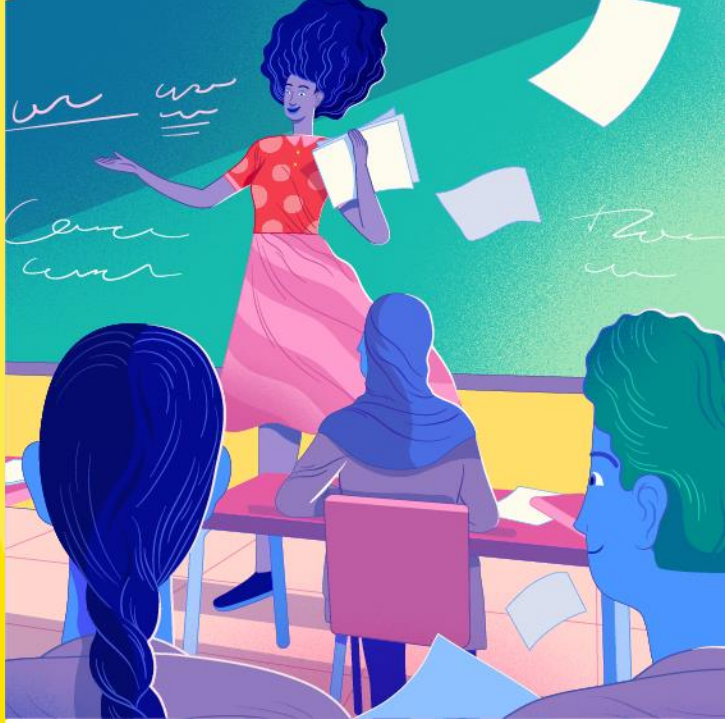
Implementation of NAMs in a Next Generation Risk Assessment

Maria Baltazar



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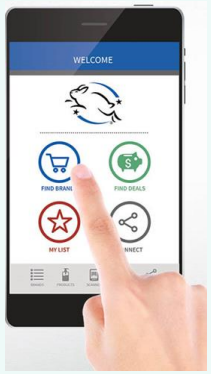
Increasing numbers of global consumers want their consumer products not tested on animals+ transparency



Scientific, societal, regulatory and ethical reasons are demanding change; calls for non-animal, next generation risk assessments

PETA Cruelty-Free and Vegan

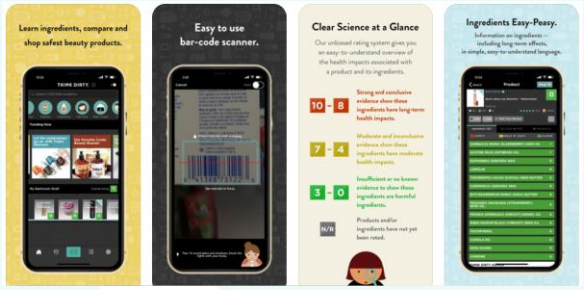
PETA Cruelty-Free

EUROPEAN VEGETARIAN UNION V-LABEL.EU VEGAN

NOT TESTED ON ANIMALS

NO CRUEL COSMETICS

Shop Cruelty-Free www.LeapingBunny.org



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RISK ASSESSMENT GOAL: Can we use a new ingredient safely?

Can we safely use X% of ingredient Y in product Z?

All safety assessments of cosmetic ingredients are exposure-driven:

Consumer Exposure

Potential hazards of the ingredients

Risk Assessment

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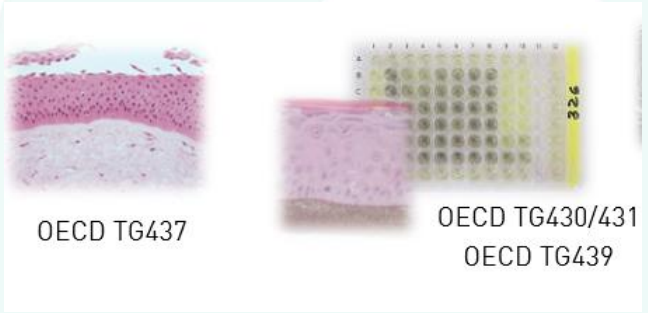
Maximising use of existing information and non-animal approaches

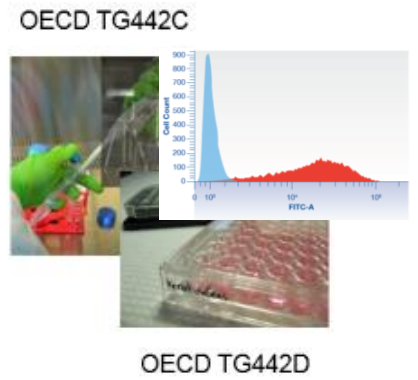
1. All available safety data
2. *In silico* predictions
3. Exposure-based waiving approaches[1]
4. History of safe use[2]
5. Read across
6. Use of *existing OECD in vitro approaches*

(Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity)

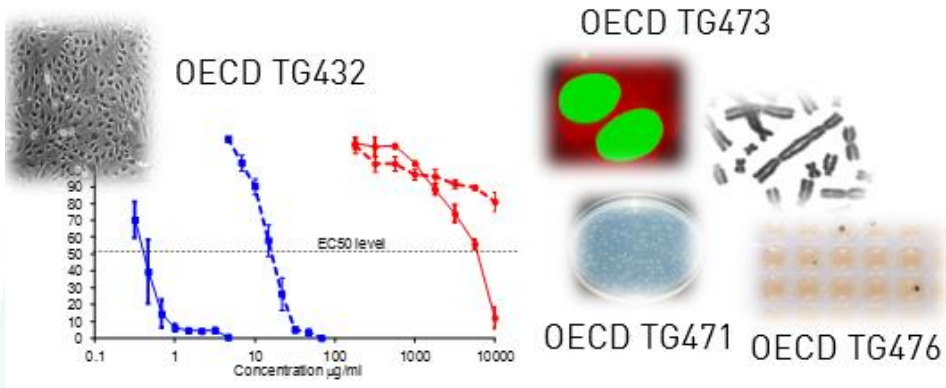
OECD test methods



Skin and eye irritation



Skin sensitisation



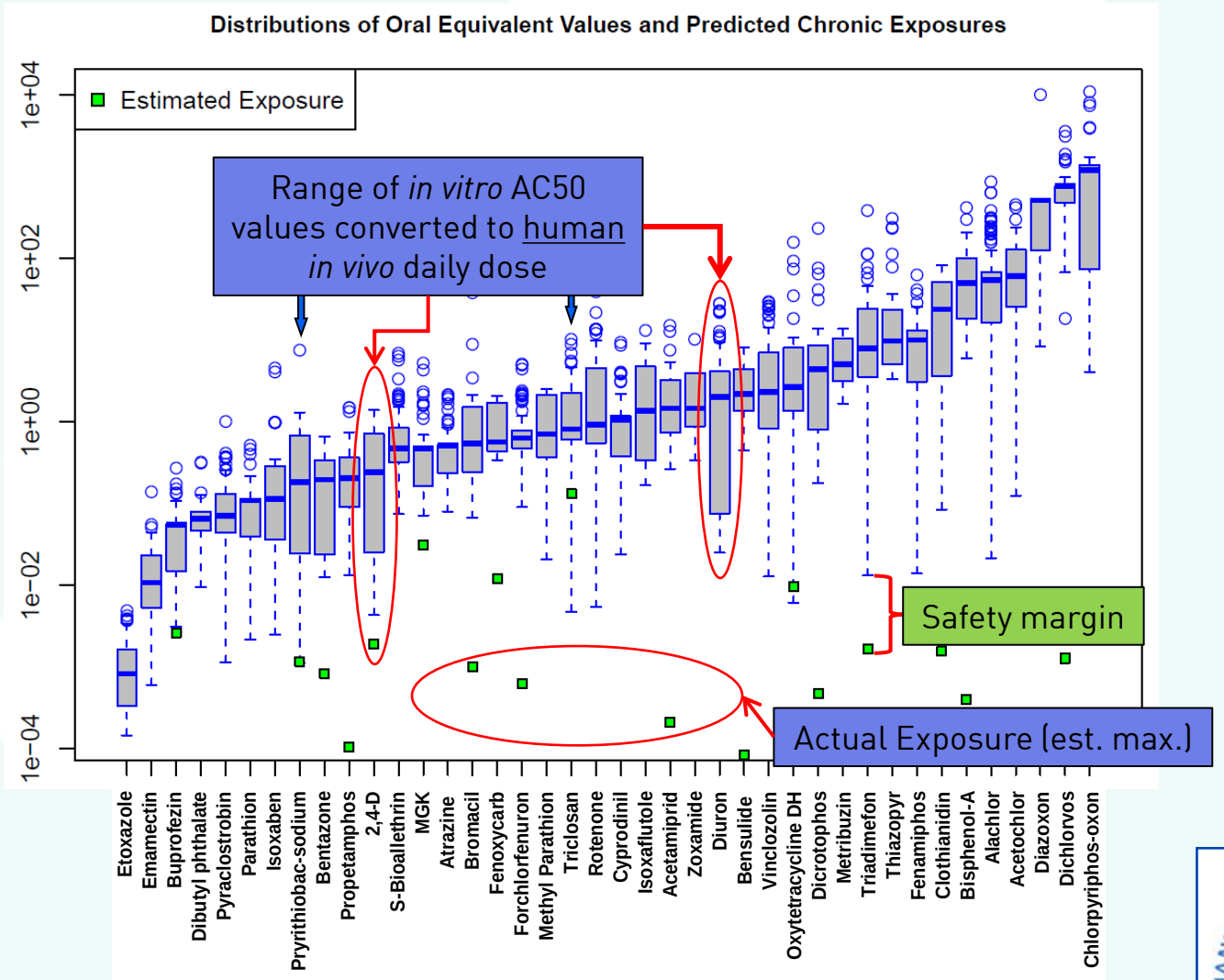
Phototoxicity

Genotoxicity

[1] Yang C, Barlow SM, Muldoon Jacobs KL, et al. Thresholds of Toxicological Concern for cosmetics-related substances: New database, thresholds, and enrichment of chemical space. *Food Chem Toxicol*. 2017;109(Pt 1):170-193. doi:10.1016/j.fct.2017.08.043

[2] Neely, T et al. "A multi-criteria decision analysis model to assess the safety of botanicals utilizing data on history of use." *Toxicology international* vol. 18,Suppl 1 (2011): S20-9. doi:10.4103/0971-6580.85882

In Vitro Bioactivity vs Bioavailability



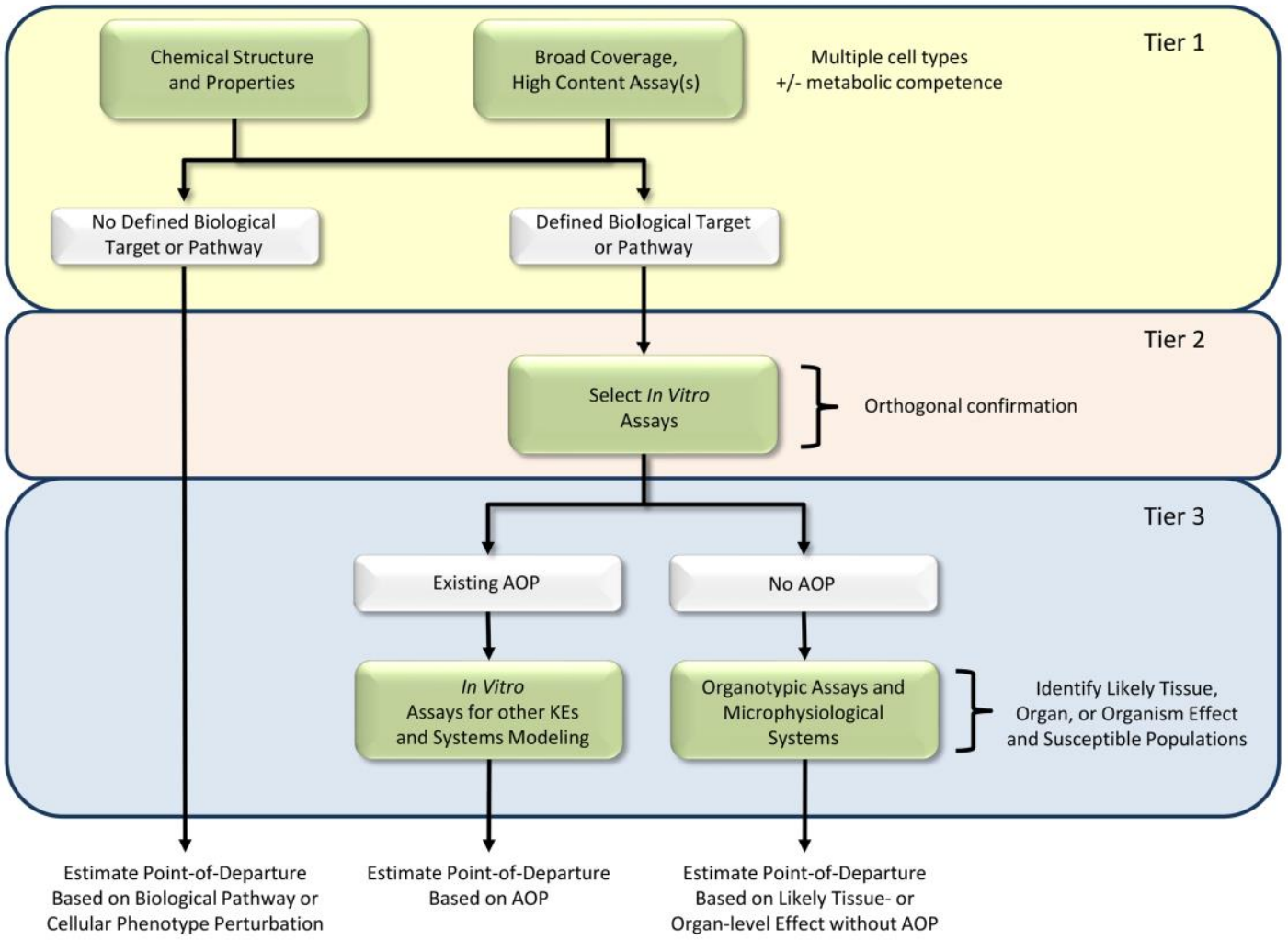
Slide from Dr Rusty Thomas, EPA, with thanks

Rotroff, *et al.* Tox.Sci 2010

The philosophy behind this type of risk assessment aimed at preventing harm is **based on the premise of "Protection not Prediction".**

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.**

The EPA Blueprint – A tiered approach to testing a novel chemical



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

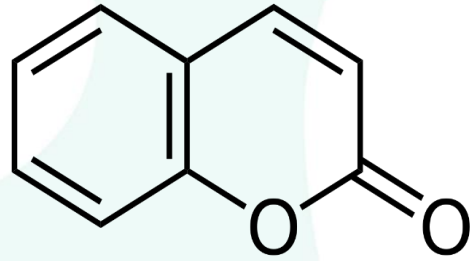
The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency

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

Russell S Thomas et al., 2019. The Next Generation Blueprint of Computational Toxicology at the U.S. Environmental Protection Agency. Tox Sci 169(2):317-332.

A case study approach – human health safety assessment required for…

0.1% COUMARIN IN FACE CREAM FOR EU MARKET (NEW FRAGRANCE)



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

Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

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 transparently and documented

ICCR
International Cooperation on Cosmetics Regulation

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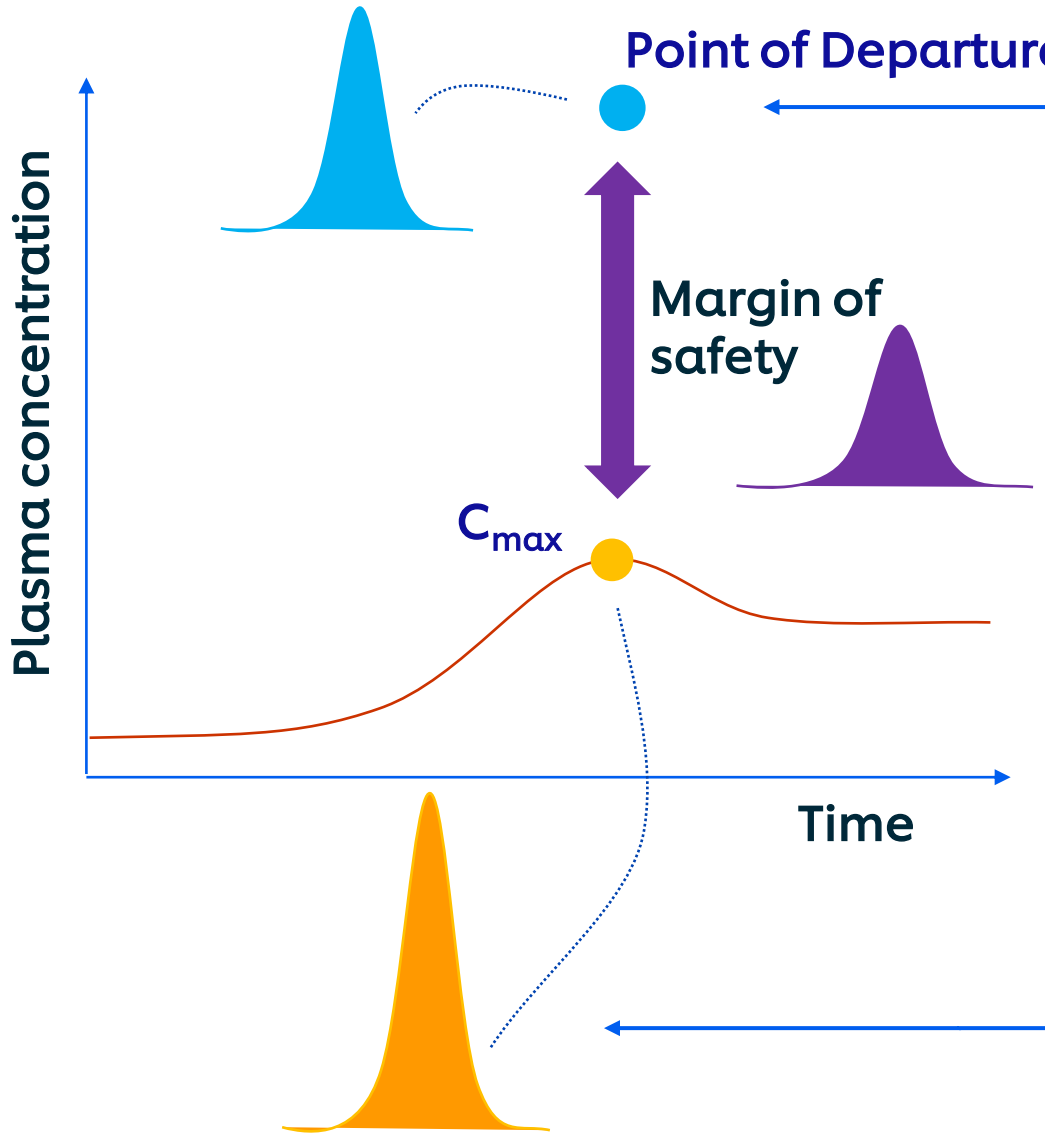
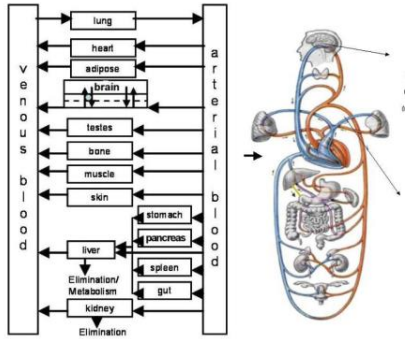
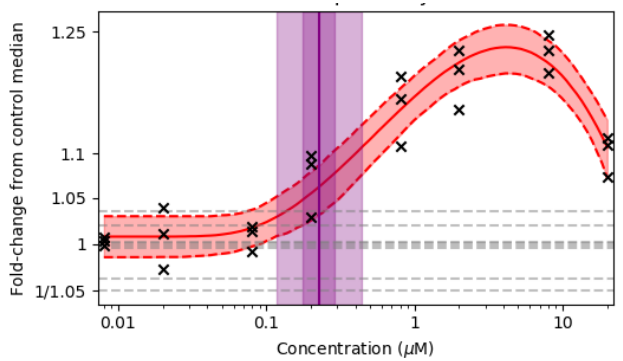
The Margin of Safety Approach

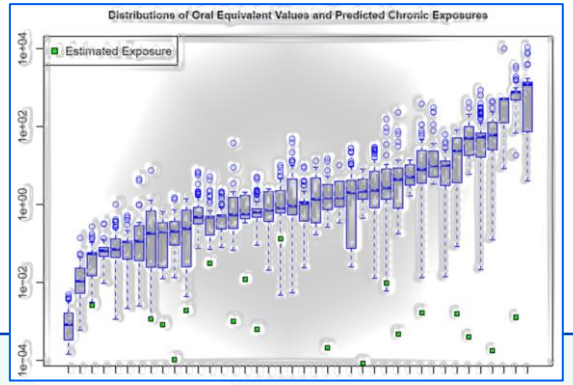


Exposure models (PBK, free/total concentration)

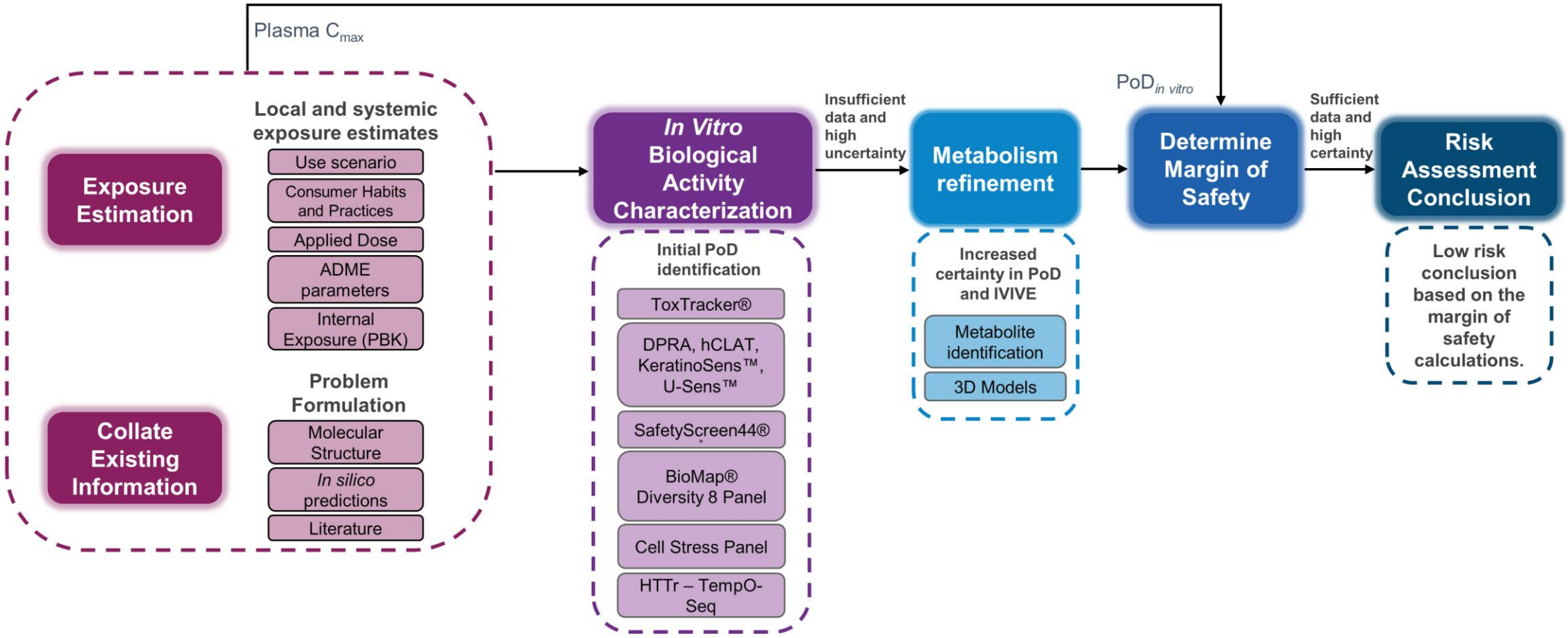


NAM Point of departure derived from *in vitro* concentration-response





Next-Generation Risk Assessment case study workflow for 0.1% coumarin in face cream



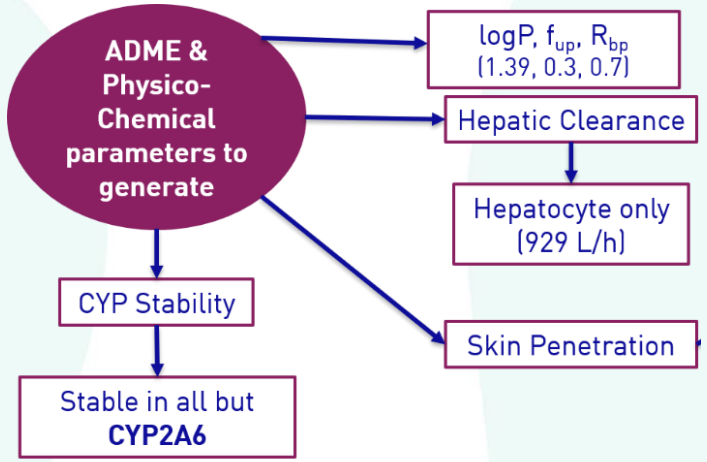
Baltazar *et al.*, (2020) *Tox Sci* Volume 176, Issue 1, 236–252

NAMS used to estimate internal concentration

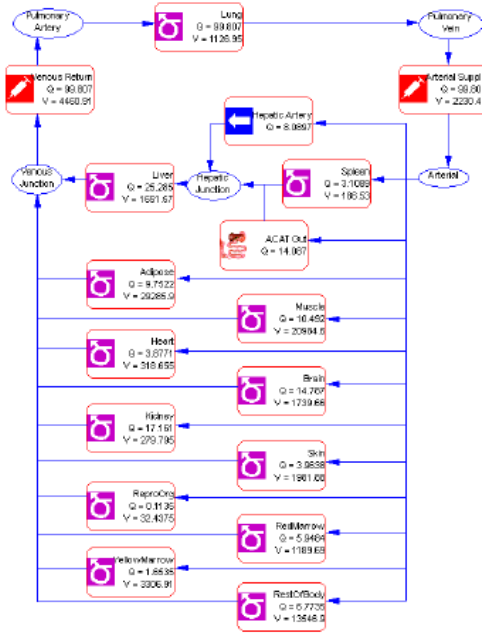
Exposure Estimation

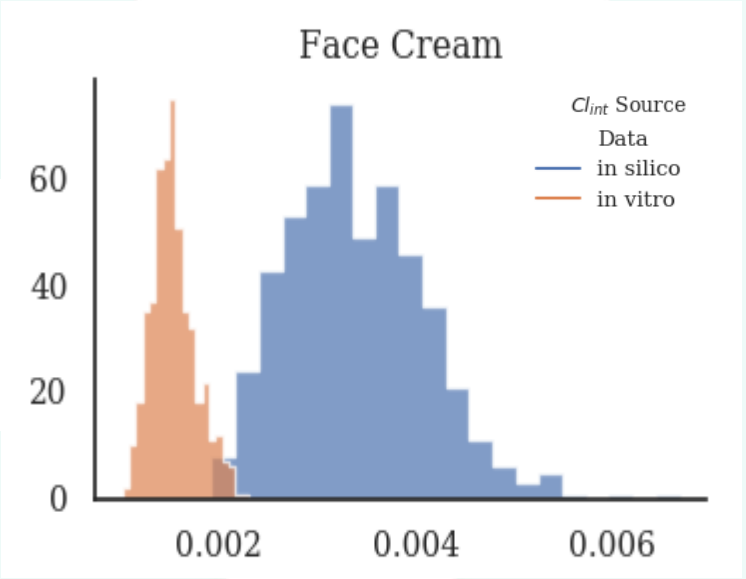
Local and systemic exposure estimates

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



GastroPlus® (Simulations Plus)





Simulated plasma concentration of coumarin after dermal exposure.

Moxon *et al.*, (2020). Application of physiologically based kinetic (PBK) modelling in the next generation risk assessment of dermally applied consumer products. Toxicology in Vitro Volume 63

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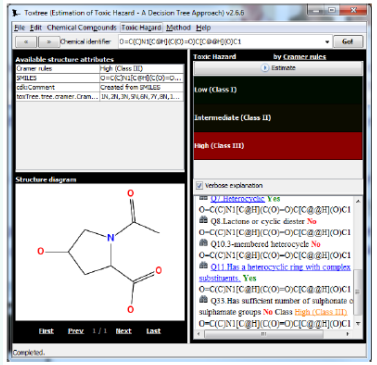
NAMS used to predict biological activity based on chemical structure

Collate Existing Information

Problem Formulation

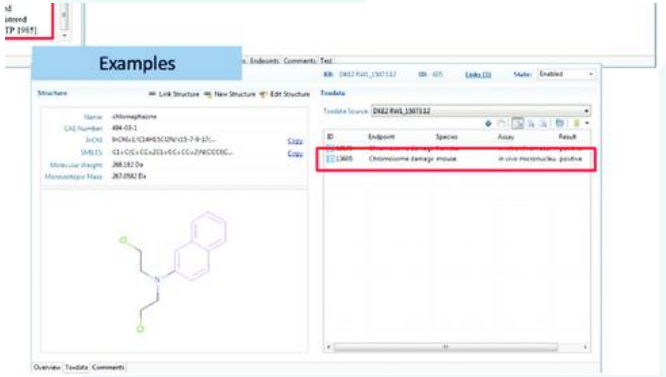
- Molecular Structure
- *In silico* predictions
- Literature

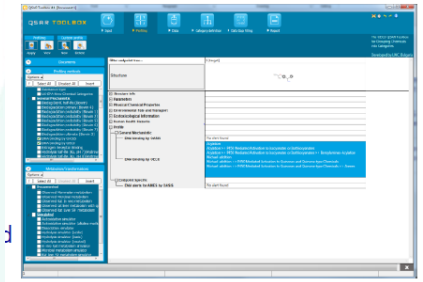
ToxTree



Derek nexus

OECD QSAR TOOLBOX





In silico models to predict Molecular initiating events (MIEs)

OXFORD SOT Society of Toxicology www.toxsci.oxfordjournals.org ToxSci 20 Years

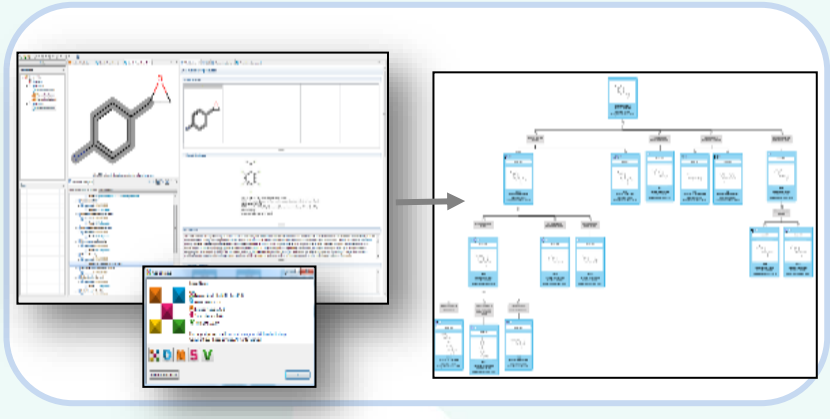
TOXICOLOGICAL SCIENCES, 165(1), 2018, 213–223

doi: 10.1093/toxsci/kfy144
Advance Access Publication Date: July 18, 2018
Research Article

Using 2D Structural Alerts to Define Chemical Categories for Molecular Initiating Events

Timothy E. H. Allen,* Jonathan M. Goodman,*,1 Steve Gutsell,† and Paul J. Russell†

Meteor nexus



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NAMS used to characterize the biological activity of coumarin

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

***In Vitro* Biological Activity Characterization**

Initial PoD identification

- ToxTracker®
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

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.

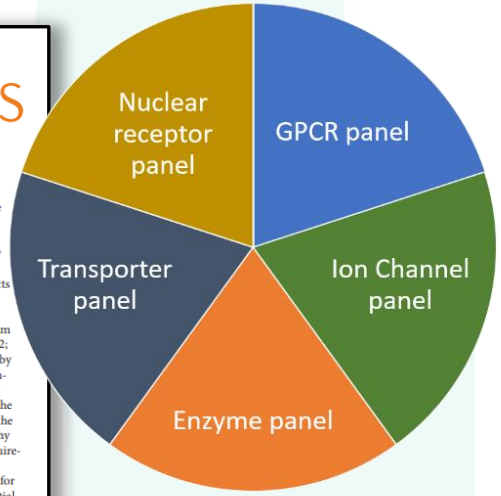
In vitro pharmacological profiling involves the screening of compounds against a broad range of targets (receptors, ion channels, enzymes and transporters) that are distinct from the intended

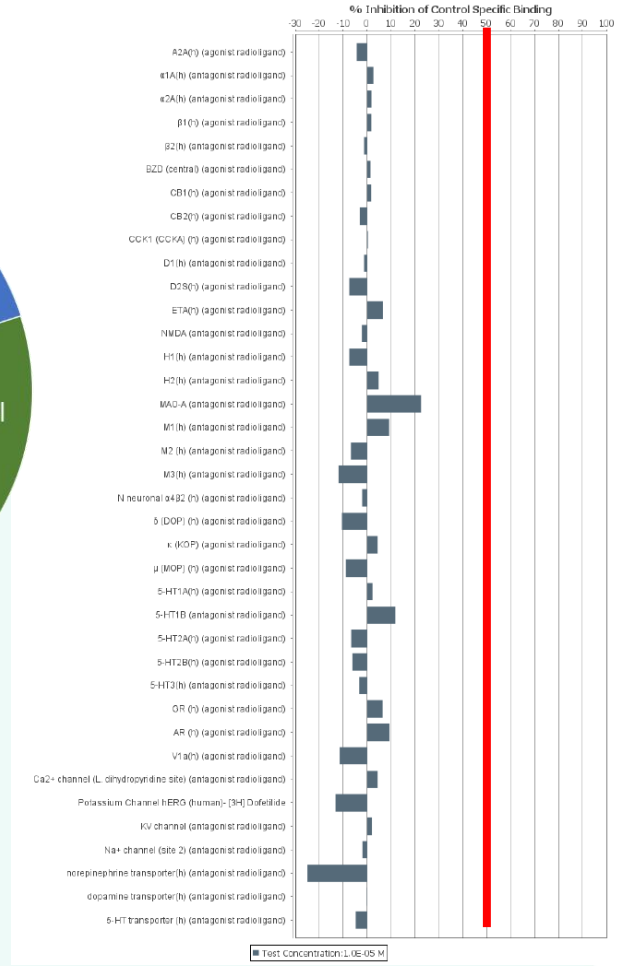
safety testing of drug candidates and are designed to prevent serious ADRs from occurring in clinical studies.

The only *in vitro* pharmacology assay that is absolutely required by regulatory authorities is one that measures the effects of new chemical entities on the ionic current of native (I_{Kr}) or heterologously expressed human voltage-gated potassium channel subfamily H member 2 (KCNH2; also known as hERG)[2]. The mechanism by which blockade of hERG can elicit potentially fatal cardiac arrhythmias (torsades de pointes) following a prolongation of the QT interval is well characterized[2,3], and the seriousness of this ADR is one reason why this assay is a mandatory regulatory requirement. Receptor binding studies are also recommended as the first-tier approach for the assessment of the dependence potential of novel chemical entities[3].

However, current regulatory guidance does not describe which targets should constitute an *in vitro* pharmacological profiling panel and does not indicate the stage of the discovery process at which *in vitro* pharmacological profiling should occur. 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





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NAMS used to characterize the biological activity of coumarin

In Vitro Biological Activity Characterization

Initial PoD identification

- ToxTracker®
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

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

- Mitochondrial Toxicity
- Oxidative Damage
- DNA damage
- Inflammation
- ER stress
- Metal stress
- Heat Shock
- Hypoxia
- Cell Health

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academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 2020, 1–23
doi: 10.1093/toxsci/kfaa054
Advance Access Publication Date: May 6, 2020
Research article

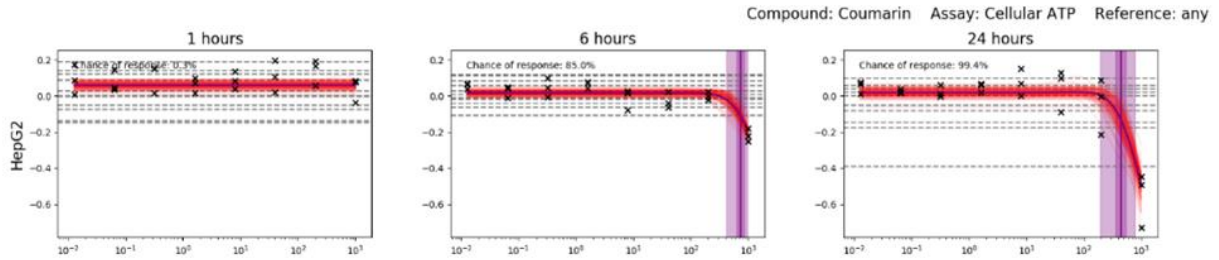
FEATURED

Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

Sarah Hatherell,* Maria T. Baltazar,* Joe Reynolds,* Paul L. Carmichael,* Matthew Dent,* Hequn Li,* Stephanie Ryder,† Andrew White,* Paul Walker ,† and Alistair M. Middleton*,1

*Unilever Safety and Environmental Assurance Centre, Colworth Science Park, Sharnbrook, Bedfordshire

Dose-response analysis and in vitro PoD derivation



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

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NAMS used to characterize the biological activity of coumarin

In Vitro Biological Activity Characterization

Initial PoD identification

- ToxTracker®
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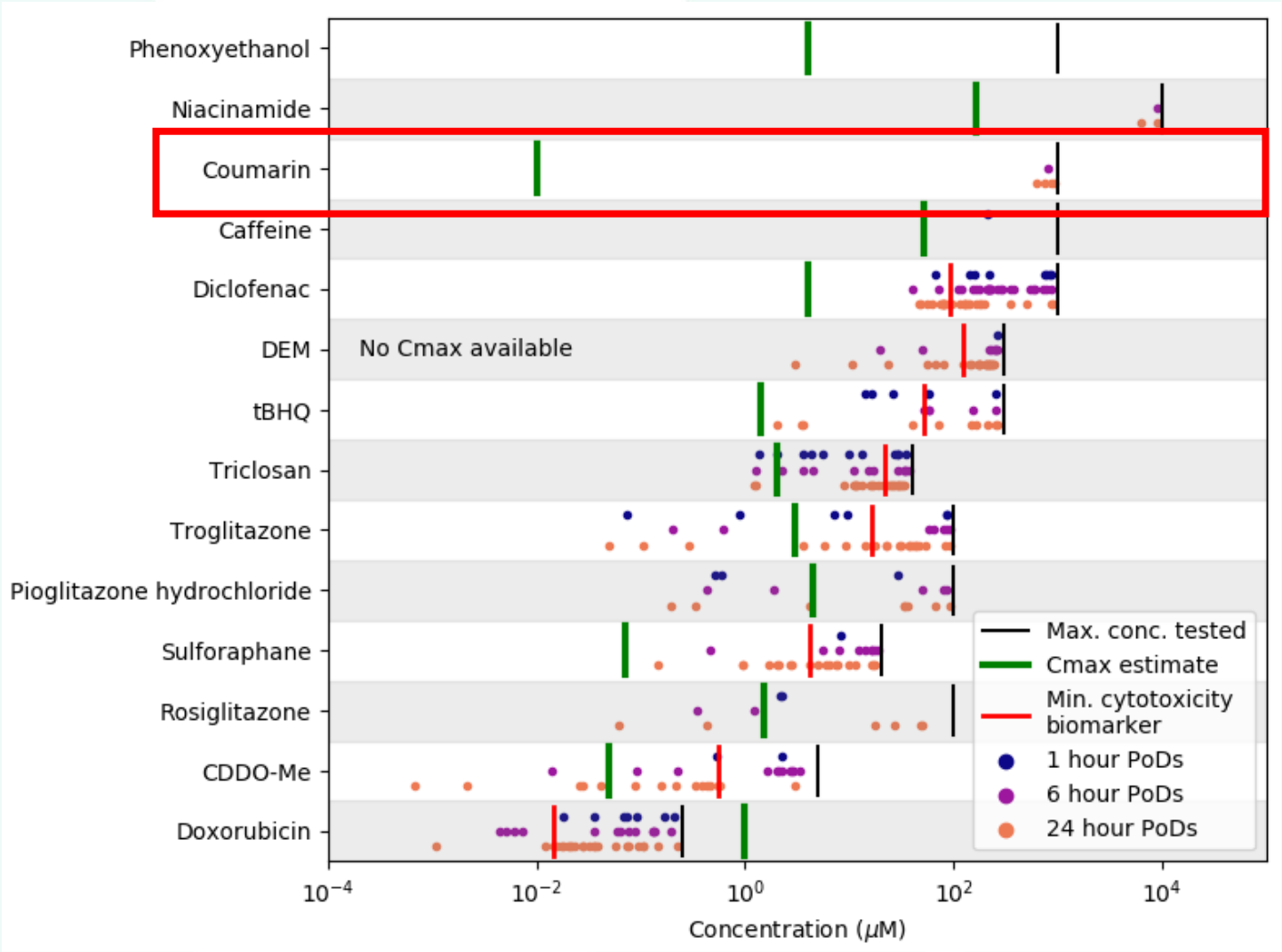


Hatherell *et al.*, 2020, Identifying and characterizing stress pathways of concern for consumer safety in next generation risk assessment, Tox. Sci. in Press. https://doi.org/10.1093/toxsci/kfaa054

NAMS used to characterize the biological activity of coumarin

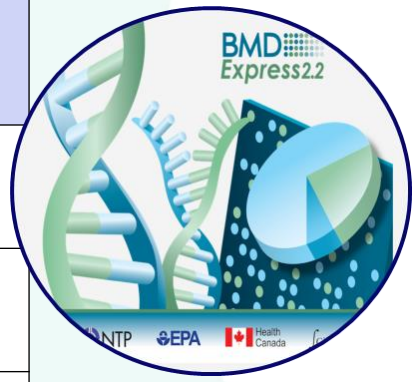
In Vitro Biological Activity Characterization

Initial PoD identification

- ToxTracker®
- SafetyScreen44®
- BioMap® Diversity 8 Panel
- Cell Stress Panel
- HTTr – TempO-Seq

Transcriptomics can be applied as a broad nontargeted biological screen – PoD determination using BMDexpress

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



Farmahin, R., Williams, A., Kuo, B. *et al.* Recommended approaches in the application of toxicogenomics to derive points of departure for chemical risk assessment. *Arch Toxicol* **91,** 2045–2065 (2017). https://doi.org/10.1007/s00204-016-1886-5

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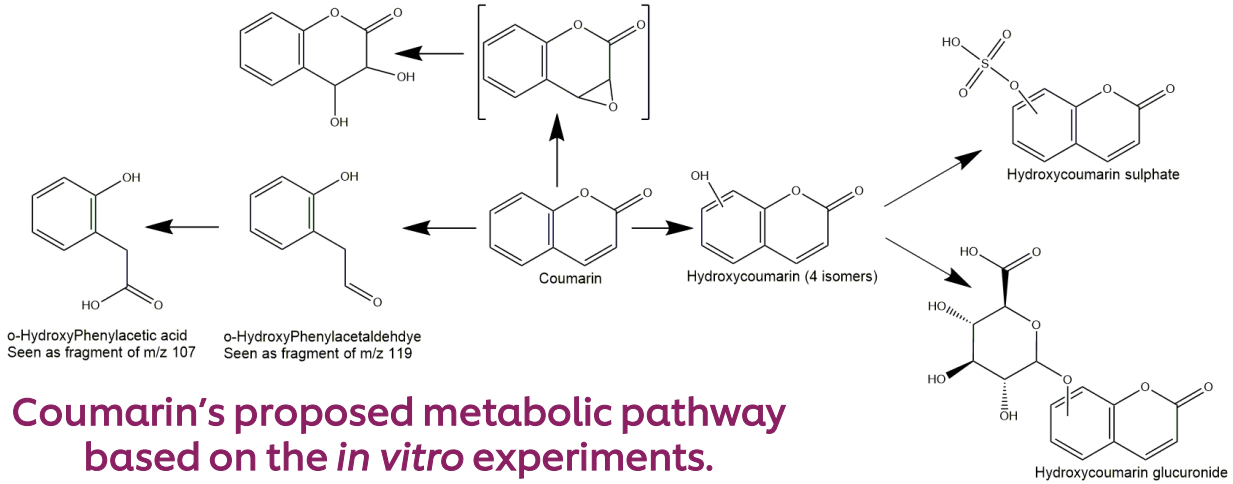
NAMS used in Refinement Steps depend on the problem formulation and remaining uncertainties

Metabolism refinement

Increased certainty in PoD and IVIVE

- Metabolite identification
- 3D Models

Human *In vitro* metabolism



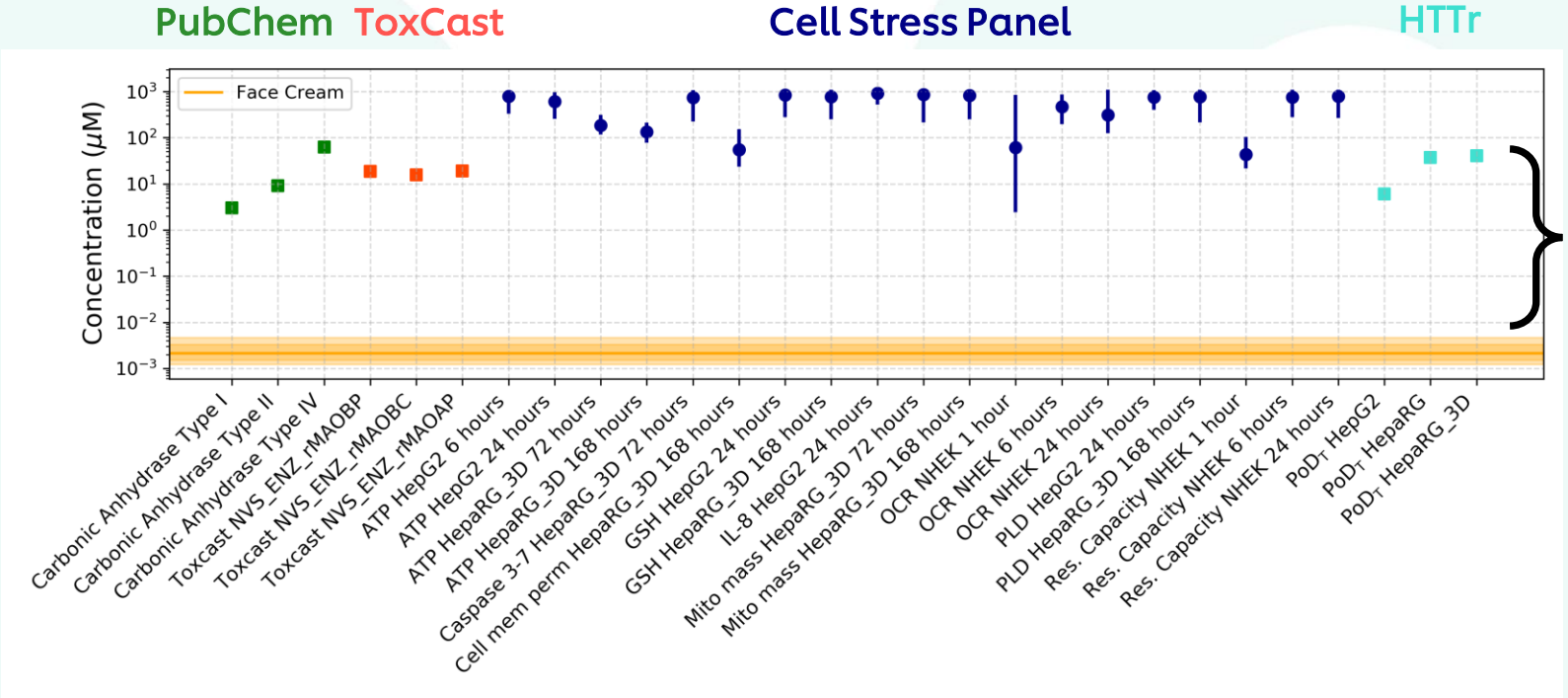
Coumarin's proposed metabolic pathway based on the *in vitro* experiments.

Cell stress & HTTr in 3D HepaRG models

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

Determination of MoS using NAMs and risk assessment conclusion

Determine Margin of Safety



Margin of safety

The 5th percentile of the MoS distribution ranged between 706 and 96738

In this case study:

- **Weight of evidence suggested that the inclusion of 0.1% coumarin in face cream is safe for the consumer**

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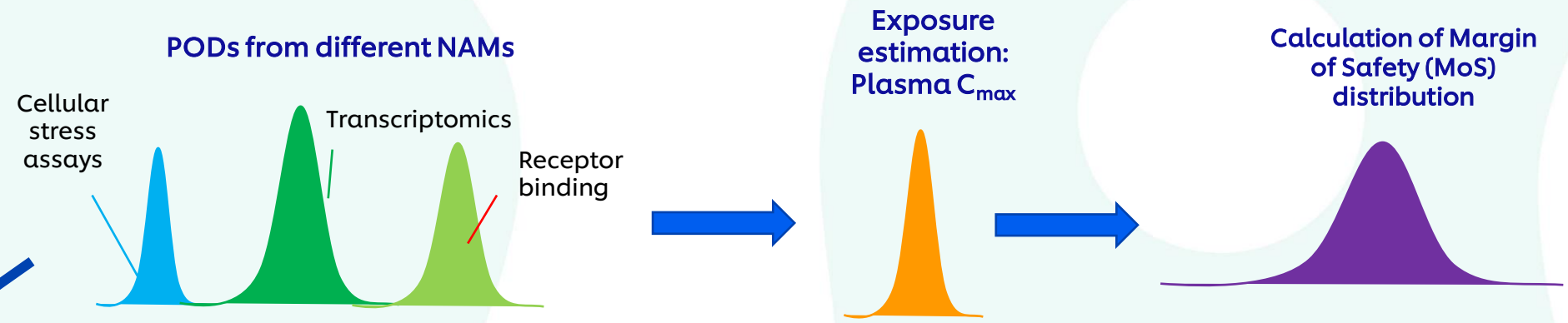
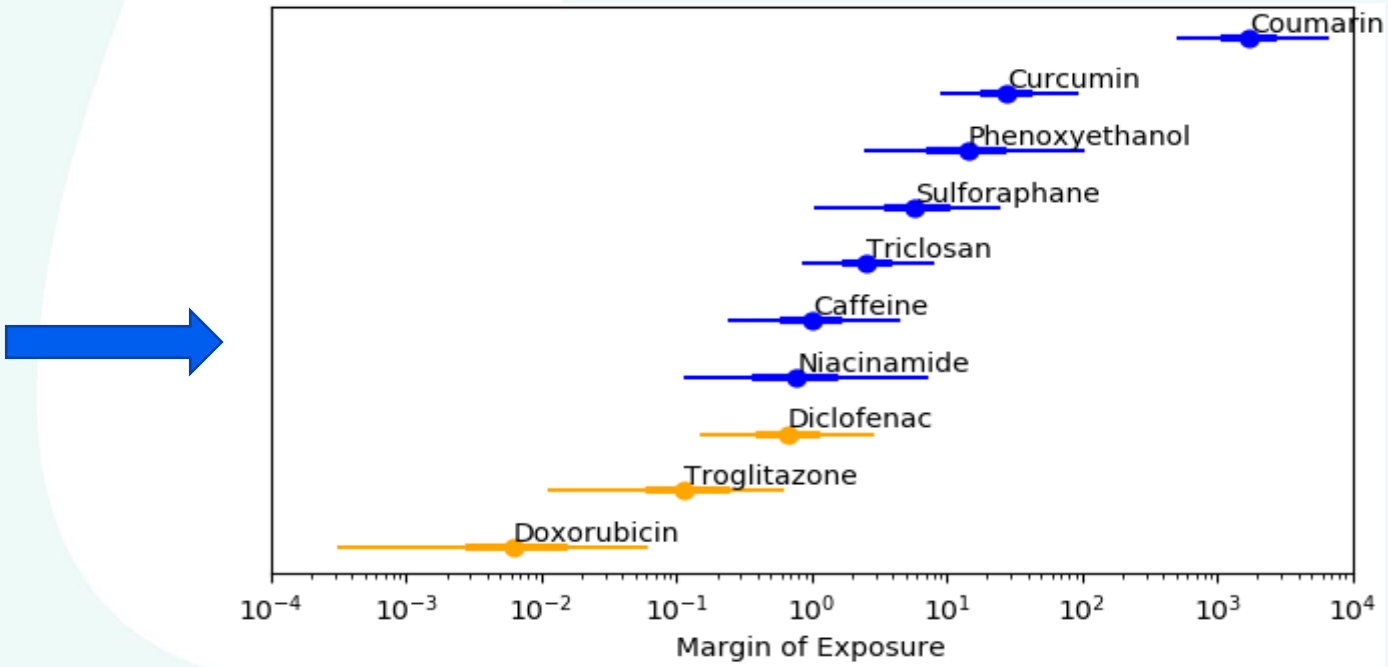
Ongoing research: How can we conclude what MoS derived from NAMs is large enough to be protective of human health?



Chemical exposures

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



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Concluding remarks

1. Available tools can be integrated to make a safety decision; multidisciplinary team needed!
2. NGRA is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions
3. Need to ensure quality/robustness of the non-standard (non-TG) work and to characterise uncertainty to allow informed decision-making
4. Rethinking MoS/MoE – future evaluation of the approach to infer a low risk space
5. Shortcomings will be addressed by current and future research
6. More research, creativity and examples needed to land this successfully across the community

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Acknowledgements

Core Team:

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Extended Team:

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