Using next generation risk assessment to make safety decisions for consumer products

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26/11/2021

Unilever

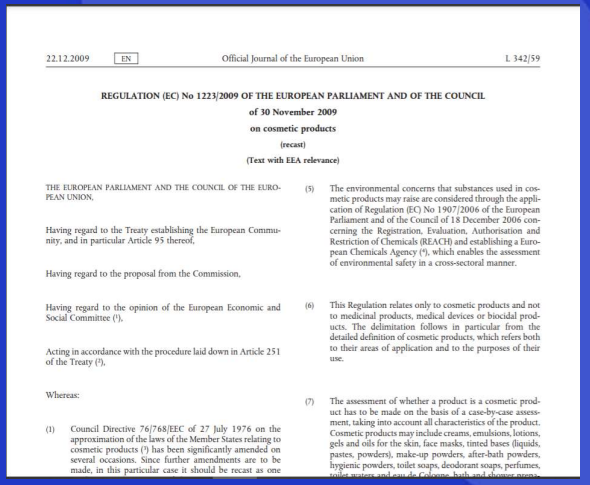
Outline

- Why is NGRA important?
- What is it?
- How is it being applied today?
- Where next?

The need for non-animal approaches

Societal Attitudes/Consumer Preference

Human Relevance



22.12.2009 EN Official Journal of the European Union L 342/59

REGULATION (EC) No 1223/2009 OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL
of 30 November 2009
on cosmetic products
(recast)
(Text with EEA relevance)

THE EUROPEAN PARLIAMENT AND THE COUNCIL OF THE EUROPEAN UNION,

Having regard to the Treaty establishing the European Community, and in particular Article 95 thereof,

Having regard to the proposal from the Commission,

Having regard to the opinion of the European Economic and Social Committee (1),

Acting in accordance with the procedure laid down in Article 251 of the Treaty (2),

Whereas:

(1) Council Directive 76/768/EEC of 27 July 1976 on the approximation of the laws of the Member States relating to cosmetic products (3) has been significantly amended on several occasions. Since further amendments are to be made, in this particular case it should be recast as one

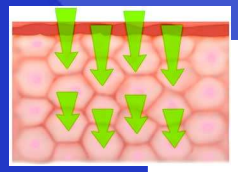
(5) The environmental concerns that substances used in cosmetic products may raise are considered through the application of Regulation (EC) No 1907/2006 of the European Parliament and of the Council of 18 December 2006 concerning the Registration, Evaluation, Authorisation and Restriction of Chemicals (REACH) and establishing a European Chemicals Agency (4), which enables the assessment of environmental safety in a cross-sectoral manner.

(6) This Regulation relates only to cosmetic products and not to medicinal products, medical devices or biocidal products. The delimitation follows in particular from the detailed definition of cosmetic products, which refers both to their areas of application and to the purposes of their use.

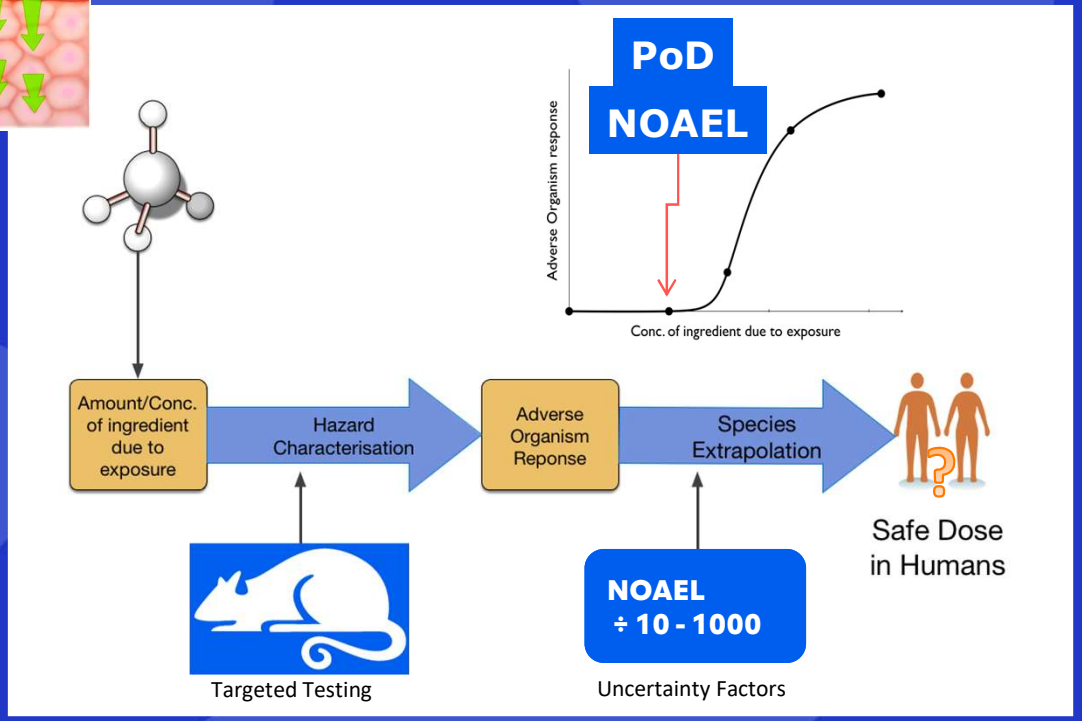
(7) The assessment of whether a product is a cosmetic product has to be made on the basis of a case-by-case assessment, taking into account all characteristics of the product. Cosmetic products may include creams, emulsions, lotions, gels and oils for the skin, face masks, tinted bases (liquids, pastes, powders), make-up powders, after-bath powders, hygienic powders, toilet soaps, deodorant soaps, perfumes, toilet waters and eau de Cologne, bath and shower prepar-

Regulatory Change

The Systemic Challenge



Is it safe?



~~e.g. 90 Day Repeat Dose Study~~

A new non-animal paradigm is needed...

...but replacement of animal test data is not the answer

Existing approaches

Threshold of Toxicological Concern

(Yang et al 2017)

https://doi.org/10.1016/j.fct.2017.08.043

Read across

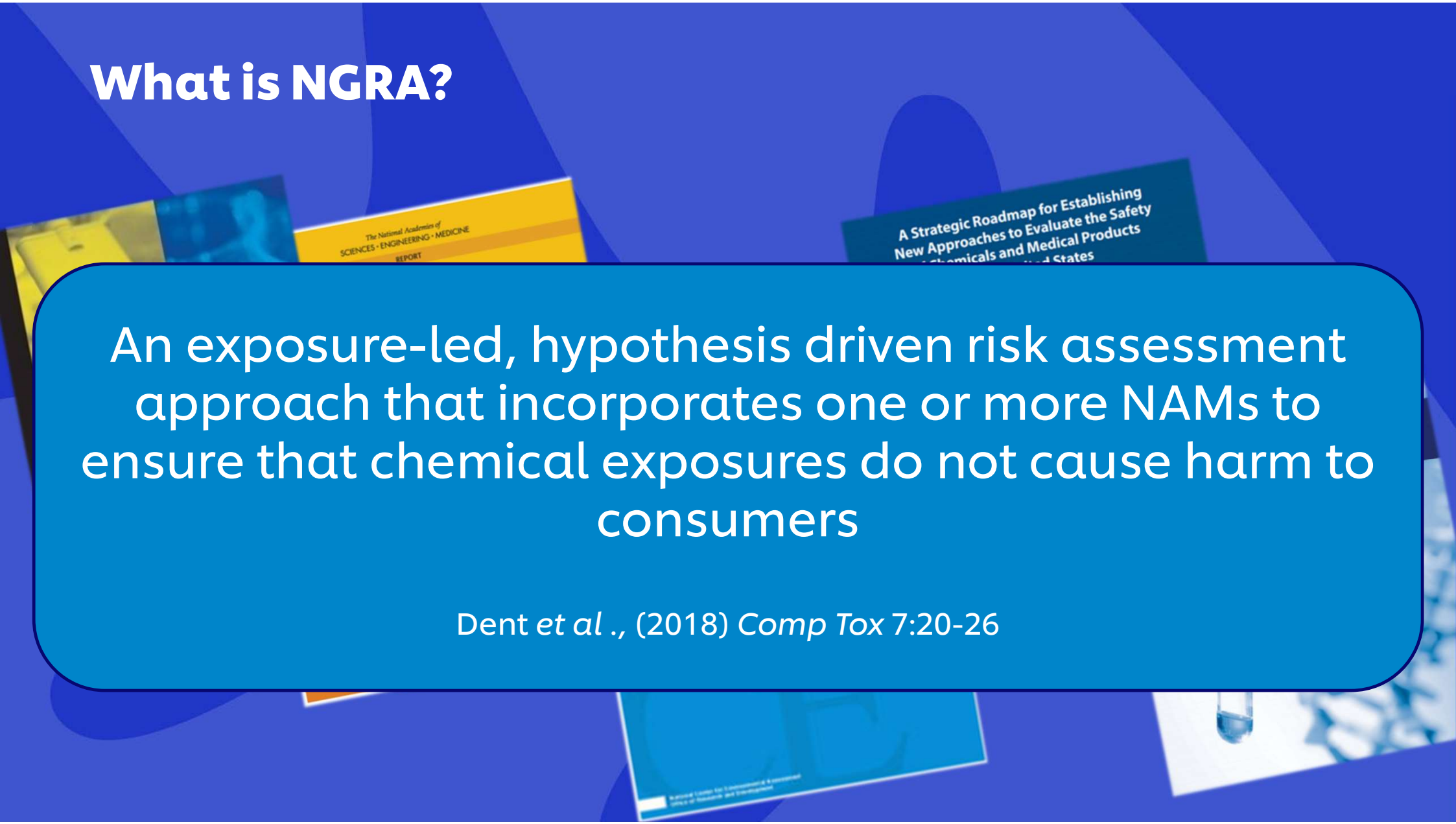
History of Safe Use

(Neely et al 2011)

https://doi.org/10.4103/0971-6580.85882

→NGRA

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

Principles of NGRA from ICCR

4 Main overriding principles:

- The overall goal is a human safety risk assessment
- The assessment is exposure led
- The assessment is hypothesis driven
- The assessment is designed to prevent harm

3 Principles describe how a NGRA should be conducted:

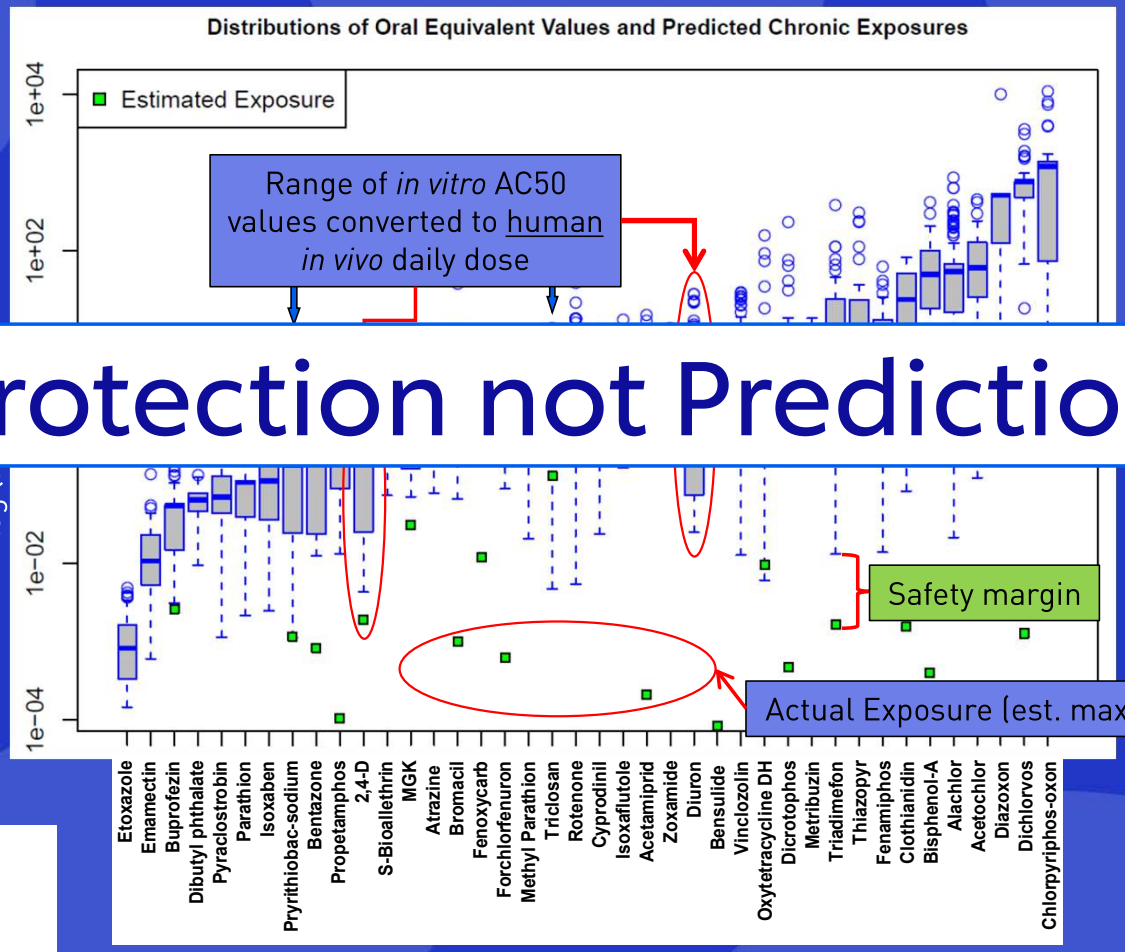
- Following an appropriate appraisal of existing information
- Using a tiered and iterative approach
- Using robust and relevant methods and strategies

2 Principles for documenting NGRA:

- Sources of uncertainty should be characterized and documented
- The logic of the approach should be transparent and documented

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

In Vitro Bioactivity vs Bioavailability



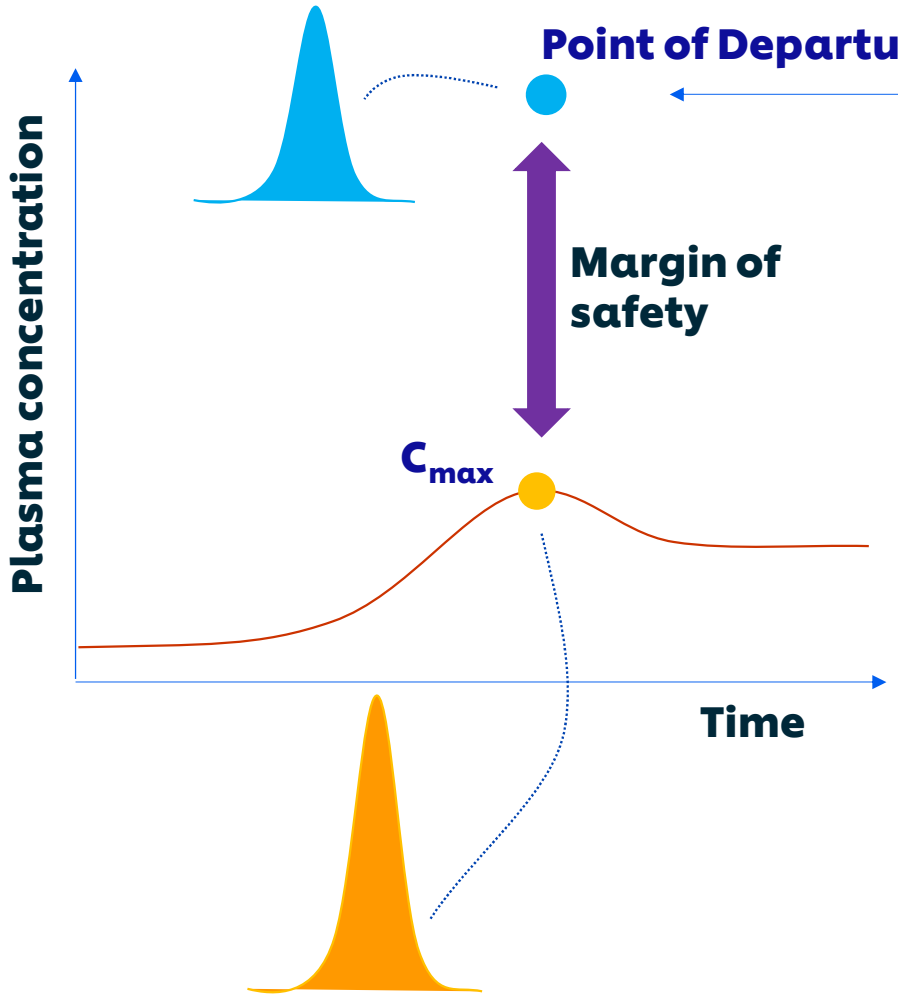
"Protection not Prediction"

Hepatic clearance and plasma protein binding determinations

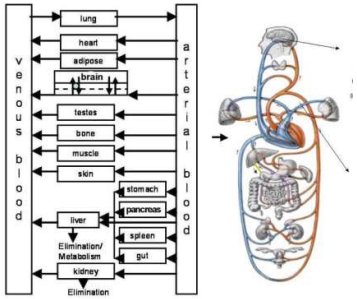
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010 Vol 117/2 348-358
https://doi.org/10.1093/toxsci/kfq220

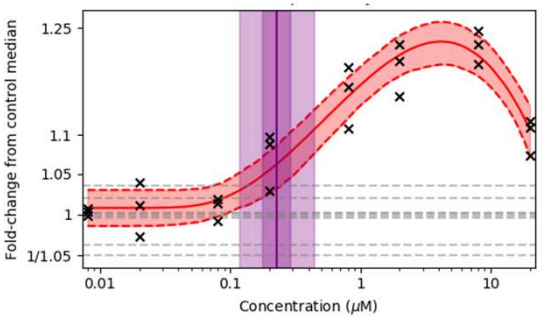
The Margin of Safety Approach



Exposure models (PBK, free/total concentration)

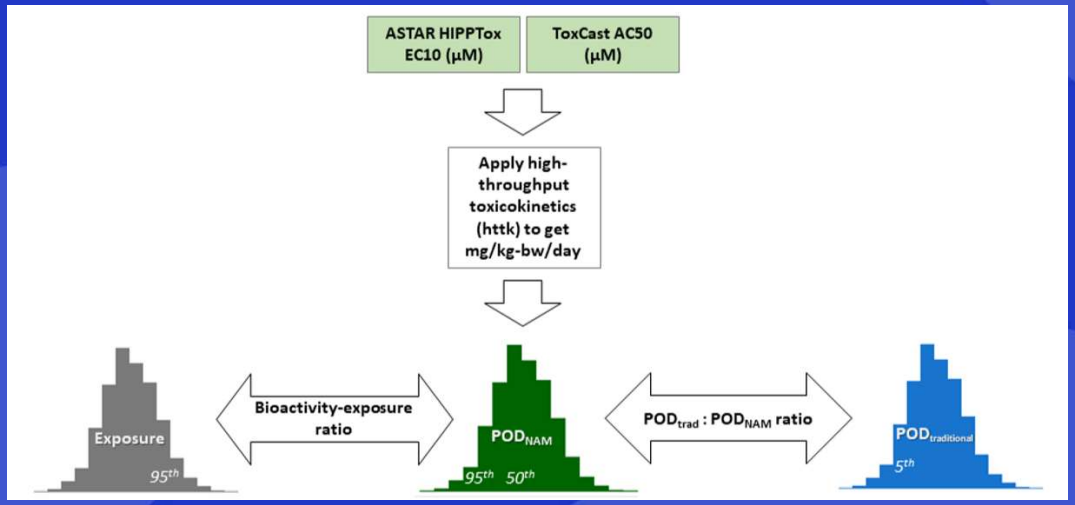


Point of departure derived from *in vitro* concentration-response

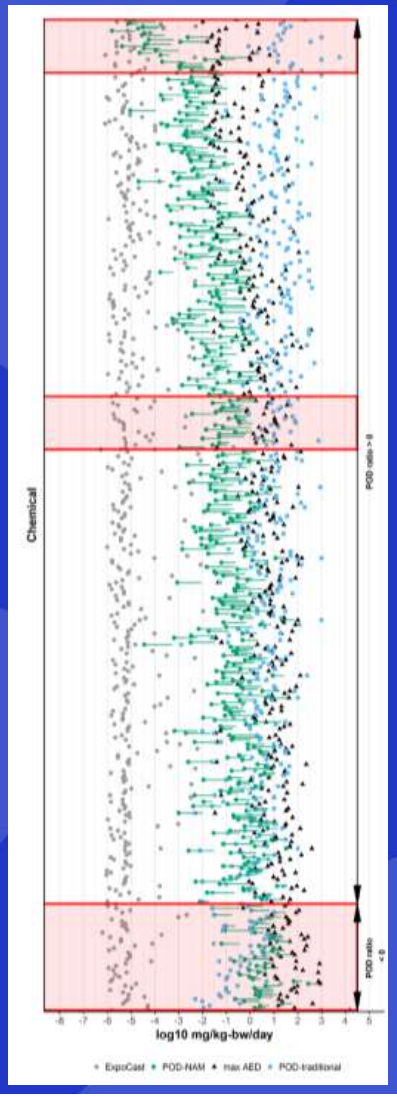


Are in vitro PoDs protective and useful?

EPA, NTP, HC, A*STAR, ECHA, EFSA, JRC, RIVM…



Katie Paul-Friedman *et al.* 2019 *Tox Sci* 173(1): 202-225



414/448 chemicals =
92% of the time this naïve approach appears conservative

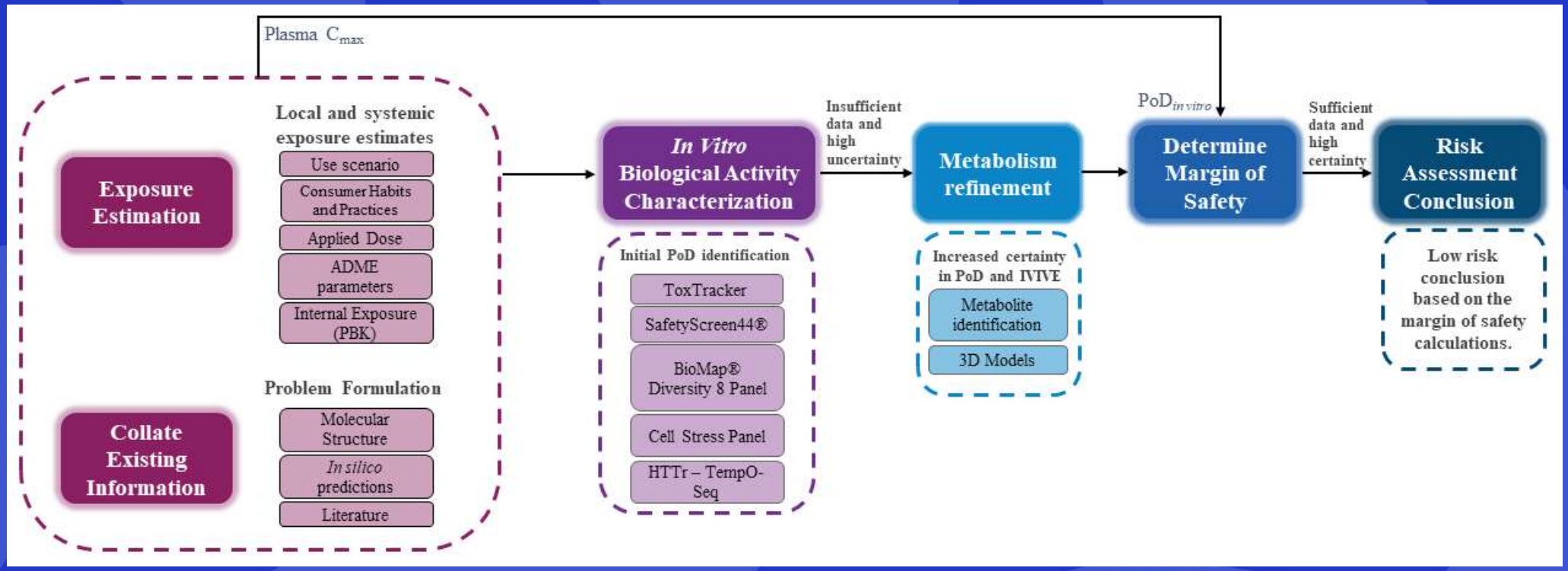
Case Study Approaches… Imagine we have no data for: Coumarin

Safety assessment required for 0.1% coumarin in Body Lotion

Safety assessment required for 0.1% coumarin in Face Cream

Baltazar *et al.*, (2020) *Tox Sci* https://doi.org/10.1093/toxsci/kfaa048

Case Study Framework



Baltazar *et al.*, (2020) *Toxicological Sciences* 176(1): 236-252
https://doi.org/10.1093/toxsci/kfaa048

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Collection of Existing Data and ADME Parameters

Name	Coumarin
CAS	91-64-5
MW	146.14 g/mol
Log P	1.39
Solubility	0.96 mg/mL in phosphate buffer
ECCS Class	Class 2 (Metabolism)
R_{b2p}	0.7
F_{ub}	0.31
Cl_{int}	929 L/h

Chemistry determinations:

- Partition coefficient logP
- Peptide binding potential

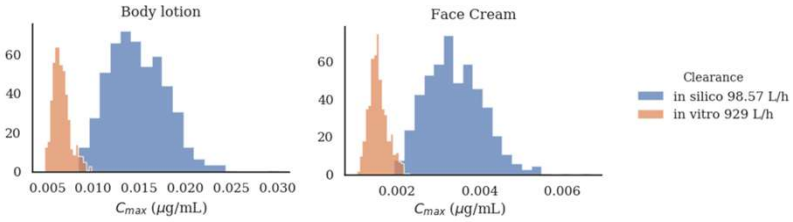
In vitro determined:

- Kinetic solubility
- Thermodynamic solubility
- Metabolic & chemical stability
- Stability in human plasma
- Plasma protein binding
- Partitioning in blood
- Skin penetration parameters

Systemic Bioavailability using PBK Modelling

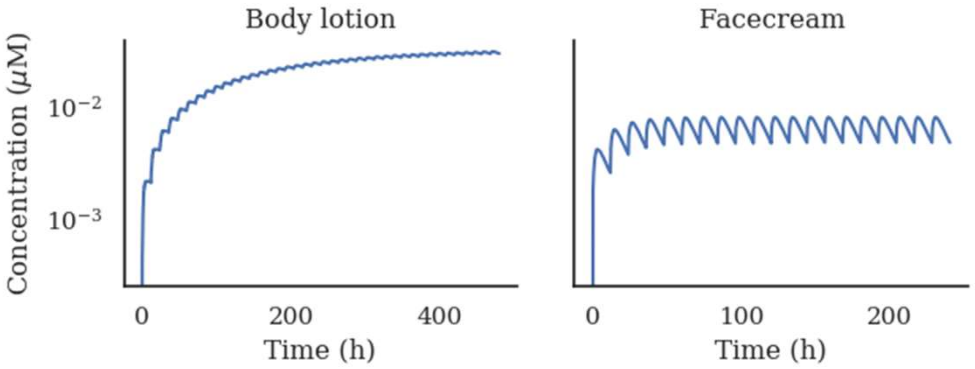
Key output parameters from uncertainty analysis:

Parameter	Face cream (applied 2x/day)	Body lotion (applied 2x/day)
Plasma Cmax total (µM)	0.023	0.10
95th percentile Cmax (µM)	0.032	0.14



Uncertainty & Population Variability

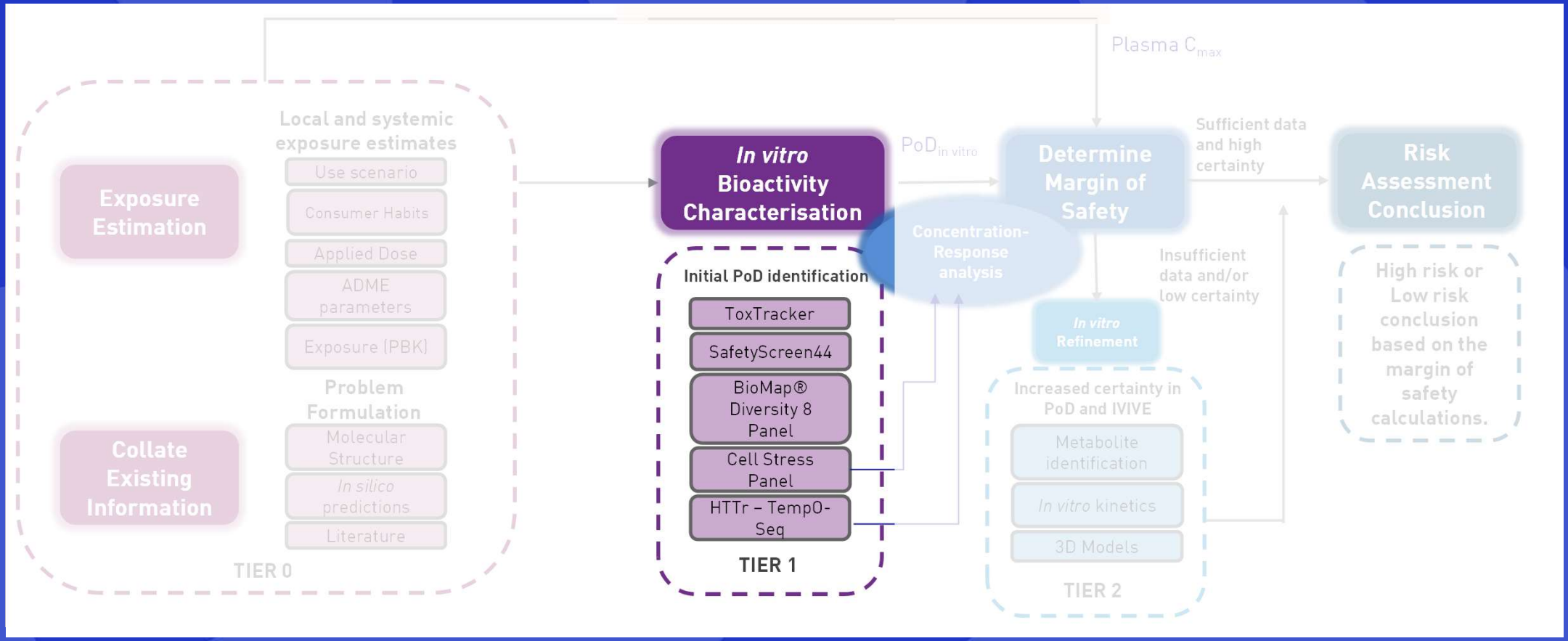
0.1% Face cream & body lotion in Europe



Physiologically-based kinetic modelling using GastroPlus® v9.5. Estimations based on experimental data (Clint, fup, bpr, solubility, LogP). Skin penetration parameters were fitted against skin penetration data.

Moxon *et al.*, (2020) TIV 63: 104746 https://doi.org/10.1016/j.tiv.2019.104746

Ab Initio NGRA Framework

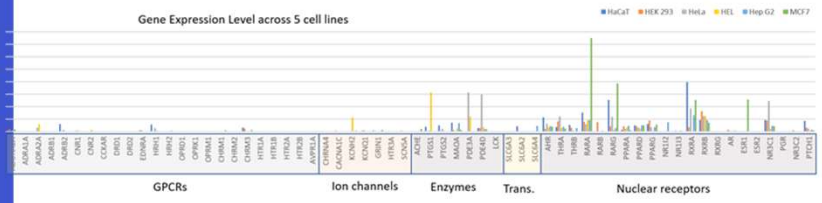


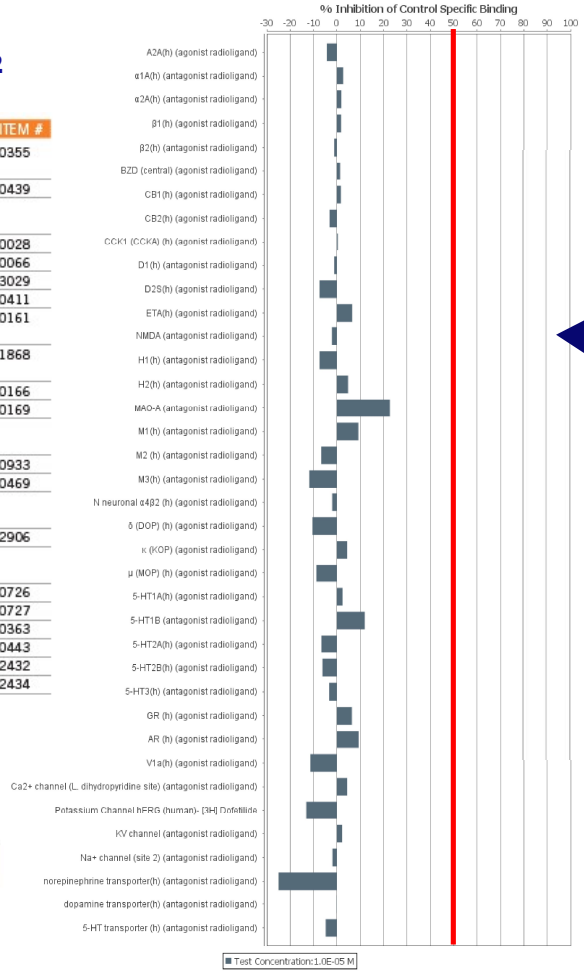
In Vitro Bioactivity: Safety Screen

eurofins | Cerep

Bowes et al 2012. Nature Reviews: Drug Discovery 11 909-922

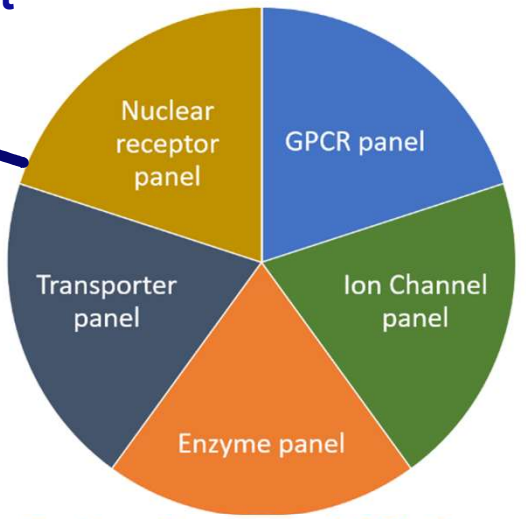
FAMILY	ASSAY	FORMAT	ITEM #
■ GPCR			
ADENOSINE	A_{2A}	•	0004
ADRENERGIC	$alpha_{1A}$		2338
	$alpha_{2A}$		0013
	$beta_1$	•	0018
	$beta_2$	•	0020
CANNABINOID	CB_1	•	0036
	CB_2	•	0037
CHOLECYSTOKININ	CCK_1 (CCK_A)	•	0039
DOPAMINE	D_1		0044
	D_{2S}	•	1322
ENDOTHELIN	ET_A	•	0054
HISTAMINE	H_1		0870
	H_2		1208
MUSCARINIC	M_1		0091
	M_2		0093
	M_3		0095
OPIOID & OPIOID-LIKE	$delta_2$ (DOP)	•	0114
	kappa (KOP)	•	1971
	mu (MOP)	•	0118
SEROTONIN	$5\text{-}HT_{1A}$	•	0131
	$5\text{-}HT_{1B}$		0132
	$5\text{-}HT_{2A}$	•	0471
	$5\text{-}HT_{2B}$	•	1333
VASOPRESSIN	V_{1a}	•	0159
■ TRANSPORTERS			
DOPAMINE	dopamine transporter		0052
NOREPINEPHRINE	norepinephrine transporter		0355
SEROTONIN	5-HT transporter		0439
■ ION CHANNELS			
GABA CHANNELS	BZD (central)	•	0028
GLUTAMATE CHANNELS	NMDA		0066
NICOTINIC CHANNELS	N neuronal α4β2	•	3029
SEROTONIN CHANNELS	$5\text{-}HT_3$		0411
Ca^{2+} CHANNELS	Ca^{2+} channel (L, dihydropyridine site)		0161
K^+ CHANNELS	hERG (membrane preparation)		1868
	K_V channel		0166
Na^+ CHANNELS	Na^+ channel (site 2)		0169
■ NUCLEAR RECEPTORS			
STEROID NUCLEAR RECEPTORS	AR	•	0933
	GR	•	0469
■ KINASES			
CTK	Lck kinase		2906
■ OTHER NON-KINASE ENZYMES			
AA METABOLISM	COX_1		0726
	COX_2		0727
MONOAMINE & NEUROTRANSMITTER	acetylcholinesterase		0363
	MAO-A		0443
PHOSPHODIESTERASES	PDE3A		2432
	PDE4D2		2434





All binding and enzymatic assay results were negative at 10 µM

No receptor/target-led pharmacological effect



SafetyScreen44™ Panel

In Vitro Bioactivity: Cell Stress Panel

Hatherell et al., 2020 *Tox Sci* 176(1): 11-33 https://doi.org/10.1093/toxsci/kfaa054

cyprotex AN EVOTEC COMPANY

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

Step 1

Selection of stress pathways

Mitochondrial Toxicity , Oxidative Stress, DNA damage, Inflammation, ER Stress, Metal Stress, Heat Shock, Hypoxia, Cell Health

Selection of biomarkers, probes or antibodies and optimisation of high-content imaging

Selection of cell line, exposure scenario and timepoints

HepG2 cell line, single exposure, 1h, 6h and 24h

Step 2

Selection of chemicals according to different classes and exposure scenarios (based on typical use of compound)

Non-stress inducers
- Caffeine (beverages, cosmetics)
- Coumarin (food, cosmetics)
- Niacinamide (food, cosmetics)
- Phenoxyethanol (cosmetics)

Stress inducers
- CDDO-Me (drug)
- Sulforaphane (food)
- DEM (industrial chemical)
- tBHQ (antioxidant)
- Doxorubicin (drug)
- Diclofenac (drug)
- Triclosan (antimicrobial)
- Troglitazone (drug)
- Pioglitazone (drug)
- Rosiglitazone (drug)

Step 3

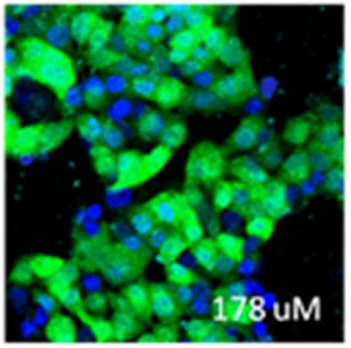
Selection of *in vitro* concentrations based upon realistic human exposures

Information on human exposure obtained from human clinical trials or PBK modelling

Selection of 8 *in vitro* concentrations (upper bound limited by ~20% cytotoxicity

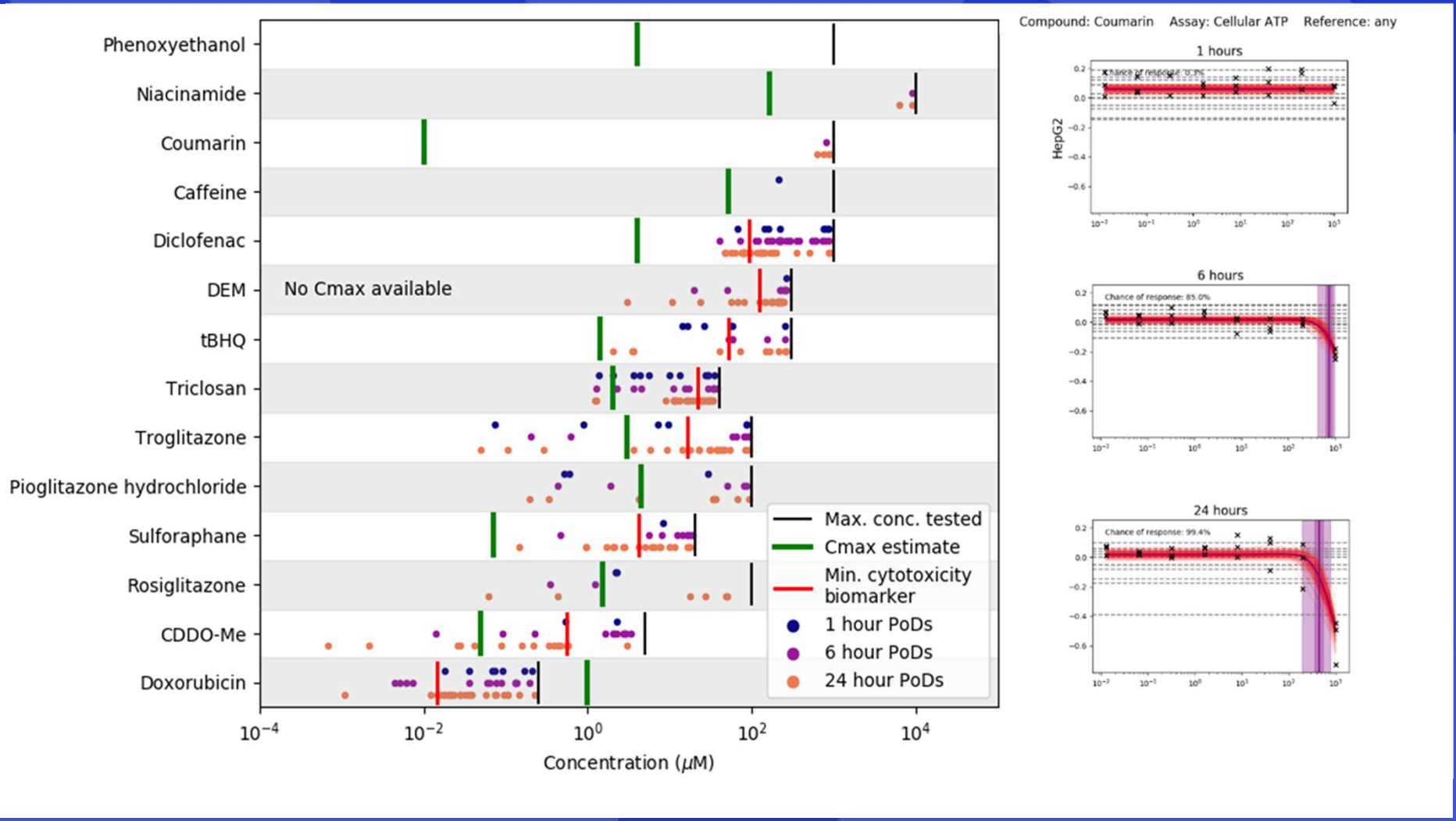
Key
- Exposure scenario adopted for chemical is 'high risk' (from consumer goods perspective).
- Exposure scenario adopted for chemical is 'low risk' (from consumer goods perspective).

- Mitochondrial Toxicity
- Oxidative Stress
- DNA damage
- Inflammation
- ER Stress
- Metal Stress
- Osmotic Stress
- Heat Shock
- Hypoxia
- Cell Health



***now conducted in HepaRG spheroids**

In Vitro Bioactivity: Cell Stress Panel



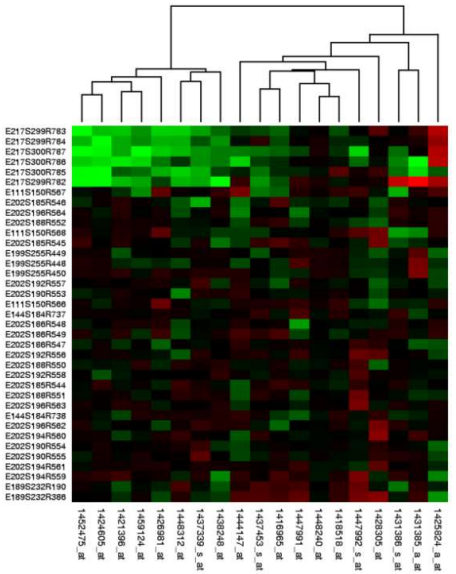
In Vitro Bioactivity: Tempo-Seq Technology

BioSpyder

High-Throughput Transcriptomics Gene Expression Profiling (HTTr)

1. Defining a safe operating exposure for systemic toxicity using a **NOTEL** (No Transcriptional Effect Level)
2. Defining compound similarity grouping (Read Across)

NOTEL is the derived concentration of a compound that does not elicit a meaningful change in gene expression (i.e. the threshold of the concentration that elicits minimal mechanistic activity)



Cell lines (chosen to express a range of relevant receptors)

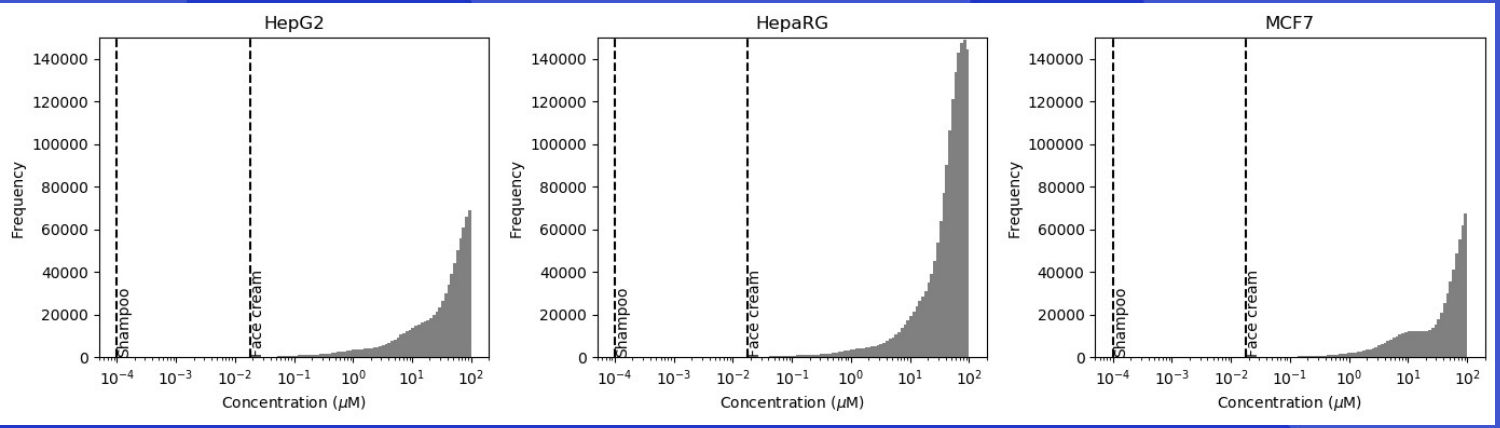
MCF-7 – human breast adenocarcinoma cell line

HepG2 – human liver carcinoma

HepaRG – terminally differentiated hepatic cells that retain many characteristics of primary human hepatocytes + as spheroids

N-HEK – primary normal human epidermal keratinocytes

In Vitro Bioactivity: Tempo-Seq Technology



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

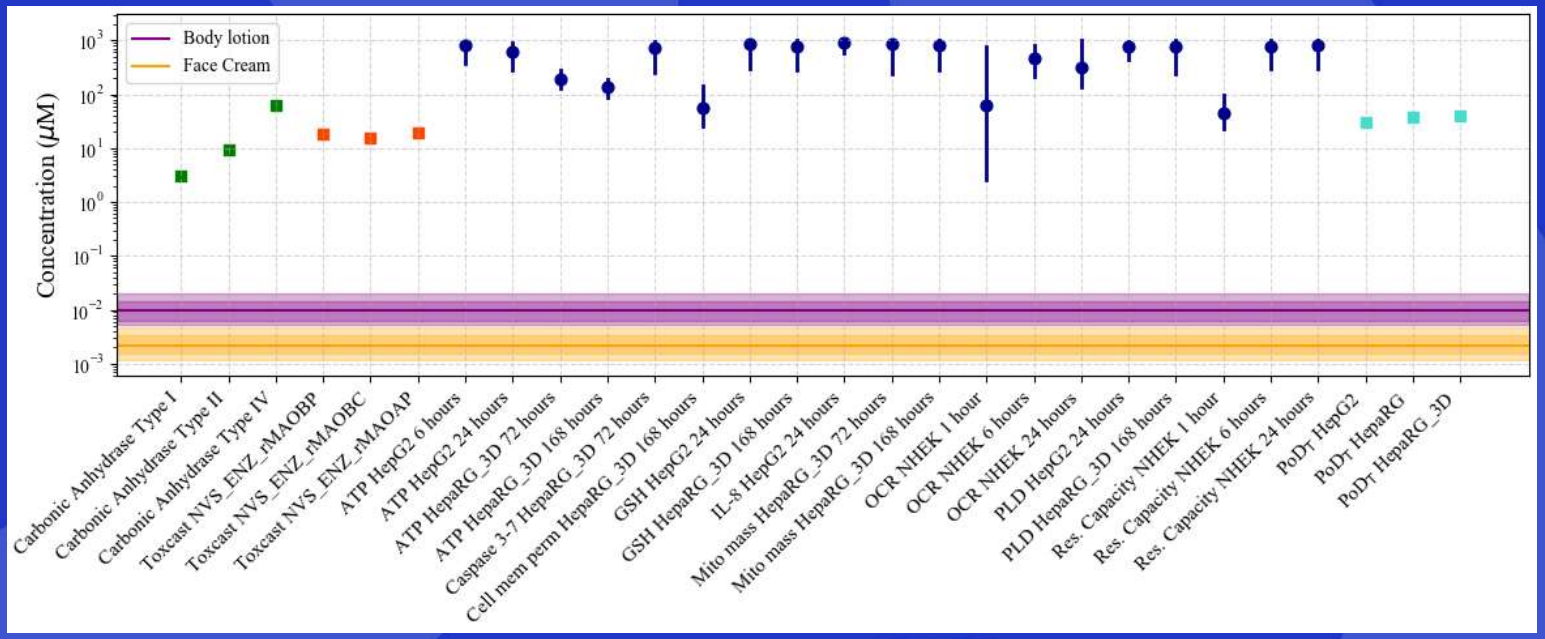
Cell Model	HepG2	MCF7	HepaRG 2D
Pathway Level Tests	(308 pathways)	(0 pathways)	(17 pathways)
20 pathways with the lowest pvalue Reactome	70	NA	58*
20 pathways with the lowest BMD Reactome	44	NA	58*
BMD of Reactome pathway with lowest BMD that meets significance threshold criteria	31	NA	38
Gene Level Tests	(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

Margin of Safety considering PODs and Exposure

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

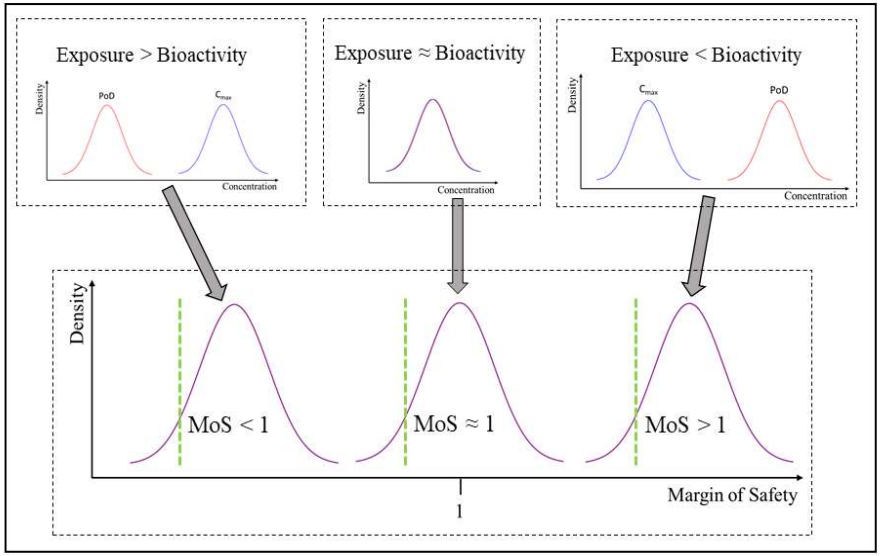
C_{max} expressed as a distribution:

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



Application of *Ab Initio A*pproach: Risk Assessment (NGRA)

Margin of safety or bioactivity:exposure ratio 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 (rat bain)	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

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Broader application and acceptance

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 Testing and Assessment,
No. 349

Regulatory Toxicology and Pharmacology 125 (2021) 105026

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

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

Paving the way for application of next generation risk assessment to safety decision-making for cosmetic ingredients

M.P. Dent [a,*], E. Vaillancourt [b], R.S. Thomas [c], P.L. Carmichael [a], G. Ouedraogo [d], H. Kojima [e], J. Barroso [f], J. Ansell [g], T.S. Barton-Maclaren [b], S.H. Bennekou [h], K. Boekelheide [i], J. Ezendam [j], J. Field [b], S. Fitzpatrick [k], M. Hatao [l], R. Kreiling [m], M. Lorencini [n,1], C. Mahony [o], B. Montemayor [p], R. Mazaro-Costa [q], J. Oliveira [r], V. Rogiers [s], D. Smegal [k], R. Taalman [t], Y. Tokura [u], R. Verma [k], C. Willett [v], C. Yang [w]

Highlights

- Next generation risk assessment (NGRA) is exposure-led and hypothesis-driven.
- NGRA has the potential to support safety decision making without animals.
- Some examples of NGRA are available, but more are needed.
- Effort is needed to develop and test NGRA for different decision contexts.
- Seven areas are identified to help develop NGRA as a robust and protective approach.

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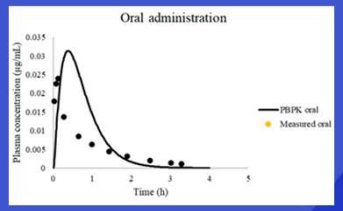
Evaluating the level of protection

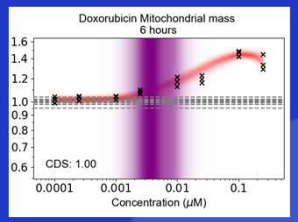
Chemical exposures scenarios

- ‘Low’ risk (from consumer goods perspective) – e.g. foods, cosmetics
- ‘High’ risk (from consumer goods perspective) – e.g. drugs

Define typical use-case scenarios benchmark chemical-exposures



PBK models of systemic exposure



Calculate the PoDs

Calculate BER

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Evaluating the level of protection

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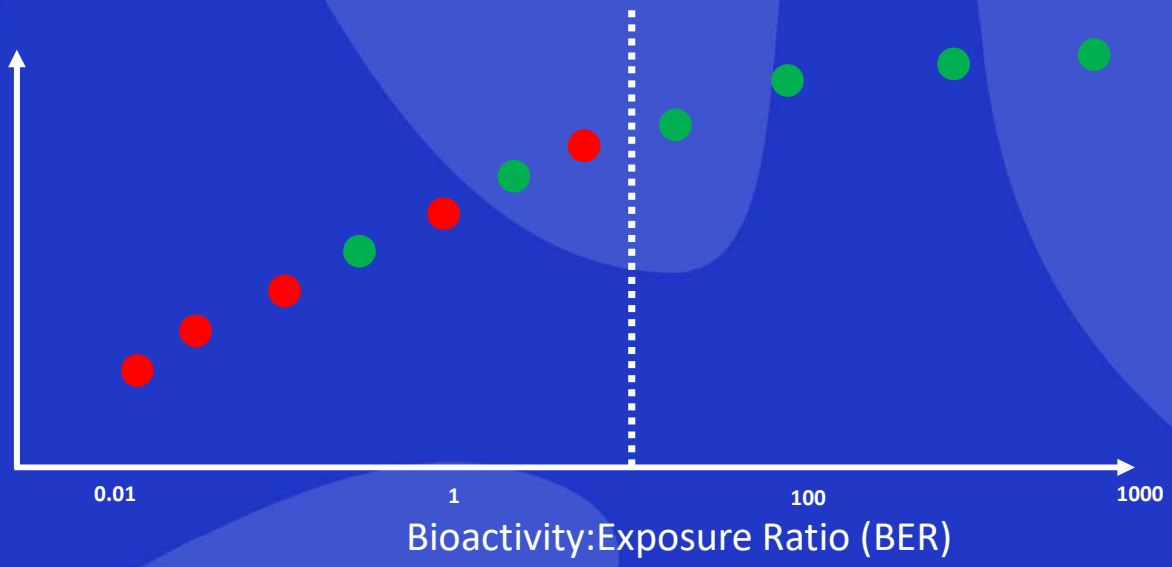

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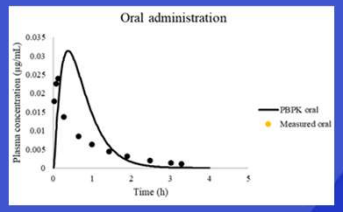
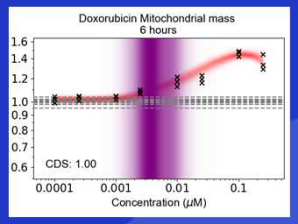


PBK models of systemic exposure



Calculate the PoDs

Calculate BER

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Where next?

- Clarity on the level of protection offered by this approach
  - Bioactivity *vs.* Adversity
- Adequacy of cell lines, timepoints, study designs – what to do when the 'protective not predictive' NGRA fails
- Role of metabolism
- Translating principles to other sectors/chemistries
  - Regulation keeping pace with science

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Conclusions

- We are seeing increased pace of development and application of next generation risk assessments in the consumer products industry
- NGRA is exposure-led, hypothesis driven, and requires clear articulation of the risk assessment question
- Progress has been possible with a change in mindset (protection not prediction)
- Once we understand the strengths and limitations why shouldn't the same approach be useful in different contexts?

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Acknowledgements

Maria Baltazar
Sophie Cable
Paul Carmichael
Richard Cubberley
Tom Cull
Julia Fentem
Sarah Hatherell
Jade Houghton
Predrag Kukic
Juliette Pickles
Hequn Li
Sophie Malcomber
Alistair Middleton
Tom Moxon
Alexis Nathanail
Beate Nicol
Ruth Pendlington
Sam Piechota
Georgia Reynolds
Joe Reynolds
Paul Russell
Nikol Simecek
Andy Scott
Carl Westmoreland
Andy White