Use of New Approach Methodologies in the Safety Assessment of Dietary Supplements – Part 2

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Overview

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NAMs and NGRA: scope and relevance to food supplements

Why new non-animal approaches are needed

Scientific enablers of non-animal risk assessment

Unilever's tiered NGRA framework

Cyclamate case study

Challenges, guidance, and the path forward

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Recap: NGRA and New Approach Methodologies (NAMs)

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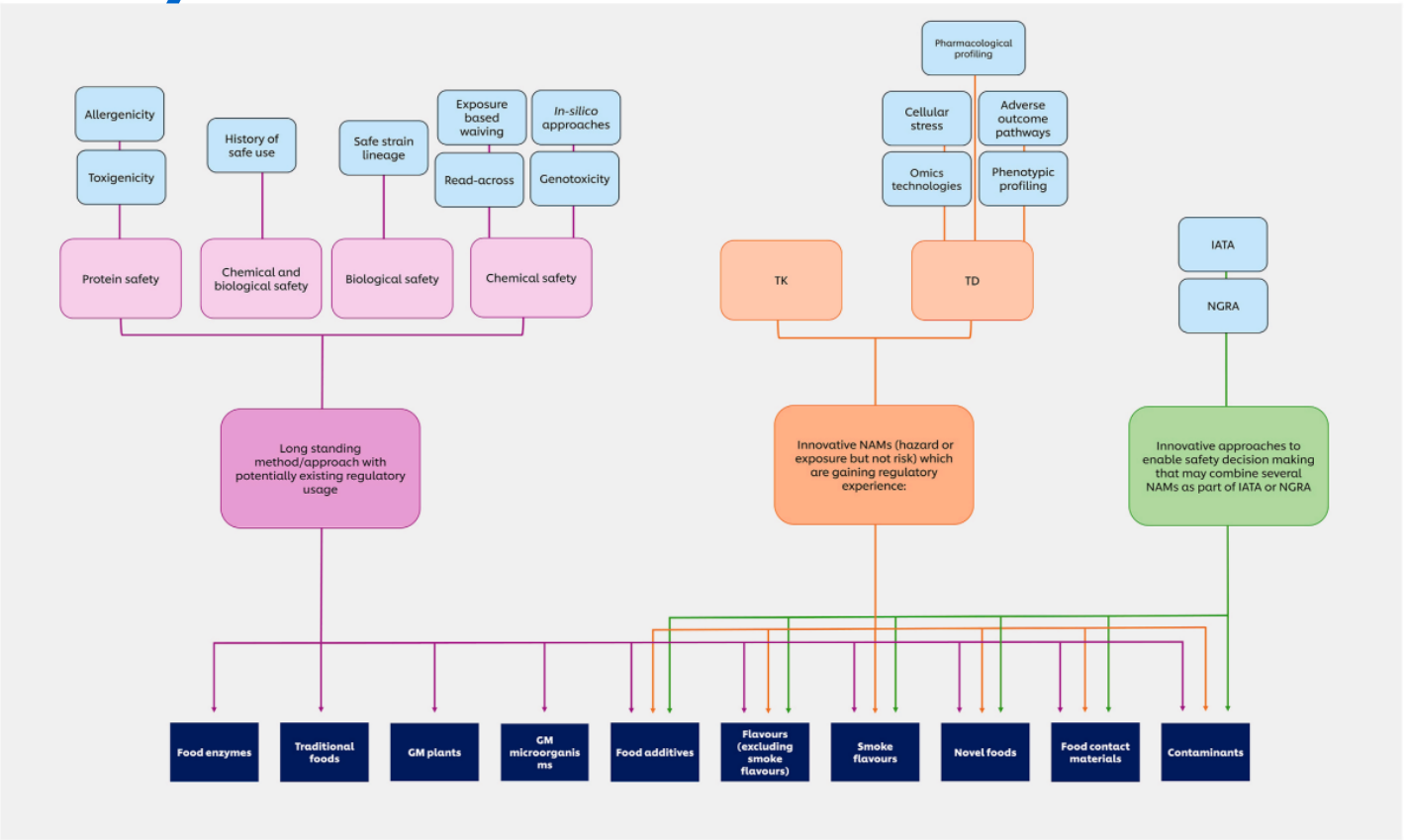
NAMs - Approaches that do not rely on generating new experimental animal data (though including those which use historical animal data) and comprising:

In vitro, *in silico*, *in chemico* and *ex vivo* human models

Such approaches may be used to provide information on hazard or exposure or used in combination.

Some NAMs are long-standing (history of use), others are more-recent (transcriptomics).

Their use in risk assessment is considered the next generation of risk assessment (NGRA)



Wood et al., (2025). Regulatory Toxicology and Pharmacology, Volume 162, November 2025

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Many NAMs of relevance to food supplement safety exist. Many could find use across multiple types of food supplement ingredients (e.g. botanicals, proteins etc)

Part 1 » Part 2: The Need for New Approaches

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Outside of risk assessment based on substance specific data, other assessment methods include:

Threshold of Toxicological Concern

(e.g. Yang et al 2017)
https://doi.org/10.1016/j.fct.2017.08.043

Read across

(e.g. Alexander-White et al 2022)
https://doi.org/10.1016/j.yrtph.2021.105094

History of Safe Use

(e.g. Neely et al 2011) PMID: 22025816

Food and Chemical Toxicology 109 (2017) 170–193

Contents lists available at ScienceDirect

ELSEVIER

Threshold
New data

Chihae Yang
Alan R. Bool
Steven Enoc

ARTICL

Regulatory Toxicology and Pharmacology 129 (2022) 105094

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

A 10-step framework for use of read-across (RAX) in next generation risk

Research Article

A Multi-Criteria Decision Analysis Model to Assess the Safety of Botanicals Utilizing Data on History of Use

T. Neely, B. Walsh-Mason, P. Russell, A. Van Der Horst, S. O'Hagan, P. Lahorkar[1]

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ABSTRACT

Botanicals (herbal materials and extracts) are widely used in traditional medicines throughout the world. Many have an extensive history of safe use over several hundreds of years. There is now a growing consumer interest in food and cosmetic products, which contain botanicals. There are many publications describing the safety assessment approaches for botanicals, based on the history of safe use. However, they do not define what constitutes a history of safe use, a decision that is ultimately a subjective one. The multi-criteria decision analysis (MCDA), is a model

Key words: Botanicals, *Brahmi*, history of safe use, multi multi-criteria decision analysis, safety assessment, similarity score

For 'significant' exposures to a novel ingredient a new non-animal paradigm is needed...

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Scientific Progress – Opening Possibilities to Replace Animal Testing at Scale

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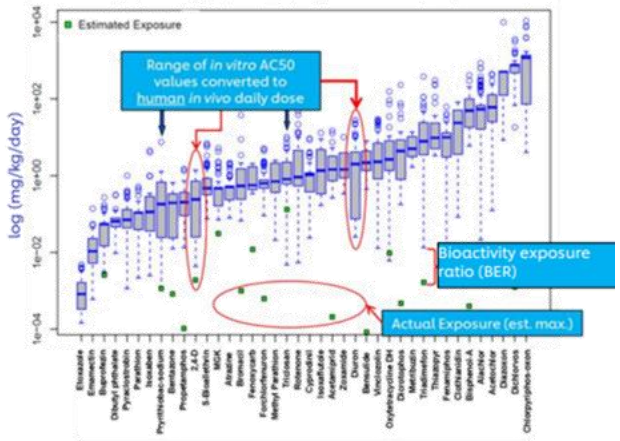
Recent scientific advancements in e.g. high-throughput screening (HTS) and computational sciences have opened new possibilities that have collectively shifted the dial in terms of our ability to demonstrate safety using non-animal methods



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

$$BER = \frac{Lowest\ bioactivity\ POD}{Internal\ in\ vivo\ exposure\ (Cmax)} (uM)$$

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Building blocks of a non-animal systemic toxicity risk assessment – tiered frameworks

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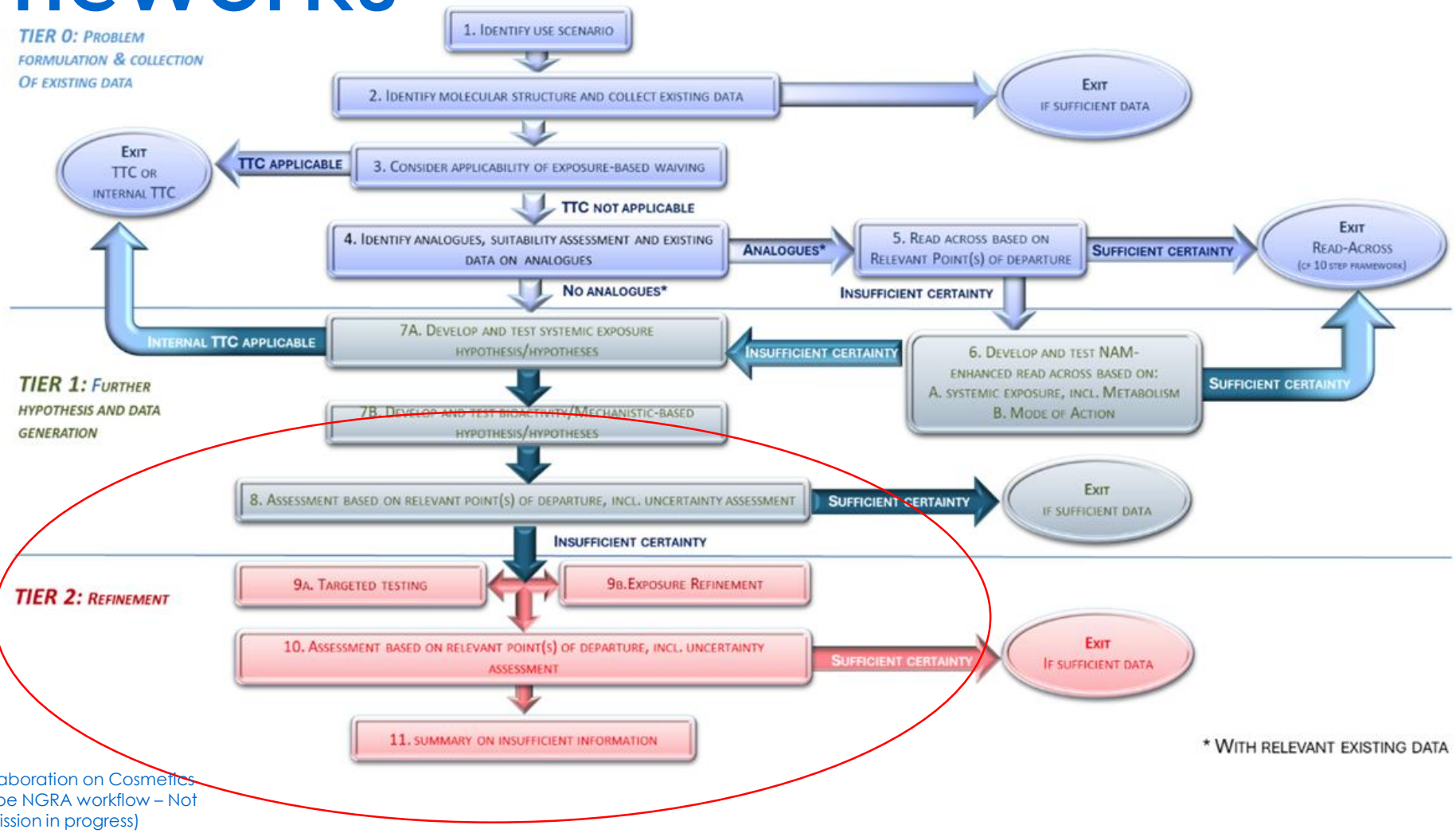

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Draft International Collaboration on Cosmetics Safety/Cosmetics Europe NGRA workflow – Not for reproduction (submission in progress)

Unilever Tiered NGRA Framework

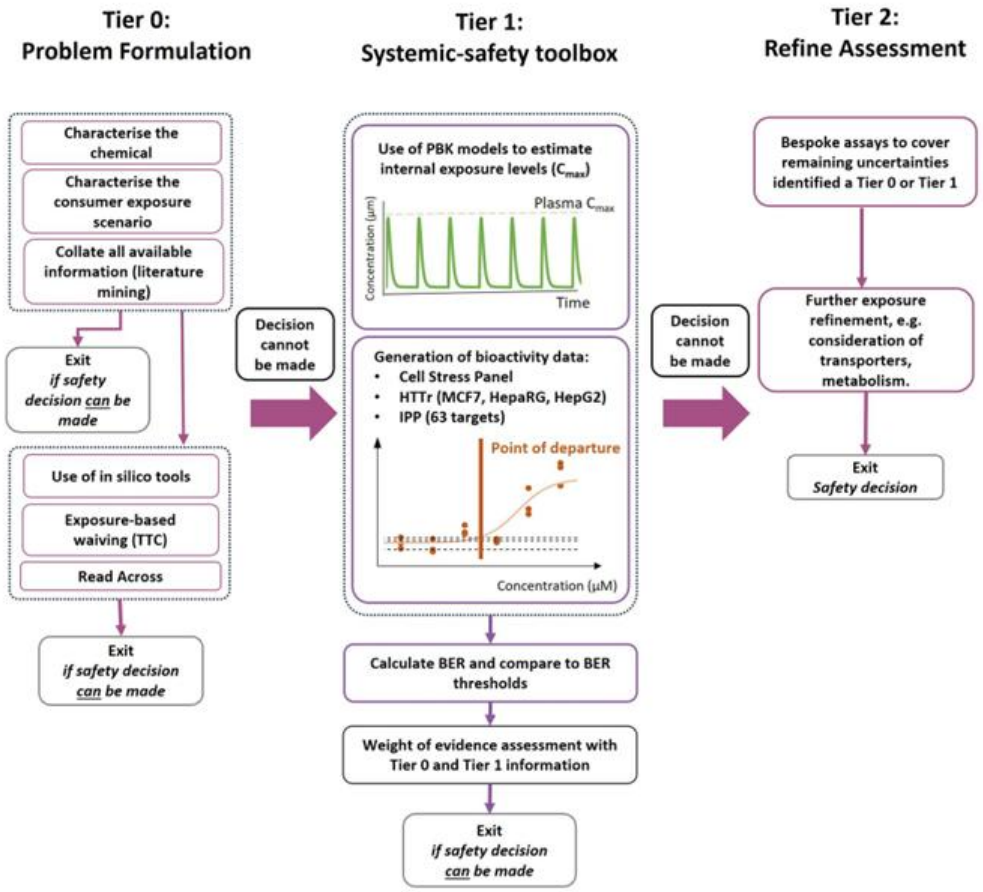
- First, it should be determined whether experimental data generation is required at all - A safety decision could be reached through e.g. TTC or read-across without new studies being performed (part 1!)

- If data generation is needed, **NAMs are used in a tiered way:**

Broad coverage (protective) NAMs

↓

Specific (predictive) NAMs



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

Toxicological Sciences, 2025, 204(1), 79–95
https://doi.org/10.1093/toxsci/kfae159
Advance Access Publication Date: December 18, 2024
Research article

Advancing systemic toxicity risk assessment: Evaluation of a NAM-based toolbox approach

Sophie Cable*, Maria Teresa Baltazar, Fazila Bungawala, Paul L. Carmichael, Leonardo Contreas, Matthew Philip Dent, Jade Houghton, Predrag Kukic, Sophie Malcomber, Beate Nicol, Katarzyna R. Przybylak, Ana Punt, Georgia Reynolds, Joe Reynolds, Sharon Scott, Dawei Tang, Alistair M. Middleton

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Abstract

For many years, a method that allowed systemic toxicity safety assessments to be conducted without generating new animal test data, seemed out of reach. However, several different research groups and regulatory authorities are beginning to use a variety of in silico, in chemico, and in vitro techniques to inform safety decisions. To manage this transition to animal-free safety assessments responsibly, it is important to ensure that the level of protection offered by a safety assessment based on new approach methodologies (NAMs), is at least as high as that provided by a safety assessment based on traditional animal studies. To this end, we have developed an evaluation strategy to assess both the level of protection and the utility offered by a NAM-based systemic safety "toolbox." The toolbox comprises physiologically based kinetic models to predict internal exposures, and bioactivity NAMs designed to give broad coverage across many different toxicity modes of action. The output of the toolbox is the calculation of a bioactivity:exposure ratio (analogous to a margin of internal exposure), which can be used to inform decision-making. In this work, we have expanded upon an initial pilot study of 10 chemicals with an additional 38 chemicals and 70 consumer exposure scenarios. We found that, for the majority of these (~90%), the NAM-based workflow is protective of human health, enabling us to make animal-free safety decisions for systemic toxicity and preventing unnecessary animal use. We have also identified critical areas for improvement to further increase our confidence in the robustness of the approach.

Keywords: new approach methodologies; risk assessment; systemic toxicity.

A major challenge in the evaluation of nonanimal safety assessments is establishing that they are truly protective of human health. Chemical risk assessment for systemic toxicity has traditionally relied on the use of repeated-dose in vivo tests to derive protective thresholds, mostly based on nonspecific bioactivity observed in animals (Browne et al. 2024). These no-observed (-adverse)-effect-levels (NO(A)EL) or benchmark dose levels (BMDLs) from chronic and subchronic animal tests are then compared with the estimated exposure in humans. If there is a sufficiently large margin between them, which is determined by using intra- and interspecies uncertainty factors (or other appropriate uncertainty factors arising from consideration of matrices differences, the exposure estimation etc), then the human exposure can be considered low risk.

The objective of using bioactivity data to derive thresholds that are protective of human health remains the goal of next-generation risk assessment (NGRA) (Dent et al. 2018), i.e. chemical risk assessment that does not involve the generation of new animal data. Where there are data gaps in existing information, or for de novo risk assessments, NGRA assesses bioactivity by estimating points of departure (PoDs) from a range of different in vitro studies and compares them with internal exposure concentrations (e.g. plasma $C_{max,u}$) to calculate a bioactivity:exposure ratio (BER, analogous to the calculation of a margin of safety/exposure (MoS/E).

The overall approach to such a tiered, nonanimal, exposure-led assessment (Fig. 1) has been described in the literature (Berggren et al. 2017, Thomas et al. 2019), adapted and incorporated into published NGRA case studies (Baltazar et al. 2020, OECD 2021, Ebmeyer et al. 2024, Wood et al. 2024). These case studies demonstrated that NGRA is a flexible approach to integrating multiple lines of evidence to reach a safety decision. However, in order to build confidence in new approach methodologies (NAMs) for systemic toxicity, there is a need to evaluate their robustness and performance in a systematic manner for a wide range of chemicals if they are to be considered for regulatory applications (Dent et al. 2021, Westmoreland et al. 2022). Recent validation frameworks emphasize the need for defining the context of use as a first step in establishing a validation strategy (Van der Zalm et al. 2022, ICCVAM 2024). In our previous work (Middleton et al. 2022), we outlined an approach to evaluating a NAM-based systemic-safety toolbox and workflow comprising bioactivity and exposure modules, which could form a basis for estimating BERs at Tier 1 of an NGRA framework (Fig. 1). Based on the proposed validation frameworks, the "context of use" for this toolbox is defined as an early-tier approach for conducting systemic toxicity risk assessments for consumer goods which aims at being protective of human health rather than predicting specific hazards, similar to the traditional approach utilizing in vivo data (Browne et al. 2024). The

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The Importance of Problem Formulation

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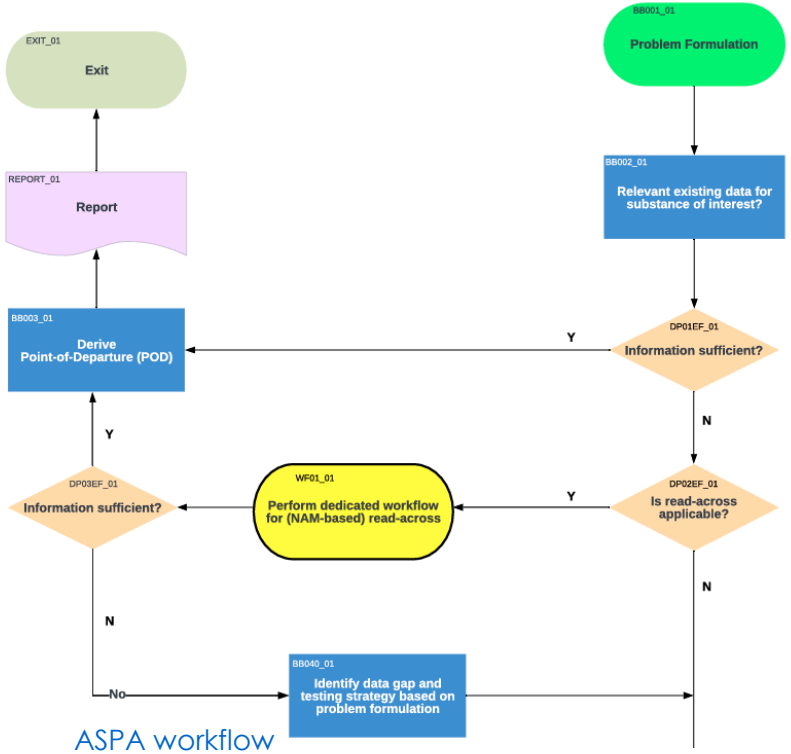

ASPA workflow
(https://doi.org/10.5281/zenodo.18682569)

Concept Article

An Alternative Safety Profiling Algorithm (ASPA) to Transform Next Generation Risk Assessment into a Structured and Transparent Process

Marcel Leist[1,2], Silvia Tangianu[1,2], Femke Affourtit[3], Hedwig Braakhuis[4], John Colbourne[5], Eike Cöllen[3], Nadine Dreser[4], Sylvia E. Escher[6], Iain Gardner[7], Stefan Hahn[6], Barry Hardy[8], Matthias Herzler[9], Barira Islam[7], Hennicke Kamp[10], Viktoria Magel[3], Philip Marx-Stoelting[9], Martijn J. Moné[11], Patrik Lundquist[12], Ilse Ottenbros[4], Gladys Ouedraogo[13], Giorgia Pallocca[14], Bob van de Water[11], Mathieu Vinken[15], Andrew White[16], Manuel Pastor[5,17] and Mirjam Luijten[5,18]

Exposure	• Cmax or AUC? • Population group? • Exposure scenario?
Hazard ID	• Biological coverage of systems? • Phys-chem properties of substance? • Metabolically activated?
Hazard characterisation	• Protection goal? • Biological/toxicological significance?
Risk characterisation	• What BER is sufficient?

Non-exhaustive issues to consider prior to *in vitro* testing

- The availability of a 'default' toolbox does not waive the need for a solid problem formulation before testing.
- Issues to consider can be split into those influencing the 4 pillars of risk assessment process.
- Emerging workflows can help work through the problem formulation to guide appropriate study design.

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Case study - Cyclamate

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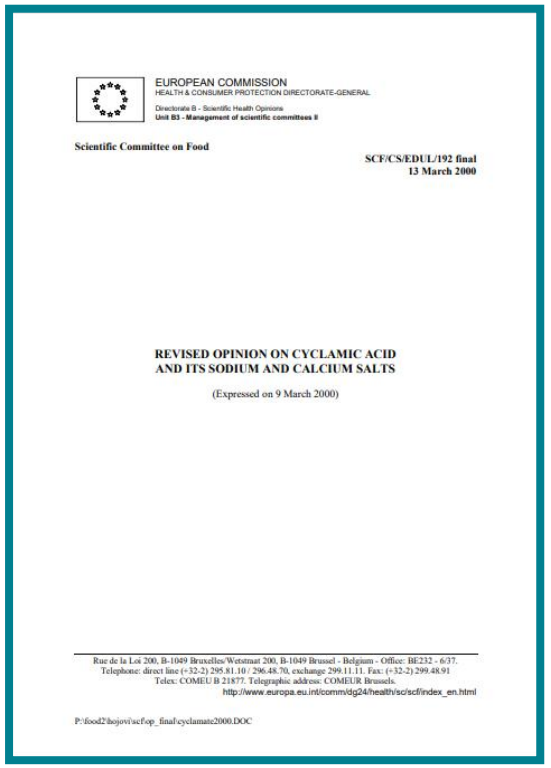
- Sodium cyclamate, also called E952 (ii), is used as an artificial sweetener.
- Has been reviewed by JECFA (1982) and the SCF (2000) with ADIs established as 0-11 mg/kg (JECFA) and 0-7 mg/kg (SCF). **Re-evaluation currently underway by EFSA.**
- ADI is based on a NOAEL of 100 mg/kg (90-day rats - administered cyclohexylamine (CHA: the major metabolite of cyclamate).



EUROPEAN COMMISSION
HEALTH & CONSUMER PROTECTION DIRECTORATE-GENERAL
Directorate B - Scientific Health Opinions
Unit B3 - Management of scientific committees II

Scientific Committee on Food

SCF/CS/EDUL/192 final
13 March 2000

REVISED OPINION ON CYCLAMIC ACID AND ITS SODIUM AND CALCIUM SALTS

(Expressed on 9 March 2000)

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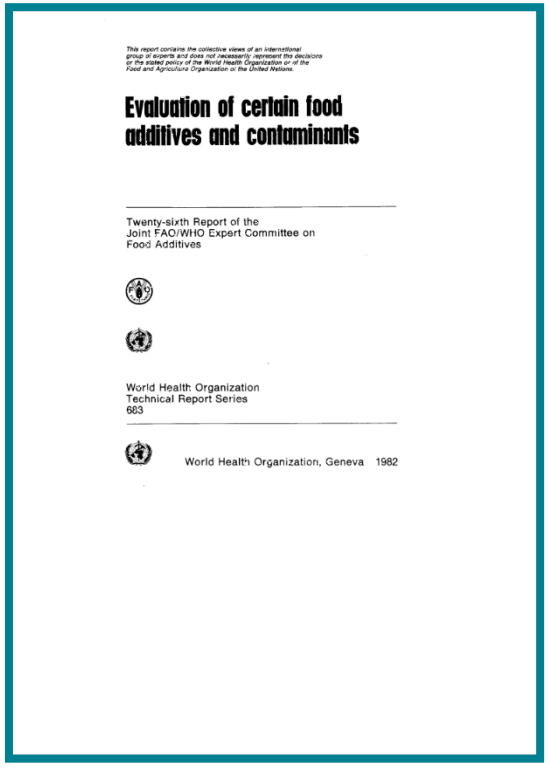


This report contains the collective views of an international group of experts and does not necessarily represent the decisions or the stated policy of the World Health Organization or of the Food and Agriculture Organization or the United Nations.

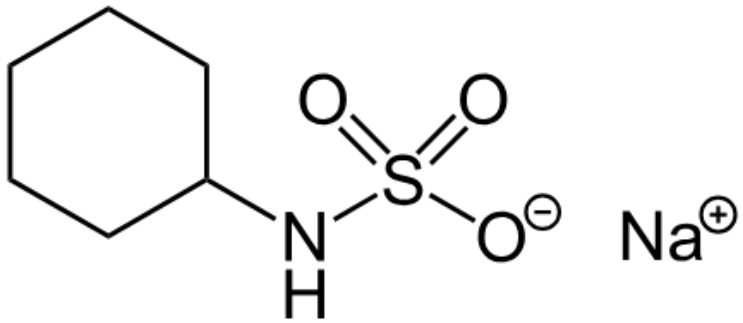
Evaluation of certain food additives and contaminants

Twenty-sixth Report of the
Joint FAO/WHO Expert Committee on
Food Additives

World Health Organization
Technical Report Series
683

World Health Organization, Geneva 1982

[All data are available in Cable et al., Toxicological Sciences, 2025, 204(1), 79–95]



E Number	Name	EFSA's Assessment
E 420	Sorbitols	Re-evaluation ongoing
E 421	Mannitol	Re-evaluation ongoing
E 950	Acesulfame K	Re-evaluation completed in 2025
E 951	Aspartame	Re-evaluation completed in 2013
E 952	Cyclamates	Re-evaluation ongoing
E 953	Isomalt	Re-evaluation ongoing
E 954	Saccharins	Re-evaluation completed in 2024
E 955	Sucralose	Re-evaluation ongoing
E 957	Thaumatin	Re-evaluation completed in 2021
E 959	Neohesperidine DC	Re-evaluation completed in 2022
E 960a	Steviol glycosides from Stevia	First evaluated in 2010
E 960c	Enzymatically produced steviol glycosides	Evaluated in 2019
E 960d	Glucosylated steviol glycosides	Evaluated in 2022
		First evaluated in 2007

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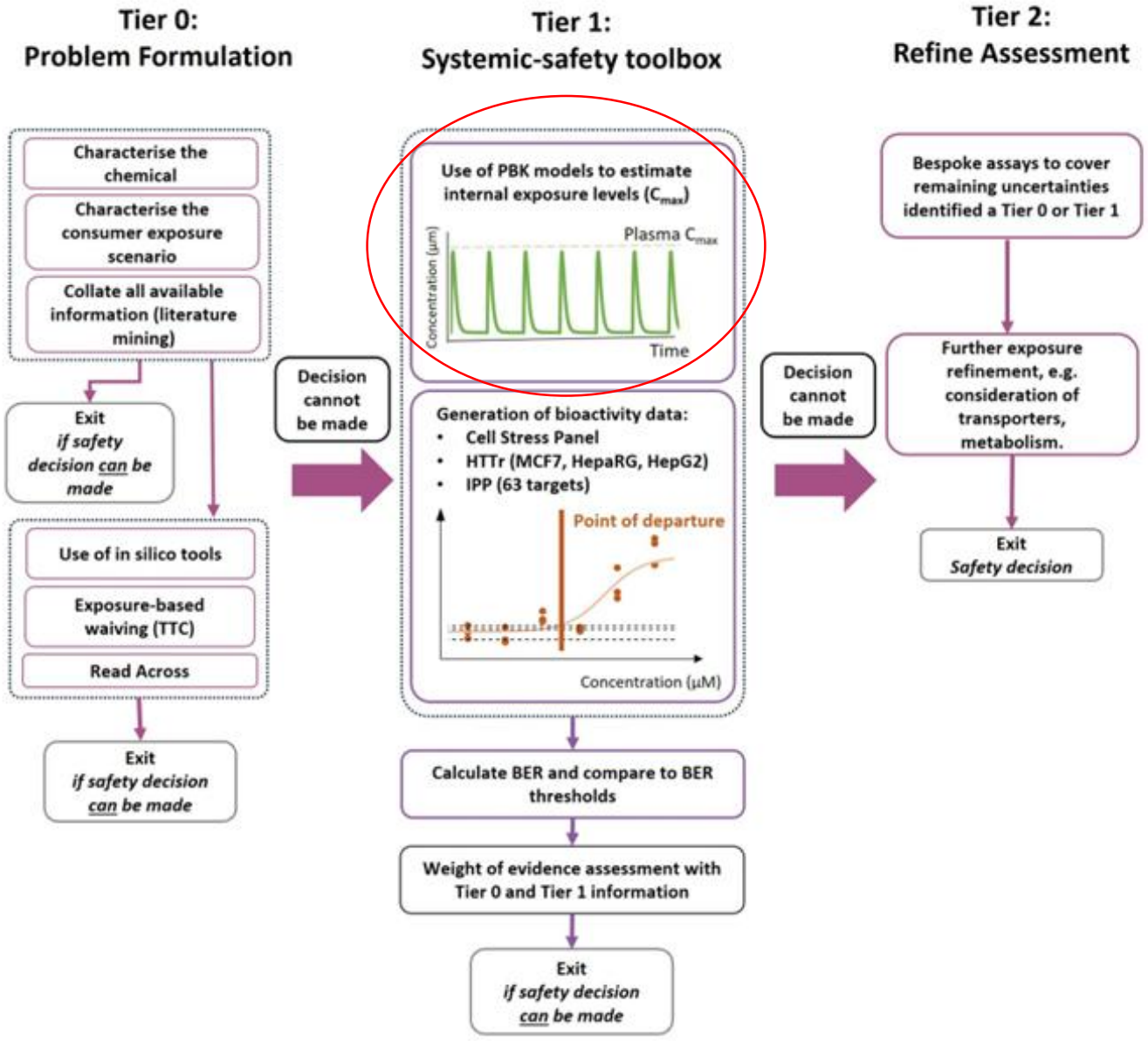
Unilever Tiered NGRA Framework



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Step 1 – Exposure Estimation – An intro to PBK Modelling

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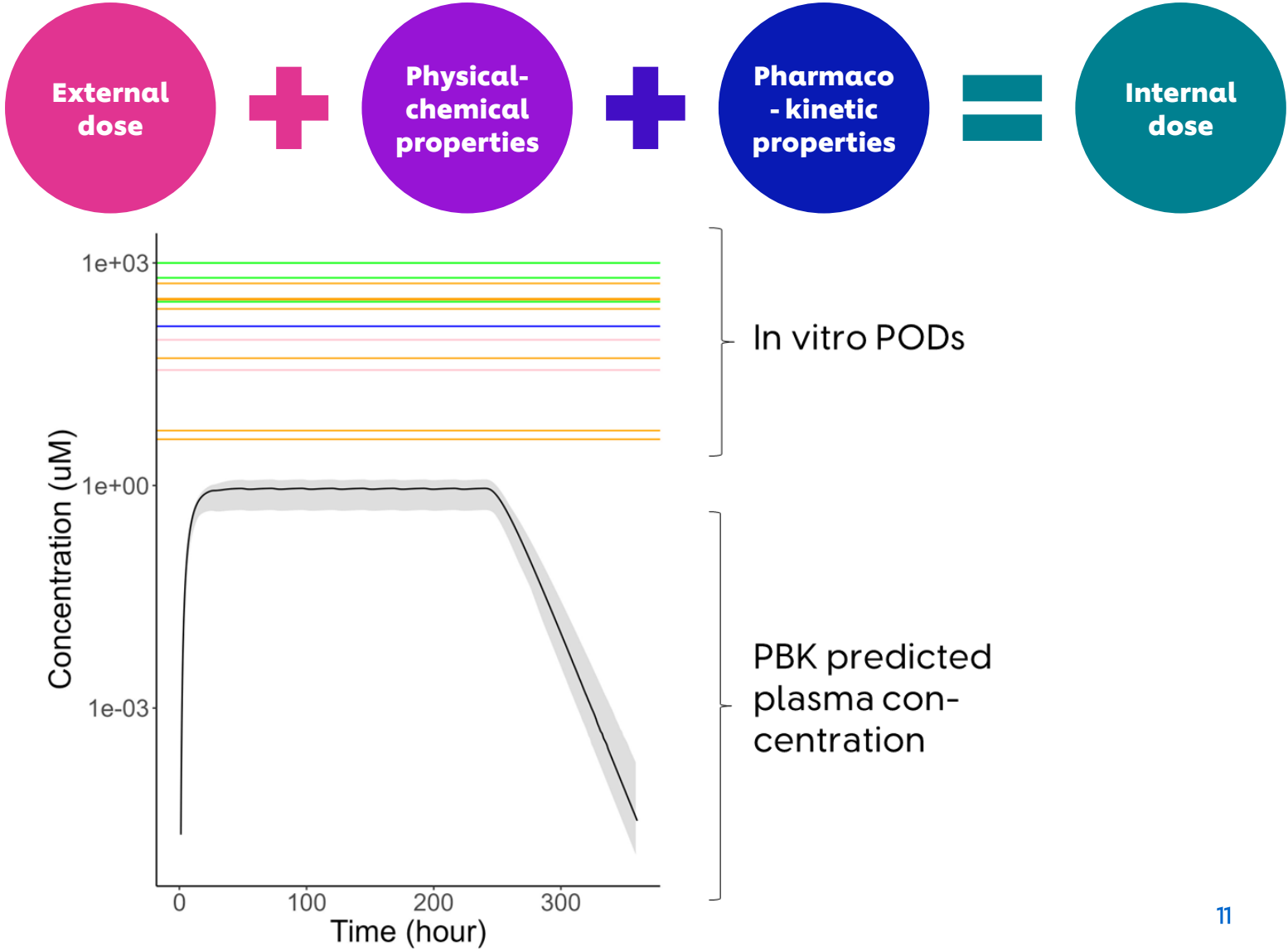
- Historically, risk assessments have compared human and animal external doses (i.e. in mg/kg bw/day).
- Use of *in vitro* models generates points of departures (PoDs) in µM.
- Risk characterisation requires unit harmonisation.
- PBK modelling is the key to either estimate internal exposures from an external dose (forward dosimetry) or vice versa (reverse dosimetry).



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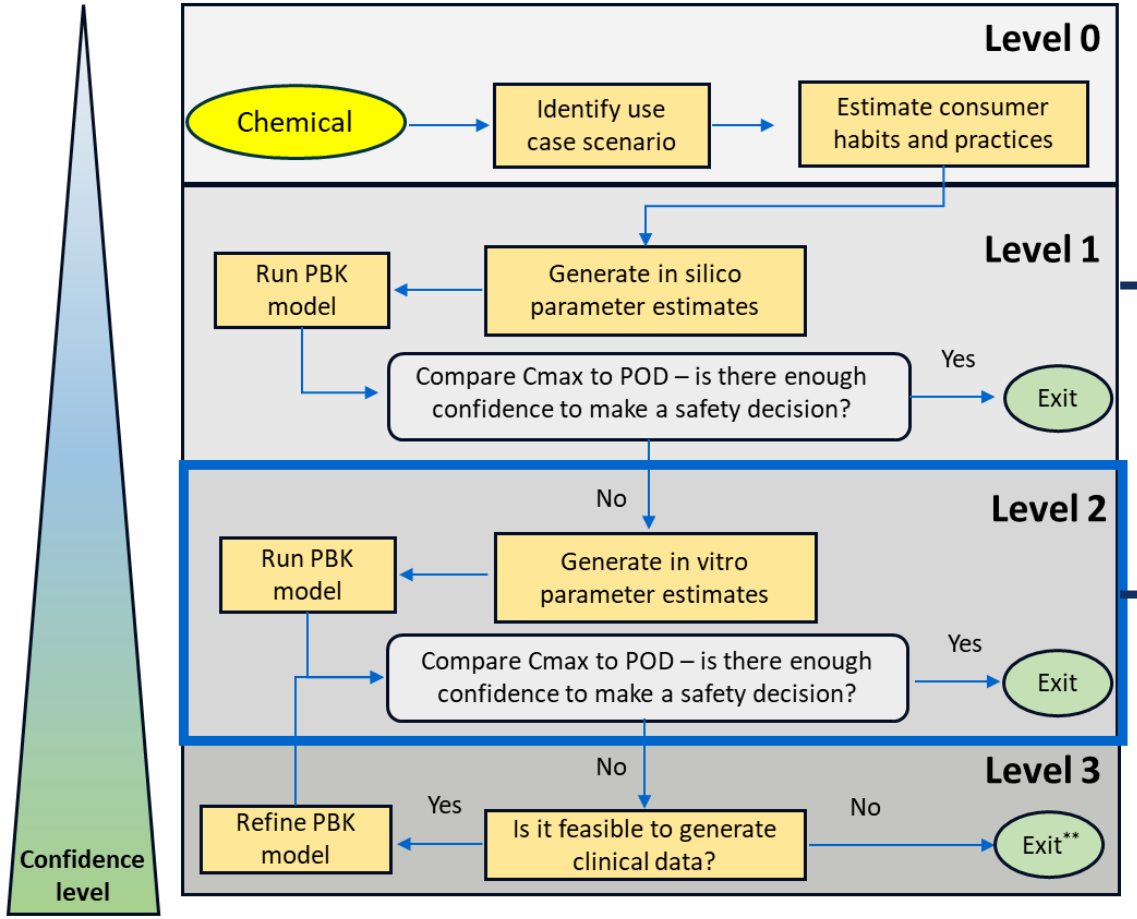
Cyclamate PBK – Inputs/Outputs



PBK model built **using *in silico*** parameter estimates (phys-chem + ADME)

PBK model built **using some *in vitro*** parameter estimates (phys-chem + ADME)

Cmax (µM) (from a 7 mg/kg bw/day/dose of cyclamate)

~ 69

~ 68

Taken forward to be compared with in vitro PoDs

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Unilever Tiered NGRA Framework

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TIER 0: Problem Formulation

- Characterise the chemical
- Characterise the consumer exposure scenario
- Collate all available information (literature mining)

Exit *if safety decision can be made*

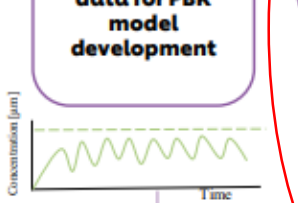
- Use of predictive tools (i.e. *in silico* QSAR models)
- Use of exposure-based waiving approaches such as Threshold of Toxicological Concern (TTC)
- Read Across

Exit *if safety decision can be made*

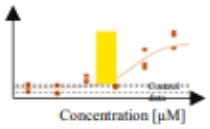
Progress if safety decision can't be made

TIER 1: Data Generation

- Exposure refinement including generation of relevant ADME parameter data for PBK model development
- Bioactivity data generation



Plasma Cmax



Lowest platform PoD

- Calculate BER

Exit *if safety decision can be made*

Progress if safety decision can't be made

TIER 2: Refine Assessment to Increase Decision Certainty

- Bespoke assays to cover remaining uncertainties identified a Tier 0 or Tier 1
- Further exposure refinement, e.g. consideration of transporters, metabolism ..etc...

Exit *Safety Decision*

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Step 2 – Bioactivity Characterisation - Approaches

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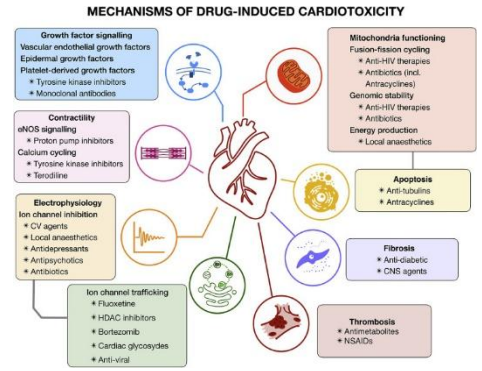
Two different but complementary approaches for using NAMs in risk assessments:

1.) NAMs developed to predict (possibly quantitatively) adverse effects

2.) NAMs developed to measure bioactivity (quantitatively) without classification as adversity or not.

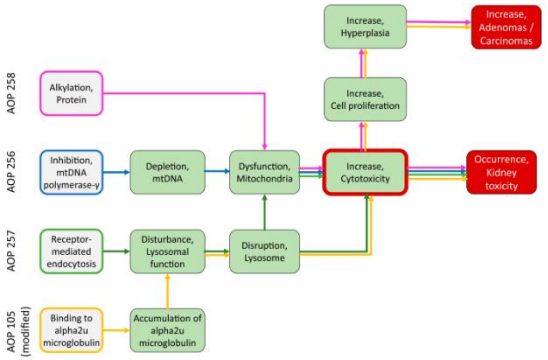
Both have a place in **future** risk assessment. **Unilever have invested significant resource into 2 (aka 'protective' NAMs)**



Manoshina et al., (2021). Cell Reports Medicine. 2:3 100216

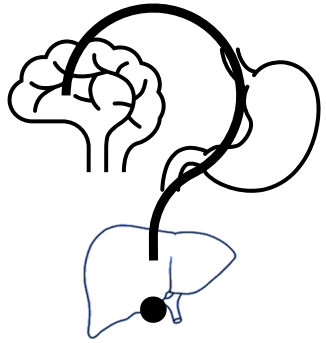
Prediction ← → Protection

NAMs capturing early biological changes protective of apical effects



Mally and Jarzina (2022). Frontiers in Toxicology

Prediction ← → Protection



- Limited coverage approaches
- Cell based/reporter assays
- Data rich approaches
- Transcriptomics
- Cell painting

State of the art (>20-years)

There are 78 major human organs; let's say there are five different ways in which chemicals could be toxic to each one (an underestimate); and let's say we need five key events (including a molecular initiating event) measured across each IATA with new in vitro tests. That's around 2000 assays conducted at just one dose and at one time point for complete human AOP-driven biological coverage.

Carmichael et al., (2022). Altex, 39:3

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Step 2 – Bioactivity Characterisation – Unilever Approaches

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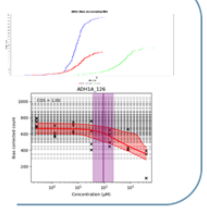
Point of Departure (PoD) determination from Bioactivity assays

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

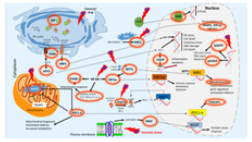
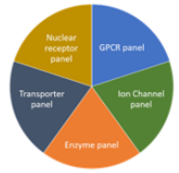


Image kindly provided by Paul Walker (Cyprotex)

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

Specific effects

In vitro pharmacological profiling



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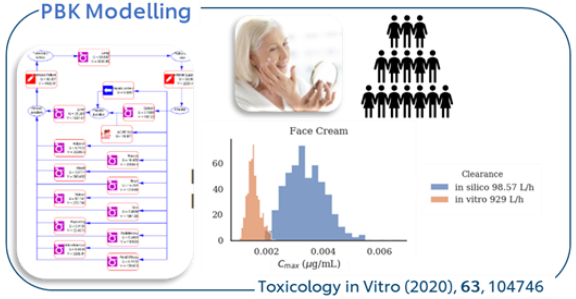
PBK Modelling

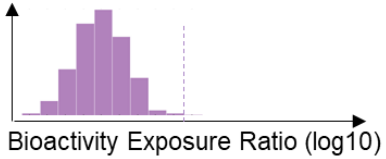


Toxicology in Vitro (2020), **63**, 104746

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

(Bayesian model)

Bioactivity Exposure Ratio (BER) Distribution



BIOACTIVITY EXPOSURE RATIO = BIOACTIVITY / EXPOSURE

The bigger the BER, the greater the confidence that bioactivity will not occur in exposed consumers

- Unilever's basic NAM toolbox uses **non-specific (protective) and specific (predictive) NAMs.**

- PoDs from these are compared with PBK model estimates of internal exposure to enable risk characterisation (through a **bioactivity exposure ratio/BER**).

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Other tools are available

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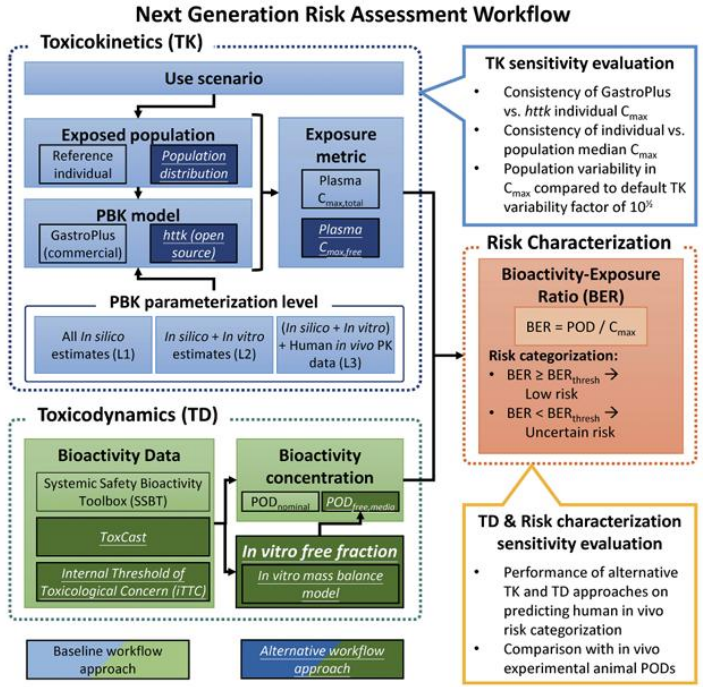
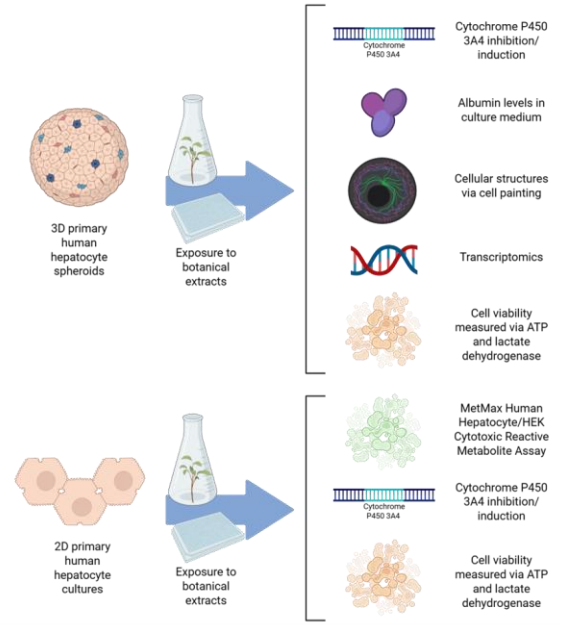
- Unilever basic toolbox relies on a fixed set of tools and methods **(suitability judged case-by-case)**
- Alternate methods/tools are available that cover 'similar' early, critical 'key events'.
- Substituting methods/tools requires case-by-case consideration.

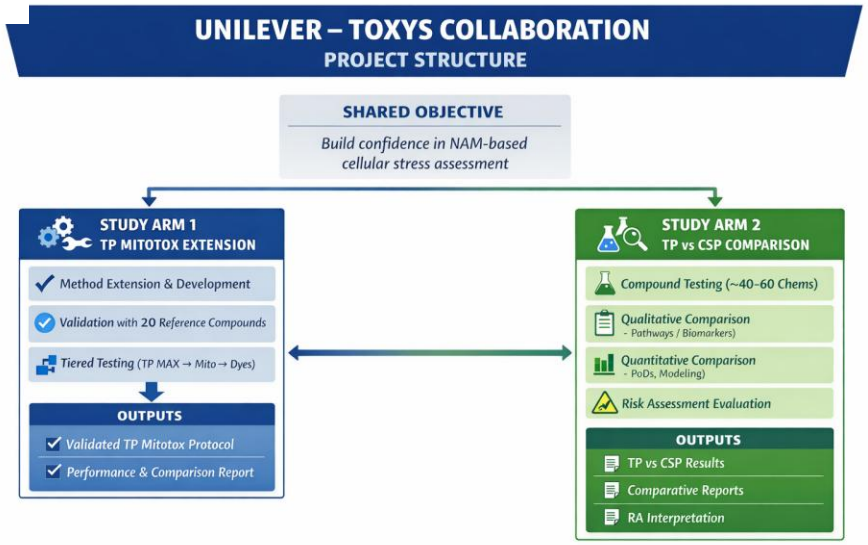
Work underway to verify influence of…

1. Different analysis methods/pipelines (genes vs pathways vs signatures etc).
2. Different assays/methods (ToxProfiler vs Cell stress panel, transcriptomics vs cell painting etc).



H.-C. Lin et al. NAM Journal 1 (2025) 100056





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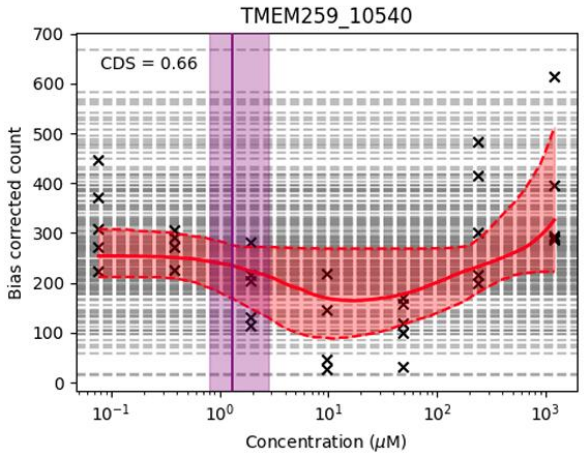
2 NGRA case studies – 2 different workflows.
What would happen if the same chemical went through both workflows?

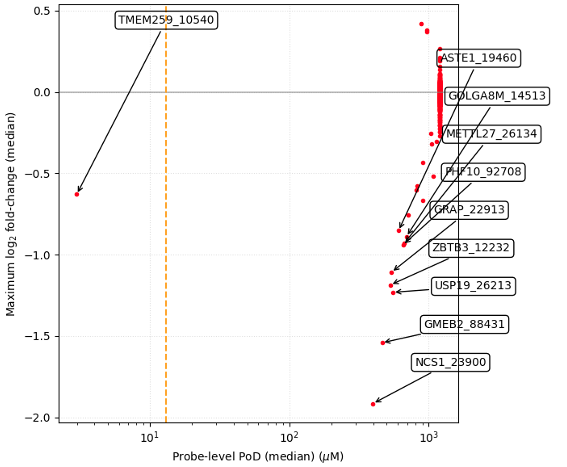
Step 3 – Risk Characterisation and Summary

- PBK estimates of internal exposure – at the ADI, Cmax = ~68 uM.
- Lowest PoD from the NAMs (~ 442 uM) came from the transcriptomics study (HepaRG cells) when looking for the lowest responding pathway.
- NAM PoDs higher than Cmax (BER ~6.4).

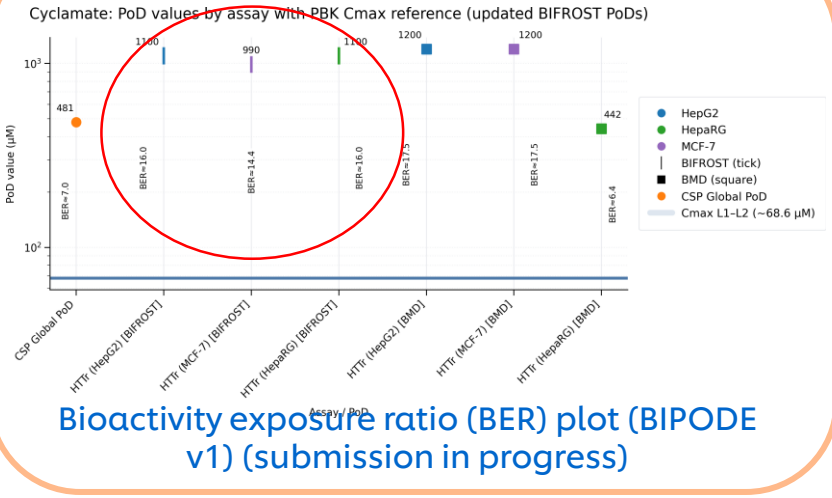
Methods continue to be refined; broader external consensus on data analysis (HTTr especially) is still in progress.



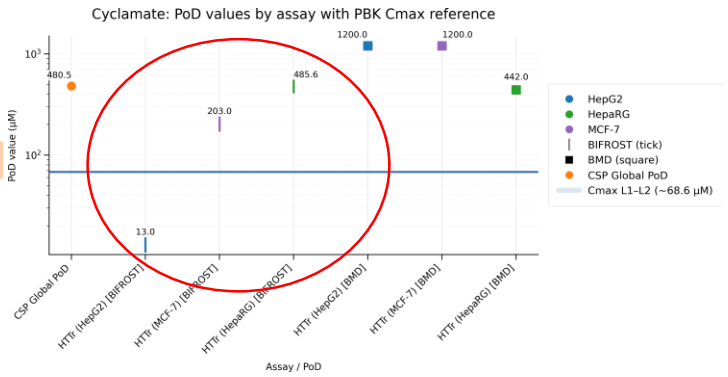
Lowest responding single probe (HepG2s) (Cable et al. 2025) BIFROST v2



Probe level PoD distribution (HepG2s) (Cable et al. 2025) BIFROST v2



Bioactivity exposure ratio (BER) plot (BIPODE v1) (submission in progress)



Bioactivity exposure ratio (BER) plot (Cable et al. 2025) BIFROST v2

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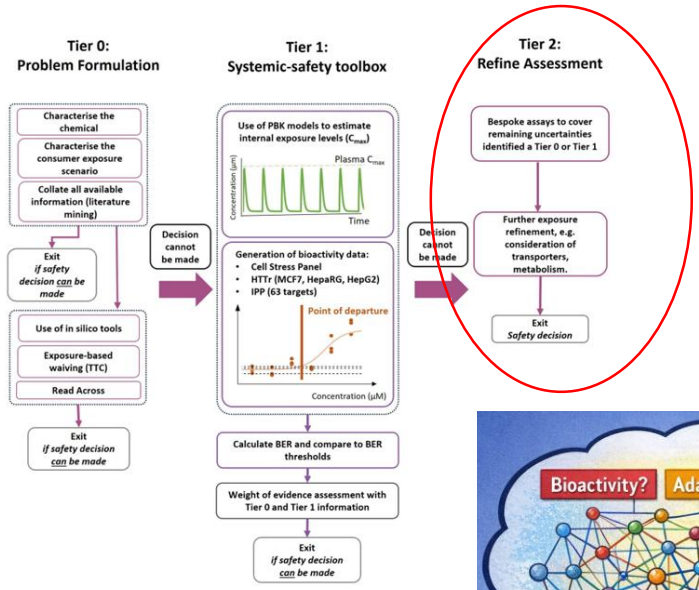
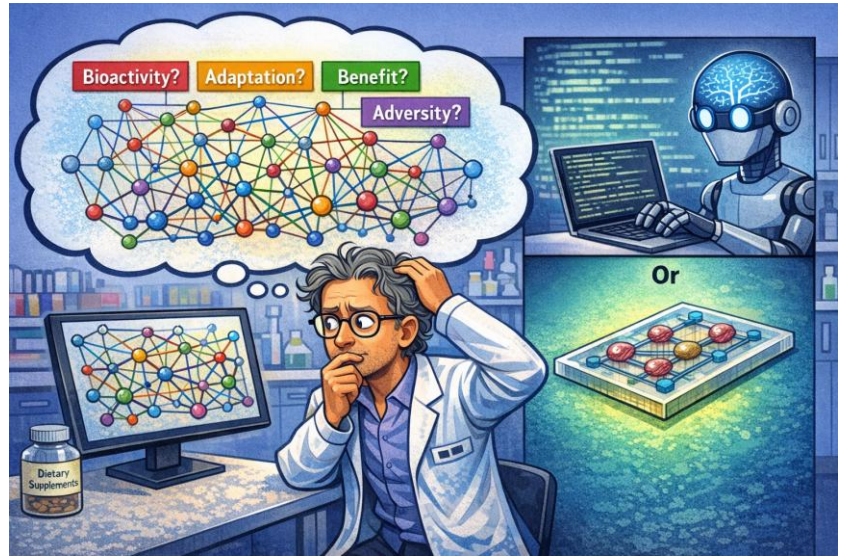
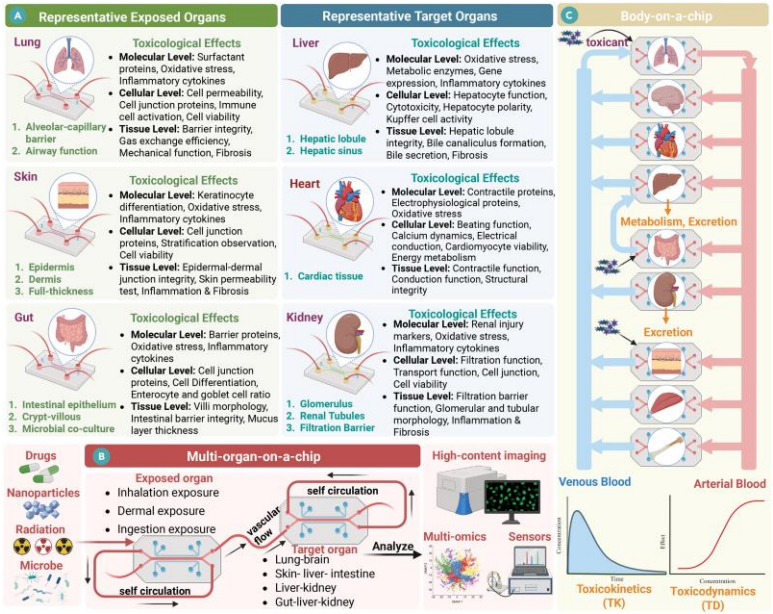
Looking Forward

- Philosophy of protective NGRA = **identifying the most sensitive, credible change in bioactivity from baseline**.
- Bioactivity in these NAMs may represent adaptation, adversity or (for functional ingredients) efficacy - differentiation therefore needed for this approach to be useful for functional ingredients.
- Considerable progress in recent years in advanced *in silico*, *in vitro* and computational approaches, which will further support ability to disentangle **bioactivity > adaptation > benefit > adversity**







Yang et al., The Innovation, 2026; 0

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Conclusions

- Advances in toxicological science now enable robust safety evaluations without animal testing.
  - Pace of change has never been faster than it is currently, and emerging topics (AI) are an opportunity to enable the greatest shift in the history of toxicology.
- Widespread adoption of non-animal approaches requires regulatory and stakeholder confidence.
- Current US regulatory reforms create a timely window to embed NAMs into safety decision-making

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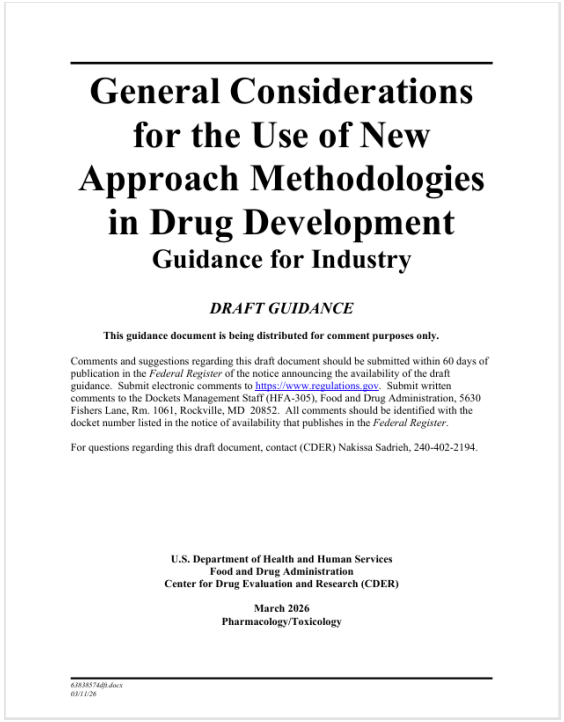


General Considerations for the Use of New Approach Methodologies in Drug Development

Guidance for Industry

DRAFT GUIDANCE

This guidance document is being distributed for comment purposes only.

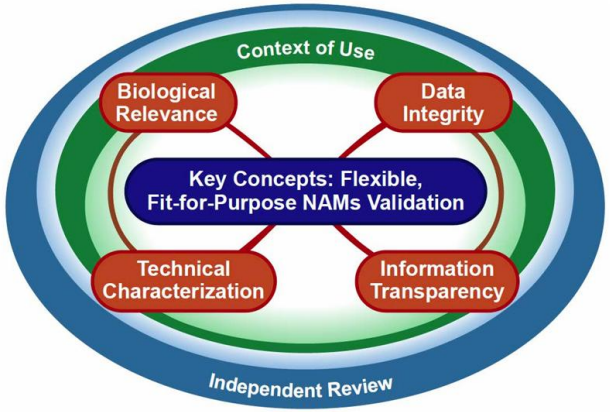
Comments and suggestions regarding this draft document should be submitted within 60 days of publication in the *Federal Register* of the notice announcing the availability of the draft guidance. Submit electronic comments to https://www.regulations.gov. Submit written comments to the Dockets Management Staff (HFA-305), Food and Drug Administration, 5630 Fishers Lane, Rm. 1061, Rockville, MD 20852. All comments should be identified with the docket number listed in the notice of availability that publishes in the *Federal Register*.

For questions regarding this draft document, contact (CDER) Nakissa Sadrieh, 240-402-2194.

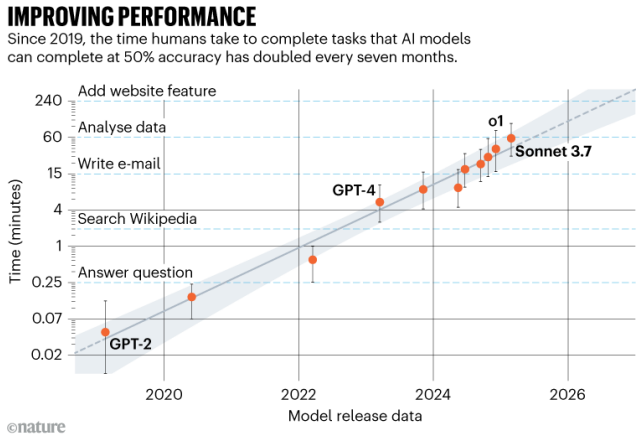
U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)

March 2026
Pharmacology/Toxicology

[General Considerations for the Use of New Approach Methodologies in Drug Development | FDA]



ICCVAM, 2024: https://doi.org/10.22427/NICEATM-2



Credit Garrison Lovely , 2025 (*doi: https://doi.org/10.1038/d41586-025-00831-8)*

Table 3: Safety Testing Recommendations Matrix

Documented Historical Use	Proposed Use of the NDI	Two-Study Genetic Toxicity Battery[1]	Three-Study Genetic Toxicity Battery[1]	14-Day Range-Finding Oral Study in Animals	90-Day Subchronic Oral Study in Animals[3]	One-Generation Rodent Reproductive Study [2]	Multi-Generation Rodent Reproductive Study[2]	Teratology Study in Animals[2]	One-Year Chronic Toxicity or Two-Year Carcinogenesis Study in Animals*	Single-Dose Tolerability and/or ADME Study in Animals and/or Humans*	Repeat-Dose Tolerability and/or ADME Study in Animals and/or Humans*
Daily Chronic	Intermittent; Less Than Historical Use (see question V.I.B.14)	Documented history of use should be sufficient as evidence of safety.									
Daily Chronic	Intermittent; Greater Than Historical Use (see question V.I.B.19)	X		X	X			X		X	
Daily Chronic	Daily Chronic; Less Than Historical Use (see question V.I.B.14)	Documented history of use should be sufficient as evidence of safety.									
Daily Chronic	Daily Chronic; Greater Than Historical Use (see question V.I.B.16)	X		X	X	X		X	X	X	X

'Dietary Supplements: New Dietary Ingredient Notifications and Related Issues: Guidance for Industry' (FDA, 2024)