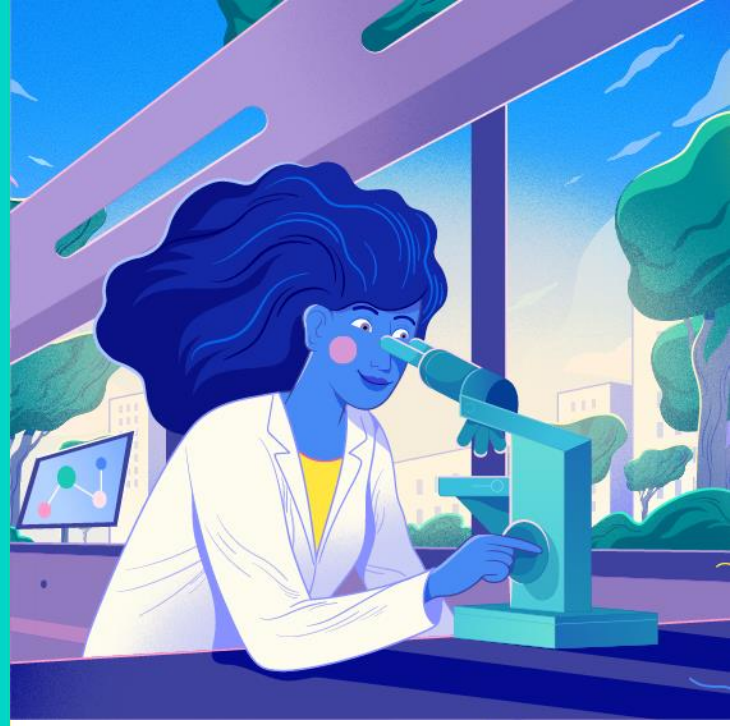
Development and Application in the use of NAMs for Next Generation Risk Assessment

Learnings from Industry case studies

Andrew White
Safety & Environmental Assurance Centre (SEAC)



Unilever

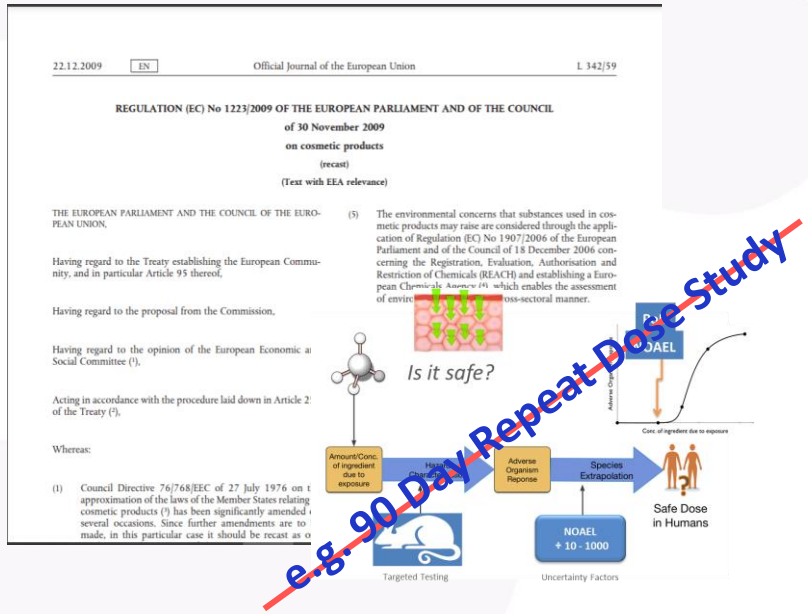
Outline

- What is NGRA?
- Examples of how it could be applied?
- How Protective is this?

The need for non-animal approaches

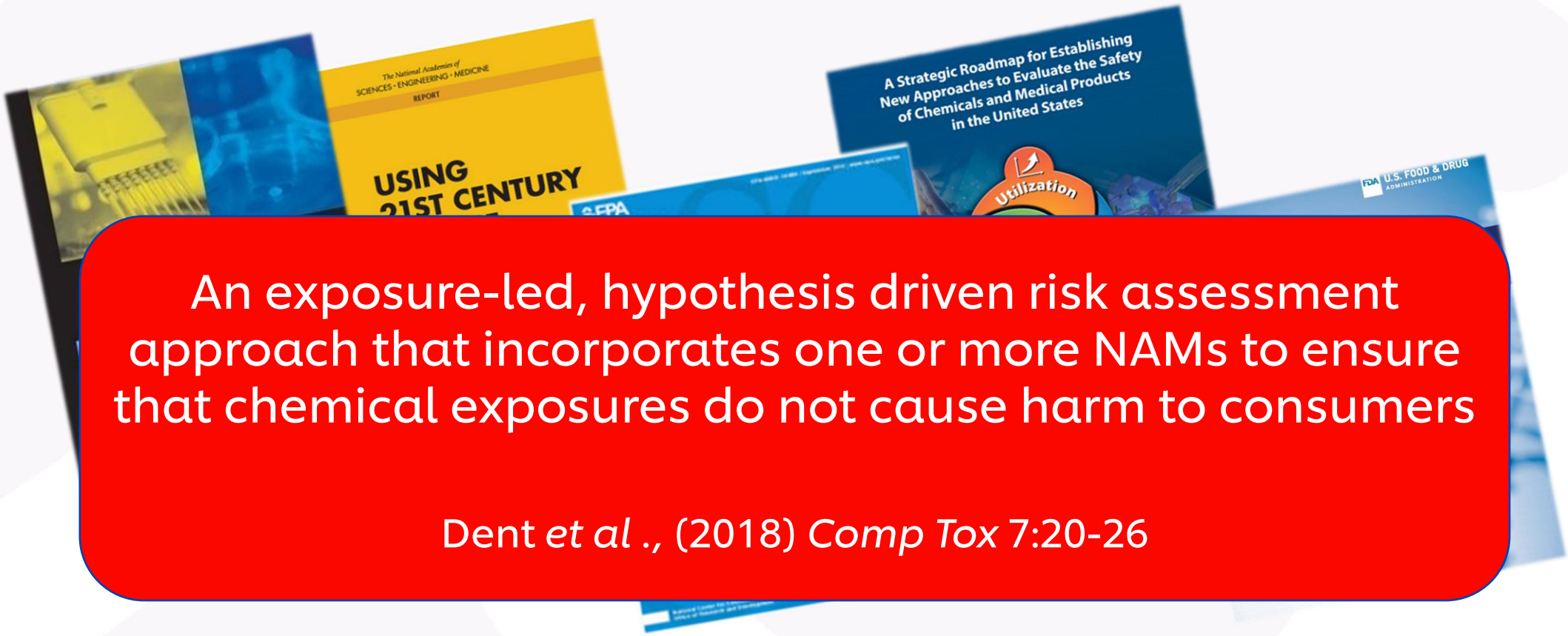
Societal Attitudes/Consumer Preference

Scientific Relevance



Regulatory Change

What is NGRA?

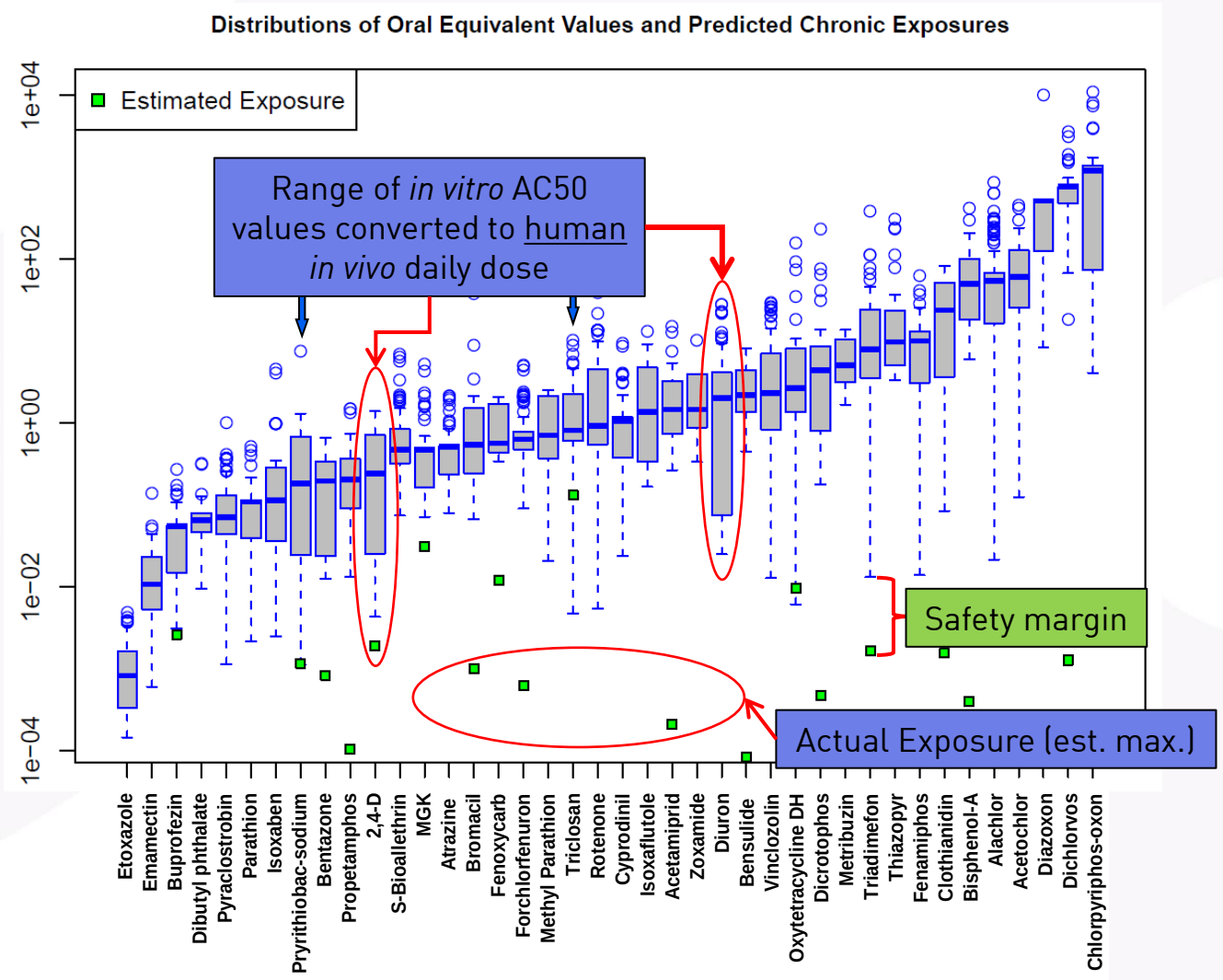


An exposure-led, hypothesis driven risk assessment approach that incorporates one or more NAMs to ensure that chemical exposures do not cause harm to consumers

Dent *et al* ., (2018) *Comp Tox* 7:20-26

Unilever

Paradigm shift for systemic safety - Protection not Prediction



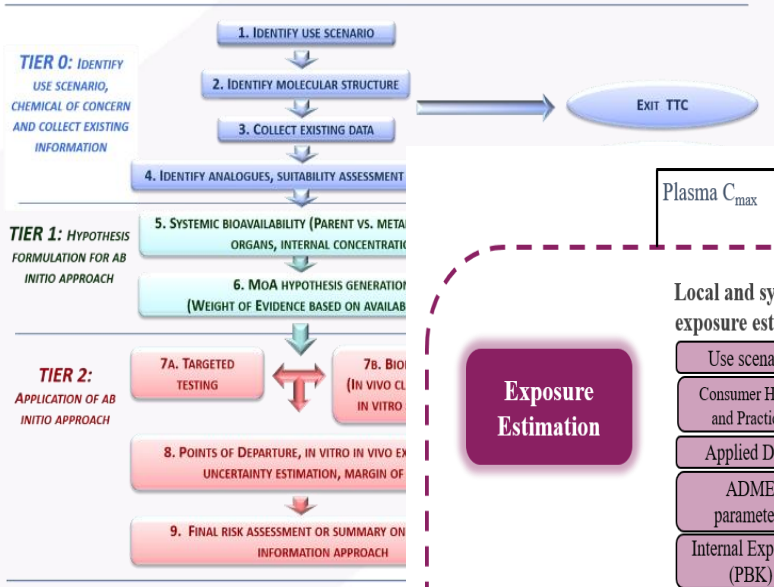
Slide from Dr Rusty Thomas, EPA, with thanks

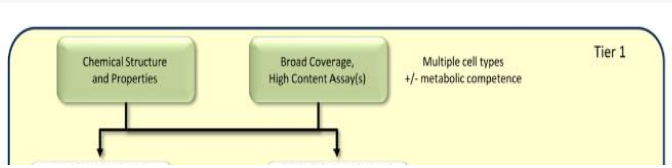
Rotroff, *et al.* Tox.Sci 2010

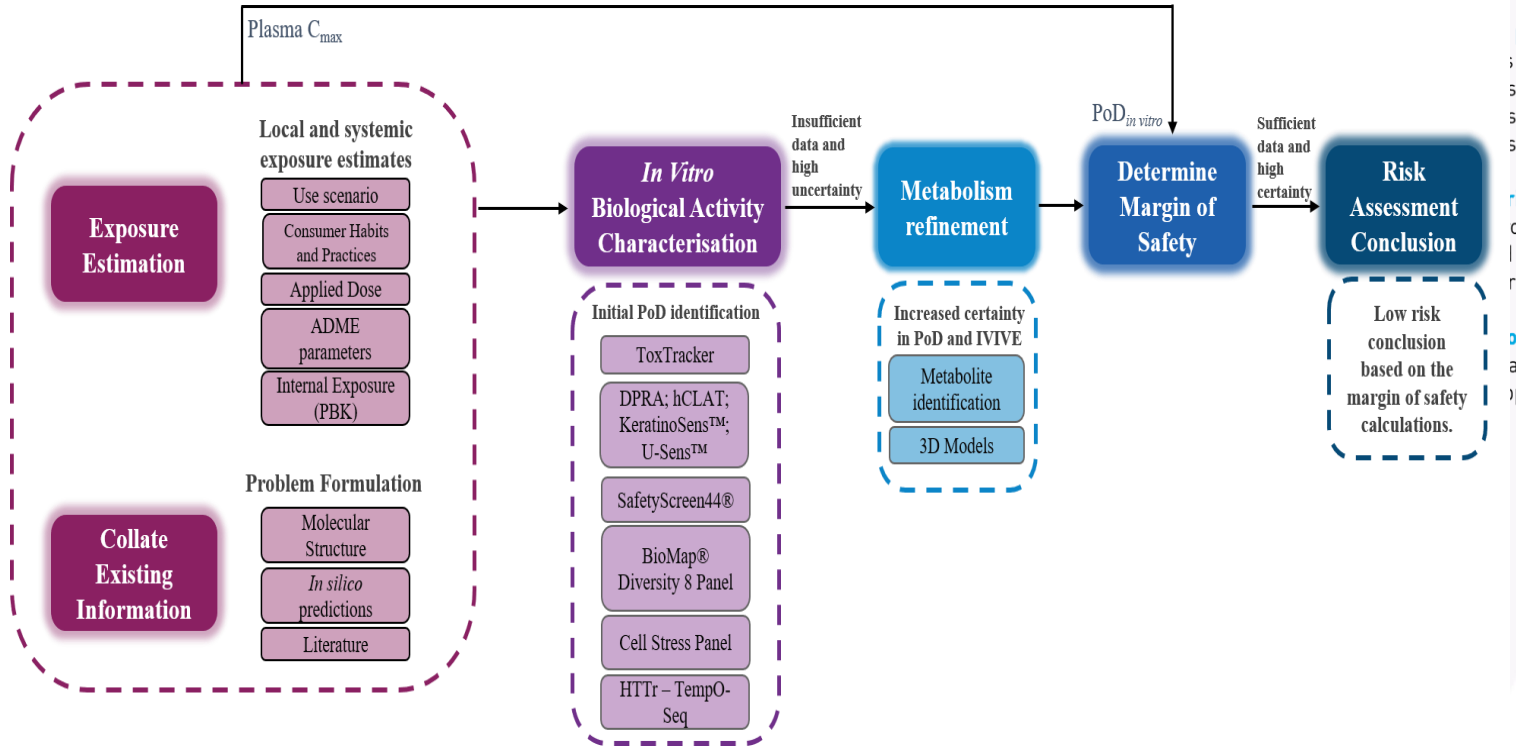
The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

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

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







ICCR 9 principles of NGRA

ICCR NINE PRINCIPLES OF NGRA

principles:
- a human safety risk assessment
- exposure led
- hypothesis driven
- designed to prevent harm

ribe how a NGRA should be conducted:
- opriate appraisal of existing information
- iterative approach
- relevant methods and strategies

ocumenting NGRA:
- ainty should be characterized and documented
- pproach should be transparent and documented

Dent *et al.* 2018 *Computational Toxicology*, 7, 20-26.

Case Study approach – Human Health Safety Assessment required for …

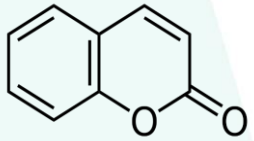
Problem Formulation

0.1% COUMARIN IN FACE CREAM

Can we safely use **x**% of ingredient **y** in product **z**?

Assumed that:

- **Coumarin was 100% pure**
- **no *in vivo* data was available such as animal data, History of Safe Use (HoSU) info. or Clinical data**
- **no use of animal data in Read Across**
- ***In silico* alerts known to be based on animal or *in vivo* data or on the structure of Coumarin itself were excluded**



Exposure Led

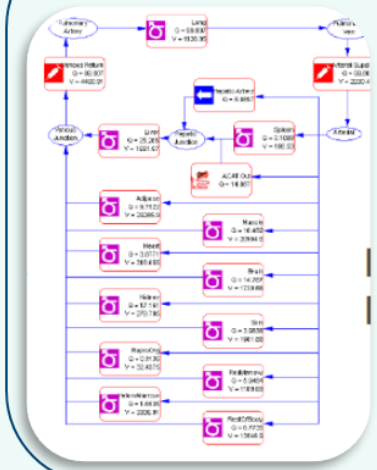
All safety assessments of cosmetic ingredients are exposure-driven:

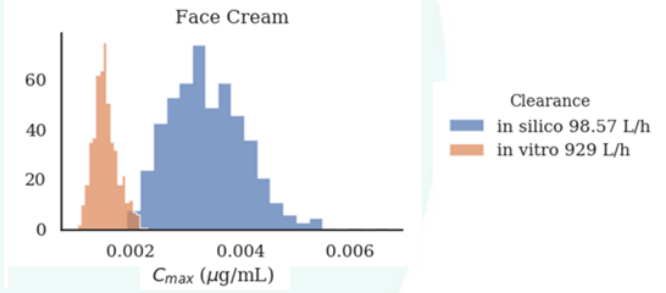
Baltazar *et al.*, (2020) *Tox Sci* (vol 176: 236–252) https://doi.org/10.1093/toxsci/kfaa048

Unilever

Some key elements in the NGRA toolbox

PBK Modelling





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

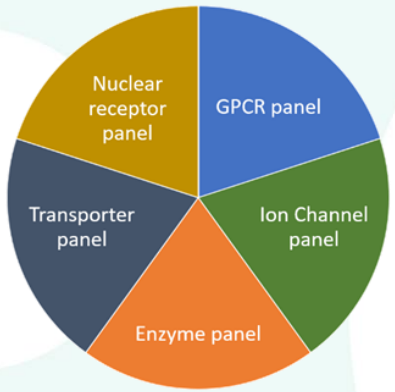
In vitro pharmacological profiling


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

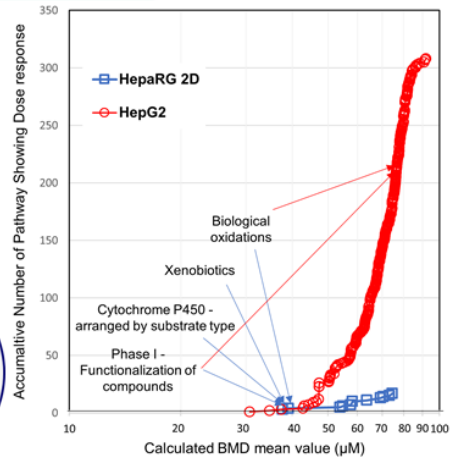


eurofins | Cerep

Transcriptomics

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

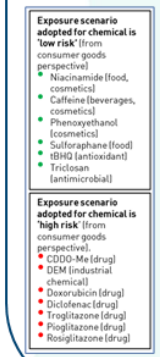
BMDExpress 2

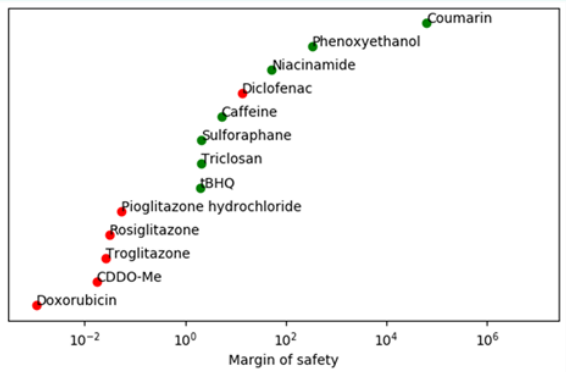


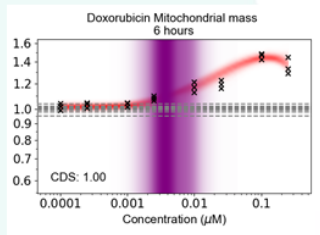
Cellular Stress Pathways

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

cyprotex AN EVOTEC COMPANY







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

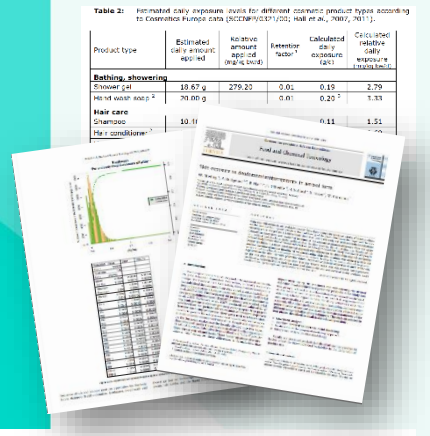
NGRA for 0.1% coumarin in face cream: exposure estimation

Exposure Estimation

Local and systemic exposure estimates

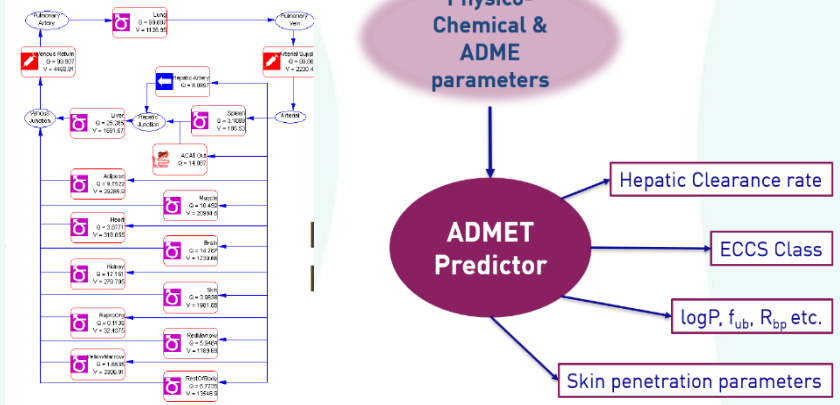
- Use scenario
- Consumer Habits and Practices
- Applied Dose
- ADME parameters
- Internal Exposure (PBK)

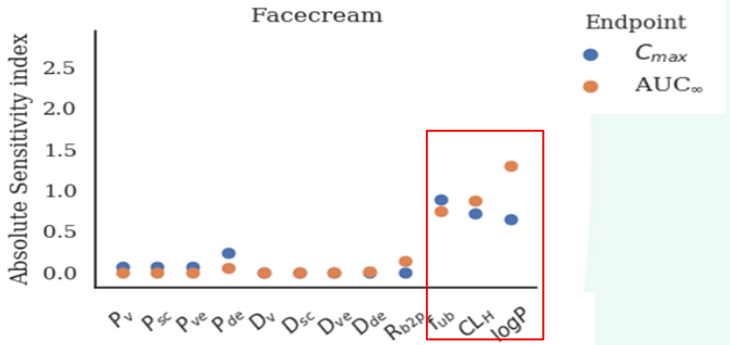
Creme GLOBAL



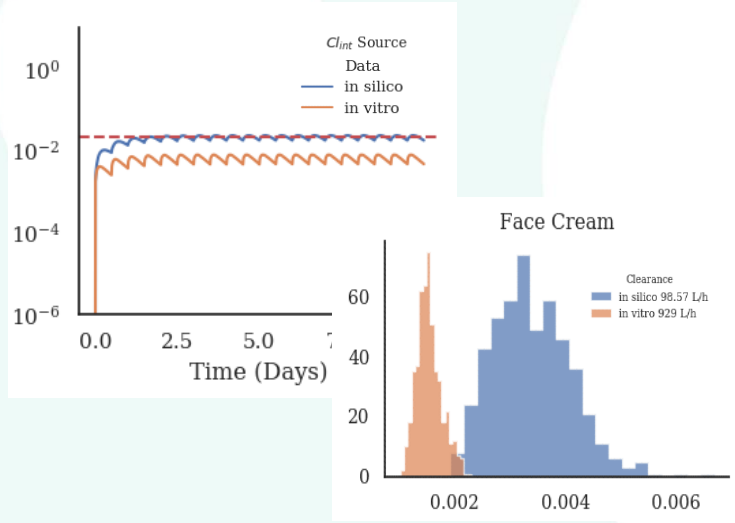
B. Hall et al./Food and Chemical Toxicology 49 (2011) 408–422

GastroPlus® (Simulations Plus)





Level 2- Simulated plasma concentration of coumarin after dermal exposure.

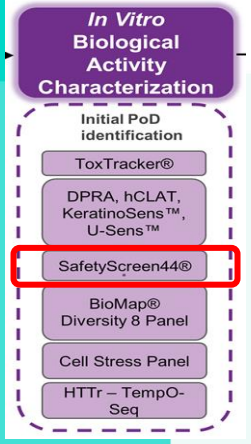


Level 2. Uncertainty and population variability

Distribution of Cmax values after performing Monte Carlo simulation.

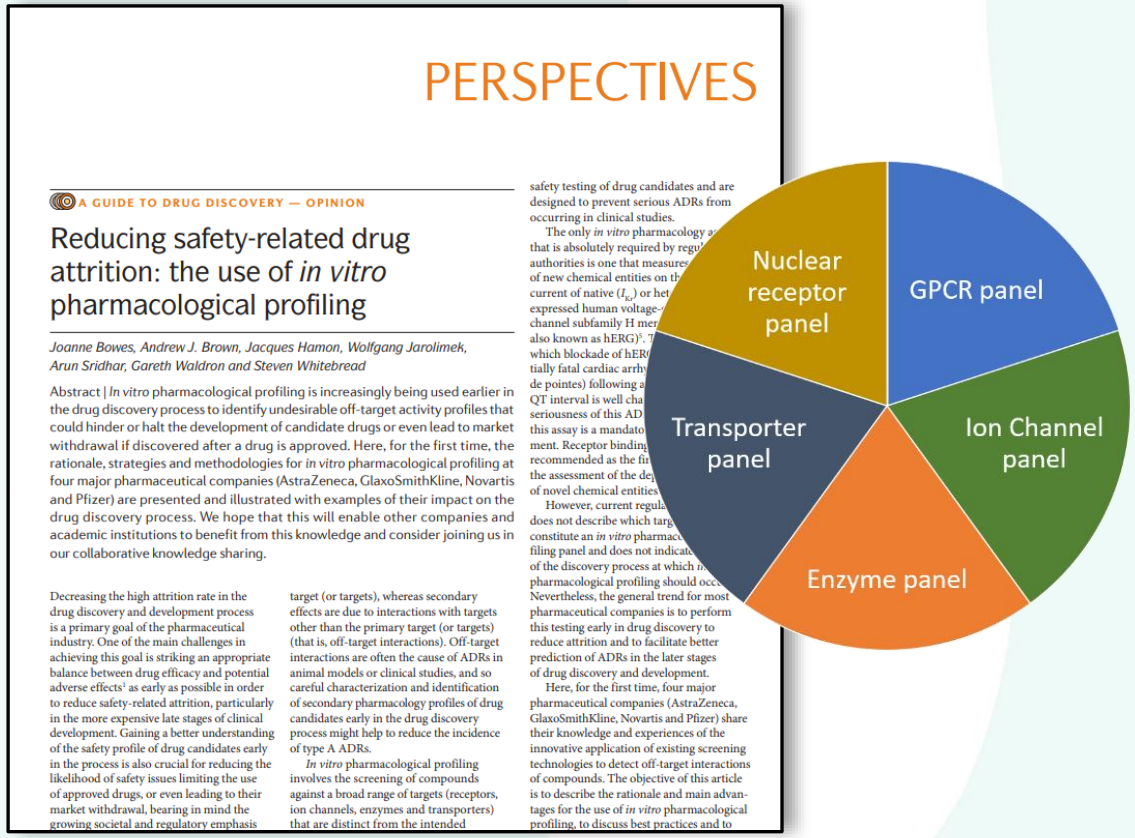
Total Plasma C_{max} (µM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005

Unilever



NGRA for 0.1% coumarin in face cream: In vitro biological activity characterisation: In vitro binding and enzymatic assays: Eurofins SafetyScreen44

To investigate possible interactions between coumarin and the 44 key targets involved in drug attrition



PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

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

Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread

Abstract | *In vitro* pharmacological profiling is increasingly being used earlier in the drug discovery process to identify undesirable off-target activity profiles that could hinder or halt the development of candidate drugs or even lead to market withdrawal if discovered after a drug is approved. Here, for the first time, the rationale, strategies and methodologies for *in vitro* pharmacological profiling at four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) are presented and illustrated with examples of their impact on the drug discovery process. We hope that this will enable other companies and academic institutions to benefit from this knowledge and consider joining us in our collaborative knowledge sharing.

Decreasing the high attrition rate in the drug discovery and development process is a primary goal of the pharmaceutical industry. One of the main challenges in achieving this goal is striking an appropriate balance between drug efficacy and potential adverse effects[1] as early as possible in order to reduce safety-related attrition, particularly in the more expensive late stages of clinical development. Gaining a better understanding of the safety profile of drug candidates early in the process is also crucial for reducing the likelihood of safety issues limiting the use of approved drugs, or even leading to their market withdrawal, bearing in mind the growing societal and regulatory emphasis

target (or targets), whereas secondary effects are due to interactions with targets other than the primary target (or targets) (that is, off-target interactions). Off-target interactions are often the cause of ADRs in animal models or clinical studies, and so careful characterization and identification of secondary pharmacology profiles of drug candidates early in the drug discovery process might help to reduce the incidence of type A ADRs.

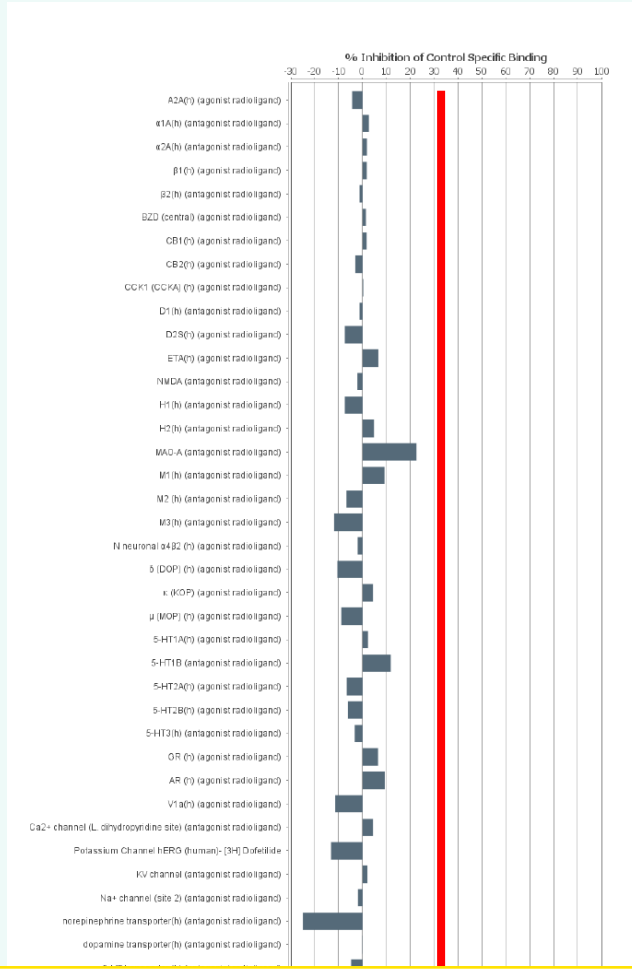
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology a
that is absolutely required by regu
authorities is one that measures
of new chemical entities on th
current of native (I_{Kr}) or het
expressed human voltage-
channel subfamily H mer
also known as hERG)[5, 7
which blockade of hER
tially fatal cardiac arrh
de pointes) following a
QT interval is well cha
seriousness of this AD
this assay is a mandato
ment. Receptor binding
recommended as the fir
the assessment of the de
of novel chemical entities

However, current regula
does not describe which targ
constitute an *in vitro* pharmac
filing panel and does not indicat
of the discovery process at which *in*
pharmacological profiling should occ
Nevertheless, the general trend for most
pharmaceutical companies is to perform
this testing early in drug discovery to
reduce attrition and to facilitate better
prediction of ADRs in the later stages
of drug discovery and development.

Here, for the first time, four major pharmaceutical companies (AstraZeneca, GlaxoSmithKline, Novartis and Pfizer) share their knowledge and experiences of the innovative application of existing screening technologies to detect off-target interactions of compounds. The objective of this article is to describe the rationale and main advantages for the use of *in vitro* pharmacological profiling, to discuss best practices and to

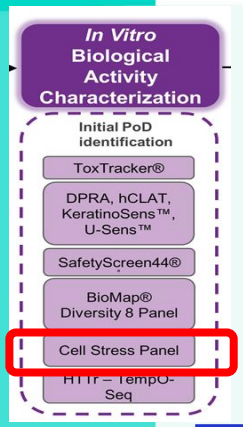


Results:

All binding and enzymatic assay results were negative at 10 µM

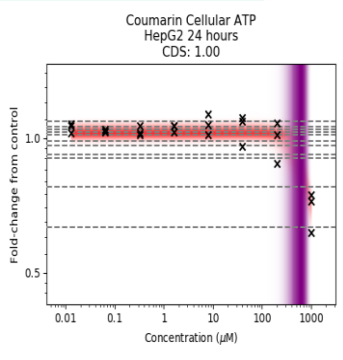
Unilever

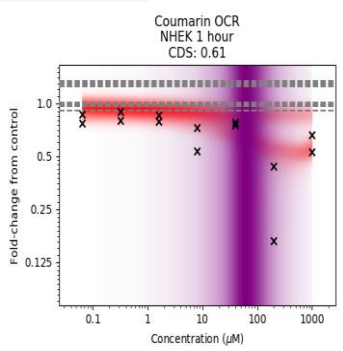
In vitro biological activity characterisation: In vitro cell stress panel



- Cellular stress response assays are useful to **characterize non-specific biological activity** which is not mediated via a specific protein/receptor interaction
- Measures a range of biomarkers covering **~10 cell stress pathways**
- Single exposure; 8 concentrations; 1h, 6h & 24hr timepoints; HepG2 & NHEK cells

- Mitochondrial Toxicity: MitoSOX, PGC1α, MMP, ATP, Glu/Gal
- Oxidative Stress: GSH, ROS, SRXN1, NRF2
- DNA damage: pH2AX, p53
- Inflammation: TNFAIP3, ICAM1, NFkB p65, IL-1β, IL-8, HMGB1
- ER Stress: PERK, ATF4, CHOP, XBP1, BiP, ER Tracker
- Metal Stress: MTF-1, Metallothionein
- Osmotic Stress (NFAT5);
- Heat Shock (HSP70);
- Hypoxia (HIF1α)
- Cell Health: LDH, Phospholipidosis, Steatosis, pH rodo indicator, apoptosis (caspase-3/7) & necrosis (ToPro-3)

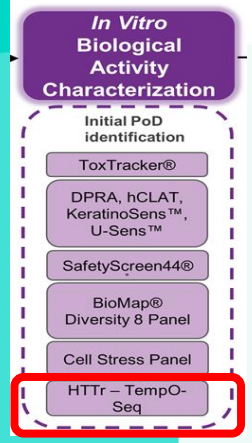




Biomarkers	Cell type	Stress pathway	PoD (μM)	Effect	Concentration dependency score (CDS)
ATP (6h)	HepG2	cell health	794 (363-977)	down	0.98
ATP (24h)			617 (282-891)	down	1
Phospholipidosis (24h)	HepG2	cell health	759 (437-977)	down	0.93
GSH (24h)	HepG2	oxidative stress	851 (301-1000)	up	0.92
IL-8 (24h)	HepG2	inflammation	912 (575-1000)	down	0.61
OCR (1h)	NHEK	mitochondrial toxicity	62 (2.6-776)	down	0.6
OCR (6h)			468 (214-794)		1
OCR (24h)			309 (138-1000)		0.52
Reserve capacity (1h)	NHEK	mitochondrial toxicity	44 (23-96)	down	1
Reserve capacity (6h)			759 (302-1000)		0.9
Reserve capacity (24h)			794 (295-1000)		0.55

Hatherell *et al.*, 2020, https://doi.org/10.1093/toxsci/kfaa054

Unilever



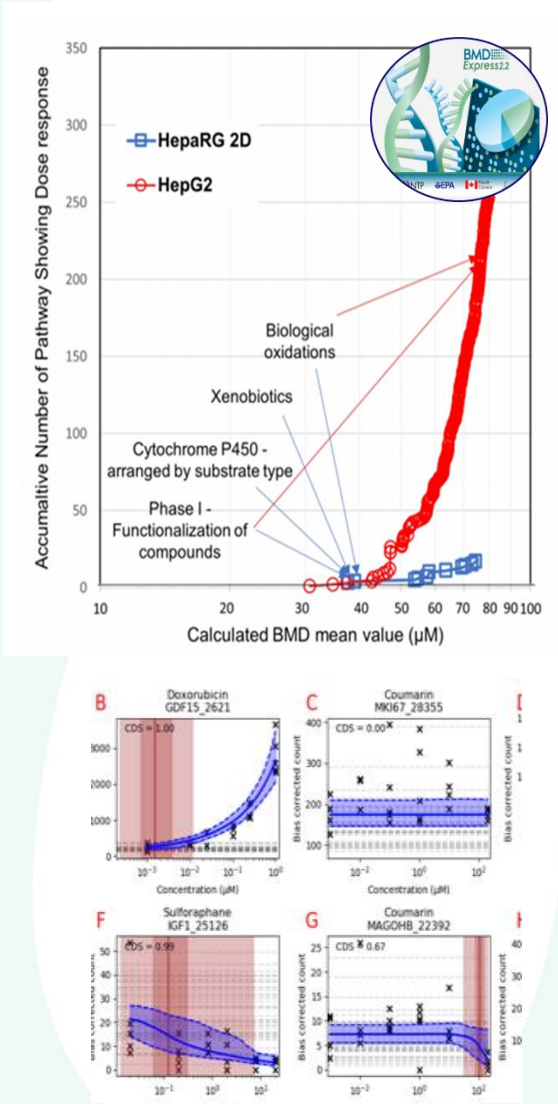
In vitro biological activity: High-Throughput Transcriptomics (HTTr)

Provide screen for biological activity across a broad biological coverage

- *Tempo-Seq*
- *Human gene panel ver1 ~ 21k*
- *3 cell lines*

Results:

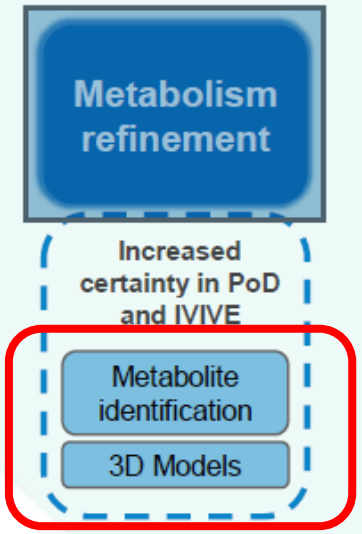
- The MCF7 PoD_T were not considered to be sufficiently robust to derive a MoS
- The lowest PoD_T for each cell model was selected for the MoS calculation

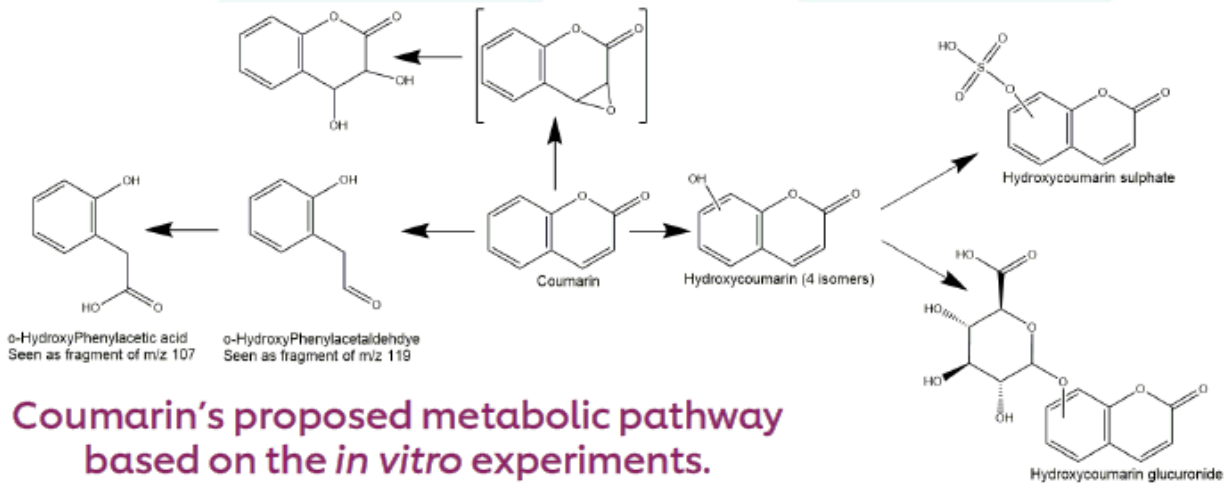


Cell model	HepG2	MCF7	HepaRG 2D
Pathway level tests PoD_T (µM)	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest p value Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38
Gene level tests PoD_T (µM)	(1570 genes)	(47 genes)	(87 genes)
Mean BMD of 20 genes with largest fold change	6	3	54
Mean BMD of genes between 25th and 75th percentile	17	1	59

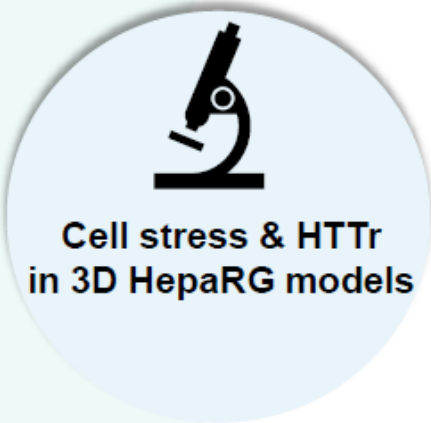
Reynolds et al. A Bayesian approach for inferring global points of departure from transcriptomics data CompTox 2020 https://doi.org/10.1016/j.comtox.2020.100138

Tier 2 refinement: Metabolism prediction and activity





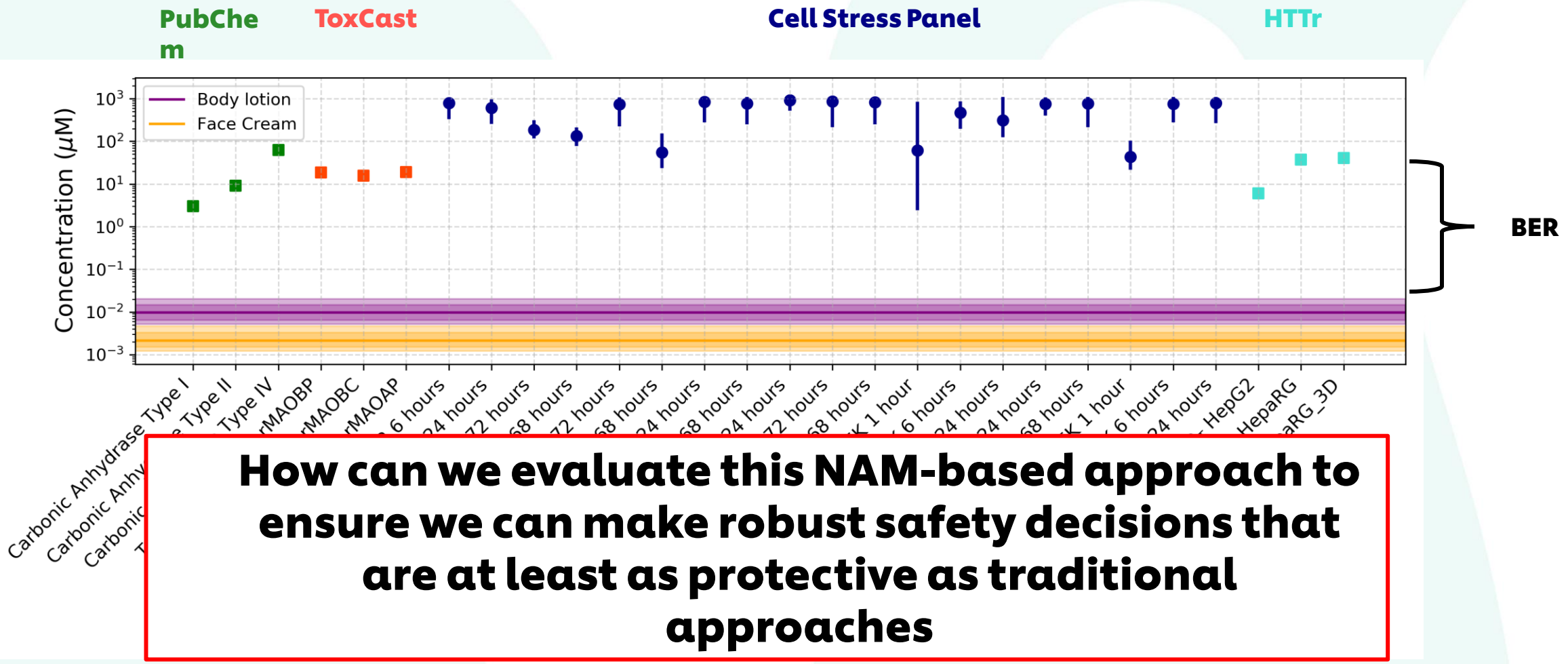
Coumarin's proposed metabolic pathway based on the *in vitro* experiments.



- Low bioactivity also found in a metabolic competent cell model (HepaRG 3D)
- PoDs range: 41-871 µM – not very different from 2D cells

Unilever

Exposure and PoD are plotted and used to derive a Bioactivity-Exposure Ratio (BER)

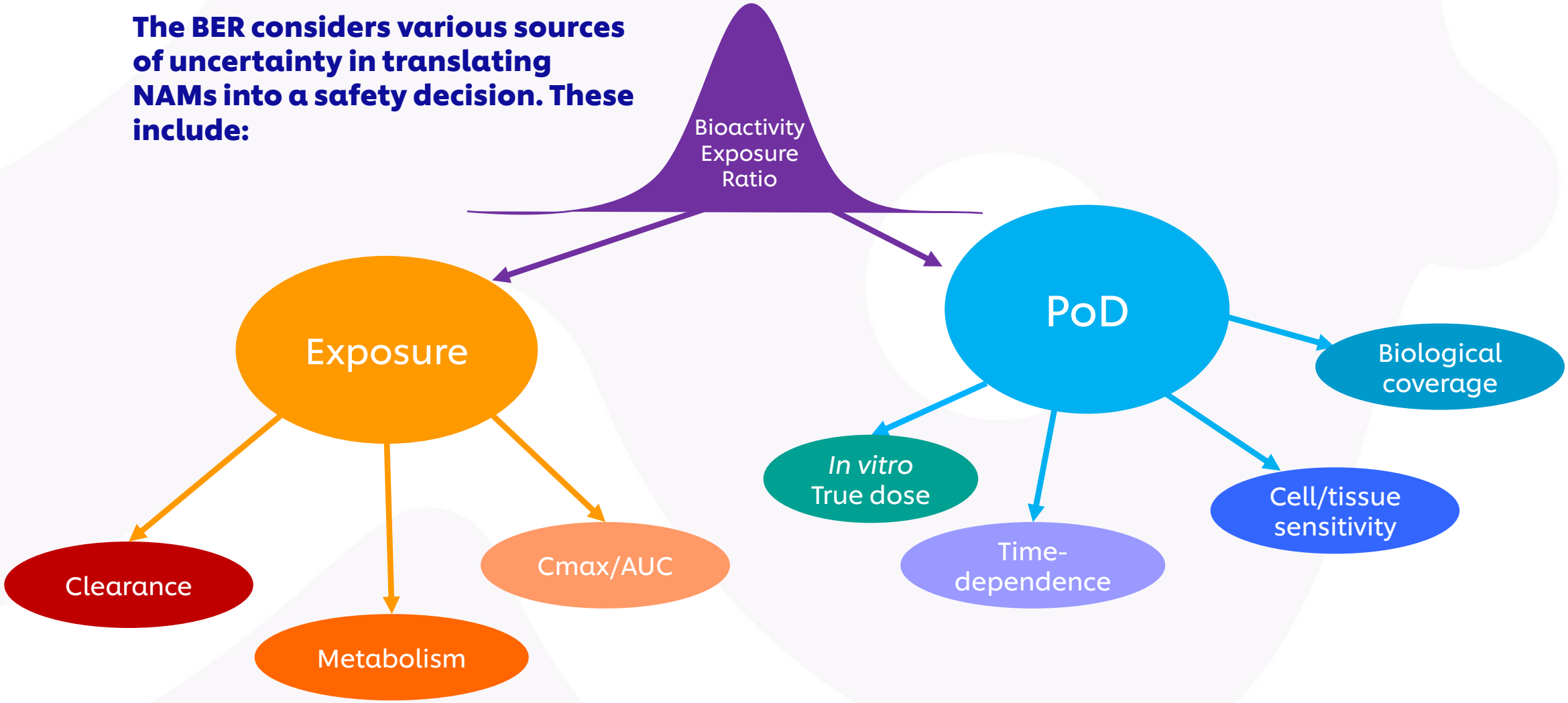


How can we evaluate this NAM-based approach to ensure we can make robust safety decisions that are at least as protective as traditional approaches

Unilever

Integrating Exposure and Bioactivity Data from NAMs to Make Safety Decisions

The BER considers various sources of uncertainty in translating NAMs into a safety decision. These include:



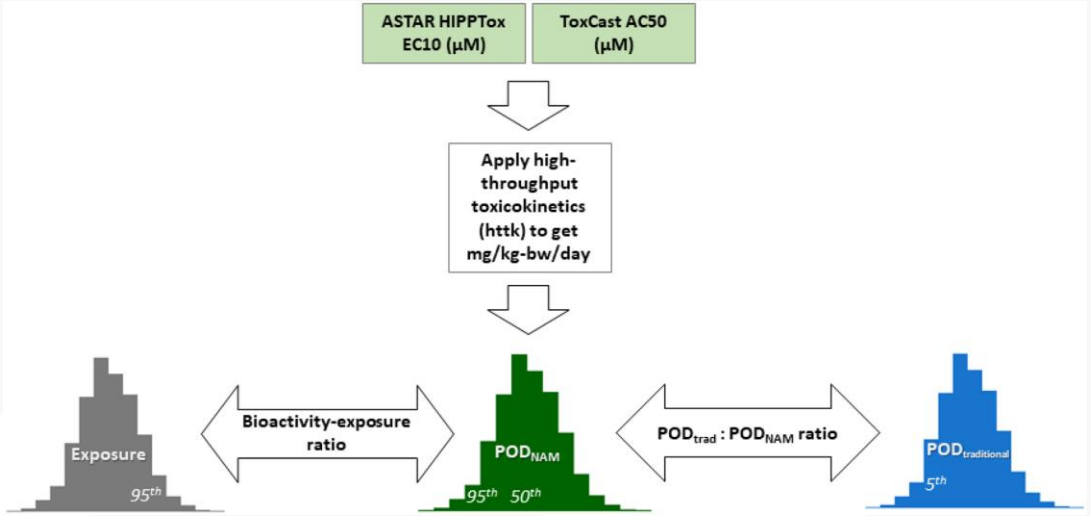
How protective are the NAMs?
Example from the Accelerating the Pace of Chemical Risk Assessment (APCRA) initiative

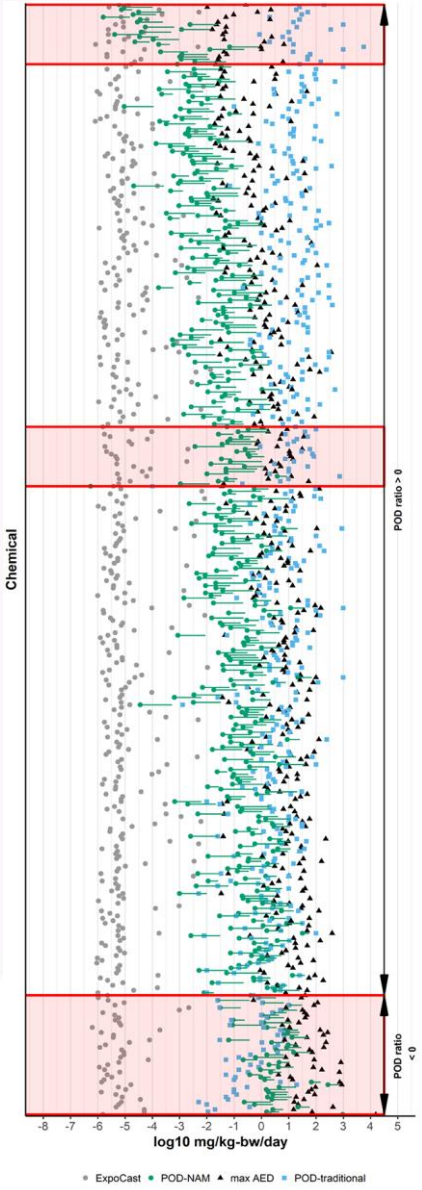
OXFORD SOT Society of Toxicology academic.oup.com/toxsci Tox Spotlight article

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 *





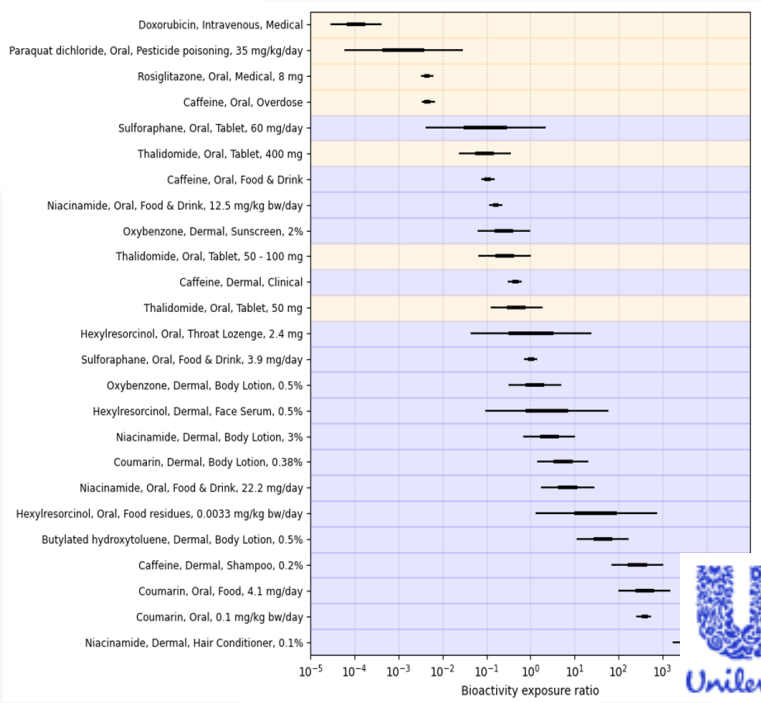
Of the 448 substances, 89% had a $POD_{NAM,95}$ that was less than the traditional POD ($POD_{traditional}$) value.

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.

Unilever

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

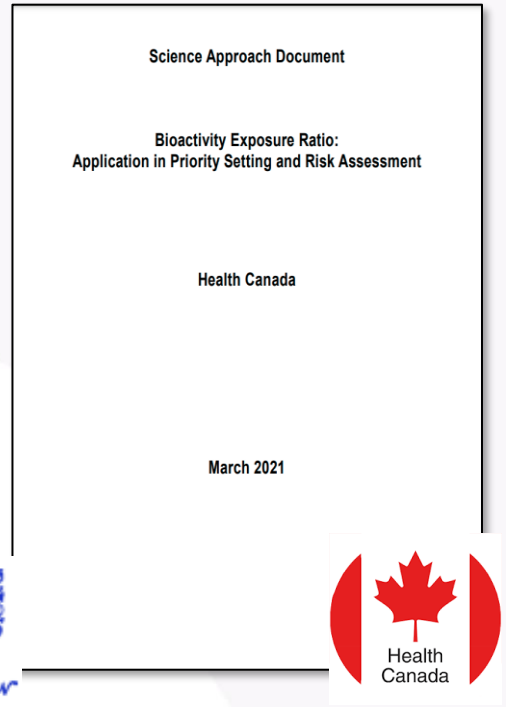
>85 scenarios
Pilot + Full study



Unilever

Benchmark BER against risk category for each exposure scenario

46 compounds



Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

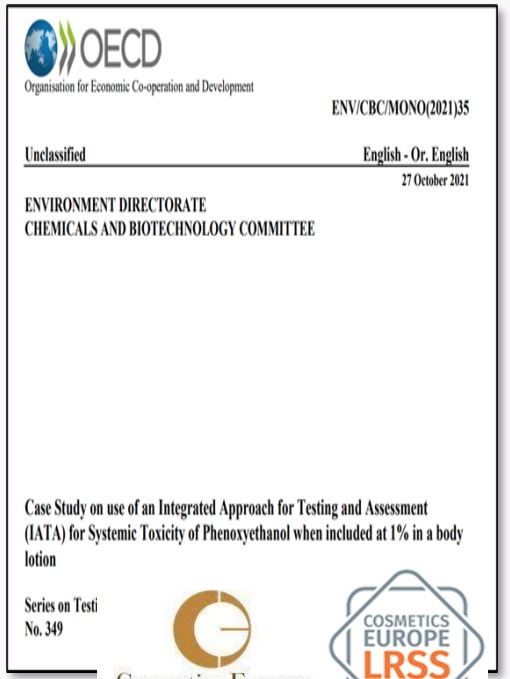
Health Canada

March 2021

Health Canada

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

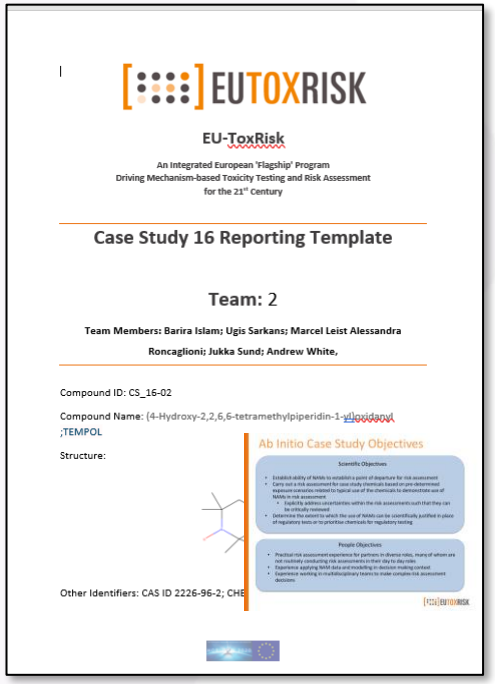
Case Study on use of an Integrated Approach for Testing and Assessment (IATA) for Systemic Toxicity of Phenoxyethanol when included at 1% in a body lotion

Series on Testi
No. 349

Cosmetics Europe
the personal care association

COSMETICS EUROPE LRSS

>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

CERTARA
Simcyp
Universiteit Leiden
Bio Clavis
BDS
BioDetection Systems

Unilever

Summary

- Exposure-led approach to determine protection through a BER (MoS)
- Focus on weight of evidence to show tools can be integrated to make a safety decision - requires diverse expertise
- Strength derived from a combination of targeted and broad unbiased tools – hypothesis led
- NAMs not standard - need to ensure robustness/quality of tools and include estimations of uncertainty to aid acceptance
- Utilise NAMs for further targeted follow where required to refine uncertainty e.g. metabolism
- Further evaluation, additional case studies internal/ in collaboration eg EPA, CosEU, EU-ToxRisk – as well as APCRA
- Dissemination required to progress assessment and build out confidence for broader stakeholder community on applicability domains/ remaining gaps

Unilever

Acknowledgements

Maria Baltazar, Alistair Middleton, Sophie Cable, Joe Reynolds, Hequn Li , Matthew Dent, Predrag Kukic, Paul Carmichael, Beate Nicol, Sharon Scott, Sophie Malcomber, Annabel Rigarlsford, Chris Sparham, Trina Barritt, Katarzyna Przybylak, Georgia Reynolds, Sarah Hatherell, Richard Cubberley, Carl Westmoreland

cyprotex AN EVOTEC COMPANY

toxys®

eurofins

Bio:Clavis™

COSMETICS EUROPE LRSS

EUTOXRISK

EPA

Unilever