The value of understanding

ReproTracker: Human Stem Cell-Based Biomarker Assay for Screening of Developmental Toxicity

EPAA meeting- 15/11/2022

Toxys B.V.
Leiden, The Netherlands

Animal testing for DART

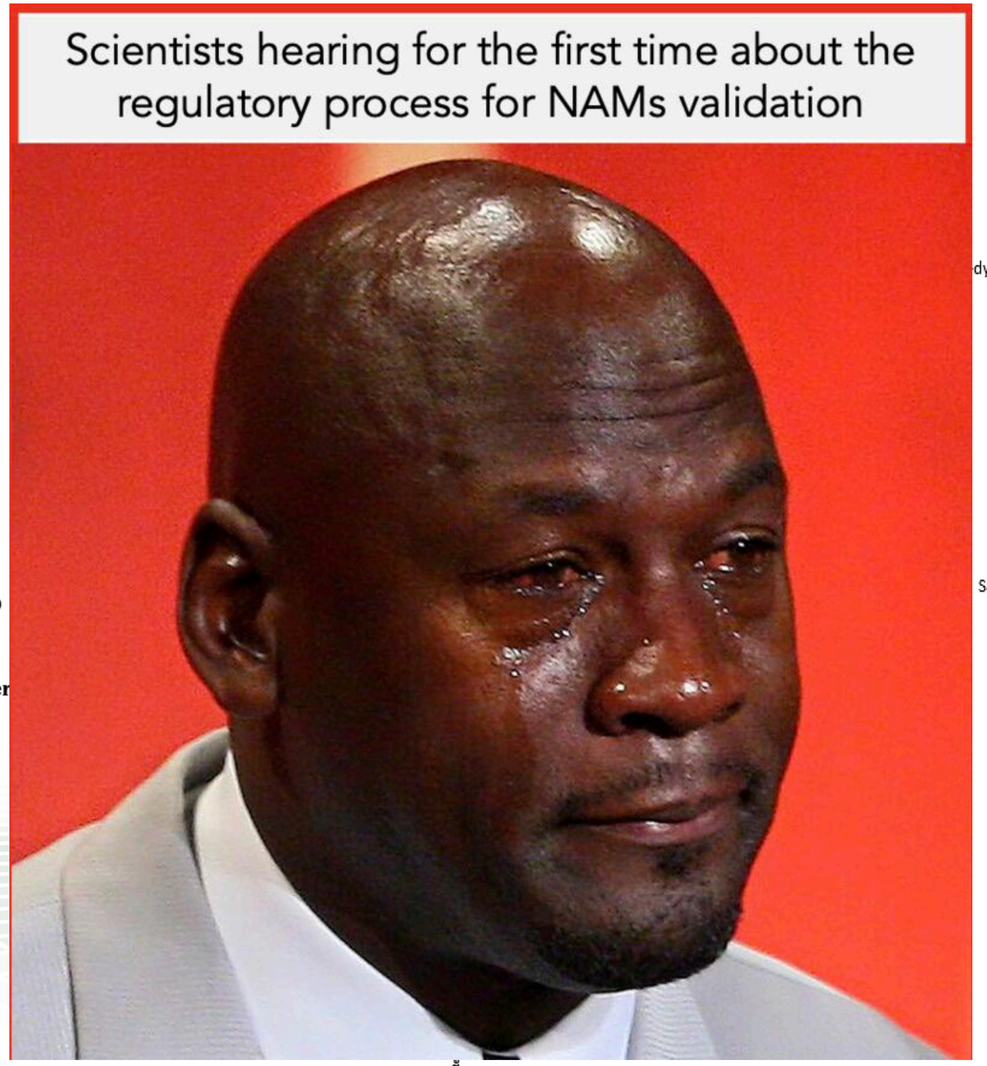
EUROPEAN COMMISSION

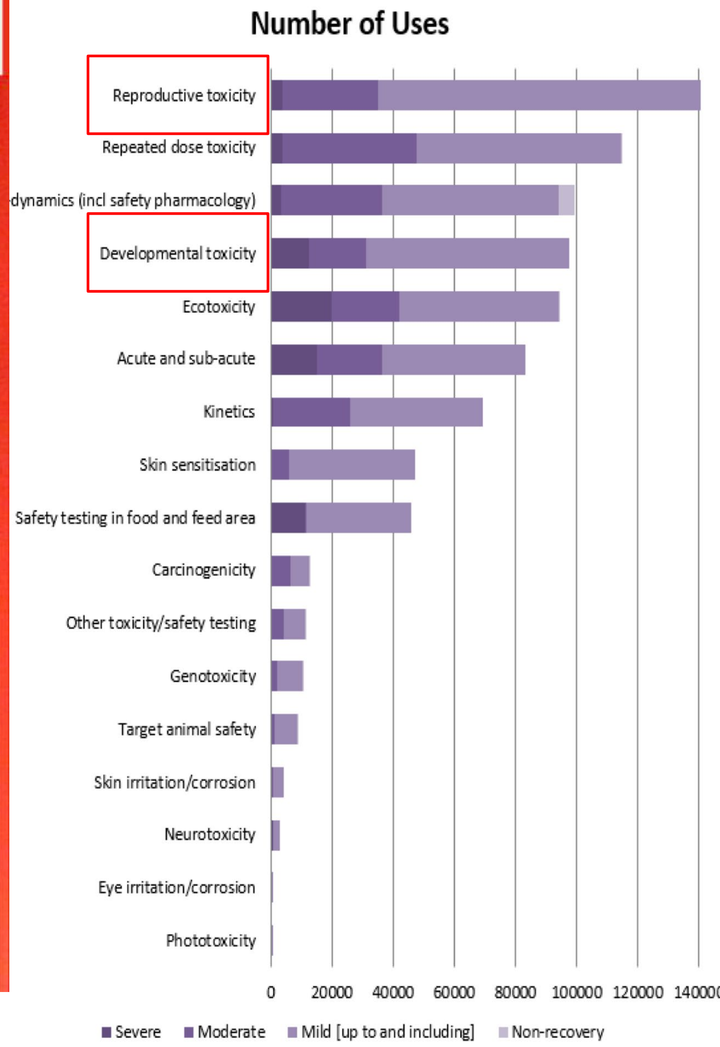
Brussels, 5.2.2020
COM(2020) 16 final

REPORT FROM THE COMMISSION TO THE EUROPEAN PARLIAMENT AND THE COUNCIL

2019 report on the statistics on the use of animals for scientific purposes in the Member States of the European Union in 2015-2017

{SWD(2020) 10 final}



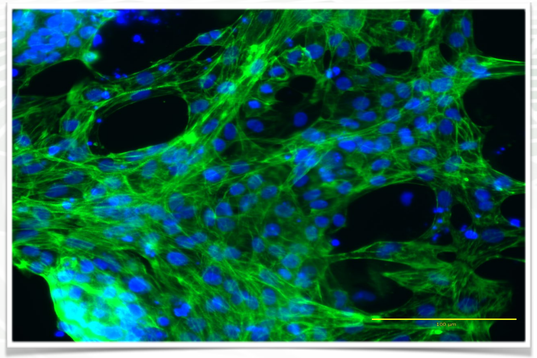


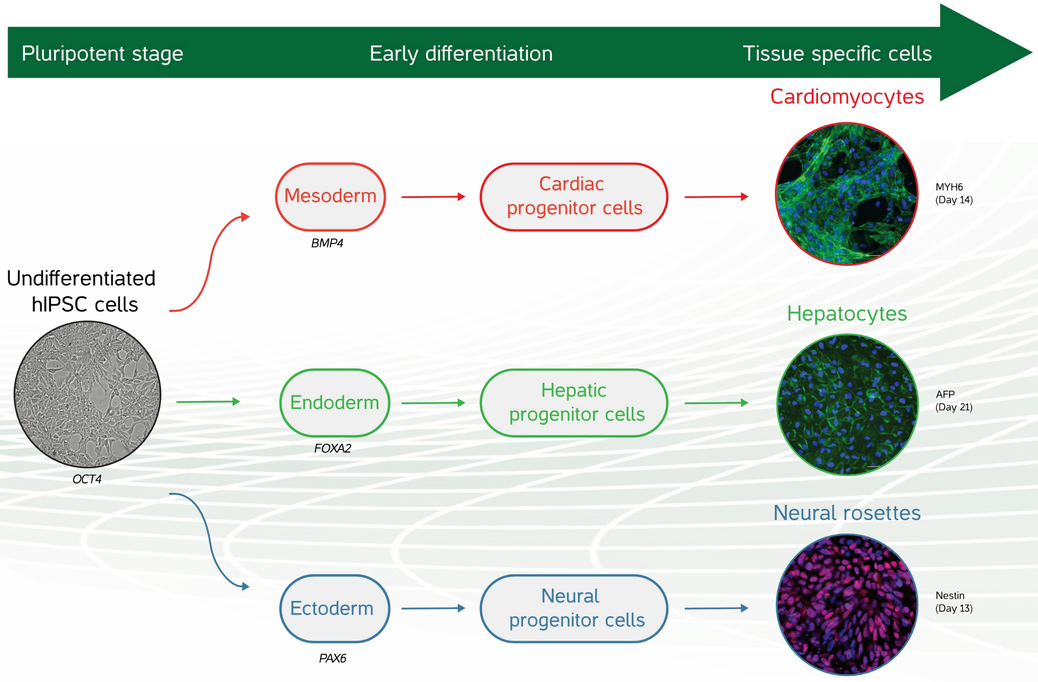
The ReproTracker® assay

Key features:

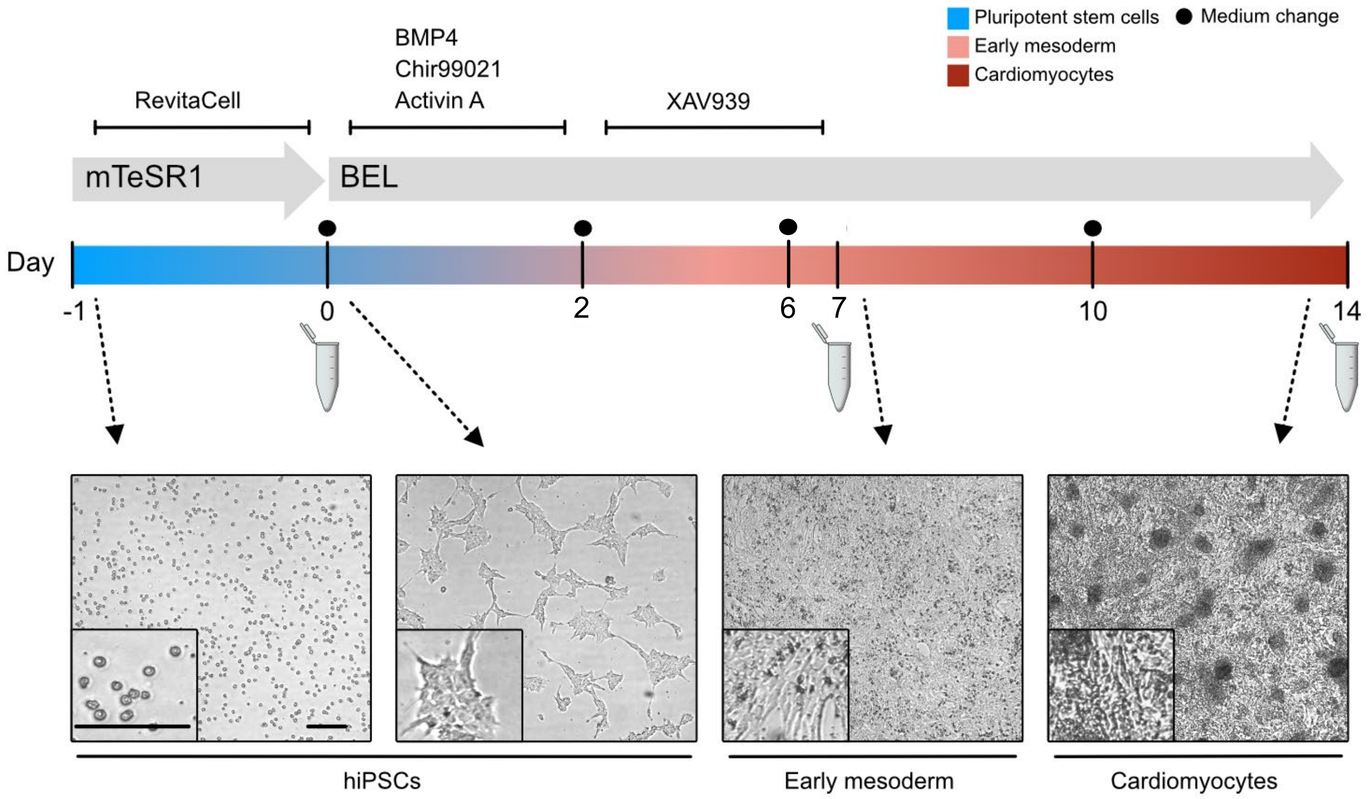
- Human test system
- *In vitro* development of functional heart, liver and neural tissues
- Visualization of the key cellular events of early embryonic development
- Detect disruption of developmental program based on morphological and molecular read-out

REPROTRACKER®

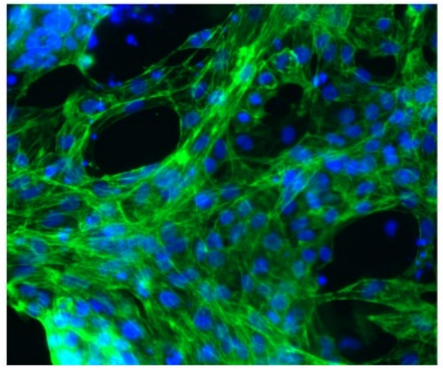




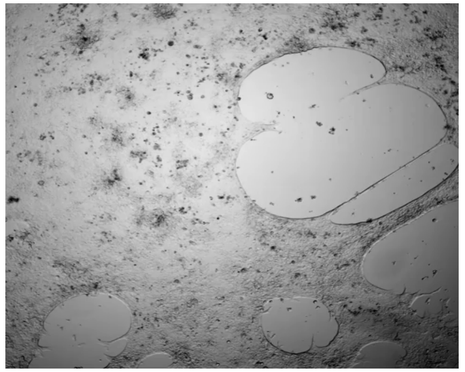
ReproTracker® assay – Cardiomyocyte differentiation

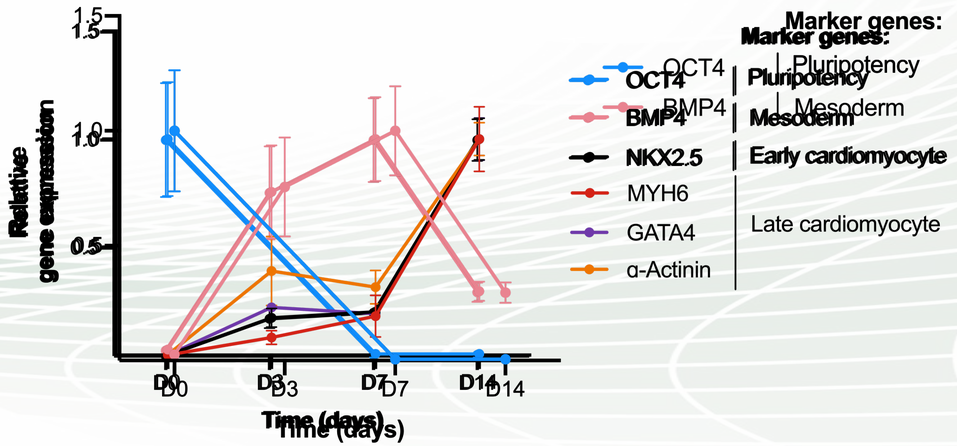


TNNT2



Cardiomyocyte beating

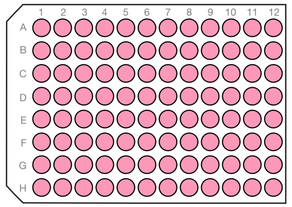




ReproTracker protocol

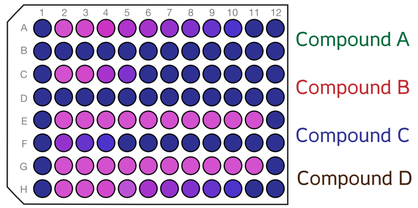
1. Dose range finding

Undifferentiated human iPSC

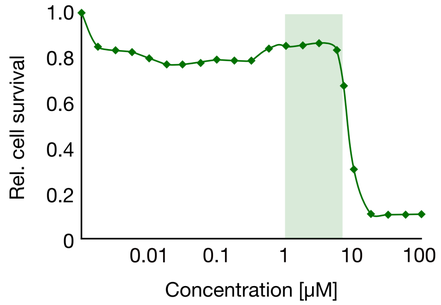


Expose hiPSC to 20 conc. for 7 days

Alamar Blue cell viability assay

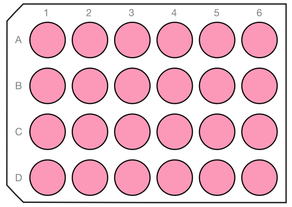


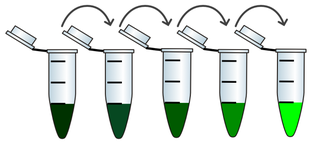
Dose selection



2. Stem cell differentiation

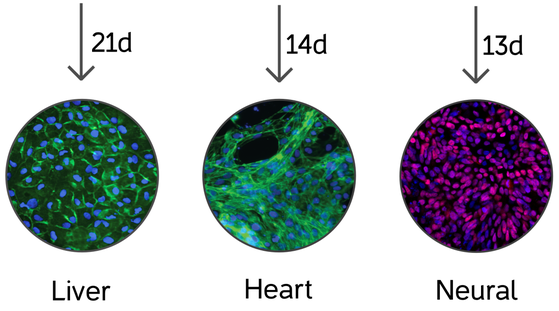
Undifferentiated human iPSC





Expose hiPSC to 5 conc.

Differentiate pluripotent stem cells



3. Biomarker analysis

Morphological profiling

- Tissue morphology
- Beating cardiomyocytes
- Liver/neural marker expression
- Toxicity

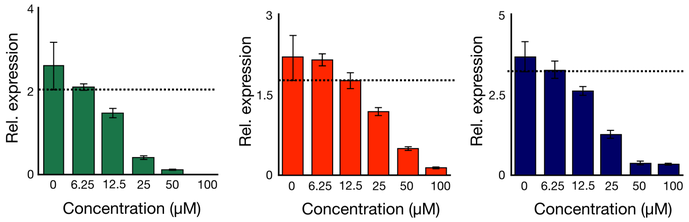
Biomarker expression

- Multiple time points
- RNA isolation
- Quantitative real time PCR

Hepatocytes (AFP)

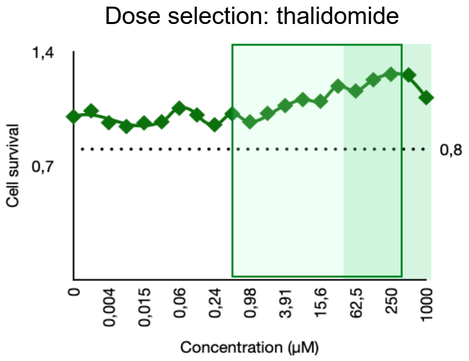
Cardiomyocytes (MYH6)

Neural rosettes (PAX6)

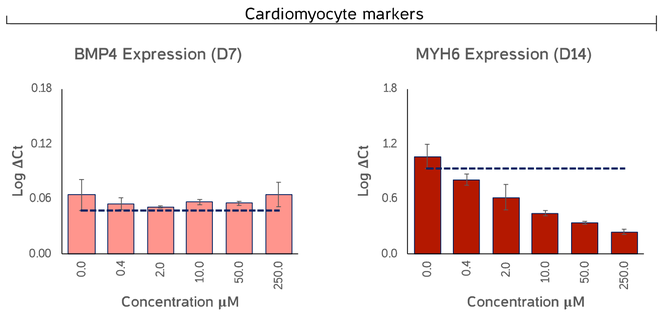


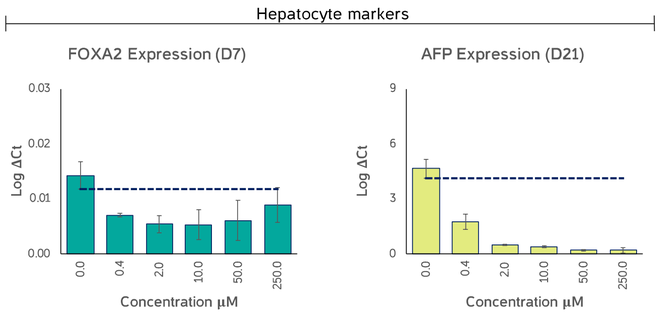
ReproTracker® examples - Teratogens

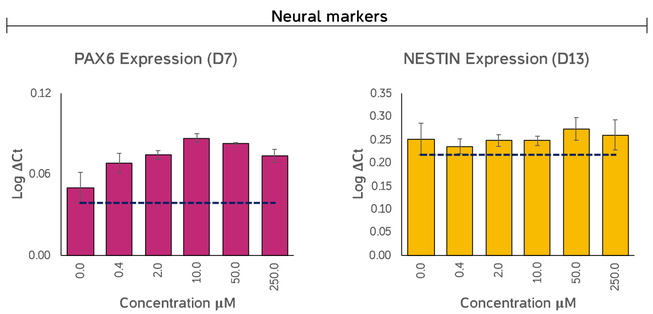
Dose selection



Biomarker analysis

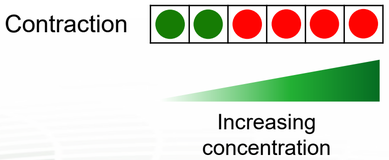


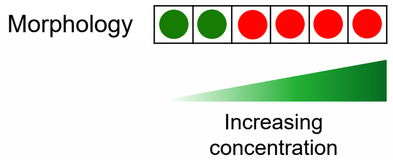


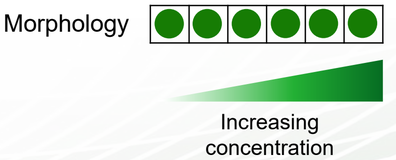


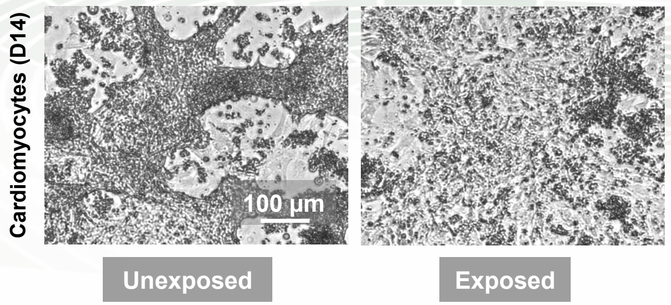
Clinically relevant concentration is between 1-6 µM

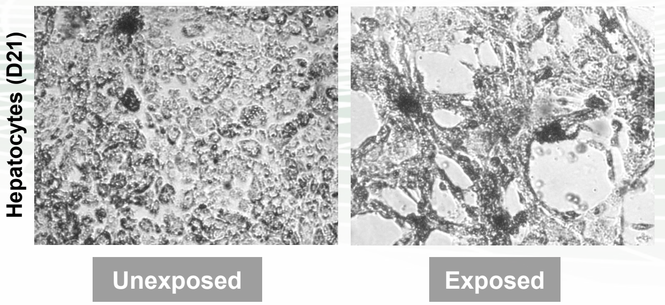
Morphology analysis

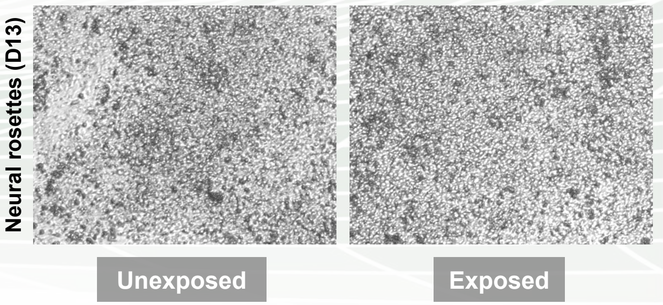






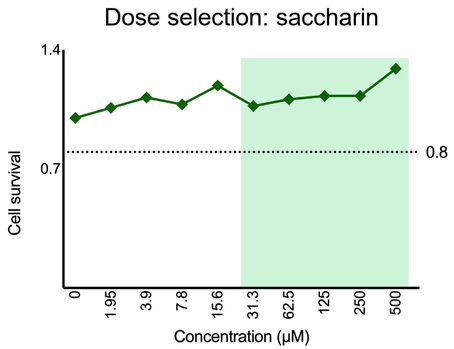




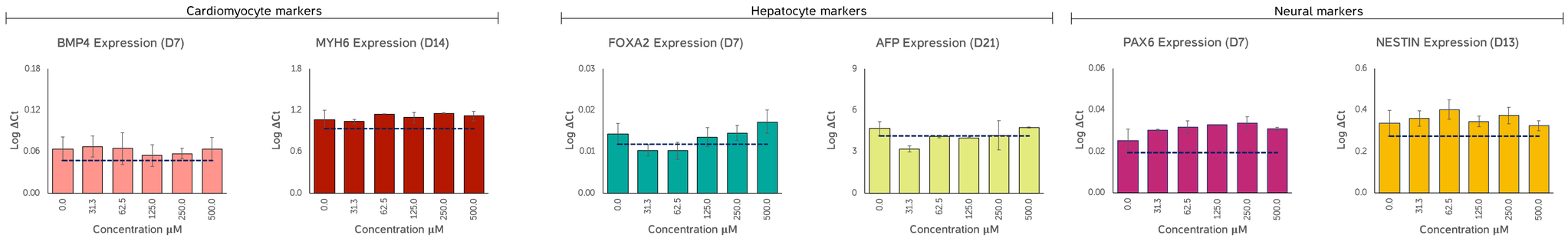


ReproTracker® examples – Non-teratogens

Dose selection

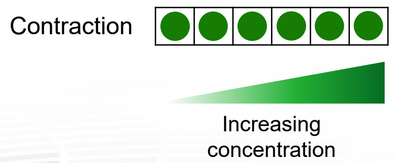


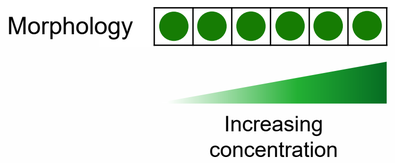
Biomarker analysis

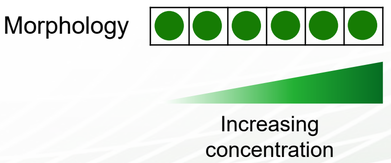


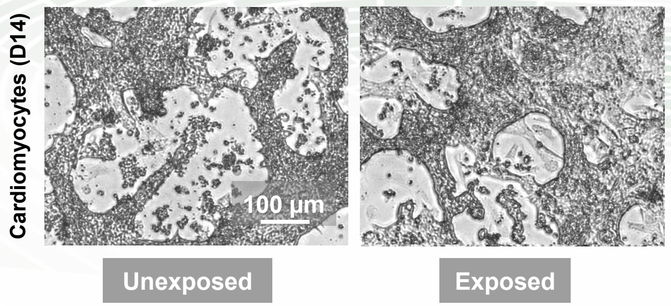
Morphology analysis

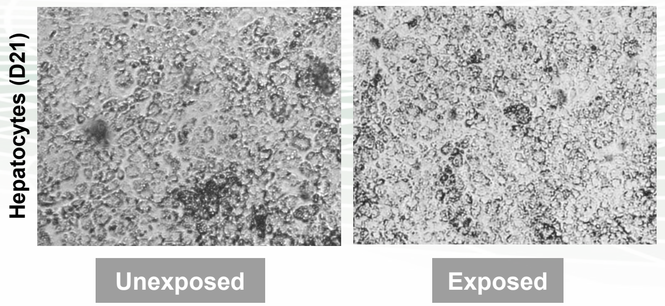
Clinically relevant concentration is 1.5 µM

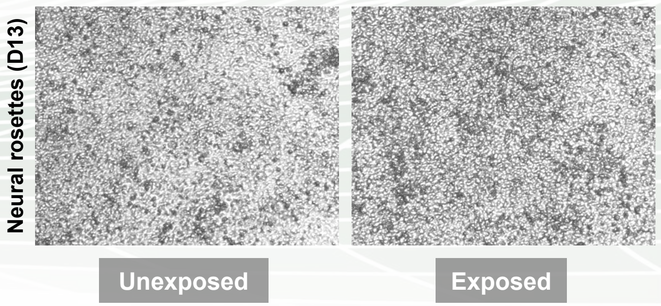












Extended validation of the ReproTracker assay

- > 100 compounds have been tested so far
- Validated with ICH S5 and EURL ECVAM-suggested libraries of teratogens and non-teratogens

Model system	Model accuracy (%)	Reference
ReproTracker	85%	A. Jamalpoor et al., 2022
Mouse EST	78%	A. Seiler et al., 2011
Whole Embryo Culture	68%	K. Augustine-Rauch et al., 2010
Micromass	70%	I. Wilk-Zasadna et al., 2009

EUROPEAN MEDICINES AGENCY
SCIENCE MEDICINES HEALTH

29 August 2017
EMA/CHMP/ICH/544278/1998
Committee for Human Medicinal Products

ICH S5 (R3) guideline on reproductive toxicology: detection of toxicity to reproduction for human pharmaceuticals

ReproTracker as a late phase verification test for animal testing outcomes

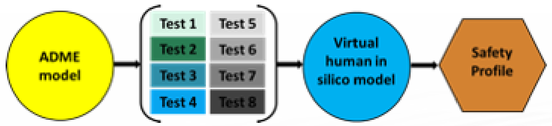
Compound	Therapeutic Cmax (µM)	FDA label	Humans	Rodent	Rabbit	mEST	WEC	True classification	ReproTracker classification
Sitagliptin	1	B				n.d.	n.d.		
Thalidomide	1-6	X							
Warfarin	25	X					n.d.		
Imatinib	2-4	D	n.d.			n.d.	n.d.		
Bosentan	2	X	n.d.			n.d.	n.d.		

- ReproTracker utilizes human material (hiPSCs) and hence can be more predictive of responses in humans.
- ReproTracker can resolve the outcome differences in animal testing.

A path towards regulatory awareness and acceptance

Dose range finding

- PKPB models
- Placenta transfer
- Cell viability



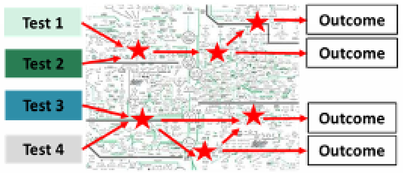
iPSCs differentiation

- Hepatocytes
- Cardiomyocytes
- Neural rosette
- Osteoblasts

ReproTracker®

Mechanistic insight

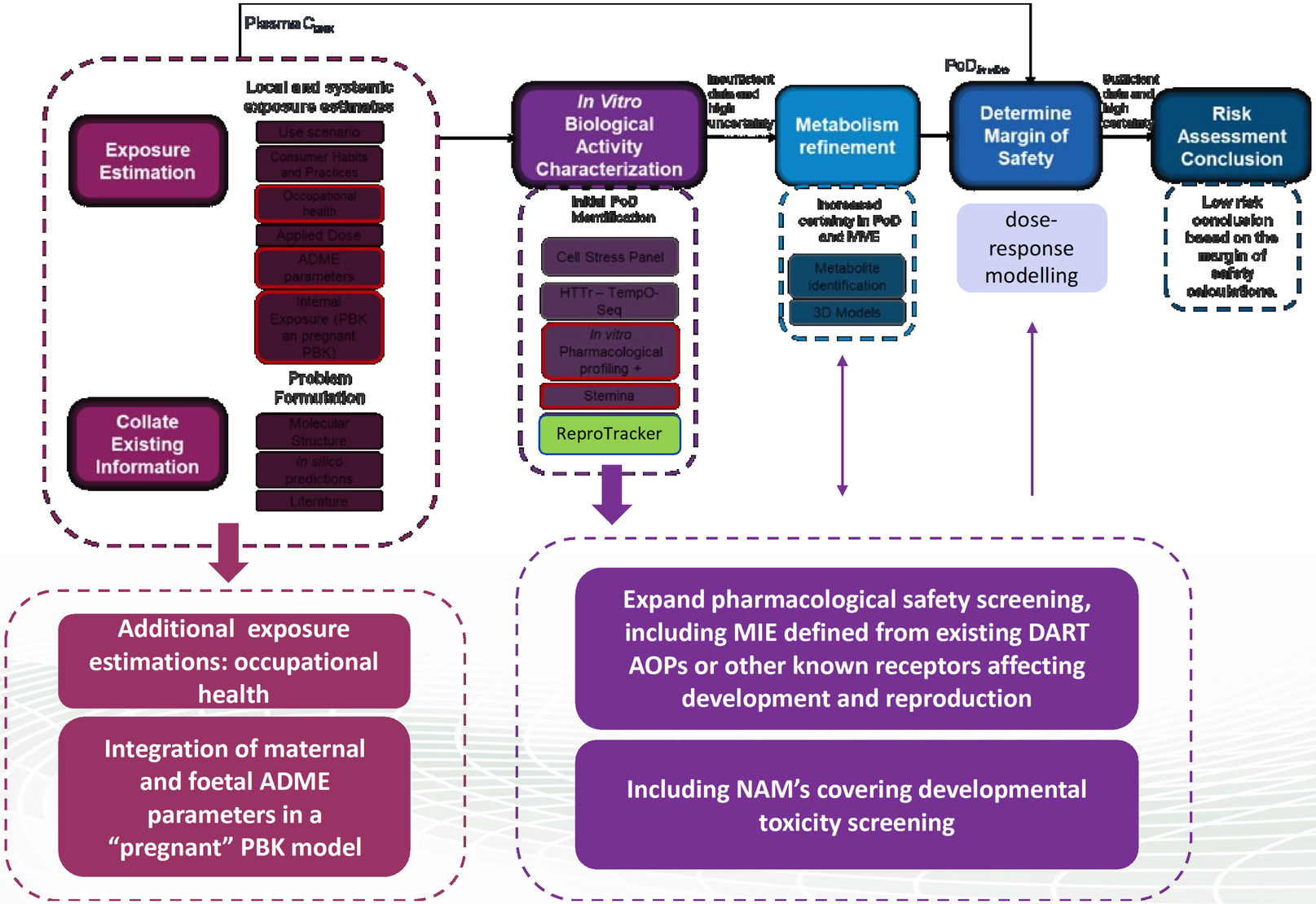
- Omics technologies
- POD
- IVIVE
- AOP mapping



Impacts

- Increase confidence in predictive value of exposure informed in vitro developmental toxicity assay
- Increased experience among industry and regulatory in interpretation and evaluation of in vitro data
- Reduction in use of animals
- Replacement of 2nd animal species

Joining forces: The way forward



Integrating ReproTracker endpoints into Unilever's NGRA framework.

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

Conclusion

- Human stem cell-based test system
- Combines functional/morphological profiling and expression pattern of selected biomarker genes
- Biomarker based approach – a way to understand biological responses
  - Insight into the molecular mode of action and key events
  - Time-window sensitive gene-biomarkers

Predictability of ReproTracker assay

- Sensitive enough to predict compounds' adverse effects on early embryonic development
- Potency ranking

Applicability

- As part of early drug development phase
- Alternative for animal-free teratogenicity testing of chemicals
- Investigate the mode-of action of teratogenic compounds
- Extrapolate animal-derived results to humans

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The value of understanding

Thank you!

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