Advancing the Application of New Approach Methodologies (NAMs) for Systemic Toxicity Assessment of Chemicals

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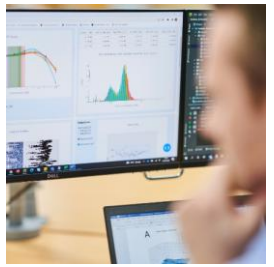
SERS
Safety, Environmental & Regulatory Science



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The Need for Implementation of NAM-Based Safety Assessments

Societal attitudes/ consumer preference

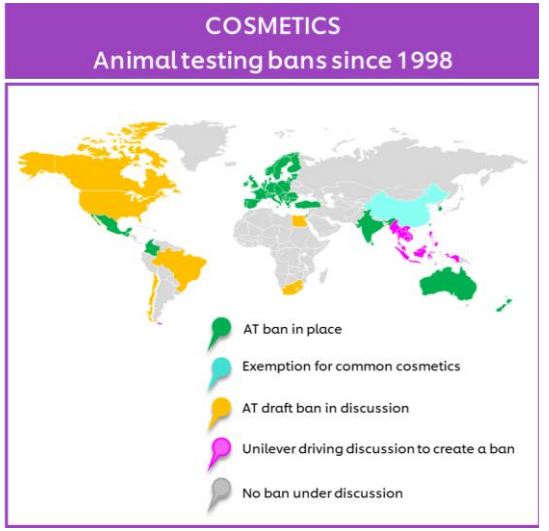
Resource constraints

Regulatory change

(e.g., bans on animal testing – cosmetic products in > 40 countries and >10 US states)

Human relevance & science evolution

PETA APPROVED
Global Animal Test Policy



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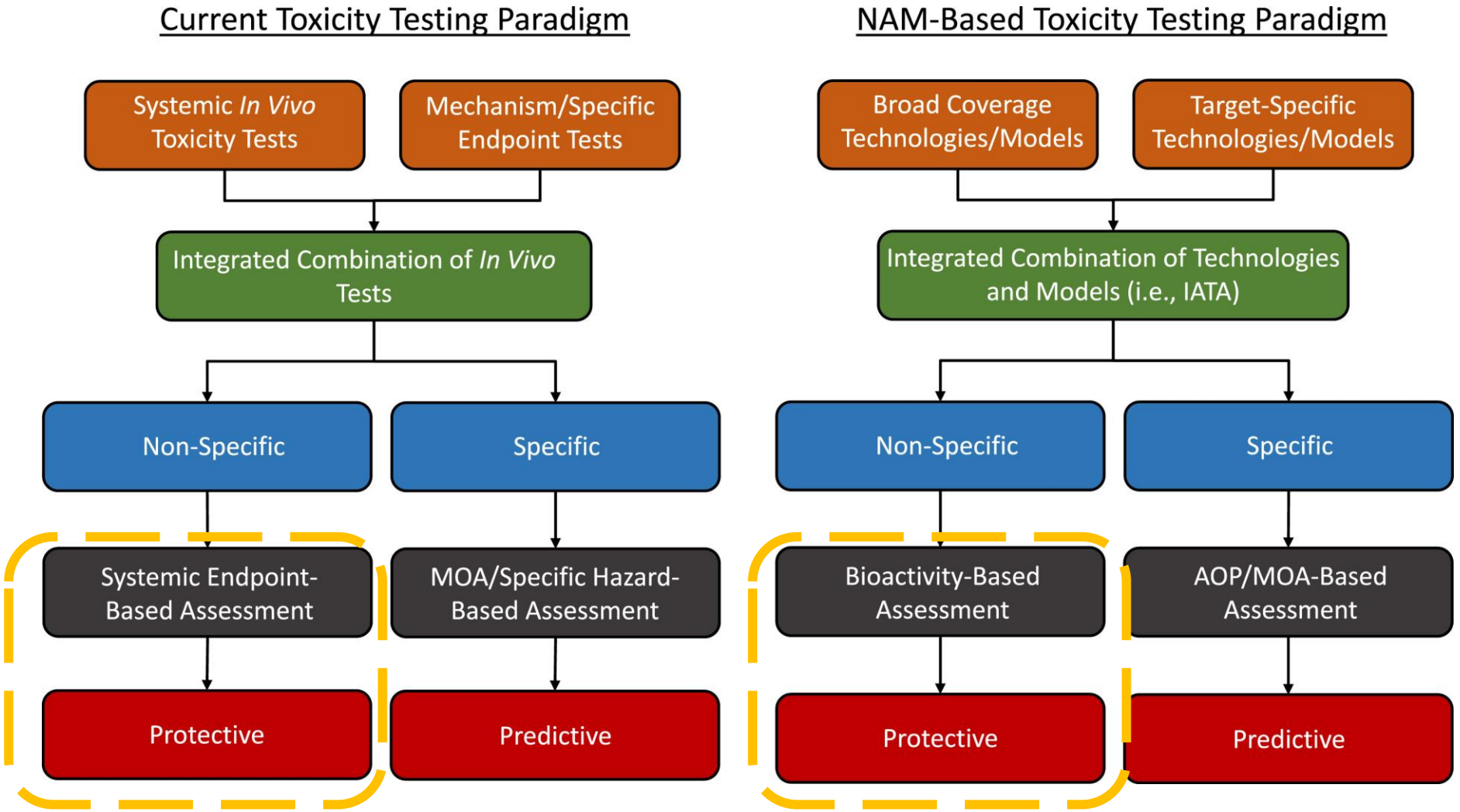
Context of use of a NAM- Systemic toolbox

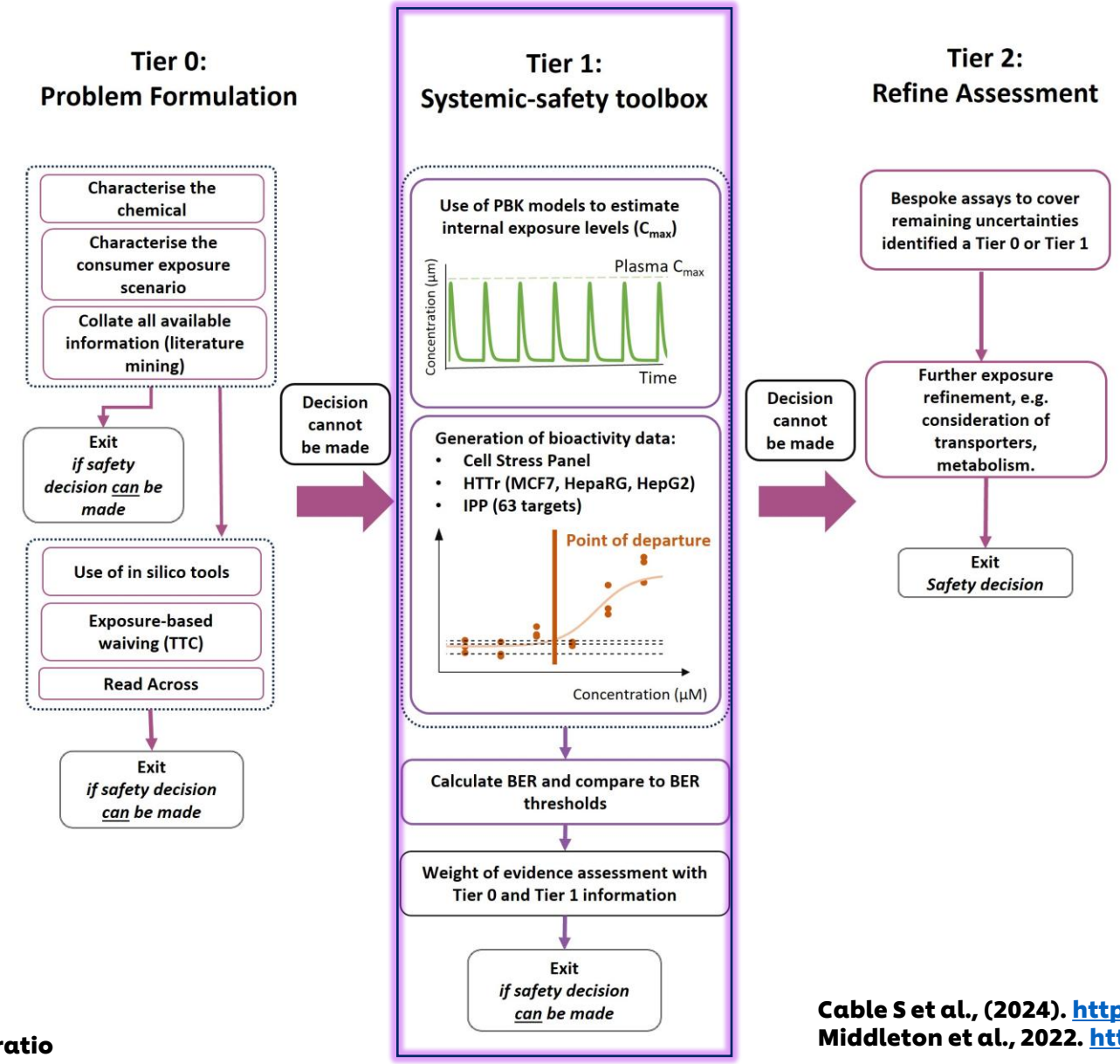
- A NAM-based toolbox intended to be used as **a Tier 1 within a NGRA/IATA framework** for systemic toxicity (i.e. quantitative risk assessment of ingredients in consumer goods products).
- A systemic toolbox which provides **protective thresholds (PoDs) for systemic toxicity**.
- A systemic toolbox that provides **better or equivalent levels of protection of human** health and useful for risk assessment which integrates bioactivity and exposure -> derive protective decision thresholds (BER)

Context of use: bioactivity based-assessment and protection of human health



Browne et al., 2024 Reg Tox Pharm https://doi.org/10.1016/j.yrtph.2024.105579

Context of Use: Tier 1 within NGRA framework



BER= bioactivity: exposure ratio

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159;
Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

Evaluation strategy for the context of use of protection of human health

Define the toolbox components

Choose a set of NAMs covering exposure modelling and **bioactivity which provide wide biological coverage**

Set performance criteria

1) The performance of the NAM toolbox is **assessed against historical safety decisions**
2) How do *in vitro* PoDs compare to *in vivo* PoD?

Select test chemicals

Maximise coverage of different **chemistries and biological effects/toxicity**

Define prototype decision model for determining the BER threshold

Data-driven derivation of protective bioactivity: exposure ratio (BER) thresholds

Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159;
Middleton et al., 2022. https://doi.org/10.1093/toxsci/kfac068

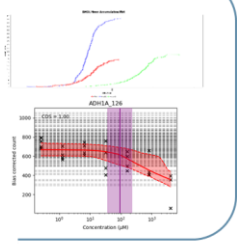
A set of NAMs covering exposure modelling and bioactivity which provide wide biological coverage

Point of Departure (PoD) determination from Bioactivity assays

Non-specific effects

High-Throughput transcriptomics (HTTr)

- TempO-seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
- HepG2
- 24hr exposure
- 8 concentrations
- Dose-response analysis using BIFROST model

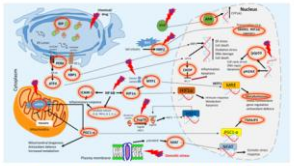
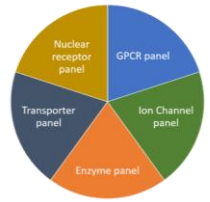


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

Specific effects

In vitro pharmacological profiling



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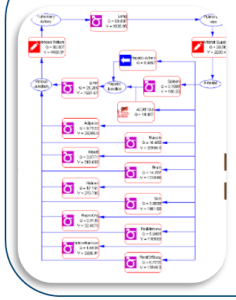
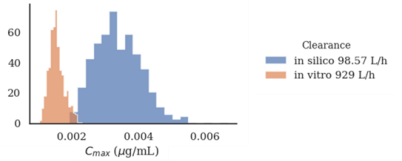
PBK Modelling



Face Cream

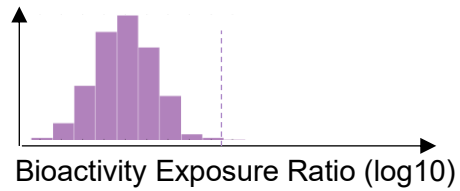


Toxicology in Vitro (2020), **63**, 104746

Plasma C_{max} estimate → **C_{max} Error Distribution model (CMED)**

(Bayesian model)

Bioactivity Exposure Ratio (BER) Distribution



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The performance criteria assumes that current risk assessments are protective for human health

What we are trying to test: Are the decisions made with a Tier 1 toolbox equivalent or better than the decisions we have been making with animal data?

What we are not trying to test: is the toolbox predictive of all possible adverse effects for a given chemical?

Set performance criteria for evaluating the protectiveness and utility of the toolbox

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available (e.g. regulatory opinions)
- Risk benchmarked to acceptability in a consumer product context

Protectiveness

How many of the high risk exposure scenarios are identified as uncertain/high risk
(i.e. BER < threshold)

Utility

How many of the low risk scenarios are identified as low risk at this early tier stage in a risk assessment framework
(i.e. BER > threshold)

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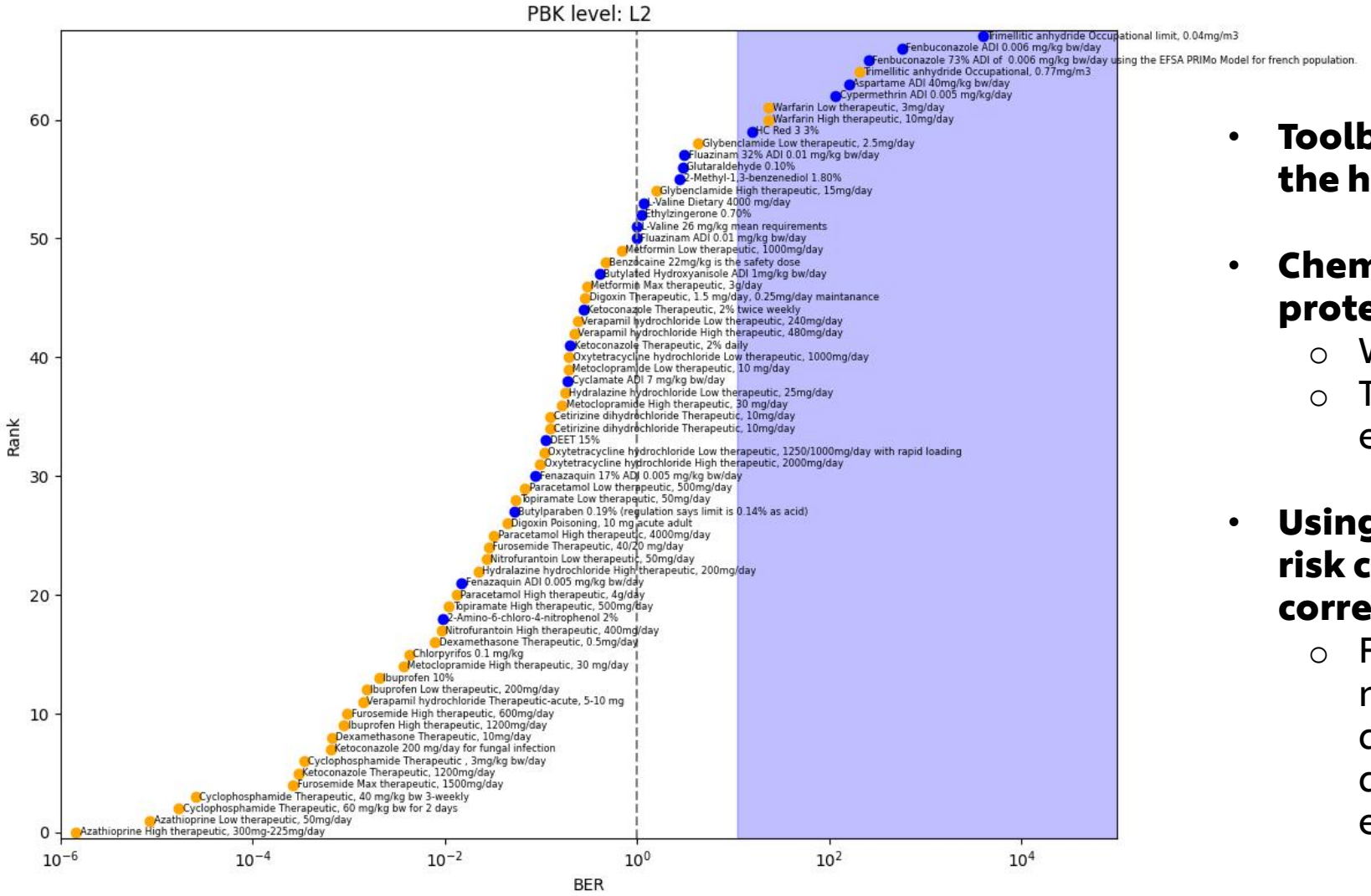
NAM Systemic toolbox provides similar level of protection

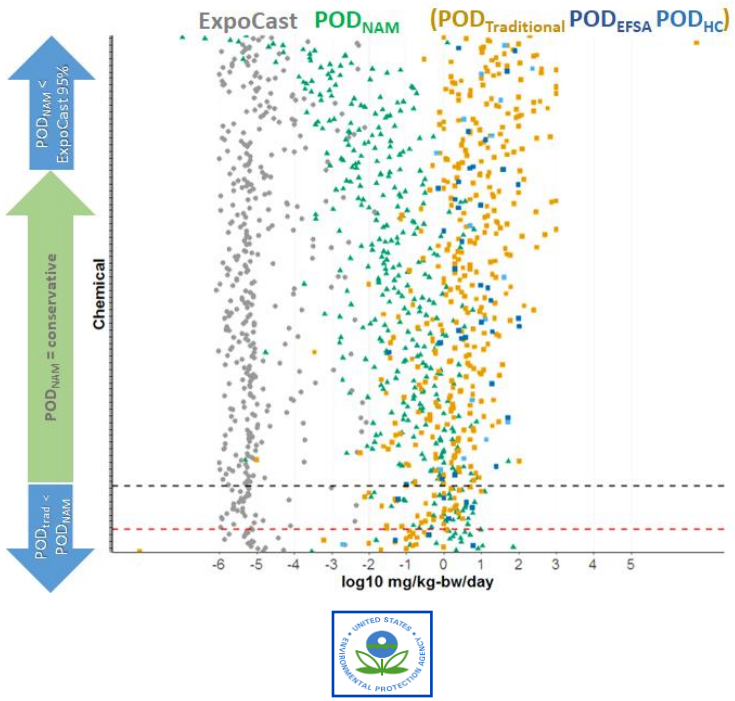


- **Toolbox not protective for 3/46 of the high-risk exposure scenarios**
- **Chemical- Exposure scenarios not protective for:**
  - Warfarin therapeutic oral dose
  - Trimellitic anhydride inhalation exposure
- **Using BER >11, only 27% of the low-risk chemical-scenarios would be correctly identified as such**
  - For the other 73%, refinement is needed (i.e. Approaches to distinguish bioactivity from adversity; refine exposure estimates etc.).

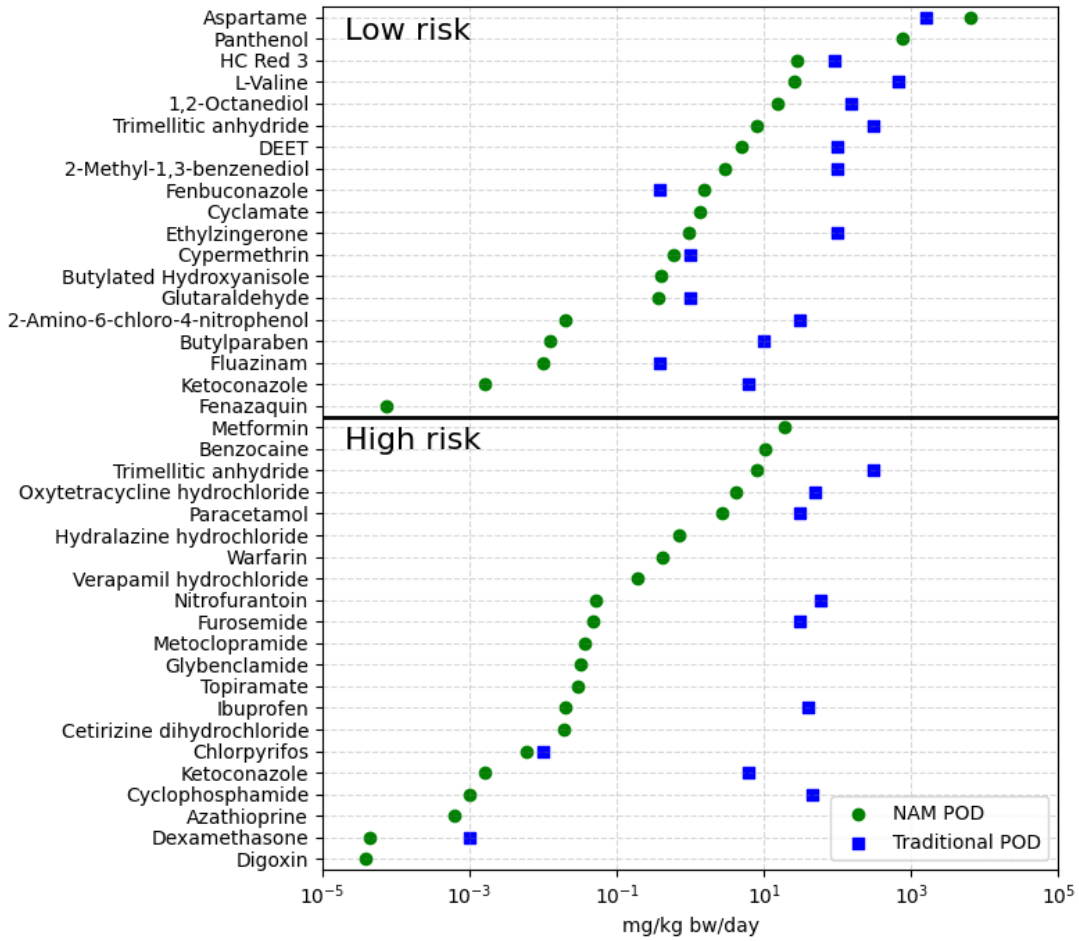
Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159

Other studies also shown that *in vitro* PoDs are more conservative (i.e. lower) than the minimum *in vivo* PoD

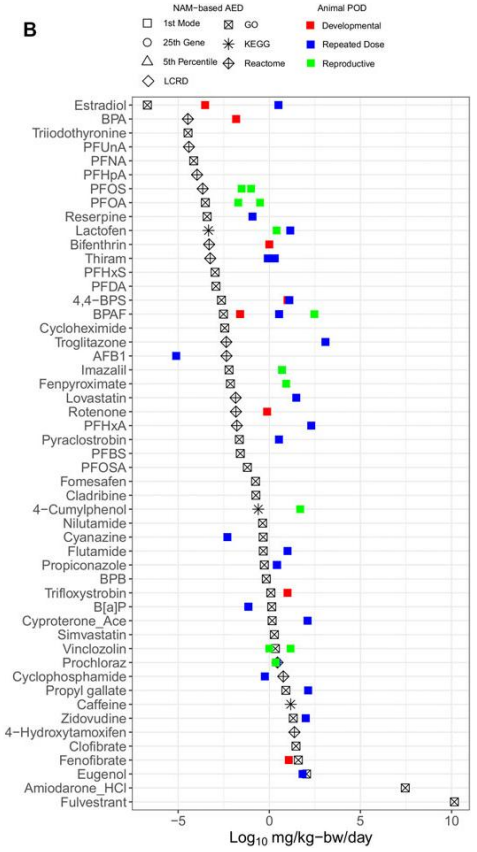
Paul-Friedman (2020) – 448 chemicals



Cable S et al., (2024) – 25 chemicals

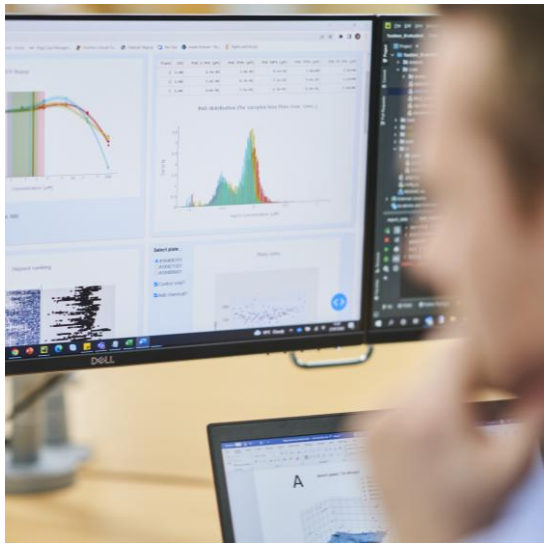


Reardon et al., (2023) – 54 chemicals



Cable S et al., (2024). https://doi.org/10.1093/toxsci/kfae159; Reardon A et al., 2023 https://doi.org/10.3389/ftox.2023.1194895; Paul-Friedman K et al., 2020: https://doi.org/10.1093%2Ftoxsci%2Fkfz201;

Example of selecting NAMs and application of the tiered framework



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Example 1: Higher Tier Tools for input into bioactivity assessment

Renal exposure & Effects

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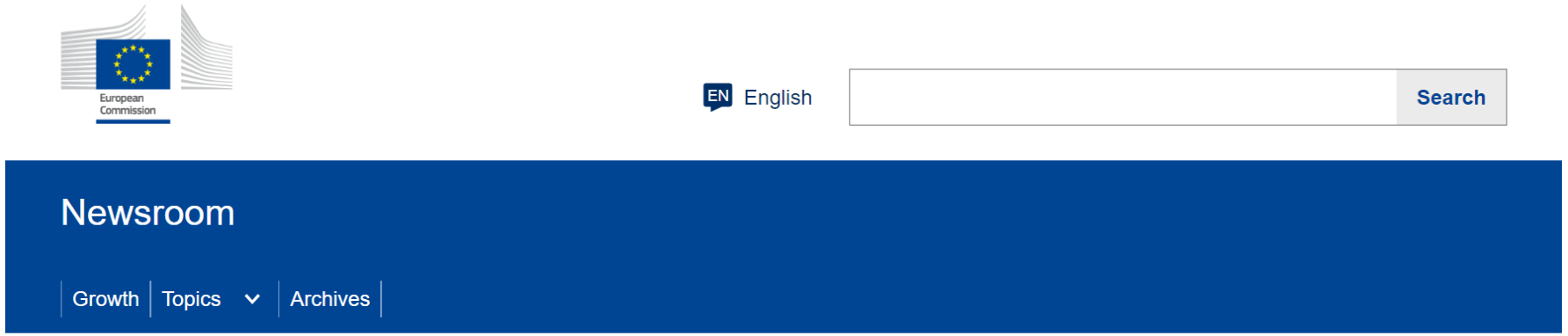
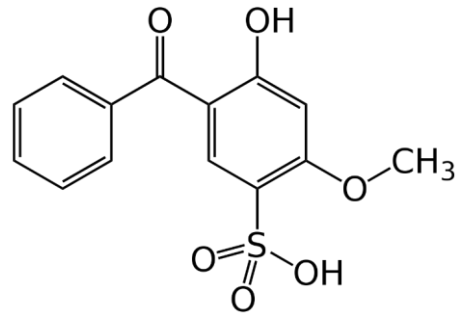
Benzophenone-4 (BP4) case study safety assessment

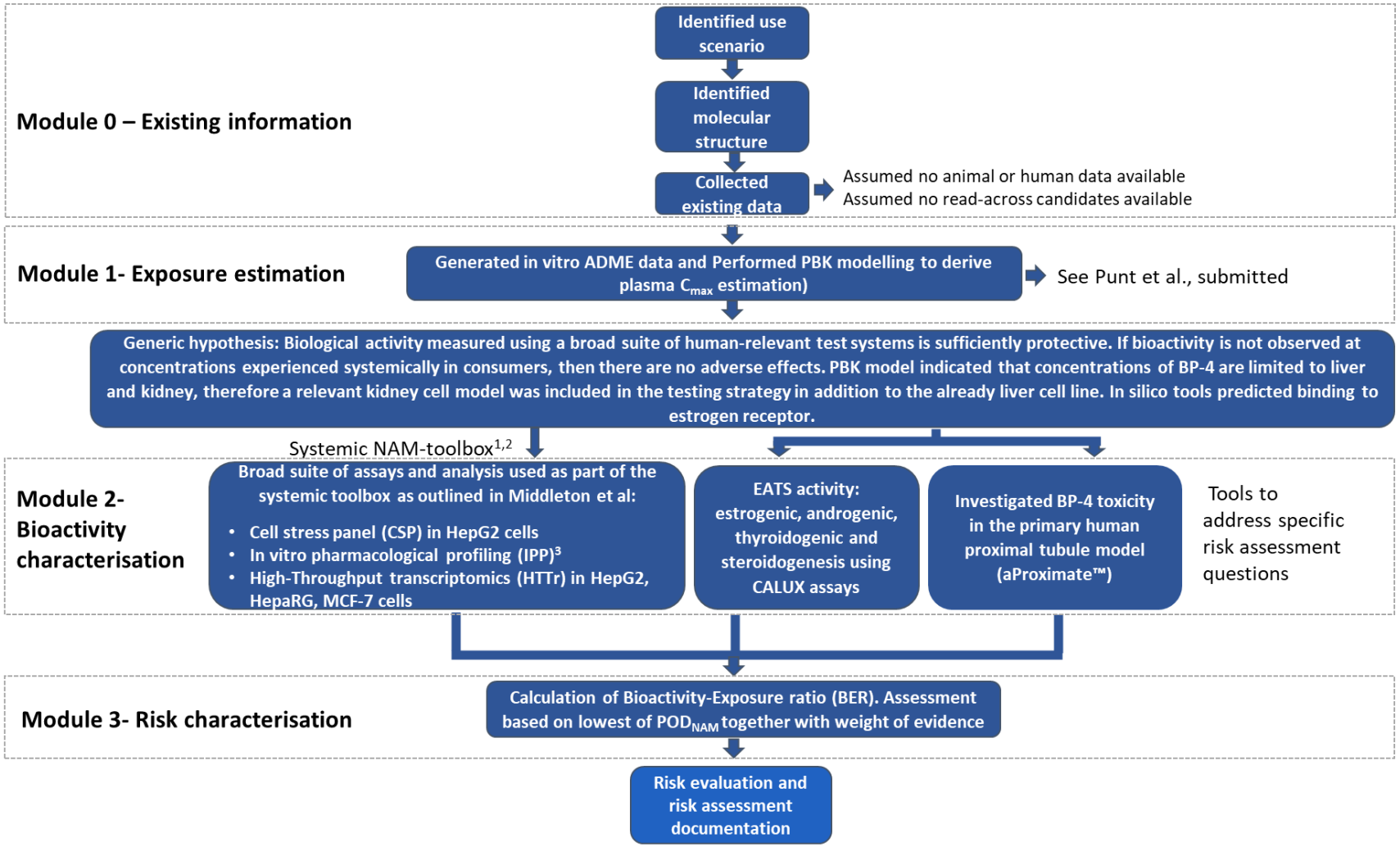




Is a tiered NGRA approach sufficiently protective and useful to answer a real-life question?

Baltazar MT et al., 2025 https://www.altex.org/index.php/altex/article/view/2934/version/2996

BP4 risk assessment framework

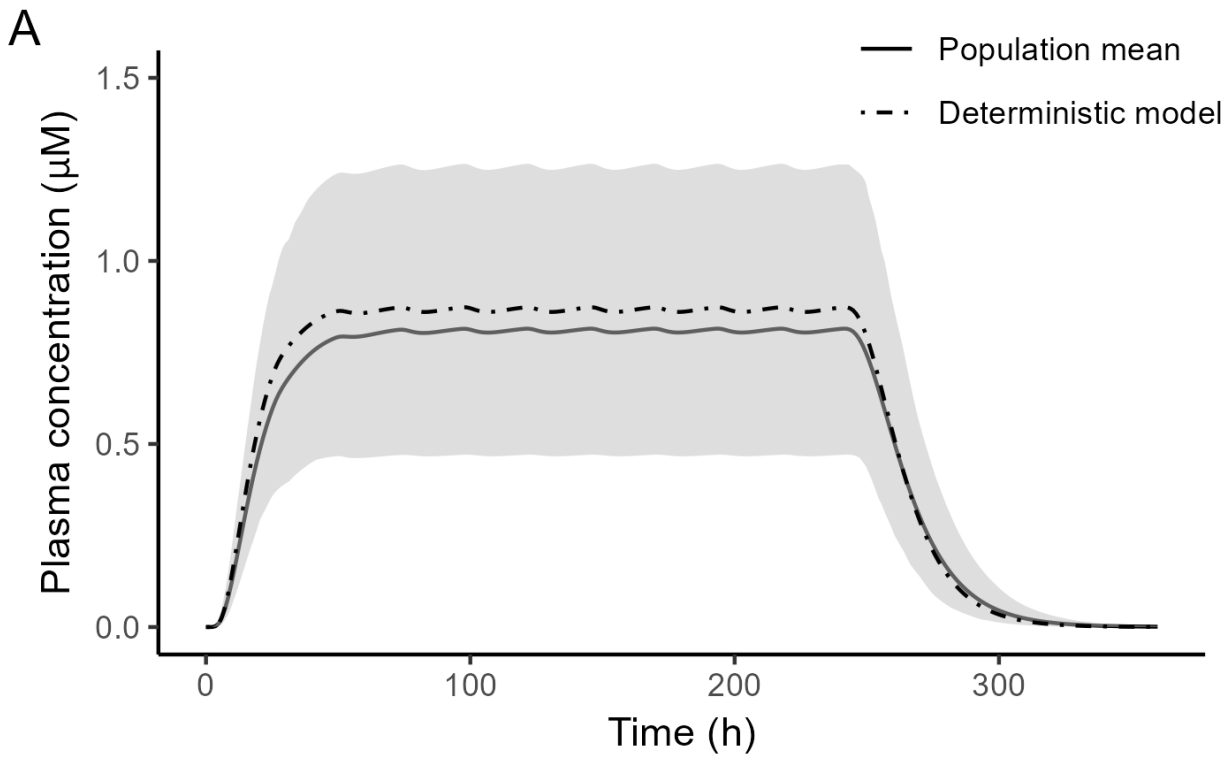


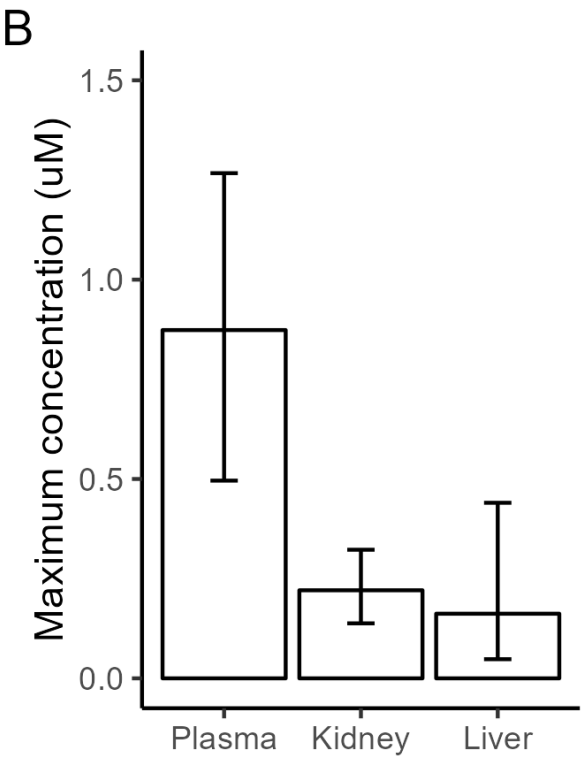
Baltazar MT et al., 2025 https://www.altex.org/index.php/altex/article/view/2934/version/2996

Exposure first: ADME results indicated limited organ distribution with exception of liver & kidney

***In vitro* ADME package**

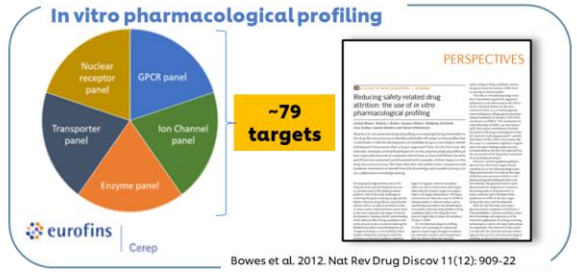
- **Skin absorption**
- **Hepatic clearance**
- **Plasma protein binding**
- **Blood: plasma**
- **Membrane permeability**
- **Transporter kinetics**





Baltazar MT et al., 2025 https://www.altex.org/index.php/altex/article/view/2934/version/2996

In addition to the core NAM-systemic toolbox, higher tier tools were required to cover for potential renal exposure and effects



Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-Seq technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
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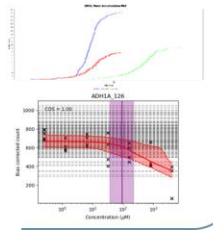

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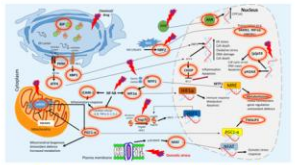


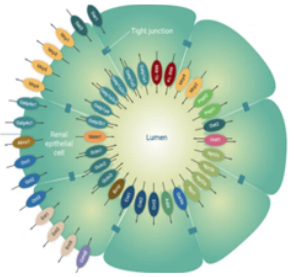
Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

- Cell models in the Tier 1 toolbox have limited expression of the relevant transporters
- Toolbox does not include kidney cells

Renal Toxicity

Renal biomarkers (3 donors, duplicate per donor), 8 concentrations, 24h and 72h timepoints in primary proximal tubule cell:



Newcells aProximate™ platform

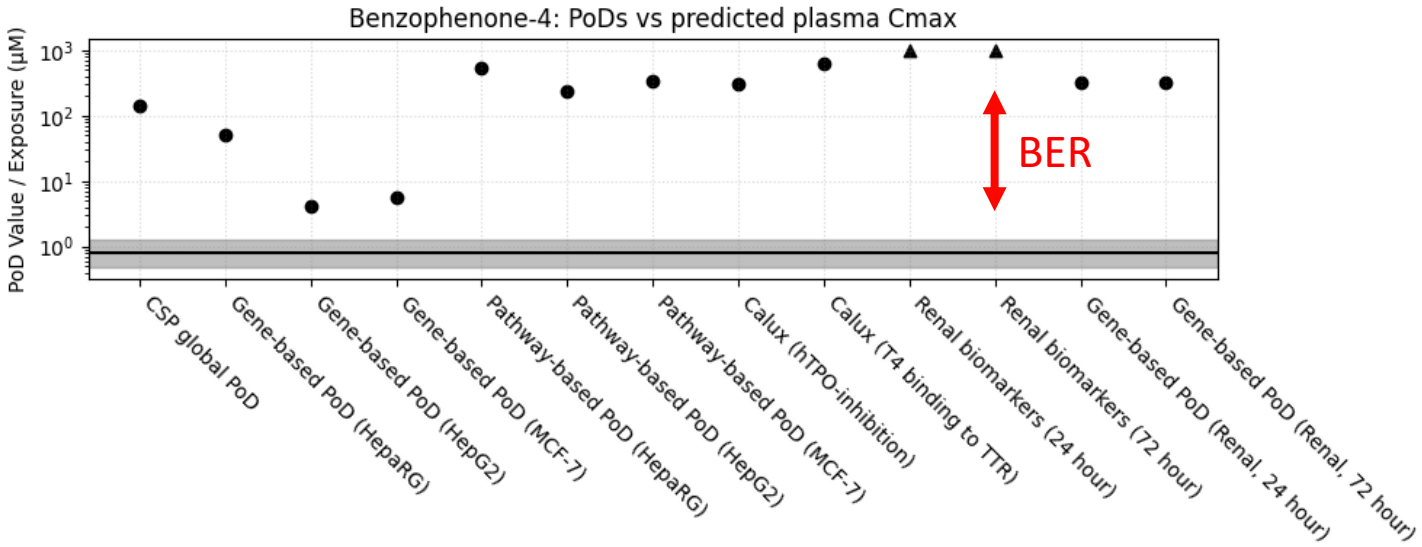
- KIM-1
- NGAL
- Clusterin
- TEER (Day 0 and Day 3)
- ATP
- LDH
- Toxicogenomics (3 donors, 2 duplicates per donor), 8 concentrations, 24h and 72h timepoints

- Omeprazole and cisplatin added as benchmarks/positive controls

Piyush Bajaj et al. 2020. Toxicology. 442, 152535

Baltazar MT et al., 2025 https://www.altex.org/index.php/altex/article/view/2934/version/2996

NAM-based risk assessment more conservative than the current regulatory risk assessment



BIOACTIVITY EXPOSURE RATIO = $\frac{\text{BIOACTIVITY}}{\text{EXPOSURE}}$

NAM-based assessment for 5% inclusion of BP-4

Lowest BER= 3.4

BER range= 3.4-508

Conclusion

Low risk considering weight of evidence and model/PoD relevance

Traditional animal assessment for 5% inclusion of BP-4

Margin of Safety (MoS)= 8986

Conclusion

Low risk – MoS >> 100

(SCCS opinion)

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Example 2: Expanding the Tier One Toolbox to cover more aspects of 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

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ORIGINAL RESEARCH article

Front. Toxicol.
Sec. Developmental and Reproductive Toxicology
Volume 7 - 2025 | doi: 10.3389/ftox.2025.1602065

This article is part of the Research Topic
New Approach Methodologies in Developmental and Reproductive Toxicology

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An Advancement in Developmental and Reproductive Toxicity (DART) Risk Assessment: Evaluation of a Bioactivity and Exposure-Based NAM Toolbox

Provisionally accepted

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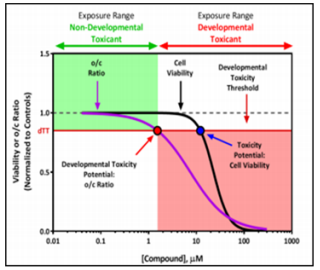
Systemic toolbox biological coverage identified needs for additional DART-specific NAMS

devTOX quickPredict™

- human iPSC cells
- metabolic perturbation of the biomarker's ornithine and cystine
- predicts concentration at which a test article shows developmental toxicity potential (dTP).

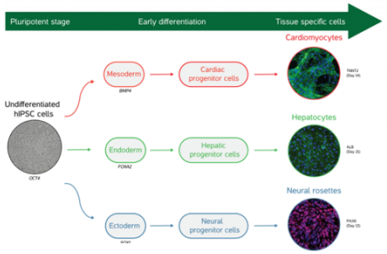
Stemina BIOMARKER DISCOVERY



Toxicology in vitro (2020) Apr 1;174(2):189-209

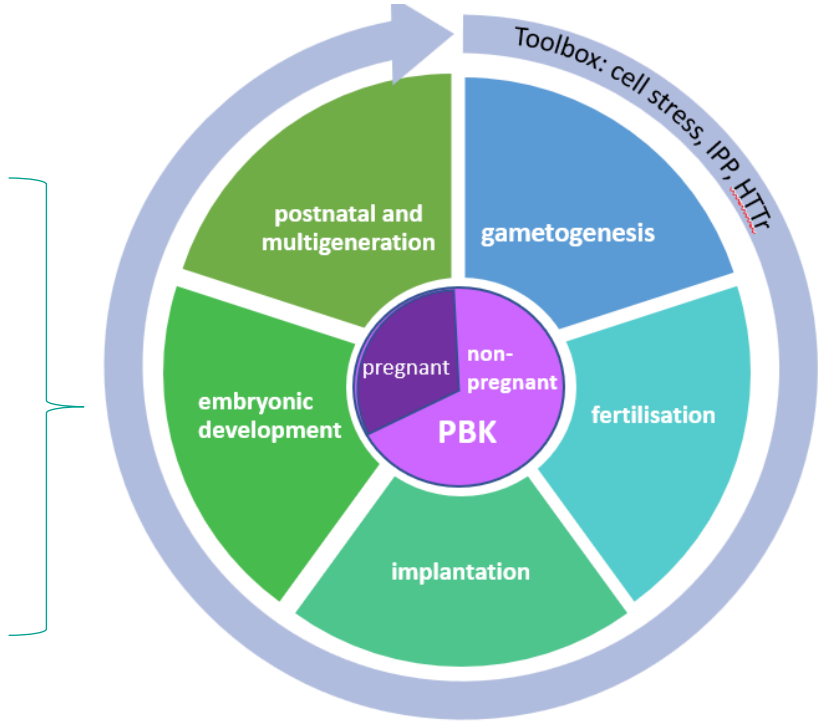
ReproTracker®

- human iPSC cells
- differentiated into cardio-myocytes, hepatocytes and neuronal rosettes
- Dose depended changes of lineage-specific gene biomarkers are measured to identify potentially teratogenic effects.



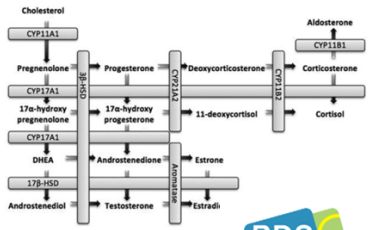
toxys

Birth defects Res. (2022) Nov 15;114(19):1210-1228.



H295R steroidogenesis assay

- human adenocarcinoma cell line NCI-H295R and U2-OS
- *in vitro* effect-based responses of compounds using the H295R steroidogenesis assay coupled to two CALUX® bioassays as a read-out: the ERα and AR CALUX®
- OECD Test No. 456



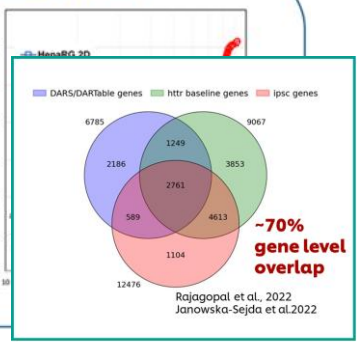
BDS

Toxicological Sciences, 2023, 194(2), 191–208

High-throughput Transcriptomics (HTTr)

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

BMDExpress 2



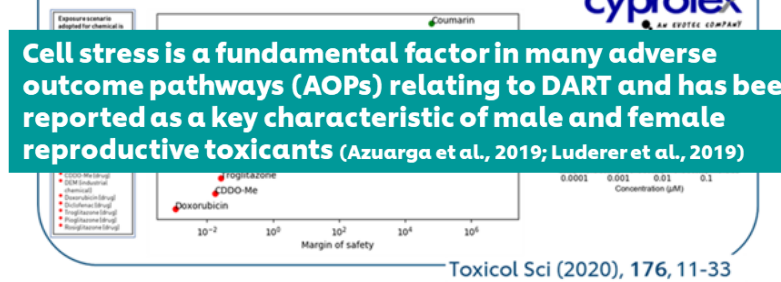
Rajagopal et al., 2022
Janowska-Sejda et al.2022

Cell Stress Panel (CSP)

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

cyprotex

Cell stress is a fundamental factor in many adverse outcome pathways (AOPs) relating to DART and has been reported as a key characteristic of male and female reproductive toxicants (Azuarga et al., 2019; Luderer et al., 2019)



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

In vitro Pharmacological Profiling (IPP)

51 out of the 75 targets are DART relevant. This includes 20 nuclear hormone receptors, 6 DNT targets, aromatase ...

eurofins | Cerep

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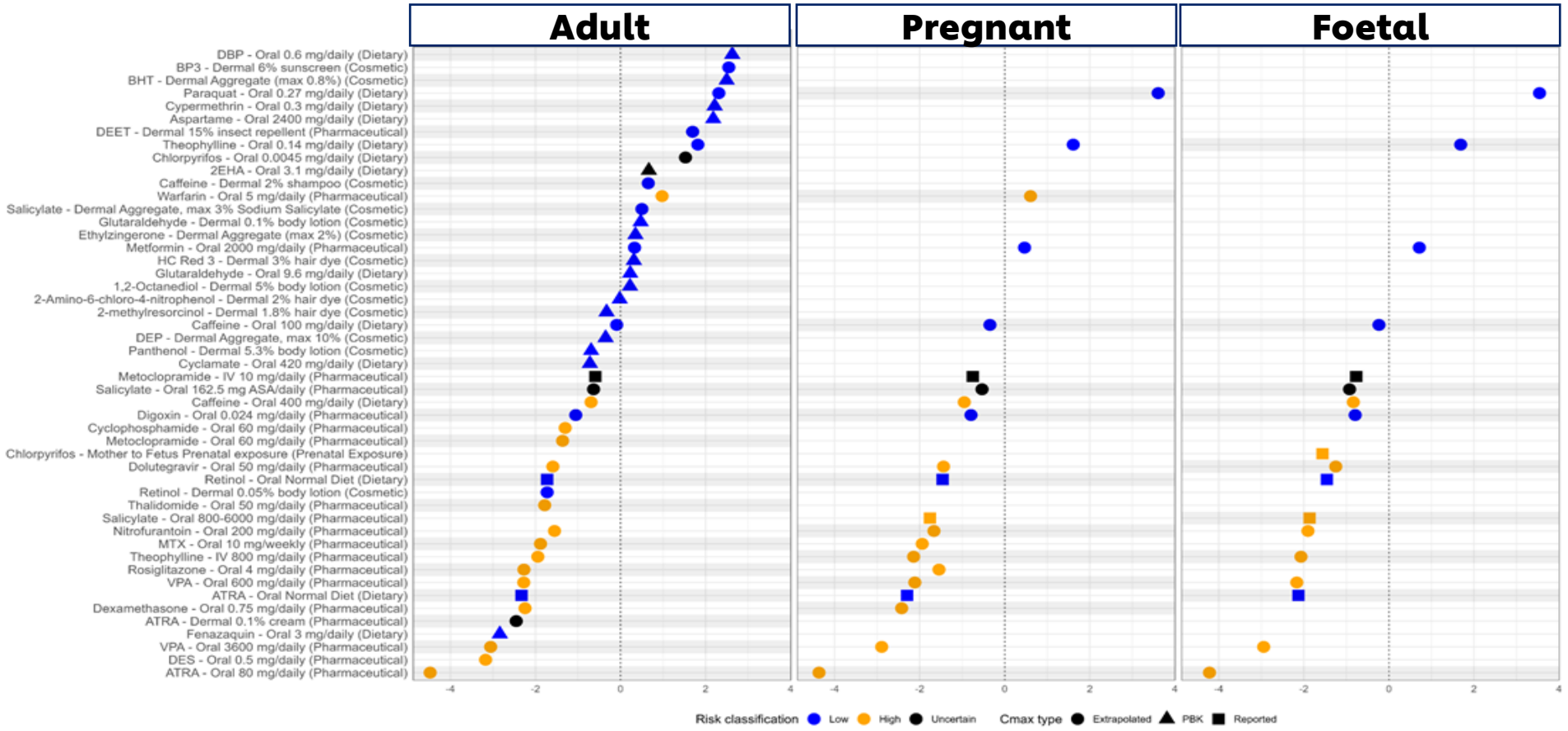
The DART framework is protective for most high-risk scenarios when using a BER threshold of 1



doi: 10.3389/ftox.2025.1602065

Opportunities to apply NAMs in the context of food safety

- **Well established non-animal methods exist to support food safety** (e.g. read across, genotoxicity, history of safe use (HoSU), Protein safety (allergenicity and toxigenicity))
- **13 food relevant materials tested in the systemic toolbox** (e.g. pesticides residues, food additives, sweeteners, flavourings**)**
  - **Results show that NAMs are applicable to these compounds, albeit conservative.**
- While novel NAMs have seen considerable uptake in cosmetic regulatory assessments, their **application in food safety remains significantly underutilized and holds substantial potential for expansion**

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Regulatory Toxicology and Pharmacology
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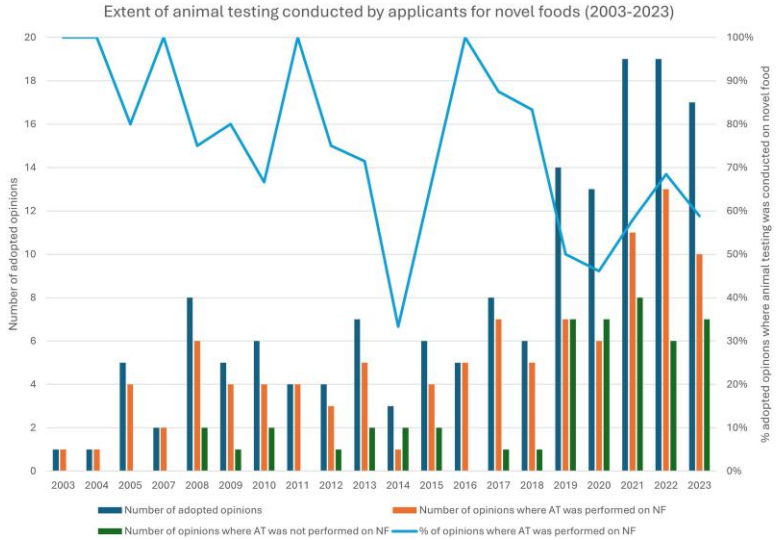
In Press, Journal Pre-proof What's this?

Countdown to 2027 – maximising use of NAMs in food safety assessment: closing the gap for regulatory assessments in Europe

Adam Wood [1], Franck Atienzar [2], Danilo Basili [3], Myriam Coulet [3], Rebeca Fernandez [4], Melina Galano [5], Maricel Marin-Kuan [3], Gina Montoya [3], Przemyslaw Piechota [3], Ans Punt [1], Elena Reale [3], Si Wang [6], Paul Hepburn [1]



Conclusions- our experience (cosmetics, detergents, biocides, foods, REACH)

- Exposure science is critical in next generation risk assessment.
- Tiered approaches unlock the potential for decision-making.
- The conservatism associated with the bioactivity PODs can be refined with higher tier *in vitro* models.
- Case studies and evaluations have helped build confidence
- Frameworks have been developed for systemic, DART, and inhalation safety[1] and skin sensitisation[2].

Fundamental change needs bold vision

1. https://doi.org/10.3389/ftox.2025.1426132
2. https://doi.org/10.1016/j.yrtph.2021.105075

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