

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

Carl Westmoreland

19th July 2022



We make many of the world's favourite brands





Unilever – Safety & Environmental Assurance Centre (SEAC)

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

Safety and Environmental Science

We want consumers to be confident that our products are safe for them and their families, and better for the environment. The scientists at Unilever’s Safety and Environmental Assurance Centre (SEAC) play a key role in ensuring that our products are safe and environmentally sustainable.



Leading safety and environmental sustainability sciences

The scientists behind our safe and sustainable products



Safe and sustainable by design

How we build safety and sustainability into every product innovation.



Keeping people and the environment safe

The science-based approaches we use to keep our consumers, workers and the environment safe.



Reducing our environmental impact

How we harness the latest science to minimise our environmental footprint.

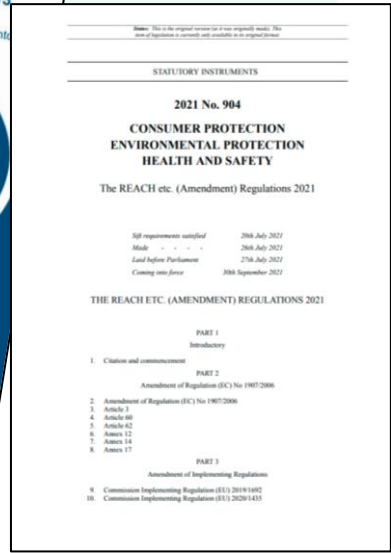
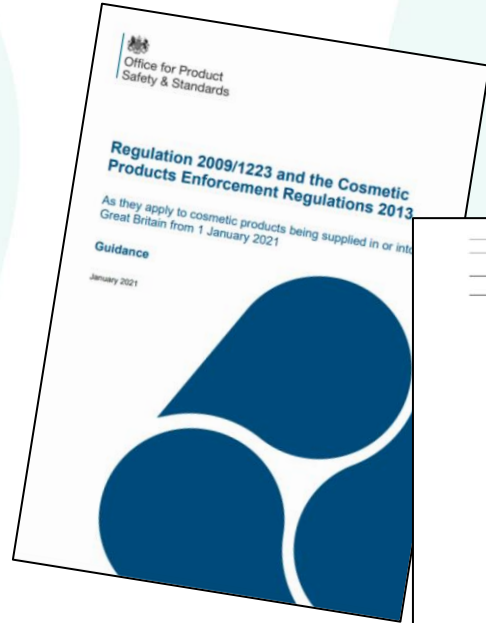
Unilever Product / Ingredient Safety Governance

- 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




**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.

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.

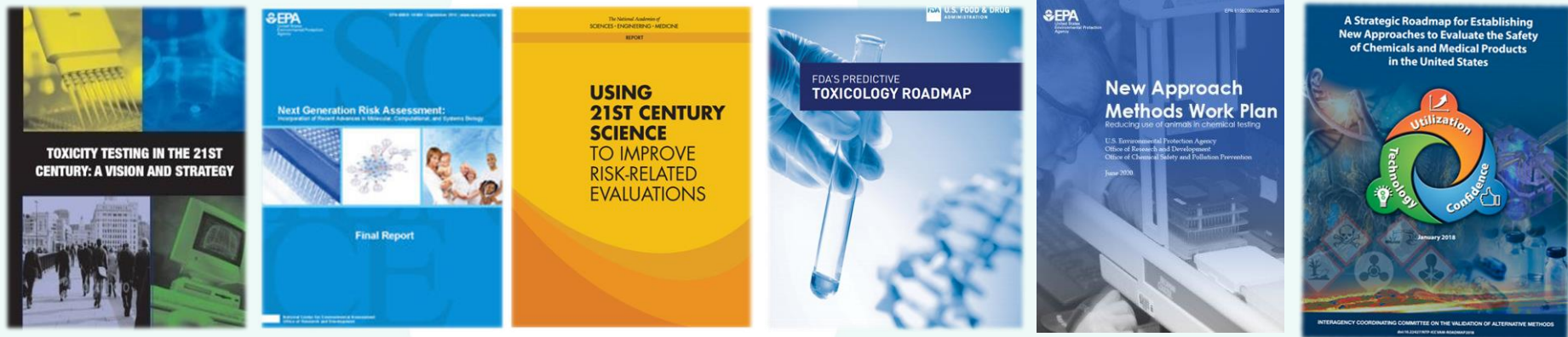


Unilever.com



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 Boislevé^d, Masato Hatao^e, Akihiko Hirose^f, Yutaka Kasai^g, Petra Kern^h, Reinhard Kreilingⁱ, Stanley Milstein^j, Beta Montemayor^k, Julcemara Oliveira^l, Andrea Richarz^m, Rob Taalmanⁿ, Eric Vaillancourt^o, Rajeshwar Verma^p, Nashira Vieira O'Reilly Cabral Posada^q, Craig Weiss^r, Hajime Kojima^s

^aUnilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire MK44 1LQ, UK
^bADIFRICE – Association of the Cosmetic, Tissue and Progress Industry (ADIFRICE), Av. Paulino, 1315 Conquistador, São Paulo, SP 01311-000, Brazil
^cUS Personal Care Products Council (PCPC), 1620 L St. NW, Suite 1200, Washington, D.C. 20036, USA
^dJohansen & Johnson Saint Bréaz France, Domaine de Malignemont, CS 10615, F-37106 VAL DE RHUÏLE, Cedex, France
^eJapan Cosmetic Industry Association (JCIA), Metro City Kamayachi 6F, 3-1-5, Toyko, Minato-ku, Tokyo 105-0001 Japan
^fNational Institute of Health Sciences, 1-18-1 Kamiyoga, Setagaya-ku, 158-8501 Tokyo, Japan
^gKao Corporation, External Relations & Government Affairs 2-1, Banba, Sumida-ku, Tokyo 131-8501 Japan
^hProcter and Gamble Services Company NY, Tomlinson 100, B-1823 Stroubeek River, Belgium
ⁱChlorian Produkte (DE) GmbH, Global Toxicology and Ecotoxicology, Am Unten Park 1, 65843 Subbach, Germany
^jUS Food and Drug Administration (US FDA), Office of Cosmetics and Colors (OCCAC), Center for Food Safety and Applied Nutrition (CFSAN), 5001 Campus Drive, College Park, MD 20740, USA
^kCosmetics Alliance Canada, 420 Britannia Road East Suite 102, Mississauga, ON L4Z 3L5, Canada
^lBrazilian Health Regulatory Agency (ANVISA), Gerência de Produtos de Higiene, Perfumes, Cosméticos e Saneamento, SIA Truão S, lote 200, Área Especial 57 – CEP 71205-650, Brazil
^mEuropean Commission, Joint Research Centre (JRC), Directorate for Health, Consumers and Reference Materials, Chemical Safety and Alternative Methods Unit, Via E. Fermi 2749, 21027 Segrate, VA, Italy
ⁿCosmetics Europe, Avenue Herrmann Doreux 40, 1160 Auderghem, Belgium
^oHealth Canada (HC), Consumer Product Safety Directorate, Health Environment and Consumer Safety Branch, 269 Laurier Ave. W., Ottawa, ON K1A 0K9, Canada
^pIndependent Cosmetic Manufacturing and Distributors (ICMDA), Suite 2015, Deer Park, IL 60010, USA

ARTICLE INFO

Keywords:
 New 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



International Cooperation on Cosmetics Regulation (2018)

SCCS/1628/21

European Commission

Scientific Committee on Consumer Safety
SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF
COSMETIC INGREDIENTS AND THEIR SAFETY

EVALUATION
11TH REVISION

Scientific Committees
 on Consumer Safety
 on Health, Environmental and Emerging Risks

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 (SCCNF/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 3-4.4. Threshold of Toxicological Concern (TTC) and internal TTC (ITC) approaches as a risk assessment tools are described in 3-5.2.



European Commission: Scientific Committee on Consumer Safety (2021)

OECD
 Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)315

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 Phenoxethanol when included at 1% in a body lotion

Series on Testing and Assessment,
 No. 349

JT03483903

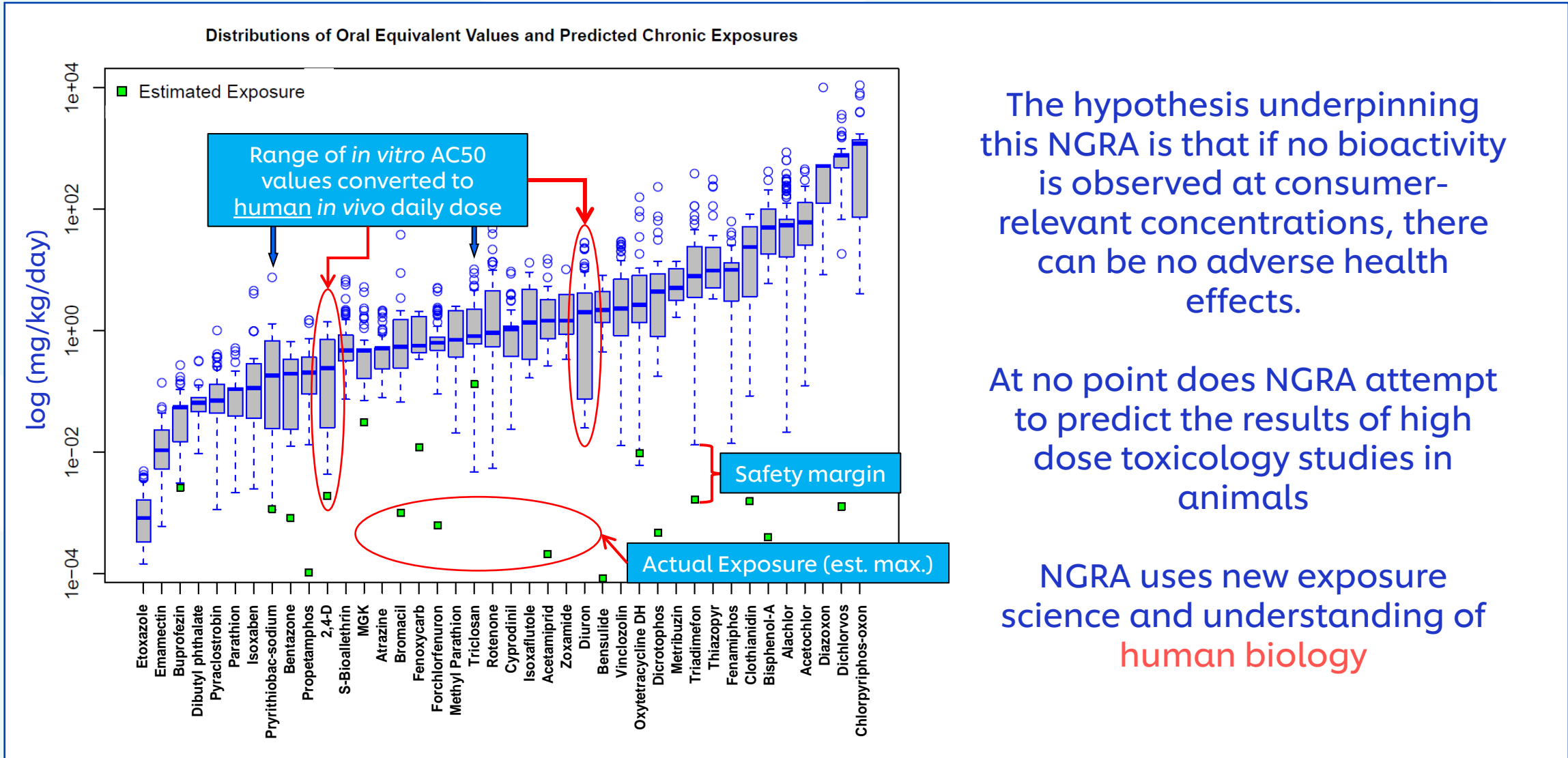
This document, as well as any data and maps included herein, are without prejudice to the status of an sovereignty over any territory, to the delimitation of international frontiers and boundaries and to the name of any territory, city or area.



OECD (2021)



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.

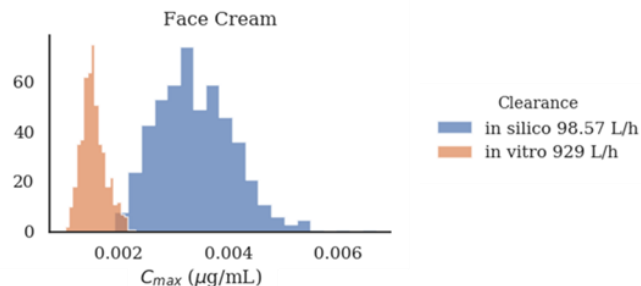
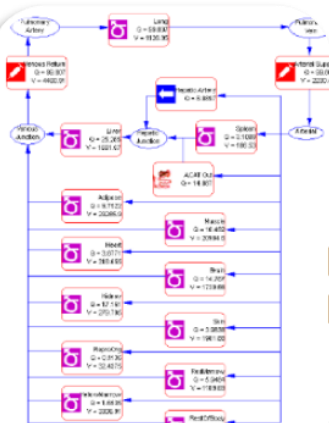
At no point does NGRA attempt to predict the results of high dose toxicology studies in animals

NGRA uses new exposure science and understanding of **human biology**



Key tools in our NGRA approach for systemic effects

PBK Modelling



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 Boveri, Andrew J. Brown, Jacques Homan, Wolfgang Juratnik, Arun Sridhar, Gareth Waldron and Steven Whitbread

Abstract In vitro pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in our collaborative knowledge sharing.

Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is creating an appropriate balance between drug efficacy and potential adverse effects as early as possible in order to reduce safety-related attrition, particularly in the more expensive late stages of clinical development. Gaining a better understanding of the safety profile of drug candidates early in the process is also crucial for reducing the likelihood of safety issues limiting the use of approved drugs, or even leading to their market withdrawal, having no impact on the patient and/or regulatory burden.

target (or targets), whose secondary effects are due to interactions with targets other than the primary target (or targets) that is off-target interactions. Off-target interactions are often the cause of ADRs in animal models or clinical studies, and careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help reduce the incidence of type A ADRs.

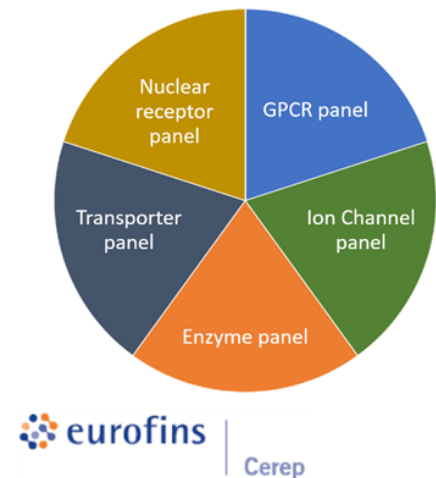
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, enzymes, ion channels, transporters, etc.) that are chosen from the scientific

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The *in vitro* pharmacology assay that is absolutely required by regulatory authorities is that measures the effects of new chemical entities on the ion channels of native I_{Ca} or heterologously expressed human voltage-gated potassium channel subfamily 11 member 2 (hKCNJ2), also known as hERG. The mechanism by which blockade of hERG can affect potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized^{1,2}, and the assessment of this ADR is one reason why this assay is a mandatory regulatory requirement. Receptor binding studies are also recommended as the first tier approach for the assessment of the dependence potential of novel chemical entities³.

However, current regulatory guidance does not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the stage of the discovery process at which an *in vitro* pharmacological profiling should occur. Nevertheless, the general trend for most pharmaceutical companies is to perform this testing early in drug discovery to reduce attrition and to facilitate better prediction of ADRs in the later stages of drug discovery and development.

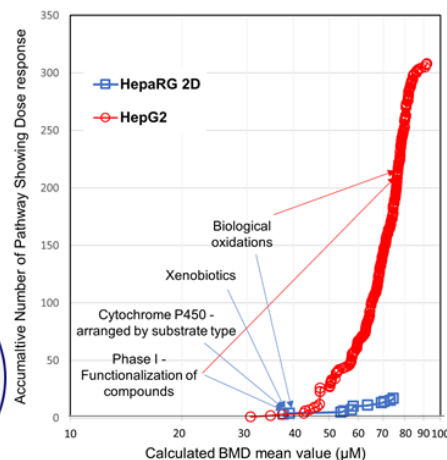
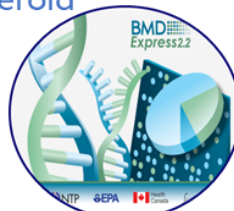
Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experience of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe the rationale and main advantages for the use of an *in vitro* pharmacological profiling panel to reduce both pre-clinical and



Transcriptomics

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

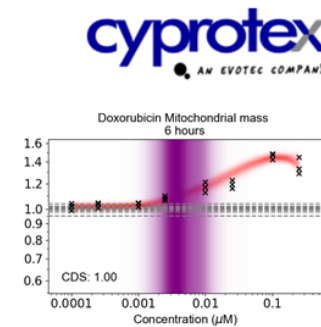
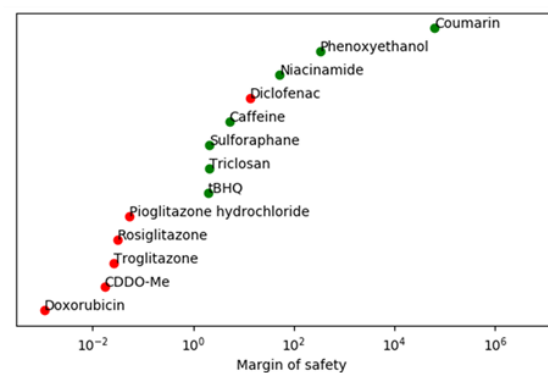
BMDexpress 2



Cellular Stress Pathways

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

- Exposure scenario adopted for chemical is 'low risk'** (from consumer goods perspective)
- Niacinamide (food, cosmetics)
 - Caffeine (beverages, cosmetics)
 - Phenoxyethanol (cosmetics)
 - Sulforaphane (food)
 - tBHQ (antioxidant)
 - Tricosan (antimicrobial)
- Exposure scenario adopted for chemical is 'high risk'** (from consumer goods perspective)
- CDDO-Me (drug)
 - DEM (industrial chemical)
 - Doxorubicin (drug)
 - Diclofenac (drug)
 - Troglitazone (drug)
 - Pioglitazone (drug)
 - Rosiglitazone (drug)



Toxicol Sci (2020), 176, 11-33



Developmental and Reproductive Toxicology (DART)

frontiers
in Toxicology

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

Check for updates

Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing

Ramya Rajagopal¹, 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.

OPEN ACCESS

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

Reviewed by: Tarje Svingsen, Technical University of Denmark, Denmark; Clavi R. Peltoranta, University of Oulu, Finland; Karine Audouin, Université de Paris, France

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

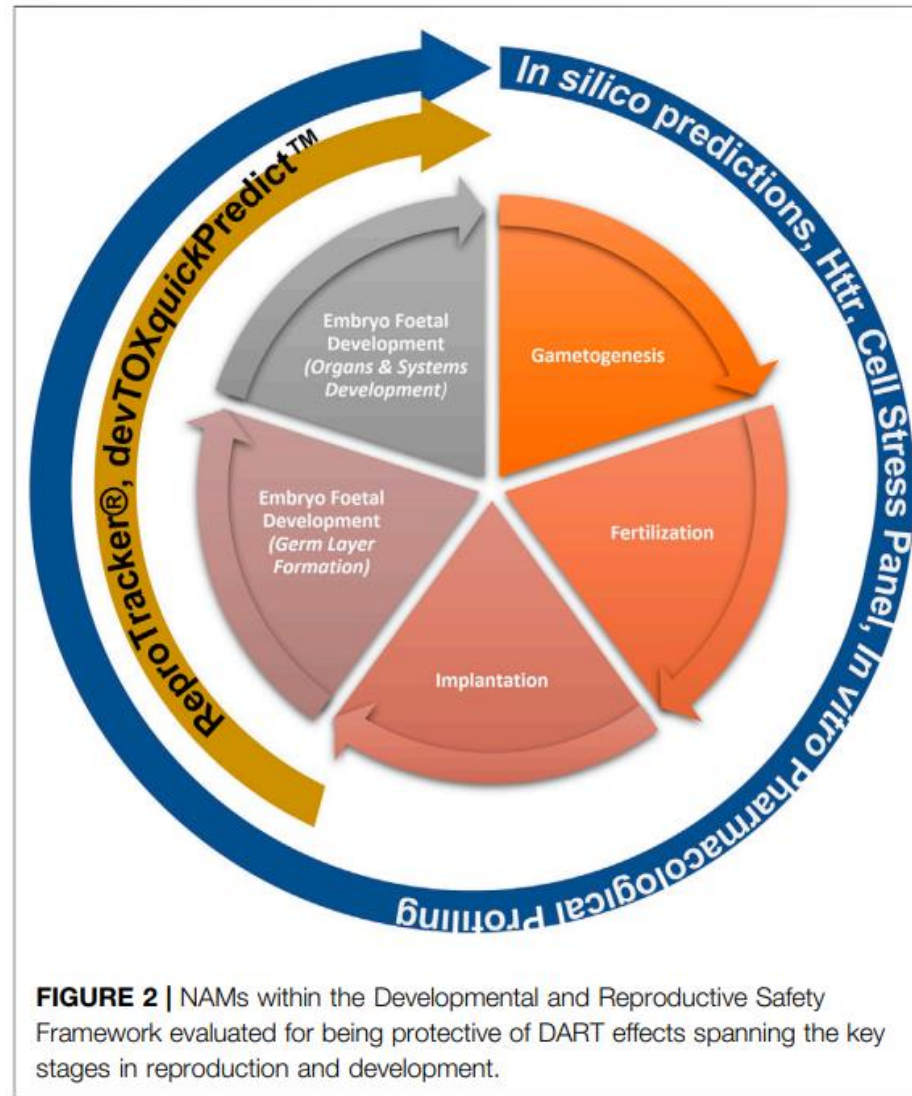
Specialty section: This article was submitted to In Vitro Toxicology, a section of the journal Frontiers in Toxicology

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/tox.2022.838466

Frontiers in Toxicology | www.frontiersin.org

March 2022 | Volume 4 | Article 838466



NGRA Framework for DART endpoints

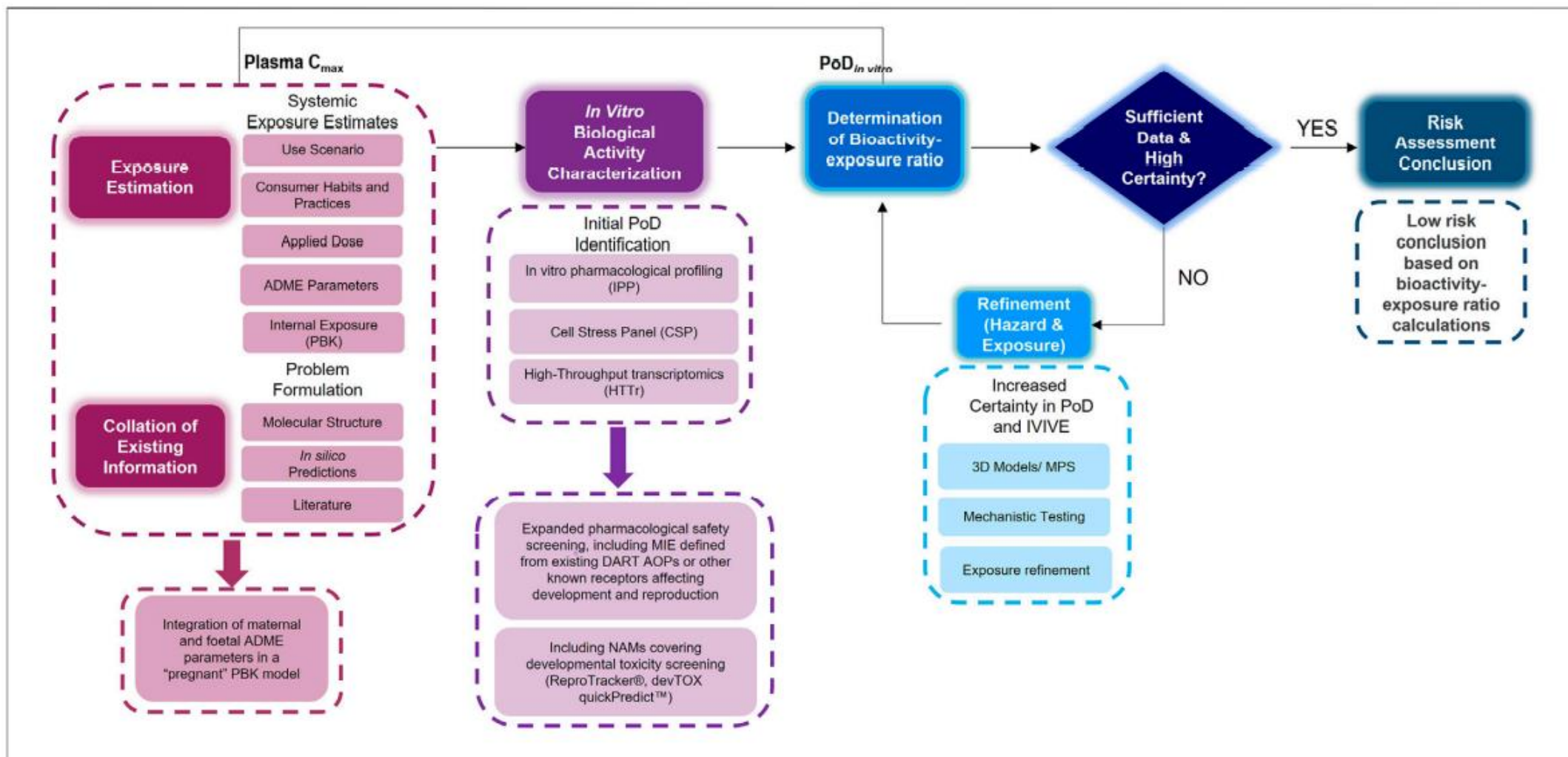
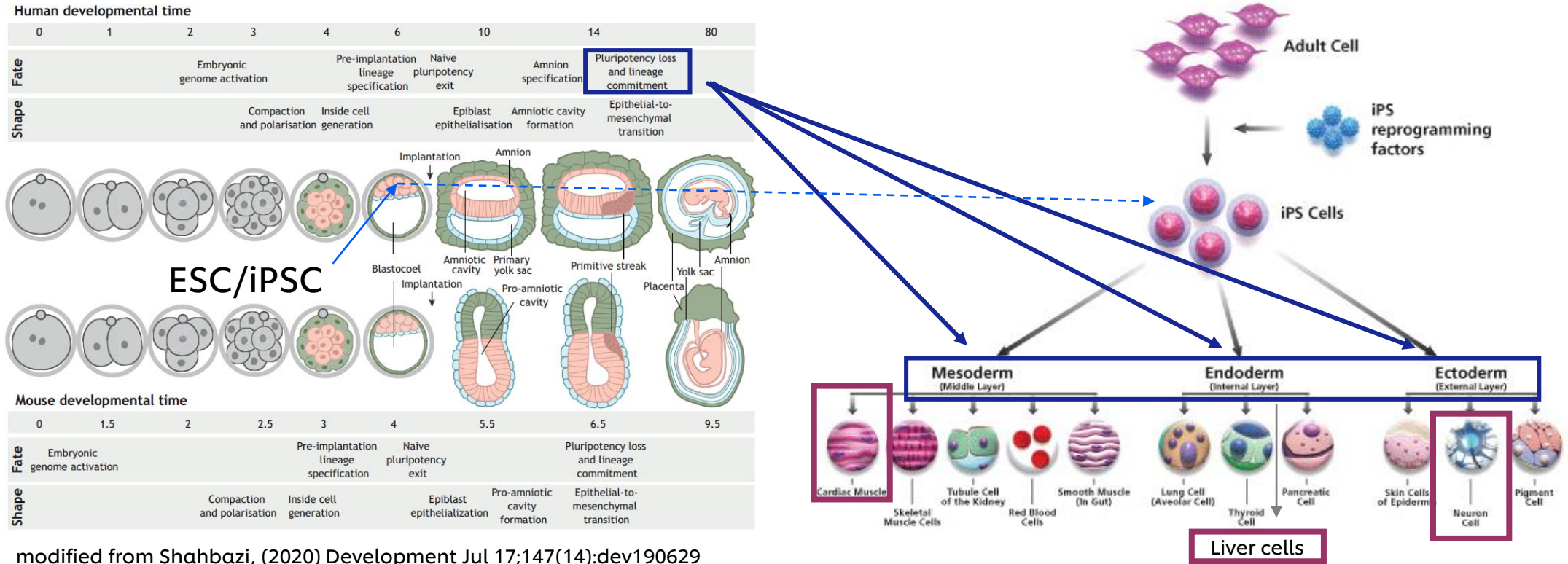


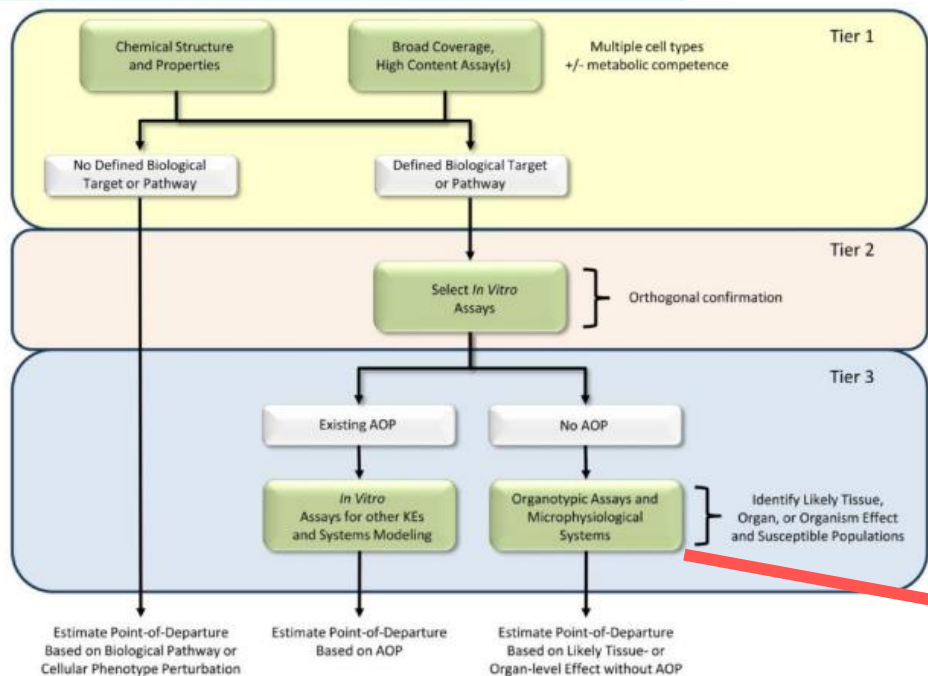
FIGURE 3 | An NGRA framework outlining the consideration of any existing information with exposure estimation including maternal and foetal ADME parameters with *in vitro* biological activity characterisation including additional NAMs relevant for DART endpoints to determine the bioactivity exposure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

Induced pluripotent stem cells (iPSCs) to detect developmental toxicity



- 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

US EPA and NGRA



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

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.

Organotypic Assays and Microphysiological Systems

Principles of Next Generation Risk Assessment from ICCR



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

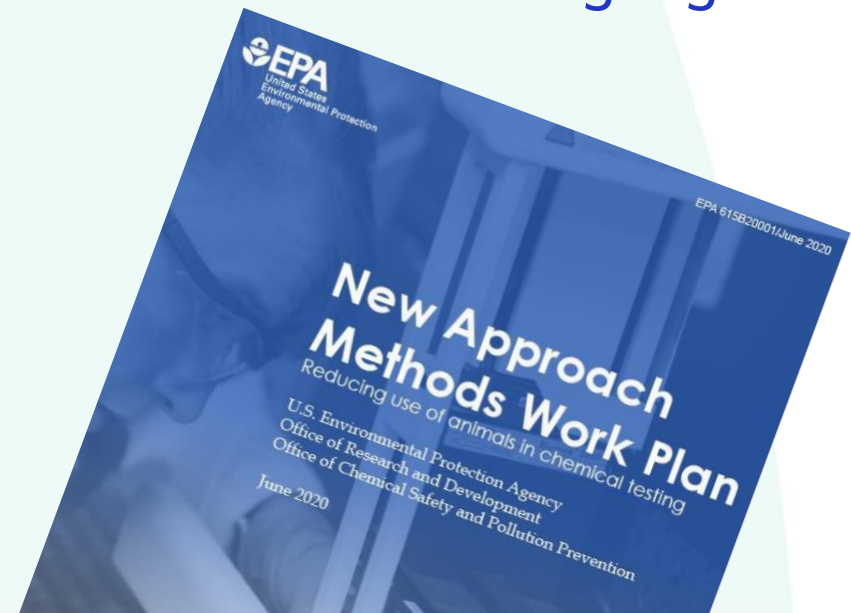
2 Principles for documenting NGRA:

- » 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>

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

Sophie Malcomber
Deborah Martin
Gavin Maxwell
Alistair Middleton
Iris Muller
Beate Nicol
Claire Peart
Ruth Pendlington
Ramya Rajagopal
Georgia Reynolds
Joe Reynolds
Annabel Rigarlsford
Gordon Riley
Paul Russell
Andy Scott
Sharon Scott
Nikol Simecek
Wendy Simpson
Chris Sparham
Sandrine Spriggs

Charlotte Thorpe
Erica Vit
Andy White
Sam Windebank