indicates bioactivity (and therefore possible toxicity) at that chemical exposure. Therefore, in our evaluation of protectiveness we looked to see if our NGRA framework allowed us to calculate a BER of 1 or below for all our high risk chemical exposure scenarios.

A)

In Silico Profiling the 37 Benchmark Compounds

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					

B)

PREFERRED_NAME	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												
Aspartame												
ATRA												
BHT												
BP3												
Caffeine												
Chlorpyrifos												
Cyclophosphamide monohydrate												
Cypermethrin												
DBP												
DEET												
DEP												
DES												
Digoxin												
Glutaraldehyde												
MTX												
Nitrofurantoin												
Retinol												
Thalidomide												
Theophylline												
VPA												
Warfarin												

Figure 2: In Silico Profiling **A)** The benchmark compounds were assigned a hazard classification as toxic or nontoxic with respect to DART. For a chemical to be assigned as toxic there had to be some evidence of DART in either animal or human studies. Most of the DART toxic chemicals have been correctly identified by at least one of the general DART in silico models. Only one toxicant (Metoclopramide) has been falsely predicted as nontoxicant by all models. **B)** Toxcast AR/ER pathway models were used as our source of truth for ER/AR activity. The Pathway Model provides a value (from 0 to 1) for activity. Value >0.1 equals significant interaction. 2/37 compounds had significant interaction with ER. In silico models identified interaction for 1 of the 2.

Is Our DART NGRA Framework Protective for Human Health?

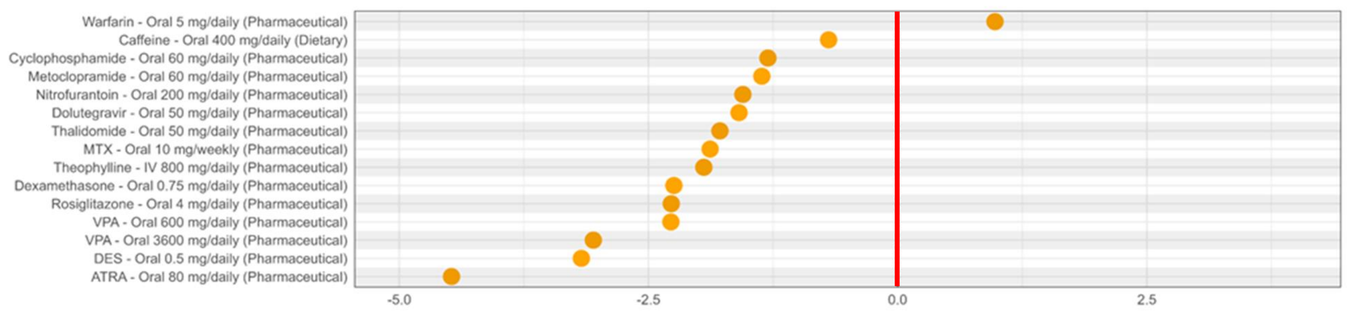

Figure 3: High risk chemical exposure scenarios The above chemical exposures are considered high risk for DART via traditional risk assessment. The red line indicates a BER of 1. Conceptually a BER <1 (left of the red line) indicates bioactivity (and therefore possible toxicity) at that chemical exposure. An optimal outcome for protectiveness would be for all high risk exposures to have a BER <1. As seen above, the majority of high risk exposures have a BER <1. Only one high risk chemical exposure (Warfarin, 5mg/daily oral, pharmaceutical) was missed by Tier 1 of our NGRA framework. Warfarin has a BER of >1 and is shown to the right of the red line.

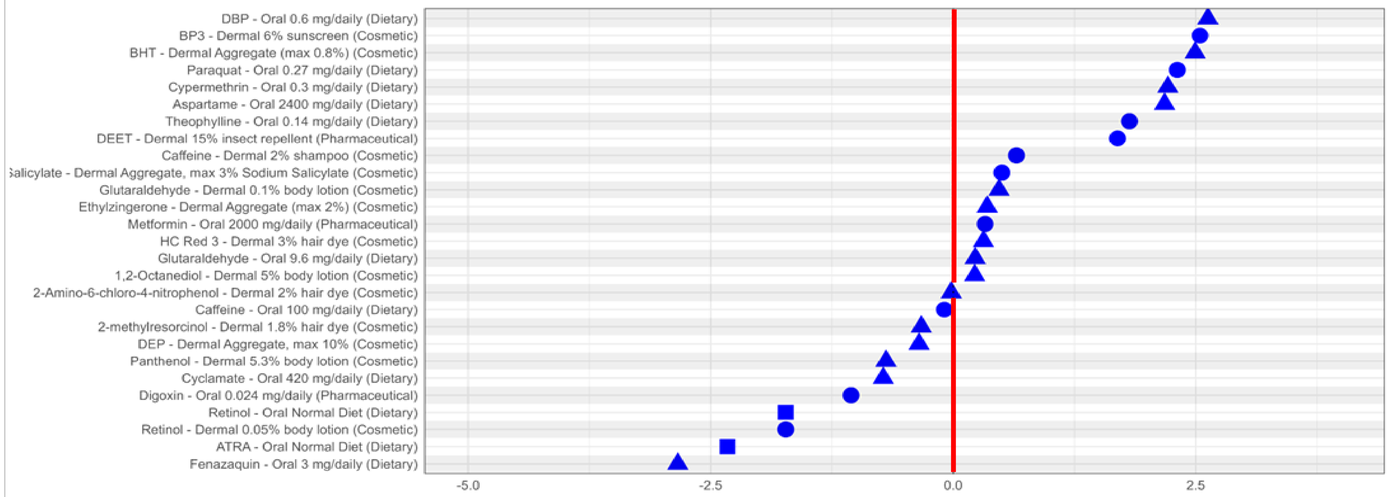

Figure 4: Low risk chemical exposure scenarios The above chemical exposures are considered low risk for DART via traditional risk assessment. The red line indicates a BER of 1. In addition to being protective for high risk chemical exposures we wanted to design a framework that could separate true low risk exposures. The utility of the framework is a measure of this. In our evaluation of utility, we looked to see if we could calculate a BER of above 1 (right of the red line) for our low risk exposures. 16/27 low risk exposures have a BER of above 1, indicating lack of bioactivity at human exposure.

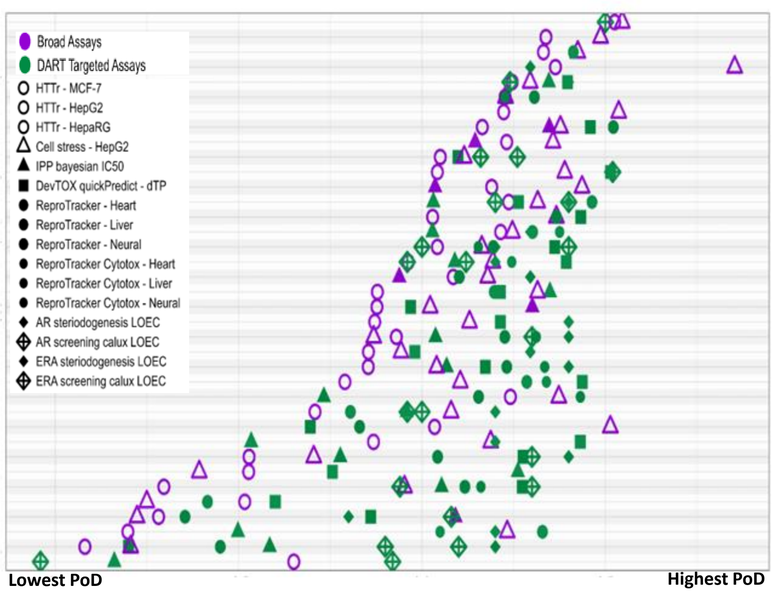

Figure 5: How do we achieve our protection goal? *Y axis each row is a chemical exposure Compound labelling is not included. This overview shows the lowest PoD from each NAM corresponding to dose - concentration calculations. NAMs are separated as DART targeted (green) versus broad screening (purple). IPP was split in two NAMs (general targets and targets associated with DART). Figure 5 shows that protectiveness is achieved through a combination of broad screening tools and more targeted/specific NAMs*

Summary

- **NGRA uses tiered and iterative frameworks to make safety decisions that are designed to prevent harm. The initial tier of such approaches (as presented here) are constructed to provide a conservative and protective approach.**
- **This is the first proof of concept study which demonstrates that a DART NGRA framework can provide a protective approach for human health risk assessment.**
- **Further work is needed to improve the protectiveness of tier one of this framework. Extended testing with more substances with different modes of action is needed to build scientific confidence and to fill existing gaps**
- **Development of advanced MPS systems is required for testing in additional tiers of the framework**

Literature

1) Paul-Friedman et al., Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization. Toxicol Sci. 2020
2) Middleton et al., Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow. Toxicol Sci. 2022
3) Rajagopal et al., Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing. Front. Toxicol. 2022

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