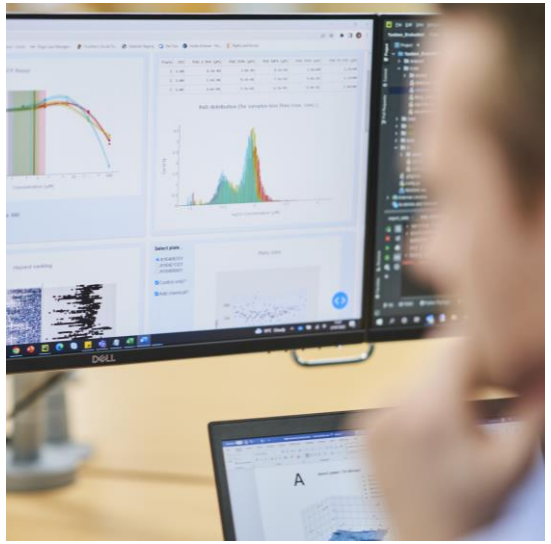
Next generation risk assessment – principles and tools

Matt Dent, Unilever Safety and Environmental Assurance Centre, UK



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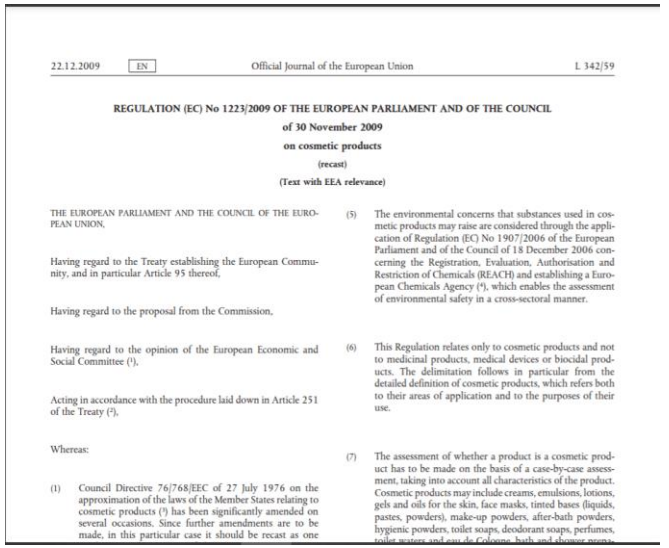
The need for non-animal safety assessments

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 prepa-

Regulatory Change

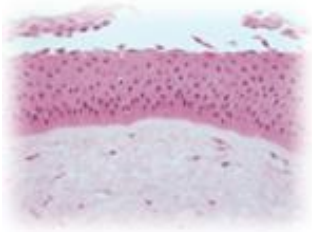
Cosmetic safety assessment: key safety considerations

Exposure data (external/applied dose and internal exposure)

Corrosion/irritation (skin/eye)
Phototoxicity
Mutagenicity/genotoxicity
Skin sensitisation
Systemic toxicity (focus on repeat dose)
Reproductive toxicity
Carcinogenicity

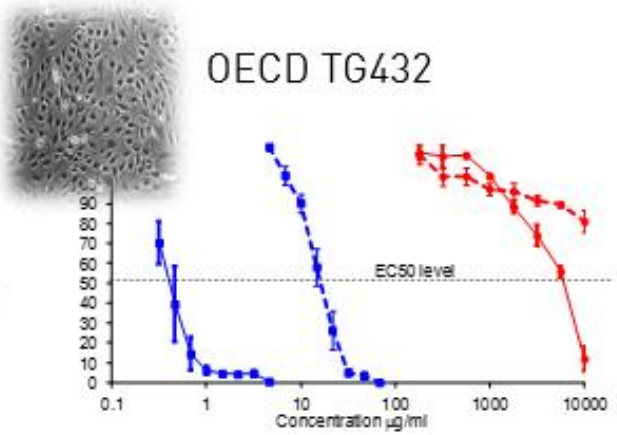
SCCS Notes of Guidance, 12th Revision

Use of Existing OECD *In Vitro* Approaches

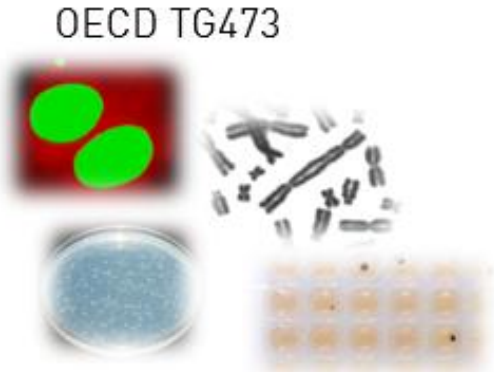


OECD TG437

OECD TG430/431
OECD TG439



OECD TG432



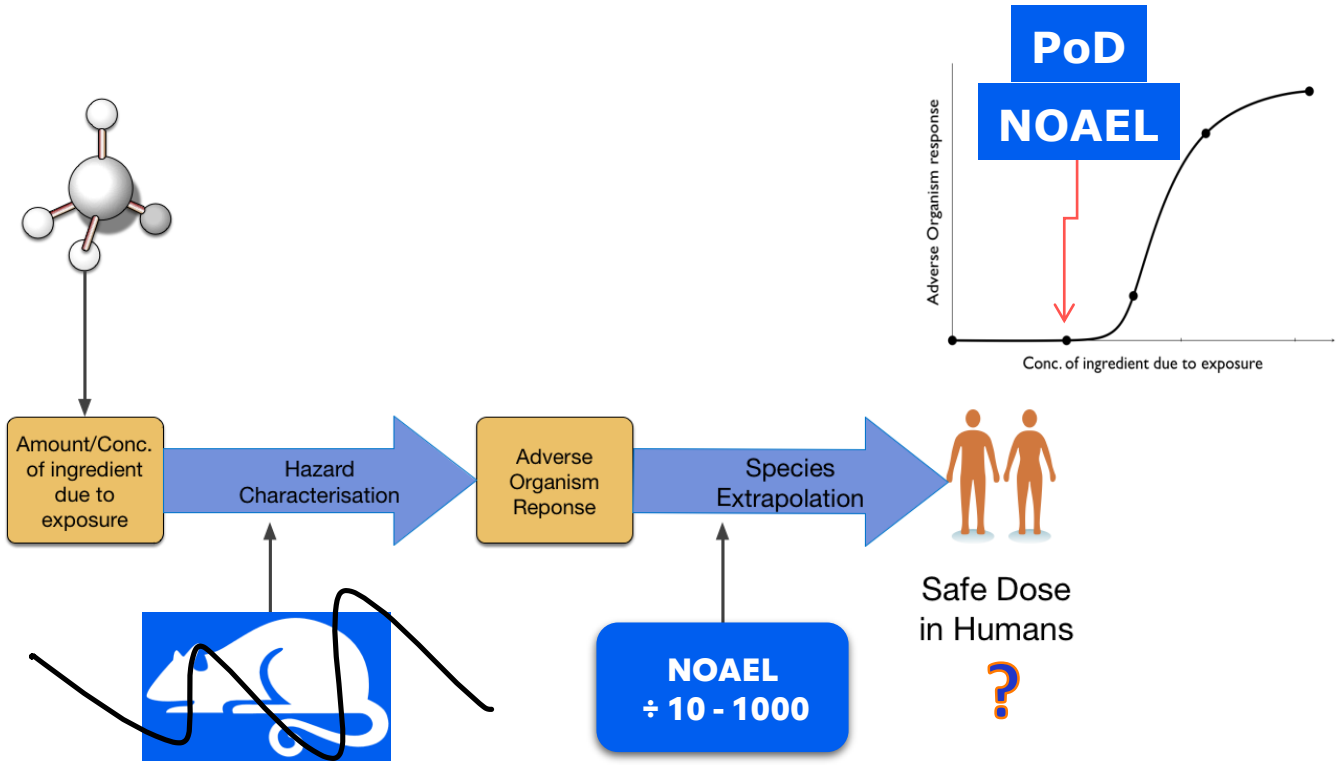
OECD TG473
OECD TG471
OECD TG476

Skin and eye irritation; skin sensitization; phototoxicity; mutagenicity…

…what about systemic effects?

Are non-animal safety assessments even possible for systemic toxicity?

Systemic toxicity isn't like local toxicity



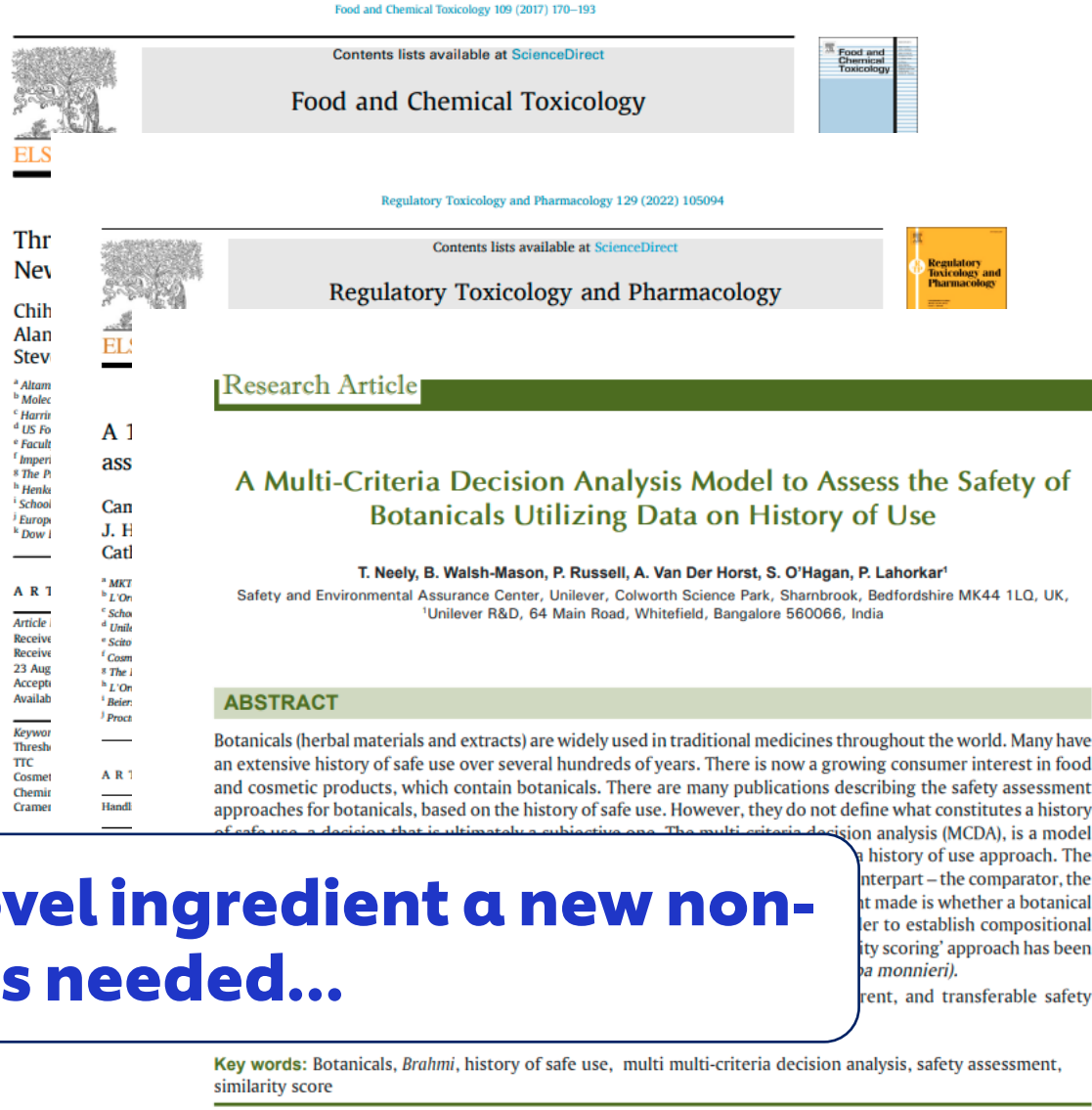
Many possible adversities...ADME considerations...Homeostasis

Well-established approaches for systemic toxicity

Threshold of Toxicological Concern
(Yang et al 2017) https://doi.org/10.1016/j.fct.2017.08.043

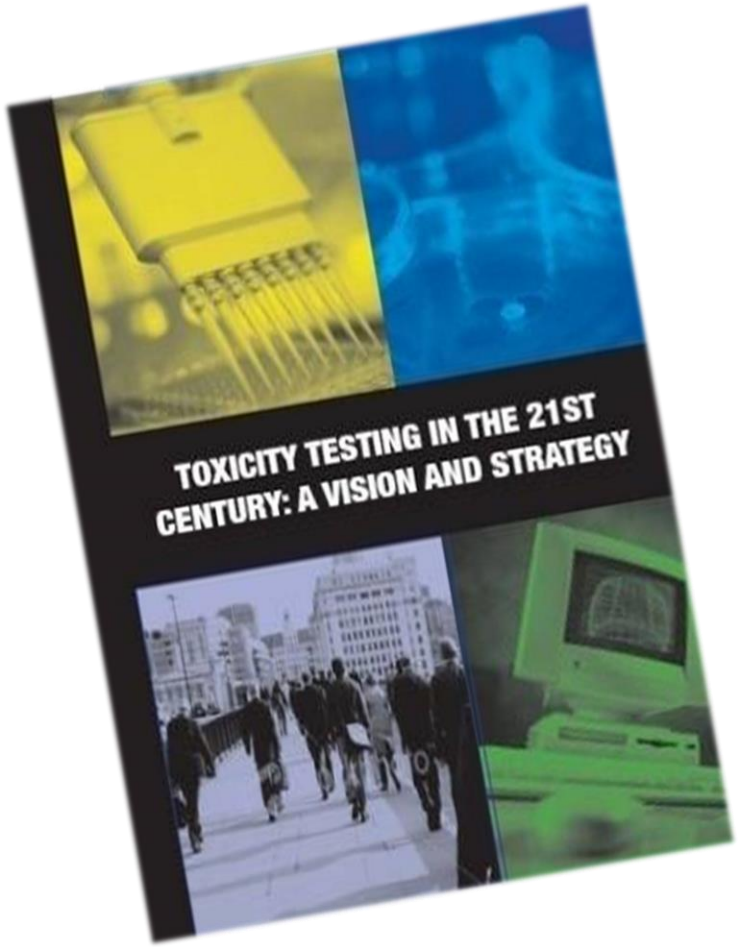
Read across
(Alexander-White et al 2022)
https://doi.org/10.1016/j.yrtph.2021.105094

History of Safe Use
(Neely et al 2011) PMID: 22025816



For 'significant' exposures to a novel ingredient a new non-animal paradigm is needed…

2007 Toxicity Testing in the 21st Century (TT21C)



"Advances in toxicogenomics, bioinformatics, systems biology, and computational toxicology could transform toxicity testing from a system based on whole-animal testing to one founded primarily on in vitro methods that evaluate changes in biologic processes using cells, cell lines, or cellular components, preferably of human origin."

Perturbation of 'toxicity pathways' and stress responses

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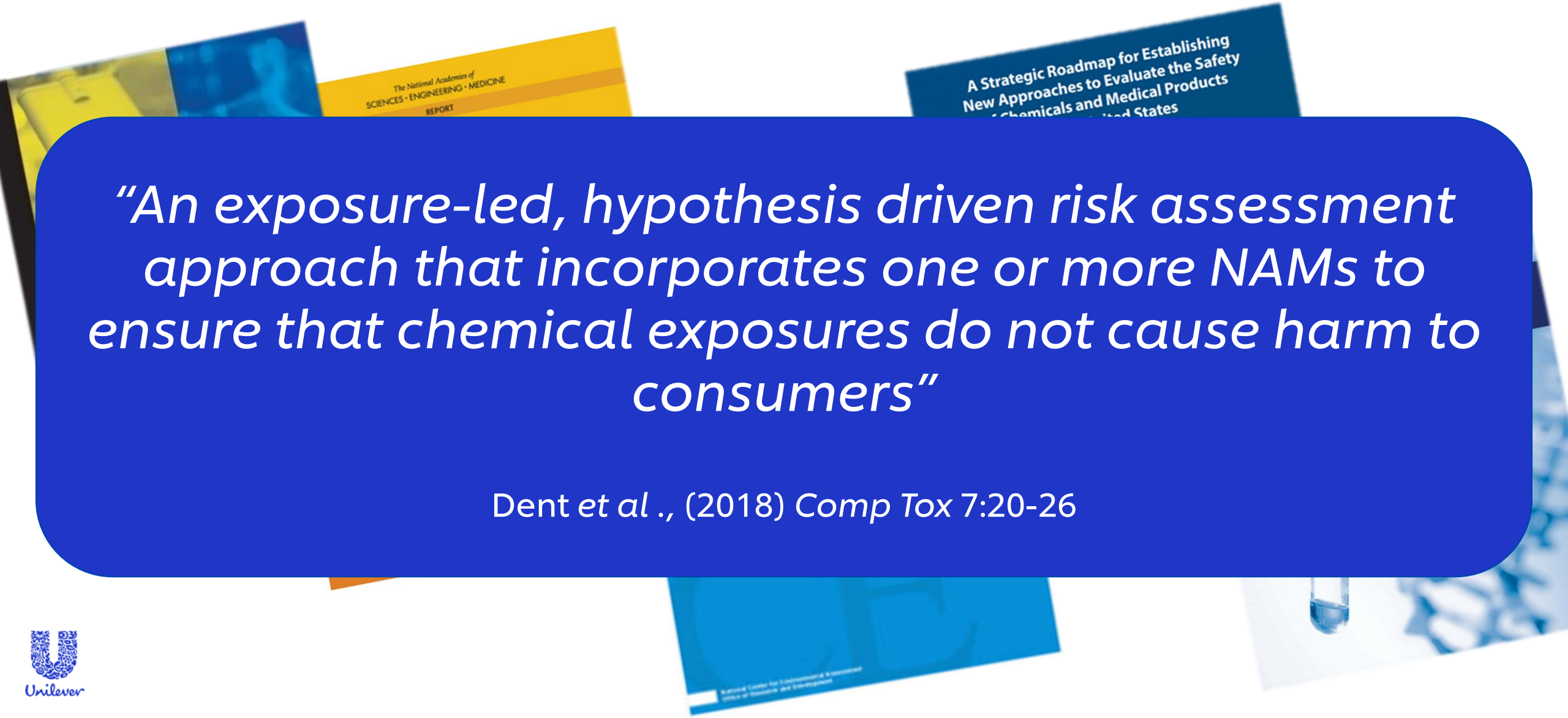
What is next generation risk assessment (NGRA)?



"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

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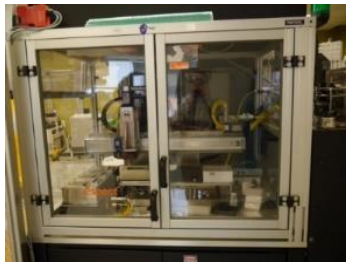
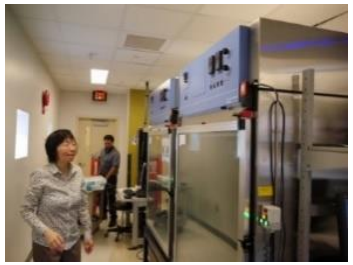
One Interpretation: Tox21/ToxCast ~700 HTS Biological Pathways Assays



https://www.epa.gov/chemical-research/toxicity-forecasting

National Institute of Environmental Health Sciences (NIEHS) / National Toxicology Program (NTP)

National Center for Advancing Translational Sciences (NCATS)

U.S. Food and Drug Administration (FDA)

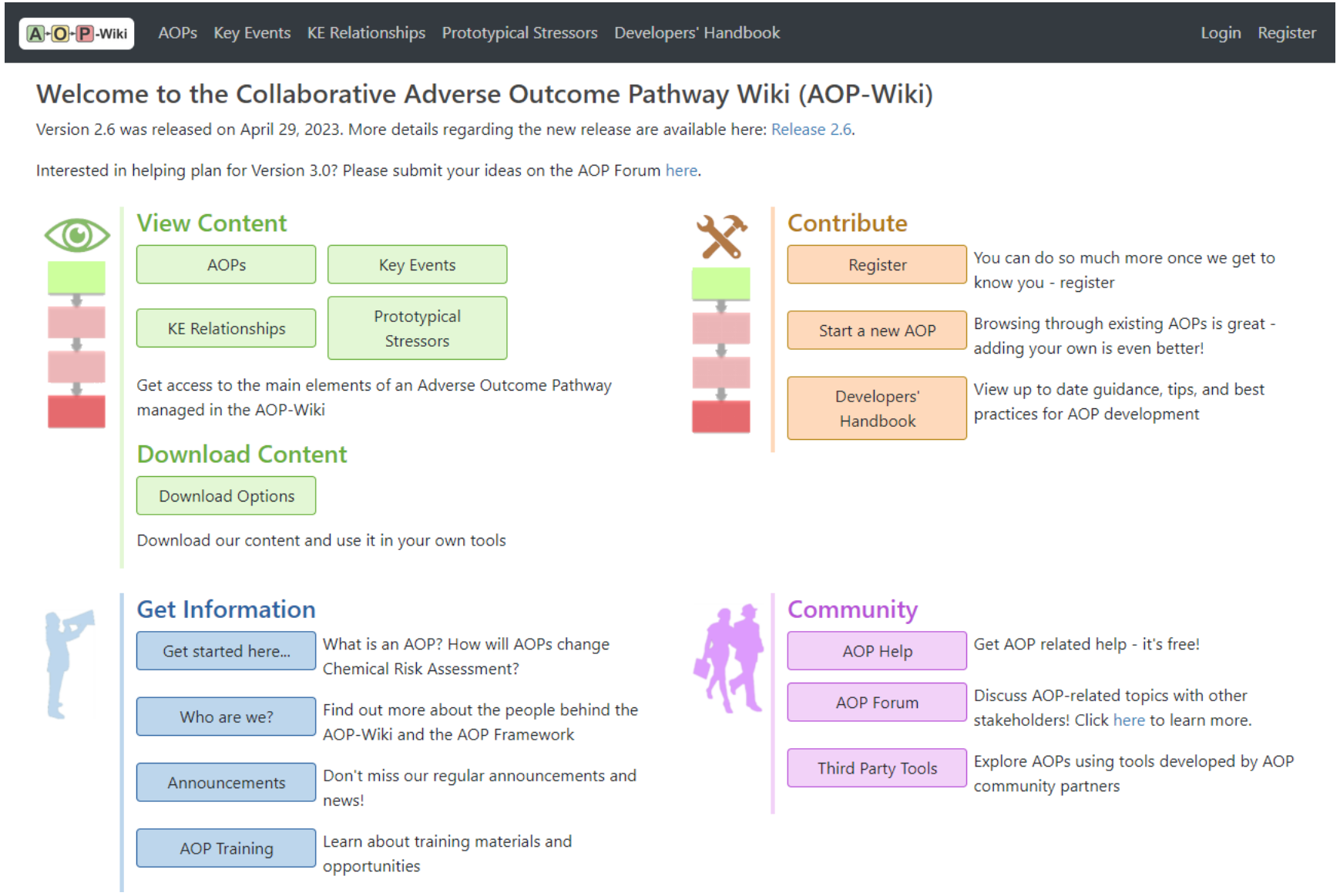
National Center for Computational Toxicology (EPA)

What to do with all these data?!

The adverse outcome pathway concept (AOPs)

Examples:	Nuclear receptor binding	Altered hormone levels	Cell proliferation	Cell transformation	Increased tumour incidence

AOP-Wiki (aopwiki.org)



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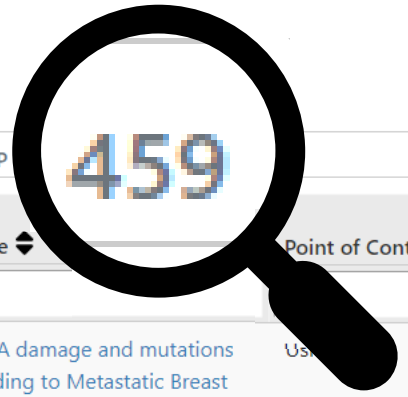

AOPs

Filtered AOP 459

ID	Title	Point of Contact	License	MIE	AO	OECD Status	OECD Project
			Clear			Clear	
443	DNA damage and mutations leading to Metastatic Breast Cancer	Usi	BY-SA	• Increased, DNA damage and mutation	• metastatic breast cancer	Under Development	1.103
450	Inhibition of AChE and activation of CYP2E1 leading to sensory axonal peripheral neuropathy and mortality	SAROJ AMAR	BY-SA	• Acetylcholinesterase (AchE) Inhibition	• Sensory axonal peripheral neuropathy • Increased Mortality		
202	Inhibitor binding to topoisomerase II leading to infant leukaemia	Andrea Terron	BY-SA	• Binding to (interferes with) topoisomerase II enzyme	• Infant leukaemia	WPHA/WNT Endorsed	1.53
389	Oxygen-evolving complex damage leading to population decline via inhibition of photosynthesis	Knut Erik Tollefsen	BY-SA	• Increase, Oxygen-evolving complex damage	• Decrease, Reproduction • Decrease, Population growth rate		

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The adverse outcome pathway concept (AOPs)

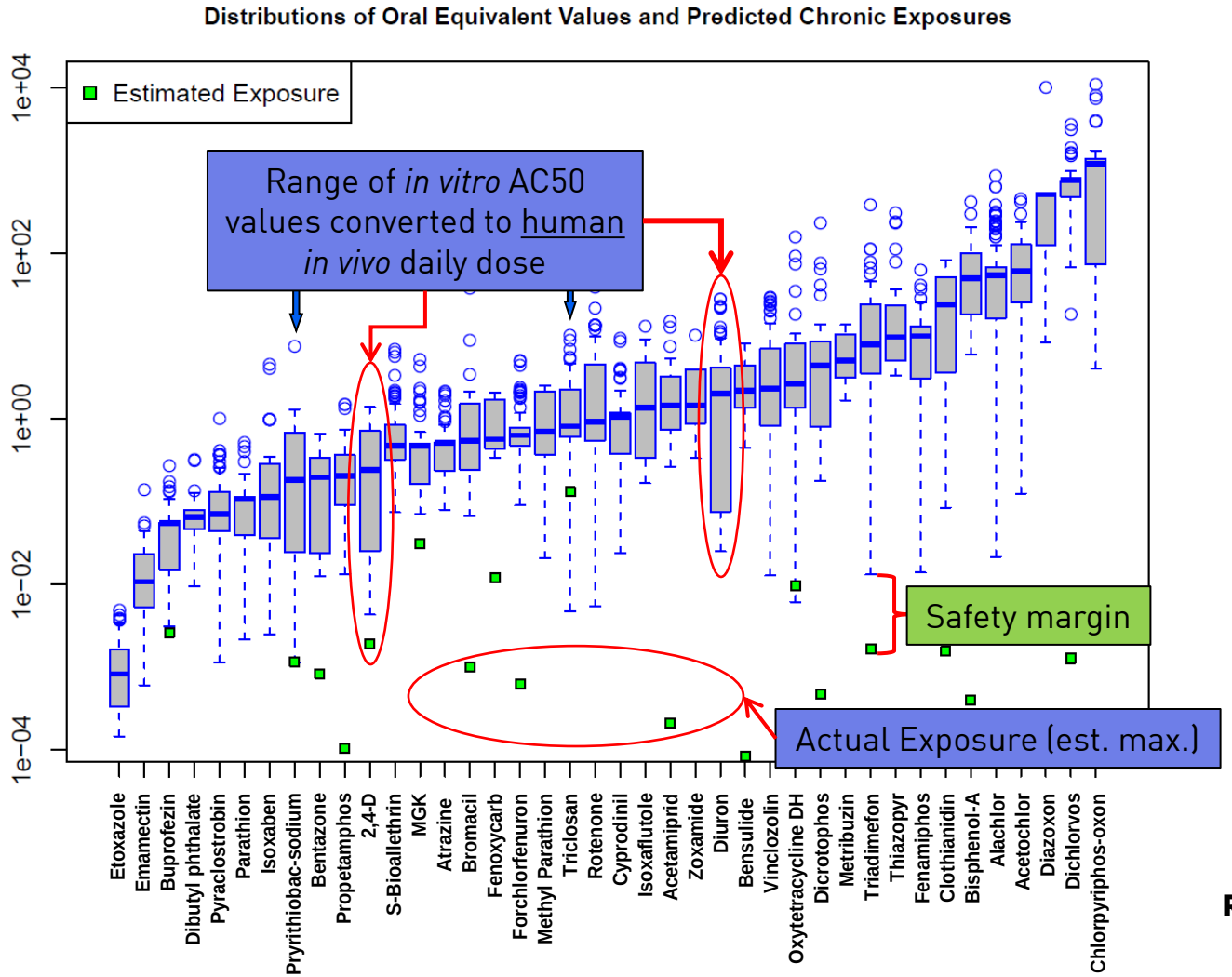
Examples:	Nuclear receptor binding	Altered hormone levels	Cell proliferation	Cell transformation	Increased tumour incidence

~~**~78 Major human organs × 5 ways a chemical could be toxic to each one × 5 Key Events ≈ 2000 assays**~~
(Carmichael et al., 2022)

If the MIE does not occur at relevant doses, neither can the AO

If the MIE occurs, this may or may not lead to the AO

Paradigm shift for systemic safety - Protection not Prediction

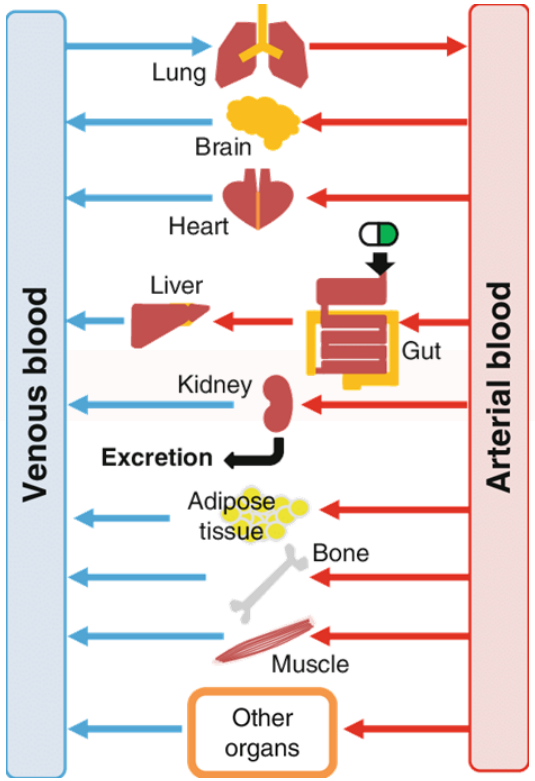


Graphic from Dr Rusty Thomas, EPA, with thanks

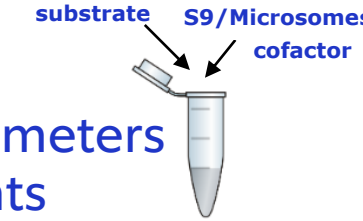
The hypothesis underpinning this type of NGRA is that **if there is no bioactivity observed at consumer-relevant concentrations, there can be no adverse health effects.**

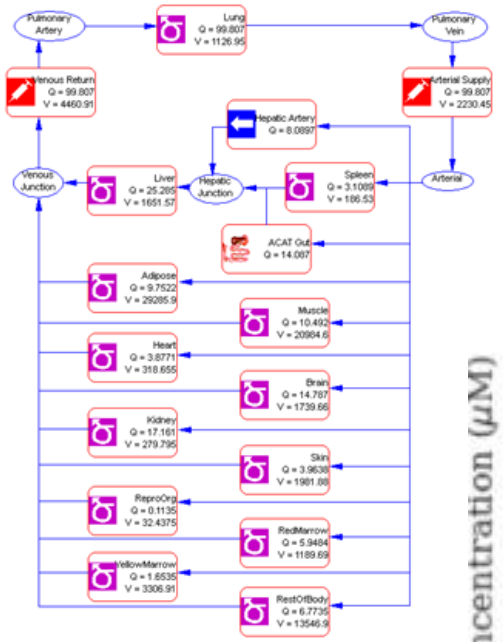
Rotroff, *et al.* Tox.Sci 2010

PBK (Physiologically Based Kinetic) Modelling



Model Input:
Physiological parameters
Partition coefficients
Kinetic constants (in vitro)



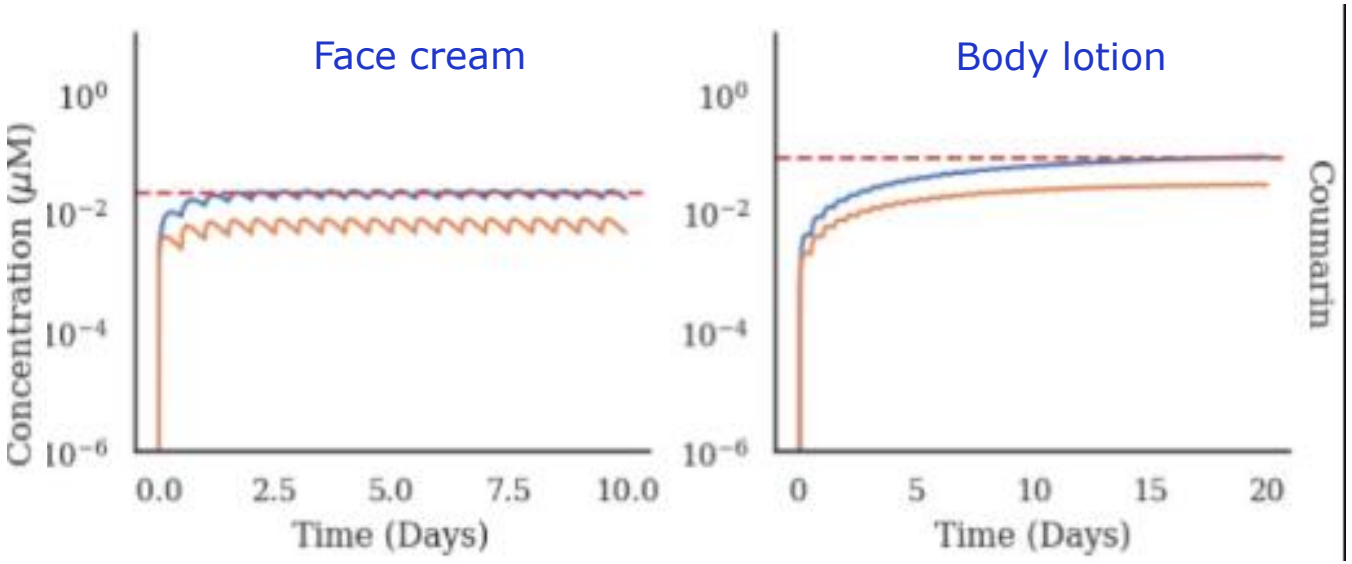


Uptake

Transport from arterial to venous blood

Metabolism

$$dA/dt = + K_A * A_{GI} + QL * (CA - CV) - V_{max} * CL / (K_m + CL)$$



Moxon *et al.*, (2020) *TIV* 63

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

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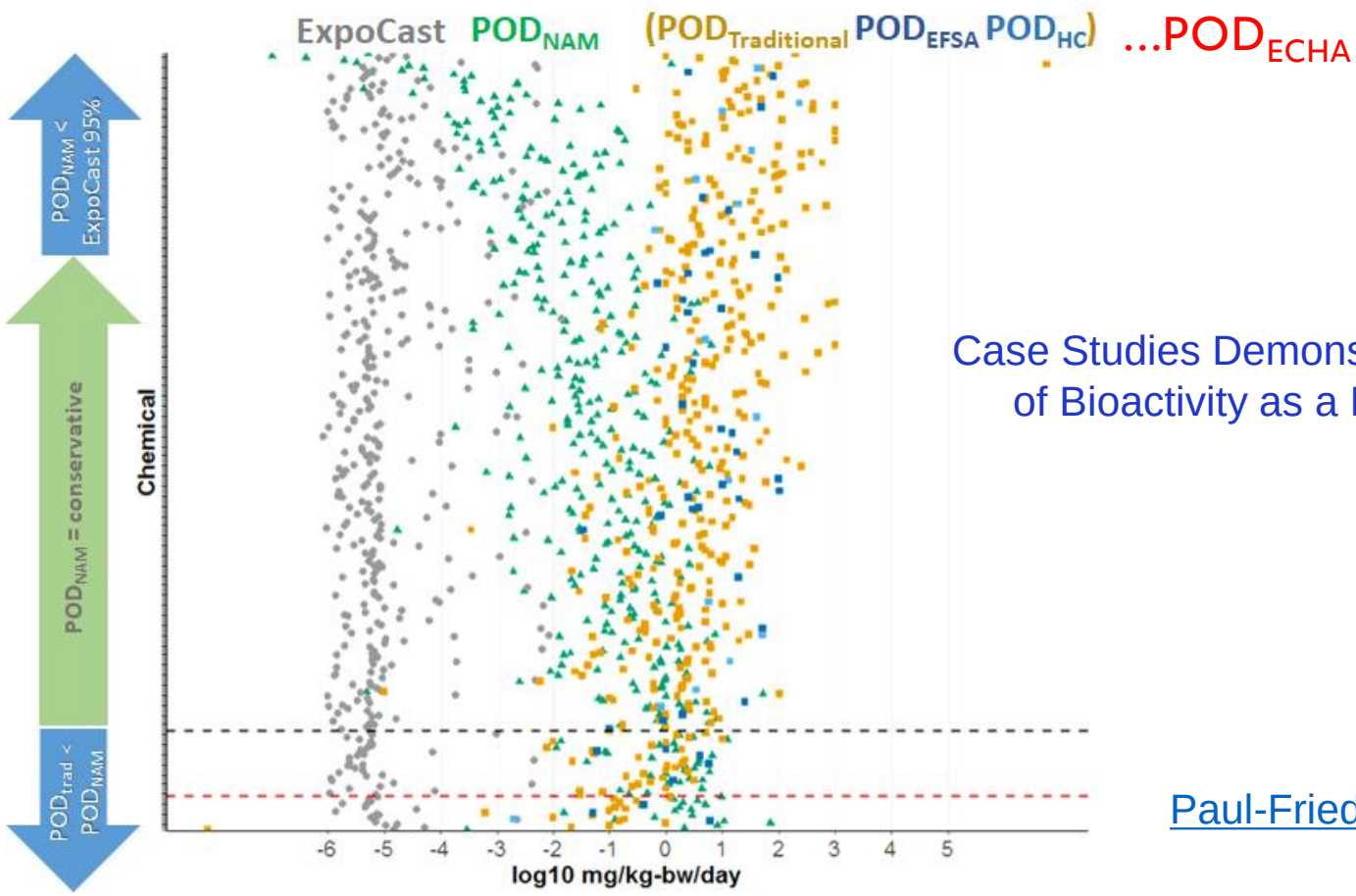
Dent *et al .*, (2018) *Comp Tox* 7:20-26

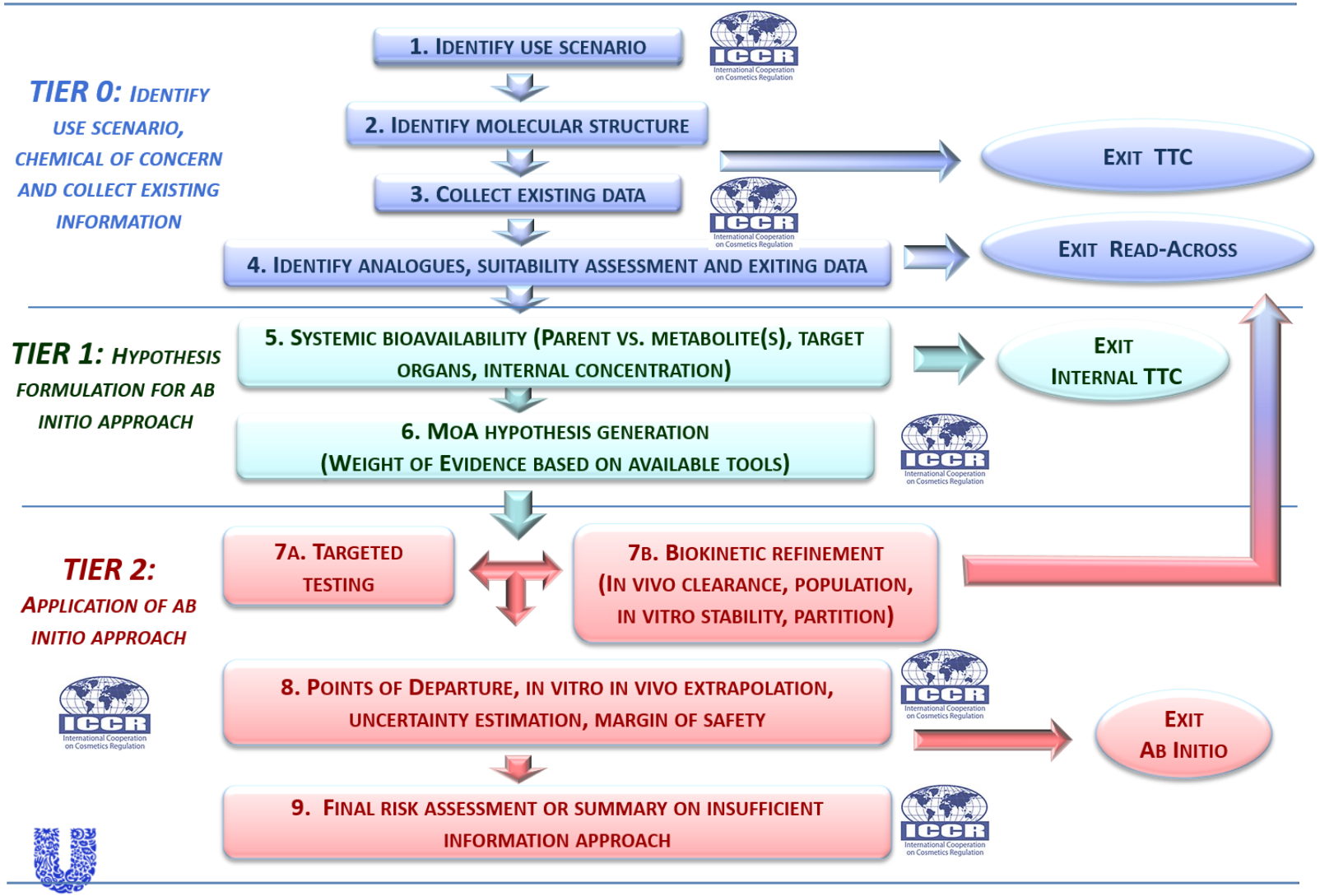
Points of Departure from NAMs can be protective



Case Studies Demonstrating Application of Bioactivity as a Protective POD

Paul-Friedman et al., 2020

First workflow for *ab initio* NGRA

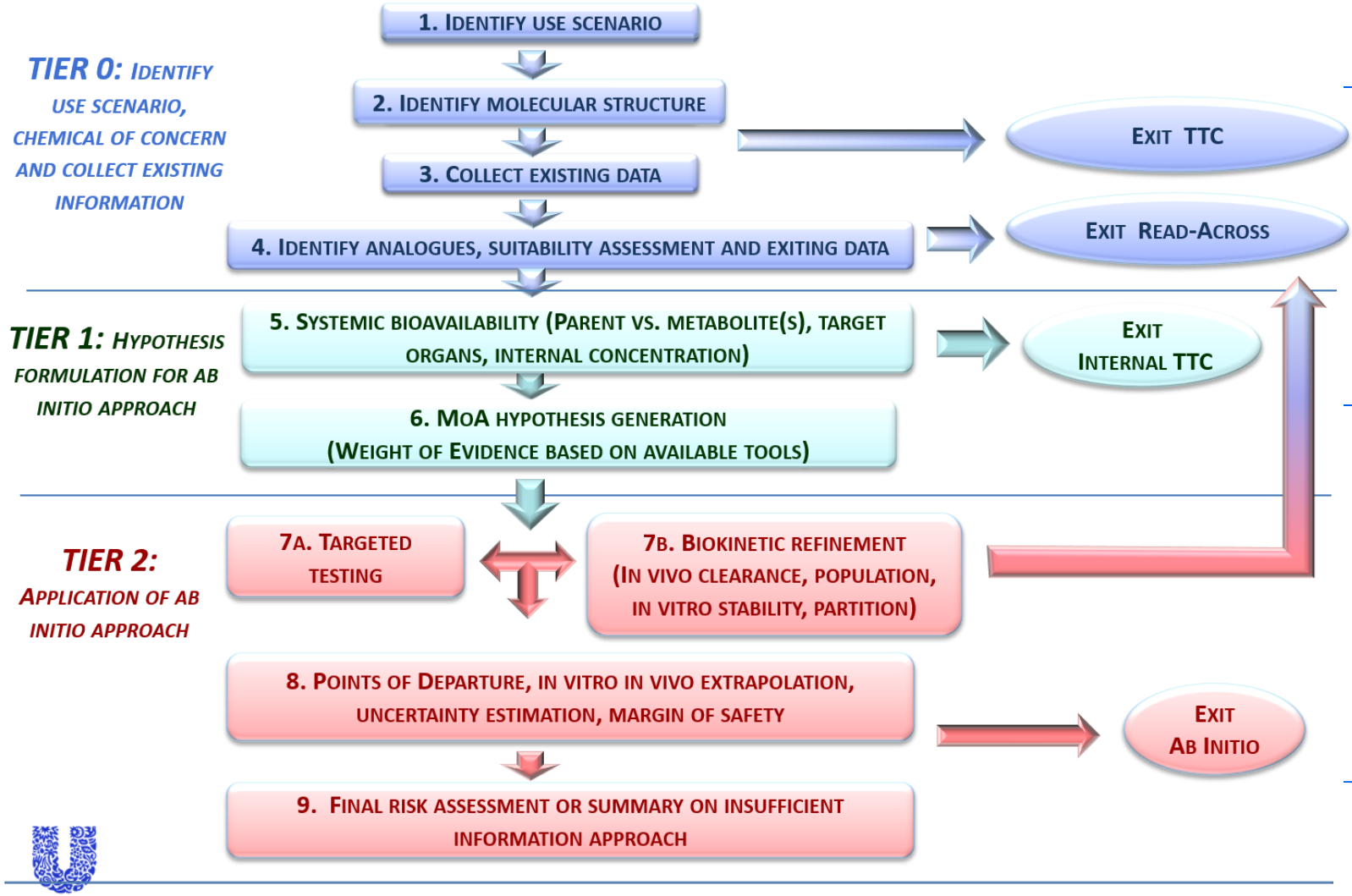


SEURAT-1

Continue through tiers until enough information to make a decision: assessment may be complete at any tier

Berggren *et al.*, (2017) *Computational Toxicology* 4: 31-44. https://doi.org/10.1016/j.comtox.2017.10.001

From principles to application



Read across

Exposure-based waiving

In silico tools

Metabolism and metabolite identification

Physiologically-based kinetic modelling

In chemico assays

'Omics

Reporter gene assays

In vitro pharmacological profiling

3D culture systems

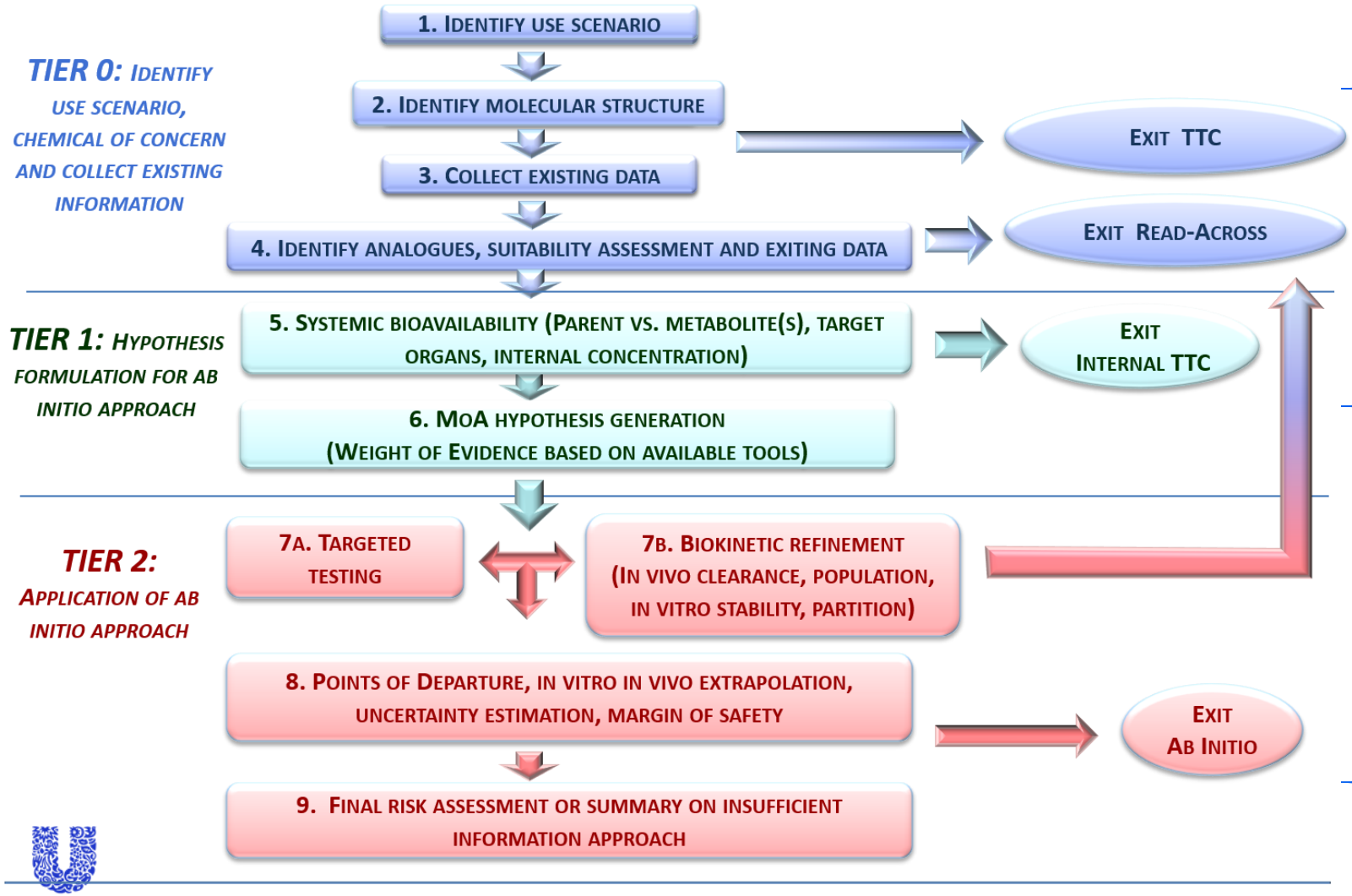
Organ-on-chip

Zebrafish larva assays

Pathways modelling

Human studies

From principles to application



Readiness judged by ICCR in 2018:

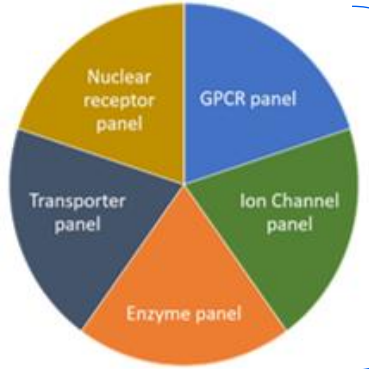
(ICCR IS JWG Part 2 FINAL (iccr-cosmetics.org)

- Read across
- Exposure-based waiving
- *In silico* tools
- Metabolism and metabolite identification
- Physiologically-based kinetic modelling
- *In chemico* assays
- 'Omics
- Reporter gene assays
- *In vitro* pharmacological profiling
- 3D culture systems
- Organ-on-chip
- Zebrafish larva assays
- Pathway modelling
- Human studies

Note - not universally considered a non-animal approach

Bioactivity NAMs in our core toolbox 1/4

In vitro pharmacological profiling



~79 targets


PERSPECTIVES

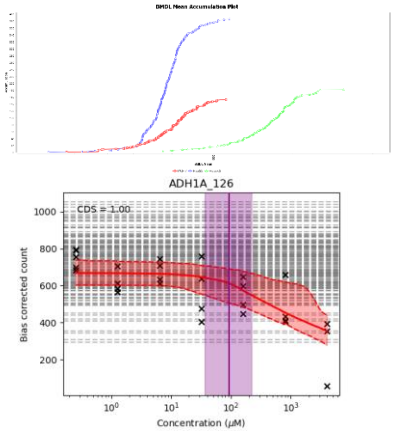
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

eurofins | Cerep

Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

High-Throughput transcriptomics (HTTr)

- TempO-seek technology – full gene panel
- 24hr exposure
- 7 concentrations
- Various cell models (e.g. HepG2, MCF7, HepaRG)
- Dose-response analysis using BMDExpress2 and BIFROST model



Reynolds et al. 2020. Comp Tox 16: 100138
Baltazar et al. 2020. Toxicol Sci 176(1): 236–252

Cell stress panel (CSP)

- 36 biomarkers covering 10 cell stress pathways
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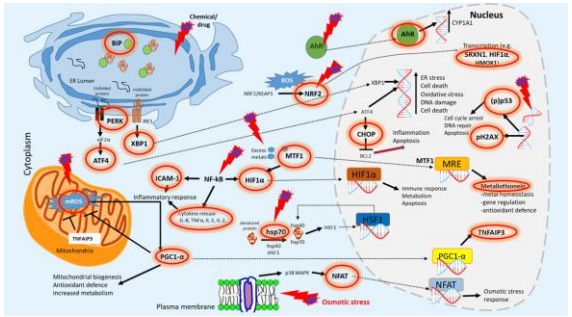

Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

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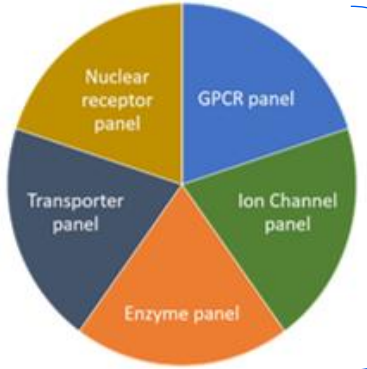
Bioactivity NAMs in our core toolbox 2/4

In vitro pharmacological profiling



eurofins | Cerep

~79 targets


PERSPECTIVES

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

Bowes et al. 2012. Nat Rev Drug Discov 11(12): 909-22

To investigate possible interactions with key targets known to be associated with adversity

Experiment in 2 phases:

Screening at a fixed concentration (10 or 100 µM)

Dose-response assays on positive hits to identify a point of departure (PoD) expressed as an IC_{50} value

How your shampoo bottle could be making you FAT: Scientists discover 11 chemicals in common plastics that contribute to weight gain

- Study has found 11 chemicals in common plastics that contribute to weight gain
- It looked at 34 different plastic products to see which chemicals they contained
- These included yoghurt containers, kitchen sponges and shampoo/drink bottles
- 11 of 55,000 chemical components in them known to interfere with metabolism

By SAM TONKIN FOR MAILONLINE
PUBLISHED: 13:00, 26 January 2022 | **UPDATED:** 13:55, 26 January 2022

European Commission

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Call for data on ingredients with potential endocrine-disrupting properties used in cosmetic products

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Bioactivity NAMs in our core toolbox 3/4

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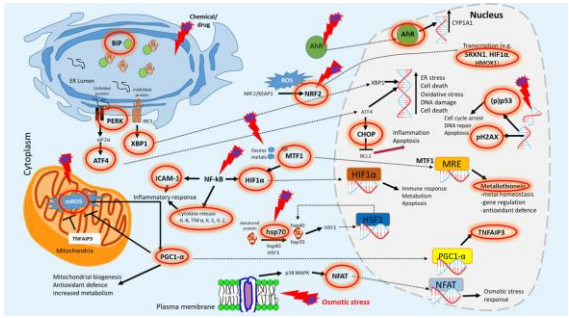
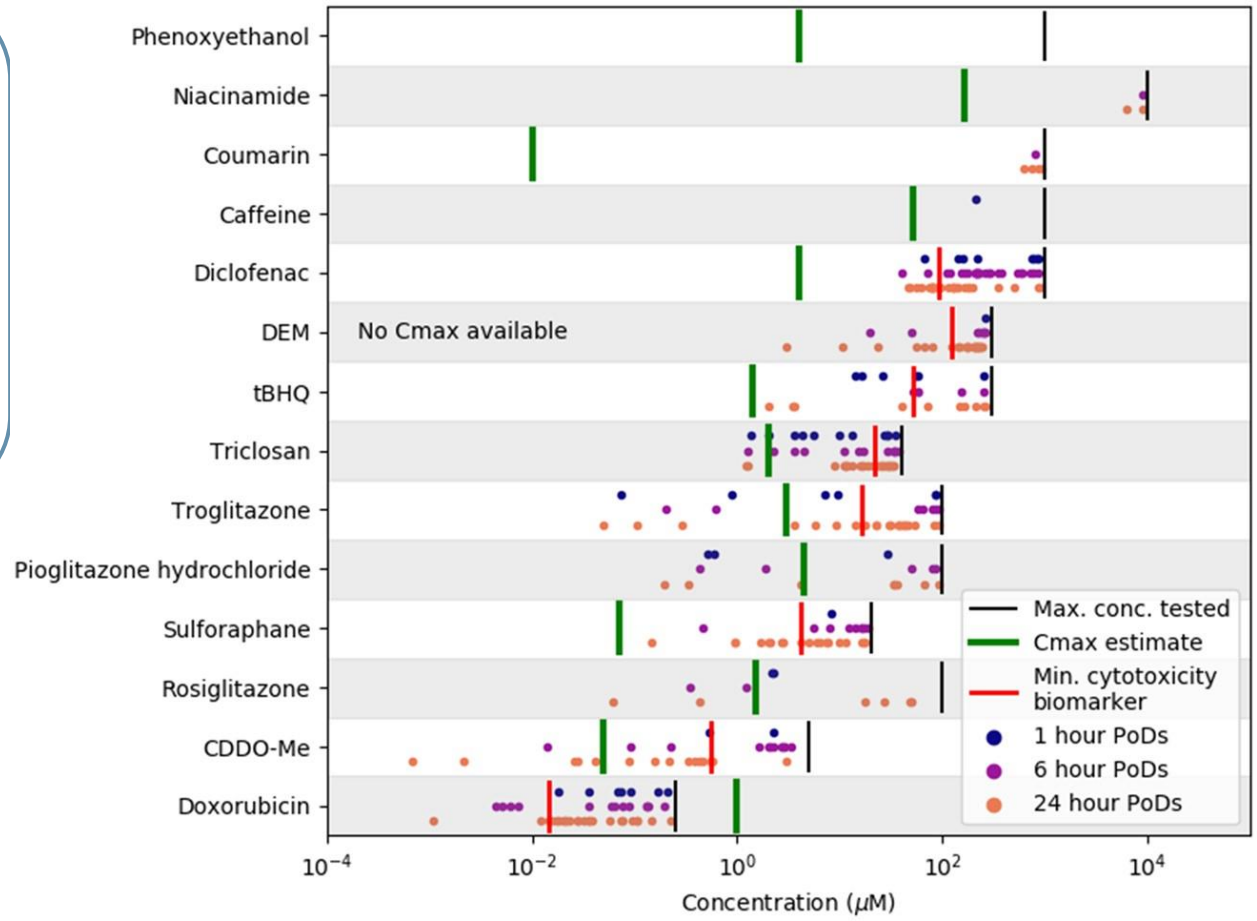


Image kindly provided by Paul Walker (Cyprotex)

Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

To characterize non-specific biological activity which is not mediated via a specific protein/receptor interaction

Cell stress can cause any number of target organ pathologies if present in the wrong place at the wrong time



https://doi.org/10.1093/toxsci/kfaa054

Bioactivity NAMs in our core toolbox 4/4

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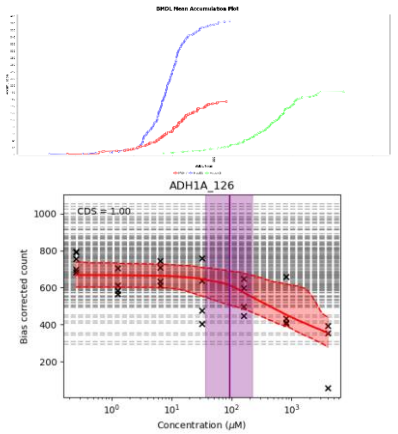

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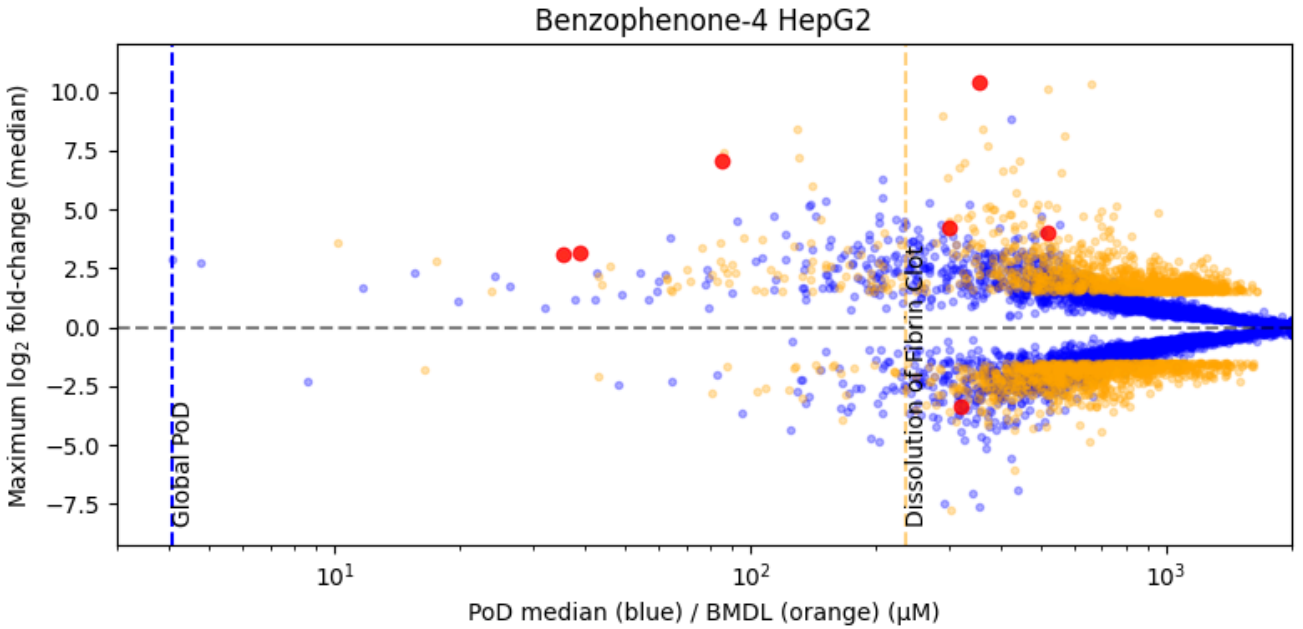
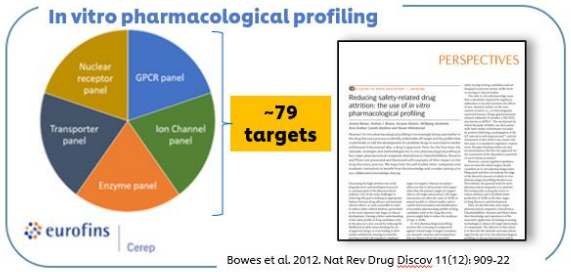


Transcriptomics as a broad non-targeted biological screen may be used in NGRA in several ways:

1. **Informing read across (based on similarity of genes affected) (De Abrew Tox Sci 2016 https://doi.org/10.1093/toxsci/kfw058)**
2. **Testing mode of action hypotheses (Catlett et al BMC Bioinf 2013 https://doi.org/10.1186/1471-2105-14-340)**
3. **Identifying a point of departure for risk assessment/no observed transcriptional effect level (Lobenhofer et al Toxicol Pathol 2004 https://doi.org/10.1080/01926230490483324)**

Risk Assessment Outcome

BIOACTIVITY



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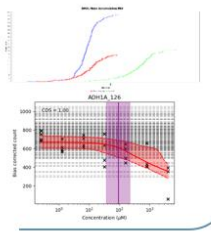

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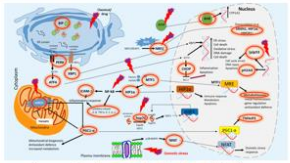

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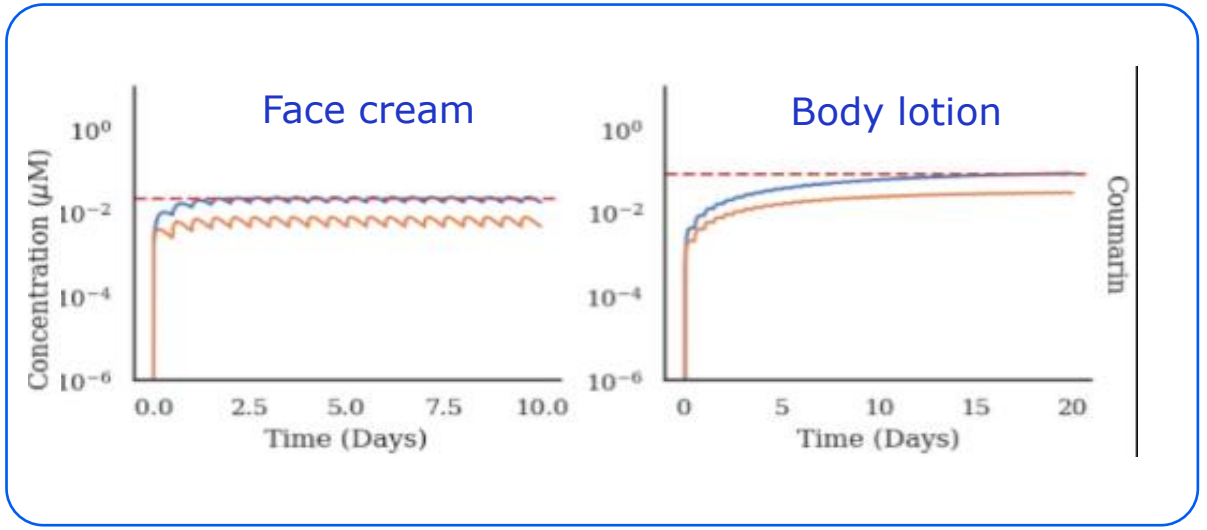
Hatherell et al. 2020. Toxicol Sci 176(1): 11-33

EXPOSURE



Identify lowest (most sensitive) point of departure, expressed in μM

Identify realistic worst-case plasma exposure (C_{max}) expressed as μM

$$\text{BIOACTIVITY EXPOSURE RATIO} = \frac{\text{BIOACTIVITY}}{\text{EXPOSURE}}$$

The bigger the BER, the greater the confidence that bioactivity will not occur in exposed consumers

Unilever

What do we still need to do?

1. Increase confidence in exposure predictions (including metabolites)
2. Determine whether tools give us enough biological coverage
3. Be explicit about the level of confidence in the assessment
4. Develop agreed standards for using tools and reporting data
5. Distinguish between adaptation and adversity
6. Develop an updated risk assessment workflow
7. More case studies

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What do we still need to do?

1. Increase **confidence** in exposure predictions (including metabolites)
2. Determine whether tools give us enough biological coverage
3. Be explicit about the level of **confidence** in the assessment
4. Develop **agreed standards** for using tools and reporting data
5. Distinguish between adaptation and adversity
6. Develop an updated risk assessment workflow
7. **More case studies**

Use of NGRA for decision making, sharing with regulators etc.

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Check for updates

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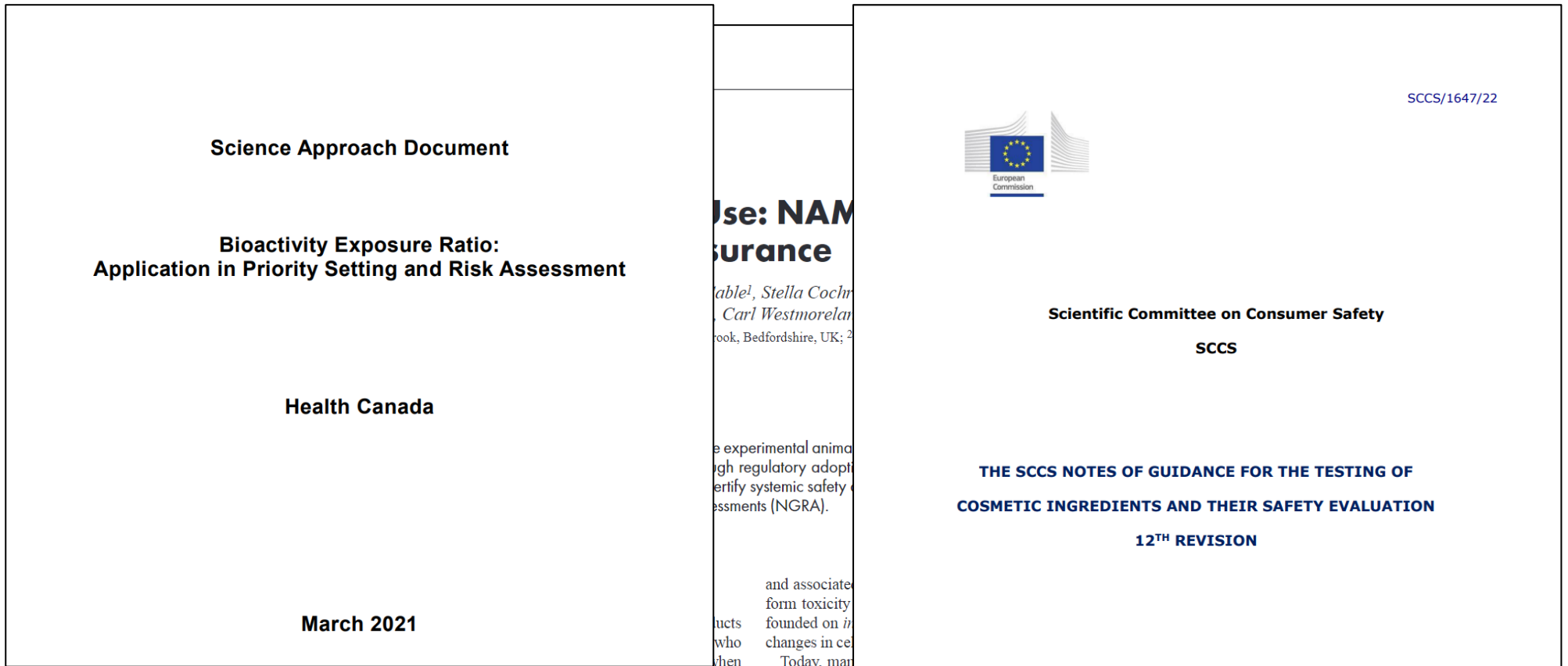
Tiered, exposure-led NGRA means we can make robust safety decisions *today*

- Increasing recognition that *in vitro* bioactivity can inform decision making (e.g. Health Canada, SCCS)
- Our knowledge will never be complete, but we know enough to start, and to ensure animal testing is only ever used as a last resort



Science Approach Document

Bioactivity Exposure Ratio:
Application in Priority Setting and Risk Assessment

Health Canada

March 2021

SCCS/1647/22

European Commission

Scientific Committee on Consumer Safety

SCCS

THE SCCS NOTES OF GUIDANCE FOR THE TESTING OF COSMETIC INGREDIENTS AND THEIR SAFETY EVALUATION

12TH REVISION

CONCLUSIONS

- The 9 ICCR Principles underpin the use of novel data in Next Generation Risk Assessment
- The Principles can be applied to improve safety decision making
- Use of tiered approaches means that gaps in some of the higher tier tools does not prevent risk assessments from being completed
- More examples of holistic risk assessments for cosmetic ingredients needed to refine and build confidence in approaches

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Sophie Malcomber
Alistair Middleton
Ruth Pendlington
Katie Przybylak
Georgia Reynolds
Sharon Scott
Sandrine Spriggs
Carl Westmoreland
Andrew White

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Thank You

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