Assuring consumer and worker safety without animal testing: Developmental and reproductive effects

Carl Westmoreland

19th July 2022

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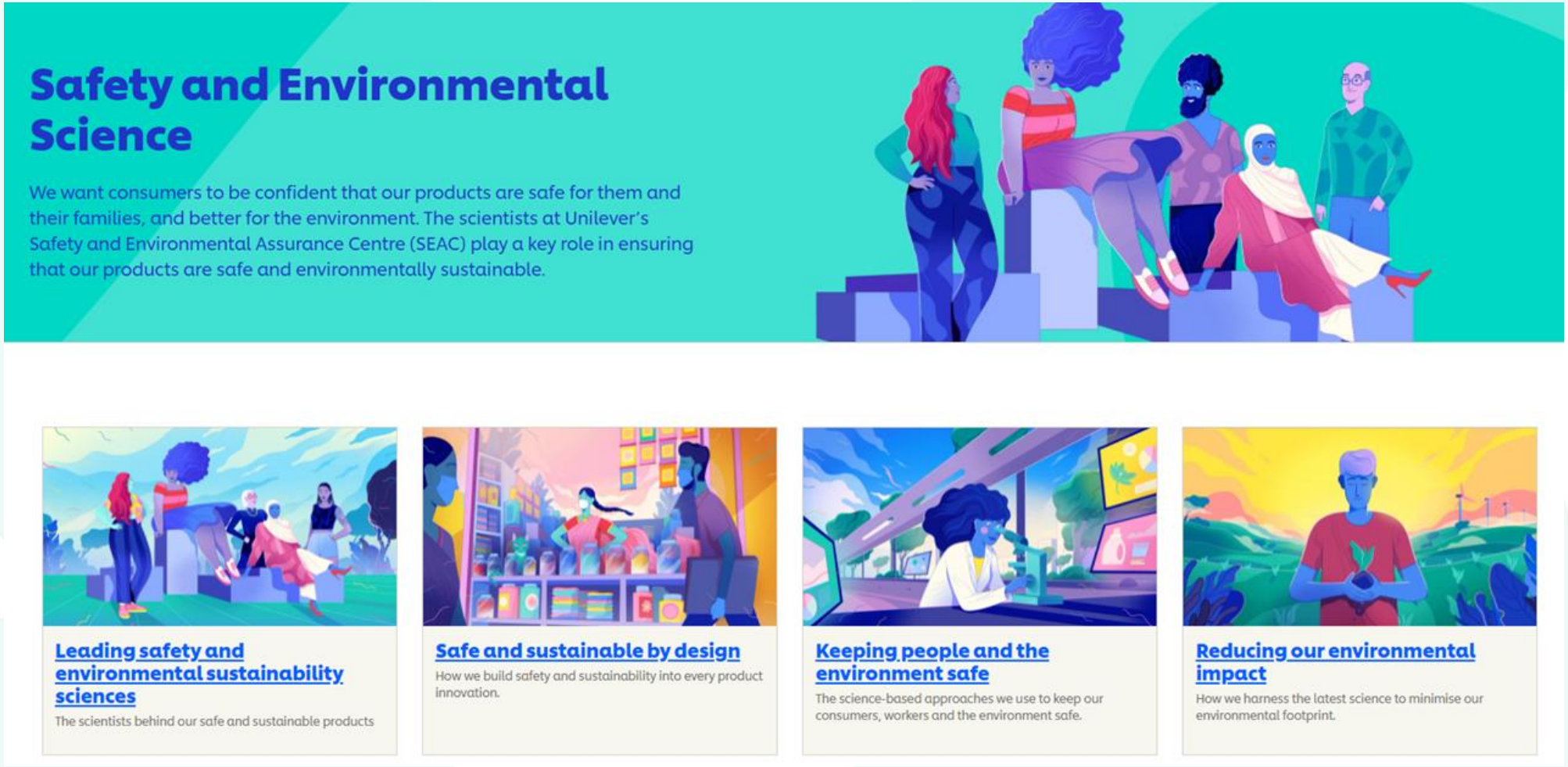
Unilever – Safety & Environmental Assurance Centre (SEAC)

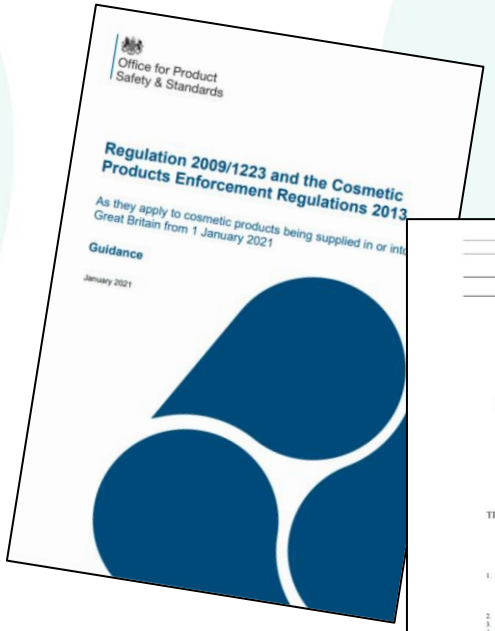
Ensuring Unilever's Innovations & Products are Safe & Sustainable by Design

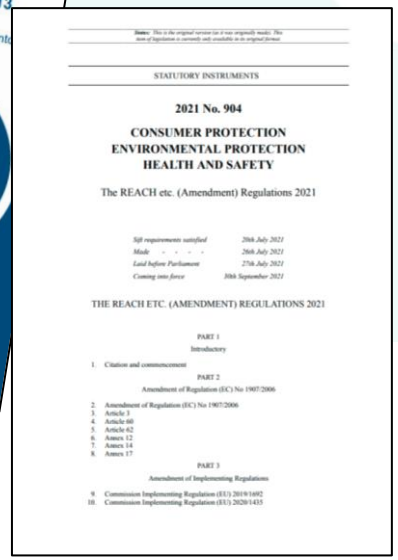


Unilever Product / Ingredient Safety Governance

- Provide scientific evidence to manage safety risks & environmental impacts

Unilever's products must be safe for the people who use and make them and for the planet





Article 18 – Animal Testing

74 Cosmetic products are not permitted on the GB market if the product's ingredients, combination of ingredients or final formulation have been the subject of animal testing used to prove their safety for the purposes of this Regulation. However, historic animal testing data from animal testing that took place before such testing was banned at EU level may still be used in order to meet the requirements of the Regulation.

Article 25

Objectives and general rules

for the purposes of this Regulation shall be undertaken only as a last resort.

PETA APPROVED

Global Animal Test Policy

We say use science.
Not animals.

Alternatives to animal testing

Our approach

We use a wide range of non-animal approaches to assess the safety of our products. Since the 1980s, our scientists have been developing and using alternatives to animal tests, e.g. computer modelling and cell culture-based experiments. We regularly present and publish our work, and continually collaborate with others to share our knowledge and apply exciting new science to assure product safety.

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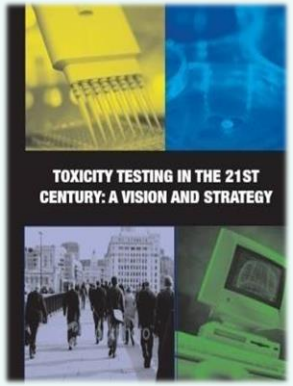
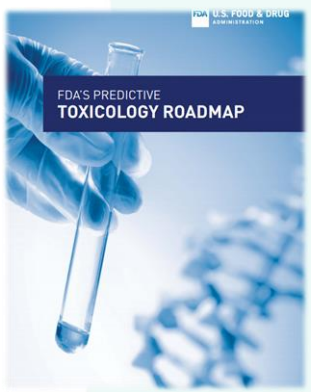
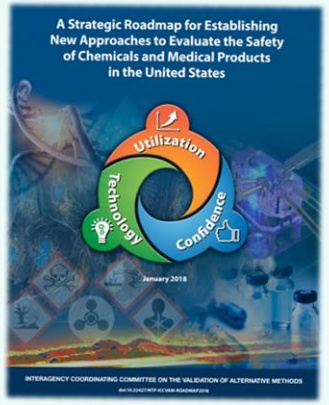
Next Generation Risk Assessment (NGRA)

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







Use of NGRA for safety assessment – Regulatory uptake

Computational Toxicology 7 (2018) 20–26

Contents lists available at ScienceDirect

Computational Toxicology

journal homepage: www.elsevier.com/locate/comtox

Principles underpinning the use of new methodologies in the risk assessment of cosmetic ingredients

Matthew Dent[a,*], Renata Teixeira Amaral[b], Pedro Amores Da Silva[b], Jay Ansell[c], Fanny Boisleve[d], Masato Hatao[e], Akihiko Hirose[f], Yutaka Kasai[g], Petra Kern[h], Reinhard Kreiling[i], Stanley Milstein[j], Beta Montemayor[k], Julcemara Oliveira[l], Andrea Richarz[m], Rob Taalman[n], Eric Vaillancourt[o], Rajeshwar Verma[l], Nashira Vieira O'Reilly Cabral Posada[l], Craig Weiss[p], Hajime Kojima[f]

ARTICLE INFO

Keywords:
Next Generation Risk Assessment
New approach methodologies
Cosmetics risk assessment

ABSTRACT

Consumer safety is a prerequisite for any cosmetic product. Worldwide, there is an ever-increasing desire to bring safe products to market without animal testing, which requires a new approach to consumer safety. 'Next Generation Risk Assessment' (NGRA), defined as an exposure-led, hypothesis driven risk assessment approach that integrates *in silico*, *in chemico* and *in vitro* approaches, provides such an opportunity. The customized nature of each NGRA means that the development of a prescriptive list of tests to assure safety is not possible, or appropriate. The International Cooperation on Cosmetics Regulation (ICCR) therefore tasked a group of scientists from regulatory authorities and the Cosmetic industry to agree on and outline the principles for incorporating these new approaches into risk assessments for cosmetic ingredients. This ICCR group determined the overall goals of NGRA (to be human-relevant, exposure-led, hypothesis-driven and designed to prevent harm); how an NGRA should be conducted (using a tiered and iterative approach, following an appropriate literature search and evaluation of the available data, and using robust and relevant methods and strategies); and how the assessment should be documented (transparent and explicit about the logic of the approach and sources of uncertainty). Those working on the risk assessment of cosmetics have a unique opportunity to lead progress in the application of novel approaches, and cosmetic risk assessors are encouraged to consider these key principles

SCCS/1628/21

Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION 11TH REVISION

The SCCS adopted this guidance document at its plenary meeting on 30-31 March 2021

3-4 RELEVANT TOXICOLOGICAL TOOLS FOR THE SAFETY EVALUATION OF COSMETIC INGREDIENTS

The SCCS has been closely following the progress made with regard to the development and validation of alternative methods and updated its NoG on a regular basis taking progress into consideration.

Besides validated alternatives, the SCCS may also accept, on a case-by-case basis, methods that are scientifically valid as new tools (*e.g.*, "-omics" technology) for the safety evaluation of cosmetic substances. Such valid methods may not have necessarily gone through the complete validation process, but the Committee may consider them acceptable when there is a sufficient amount of experimental data proving relevance and reliability and including positive and negative controls.

According to the Cosmetics Regulation, the experimental studies have to be carried out in accordance with the principles of Good Laboratory Practice (GLP)laid down in Council Directive 87/18/EEC. All possible deviations from this set of rules should be explained and scientifically justified (SCCNFP/0633/02).

3-4.1 NEW APPROACH METHODOLOGY (NAM) AND NEXT-GENERATION RISK ASSESSMENT (NGRA)

Whereas the terminology of "Alternative Test Methods (ATMs)" does not cover all available tools *e.g., in silico* methodology, the more general term, New Approach Methodology (NAM) has been introduced. As for cosmetics and their ingredients, testing and marketing bans apply with respect to animal use and also the obligation exists to only use validated replacement alternatives, the need for validated non-animal alternative methods for chemical hazard assessment is much more important in Europe for compliance with the Cosmetics Regulation than for other regulatory frameworks. NAMs may include *in vitro, ex vivo, in chemico and in silico* methods, read-across, as well as combinations thereof. Therefore, before any testing is carried out for safety evaluation, all information on the substance under consideration should be gathered from different available means. A set of criteria, universal across initiatives, to evaluate NAMs fit-for-purpose was developed by a multi-stakeholder group and may support greater consistency across different initiatives (Parish *et al.*, 2020).

Many efforts are ongoing to modernise toxicological safety evaluation and to look for non-animal methodology that can be used for the risk assessment of compounds that after long-term exposure could be at the origin of systemic toxicity. One of these approaches is referred to as NGRA (USEPA, 2014). The principles underpinning the application of an NGRA to cosmetics have been defined by the International Cooperation on Cosmetics Regulation (ICCR), a platform of regulators and cosmetics industry from the EU, the US, Japan, Canada and Brazil (Dent *et al.*, 2018). NGRA is a human-relevant, exposure-led, hypothesis-driven risk assessment designed to prevent harm. It integrates several NAMs to deliver safety decisions relevant to human health without the use of experimental animals. An NGRA should be conducted using a tiered and iterative approach, following an appropriate literature search and evaluation of the available data, and using robust and relevant methods and strategies. Given the novelty of NGRA and the current lack of regulatory guidance on the use of a variety of NAMs in decision-making, it is important that the assessment should be transparently documented and explicit about the logic of the approach and sources of uncertainty (Dent *et al.*, 2018). A general NGRA workflow is described in **Figure 5** (Berggren *et al.*, 2017). The tools useful for safety evaluation of cosmetic ingredients, which could also be used in case NGRA would be taken as a possible workflow in the future, are described in chapters 3-4.2 to 3-4.14. Treshold of Toxicological Concern (TTC) and internal TTC (iTTC) approaches as a risk assessment tools are described in 3-5.2.

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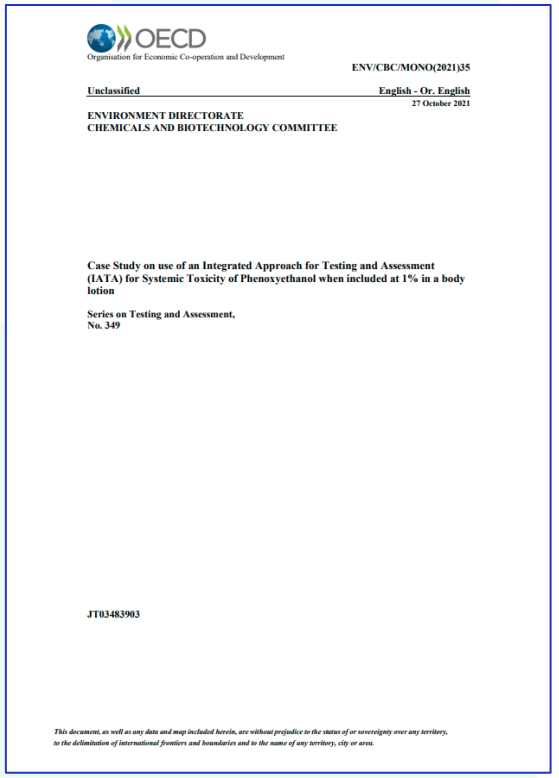

OECD

Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

Unclassified — English - Or. English

27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testing and Assessment, No. 349

JT03483903

This document, as well as any data and map included herein, are without prejudice to the status of or sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.

International Cooperation on Cosmetics Regulation (2018)

European Commission: Scientific Committee on Consumer Safety (2021)

OECD (2021)

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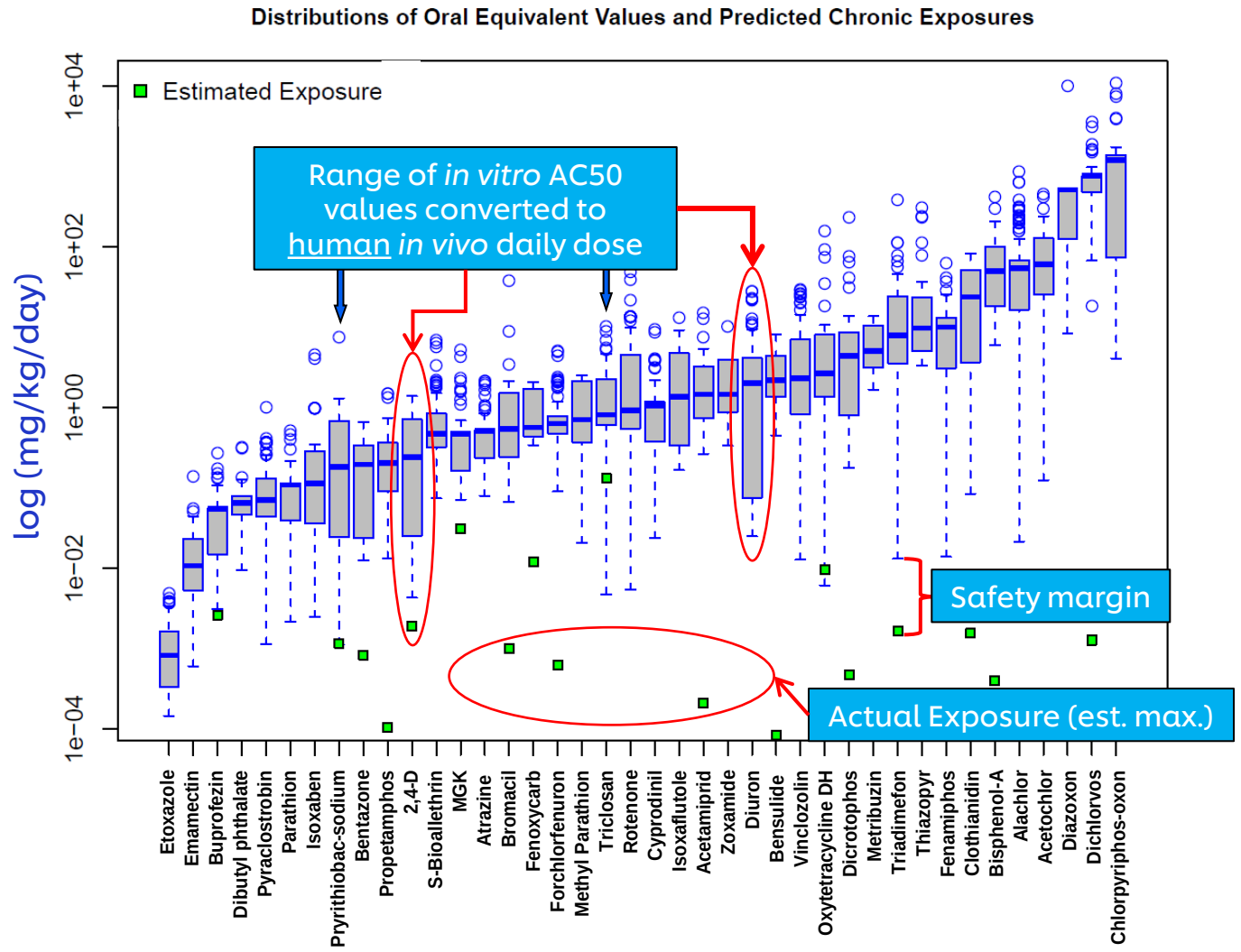
NGRA: Protection not prediction



The hypothesis underpinning this NGRA is that if no bioactivity is observed at consumer-relevant concentrations, there can be no adverse health effects.

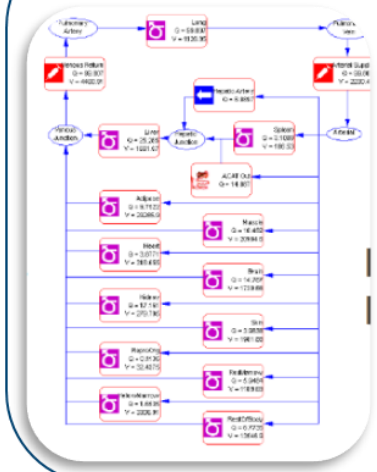
At no point does NGRA attempt to predict the results of high dose toxicology studies in animals

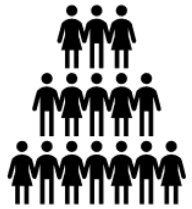
NGRA uses new exposure science and understanding of human biology

Graph from Rusty Thomas US EPA, with thanks. Rotroff *et al* (2010) Toxicological Sciences , **117**, 348-358

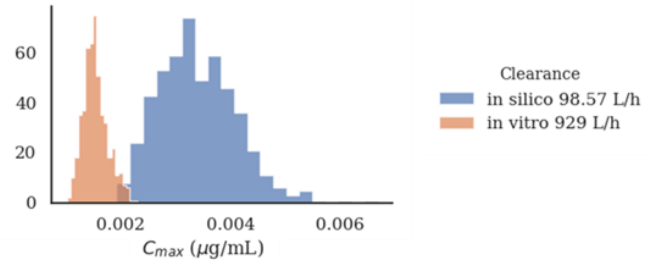
Key tools in our NGRA approach for systemic effects

PBK Modelling





Face Cream



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

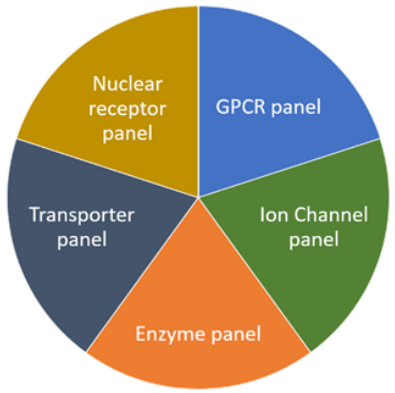
In vitro pharmacological profiling

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

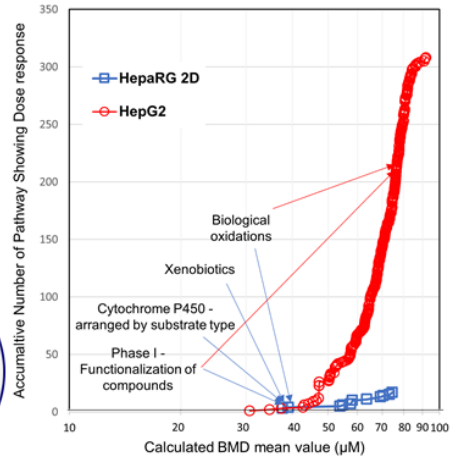


eurofins | Cerep

Transcriptomics

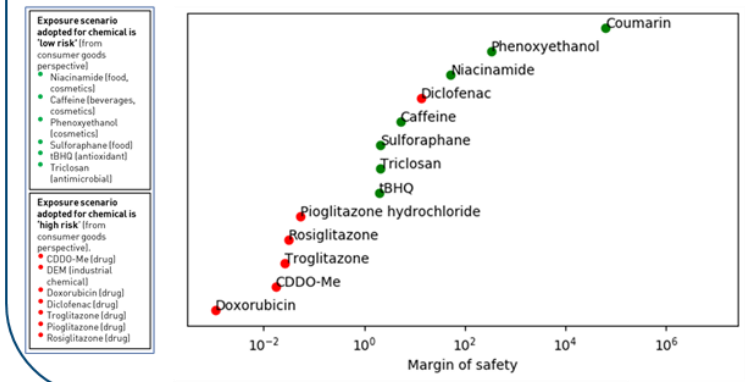
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

BMDExpress 2

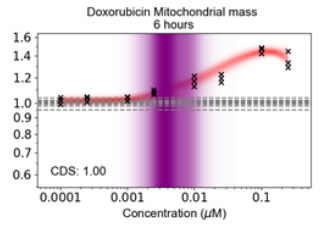


Cellular Stress Pathways

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



cyprotex AN EVOTEC COMPANY



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

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Baltazar *et al.*, (2020) *Toxicol Sci* **176**, 236–252

Developmental and Reproductive Toxicology (DART)

frontiers in Toxicology

ORIGINAL RESEARCH
published: 07 March 2022
doi: 10.3389/ftox.2022.838466

Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing

Ramya Rajagopal, Maria T. Baltazar, Paul L. Carmichael, Matthew P. Dent, Julia Head, Hequn Li, Iris Muller, Joe Reynolds, Kritika Sadh, Wendy Simpson, Sandrine Spriggs, Andrew White and Predrag Kukic*

Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, United Kingdom

New Approach Methodologies (NAMs) promise to offer a unique opportunity to enable human-relevant safety decisions to be made without the need for animal testing in the context of exposure-driven Next Generation Risk Assessment (NGRA). Protecting human health against the potential effects a chemical may have on embryo-foetal development and/or aspects of reproductive biology using NGRA is particularly challenging. These are not single endpoint or health effects and risk assessments have traditionally relied on data from Developmental and Reproductive Toxicity (DART) tests in animals. There are numerous Adverse Outcome Pathways (AOPs) that can lead to DART, which means defining and developing strict testing strategies for every AOP, to predict apical outcomes, is neither a tenable goal nor a necessity to ensure NAM-based safety assessments are fit-for-purpose. Instead, a pragmatic approach is needed that uses the available knowledge and data to ensure NAM-based exposure-led safety assessments are sufficiently protective. To this end, the mechanistic and biological coverage of existing NAMs for DART were assessed and gaps to be addressed were identified, allowing the development of an approach that relies on generating data relevant to the overall mechanisms involved in human reproduction and embryo-foetal development. Using the knowledge of cellular processes and signalling pathways underlying the key stages in reproduction and development, we have developed a broad outline of endpoints informative of DART. When the existing NAMs were compared against this outline to determine whether they provide comprehensive coverage when integrated in a framework, we found them to generally cover the reproductive and developmental processes underlying the traditionally evaluated apical endpoint studies. The application of this safety assessment framework is illustrated using an exposure-led case study.

Keywords: DART, NAMs, non-animal alternatives, NGRA, mechanistic evaluation

OPEN ACCESS

Edited by:
Daniel Villeneuve, United States Environmental Protection Agency, United States

Reviewed by:
Terje Svingen, Technical University of Denmark, Denmark
Olavi R. Pelkonen, University of Oulu, Finland
Karine Audouze, Université de Paris, France

****Correspondence:***
Ramya Rajagopal
ramya.rajagopal@unilever.com

Specialty section:
This article was submitted to In Vitro Toxicology, a section of the journal Frontiers in Toxicology

Received: *17 December 2021*
Accepted: *31 January 2022*
Published: *07 March 2022*

Citation:
Rajagopal R, Baltazar MT, Carmichael PL, Dent MP, Head J, Li H, Muller I, Reynolds J, Sadh K, Simpson W, Spriggs S, White A and Kukic P (2022) Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing. Front. Toxicology. 4:838466. doi: 10.3389/ftox.2022.838466

1 INTRODUCTION

The value of using New Approach Methodologies (NAMs) as alternatives to animal testing in the evaluation of chemical safety has gained much attention and recognition (ECC/HC, 2016; ECHA, 2016; ECHA, 2017; EPA, 2018). To foster their development and application, various agencies have put forward guidance, frameworks and workplans that ensure confidence, consistency and are fit-for-purpose, when generating NAMs hazard data for various purposes (OECD, 2005; OECD, 2017; OECD, 2018; Parish et al., 2020). The International Cooperation on Cosmetics Regulation (ICCR) has outlined key principles that guide risk assessors to use NAMs in an integrated manner for Next

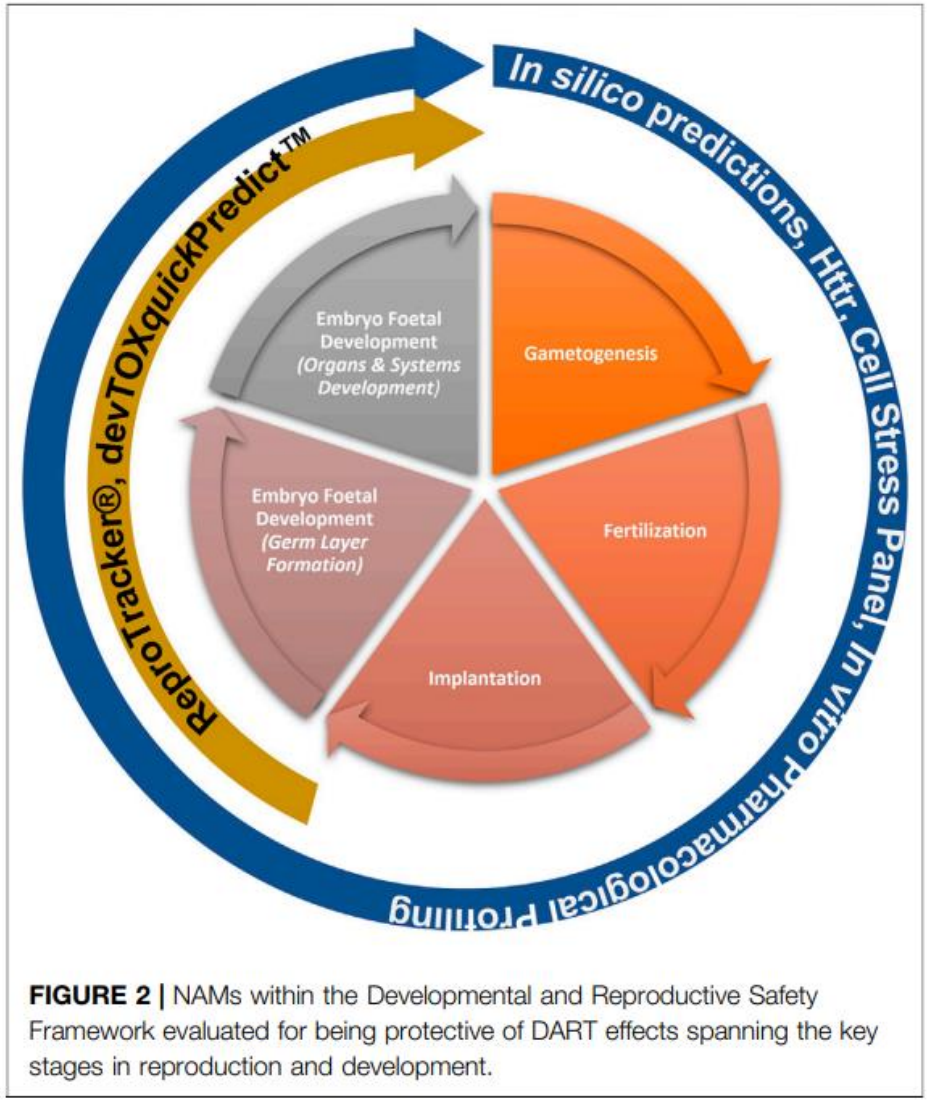

FIGURE 2 | NAMs within the Developmental and Reproductive Safety Framework evaluated for being protective of DART effects spanning the key stages in reproduction and development.

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NGRA Framework for DART endpoints

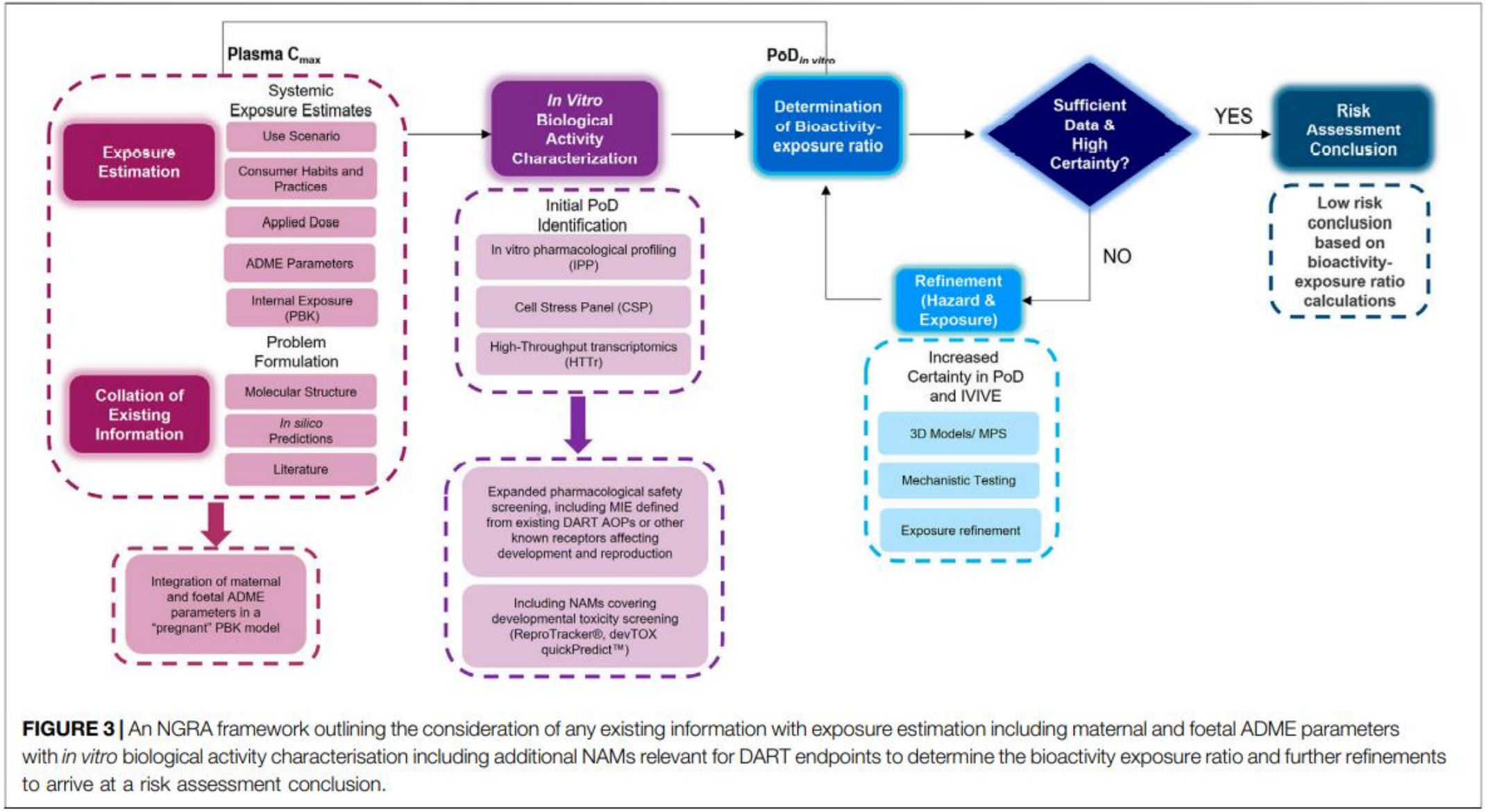
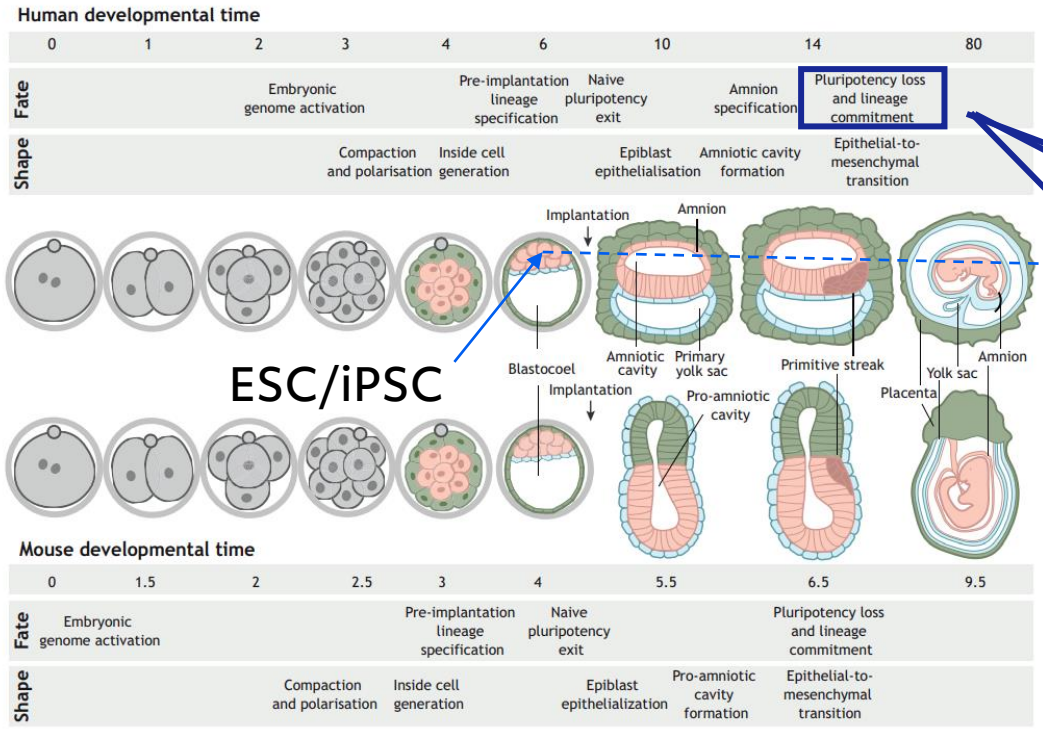
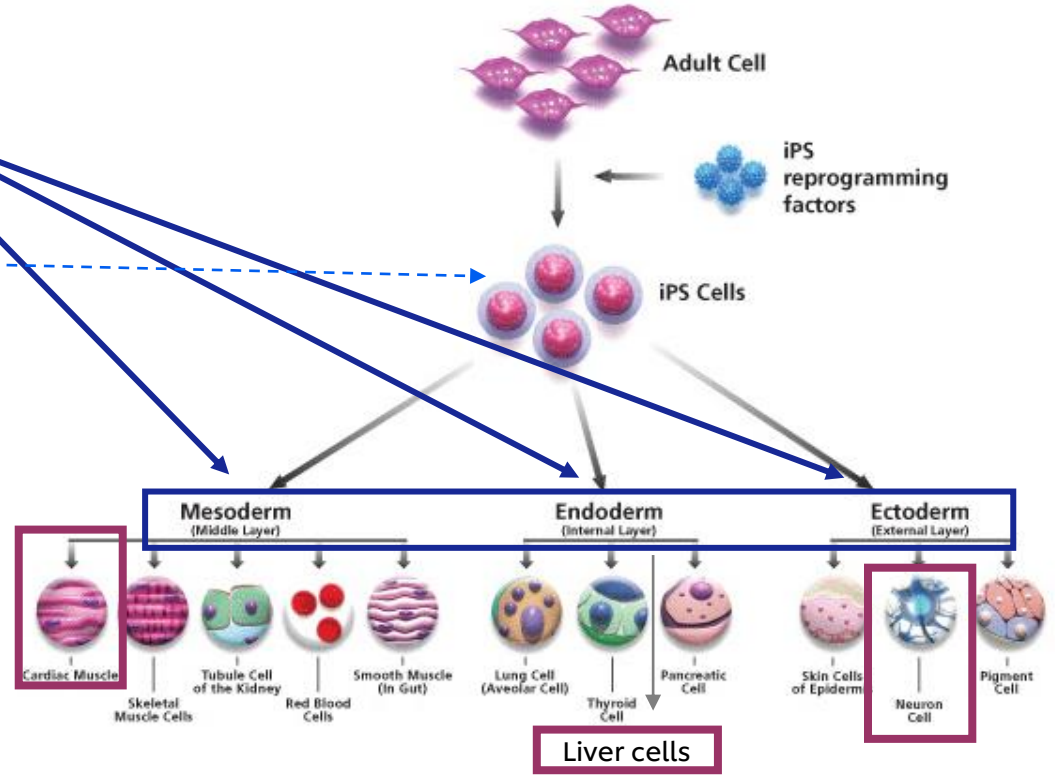


FIGURE 3 | An NGRA framework outlining the consideration of any existing information with exposure estimation including maternal and foetal ADME parameters with *in vitro* biological activity characterisation including additional NAMs relevant for DART endpoints to determine the bioactivity exposure ratio and further refinements to arrive at a risk assessment conclusion.

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

Induced pluripotent stem cells (iPSCs) to detect developmental toxicity





modified from Shahbazi, (2020) Development Jul 17;147(14):dev190629

- Assays have been developed to either use iPSCs directly (devTox quickPredict platform; Stemina) or the differentiation into heart, liver and neuronal cells (ReproTracker; Toxys) as New Approach Methodologies (NAMs) for developmental toxicity

toxys ReproTracker® Stemina BIOMARKER DISCOVERY devTOX qP quickPREDICT

US EPA and NGRA

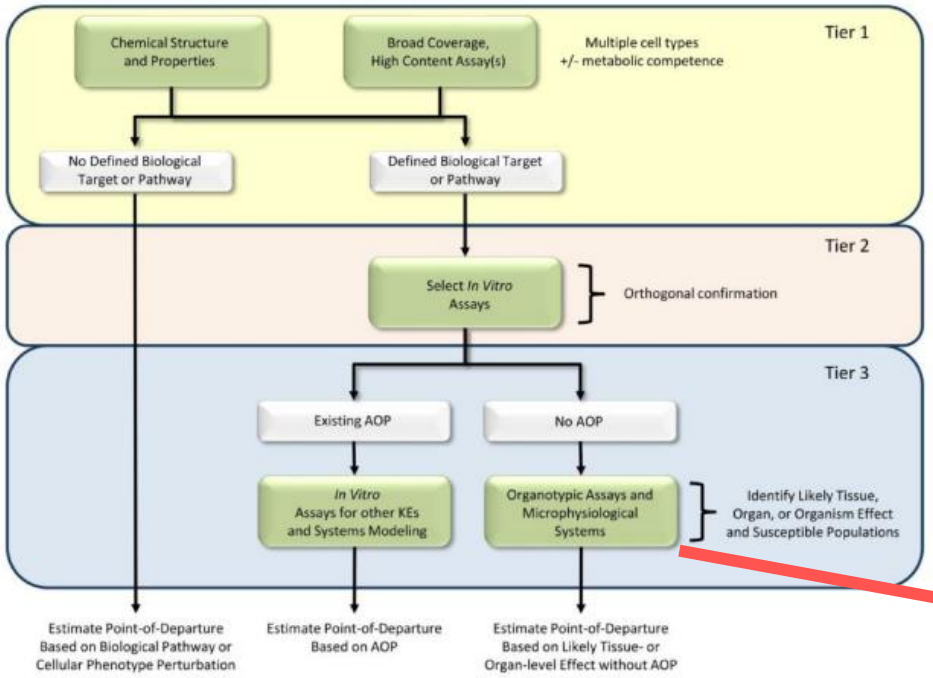
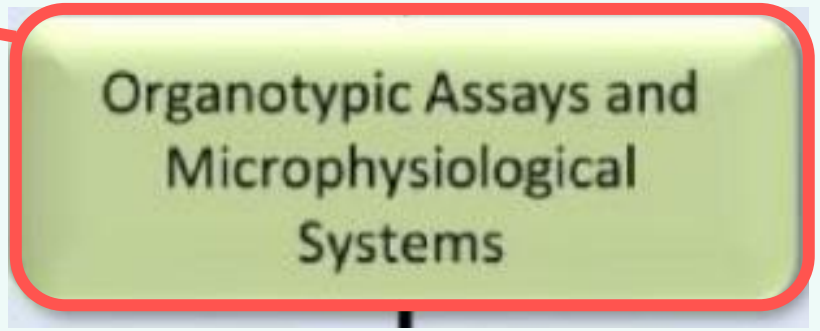


Figure 2.
Tiered testing framework for hazard characterization. Tier 1 uses both chemical structure and broad coverage, high content assays across multiple cell types for comprehensively evaluating the potential effects of chemicals and grouping them based on similarity in potential hazards. For chemicals from Tier 1 without a defined biological target/pathway, a quantitative point-of-departure for hazard is estimated based on the absence of biological pathway or cellular phenotype perturbation. Chemicals from Tier 1 with a predicted biological target or pathway are evaluated Tier 2 using targeted follow-up assays. In Tier 3, the likely tissue, organ, or organism-level effects are considered based on either existing adverse outcome pathways (AOP) or more complex culture systems. Quantitative points-of-departure for hazard are estimated based on the AOP or responses in the complex culture system.

The next generation blueprint of computational toxicology at the U.S. Environmental Protection Agency

Thomas R *et al*, (2019). Toxicol Sci. **169**, 317–332. doi:10.1093/toxsci/kfz058



Principles of Next Generation Risk Assessment from ICCR

International Cooperation on Cosmetics Regulation

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparently and documented

Dent *et al* (2018), Computational Toxicology, **7**, 20-26: https://doi.org/10.1016/j.comtox.2018.06.001

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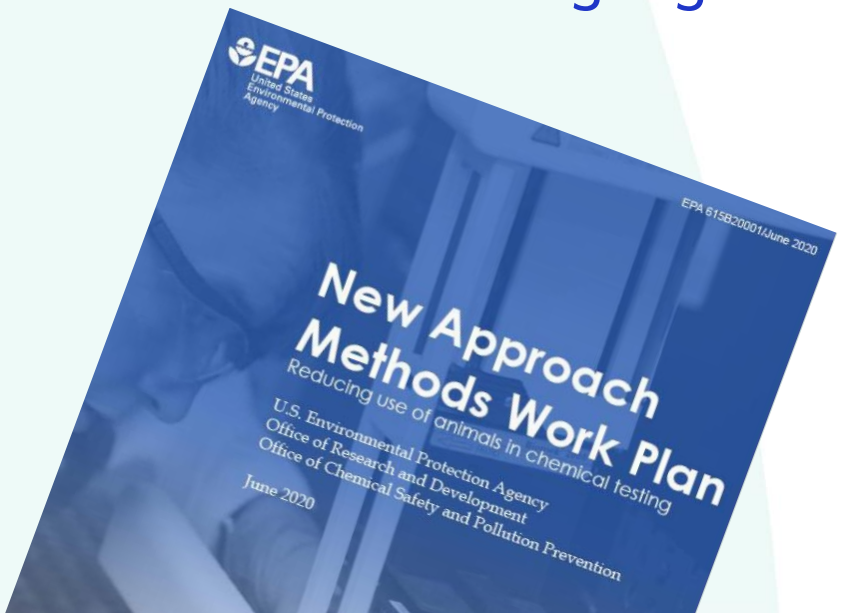
A role for reproductive organoids in NGRA?

- Organotypic assays and microphysiological systems have a key role in higher tier human-based NGRA

- NGRA needs multidisciplinary teams (Risk assessment, PBK modelling, early tier bioactivity assays, mathematical modelling, informatics etc).

- Could there be bespoke, investigative higher tier roles for reproductive organoids?
  Higher tiers will always need expertise in areas identified in earlier tiers

- Bringing complex *in vitro* biology and detailed mechanistic understanding together with regulatory requirements for safety
  Robust, reproducible models
  Transferability, reproducibility
  Good laboratory practice?



Acknowledgements

Nora Aptula
Maja Aleksic
Maria Baltazar
Trina Barritt
Danilo Basili
Sophie Cable
Paul Carmichael
Tom Cull
Matt Dent
Ellen Edwards
Julia Fentem
Nicky Gilmour
Steve Gutsell
Sarah Hatherell
Jade Houghton
Lucy Ingram
Predrag Kukic
Hequn Li
Mark Liddell
Keeley Mahwing
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