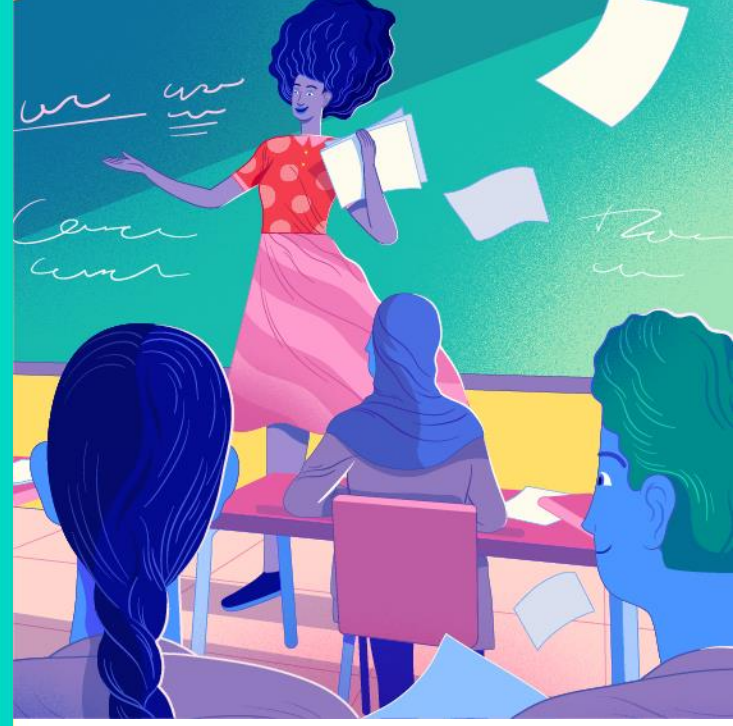
Bringing it all together:
Integration of new approach methodologies (NAMs) for cosmetic safety decision-making



Paul Russell

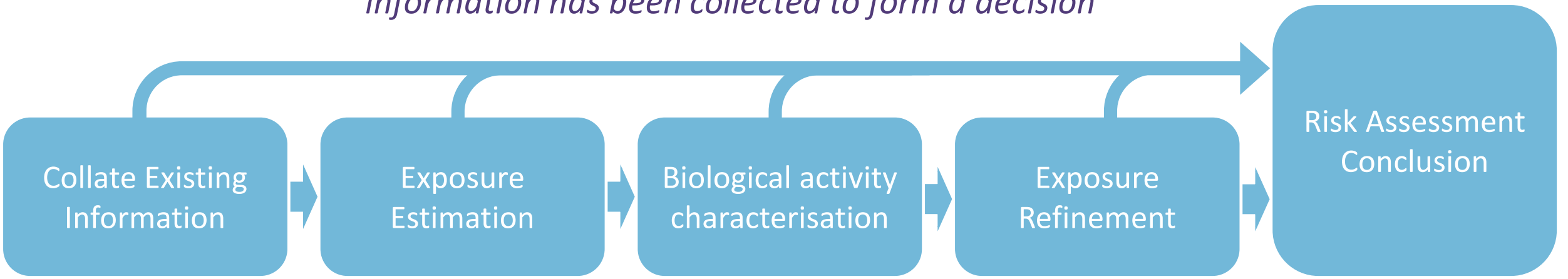
Unilever

Risk assessment process

*NGRA is defined as **an exposure-led, hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Dent et al 2018. Computational Toxicology Volume 7, August 2018, Pages 20-26

A tiered and iterative approach is needed until sufficient information has been collected to form a decision



1. Problem Formulation
2. Consumer Exposure
3. Predictive Chemistry
5. Internal Exposure
7. Integration into risk assessment
8. History of Safe Use
4. Exposure Based Waiving
6. *In Vitro* Assay Synthesis

AFSA ANIMAL-FREE SAFETY ASSESSMENT COLLABORATION Modules

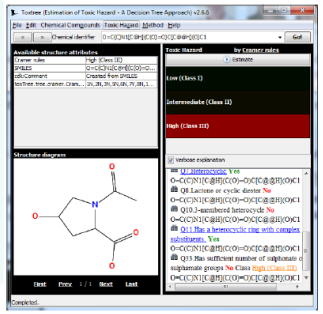
Collate existing information

Literature

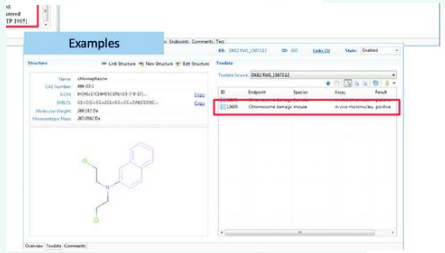
PubMed Google Scholar

In silico tools

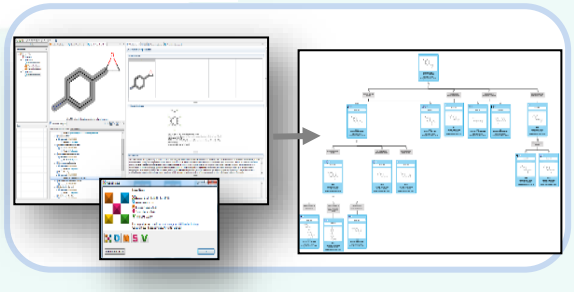
ToxTree



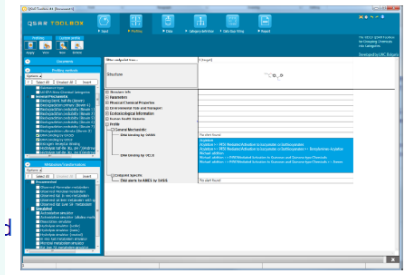
Derek nexus



Meteor nexus



OECD QSAR TOOLBOX



Metabolic fate predictions

Existing datasets

ChEMBL

EPA iCSS ToxCast Dashboard

PubChem

Bespoke models

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†

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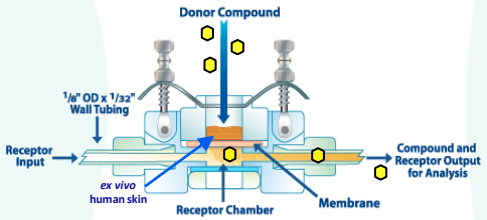
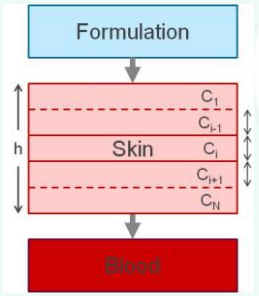
Exposure estimation & refinement

- **Route of exposure**
- **Consumer use (Habits & Practices)**
- **Applied dose (external concentration)**

ADME parameters

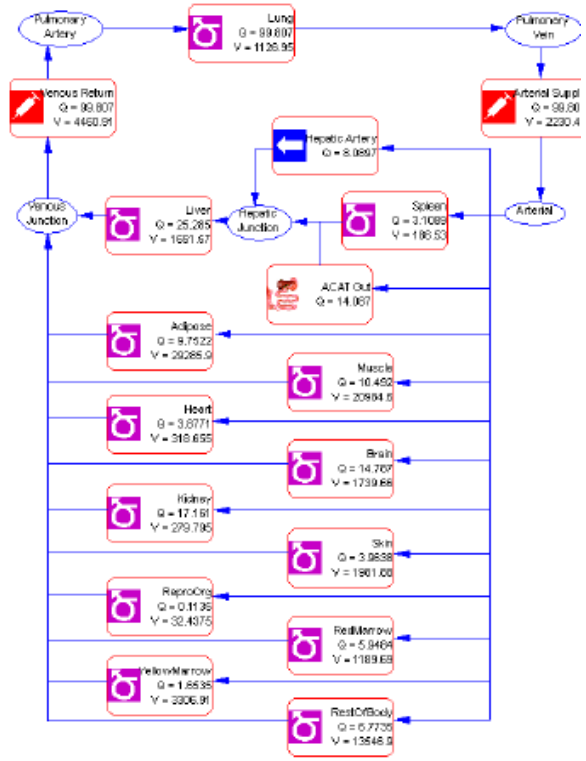




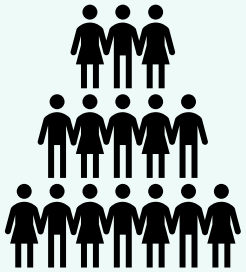
- **Skin penetration**
- **Phys-chem properties**
- **Hepatic clearance**
- **Fraction unbound**
- **blood:plasma ratio**
- **Metabolic profiling**

Opportunity for refinement

Physiologically-based kinetic (PBK) modelling – Internal concentration (plasma, urine, organ-level)



Uncertainty analysis- Population simulation



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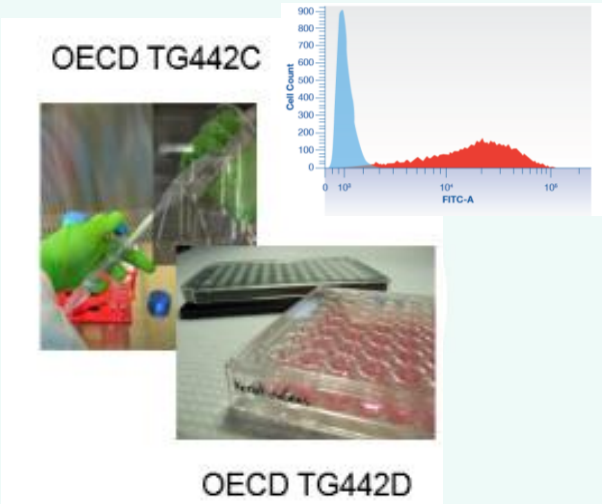
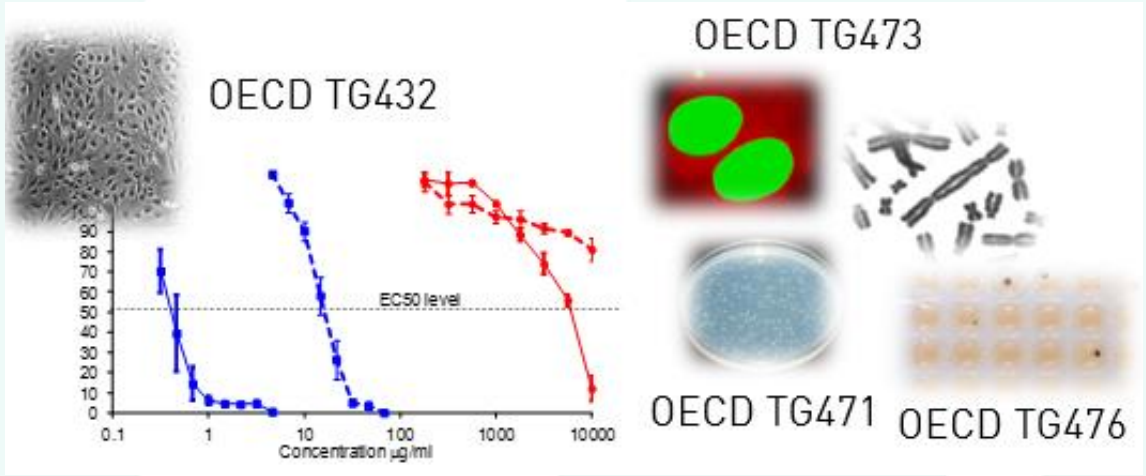
Biological activity characterisation

OECD test methods

Skin and eye irritation



Skin sensitisation



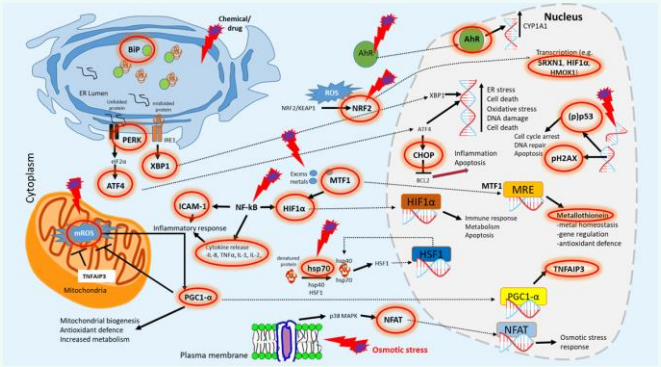
Phototoxicity

Genotoxicity

Unilever

Biological activity characterisation

Cellular stress



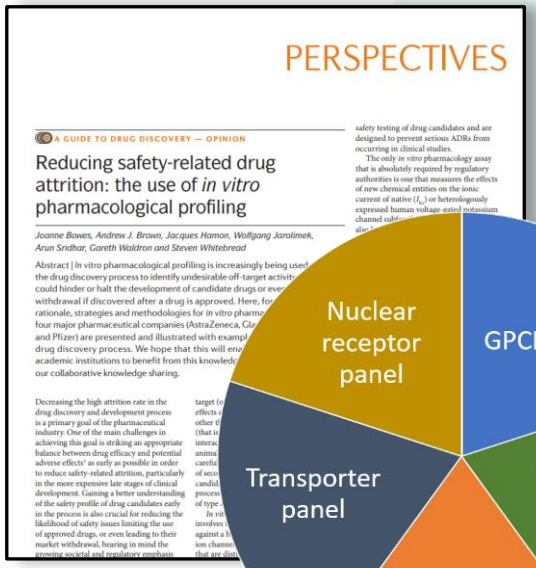
cyprotex AN EVOTEC COMPANY

Image kindly provided by Paul Walker (Cyprotex)

36 biomarkers identified that were representative of key stress pathways, mitochondrial toxicity and cell health.

Hatherell et al (2020), Toxicological Sciences, 176, 11-33

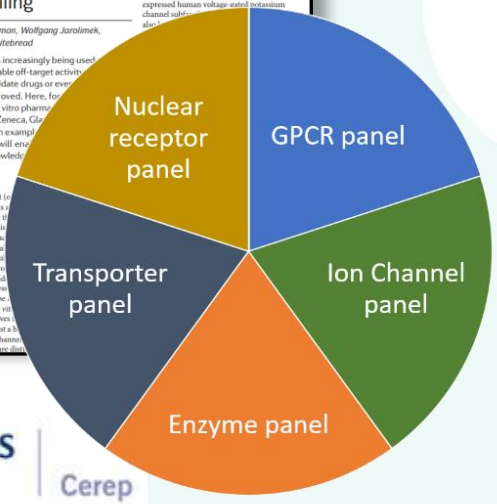
Receptor-binding assays


PERSPECTIVES

A GUIDE TO DRUG DISCOVERY — OPINION

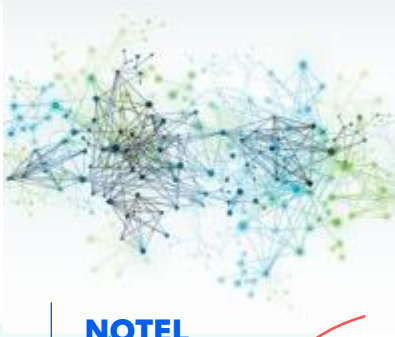
Reducing safety-related drug attrition: the use of *in vitro* pharmacological profiling

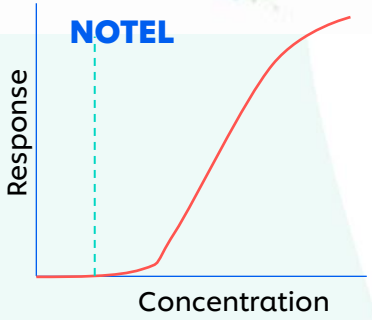
Joanne Bowes, Andrew J. Brown, Jacques Hamon, Wolfgang Jarolimek, Arun Sridhar, Gareth Waldron and Steven Whitebread



eurofins | Cerep

High throughput transcriptomics





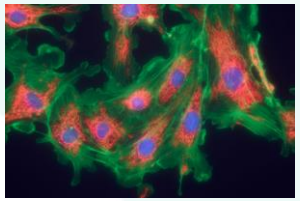
Mechanism based gentox assessment

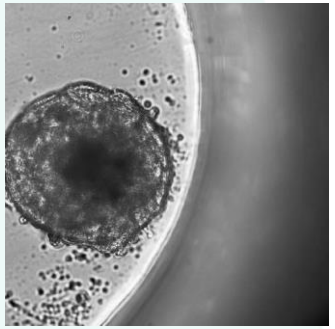
toxys®

ToxTracker®

DNA Damage
P53 Binding
Oxidative Stress
Protein Damage

Advanced cell systems and microtissues





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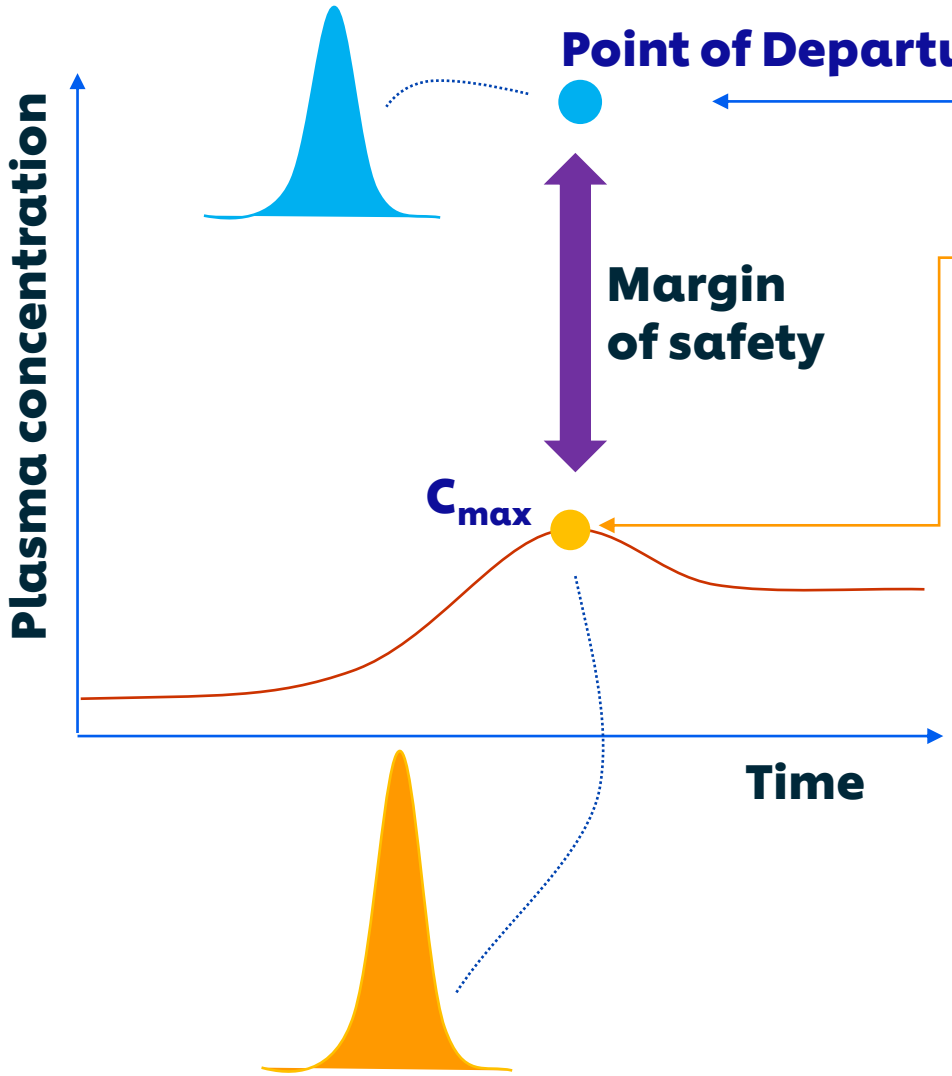
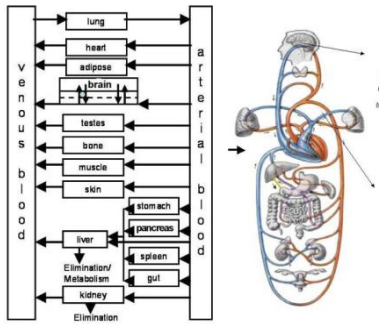
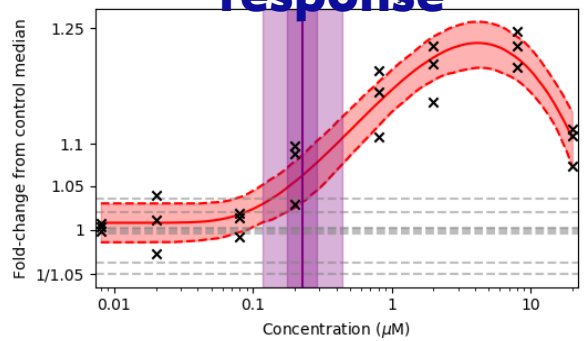
Margin of Safety



Exposure models (PBK, free/total concentration)

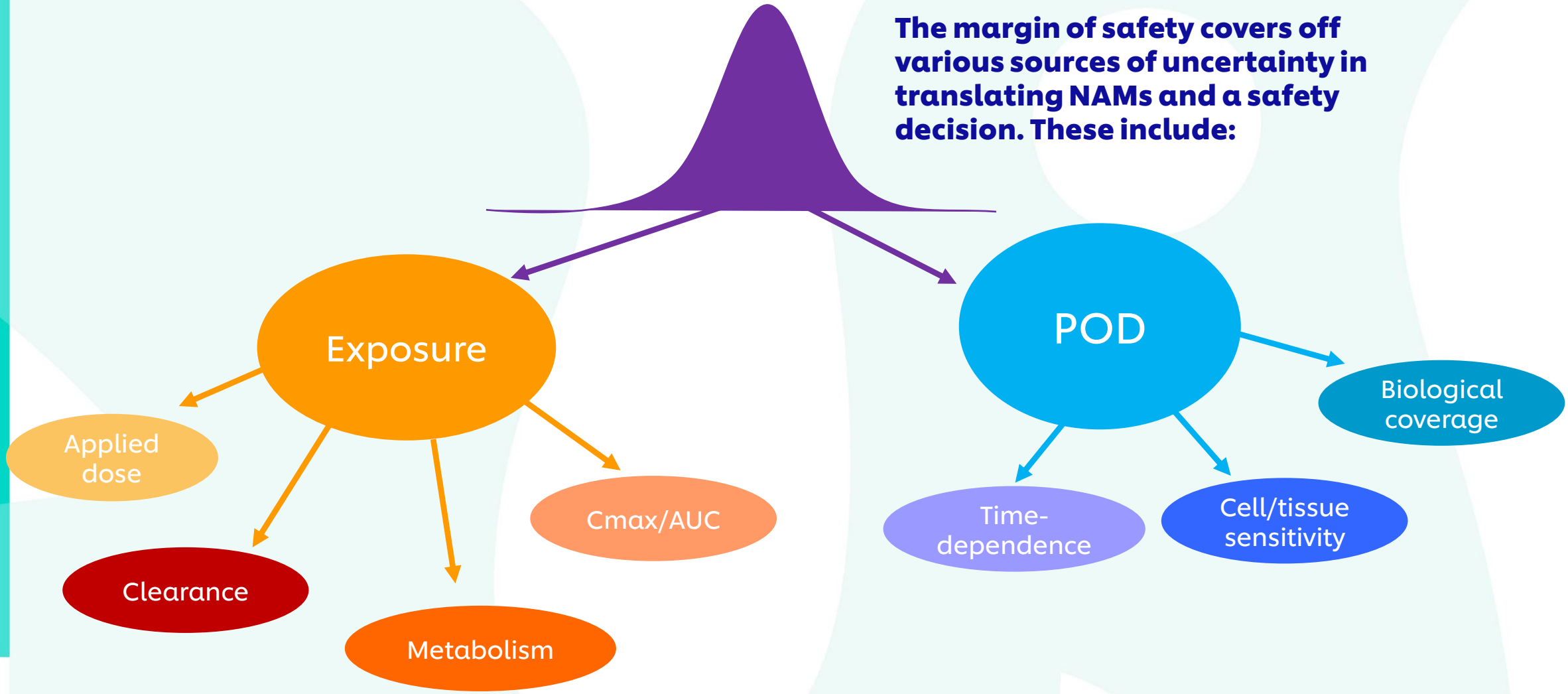


Point of departure derived from in vitro concentration-response



NGRA: Sources of uncertainty should be characterized and documented

The margin of safety covers off various sources of uncertainty in translating NAMs and a safety decision. These include:



Case study example

Baltazar *et al* (2020) A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Toxicological Sciences, 176, 236-252

OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 176(1), 2020, 236–252

doi: 10.1093/toxsci/kfaa048
Advance Access Publication Date: April 10, 2020
Research article

A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products

Maria T. Baltazar,[1] Sophie Cable, Paul L. Carmichael, Richard Cubberley, Tom Cull, Mona Delagrange, Matthew P. Dent, Sarah Hatherell, Jade Houghton, Predrag Kukic, Hequn Li, Mi-Young Lee, Sophie Malcomber, Alistair M. Middleton, Thomas E. Moxon, Alexis V. Nathanail, Beate Nicol, Ruth Pendlington, Georgia Reynolds, Joe Reynolds, Andrew White, and Carl Westmoreland

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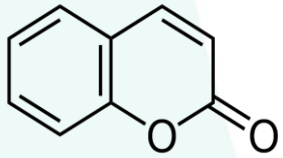
ABSTRACT

Next-Generation Risk Assessment is defined as an exposure-led, hypothesis-driven risk assessment approach that integrates new approach methodologies (NAMs) to assure safety without the use of animal testing. These principles were applied to a hypothetical safety assessment of 0.1% coumarin in face cream and body lotion. For the purpose of evaluating the use of NAMs, existing animal and human data on coumarin were excluded. Internal concentrations (plasma C_{max}) were estimated using a physiologically based kinetic model for dermally applied coumarin. Systemic toxicity was assessed using a battery of *in vitro* NAMs to identify points of departure (PoDs) for a variety of biological effects such as receptor-mediated and immunomodulatory effects (Eurofins SafetyScreen44 and BioMap Diversity 8 Panel, respectively), and general bioactivity (ToxCast data, an *in vitro* cell stress panel and high-throughput transcriptomics). In addition, *in silico* alerts for genotoxicity were followed up with the ToxTracker tool. The PoDs from the *in vitro* assays were plotted against the calculated *in vivo* exposure to calculate a margin of safety with associated uncertainty. The predicted C_{max} values for face cream and body lotion were lower than all PoDs with margin of safety higher than 100. Furthermore, coumarin was not genotoxic, did not bind to any of the 44 receptors tested and did not show any immunomodulatory effects at consumer-

0.1% COUMARIN IN FACE CREAM (NEW FRAGRANCE)



Assumptions:

- EU Market
- 100% purity
- no in vivo data was available such as animal data, History of Safe Use (HoSU) 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

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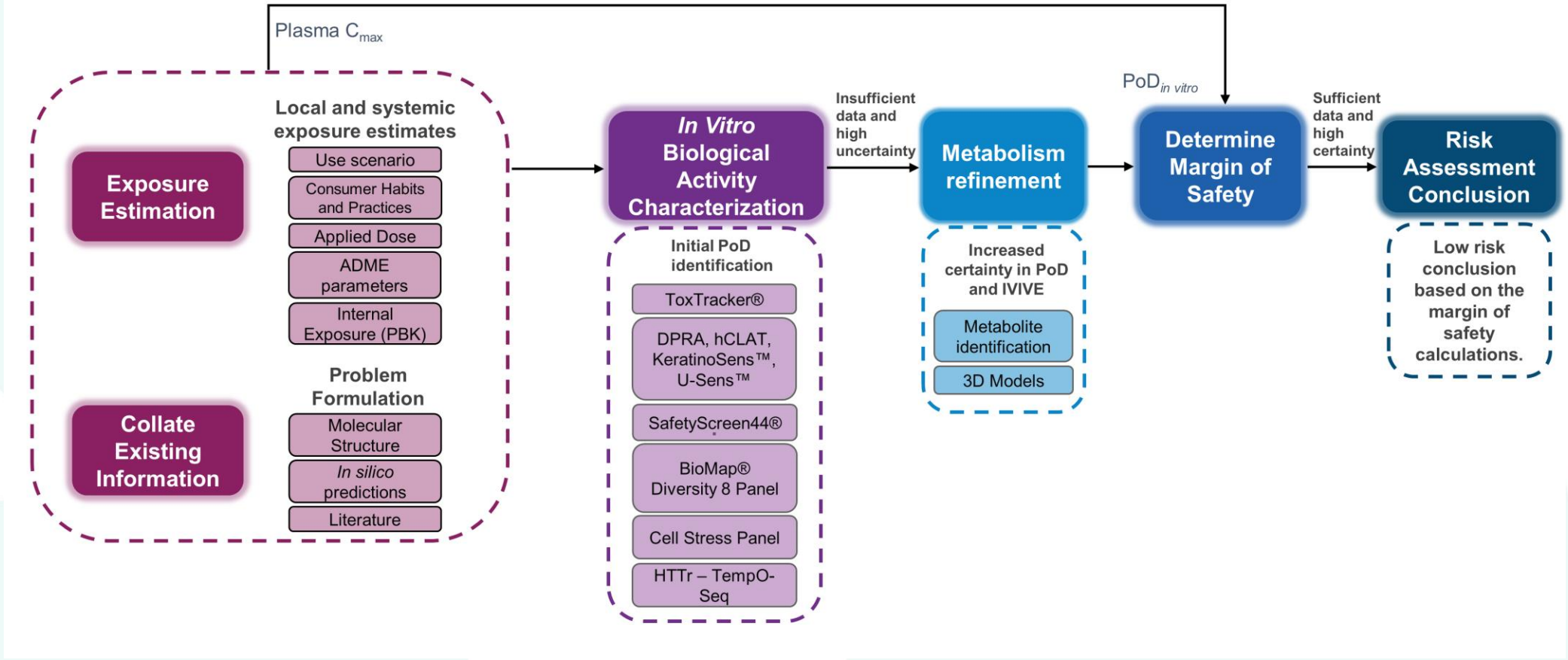
Next-Generation Risk Assessment workflow

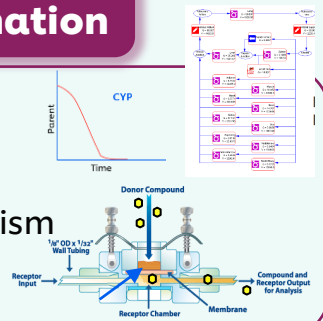


Baltazar *et al* (2020) A Next-Generation Risk Assessment Case Study for Coumarin in Cosmetic Products. Toxicological Sciences, 176, 236-252

NGRA for 0.1% coumarin in face cream:
Key results

Exposure Estimation

- Plasma Cmax 0.002- 0.02 µM
- Rapidly metabolism via CYP2A6



Collate Existing Information

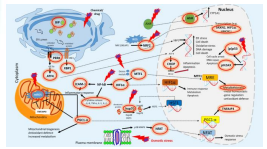
Meteor nexus · Derek nexus · QSAR TOOLBOX

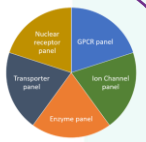
- Gentox and protein binding alerts
- Biotransformation via hydroxylation
- Reactive metabolites predicted.
- 90-100% freely available *in vitro*
- Low bioactivity in ToxCast and Pubchem

EPA iCSS ToxCast Dashboard

PubChem

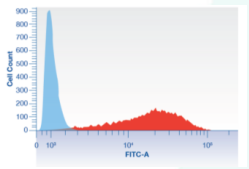
In Vitro Biological Activity Characterisation





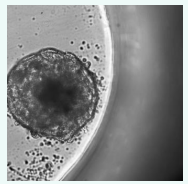
ToxTracker®

- Negative gentox
- Low probability of skin sensitisation
- No immunomodulation potential
- Low bioactivity
- PoD range: 6-912 µM



Metabolism refinement

- Low bioactivity found in a metabolic competent cell model (HepaRG 3D)



Determine Margin of Safety

Updated MoS

9538- 9601

Preliminary MoS

706 - 96738

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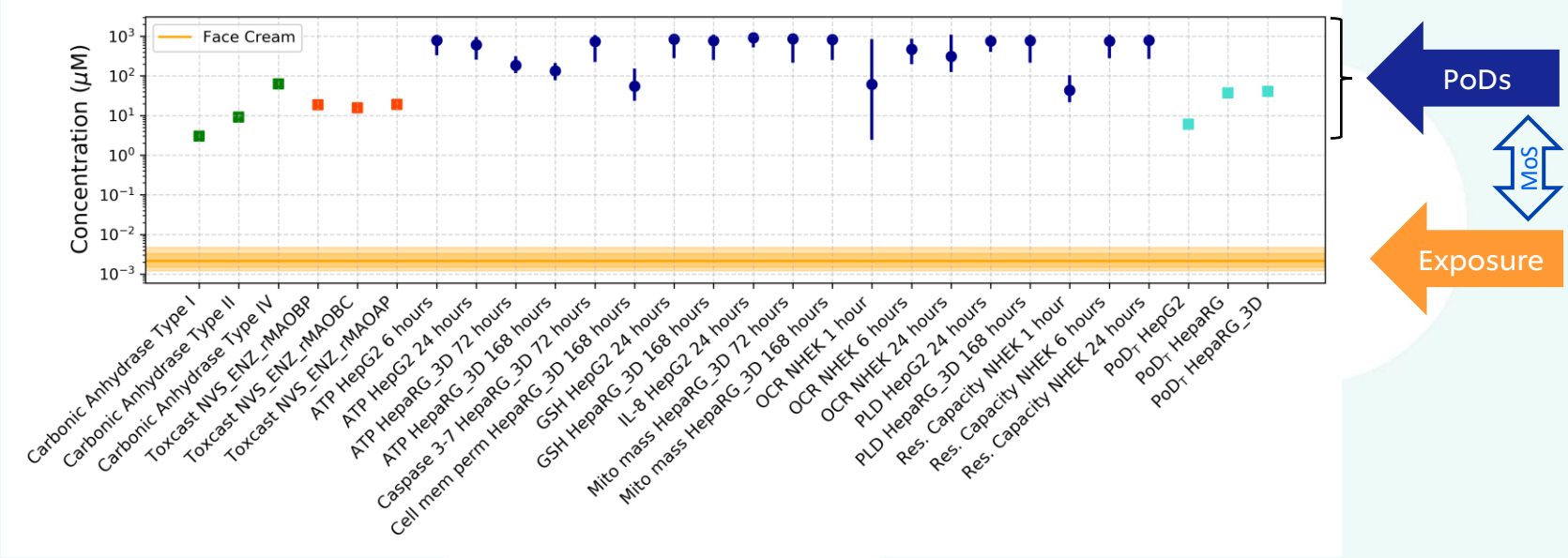
NGRA for 0.1% coumarin in face cream: Risk assessment conclusion



- The predicted C_{max} values for face cream were lower than all PoDs with a MoS (the 5th percentile) higher than 100
- Coumarin is not genotoxic, does not cause skin sensitisation, does not bind to any of the 44 targets and does not show any immunomodulatory effects at consumer relevant exposures
- **Weight of evidence suggests that the inclusion of 0.1% coumarin in face cream is safe for the consumer**

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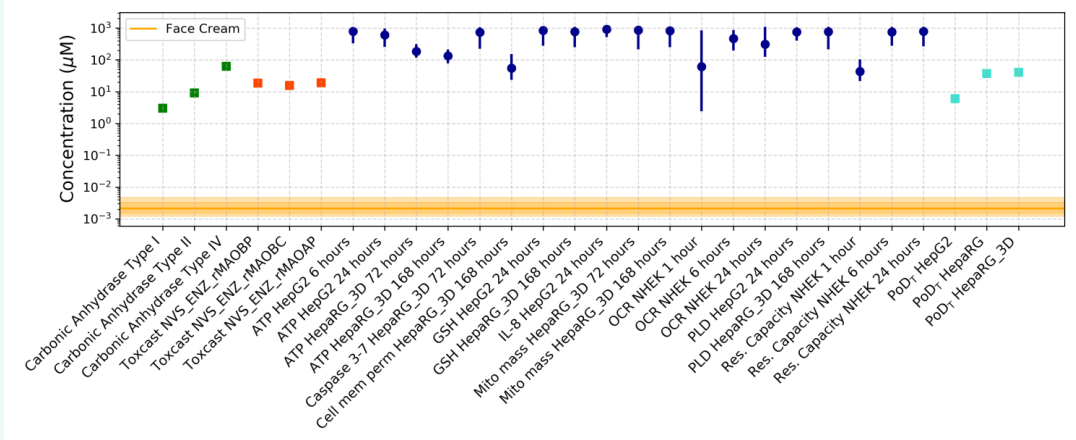
Concluding remarks

- **NAMs for decision making is a framework of non-standard, bespoke data-generation, driven by the risk assessment questions**
  - **Exposure led**
  - **Human relevant**
  - **in silico**
  - ***in vitro***
  - **weight of evidence**
- **Margin of safety is determined by the ratio of human exposure to the point of departure for the most sensitive assay, taking sources of uncertainty into account**
- **NAMs for NGRA are available now and research into more approaches continues**



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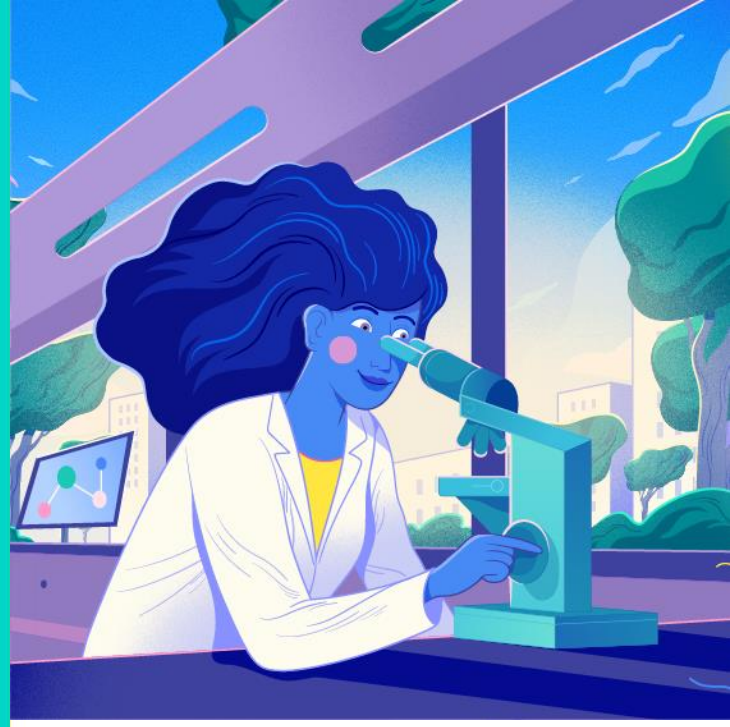
Beate Nicol
Ruth Pendlington
Sam Piechota
Julia Fentem
Georgia Reynolds
Joe Reynolds
Nikol Simicek
Andy Scott
Carl Westmoreland
Andy White

+ All our collaborators



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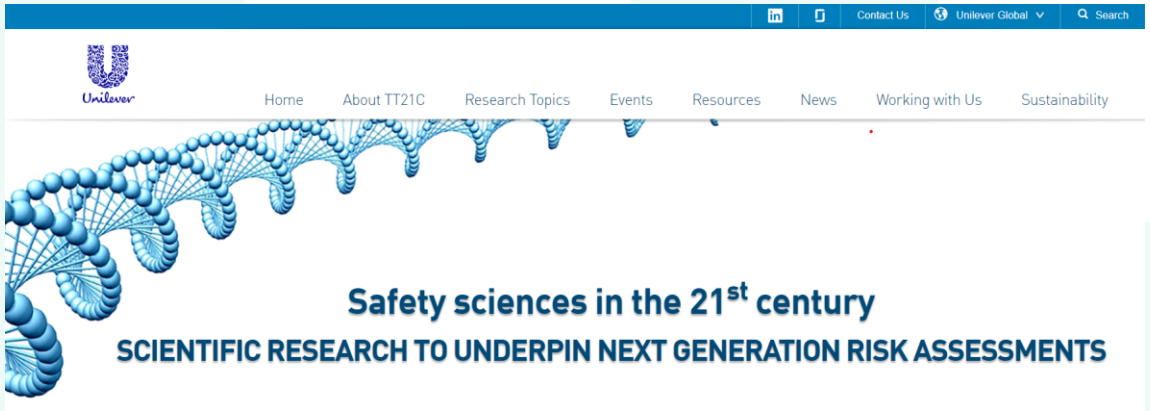
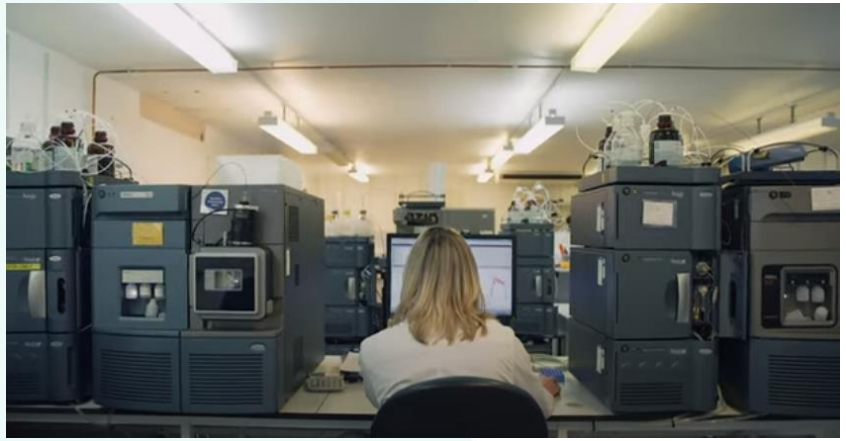
#UseScienceNotAnimals

For more information on Unilever's ongoing research to develop non-animal approaches to safety assessment visit www.tt21c.org





YouTube Implementing a Next Generation Risk Assessment

YouTube Animal Testing Alternatives in Unilever

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