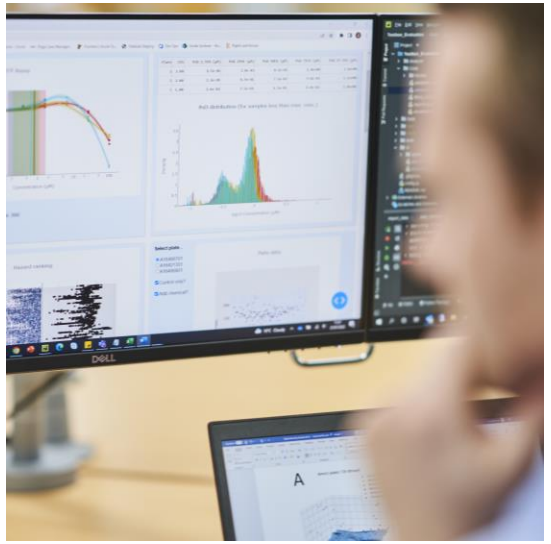
Next Generation Risk Assessment: An industry perspective on the application of toxicogenomics and the challenges for implementation

Dr Andrew White
Unilever Safety and Environmental Assurance Centre

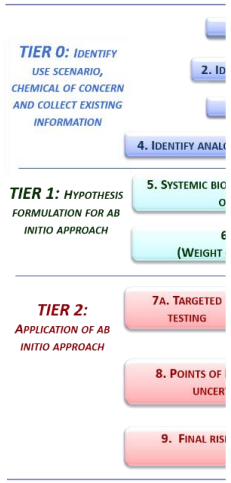
Session 24: Toxicogenomics: breaking barriers for regulatory implementation

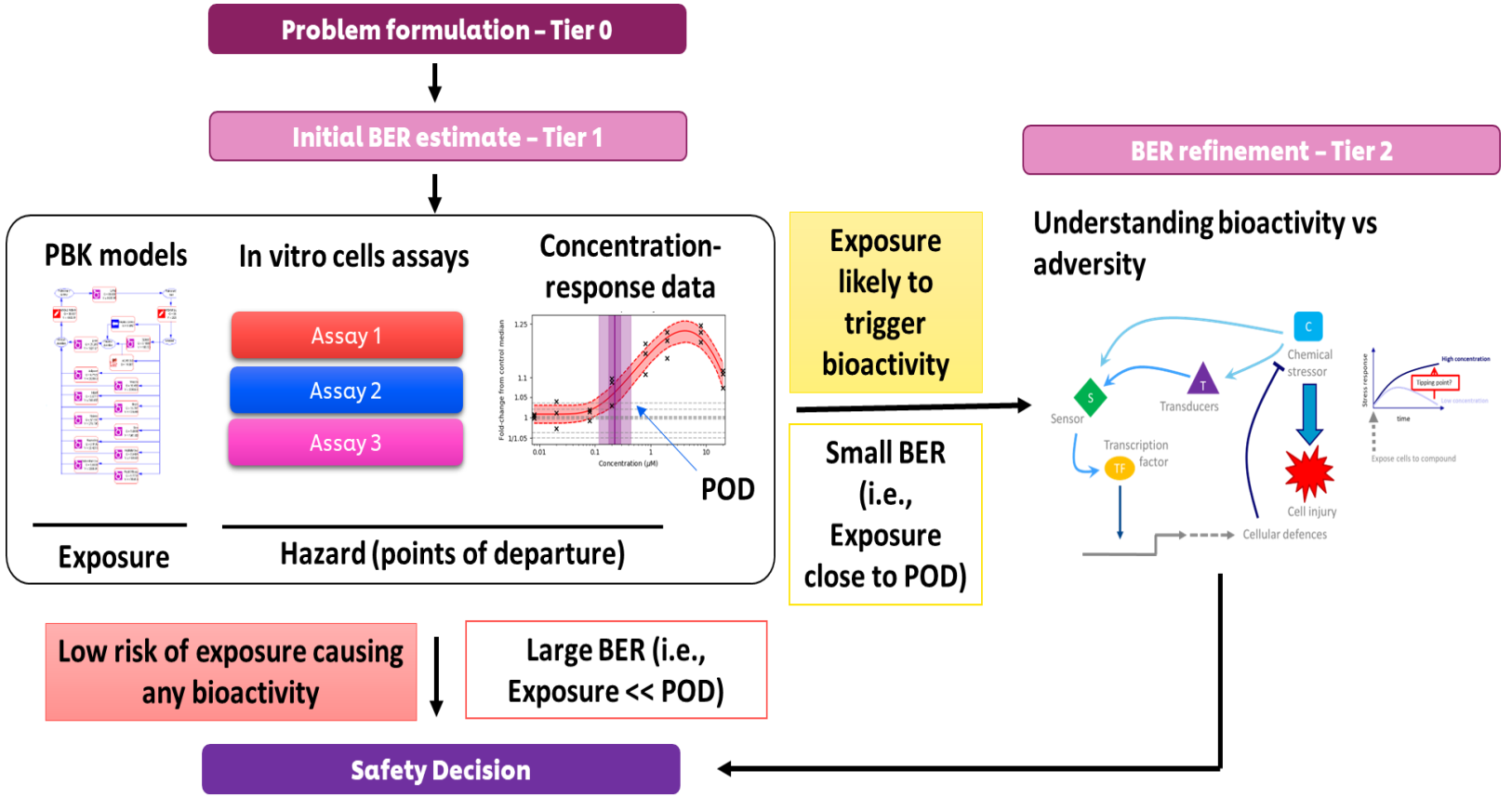
13th September 2023

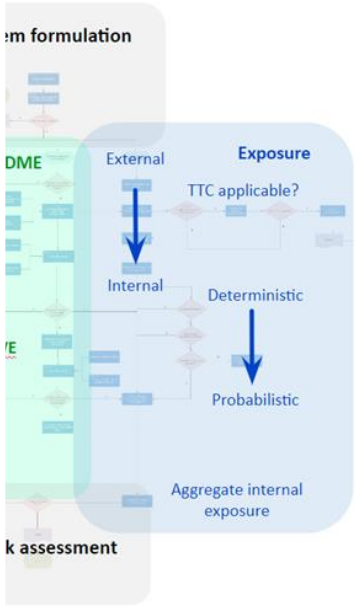


Unilever

Decision frameworks in NGRA







A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Baltazar et. al. 2020 Tox Sci 176

Are non-animal systemic safety assessments protective. A toolbox and workflow Middleton et al 2022

Objective Application of Omics for NGRA

- Determination of Compounds primary MoA through pathway analysis
- Underpinning AOP key events and MIEs

- Comparison of differentially expressed signatures from one treatment to a database of previously reported gene signatures or to another sample.

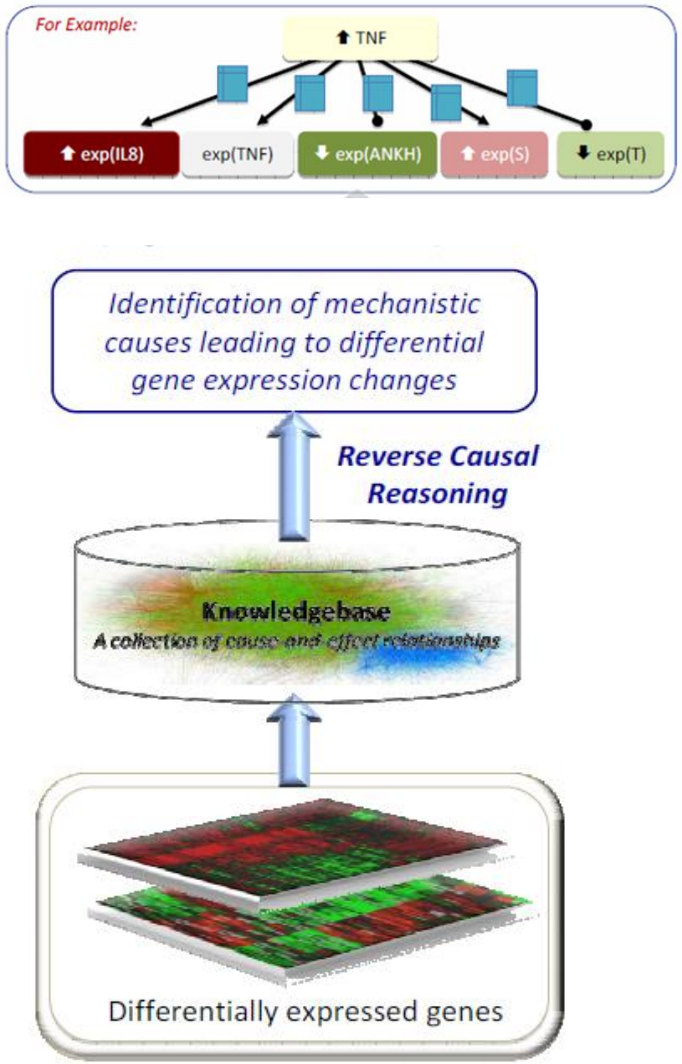
- Dose response relationship and identification of biologically relevant dose

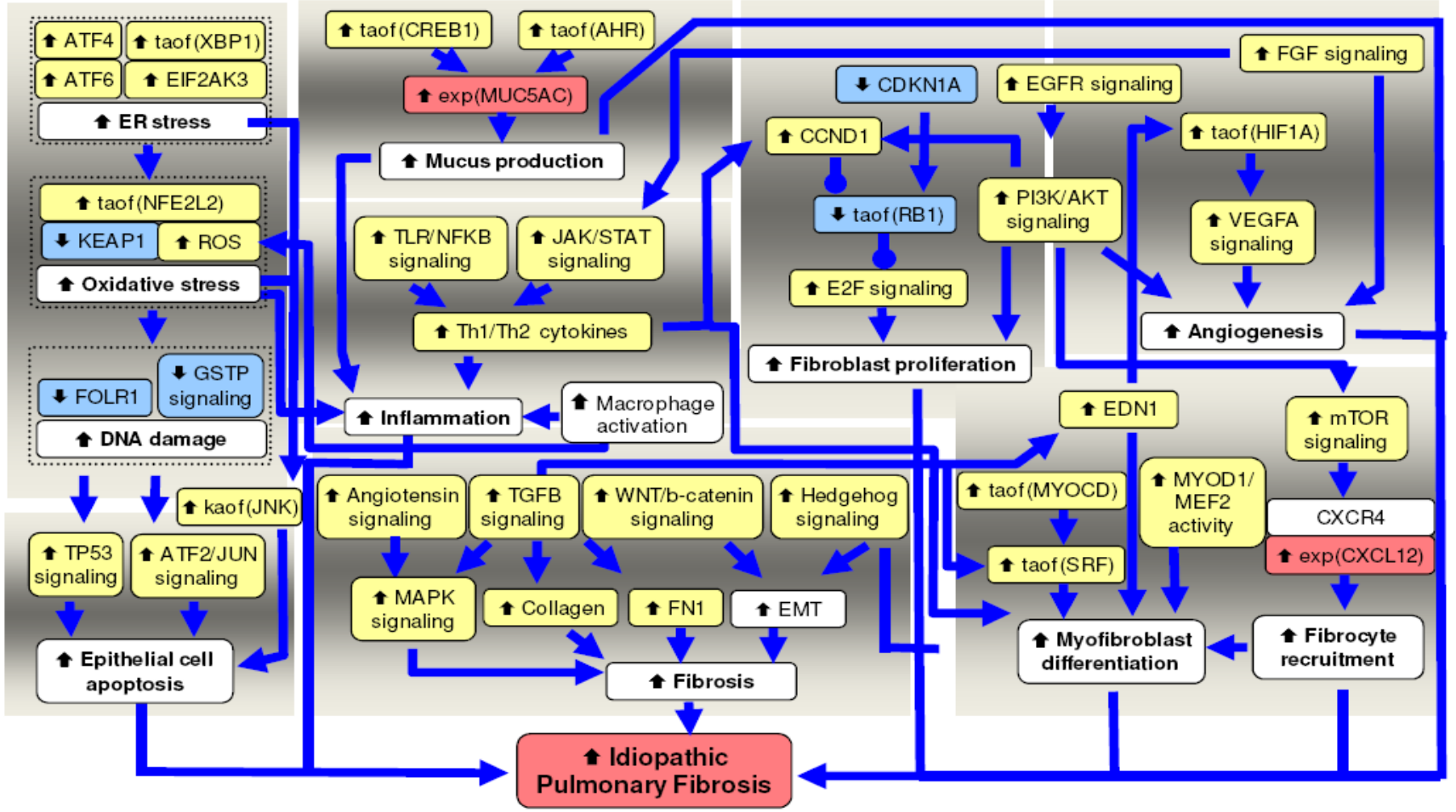
Prediction

Protection

However : acceptance of omics data to support the hazard/safety assessment is still limited. Due to a combination of complexity, rapid developments, no defined ground truth

Developing a Data driven AOP



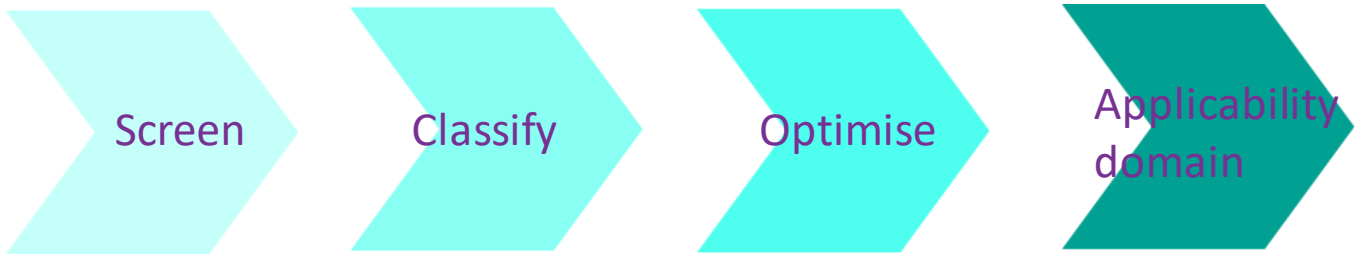


Unilever

SELVENTA™

Example Classification approaches – Gene Signature / Gene Signature Database

- Still an area of active development especially seeing new developments in use of AI
- Tends to provide clearer data on more potent compounds due to stronger signal to noise values
- Signatures for classifiers
  GARD - skin sensitisation and
  TGx-DDI - Gentox
- Concerns around transferability across different cell lines
- Requires approaches that minimises FP rate
- Platform limitations of comparative Databases
- Shown to be used for cosmetic relevant ingredients – parabens Naciff et.al 2022



Objective Application of Omics for NGRA

- Determination of Compounds primary MoA through pathway analysis
- Underpinning AOP key events and MIEs

Prediction

- Comparison of differentially expressed signatures from one treatment to a database of previously reported gene signatures or to another sample.

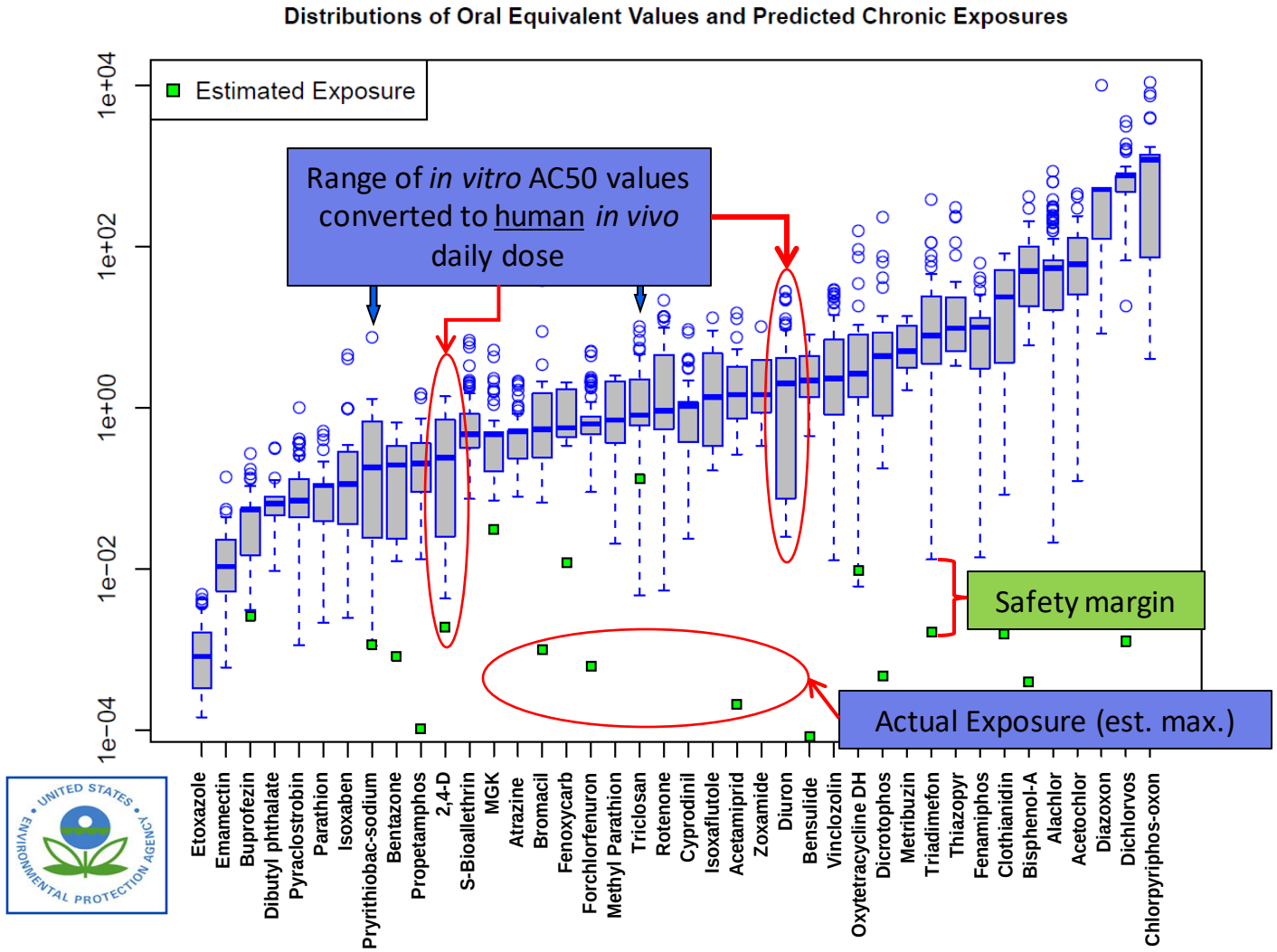
- Dose response relationship and identification of biologically relevant dose

Protection

However : acceptance of omics data to support the hazard/safety assessment is still limited. Due to a combination of complexity, rapid developments, no defined ground truth

Unilever

Paradigm shift for systemic safety - Protection not Prediction



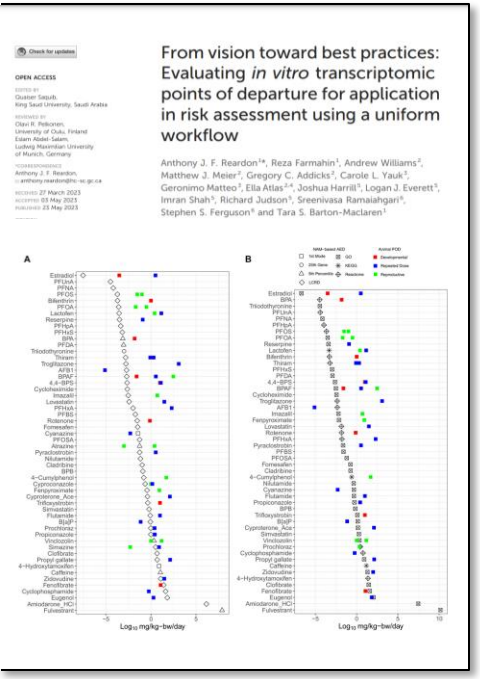
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

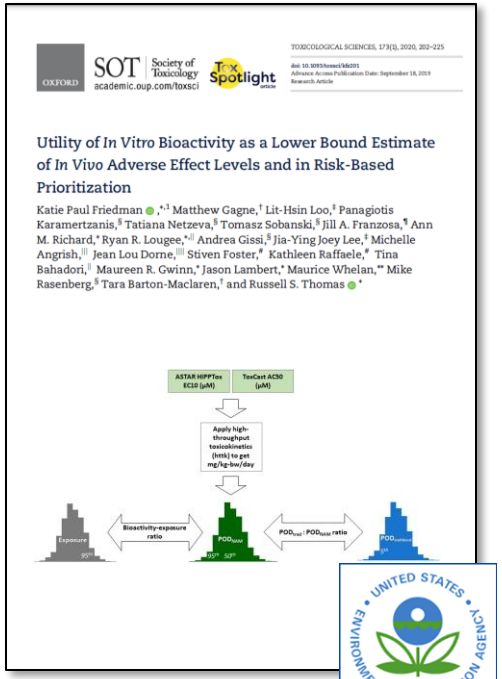
The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

Aligns to an exposure led framework where estimates of consumer product ingredients can be determined

Examples of ongoing or completed case studies for NAM/NGRA BER based risk assessment or prioritisation

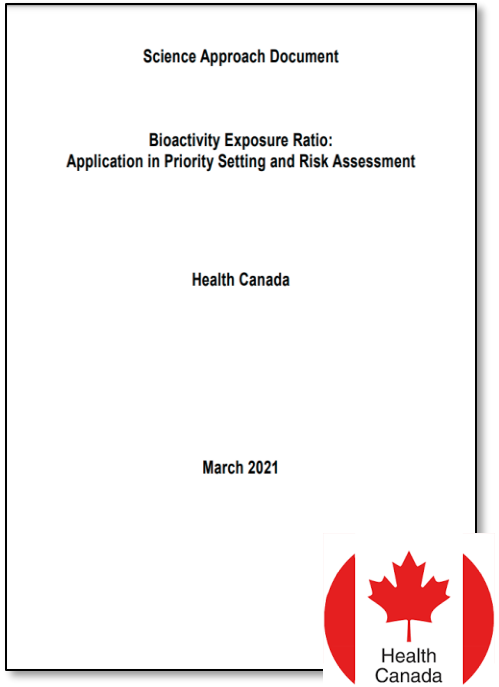

From vision toward best practices: Evaluating *in vitro* transcriptomic points of departure for application in risk assessment using a uniform workflow

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[2,4], 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]


SOT Society of Toxicology academic.oup.com/toxsci

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

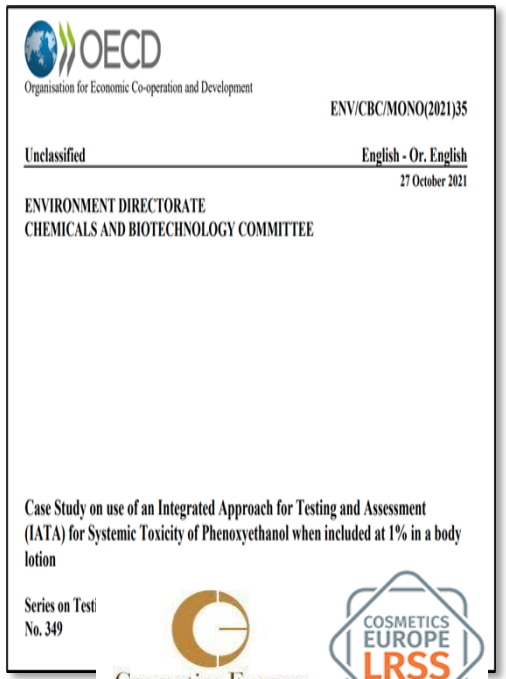
Katie Paul Friedman[*,1], Matthew Gagne[†], Lit-Hsin Loo[‡], Panagiotis Karamertzanis[§], Tatiana Netzeva[§], Tomasz Sobanski[§], Jill A. Franzosa[¶], Ann M. Richard[*], Ryan R. Lougee[*,||], Andrea Gissi[§], Jia-Ying Joey Lee[‡], Michelle Angrish[|||], Jean Lou Dorne[||||], Stiven Foster[#], Kathleen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


Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021


OECD
Organisation for Economic Co-operation and Development

ENV/CBC/MONO(2021)35

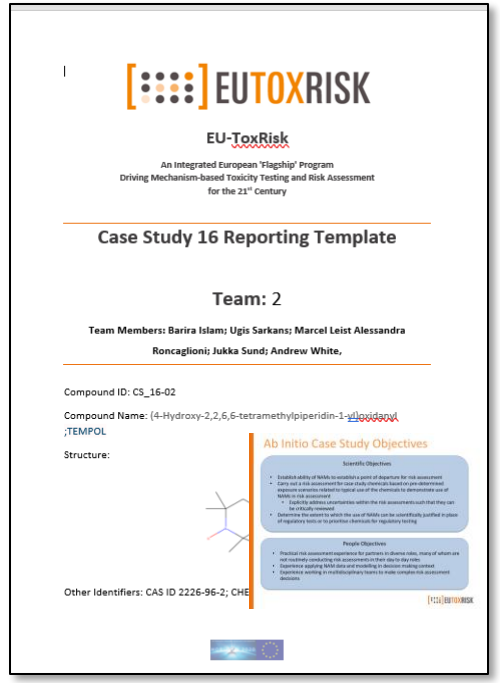
Unclassified — English - Or. English

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


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

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

Cosmetics Europe
the personal care association

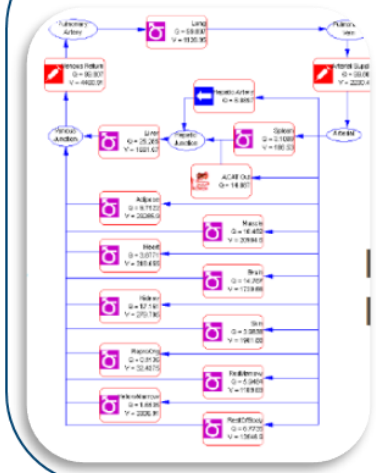
COSMETICS EUROPE LRSS

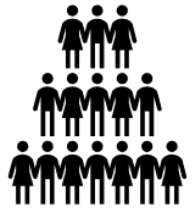
RISK HUNT3R

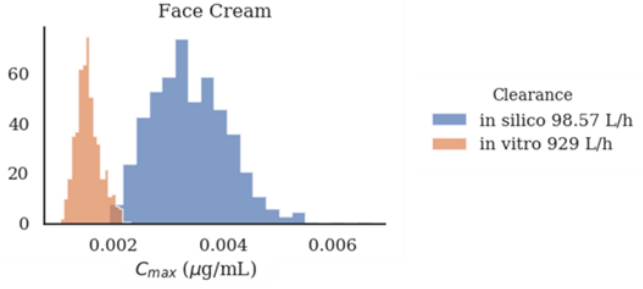
Unilever

The key NAMs in our NGRA approach

PBK Modelling







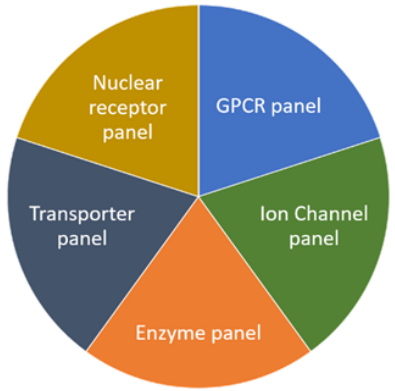
Toxicology in Vitro (2020), **63**, 104746

In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

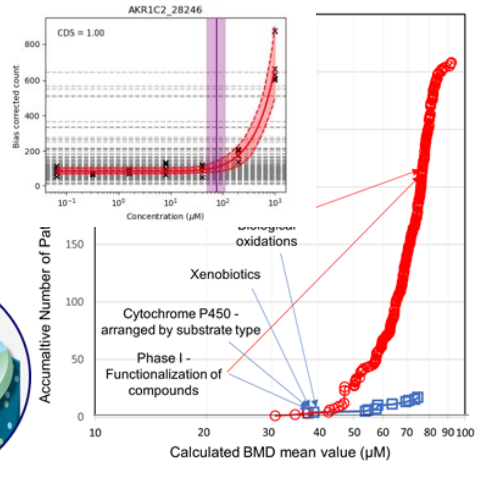
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling



eurofins | Cerep

Transcriptomics

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

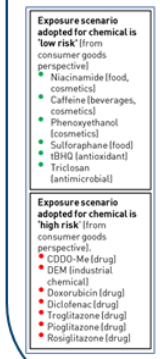


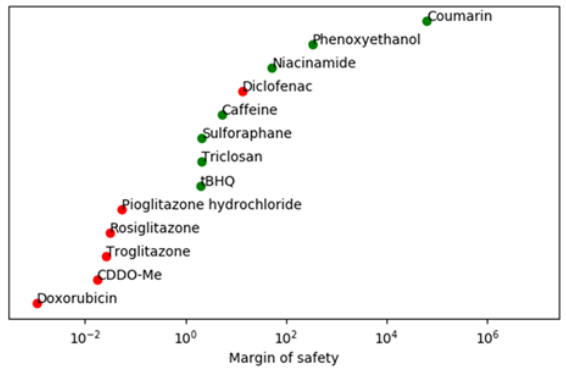
BMDexpress 2

BiFrost

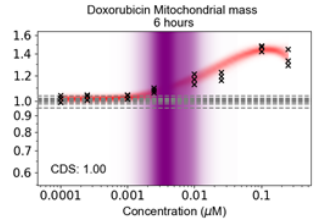
10 stress pathways, 36 Biomarkers 8 concentrations

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





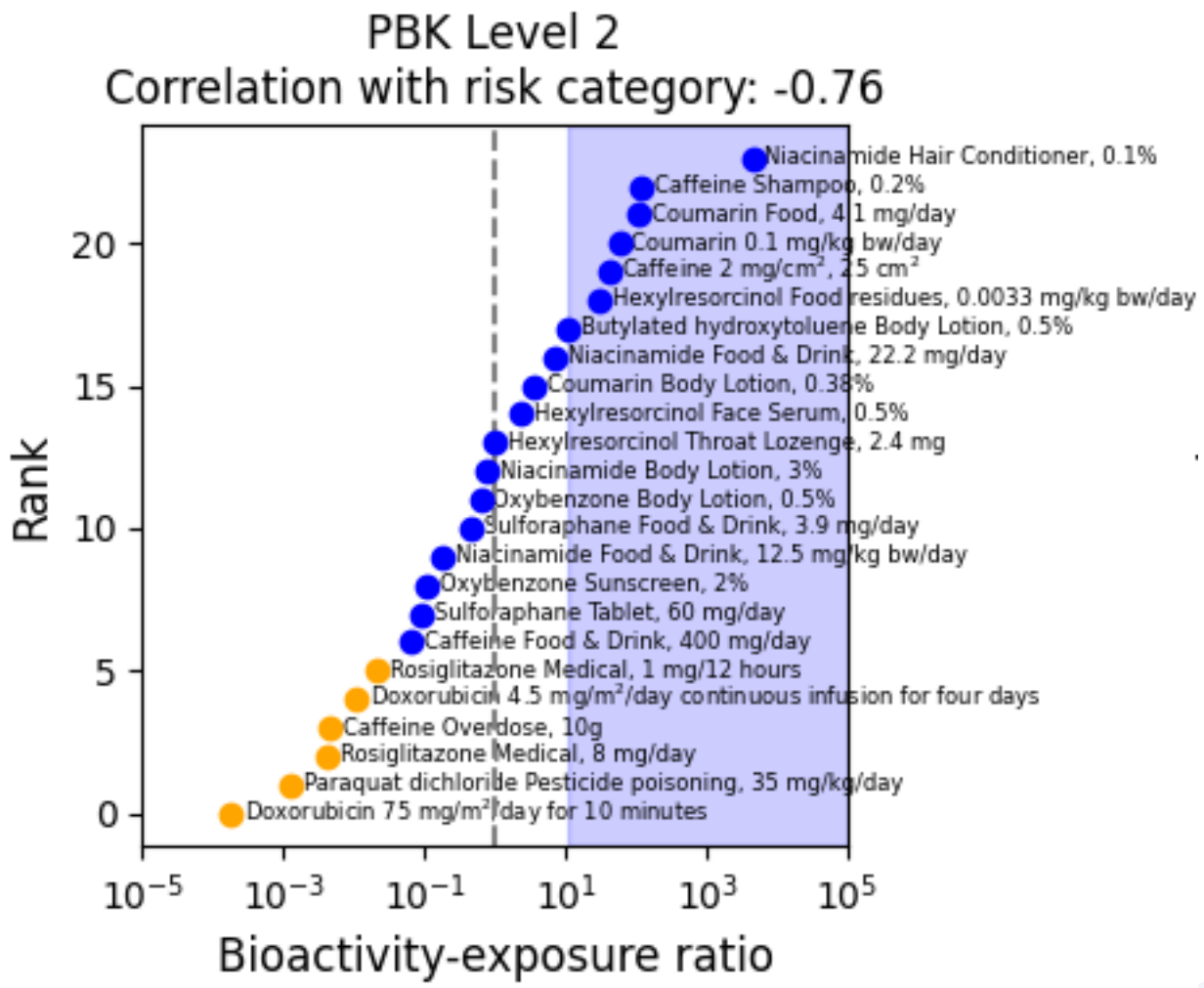
cyprotex AN EVOTEC COMPANY



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

Unilever

Visualising how the toolbox performs against the pilot study data



Blue: low risk chemical-exposure scenario

Yellow: high risk chemical-exposure scenario

Exposure scenarios within the **blue shaded region** are identified as **low risk.**

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

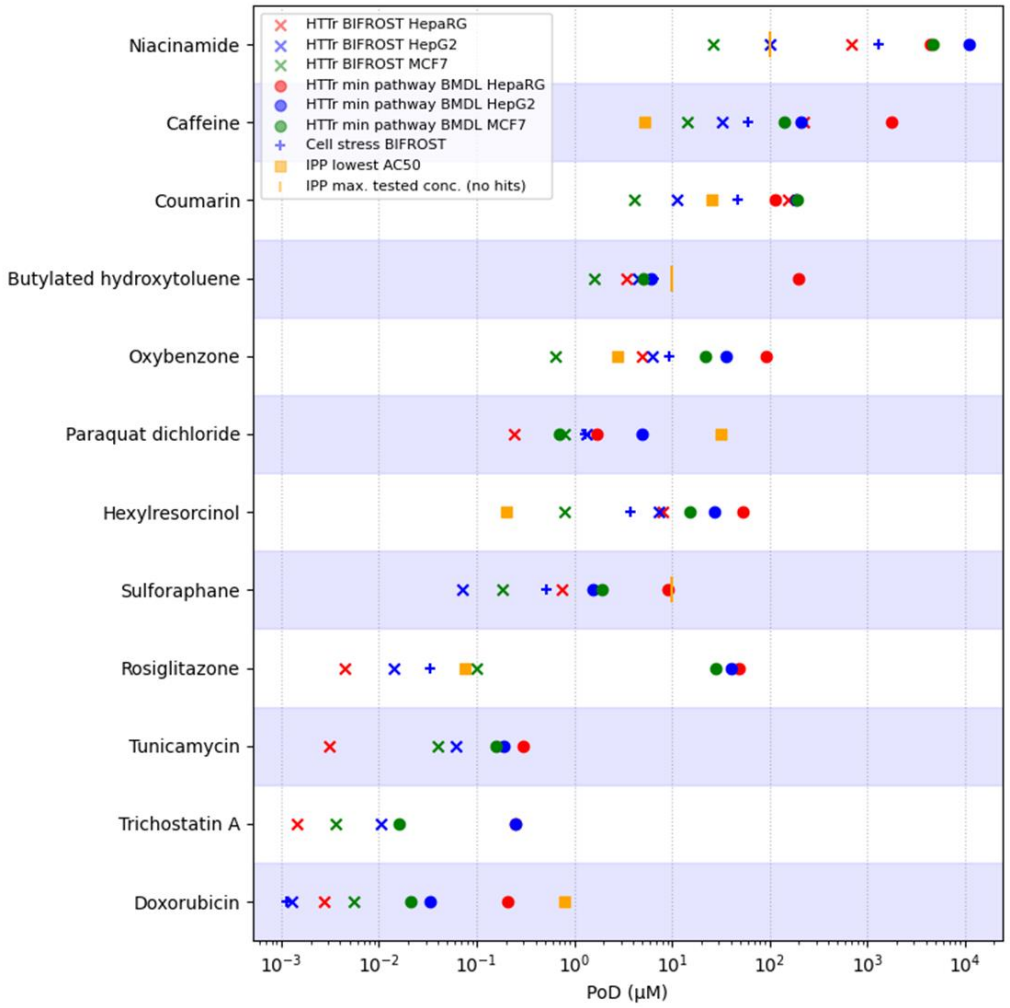
TOXICOLOGICAL SCIENCES, 189(1), 2022, 124–147
https://doi.org/10.1093/toxsci/kfac068
Dryad Digital Repository DOI: https://doi:10.5061/dryad.fbg79c
Advance Access Publication Date: 13 July 2022
Research article

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*

Unilever

Transcriptomic POD tend to be one of most sensitive especially at a gene level.

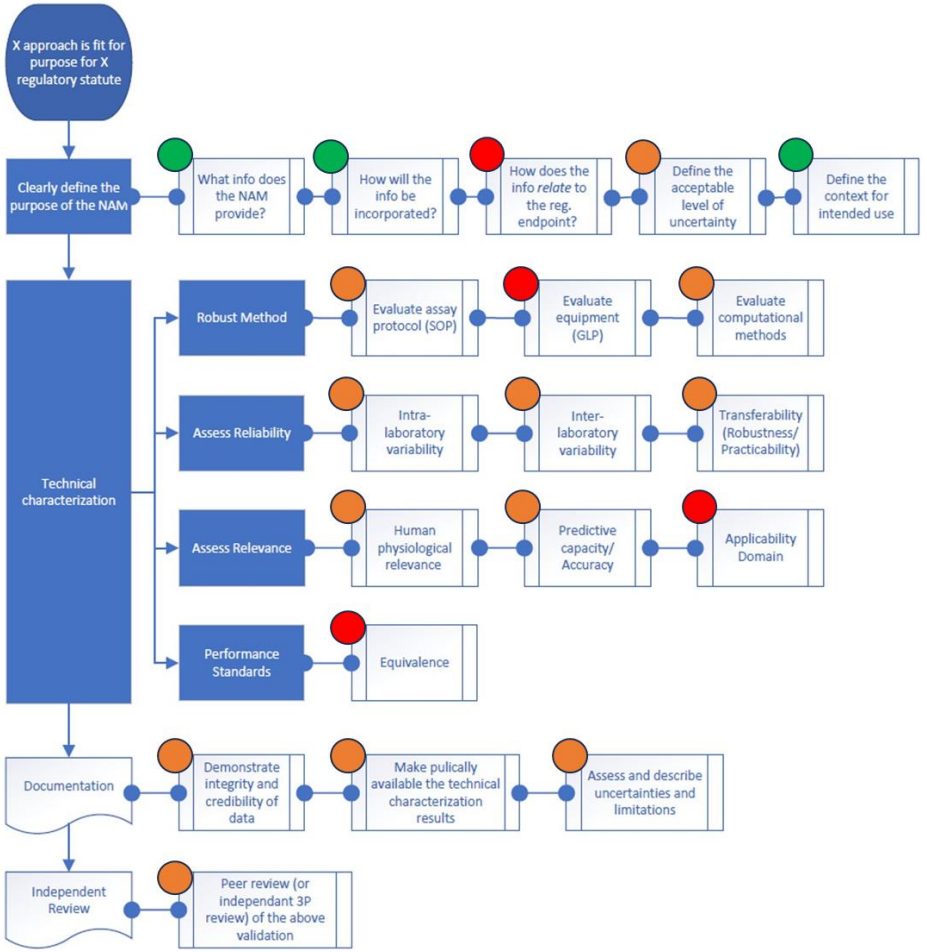


- For 60% of compounds tested to date HTTr provides the most conservative (lowest POD)
- As previously observed shifting from a gene level to a pathway based increases the predicted concentration of the POD

HTTr: High-throughput transcriptomics
IPP: In vitro pharmacological profiling
CSP: Cell Stress Panel

Unilever

How do we build scientific confidence in a systemic safety toolbox?



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]

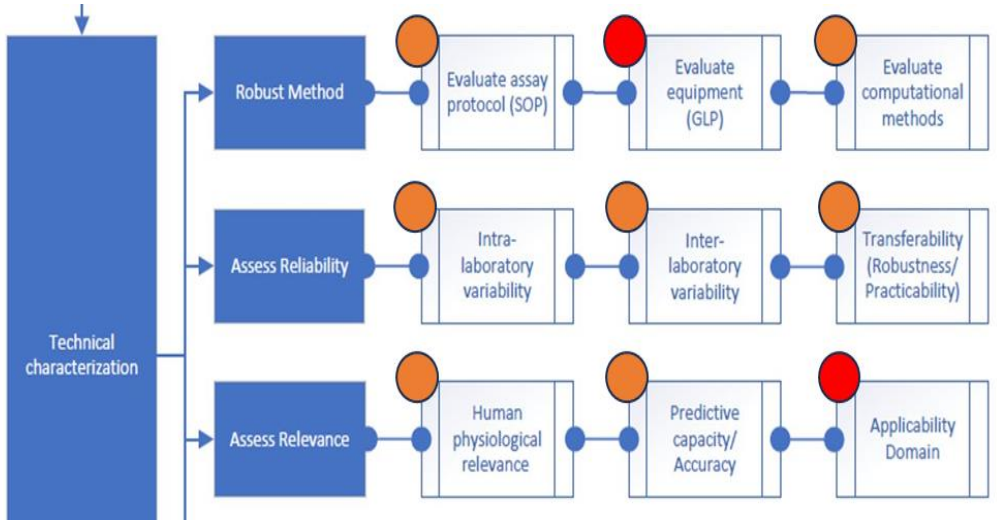
Received: 17 May 2022 / Accepted: 11 August 2022 / Published online: 20 August 2022

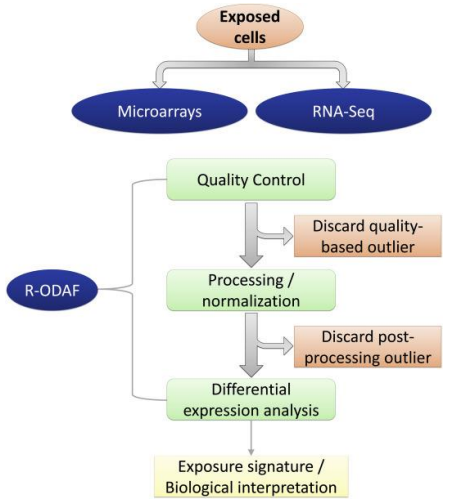
1. Determine whether the toolbox is fit for purpose.
2. Take into account human safety in assessing the approach (where possible)
3. Identify what an appropriate safety decision might be (e.g., BER threshold).

Unilever

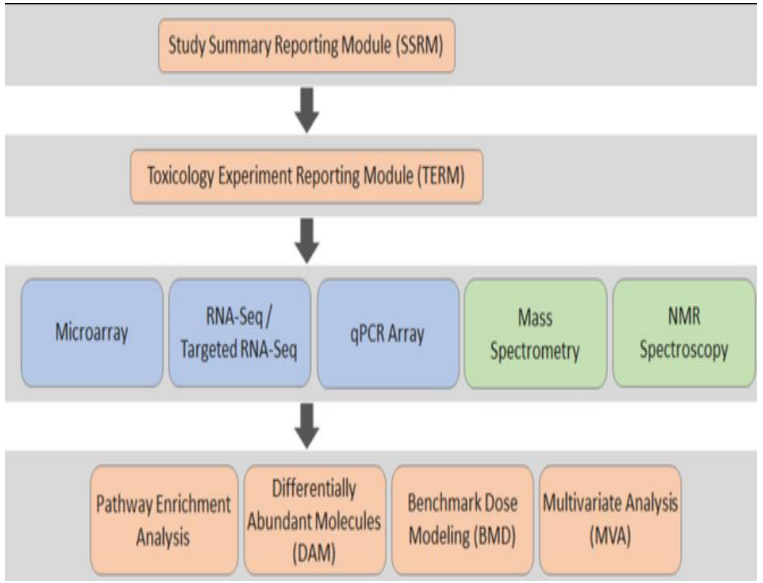
Building Scientific confidence in application of HTTr

Ability to generate reliable and consistent reproduction of results is the prerequisite for successful application of TGx results in the regulatory setting





R-ODAF –Verheijen et al 2022

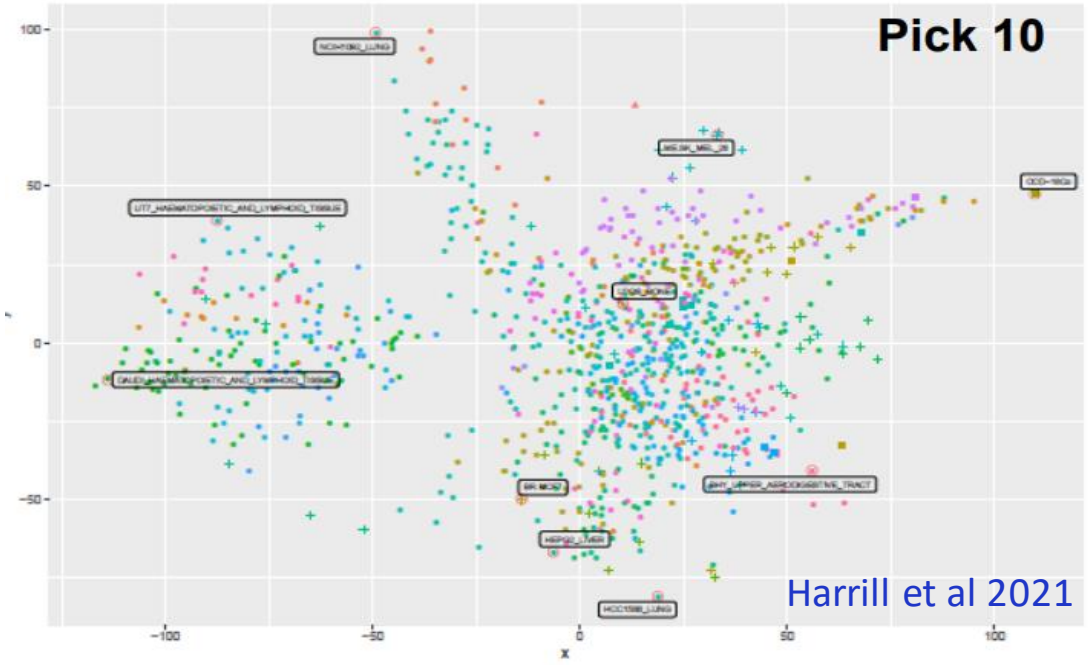


OECD -ORF –Harrill et al 2021

- **Providing supporting evidence to accelerate confidence and acceptance for decision making.**

Cellular Models incl. Treatment → **Data Acquisition** → **Data Modelling** → **Interpretation**

Cell line Applicability



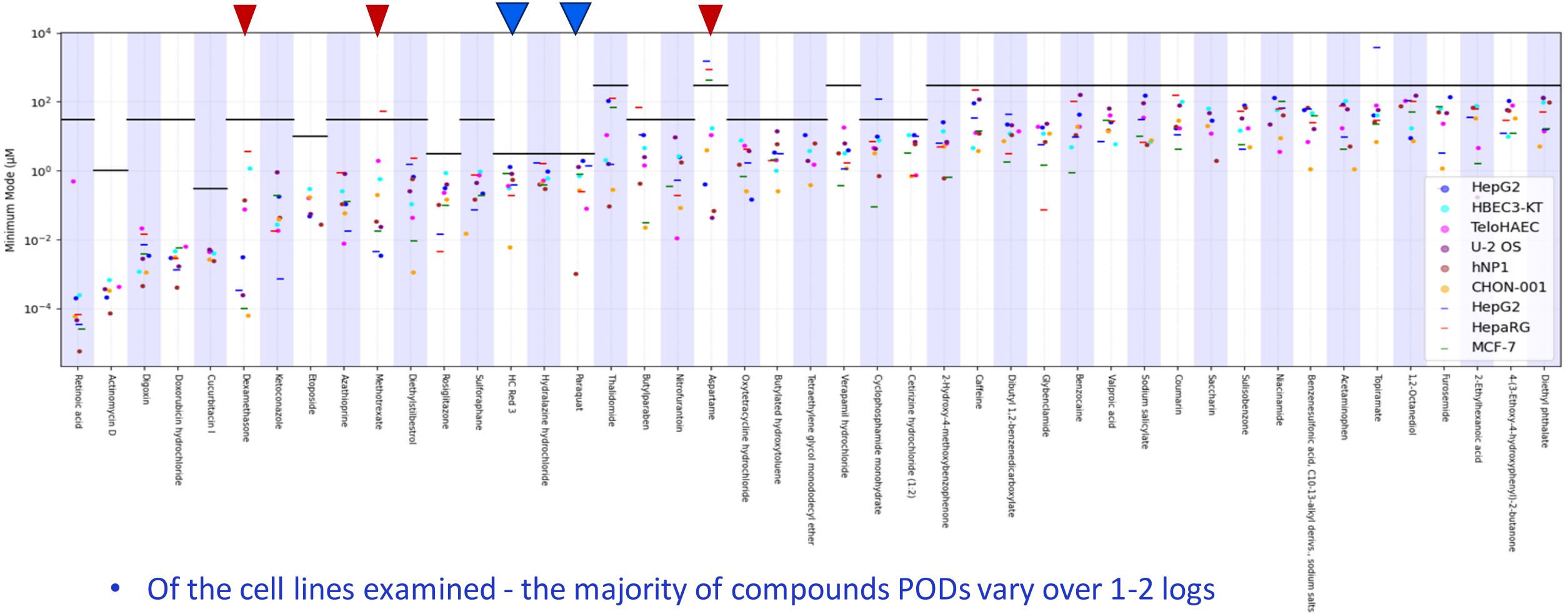
Cellular Models incl. Treatment → Data Acquisition → Data Modelling → Inter-pretation

Cell_Type	Tissue_Origin	Cell_Type	Tissue_Origin
U-2 OS	Bone	U-2 OS	Bone
MCF-7	Breast	MCF-7	Breast
HepaRG_2D	Liver	HepaRG_2D	Liver
HBEC3-KT	*Lung*	*CHON-001*	*Fibroblast*
CHON-001	*Fibroblast*	*HBEC3-KT*	*Lung*
TeloHAEC	*Vascular*	*TeloHAEC*	*Vascular*
RPTEC	*Kidney*	*RPTEC*	*Kidney*
ARPE-19	*Retina*	*ARPE-19*	*Retina*
HPNE	*Pancreas*	*HPNE*	*Pancreas*
Ker-CT	*Skin*	*CCD-18Co*	*Fibroblast*
CCD-18Co	*Fibroblast*	*Ker-CT*	*Skin*
ASC52telo	*Mesenchymal Stem Cell*	*ASC52telo*	*Mesenchymal Stem Cell*
BJ-5ta	*Fibroblast*	*BJ-5ta*	*Fibroblast*
HME-1	*Breast*	*RPE-1*	*Retina*
RPE-1	*Retina*	*HME-1*	*Breast*
TIME	*Vascular*	*TIME*	*Vascular*
HUVEC	*Vascular*	*HUVEC*	*Vascular*
HSAEC-1	*Lung*	*HSAEC-1*	*Lung*

- Breadth of coverage of biological pathways - No single cell line captures all biological variation
  - Complexity of surrogate test system compared to integrated systems
  - Acute vs chronic responses / Sensitivity
- Initial cell line use focused on historical use patterns – ie Data availability for gene signature comparisons/ Use in other ongoing assays / Metabolic competency
- Develop Data driven approach to extend and maximise the biological space covered

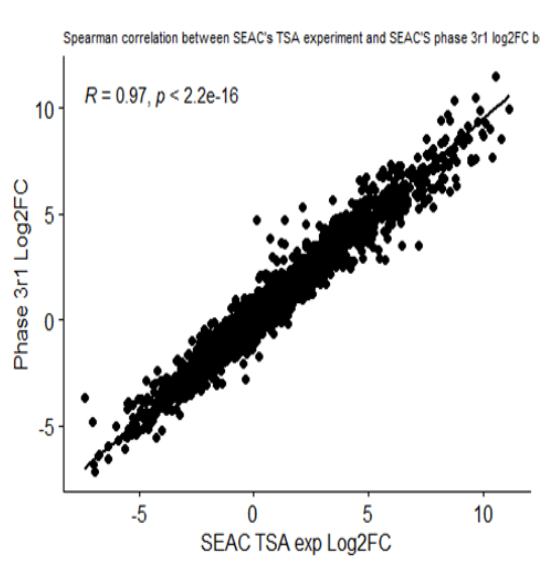
Unilever

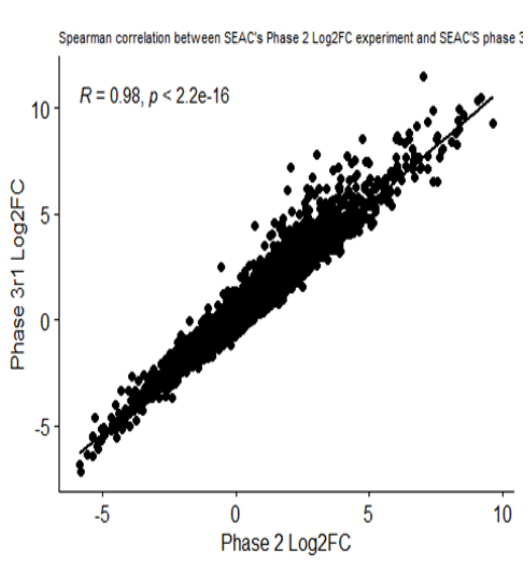
Cell line DR variability following compound treatment

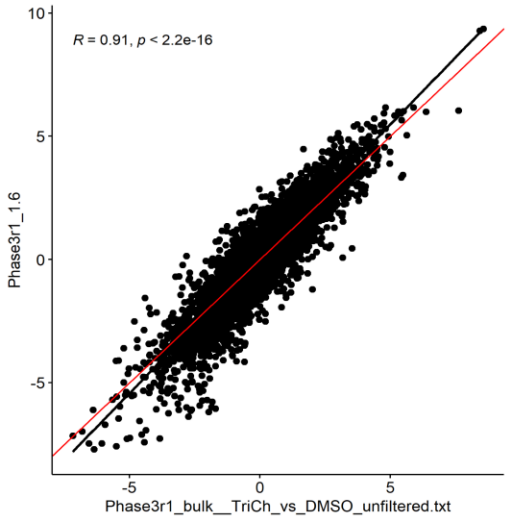


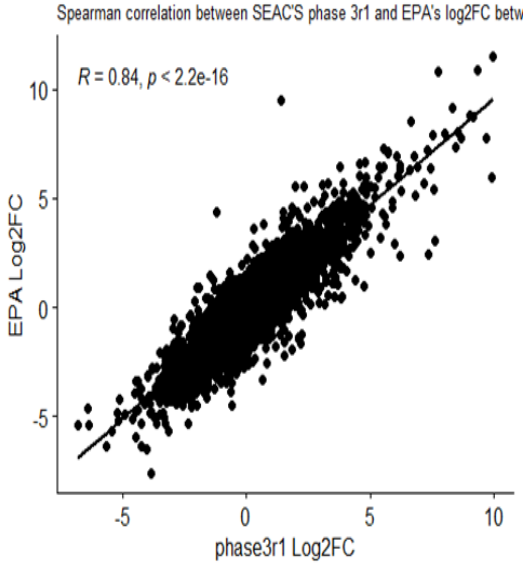
- Of the cell lines examined - the majority of compounds PODs vary over 1-2 logs
- Some cases where this variability extends over a greater range >4 logs ▼
- Few cases where single cell line is significantly more sensitive that the others ▼

- Bulk lysate samples – understand inter run variability of sequencing process
- Fresh positive control samples understand whole process variability including sample generation.
- Ensure assay variability remains within defined limits



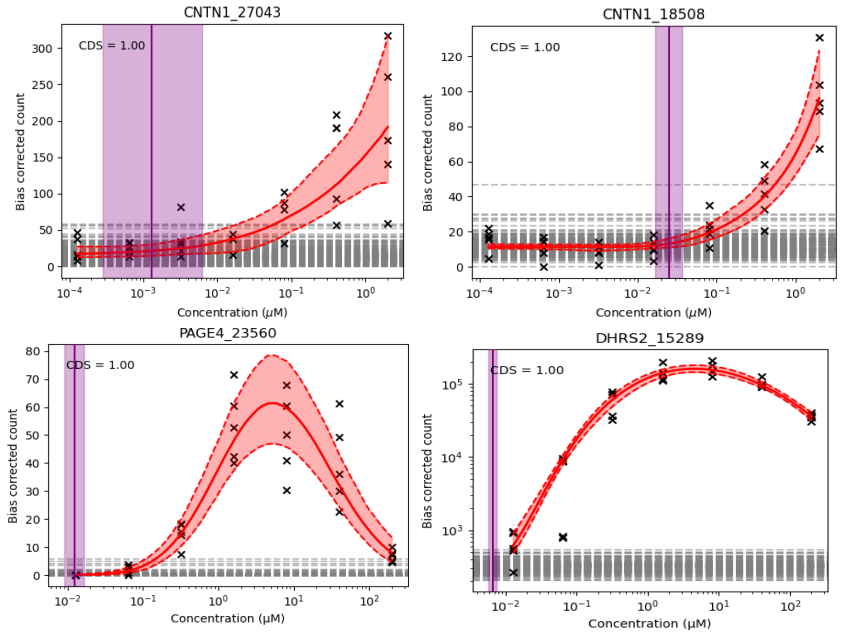






Unilever

Cellular Models incl. Treatment → Data Acquisition → **Data Modelling** → Inter-pretation



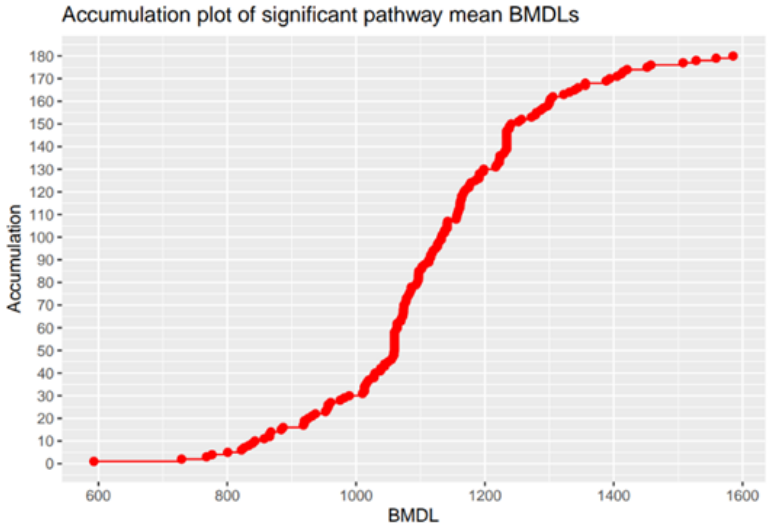
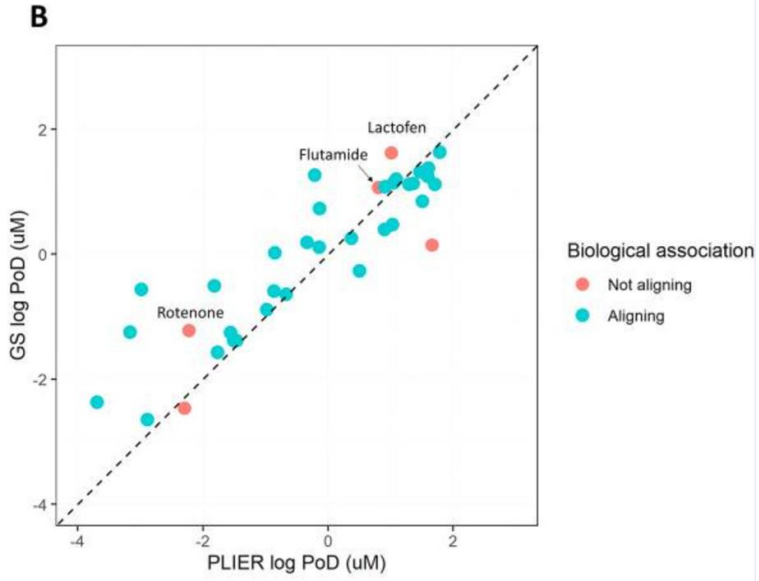


Figure 6: Significant pathway BMDL accumation plot



A Bayesian approach for inferring global points of departure from transcriptomics data

Joe Reynolds, Sophie Malcomber, Andrew White

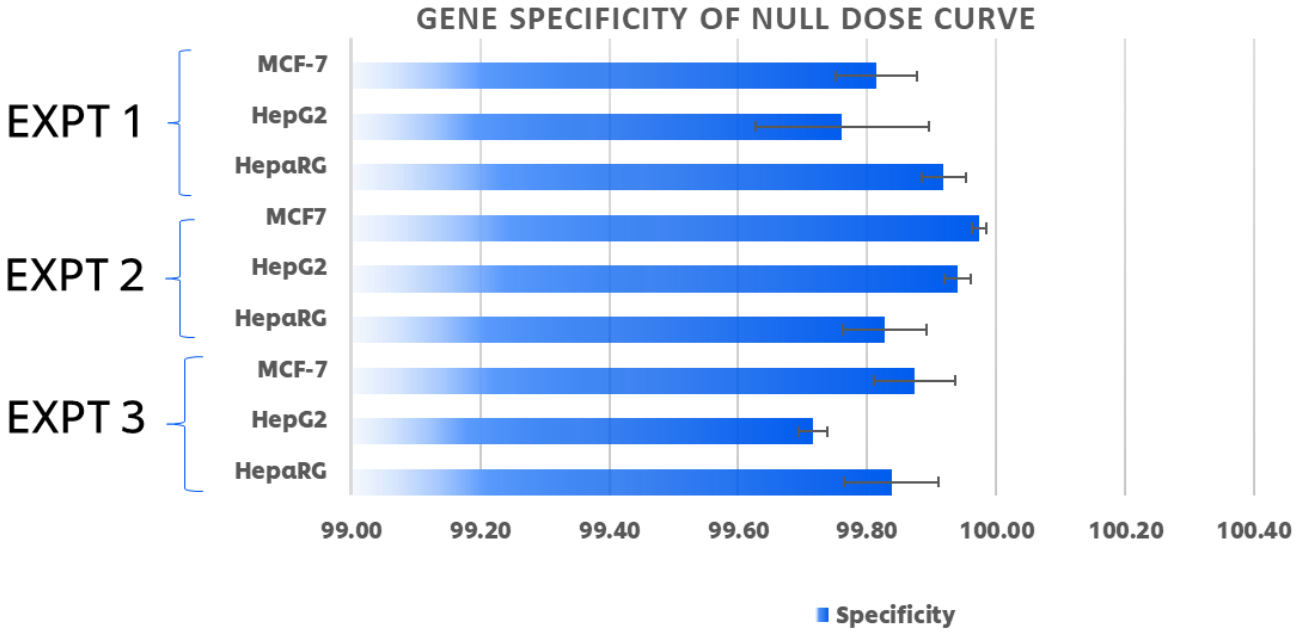
Philips et al 2019 BMDExpress 2: enhanced transcriptomic dose-response analysis workflow

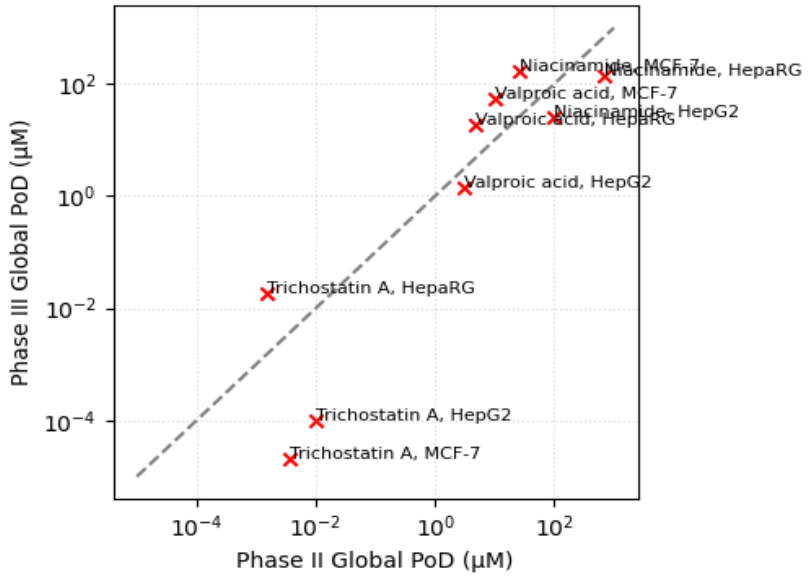
Bioinformatics 35: 1780-1782

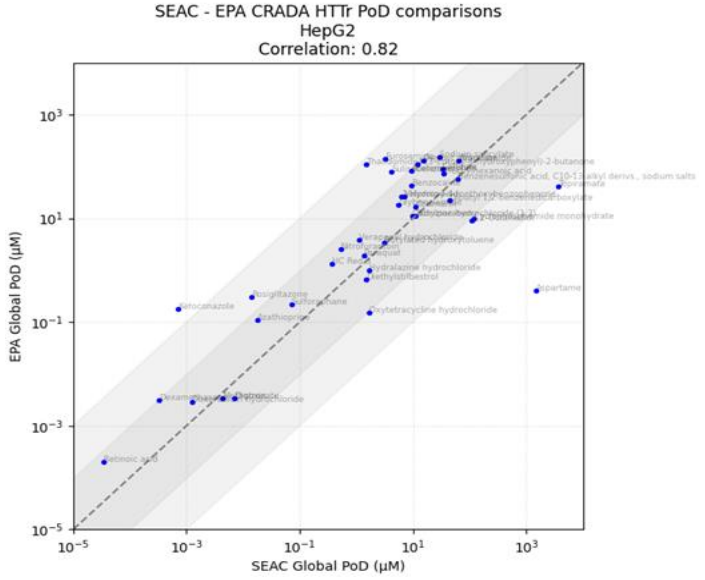
Basili et al 2022 Latent Variables Capture Pathway-Level Points of Departure in High-Throughput Toxicogenomic Data

Chem Res Toxicol. 35: 670–683

Unilever

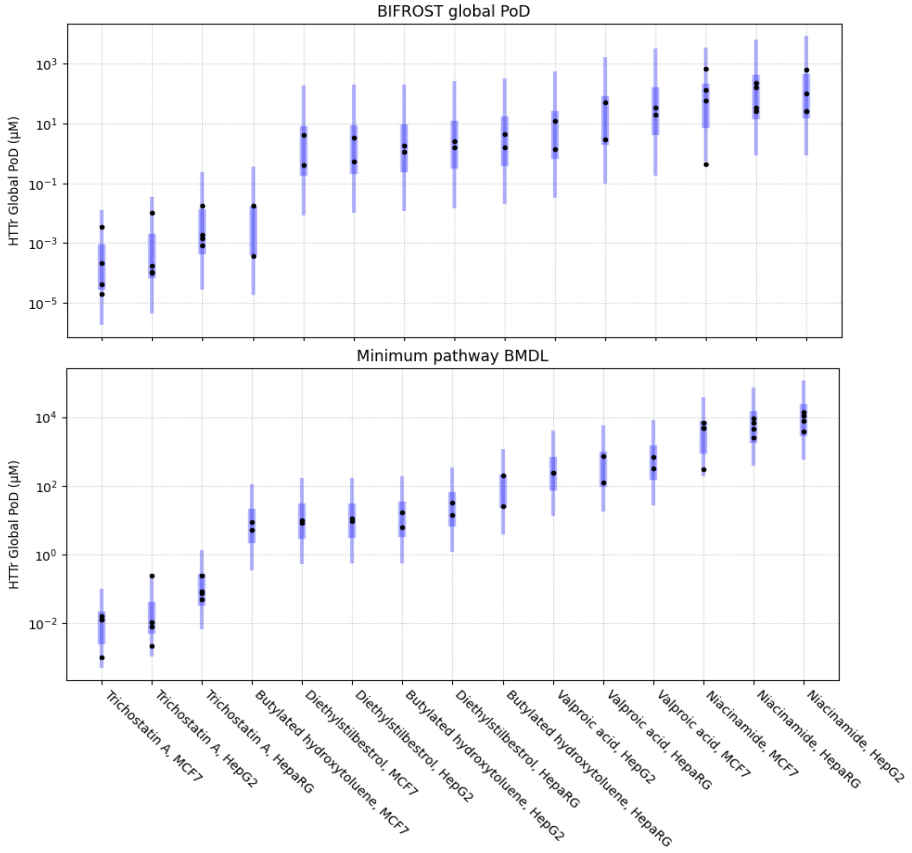




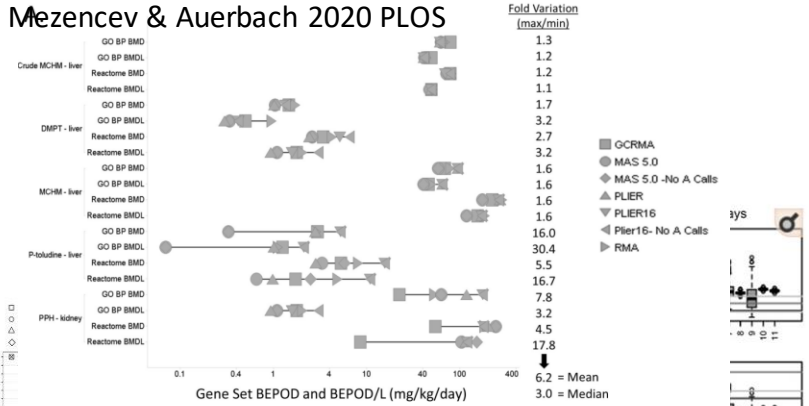


- Application of negative control data to provide a ground truth and estimate false positive predictivity
  Build synthetic data sets of known dose response profiles to estimate positive predictivity
  Reproducibility of derived PODs from replicate experiments (7 out of 9) within 1 log order magnitude

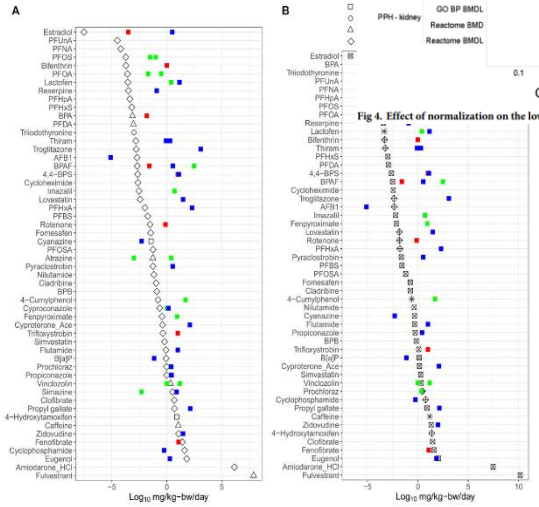
Cellular Models incl. Treatment → Data Acquisition → Data Modelling → Inter-pretation



Mezencev & Auerbach 2020 PLOS



Farmahin et al. 2017 Arch. Tox



Reardon et al 2023 Front. Toxicol

Large area of development and ongoing research to define approaches for POD estimation

Use of benchmarking to assess utility

Summary

- Exposure-led approach to determine protection through a BER (MoS) – range of different case studies now showing utility of approach
- Focus on weight of evidence to show tools can be integrated to make a safety decision - requires diverse expertise
- Strength derived from integrating a combination of targeted and broad unbiased tools – not a one to one replacement
- Utilise NAMs for further targeted follow where required to refine uncertainty e.g. metabolism
- NAMs not standardised - need to ensure robustness/quality of tools and include estimations of uncertainty to aid acceptance
- Further activity required to build evaluation data sets and ground truth to evaluate current approaches and those in the future
- Collaboration required to progress assessment and build out confidence for broader stakeholder community on applicability domains/ remaining gaps

Acknowledgements

Unilever: Maria Baltazar, Sophie Cable , Alistair Middleton, Joe Reynolds, Georgia Reynolds, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Chris Sparham, Katarzyna Przybylak, Predrag Kukic, Georgia Reynolds, Tom Moxon, Hequn Li, Dawei Tang, Jayasujatha Vethamanickam, Matthew Dent, Paul Carmichael, Sarah Hatherell, Richard Cubberley, Carl Westmoreland

US-EPA: Richard Judson, Josh Harrell, Logan Everett, Imran Shah

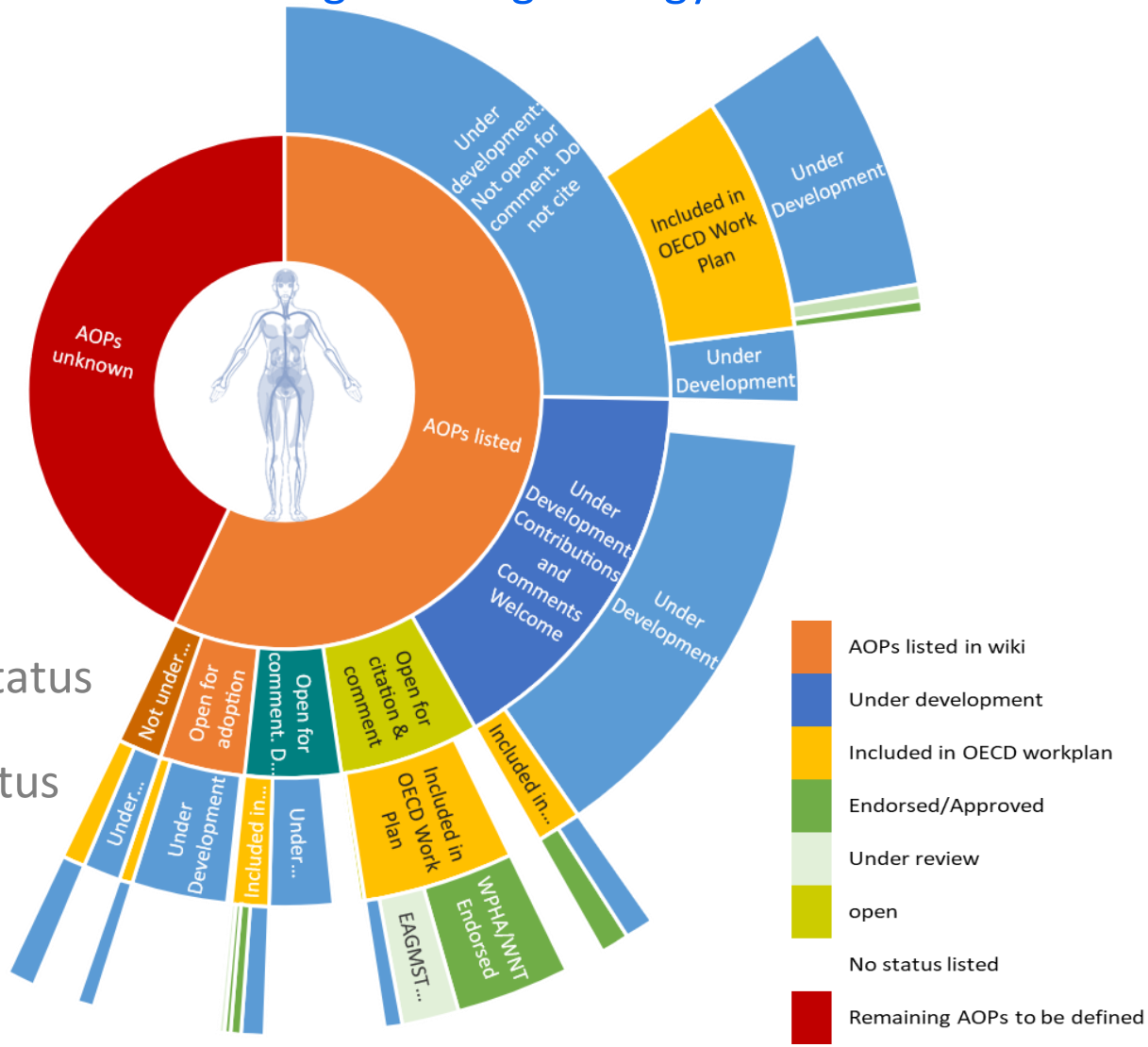
Thank You

seac.unilever.com

Unilever

Current status of AOP

At present, a decision framework based only on AOPs is not feasible. However, AOPs can used as a knowledge base for enhancing a testing strategy



Only a limited number of AOPs, many of which have not yet been verified (biological coverage).

There are 446 AOPs on AOP-Wiki. Assuming 5 KEs per AOP, that's over 2000 assays.

- Toxcast has ~ 700 assays