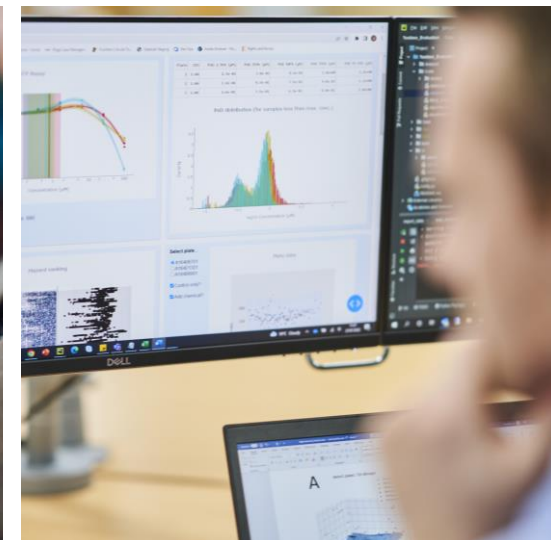


NAMs in the real world: Selecting and using NAMs in industry for safety assessment

Carl Westmoreland
Unilever Safety and Environmental Assurance Centre

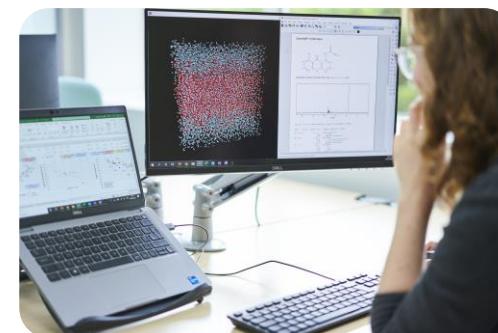
30th April 2024



Unilever's Safety and Environmental Assurance Centre (SEAC)



SEAC performs assessments across Unilever's product portfolio (Personal Care, Beauty & Wellbeing, Home Care, Nutrition and Ice Cream) and provides expert support to regulatory submissions



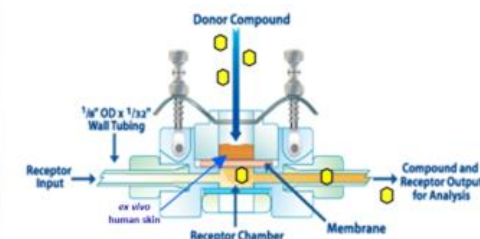
Assessing consumer safety without new animal testing – maximising use of existing information & non-animal approaches

- All our risk assessments are exposure-led

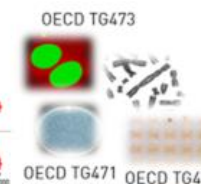
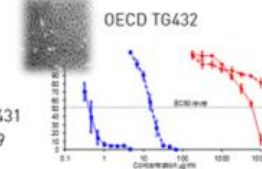
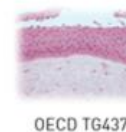


Table 2: Estimated daily exposure levels for different cosmetic product types according to Cosmetics Europe data (SCCNPP/0321/00; Hal et al., 2007, 2011).

Product type	Estimated daily amount applied	Relative amount applied (ppm% body)	Retention factor ¹	Calculated daily exposure (µg/d)	Calculated relative daily exposure (µg/kg body/d)
Bathing, showering					
Shower gel	18.67 g	279.20	0.01	0.19	2.79
Hand wash soap ²	20.00 g	-	0.01	0.20 ³	3.33
Hair care					
Shampoo	10.46 g	150.49	0.01	0.11	1.51
Hair conditioner ²	3.92 g	-	0.01	0.04	0.60
Hair styling products	4.00 g	57.40	0.1	0.40	5.74

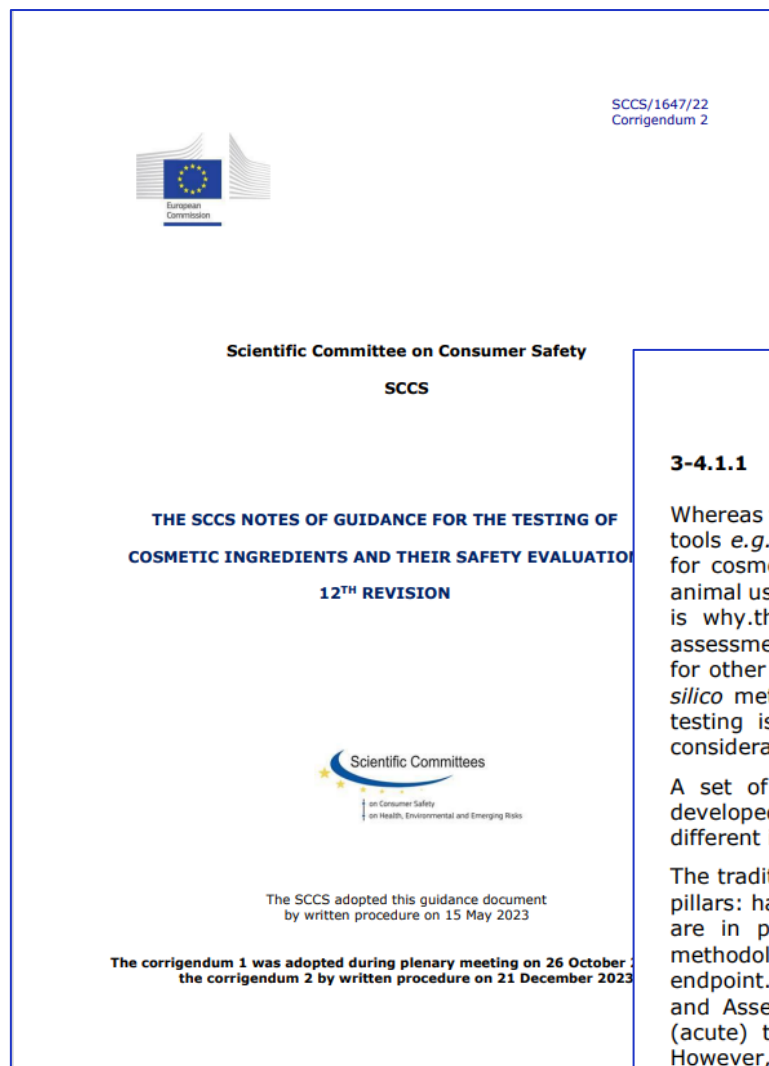


- Use all available safety data on the ingredient
 - clinical, epidemiological, animal (if dates permit), *in vitro*, etc.
- Exposure-based waiving approaches (e.g. toxicological threshold of concern)
- In silico* predictions
- History of safe use
- Read-across
- Use of existing OECD *in vitro* approaches
- Next Generation Risk Assessment (NGRA)**



OECD TG473

Next Generation Risk Assessment (NGRA) in Regulatory Guidance



3-4.1 New Approach Methodology (NAM) and Next-Generation Risk Assessment (NGRA)

3-4.1.1 DEFINITIONS

Whereas the terminology of "Alternative Test Methods (ATMs)" does not cover all available tools *e.g.*, *in silico* methodology, the more general term, NAM, has been introduced. As for cosmetics and their ingredients, testing and marketing bans apply with respect to animal use and there is an obligation to only use validated replacement alternatives, which is why the need for validated non-animal alternative methods for chemical hazard assessment is much greater 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, grouping, RAX, 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).

The traditional framework of risk assessment used for cosmetic ingredients is based on 3 pillars: hazard identification, dose assessment and exposure assessment (see 3-1). NAMs are in particular applied in hazard identification. They may represent stand-alone methodology and as such replace an *in vivo* method (one by one) for a specific toxicological endpoint. They may also be used in combination, *e.g.* as Integrated Approaches to Testing and Assessment (IATA) and Defined Approaches (DAs). In particular, NAMs for local (acute) toxicity became available and have been validated for regulatory purposes. However, a serious obstacle remains the lack of NAMs for systemic and long-term toxicity. Many efforts are ongoing to modernise toxicological safety evaluation and to look for non-animal methodology that can be used, not only for hazard assessment, but also for quantitative risk assessment of compounds that after long-term exposure could be at the origin of systemic toxicity. The whole traditional framework for risk assessment is under

Principles of NGRA from ICCR: Non-animal approaches in Cosmetic Risk Assessment



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

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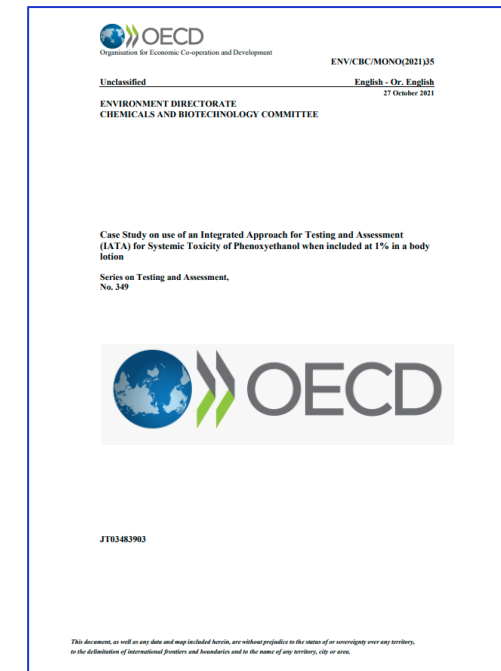
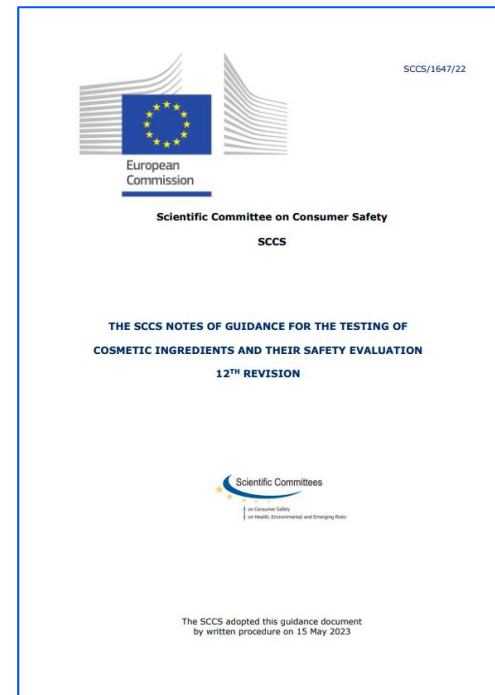
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2 Principles for documenting NGRA:

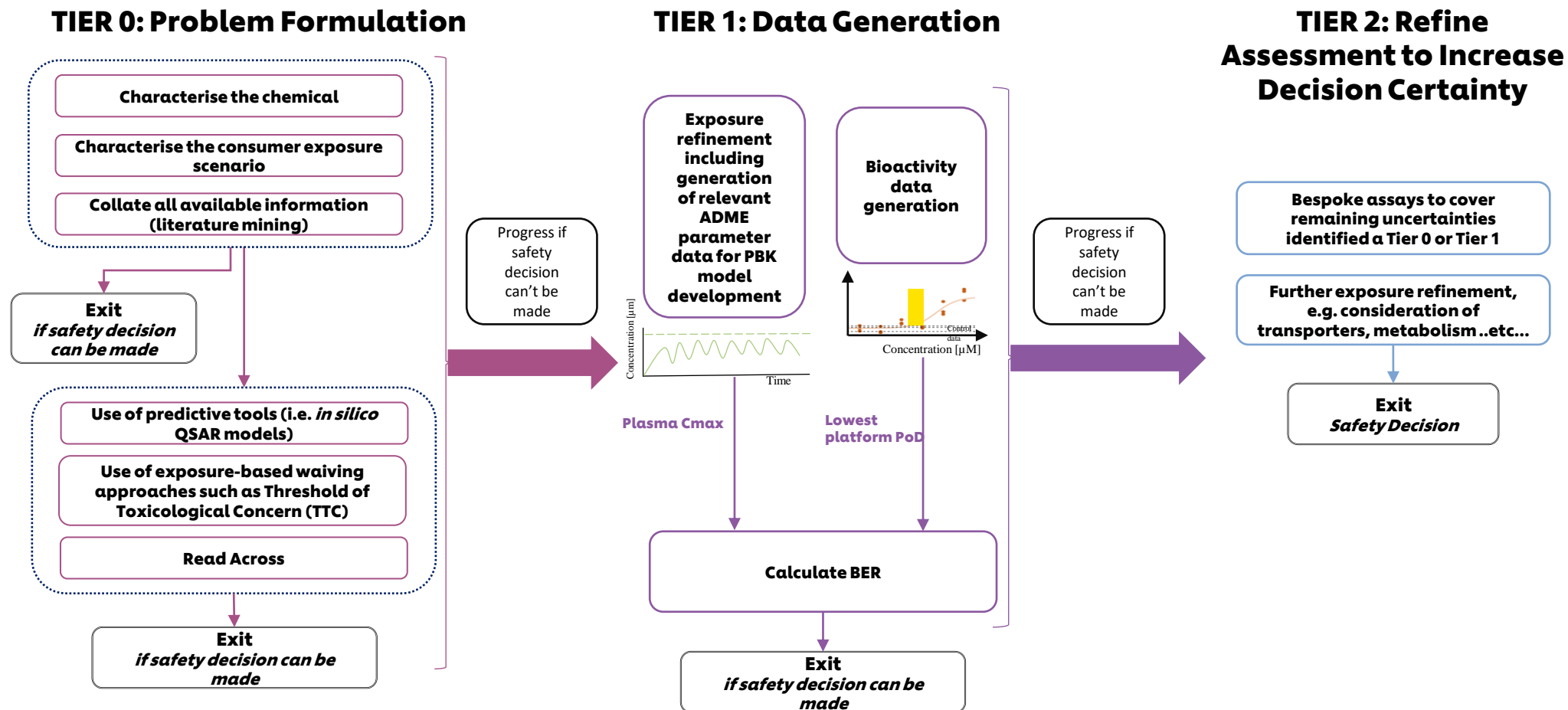
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Tiered, exposure-led NGRA means we can make robust safety decisions

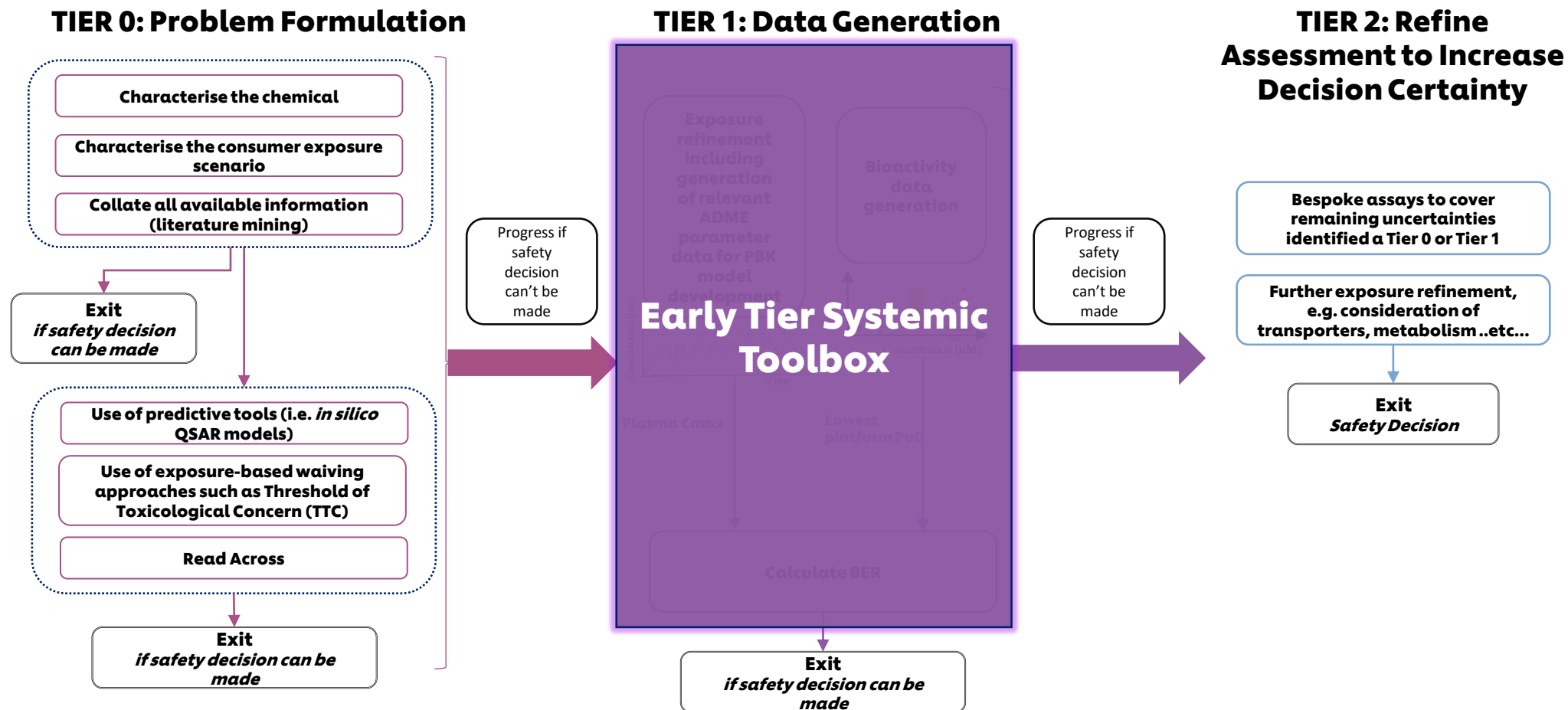
- Increasing recognition that *in vitro* bioactivity can inform decision making (e.g. Health Canada, EU SCCS)



Framework Approach: The overall goal is a human safety risk assessment



Framework Approach: The overall goal is a human safety risk assessment



A first tier systemic toolbox for safety decision making: Defining the toolbox components

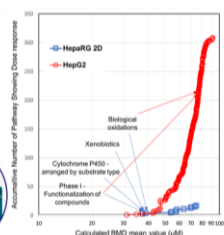
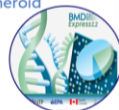
Point of Departure determination

Non-specific effects

Transcriptomics

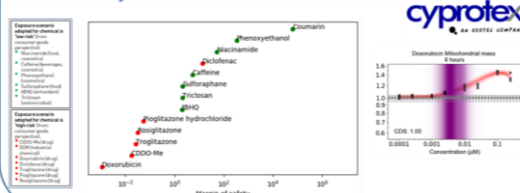
- Use of full human gene panel ~ 21k
- 24 hrs exposure
- 7 concentrations
- 3 cell lines HepG2/ HepaRG/ MCF7
- 3D HepaRG spheroid

BMDexpress 2



Cellular Stress Pathways

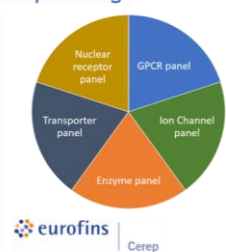
13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways



Toxicol Sci (2020), 176, 11-33

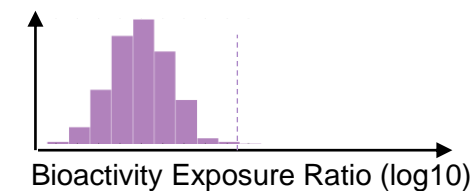
Specific effects

In vitro pharmacological profiling

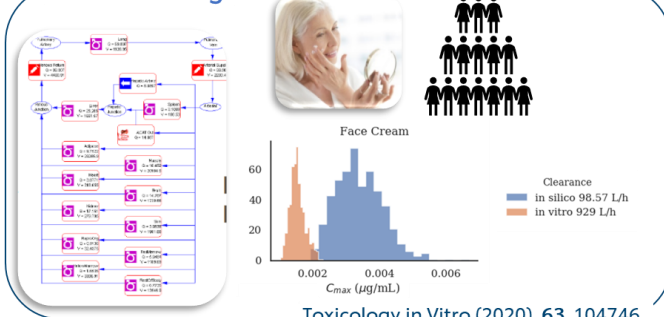


eurolins | Cerep

Bioactivity Exposure Ratio Distribution



PBK Modelling



Toxicology in Vitro (2020), 63, 104746

Plasma
 C_{max}
estimate



SOT | Society of Toxicology
academic.oup.com/toxsci



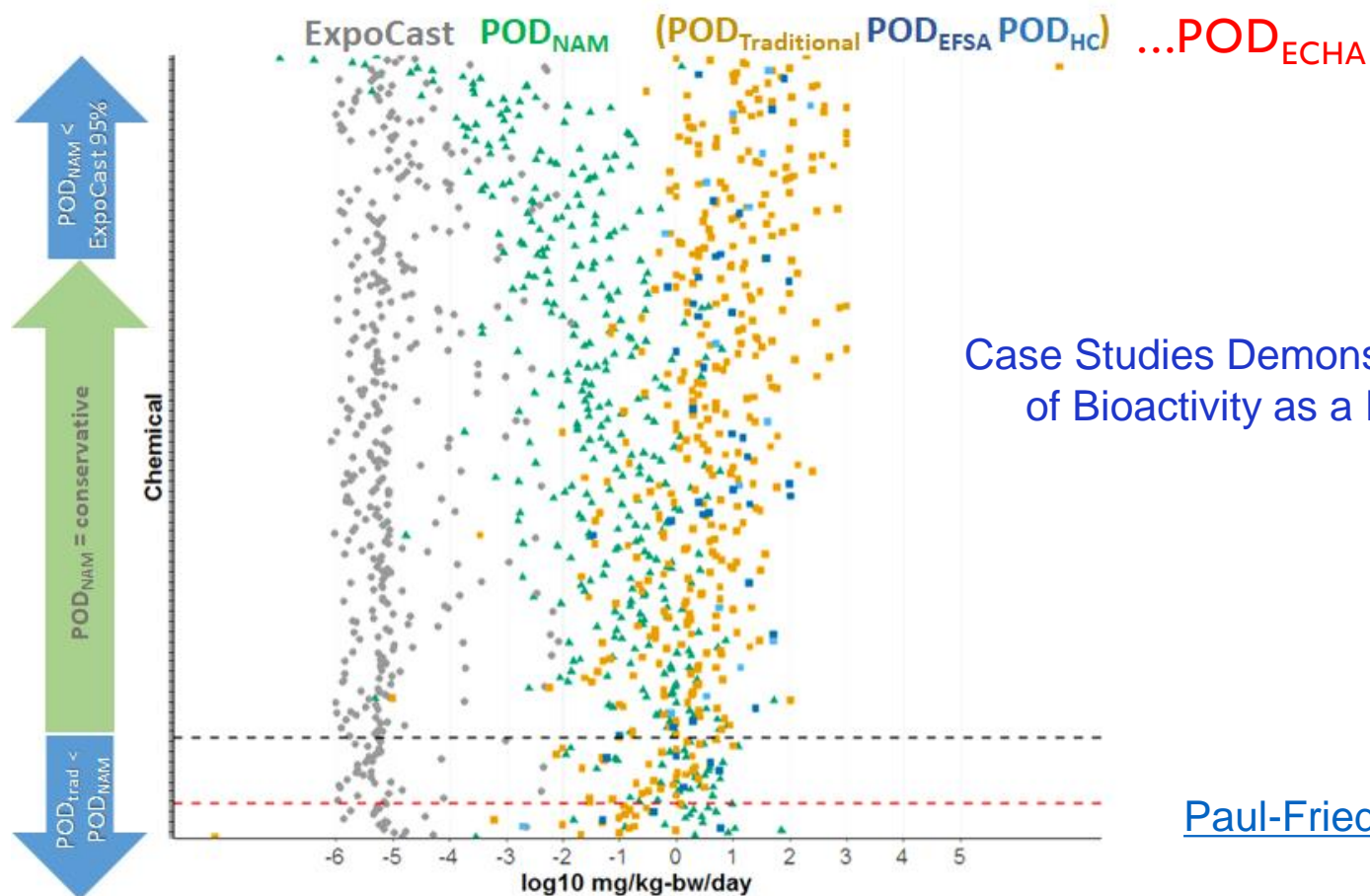
TOXICOLOGICAL SCIENCES, 189(1), 2022, 124–147

<https://doi.org/10.1093/toxsci/ktfac068>
Dryad Digital Repository DOI: <https://doi.org/10.5061/dryad.fbg79cncx1>
Advance Access Publication Date: 13 July 2022
Research article

Are Non-animal Systemic Safety Assessments Protective? A Toolbox and Workflow

Alistair M. Middleton ,^{*,†} Joe Reynolds,^{*} Sophie Cable,^{*} Maria Teresa Baltazar,^{*} Hequn Li ,^{*} Samantha Bevan,[†] Paul L. Carmichael,^{*} Matthew Philip Dent,^{*} Sarah Hatherell,^{*} Jade Houghton,^{*} Predrag Kucic,^{*} Mark Liddell,^{*} Sophie Malcomber,^{*} Beate Nicol,^{*} Benjamin Park,[†] Hiral Patel,[‡] Sharon Scott,^{*} Chris Sparham,^{*} Paul Walker ,[†] and Andrew White^{*}

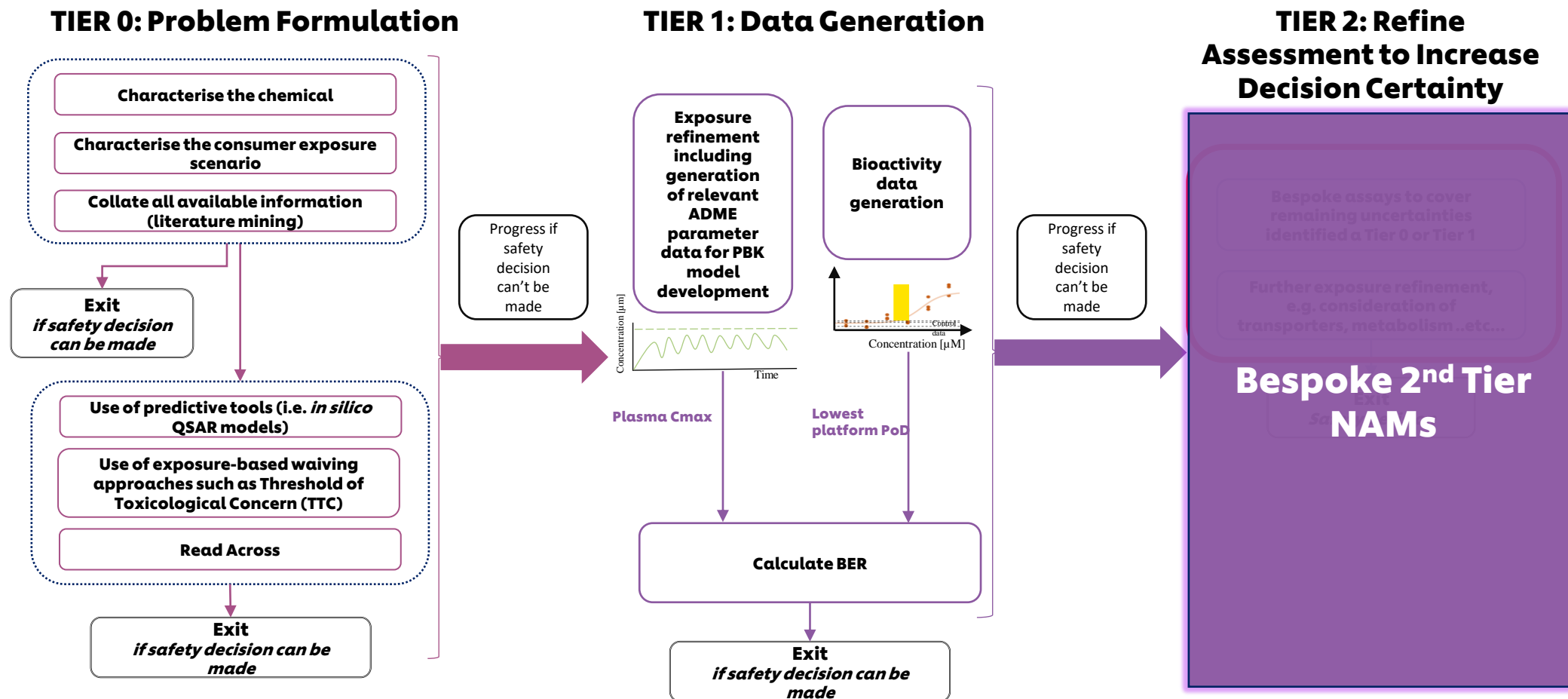
Points of Departure from NAMs can be protective



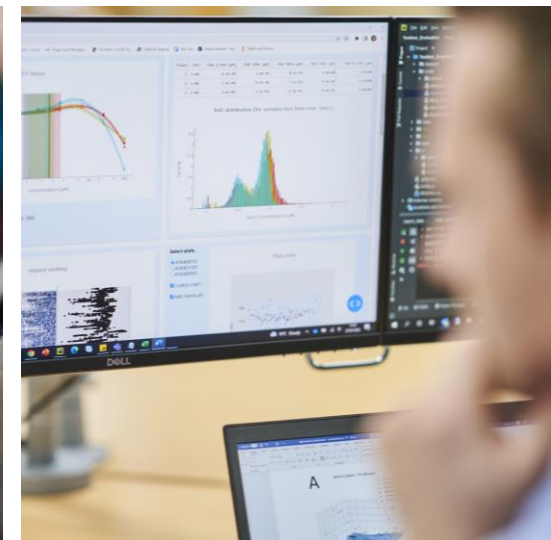
Case Studies Demonstrating Application of Bioactivity as a Protective POD

[Paul-Friedman et al., 2020](#)

Framework Approach: The overall goal is a human safety risk assessment



Examples of selecting, evaluating and using NAMs



Example 1: Higher Tier Tools for input into exposure assessments

Physiologically-based kinetic modelling (PBK) input data

From applied dose to internal exposure based on NAMs

Level 0:

- Characterise exposure scenario (who, where, how often, and how much)
- Product & chemical information

Level 1:

- Predictions from *in silico* only
- parameterisation & sensitivity

Level 2:

- PBK modelling based on *in vitro* parameterisation

Level 3:

- Generating human PK data for validation or/and calibration

- The progression between levels is closely related to the risk assessment process
- Use tools that are as complex as necessary to make the decision
- move to more complex tools if more data are needed

Example 2: Higher Tier Tools for input into bioactivity assessment

Renal Effects

Benzophenone-4 (BP4) case study safety assessment



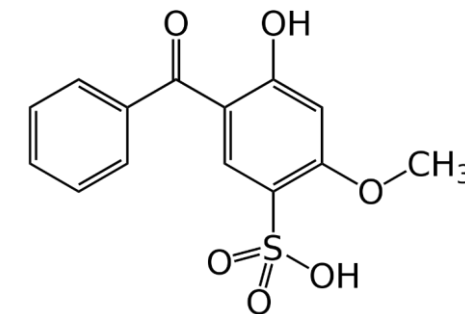
EN English

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Call for data on ingredients with potential endocrine-disrupting properties used in cosmetic products



Is a tiered NGRA approach is sufficiently protective and useful to answer a real-life question?

In silico predictions:

- BP-4 is an anion sulphonate
- Likely to be a substrate of Organic anion transporters (OATs)
- Renal clearance may be higher than $GFR \cdot f_{up}$

Two assays:

- Transporter studies in transfected kidney cells in two different assays (uptake assay and vesicular assay)
- Bidirectional permeability of BP-4 in freshly isolated kidney proximal tubule cells monolayer (aProximate™)

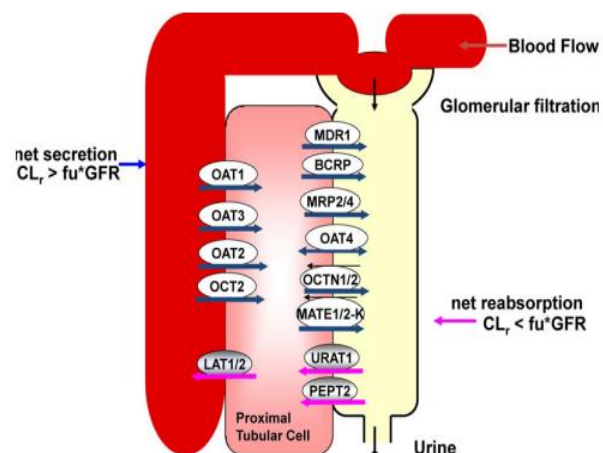
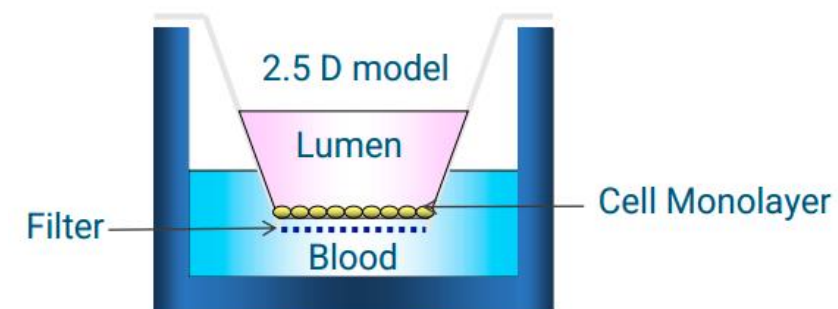


Figure 1. Mechanism of drug elimination and major transporters in the kidney. Drug elimination in the kidney is through glomerular filtration, secretion, and reabsorption process. Major transporters localized in the proximal tubule cells are depicted. The blue arrows indicate secretion, and the pink arrows indicate reabsorption.



B-A → blood to urine → active secretion
A-B → urine to blood → reabsorption

Newcells aProximate™ platform

<https://doi.org/10.1002/jcph.702>

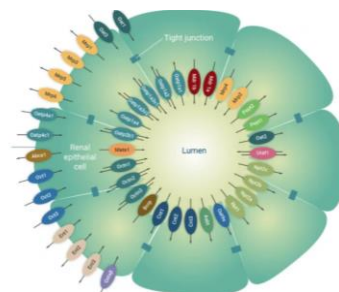
Benzophenone-4 concentration was predicted to be higher in the kidney than any other organ

Cell models in the Tier 1 toolbox have limited expression of the relevant transporters

Renal Toxicity

Renal biomarkers (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints:

- KIM-1
- NGAL
- Clusterin
- TEER (Day 0 and Day 3)
- ATP
- LDH
- Toxicogenomics (3 donors, 2 duplicates per donor), 8 concentrations, 24h and 72h timepoints
- Omeprazole and cisplatin added as benchmarks/positive controls



[Newcells aProximate™ platform](#)

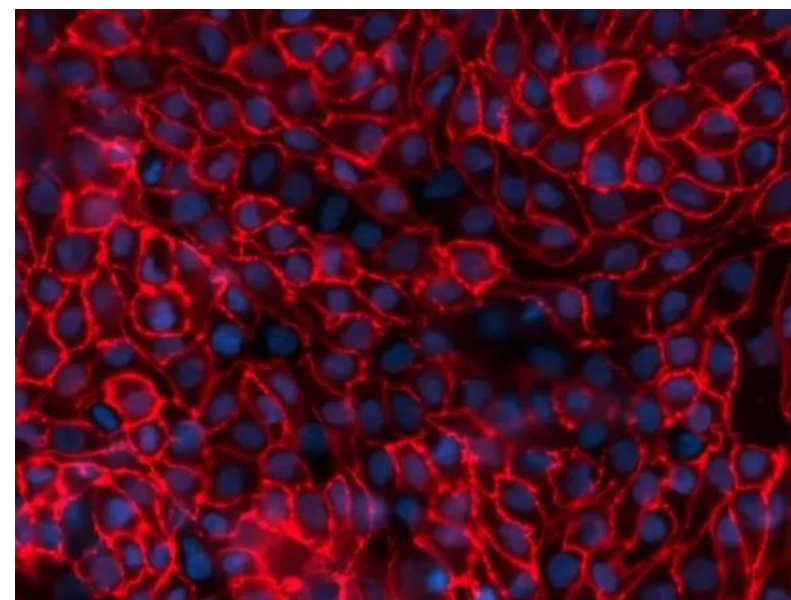


Image from
[Proximal Tubule | Newcells Biotech](#)

Example 3: Expanding the Tier One Toolbox to cover more aspects of Developmental and Reproductive Toxicology (DART)



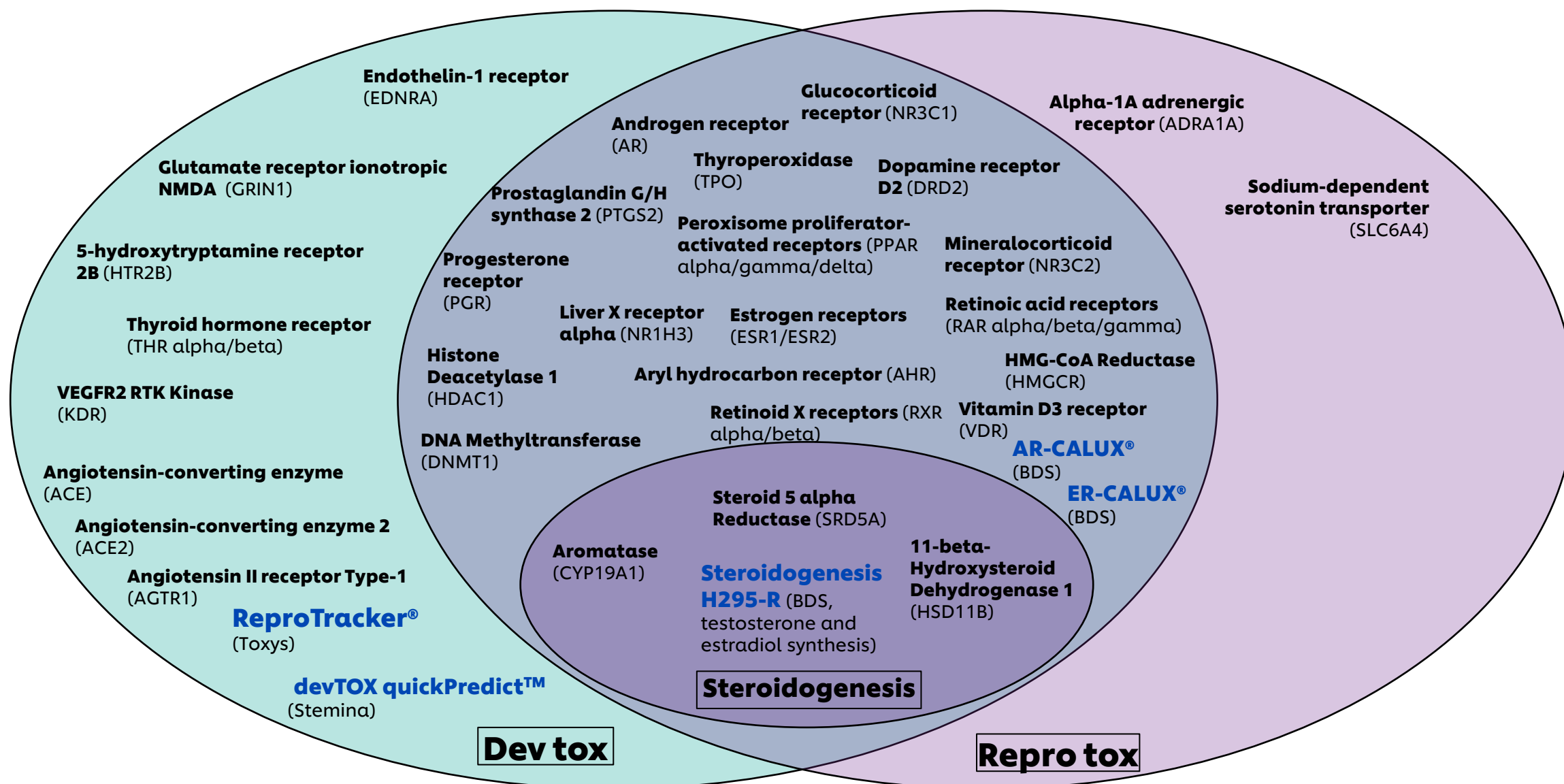
Beyond AOPs: A Mechanistic Evaluation of NAMs in DART Testing

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

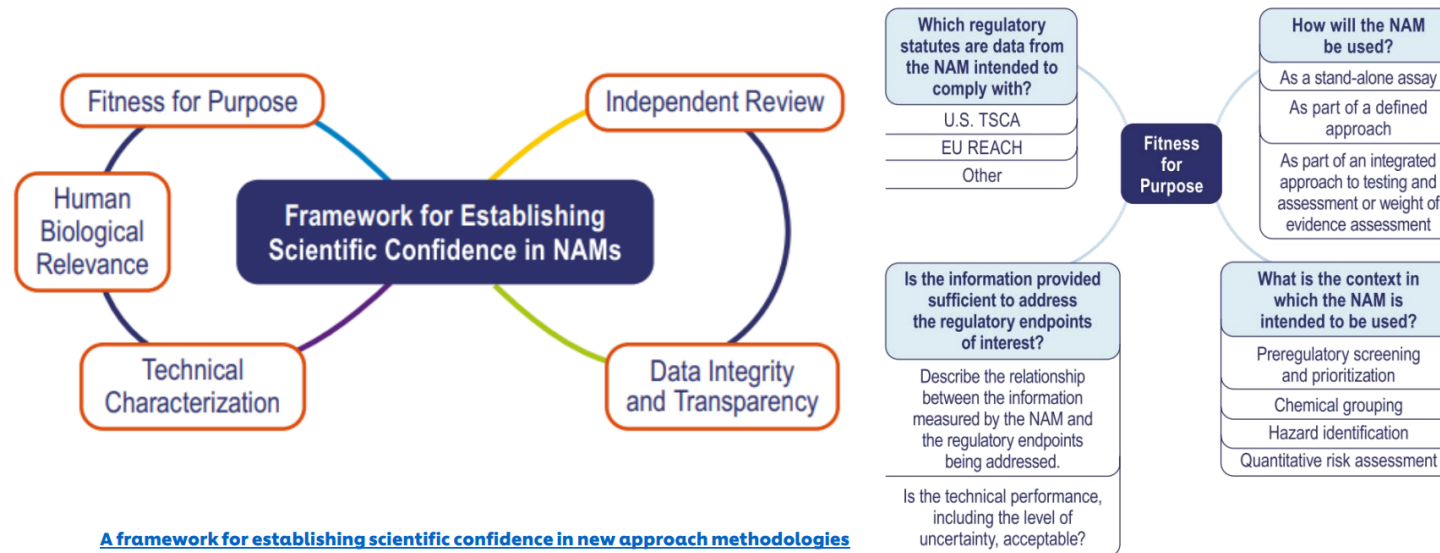
New Approach Methodologies (NAMs) promise to offer a unique opportunity to enable human-relevant safety decisions to be made without the need for animal testing in the context of exposure-driven Next Generation Risk Assessment (NGRA). Protecting human health against the potential effects a chemical may 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



Validation/Regulatory Acceptance of NAMs

- Understanding the needs for formal validation at an international level
- Current validation process is slow and based on traditional animal tests
- Need for a framework/guidance for fit for purpose validation at an international level, e.g. update of OECD GD 34



Conclusions

1. **Next Generation Risk Assessment** relies on exposure and bioactivity data generated in robust, reliable NAMs
2. The tiered and iterative nature of NGRA means that higher tiers will need **more complex human-relevant NAMs** for answering bespoke questions
3. **Choosing, evaluation and using NAMs** requires **scientific networks** and **different types of expertise**
4. **Working together across the NC3Rs NAMs Network**
 - Understanding needs
 - Understanding what's out there
 - Developing new science together

NAMs Network



The NAMs Network is a community of researchers, developers and industry and regulatory end-users working together to accelerate the use of new approach methodologies.

Network values



Support the 3Rs
in scientific
research



Be open to
collaboration and
communication
between sectors



Share new
opportunities and
information with
the community

Thank You

- Adam Wood
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- Annabel Rigarfsford
- Ashraf Abdelkhalig
- Beate Nicol
- Catherine Barratt
- Chris Sparham
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