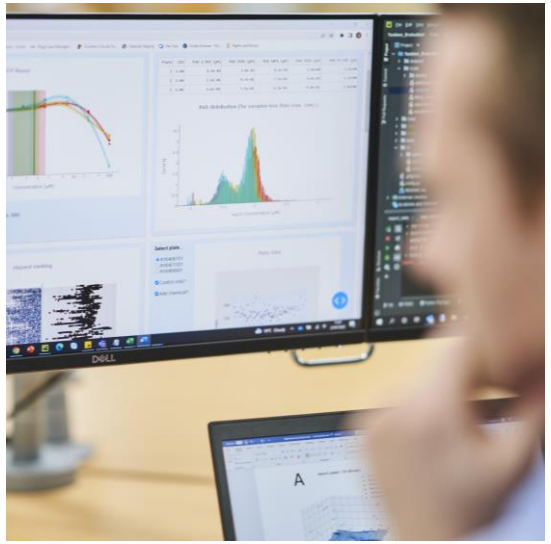
NAMs for use in NGRA for Systemic Safety:
A pragmatic approach to 'validation' by establishing protectiveness and utility

Paul Carmichael
8th April 2024



Unilever

Ensuring Safe In nks, Homecare and Cosmetic Pr

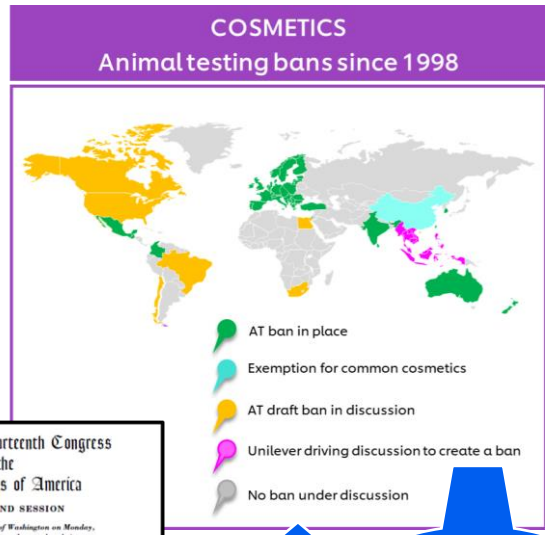
Risk Based Appr
Considers both the exposure to evalua

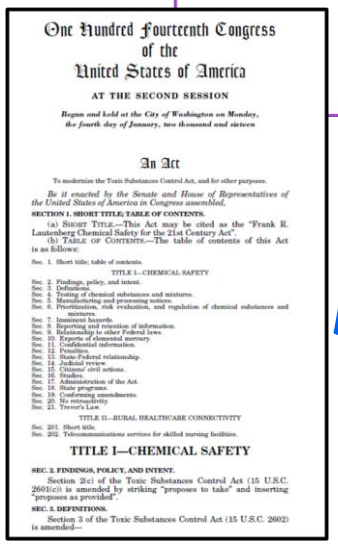
Can we safely use in product?

For **consumers**; **w** the **environment**

Unilever

The Need for Implementation of NAM-Based Safety Assessments





One Hundred Fourteenth Congress
of the
United States of America
AT THE SECOND SESSION

Begun and held at the City of Washington on Monday, the fourth day of January, two thousand and sixteen

An Act

To modernize the Toxic Substances Control Act, and for other purposes.

Be it enacted by the Senate and House of Representatives of the United States of America in Congress assembled,

SECTION 1. SHORT TITLE; TABLE OF CONTENTS.

(a) SHORT TITLE.—This Act may be cited as the "Frank R. Lautenberg Chemical Safety for the 21st Century Act".

(b) TABLE OF CONTENTS.—The table of contents of this Act is as follows:

Sec. 1. Short title; table of contents.

TITLE I—CHEMICAL SAFETY

Sec. 2. Findings, policy, and intent.
Sec. 3. Definitions.
Sec. 4. Testing of chemical substances and mixtures.
Sec. 5. Manufacturing and processing notices.
Sec. 6. Prioritization, risk evaluation, and regulation of chemical substances and mixtures.
Sec. 7. Imminent hazards.
Sec. 8. Reporting and retention of information.
Sec. 9. Relationship to other Federal laws.
Sec. 10. Exports of elemental mercury.
Sec. 11. Confidential information.
Sec. 12. Penalties.
Sec. 13. State-Federal relationship.
Sec. 14. Judicial review.
Sec. 15. Citizens' civil actions.
Sec. 16. Studies.
Sec. 17. Administration of the Act.
Sec. 18. State programs.
Sec. 19. Conforming amendments.
Sec. 20. No retroactivity.
Sec. 21. Trevor's Law.

TITLE II—RURAL HEALTHCARE CONNECTIVITY

Sec. 201. Short title.
Sec. 202. Telecommunications services for skilled nursing facilities.

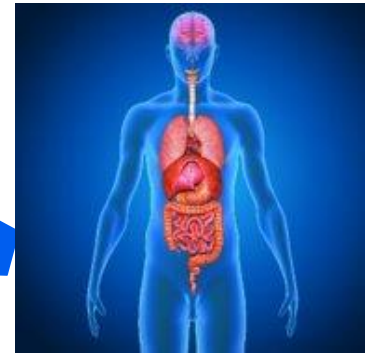
TITLE I—CHEMICAL SAFETY

SEC. 2. FINDINGS, POLICY, AND INTENT.

Section 2(c) of the Toxic Substances Control Act (15 U.S.C. 2601(c)) is amended by striking "proposes to take" and inserting "proposes as provided".

SEC. 3. DEFINITIONS.

Section 3 of the Toxic Substances Control Act (15 U.S.C. 2602) is amended—

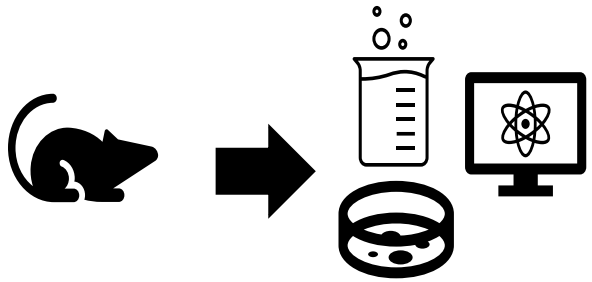


PETA APPROVED
Global Animal Test Policy

Unilever

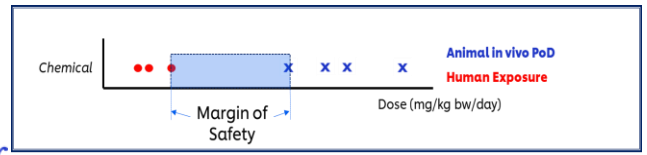
Non-Animal Protective Frameworks for Safety Decisions

Non-animal NAMs strategies for 1-2-1 replacement – prediction of animal outcome

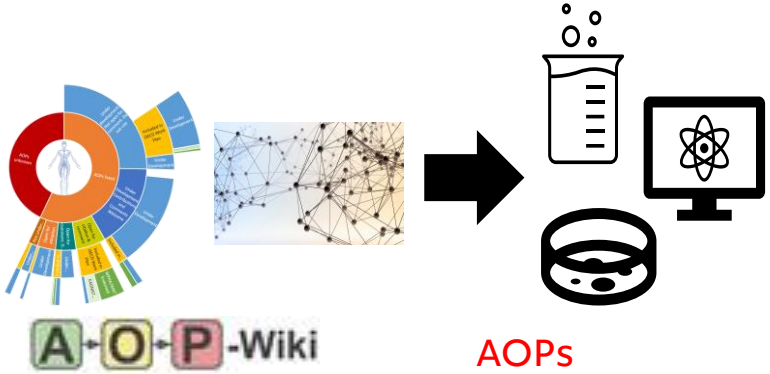


Prediction of an animal test is not necessarily relevant to assess human safety

Rodent studies have been used in a protective manner with the use of uncertainty factors rather than in a predictive way



Development of battery of assays aligned to AOPs



AOPs (currently 470 in AOP wiki)

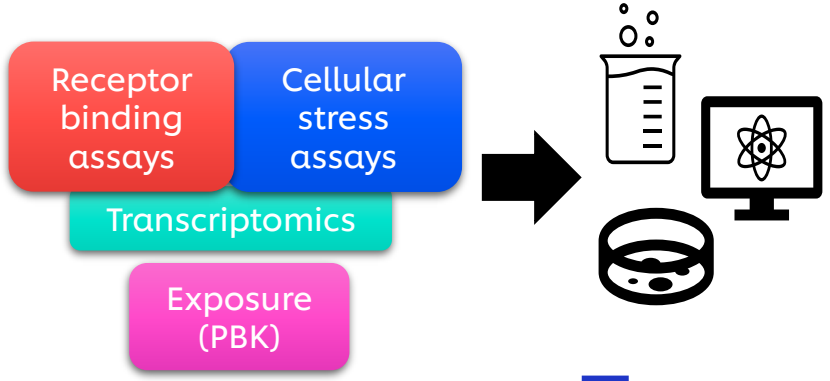
~ Multiple 1000s of assays need to be if multiple AOPs are covered

How to identify the relevant AOP?

Not feasible as a Tier 1 approach

Useful for Tier 2/bespoke safety assessment when differentiation between bioactivity & adversity is needed

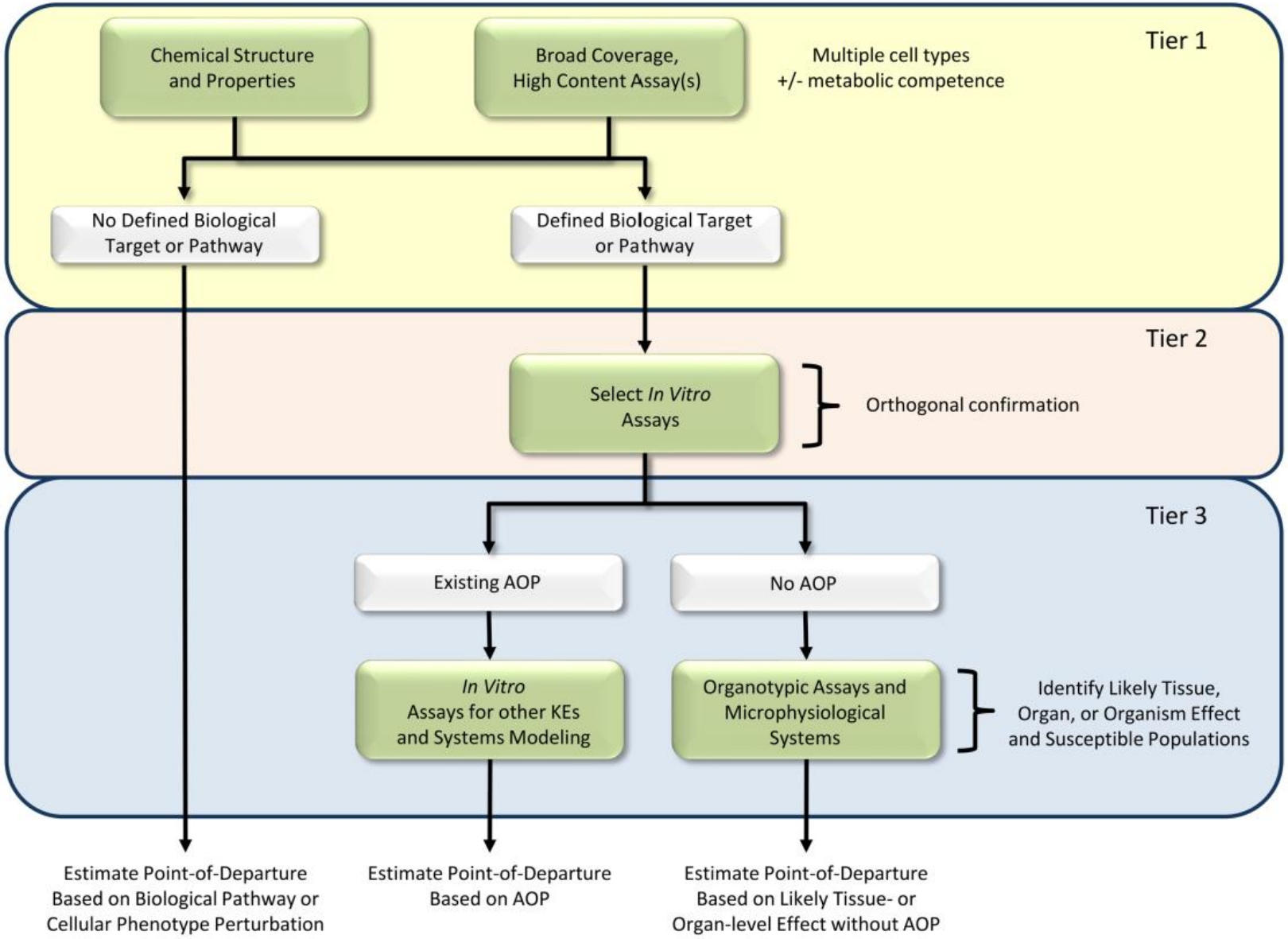
Development of high-throughput & broad coverage set of non-animal NAMs



Protection Hypothesis:

If biological activity measured using a broad suite of human-relevant test systems is above the predicted exposure in humans, then systemic adverse effects are highly unlikely

The EPA Blueprint



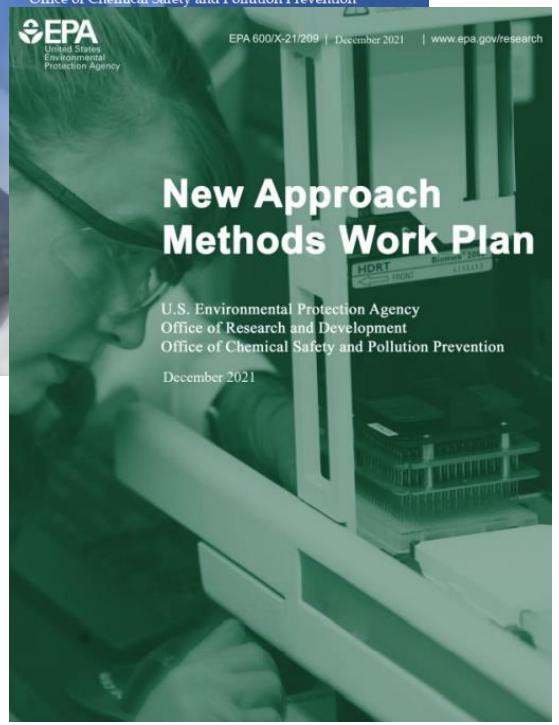
OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org

TOXICOLOGICAL SCIENCES, 169(2), 2019, 317–332
doi: 10.1093/toxsci/kfz058
Advance Access Publication Date: March 5, 2019
Forum

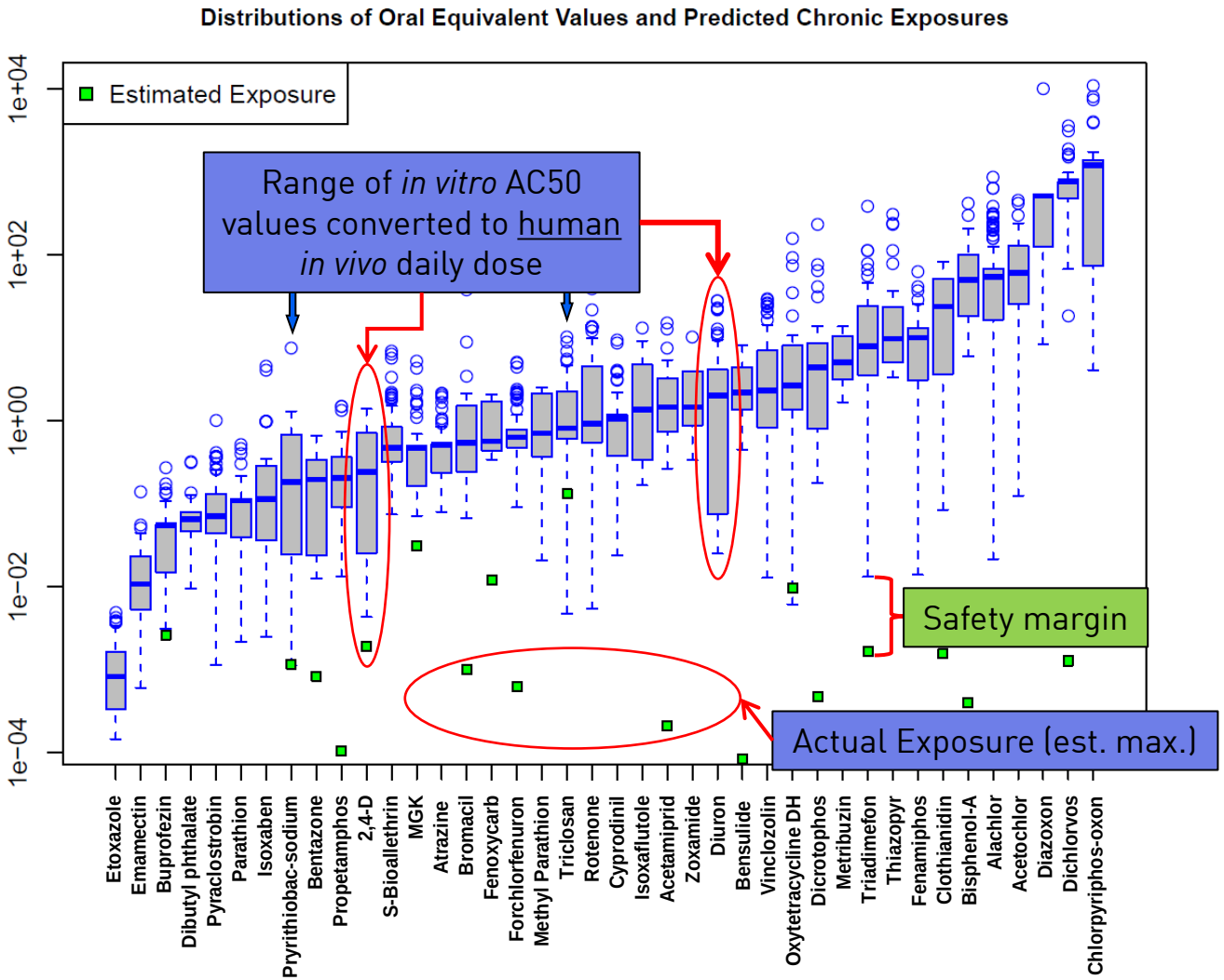
FORUM

The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

Russell S. Thomas,*,1 Tina Bahadori,† Timothy J. Buckley,‡ John Cowden,* Chad Deisenroth,* Kathie L. Dionisio,‡ Jeffrey B. Frithsen,§ Christopher M.



Next Generation Risk Assessment (NGRA) – Protection not Prediction



Slide from Dr Rusty Thomas, EPA, with thanks Rotroff, *et al.* Tox.Sci 2010

If there is no bioactivity observed at consumer-relevant concentrations, there should be no adverse health effects

Thomas RS et al., 2019. Tox Sci. 1;169(2):317-332.

How Protective are those NAMs?

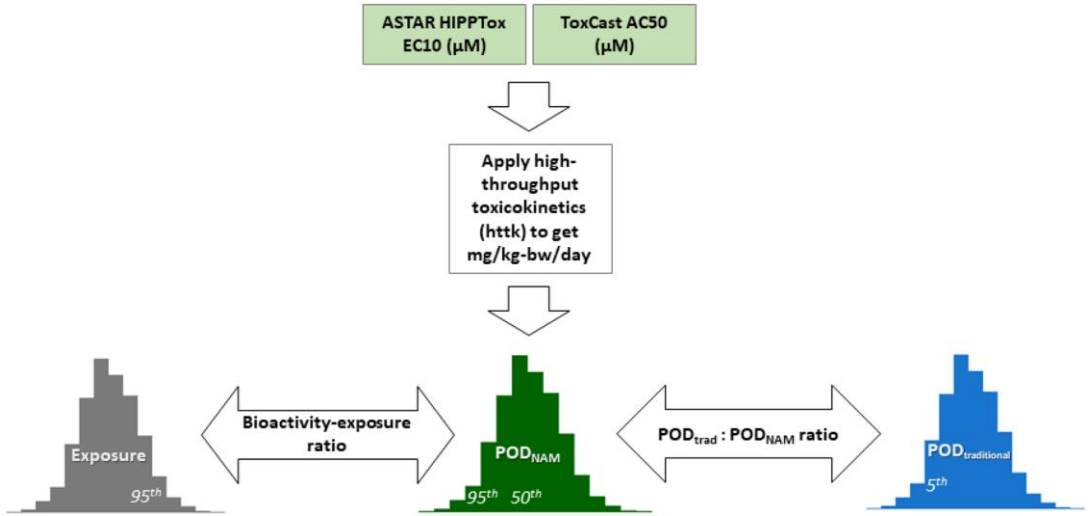
Example from the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative – a 'validation' of Protection not Prediction?

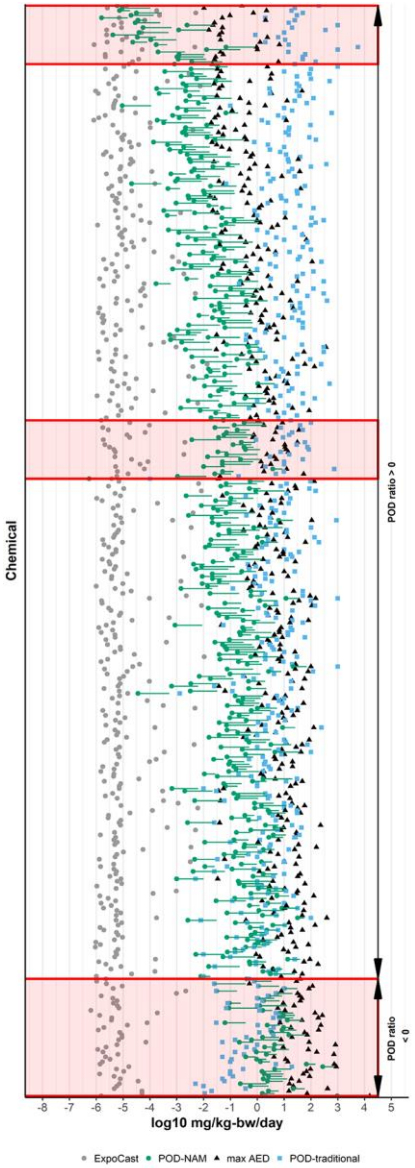
OXFORD SOT Society of Toxicology academic.oup.com/toxsci ToxSpotlight article

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman ,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Franzosa,¶ Ann M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Ying Joey Lee,‡ Michelle Angrish,|| Jean Lou Dorne,|| Stiven Foster,# Kathleen Raffaele,# Tina Bahadori,|| Maureen R. Gwinn,* Jason Lambert,* Maurice Whelan,** Mike Rasenberg,§ Tara Barton-Maclaren,† and Russell S. Thomas *



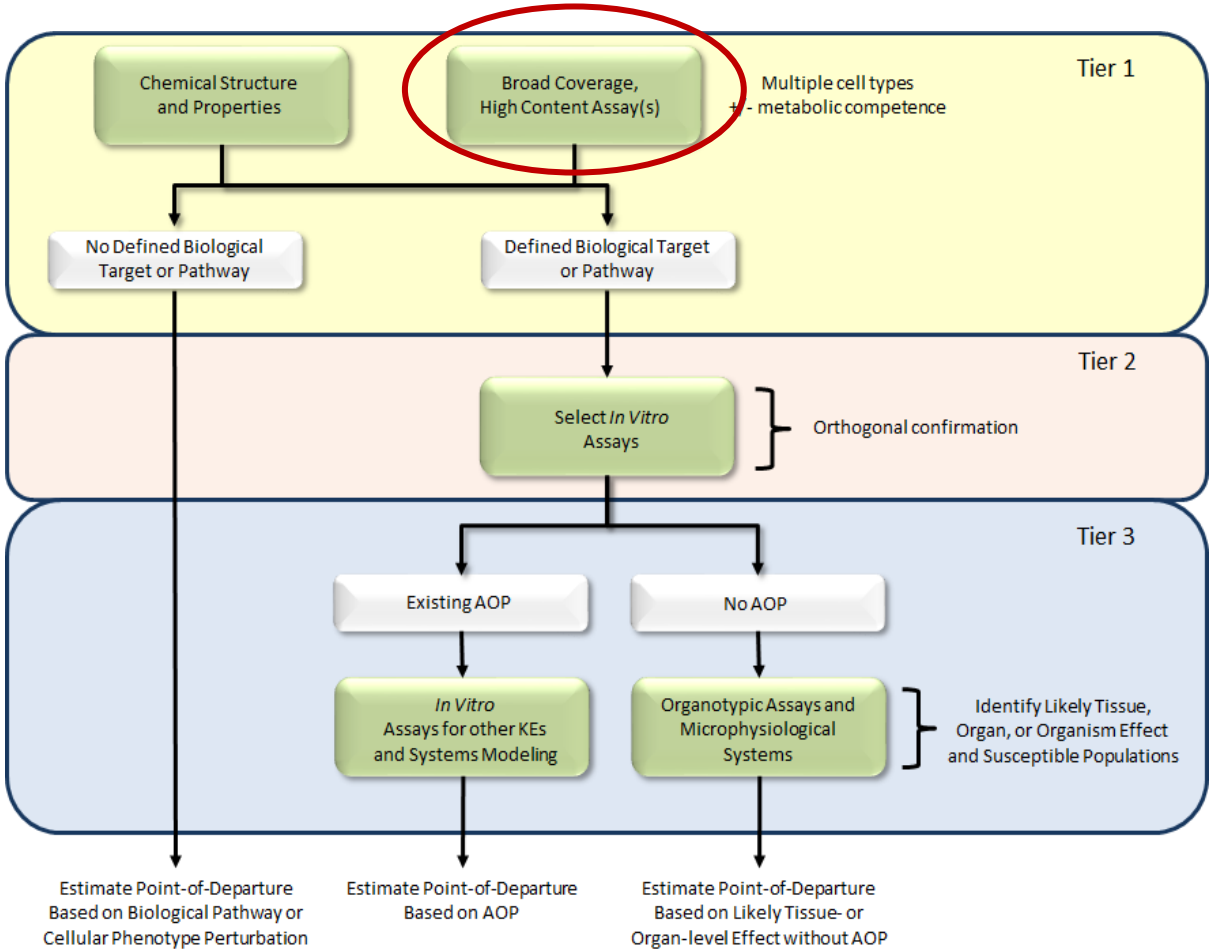


Of the 448 substances, **~90%** had a $POD_{NAM,95}$ that was less than the traditional POD ($POD_{traditional}$) value

Bioactivity:exposure ratios (BERs), useful for identification of priority substances, demonstrated that high-throughput exposure predictions were greater than the $POD_{NAM,95}$ for 11 substances

APCRA ACCELERATING THE PACE OF CHEMICAL RISK ASSESSMENT

EPA Transition from ToxCast to Broad Coverage NAM 'Product'



High throughput profiling (HTP) assays are proposed as the first tier in a NAMs-based hazard evaluation approach

HTP Assay Criteria:

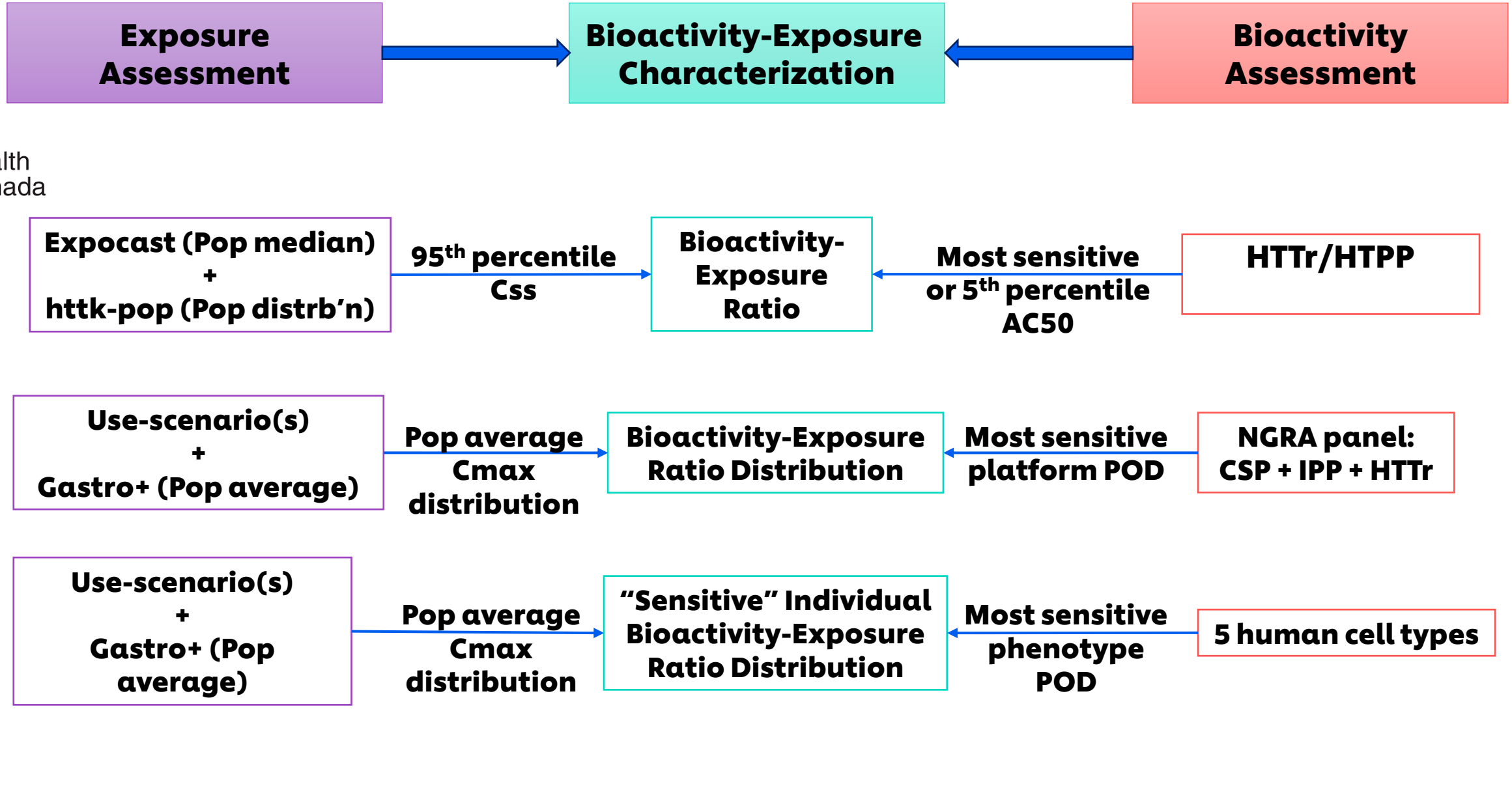
1. Yield bioactivity profiles that can be used for potency estimation, mechanistic prediction and evaluation of chemical similarity
2. Compatible with multiple human-derived culture models
3. Concentration-response screening mode
4. Potential to detect specific and non-specific bioactivity

To date, EPA has identified and implemented two HTP assays that meet this criteria:

- **High-Throughput Transcriptomics [HTTr]**
- **High-Throughput Phenotypic Profiling [HTPP]**

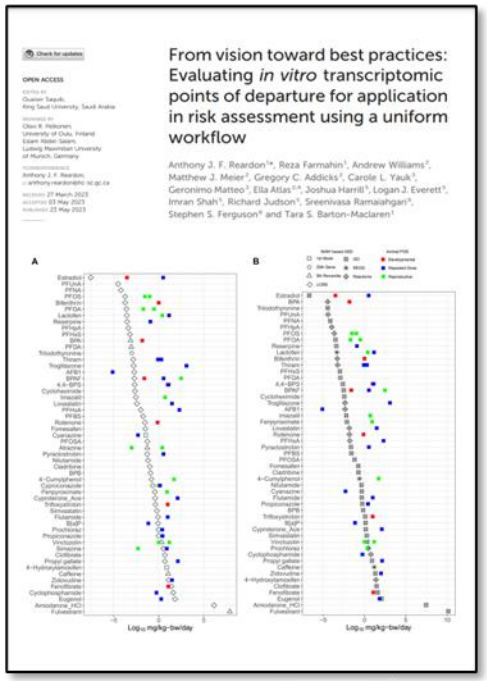
Unilever

NGRAs


Exposure Assessment
Bioactivity-Exposure Characterization
Bioactivity Assessment
Health Canada
Expocast (Pop median) + httk-pop (Pop distrb'n)
95th percentile Css
Bioactivity-Exposure Ratio
Most sensitive or 5th percentile AC50
HTTr/HTPP
Unilever
Use-scenario(s) + Gastro+ (Pop average)
Pop average Cmax distribution
Bioactivity-Exposure Ratio Distribution
Most sensitive platform POD
NGRA panel: CSP + IPP + HTTr
Use-scenario(s) + Gastro+ (Pop average)
Pop average Cmax distribution
"Sensitive" Individual Bioactivity-Exposure Ratio Distribution
Most sensitive phenotype POD
5 human cell types
Unilever

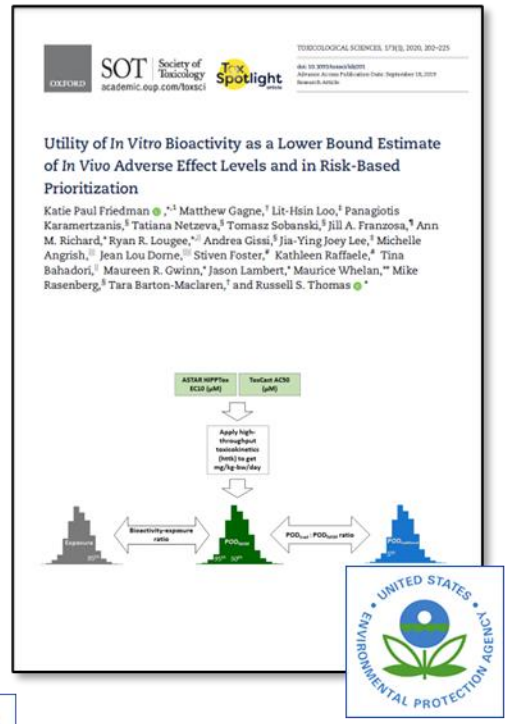
Building NAMs/NGRA Confidence: End-to-End Case Studies

≈40 compounds


From vision toward best practices: Evaluating *in vitro* transcriptomic points of departure for application in risk assessment using a uniform workflow

Anthony J. F. Reardon[1*], Reza Farmahin[1], Andrew Williams[2], Matthew J. Meier[2], Gregory C. Addicks[2], Carole L. Yauk[3], Geronimo Matteo[3], Ella Atlas[3,4], Joshua Harrill[5], Logan J. Everett[5], Imran Shah[5], Richard Judson[5], Sreenivasa Ramaiahgari[6], Stephen S. Ferguson[6] and Tara S. Barton-Maclaren[1]

448 compounds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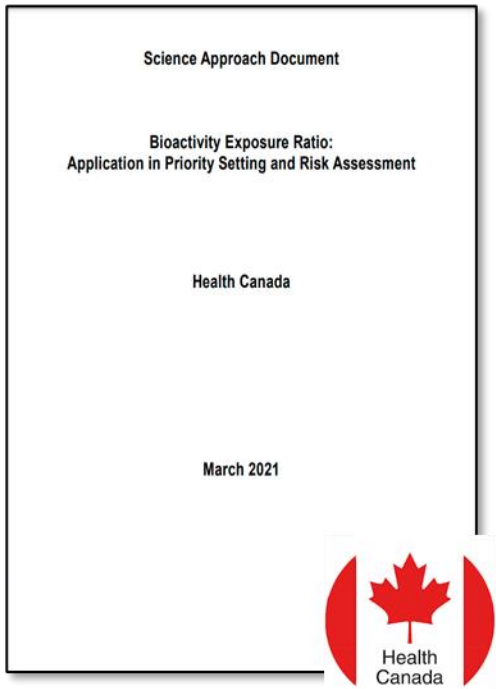
46 compounds


Science Approach Document

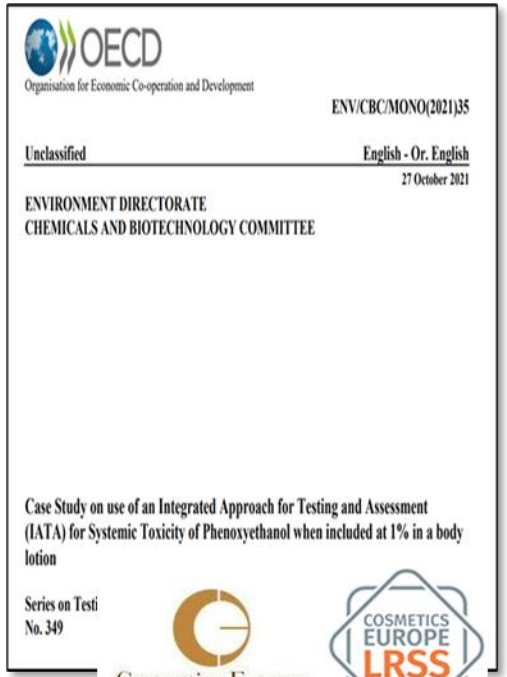
Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/science-approach-document-bioactivity-exposure-ratio-application-priority-setting-risk-assessment.html

30 compounds


OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

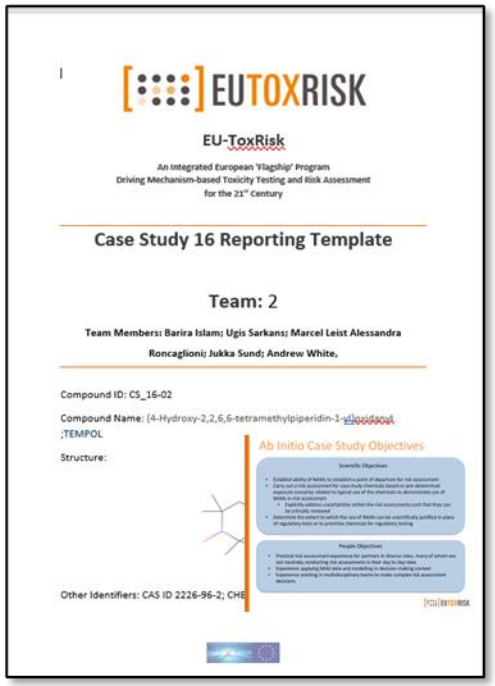
Unclassified — English - Or. English
27 October 2021

ENVIRONMENT DIRECTORATE
CHEMICALS AND BIOTECHNOLOGY COMMITTEE

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

Series on Testi
No. 349

>22 compounds


EUTOXRISK

EU-ToxRisk

An Integrated European 'Flagship' Program
Driving Mechanism-based Toxicity Testing and Risk Assessment for the 21st Century

Case Study 16 Reporting Template

Team: 2

Team Members: Barira Islam; Ugis Sarkans; Marcel Leist Alessandra Roncaglioni; Jukka Sund; Andrew White,

Compound ID: CS_16-02

Compound Name: (4-Hydroxy-2,2,6,6-tetramethylpiperidin-1-yl)oxidanyl ;TEMPOL

Structure:

Ab Initio Case Study Objectives

Other Identifiers: CAS ID 2226-96-2; CHE

Health Canada

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY

Cosmetics Europe
the personal care association

COSMETICS EUROPE LRSS

RISK HUNT3R

Unilever

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

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*

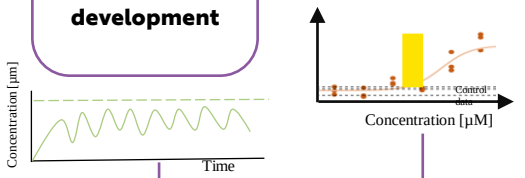
- Use of predictive tools (i.e. *in silico* QSAR models)
- Use of exposure-based waiving approaches such as Threshold of Toxicological Concern (TTC)
- Read Across

→ **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*

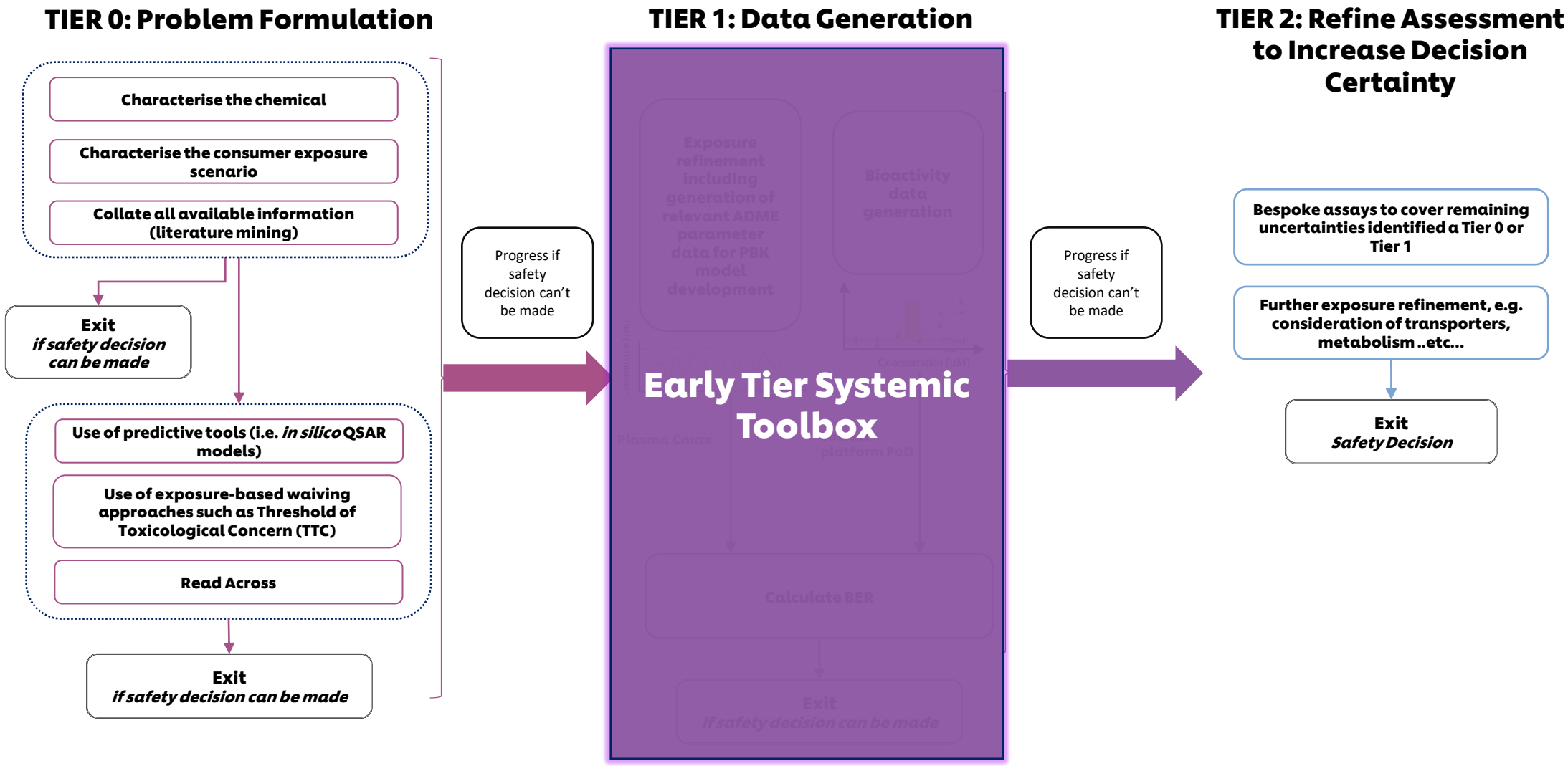
Progress if safety decision can't be made

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*

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



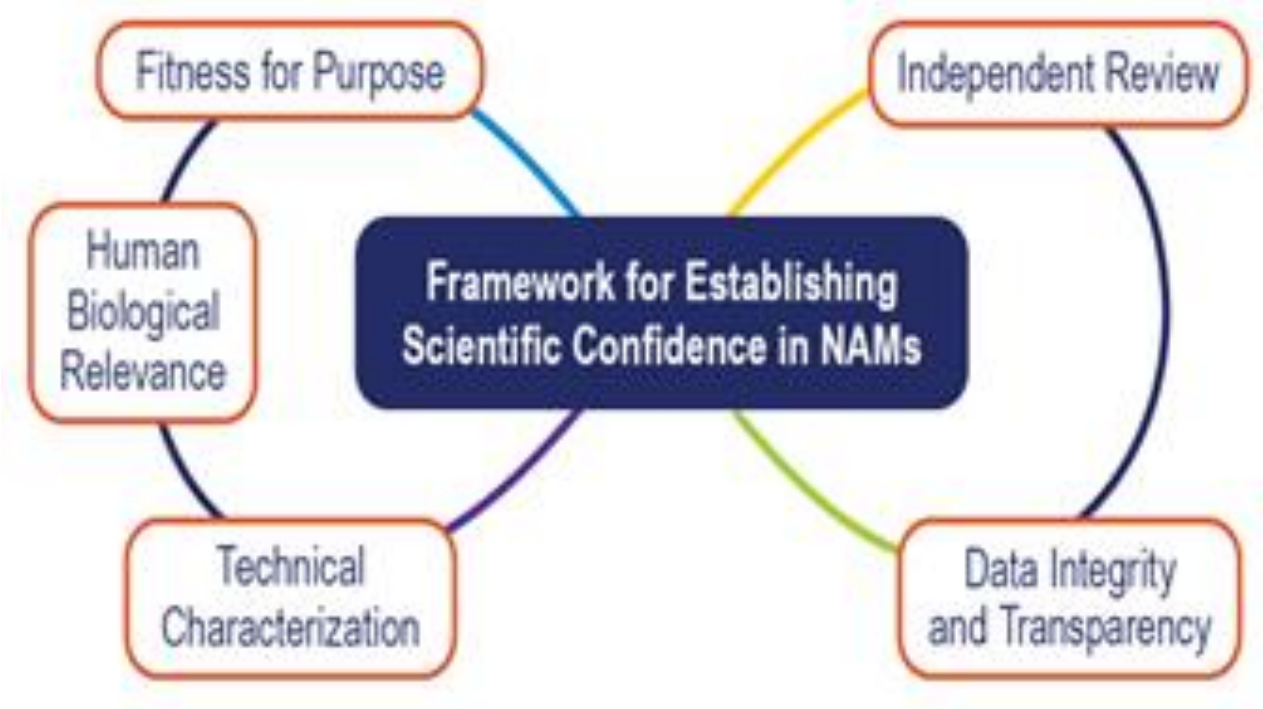
A framework for establishing scientific confidence in new approach methodologies

Anna J. van der Zalm[1] · João Barroso[2] · Patience Browne[3] · Warren Casey[4] · John Gordon[5] · Tala R. Henry[6] · Nicole C. Kleinstreuer[7] · Anna B. Lowit[6] · Monique Perron[8] · Amy J. Clippinger[1]

Archives of Toxicology (2022) 96:2865–2879
https://doi.org/10.1007/s00204-022-03365-4

Received: 17 May 2022 / Accepted: 11 August 2022 / Published online: 20 August 2022

REVIEW ARTICLE



Unilever

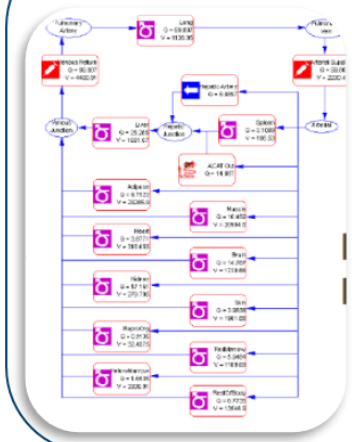
Evaluation/"Validation" of an Early Tier Toolbox for Systemic Safety

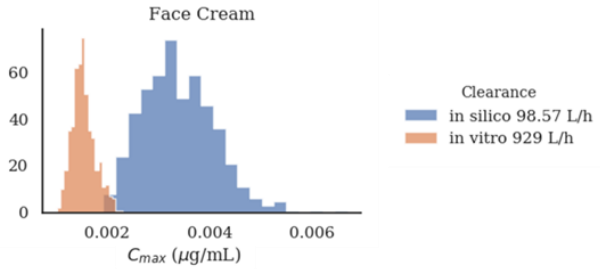
AIM: Use NAMs to ensure the protection of consumers: can the approach be used to confidently identify low risk chemical exposure scenarios?

1. **Define the toolbox components** Choose and evaluate a set of NAMs covering exposure modelling and bioactivity investigations
2. **Select test chemicals** Choose as many as practicable to maximise coverage of different chemistries and biological effects/toxicity
3. **Set performance criteria** Define the 'truth' that the performance of the toolbox will be compared to

Our Key NAMs

PBK Modelling





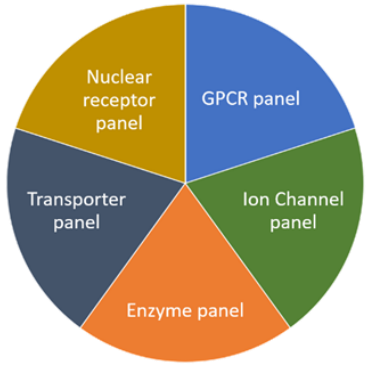
Toxicology in Vitro (2020), **63**, 104746

In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

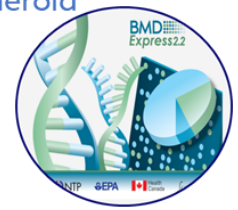


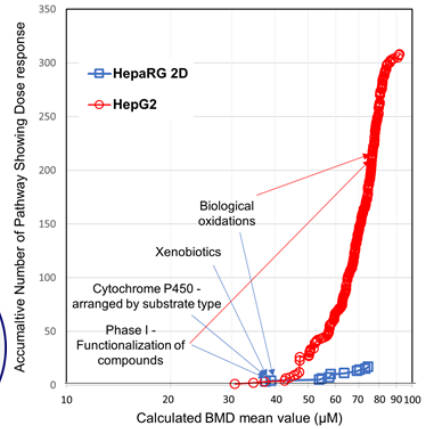
eurofins | Cerep

Transcriptomics

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

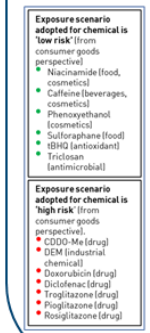
BMDExpress 2

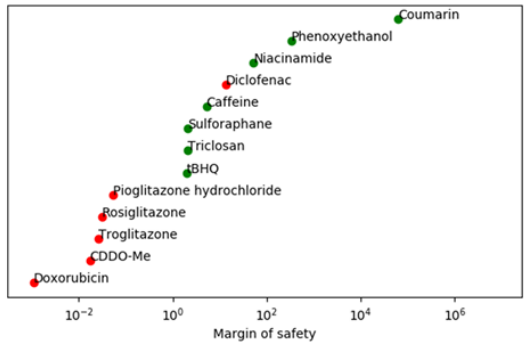




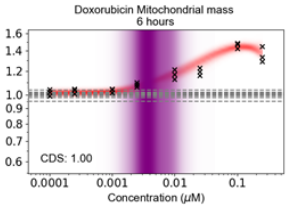
Cellular Stress Pathways

13 chemicals, 36 Biomarkers; 2 Timepoints; 8 Concentrations; ~10 Stress Pathways





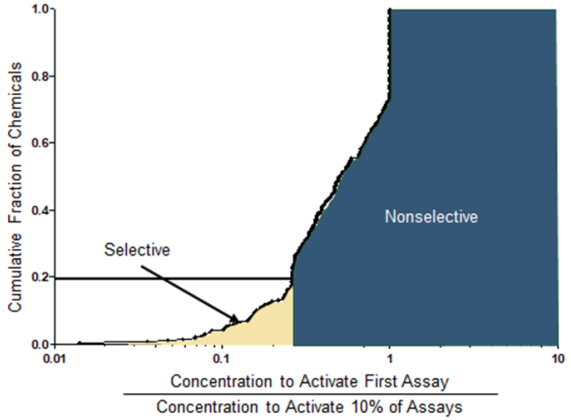
cyprotex AN EVOTEC COMPANY



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

Unilever

1. Defining the Toolbox Components



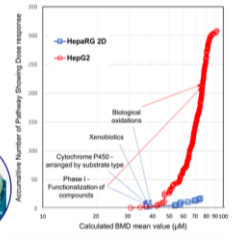
Point of Departure Determination

Nonselective 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

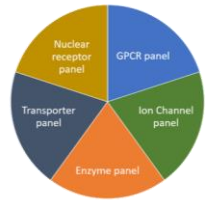
13 chemicals, 36 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

cyprotex

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

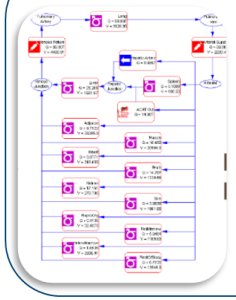
Selective Effects

In vitro pharmacological profiling

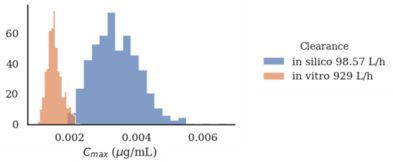


eurofins | Cerep

PBK Modelling



Face Cream

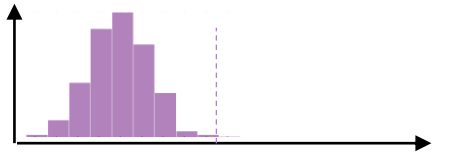


Toxicology in Vitro (2020), **63**, 104746

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

(Bayesian model)

Bioactivity Exposure Ratio Distribution



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2. Select Test Chemicals

Collate possible chemicals from databases, large-scale projects, expert opinion

Filter out chemicals that would be impractical to test

38 Test Chemicals

- 9 cosmetic ingredients, 21 drugs, 3 food additives, 5 agricultural chemicals, 1 industrial chemical

- Oral, dermal, IV and inhalation exposure scenarios

- Organ toxicities, CNS disruptions, immune system dysregulation, non-specific effects, blood-based disorders etc...

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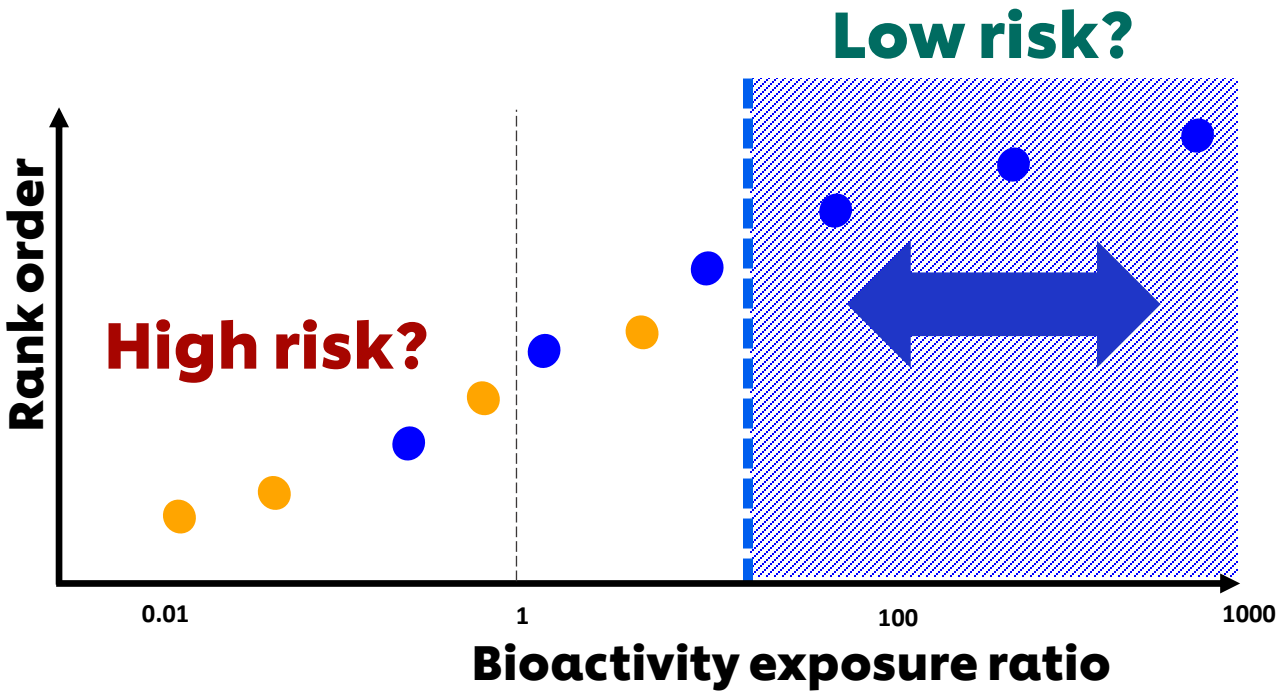
3. Set Performance Criteria

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)



‘Low’ risk for consumers from systemic perspective

‘High’ risk for consumers from systemic perspective

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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3. Set Performance Criteria

Benchmarking using chemical-exposure scenarios

- Chemicals with well-defined human exposures
- Traditional safety assessment available
- High certainty in the risk classification for each chemical-exposure scenario from a consumer goods perspective
- Risk class is relative to consumer health (N.B. drugs = high-risk)

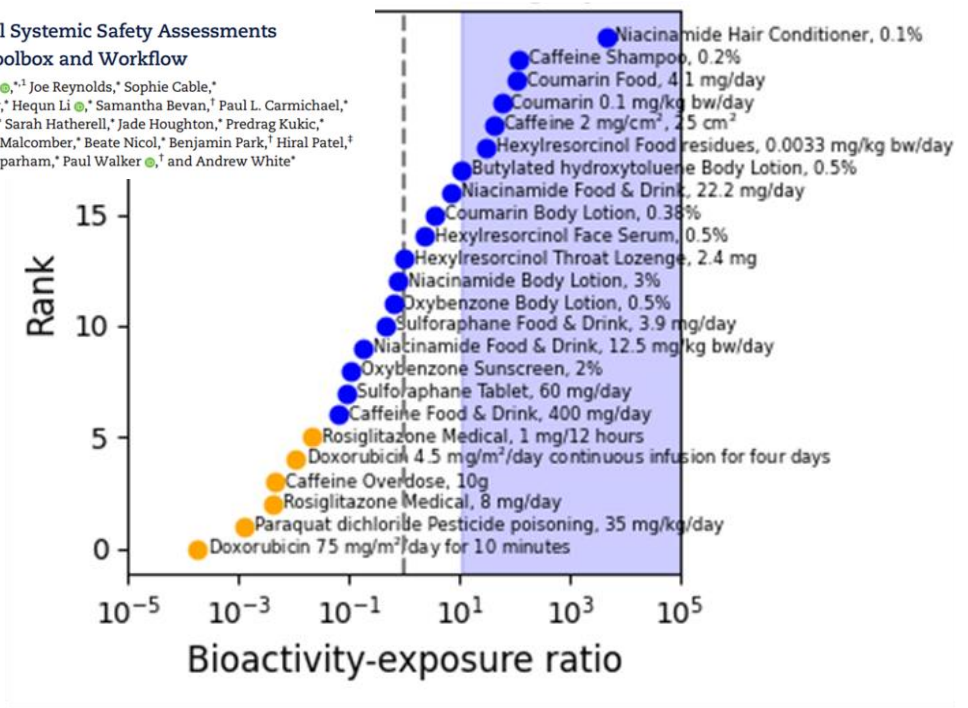
‘Low’ risk for consumers from systemic perspective

‘High’ risk for consumers from systemic perspective

OXFORD SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight
TOXICOLOGICAL SCIENCES, 189(1), 2022, 124–147

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

Alistair M. Middleton,*,1 Joe Reynolds,* Sophie Cable,* Maria Teresa Baltazar,* Hequn Li,* Samantha Bevan,† Paul L. Carmichael,* Matthew Philip Dent,* Sarah Hatherell,* Jade Houghton,* Predrag Kukic,* Mark Liddell,* Sophie Malcomber,* Beate Nicol,* Benjamin Park,† Hiral Patel,† Sharon Scott,* Chris Sparham,* Paul Walker,† and Andrew White*



Protectiveness and utility metrics

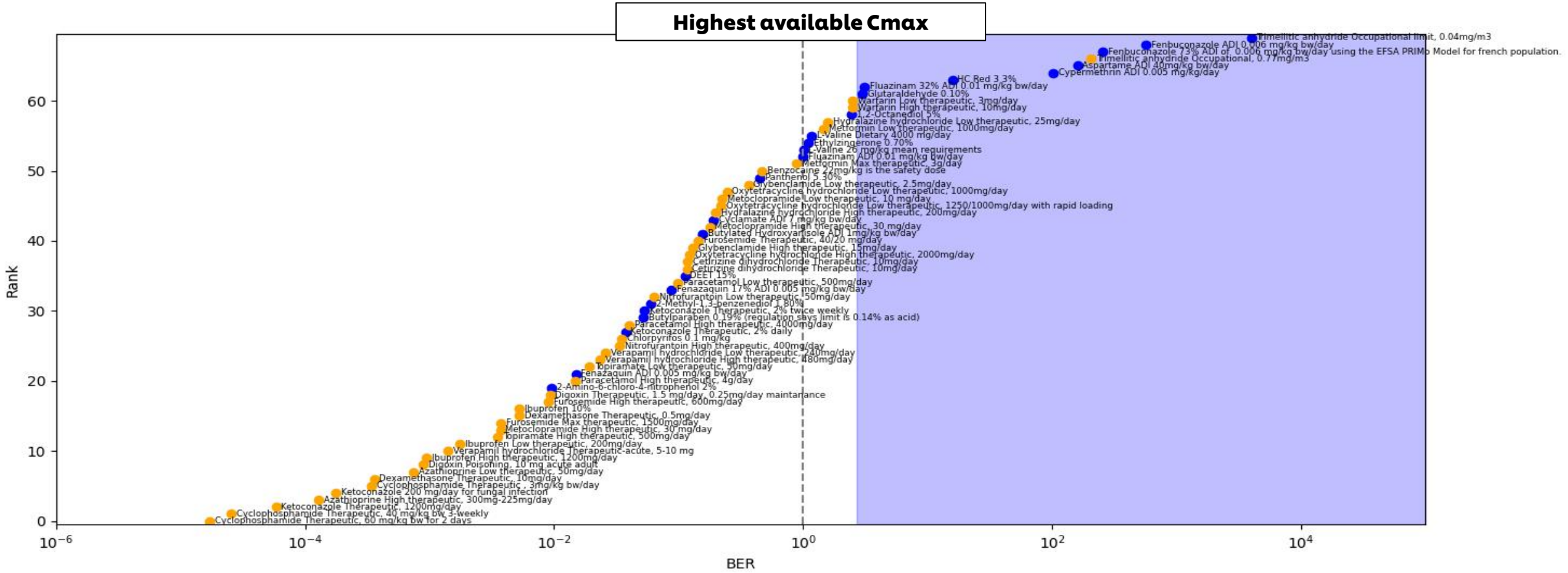
$$\text{Protectiveness} = \frac{H_U}{H_U + H_L} \qquad \text{Utility} = \frac{L_L}{L_L + L_U}$$

H_U - # of high risk exposures identified as uncertain risk
H_L - # of high risk exposures identified as low risk
L_U - # of low risk exposures identified as uncertain risk
L_L - # of low risk exposures identified as low risk

Threshold values of the BER point estimates for determining whether an exposure is low risk

PBK Level	Threshold BER Required for Exposure to Be Identified as Low Risk	Confidence Threshold ($p_{threshold}$) Required for Exposure Scenario to Be Identified as Low Risk
1	110	.98
2	11	.97
3	2.5	.95

Results for 38 Test Chemicals and 70 Exposure Scenarios



Protectiveness **98% (45 out of 46)**

High-risk exposure scenarios are identified as uncertain/high risk (i.e. BER < threshold)

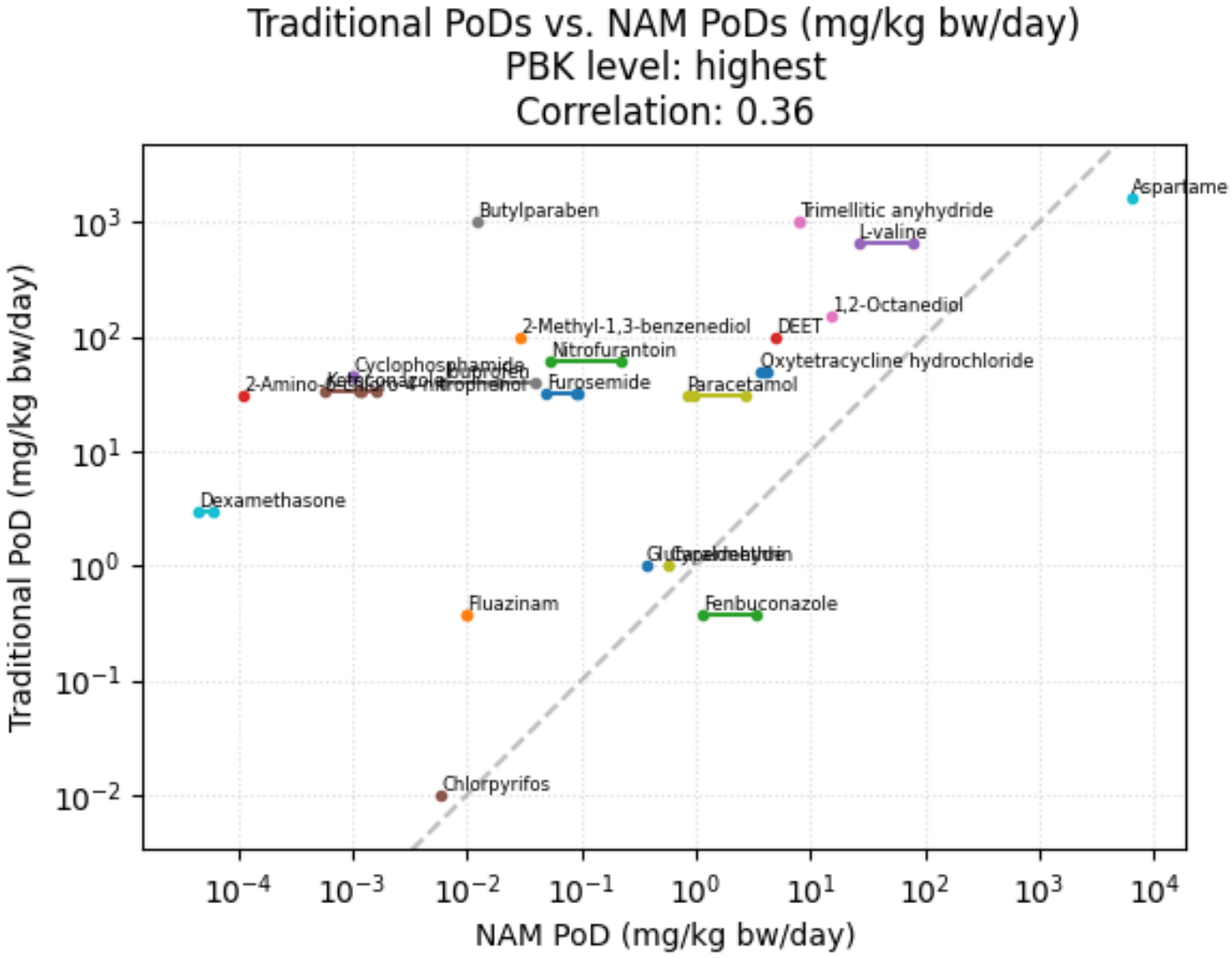
Utility **33% (8 out of 24)**

Low-risk scenarios are identified as low risk at this early tier stage in a risk assessment framework (i.e. BER > threshold)

Comparison of NAM-based Early Tier Toolbox with Decisions Made Using *in vivo* Data – Protective not Predictive

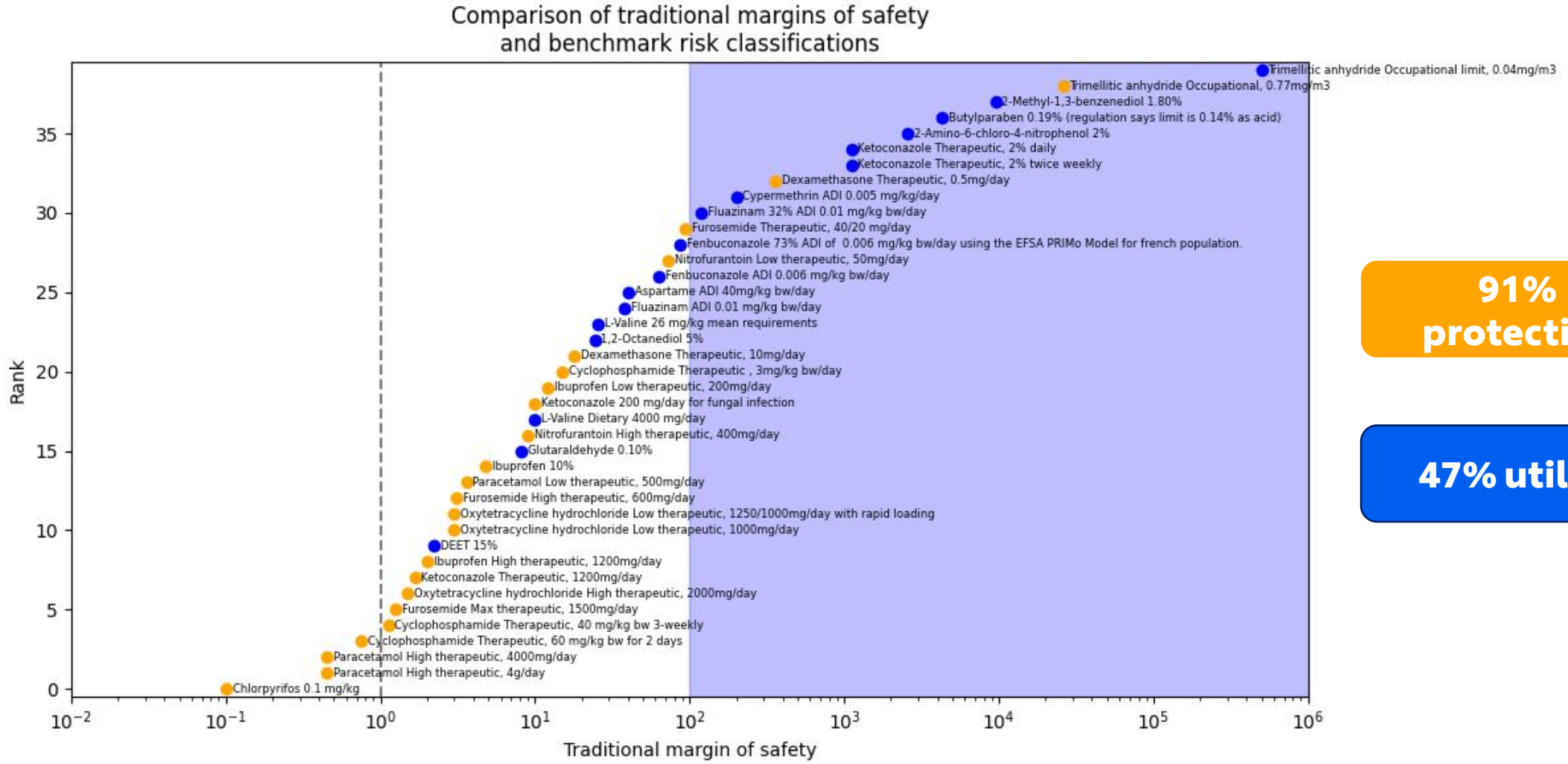
What if we took the same approach with *in vivo* data?

- Repeat dose *in vivo* data identified for 27 chemicals of the 38 tested.
- In most cases NAM PoDs are more conservative than traditional PoDs



In agreement with Paul-Friedman et al (2020)

Comparison of NAM-based Early Tier Toolbox with Decisions Made Using *in vivo* Data



91% protective

47% utility

Using the minimum of NOAELs/LOAELs identified, margins of safety plotted and threshold at MoS = 100

Regulatory Toxicology and Pharmacology 148 (2024) 105579

Contents lists available at ScienceDirect

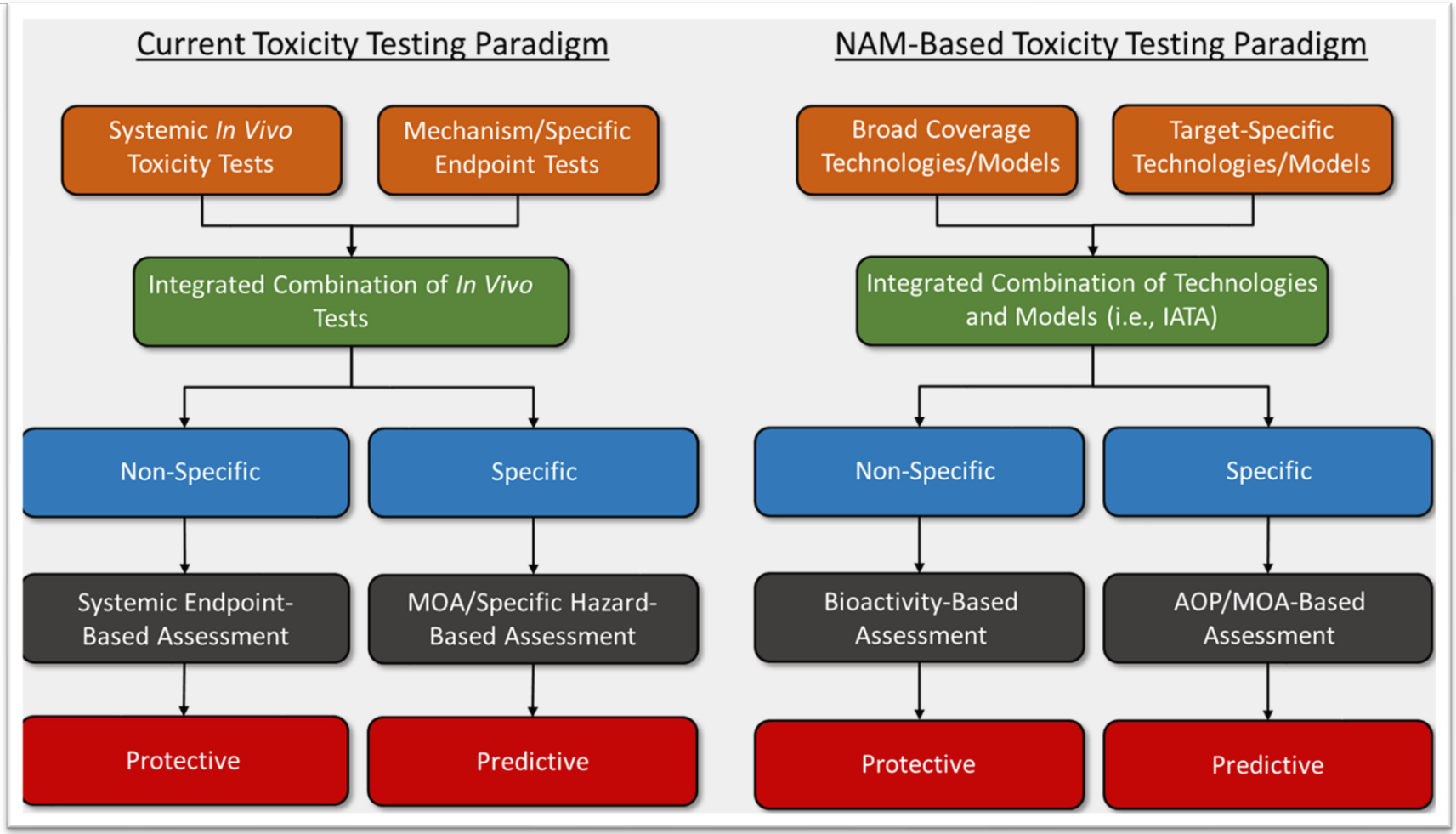
Regulatory Toxicology and Pharmacology

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

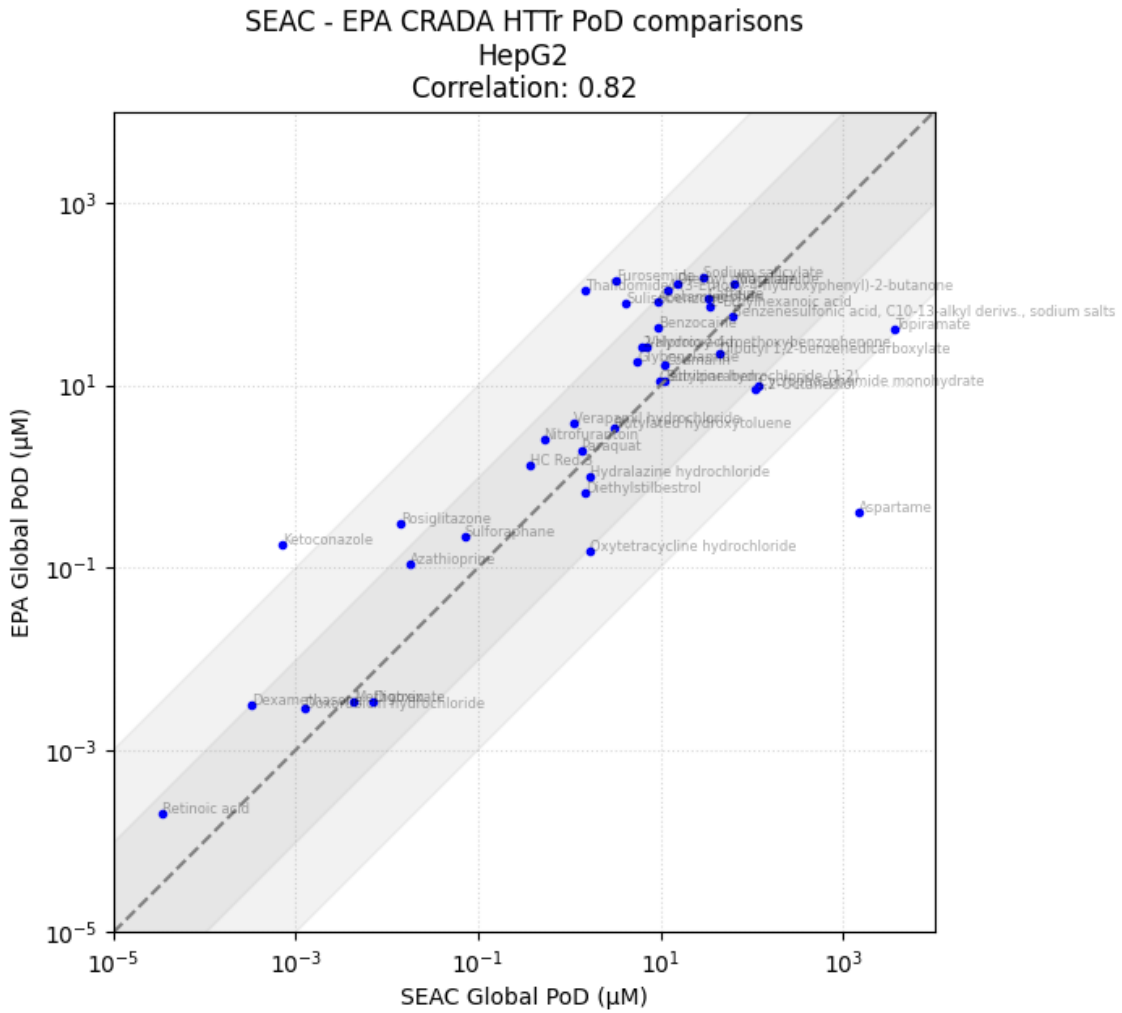
Adverse effects in traditional and alternative toxicity tests

Patience Browne [a,*], Katie Paul Friedman [b], Kim Boekelheide [c], Russell S. Thomas [b]

[a] Environment Health and Safety Division Environmental Directorate, Organisation for Economic and Cooperative Development (OECD), 2 rue André Pascal, Paris Cedex 16, 75775, France
[b] Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC, 27711, USA
[c] Department of Pathology and Laboratory Medicine, Brown University, Providence, RI, USA



Reproducibility of HepG2 BIFROST global PoD from HTTr



Subset of toolbox evaluation chemicals tested in HepG2 cell line at Unilever + US-EPA

Can compare global PoDs estimated from SEAC and EPA datasets

Moderate correlation (0.82), hampered by noticeable outliers:

Aspartame – Has a retinoic acid like signal, suspected contamination in EPA data due to proximity on dosing plate

Ketoconazole – Difference attributable to BIFROST modelling choices

SOT | Society of Toxicology

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Conclusions and Next Steps

- For the test chemicals in this evaluation, an early tier systemic toolbox is **98% protective**
- Fair to say 'overly-conservative'?

  Low utility requires higher-tier tools for bioactivity distinguishing adversity from adaption (AOP and prediction-led e.g. from ONTOX/RiskHunt3R)

- A NAM-based toolbox for systemic toxicity has comparable performance to safety decision making using traditional *in vivo* data.
- What is the applicability domain of this toolbox?

  How would the toolbox perform with a wider set of chemicals?

- What would the performance be like with a different set of assays/cells?

  Is there an optimum combination of NAMs to maximise both protectiveness and utility?

- **Assuring human safety is the most important thing**

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EPA and Unilever Announce Major Research Collaboration to Advance Non-animal Approaches for Chemical Risk Assessment

August 19, 2021

Contact Information
EPA Press Office (press@epa.gov)

WASHINGTON – Today, the U.S. Environmental Protection Agency (EPA) and Unilever announced a collaborative agreement to explore better ways to assess chemical risks associated with consumer products. This agreement builds on prior cooperation between EPA and Unilever regarding New Approach Methods (NAMs), which are a promising alternative to conventional toxicity testing that are intended to reduce reliance on the use of animals.

EPA and Unilever have been jointly evaluating and using NAMs since 2015. This collaboration is helping EPA implement its New Approach Methods Work Plan and is the foundation for new efforts to demonstrate that these novel approaches can help decision makers better protect consumers, workers and the environment.

"EPA is a pioneer in developing and applying NAMs to identify and quantify risks to human health, while reducing the use of animals in chemical toxicity testing," said **H. Christopher Frey, Deputy Assistant Administrator for Science Policy in EPA's Office of Research and Development**. "We are excited to continue the collaboration with Unilever, which enhances the robustness of our mutual research to demonstrate the use of NAMs."

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Thank You

seac.unilever.com