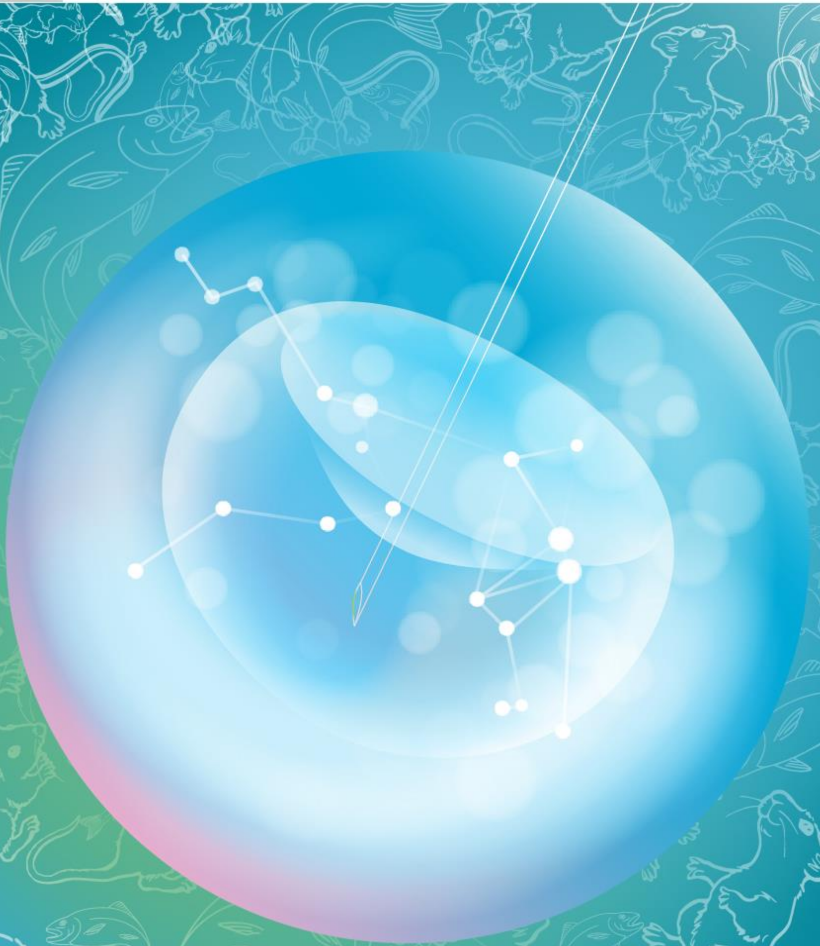
European Commission

Towards replacement of animals for scientific purposes

Scientific Conference

Brussels, 2-3 February 2021



DG Environment

Case studies for assuring safety without animal testing

Carl Westmoreland
Maria Baltazar, Paul Carmichael
Safety & Environmental Assurance Centre, Unilever

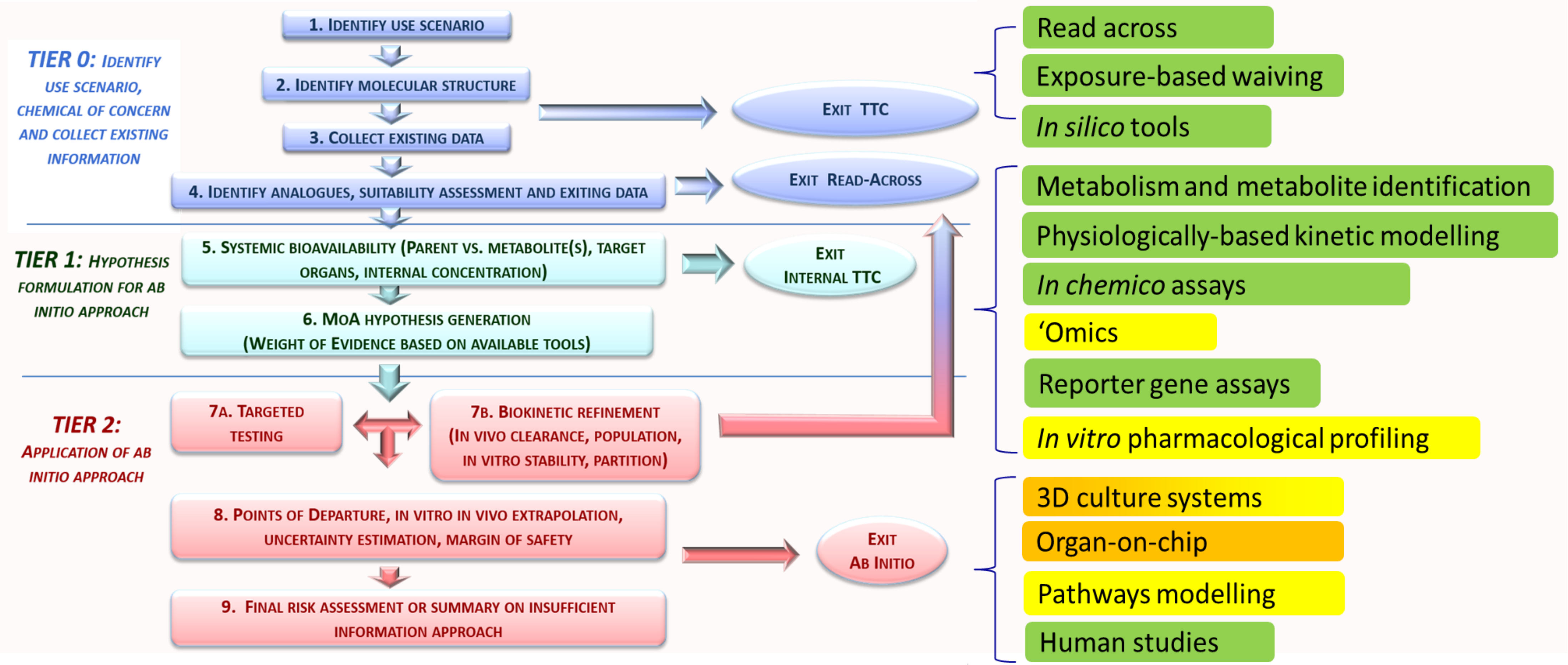
3rd February 2021

Unilever

Next Generation Risk Assessment (NGRA)

Safety without
animal testing

Next Generation Risk Assessment (NGRA)



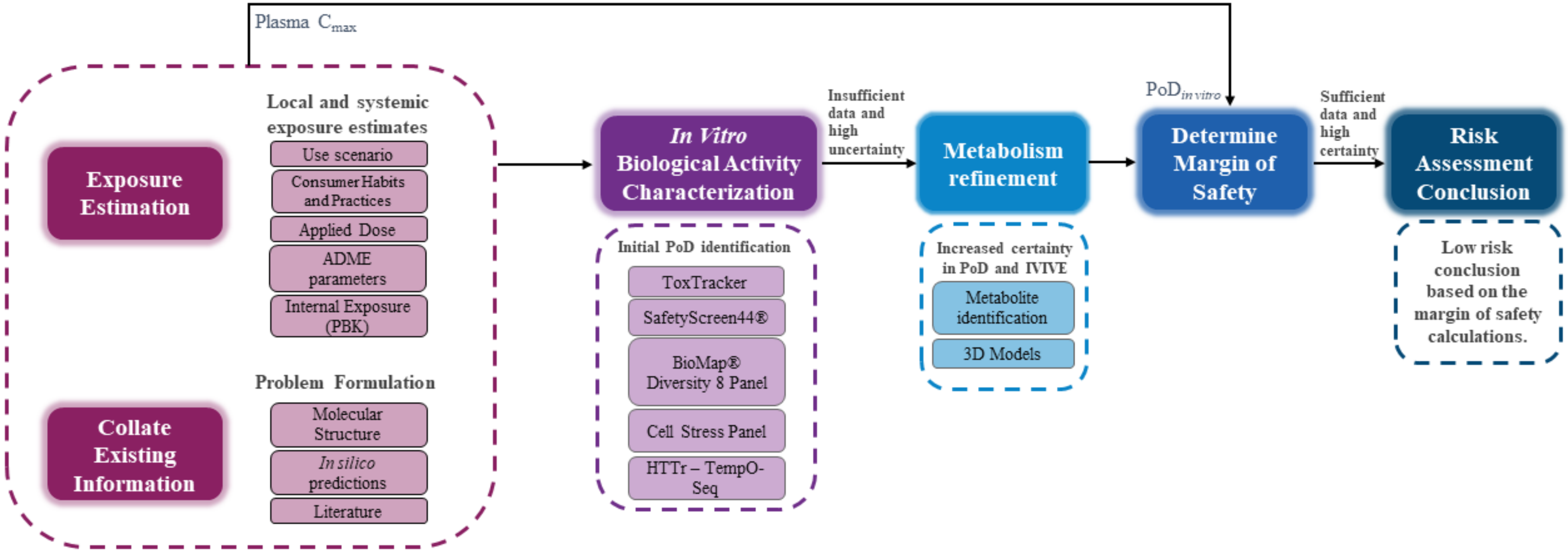
Computational Toxicology (2017) **4**, 31-44

Computational Toxicology (2017) 4, 31-44

Case Study Approach… Imagine we have no data for: Coumarin

Safety assessment for
0.1% coumarin in Face Cream

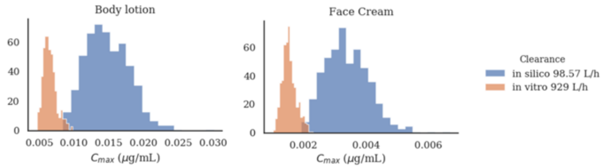
Safety assessment for
0.1% coumarin in Body Lotion



Toxicol Sci. (2020) **176**, 236–252

PBK Modelling

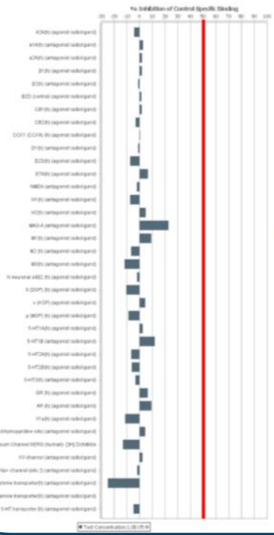
Total Plasma C_{max} (µM)	Mean	Median	90th percentile	95th percentile	97.5th percentile	99th percentile
Face Cream	0.0022	0.0021	0.004	0.0043	0.0046	0.005
Body lotion	0.01	0.01	0.018	0.019	0.02	0.022



Uncertainty & Population Variability

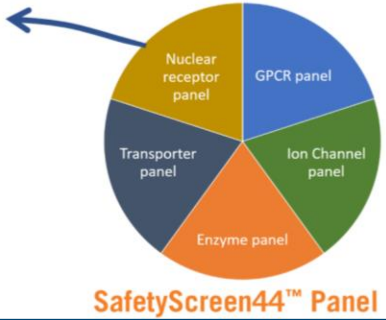
Toxicology in Vitro (2020), **63**, 104746

Safety 44 Screen



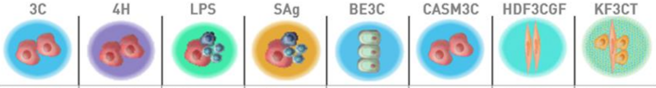
All binding and enzymatic assay results were negative at 10 uM, including COX-1 and COX-2

No receptor/target-led pharmacological effect



eurofins | Cerep

BioMap Systems



Cell System	3C	4H	LPS	SAg	BE3C	CASM3C	HDF3CGF	KF3CT
Cell System	Endothelial [IL1b+TNFa+IFNy]	Endothelial [IL4+Hist]	PBMC + Endothelial [TLR4]	PBMC + Endothelial [TCR]	Bronchial Epithelial [IL1b+TNFa+IFNy]	Coronary artery SMCs [IL1b+TNFa+IFNy]	Fibroblasts [IL1b+TNFa+IFNy +EGF +bFGF+PDGF-BB]	Keratinocytes + Fibroblasts [[IL1b+TNFa+IFNy +TGFb]
LOEL	18.5µM	500µM	>500µM	>500µM	167µM	167µM	56µM	500µM
Biomarker affected (>20%) [a]	Proliferation (-33%) Tissue Factor (-26%)	-	-	-	MMP-1 (-27%)	Proliferation (-25%)	Proliferation (-46%)	-
Biomarker affected (<20%) [b]	HLA-DR (-13%)	Eotaxin-3 (-14%) SRB (-16%)	-	-	PAI-I (-18%)	VCAM-1 (15%) TF (-17%)	MMP-1 (-14%)	IL-1a (-16%)

Biological readouts associated with anti-proliferative and tissue remodelling activities across all cell systems. No immunomodulatory effects at relevant concentrations

Data suggest that coumarin is not an anti-inflammatory compound

Stress Pathways

~40 Biomarkers; 3 Timepoints; 8 Concentrations; ~10 Stress Pathways

Biomarker	Cell type	Stress pathway	PoD (µM)
ATP (6h)	HepG2	cell health	794 (363-977)
ATP (24h)			617 (282-891)
Phospholipidosis (24h)	HepG2	cell health	759 (437-977)
GSH (24h)	HepG2	oxidative stress	851 (301-1000)
IL-8 (24h)	HepG2	inflammation	912 (575-1000)
OCR (1h)			62 (2.6-776)
OCR (6h)	NHEK	mitochondrial toxicity	468 (214-794)
OCR (24h)			309 (138-1000)
Reserve capacity (1h)			44 (23-96)
Reserve capacity (6h)	NHEK	mitochondrial toxicity	759 (302-1000)
Reserve capacity (24h)			794 (295-1000)

cyprotex AN EVOTEC COMPANY

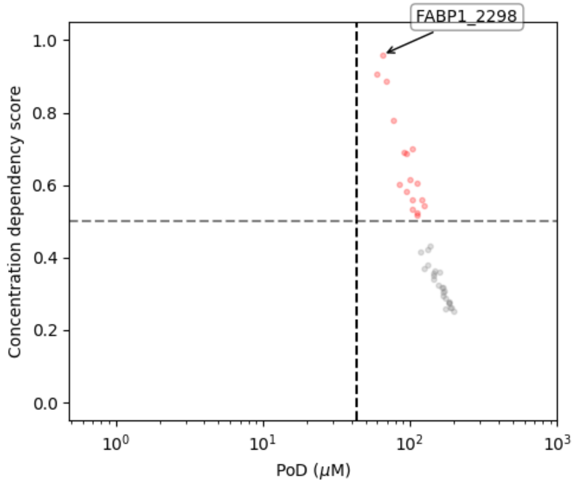
Toxicol Sci (2020), **176**, 11-33

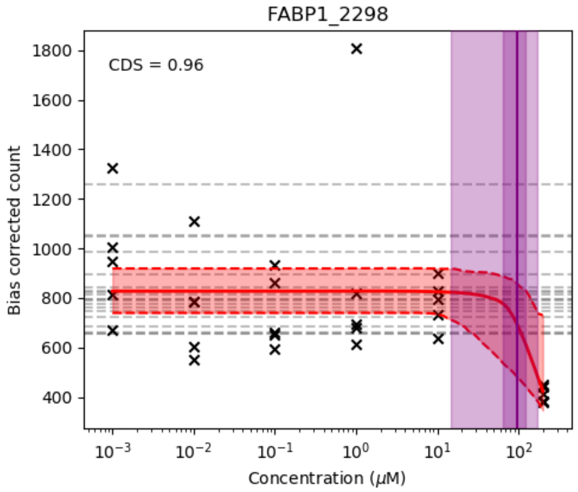
In Vitro Bioactivity: Tempo-Seq Technology

BioSpyder

High Throughput Transcriptomics (HTTr)

- Coumarin dose range 0.001uM to 100uM
- 24 hour time point
- QC and normalisation in DESeq2
- BMDExpress2 applied to determine NOTEL (3 pathway approaches)





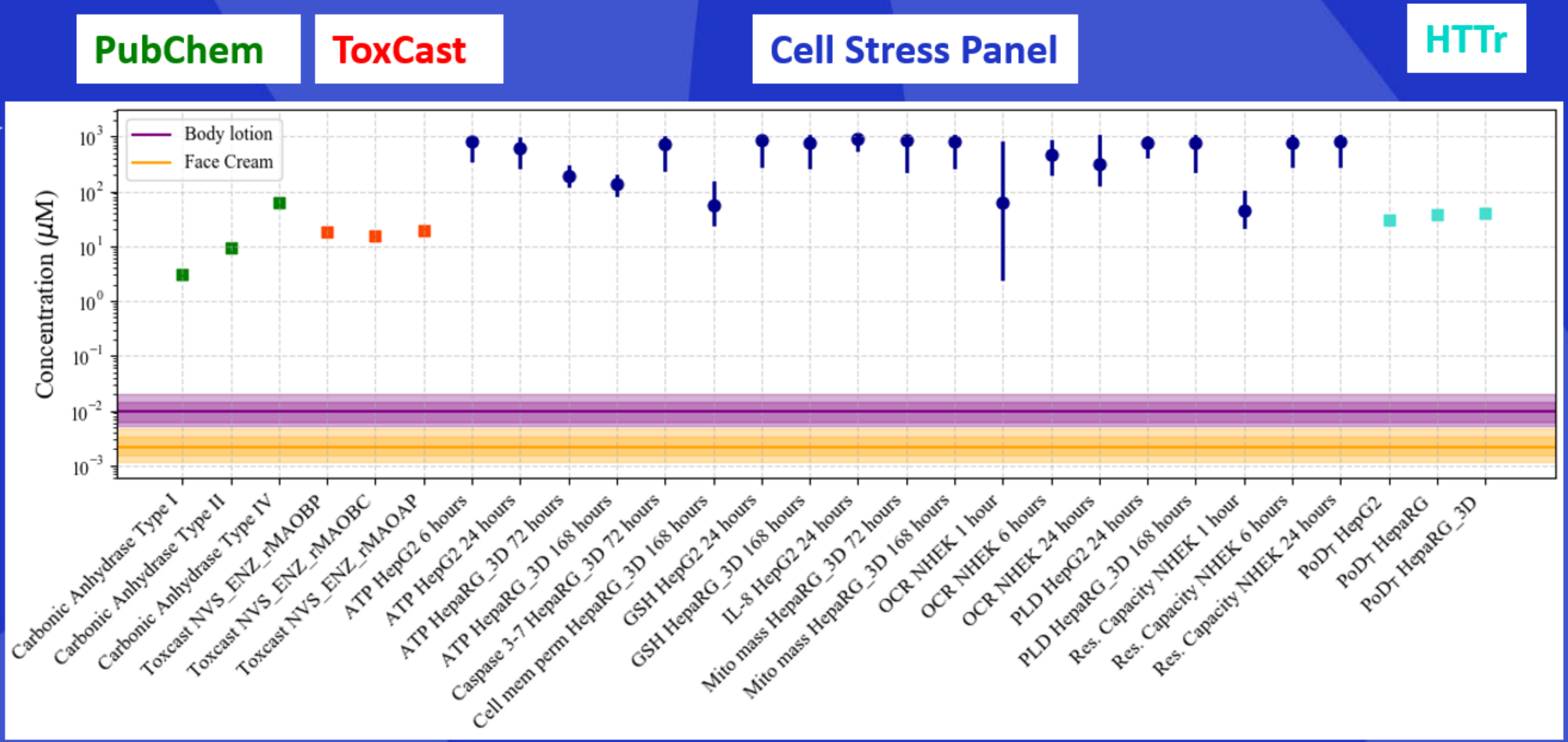
- Bayesian approach to estimate maximum no effect concentration published in 2020 (Reynolds et al. 2020)
- Method applied to multiple HTTr datasets generated with coumarin

Cell type	Global PoD (μM)
HepaRG	7.2
HepG2	6.7
MCF7	5.9
HepaRG	62
HepaRG (3D)	44

Comp Toxicol. (2020) **16**, 100138

Margin of Safety considering PODs and Exposure

PoDs and plasma C_{max} (μM) are expressed as total concentration

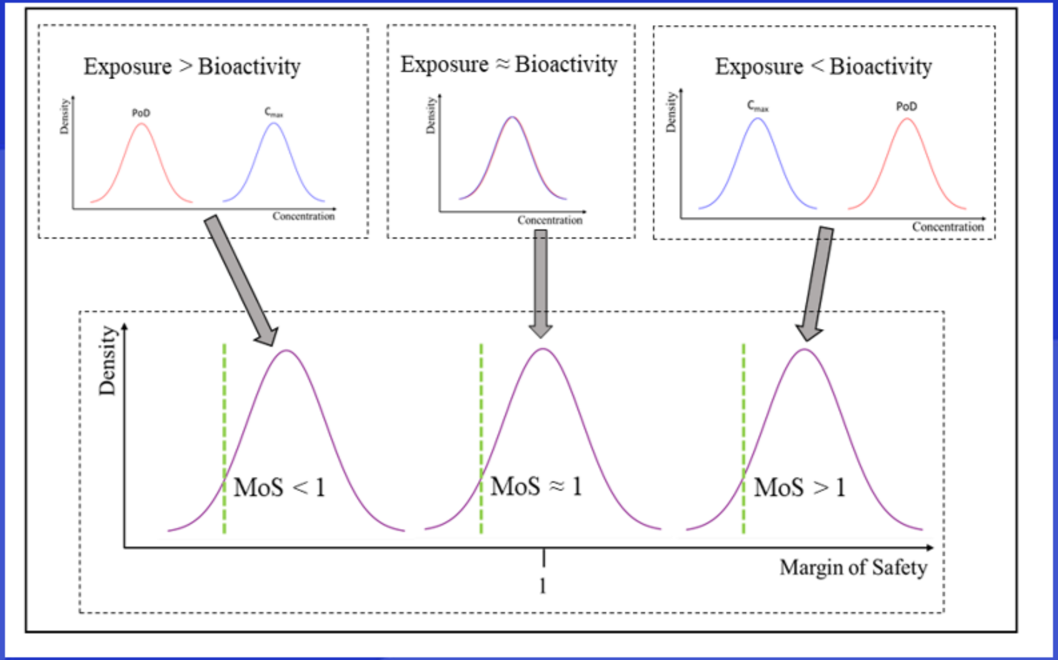


C_{max} expressed as a distribution:

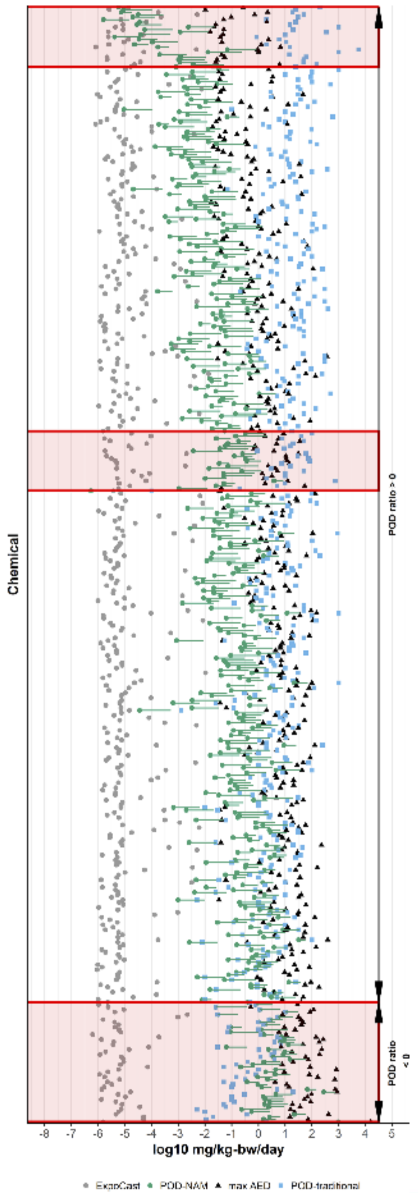
- Line = median (50th percentile)
- Inner band = 25th-75th percentile
- Outer band = 2.5th-97.5th percentile (95th credible interval)

Application of *Ab Initio* Approach: NGRA

Margin of safety is the fold difference between the Cmax and the *in vitro* POD



Technology	Cell line/ Enzyme/Biomarker	Face cream Min. 5th percentile MoS	Body Lotion Min. 5th percentile MoS
Cell stress panel	HepG2 (ATP, 24h)	96738	22048
Cell stress panel	NHEK (OCR 1h)	1330	**295**
HTTr	HepG2 (24h)	7223	1618
HTTr	HepaRG (24h)	8864	1986
Toxcast	MAO B	3711	831
PubChem	Carbonic Anhydrase Type I	**706**	**158**
PubChem	Carbonic Anhydrase Type II	2140	479
PubChem	Carbonic Anhydrase Type VI	14652	3282
Cell stress panel	HepaRG_3D (cell mem perm 168h)	9601	2197
HTTr	HepaRG_3D_24h	9538	2137



Accelerating the Pace of Chemical Risk Assessment

APCRA

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

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225

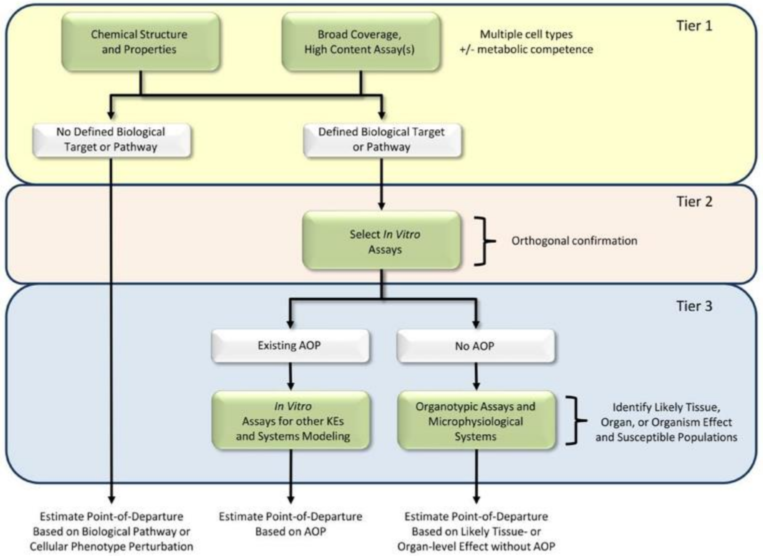
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

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,[||||] Steven Foster,[#] Kathleen Raffaele,[#] Tina Bahadori,[||] Maureen R. Gwinn,[*] Jason Lambert,[*] Maurice Whelan,[**] Mike Rasenberg,[§] Tara Barton-Maclaren,[†] and Russell S. Thomas [*]

"The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals"

United States Environmental Protection Agency



Toxicol Sci (2019) 169, 317-332

An NGRA Example from a Homecare Product

Unilever #StaySafe
@Unilever

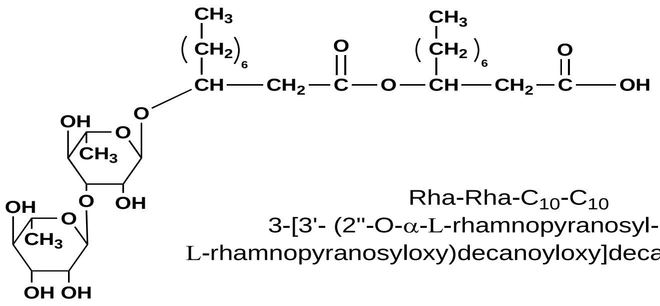
Partnering with @Evonik, we have harnessed a naturally occurring process to develop a first-of-its-kind ingredient for household cleaning products: rhamnolipids

🔹 100% renewable
🔹 100% biodegradable

First launched in our Quix dishwashing liquid in Chile.

#OurCleanFuture

10:24 am · 23 Sep 2020 · Twitter Web App



Rha-Rha-C_{10}-C_{10}
3-[3'- (2"-O-α-L-rhamnopyranosyl-α-L-rhamnopyranosyloxy)decanoyloxy]decanoic acid

NGRA included:

- Detailed consumer exposure work
- *In vitro* skin penetration work
- Understanding metabolism
- *In vitro* immunotoxicity assessment

Conclusions

Non-animal safety assessments for consumer goods are moving from '*might be possible in theory*' to '*case studies of NGRA in action*'

NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions

- Enabling a transition from using data from tests in live animals to one founded on understanding the effects of chemicals in humans using computational approaches and *in vitro* methods that evaluate changes in biologic processes using human cells
- Need to ensure quality/robustness of the non-standard (non-TG) work
- More published examples to increase confidence for regulatory application e.g. Cosmetics Europe, OECD, EUTOXRISK
- Importance of characterising uncertainty to allow informed decision-making
- Shortcomings will be addressed by current and future research; NGRA will constantly evolve.

Acknowledgements

Maria Baltazar
Catherine Barratt
Sophie Cable
Paul Carmichael
Stella Cochrane
Richard Cubberley
Tom Cull
Claire Davies
Mona Delagrange
Matt Dent
Julia Fentem
Tom Green
Sarah Hatherell
Jade Houghton
Predrag Kukic
Juliette Pickles
Hequn Li
Mi-Young Lee
Sophie Malcomber
Deborah Martin
Gavin Maxwell
Alistair Middleton
Alexis Nathanail
Tom Moxon
Beate Nicol
Ruth Pendlington
Sam Piechota
Fiona Reynolds
Georgia Reynolds
Joe Reynolds
Paul Russell
Nikol Simecek
Andy Scott
Ian Sorrell
Andy White

eurofins | Cerep
BioSpyder
cyprotex AN EVOTEC COMPANY