MPS in safety assessment for DART and endocrine disruption: an industry perspective

Iris Müller[1]*; Paul Carmichael[1]; Matthew Dent[1]; Rick Greupink[2]; Jade Houghton[1]; Hedwig van Hove[2]; Predrag Kukic[1]; Hequn Li[1]; Beate Nicol[1]; Magdalena Panczuk[3]; Gopal Pawar[1]; Claire Peart[1]; Mathew Van De Pette[3]; Damian Roelofsen[2]; Magdalena Sawicka[1]; Sandrine Spriggs[1]; Katy Wilson[1]; Kathryn Wolton[1]

[1] Unilever, Sharnbrook, United Kingdom; *Iris.Muller@unilever.com
[2] Radboud UMC, Nijmegen, Netherlands
[3] MRC tox Unit, University of Cambridge, United Kingdom

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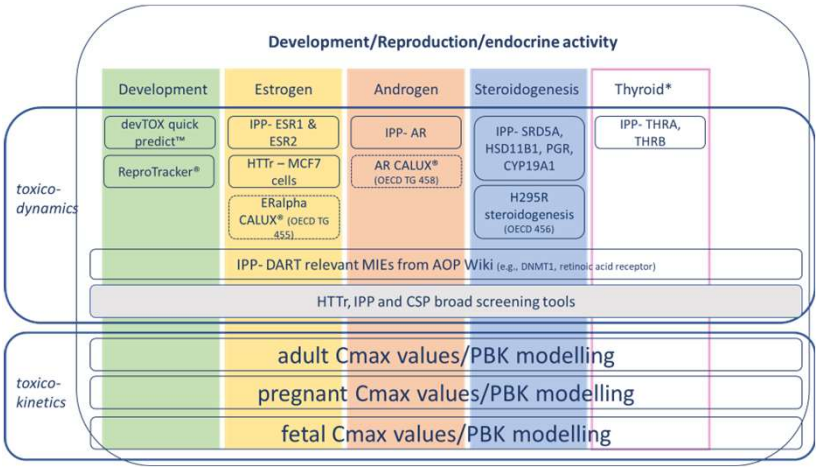
Building a" fit for purpose" toolbox ensuring sufficient protectiveness for DART/ED consumer safety assessments



The toolbox serves as a pragmatic first step to evaluate the safety of an ingredient for developmental and reproductive toxicity (DART) without the use of animal tests. A comparison of the combination of all these NAMs with known molecular events in human reproduction and embryonic development showed comprehensive biological coverage of our NAMs with traditionally evaluated endpoints[2]. We have already identified gaps in our framework; some we are looking to fill currently (e.g. placenta transfer) and others we need to consider further in future (e.g. thyroid). Advanced cell models/MPS will help to fill the gaps and to refine risk assessments in future.

Figure 1: NAM toolbox for DART following a Next Generation Risk Assessment approach. Overview of DART toolbox aligned with different NAMs for their DART/ED relevant endpoints. The toolbox has been designed to provide best biological coverage for DART/endocrine activity combining broad screening tools (HTTr - high throughput transcriptomics, CSP – cell stress panel and IPP - in vitro pharmacological profiling), which are also used within our systemic tox toolbox (poster ID #161) complemented with NAMs with DART specific endpoints (ReproTracker® (Toxys) and the devTOX quickPredict™ assay (Stemina) for developmental toxicity, DART specific IPP endpoints, CALUX® assays). The CALUX ® assays in boxes with dashed lines can be used for refinement of IPP results reflecting on cellular responses to a compound rather than receptor binding. Cmax values for adult, pregnant woman and foetus will be used to account for physiological changes in between the life stages. Bioactivity exposure ratios (BERs) will be calculated to inform risk assessment. *The testing strategy for thyroid is work in progress.

Evaluation and application of the DART framework/toolbox – first results

The protectiveness of the DART toolbox will be evaluated using ~40 benchmark compound . Exposure scenarios with known risk will be used to compare the estimated corresponding systemic exposures against PoDs from the described in vitro tools. Maximum plasma concentrations for non-pregnant and pregnant females as well as for the developing fetus are derived either on the basis of data from clinical pharmacokinetic studies or by making predictions applying PBK modelling approaches.

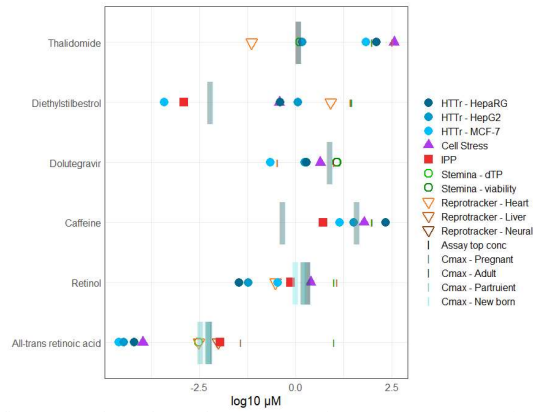
50mg oral application of Thalidomide, high risk, causing dev. toxicity.

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 .

Figure 2: Evaluation of DART toolbox for the first 6 compounds tested . *Point of Departure (PoD) values in comparison to Cmax values (graph) with relevant exposure scenarios (left) and interpretation for risk assessment (right).*

Filling gaps using advanced cell models/MPS systems – example placenta transfer

For the prediction of fetal exposures, characterisation and parameterisation of placenta transfer was identified as a gap to fill for PBK modelling. To build capability two tissue/cell models are evaluated; transfer parameters will be measured and incorporated into PBK modelling approaches. While the placenta perfusion system works as the gold standard it allows only short-term experiments. The BeWo b30 system on the other side might be a too simple cell system and an organoid or MPS system might be needed to give more physiological responses.

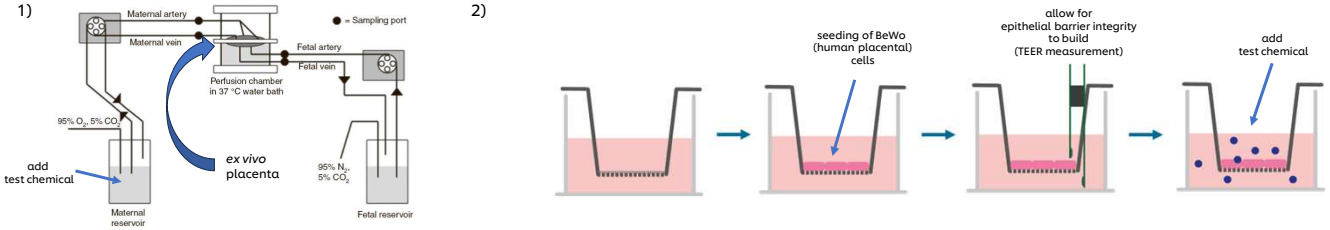


Figure 3: Tissue/cell models measuring placenta transfer. Schemes of **1)** the ex vivo placental perfusion setting and 2) BeWo b30 transwell system. Test chemical are added at the "maternal side" and samples are collected over time to measure transfer through the placenta.

Filling gaps using advanced cell models – example multigenerational inheritance

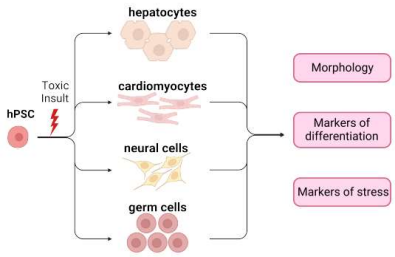


Figure 4: Comparing toxic effects in somatic versus germ cell development

- The impact of either environmental or pharmacological agents on the human germline remains poorly understood
- Traditionally, rat models have been used to assess toxic effects that might persist through generations
- Reproducibility and human relevance of these studies have been questioned
- Developing an in vitro method of assessing long lasting toxic effects will help us understand the mechanisms involved in multigenerational inheritance

Literature

1) Middleton et al., 2022 Aug 25;189(1):124-147
2) Rajagopal et al., 2022 Mar 7;4:838466
3) Berggren Soderlund et al., 2005 Mar;81(3):633-6
4) Kirkwood-Johnson et al., 2021 Nov 24;184(2):191-203

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