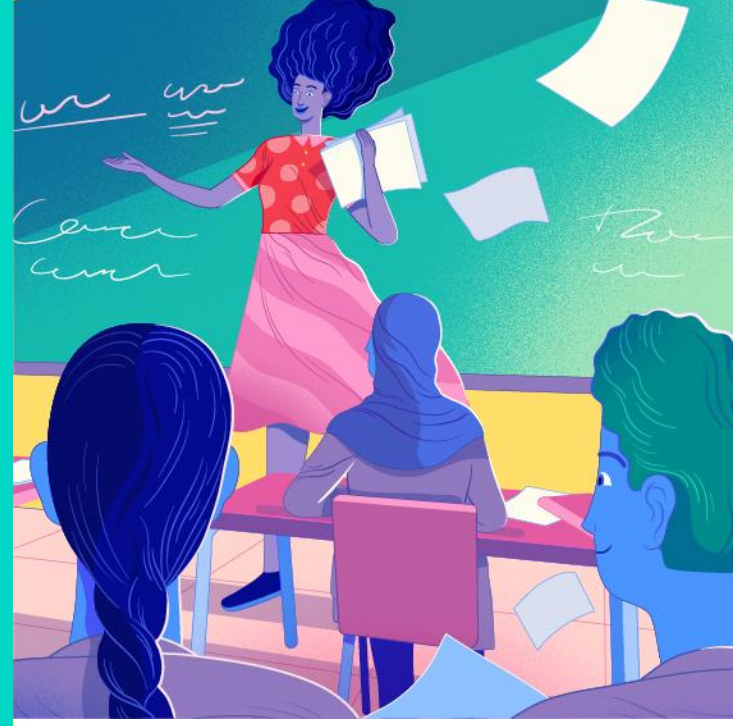
Opportunities and Challenges to the use of NAMs to support Environmental Safety: an industry perspective



Dr. Bruno Campos
bruno.campos@unilever.com

Safety & environmental Assurance Centre (SEAC) Unilever, UK

Unilever

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Who we are and what we do

Unilever

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We make many of the world's favourite brands

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Innovating boldly for people and planet

Every day, 2.5 billion people around the world use our products, and every one of these products is developed using world-class science and technology.

We invested €800m into R&D in 2020 and we have over 20,000 patents protecting the ideas, discoveries and breakthroughs that our global team of 5,000 world-leading experts produce.

"Innovating boldly for people and planet means challenging our thinking and applying real science and technology to tackle big challenges that matter"

Richard Slater, Chief R&D Officer

Unilever

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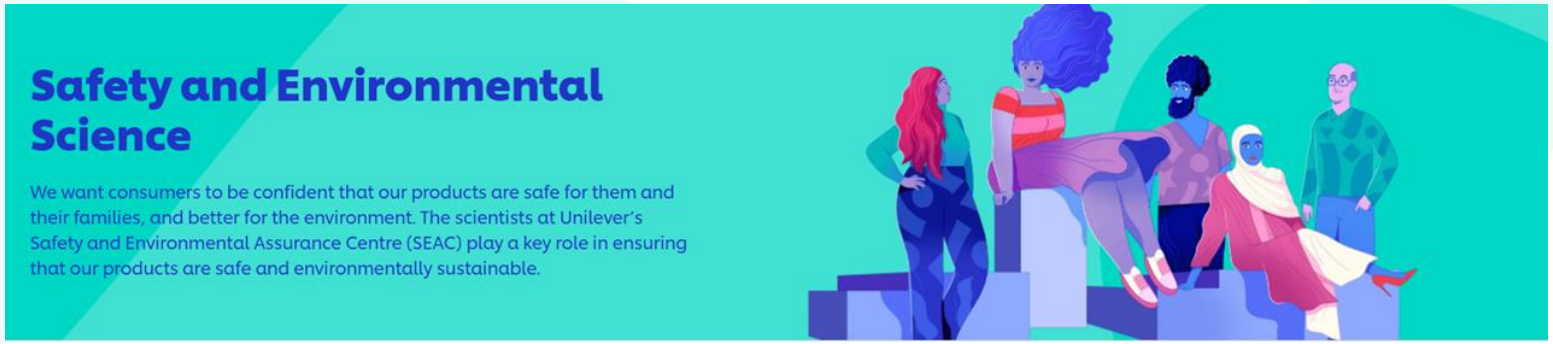
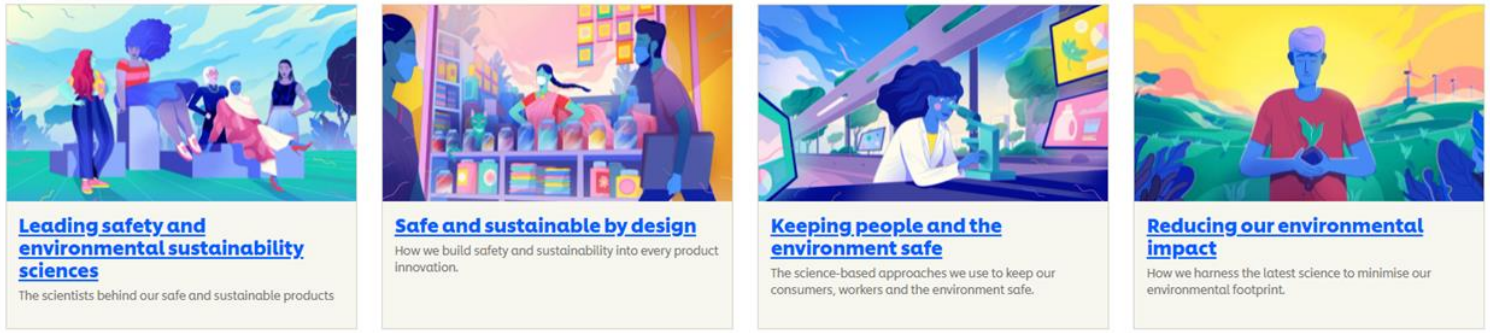
Unilever – Safety & Environmental Assurance Centre (SEAC)

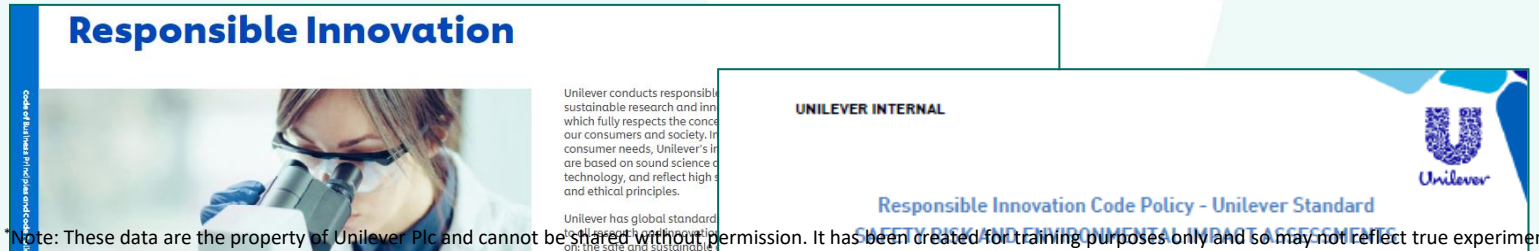
Ensuring Unilever's Innovations & Products are Safe & Sustainable by Design





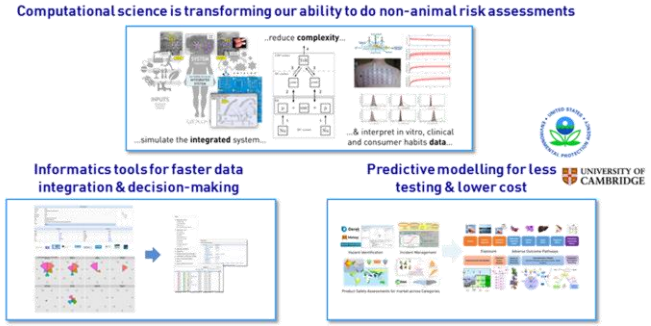
Unilever Product / Ingredient Safety Governance

- Provide scientific evidence to manage safety risks & environmental impacts



Industry-leading Safety & Environmental Sustainability Science Capability

- Deploy expertise on higher risk business projects
- Collaborate with leading external research teams to develop & apply new capability
- Leverage our science & global networks for consumer trust & freedom to operate



All underpinned by SEAC science, its scientists and our scientific partners

EPA United States Environmental Protection Agency

Search EPA.gov

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News Releases from Headquarters > Research and Development (ORD) CONTACT US

EPA and Unilever Announce Major Research Collaboration to Advance Non-animal Approaches for Chemical Risk Assessment

August 19, 2021

Contact Information
EPA Press Office (press@epa.gov)

WASHINGTON – Today, the U.S. Environmental Protection Agency (EPA) and Unilever announced a collaborative agreement to explore better ways to assess chemical risks associated with consumer products. This agreement builds on prior cooperation between EPA and Unilever regarding New Approach Methods (NAMs), which are a promising alternative to conventional toxicity testing that are intended to reduce reliance on the use of animals.

EPA and Unilever have been jointly evaluating and using NAMs since 2015. This collaboration is helping EPA implement its New Approach Methods Work Plan and is the foundation for new efforts to demonstrate that these novel approaches can help decision makers better protect consumers, workers and the environment.

"EPA is a pioneer in developing and applying NAMs to identify and quantify risks to human health, while reducing the use of animals in chemical toxicity testing," said **H. Christopher Frey, Deputy Assistant Administrator for Science Policy in EPA's Office of Research and Development**. "We are excited to continue the collaboration with Unilever, which enhances the robustness of our mutual research to demonstrate the use of NAMs."

19 Aug 2021

ICCR International Cooperation on Cosmetics Regulation

epaa The European Partnership for Alternative Approaches to Animal Testing

Details of SEAC's presentations and publications on www.tt21c.org

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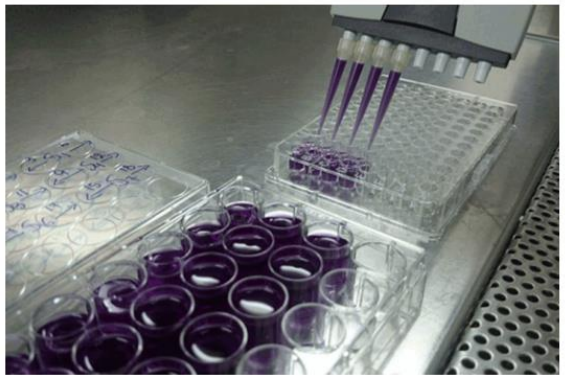
Unilever

Sharing our science



Videos

tt21c.org

Conferences and workshops

Webinar: Use of NAMs for Cosmetic Safety Assessment

Online 30th April 2020

Webinars

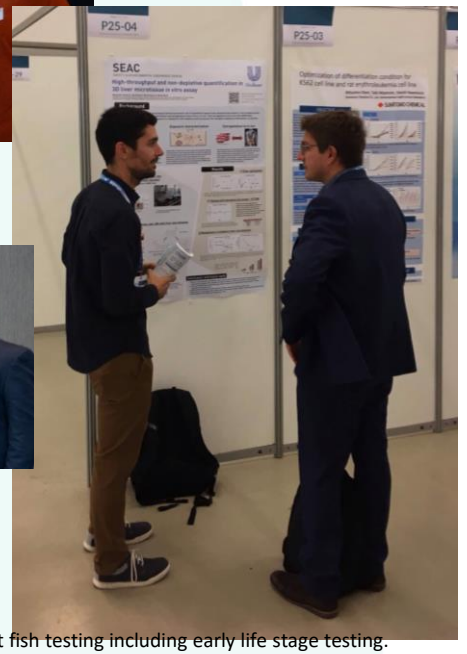
Identifying and Characterizing Stress Pathways of Concern for Consumer Safety in Next-Generation Risk Assessment

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

Computational Toxicology

Probabilistic prediction of human skin sensitiser potency for use in next generation risk assessment

Joe Reynolds*, Cameron MacKay, Nicola Gilmour, David Miguel-Vilumbrales, Gavin Maxwell



Scientific publications

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Driving Innovation of Environmental safety:

Advancing the science

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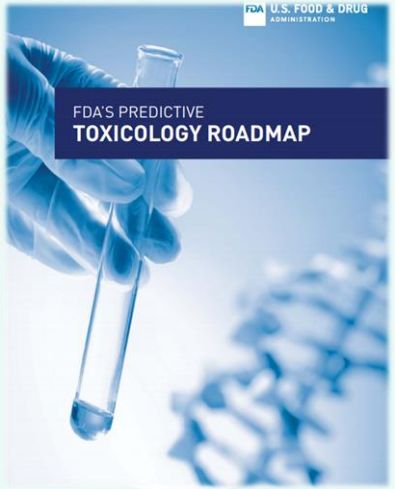
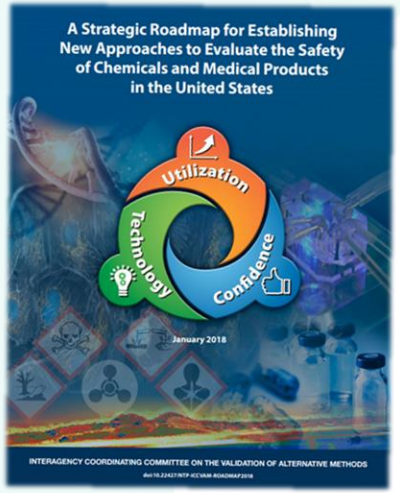
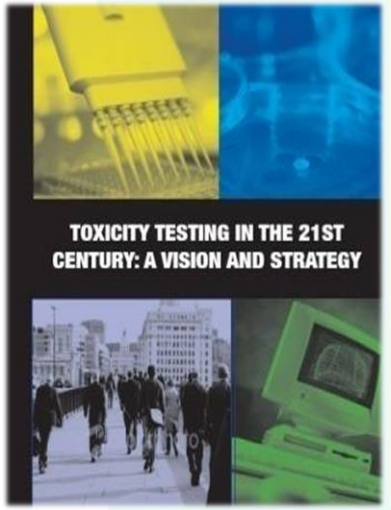
Next Generation Risk Assessment (NGRA)

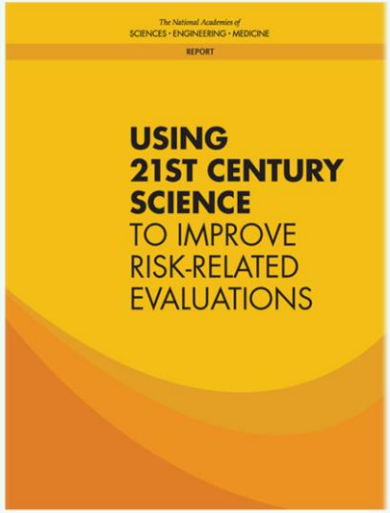
*NGRA is defined as **an exposure-led**, **hypothesis-driven** risk assessment approach that **integrates New Approach Methodologies (NAMs)** to assure **safety without the use of animal testing***

Safety without animal testing



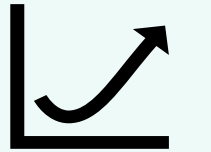




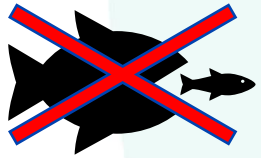


Safety science: what can we do better?

Ensuring that the use of ingredients in our products is **safe** for the receiving environment



Better, more sustainable chemicals



Moving away from animal tests

...THUS NAMs provide the opportunity for more mechanistic, higher throughput and animal-free ERA

Unilever

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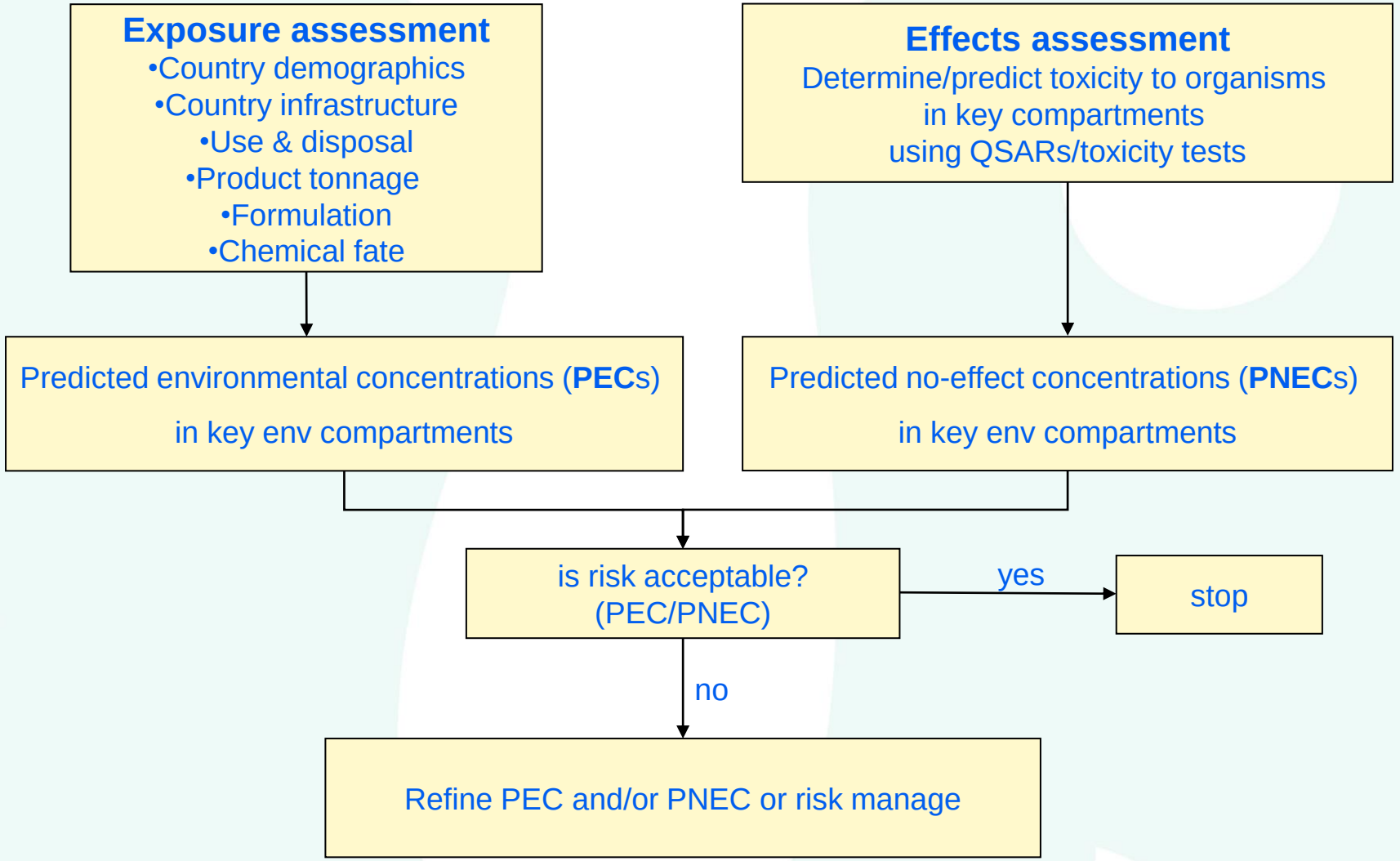
Environmental Risk Assessment (ERA) Framework

ERA is driven by the exposure



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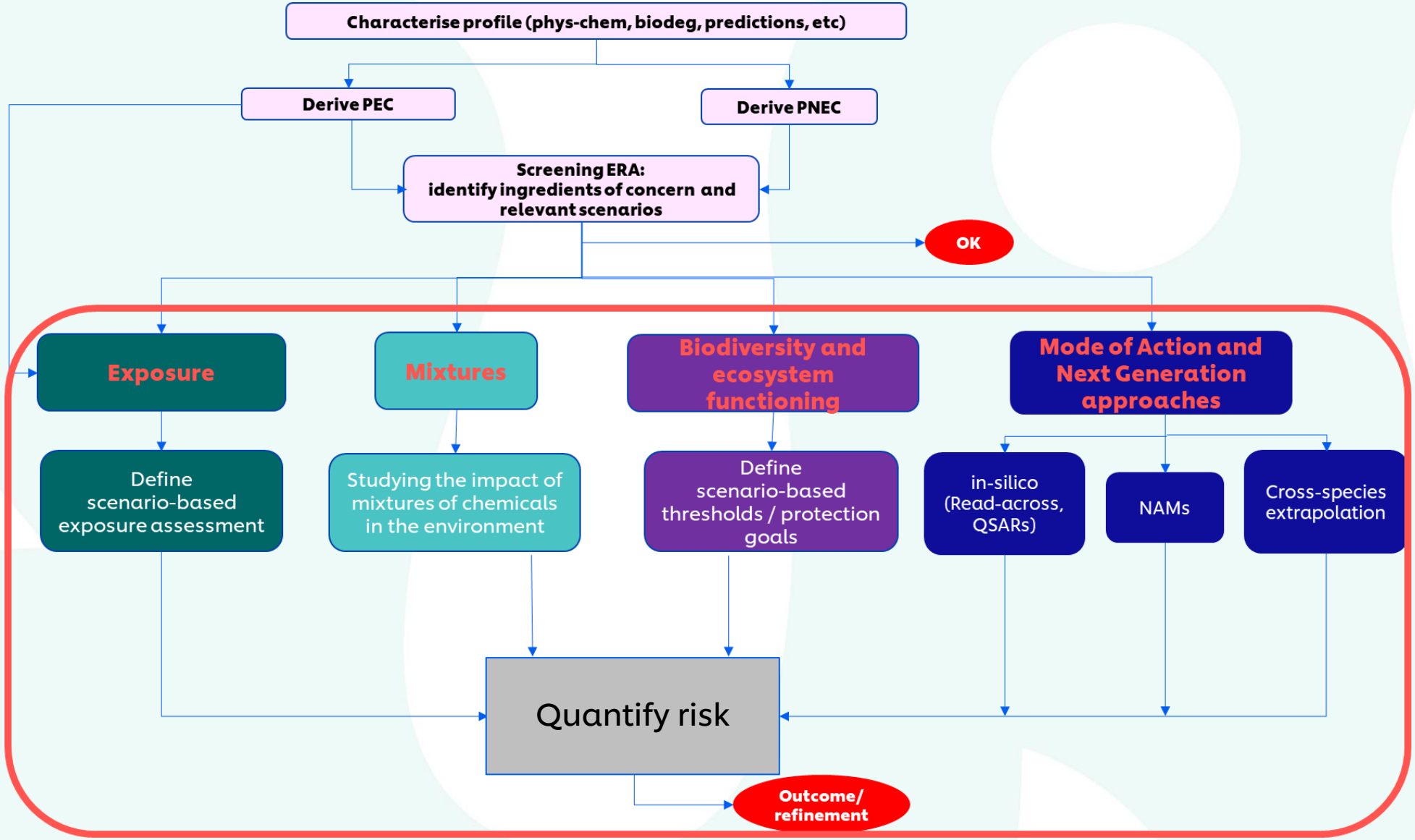
Safety science: what can we do better?



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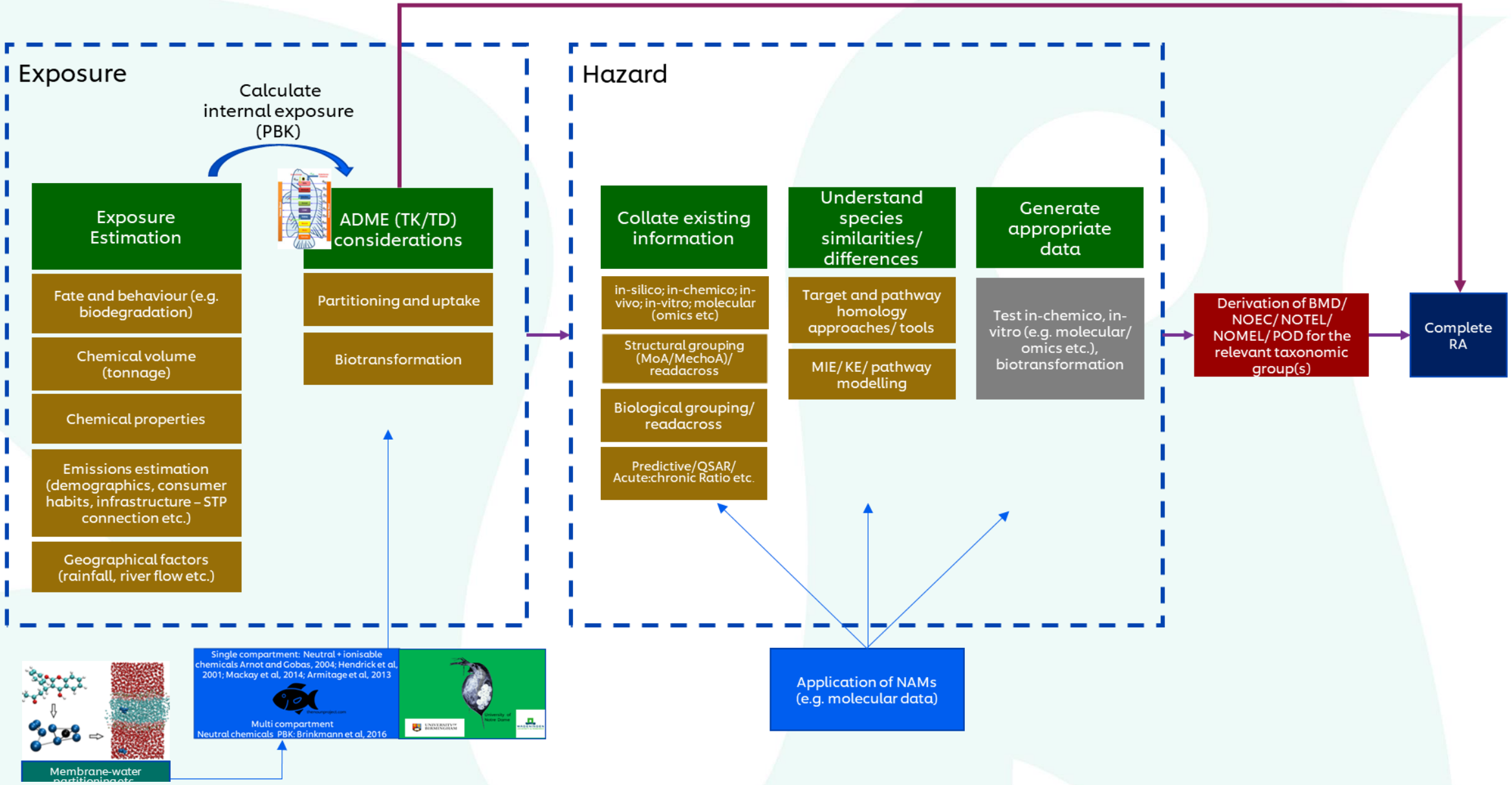
NAMs in environmental safety assessments (examples)



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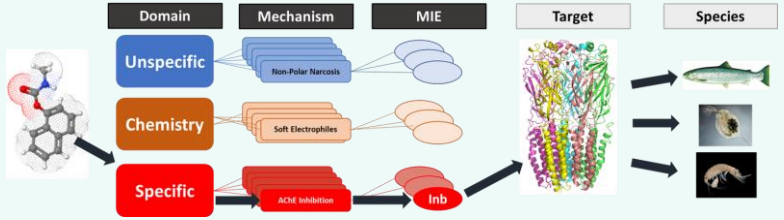
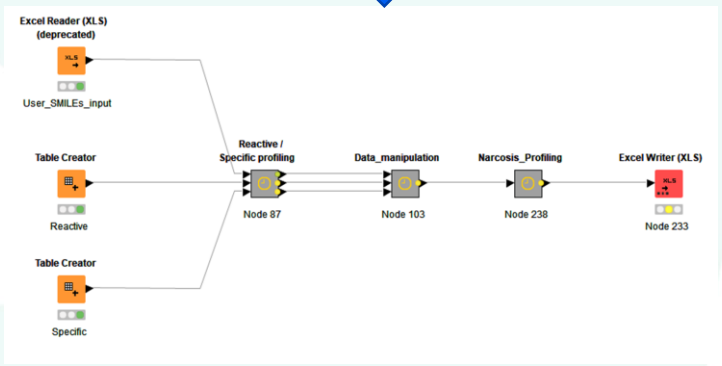
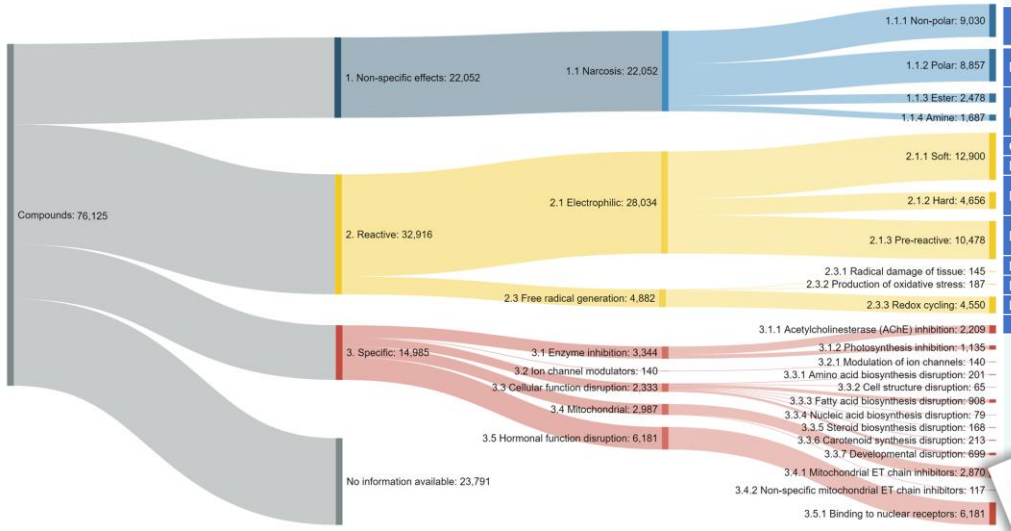
MIE/ MechoA profiling

To reduce the proportion of compounds that receive an “unclassified” by current schemes enabling more robust grouping/ read-across/ prioritisation

Domain	Mechanistic Group	
1. Unspecific	1.0 Narcosis 1.1 Uncoupling	1.0.1 Non-polar, 1.0.2 Polar, 1.0.3 Ester, 1.0.4 Amine 1.1.1 Other
2. Reactive / Chemistry based	2.1 Electrophilic 2.2 Free radical generation	2.1.1 Soft , 2.1.2 Hard, 2.1.3 Pre-reactive 2.2.1 Radical damage of tissues, 2.2.2 Production of oxidative stress, 2.2.3 Redox cycling
3. Specific	3.1 Enzyme inhibition 3.2 Ion channel modulators 3.3 Cellular function disruption 3.4 Mitochondrial 3.5 Hormonal function disruption	3.1.1 AChE inhibition, 3.1.2 Photosynthesis inhibition 3.2.1 Modulation of ion channels 3.3.1 Amino acid biosynthesis disruption, 3.3.2 Cell structure disruption, 3.3.3 Fatty acid biosynthesis disruption, 3.3.4 Nucleic acid biosynthesis disruption, 3.3.5 Steroid biosynthesis disruption, 3.3.6 Cerotenoid synthesis disruption, 3.3.7 Development disruption 3.4.1 Mitochondrial ET chain inhibitors, 3.4.2 Non-specific mitochondrial ET chain inhibitors 3.5.1 Nuclear receptors - ER, AR, TR etc.







Dataset	Origin	Number of compounds
REACH	REACH pre-registered substances (ECHA, 2008)	66832
DrugBank	Drugbank v. 5.1.8 Open Data (DrugBank, 2021)	10392
COSMOS	COSMOS DB	4303
Pharma	Firman et al., 2021	1,571
Pesticides	EU Pesticides Database (European Commission, 2021)	941
Botanicals	EFSA Compendium of Botanicals (EFSA, 2017)	899
Literature dataset	Various publications	3458
Mintel dataset	Mintel Global New Products Database	228
Monitoring dataset	Supplied by Unilever	2703
		76125

- Classified compounds
- Species coverage
- Chemical coverage
- Unique information particularly for the reactive and specific domains

MechoA

REATiS

Environment and Climate Change Canada
Environnement et Changement climatique Canada

LIVERPOOL JOHN MOORES UNIVERSITY

Unilever

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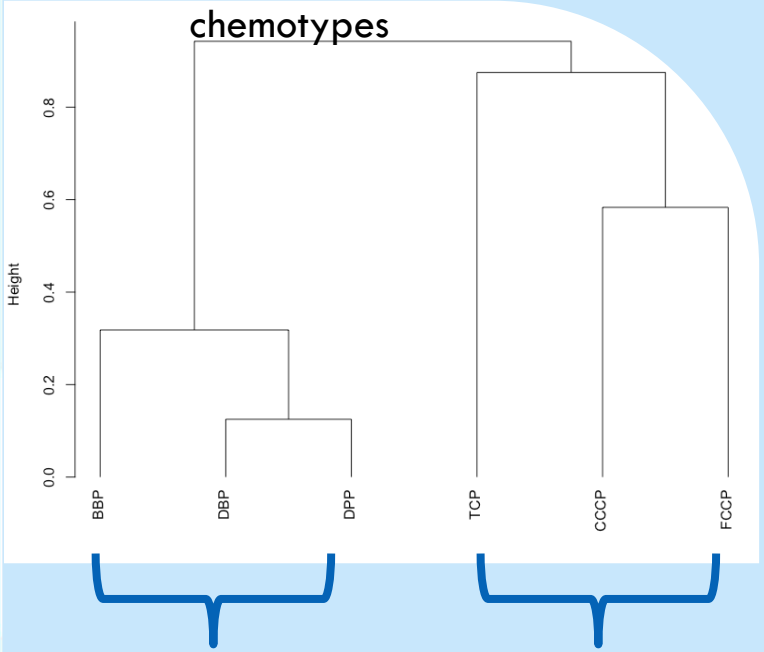
Omics based grouping for read-across

Conventional structure-based grouping hypothesis

Hierarchical clustering of ToxPrint chemotypes



Butyl phthalates

benzyl butyl phthalate (BBP)
dibutyl phthalate (DBP)
diisobutyl phthalate (DiBP)

Uncouplers of oxidative phosphorylation

2,3,4,5-tetrachlorophenol (TCP)
carbonyl cyanide 3- chlorophenylhydrazone (CCCP)
carbonyl cyanide 4-(trifluoromethoxy)phenylhydrazone (FCCP)

Omics-based chemical grouping

Focus Article on Omics-based grouping

Acute (48 h) exposure of juvenile (5 d) *D. magna* to 6 test compounds

Transcriptomics

Custom **BioSpyder** TempO seq platform covering **1991 *D. magna* genes**

Metabolomics

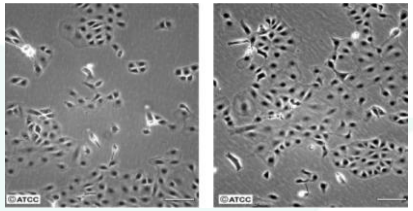
Hybrid **LC–MS(/MS)** assays by **Phenome Centre Birmingham**

Processing and statistical analysis of each omics data stream

Multi-omics based grouping

Fuse data streams and perform hierarchical cluster analysis

From *in vivo* to *in vitro*



Application of fish cell lines to inform hazard

RTgill-W1 Cell Line Assay for **Predicting Fish Acute Toxicity** in surfactants OECD 249

https://www.oecd.org/chemicalsafety/test-no-249-fish-cell-line-acute-toxicity-the-rtgill-w1-cell-line-assay-c66d5190-en.htm

CRACK-IT Challenge: Develop bioassays to report impairment of critical fish-specific pathways

Benchmark response of Human and Fish cell lines in response to impairment of **Cellular Stress**

Benchmark PODs derived to a common set of chemicals covering diverse MoA in Human and fish Cell lines

Culture cell lines without using animal compounds (e.g. **FBS**)

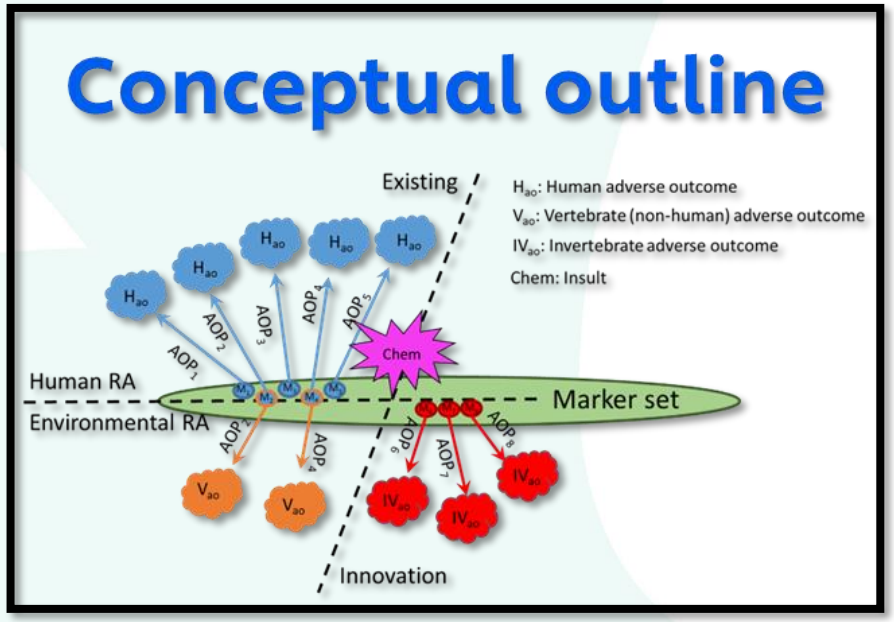
Cross-species extrapolation

Short-term → Long-term

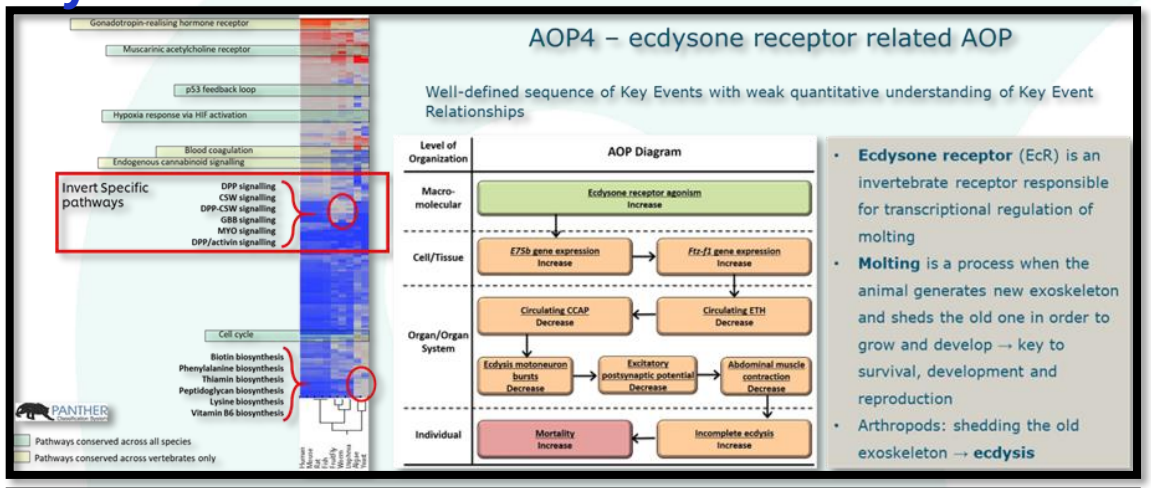
Development of invertebrate, regulatory relevant, in vitro screening panel

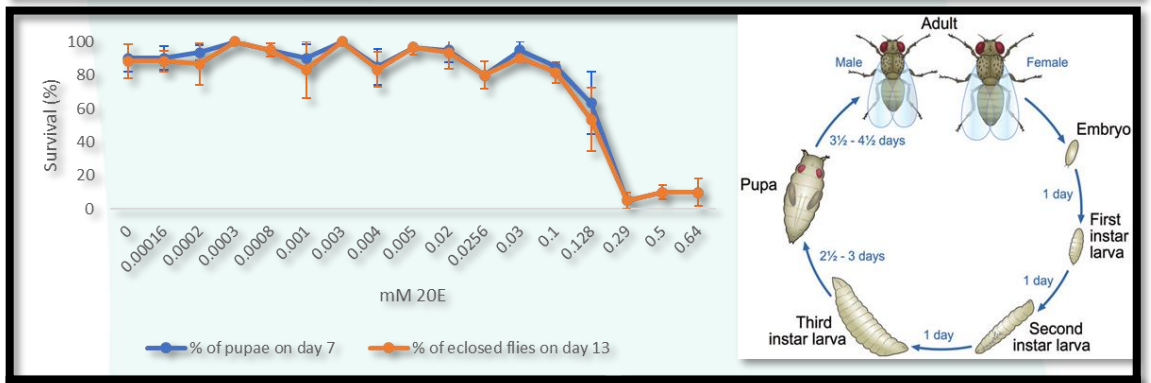
Key research objectives

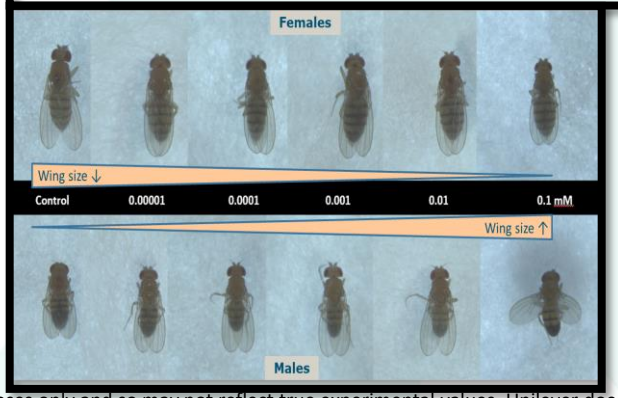
- Identification of relevant biological space and Adverse outcomes
- Quantitative understanding of key event relationships (KERs) integrating *in vitro* and *in vivo* experimental approaches
- Developing in vitro assays with biological pathway information specific for invertebrates
- Linking macroscopic (organism) adverse outcome to microscopic (cell) initiating event

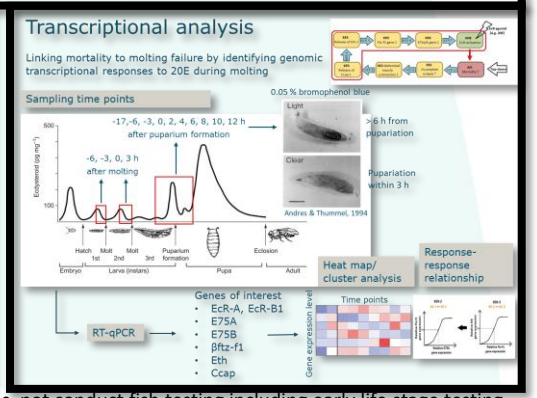


Key results









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Other relevant activities

Safety & Environmental Assurance Centre

Unilever

Durham University

Deriving baseline toxicity QSARs for ionisable organic chemicals by using experimental and computational membrane-water partition coefficients

Andrea Gredelj[1], Elin L. Barrett[1], Jayne Roberts[1], Thomas D. Potter[2], Alexandre Teixeira[1], Nicola Bettles[1], Geoff Hodges[1], Mark A. Miller[2]
[1] Safety and Environmental Assurance Centre, Unilever, Bedford, MK44 1LQ, UK [2] Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK

1. Introduction

- Quantitative Structure Activity Relationships (QSARs) provide a viable alternative to in-vivo toxicity testing for risk assessment.
- The majority of these QSARs are hydrophobicity-based relationships correlating the octanol-water partition coefficient, $\log K_{OW}$, of a chemical to its toxicity
- Despite this, determination of $\log K_{OW}$ for ionisable chemicals and surfactants is empirically difficult due to their tendency to accumulate at the octanol-water interface. Predictive methods are also often unreliable [1]
- Octanol cannot adequately describe the interactions of polar, charged, or amphiphilic compounds within ordered 3D structures of biological membranes.
- $\log K_{MW}$ provides a more biologically realistic approach for these compound types.
- Experimental determination of $\log K_{MW}$ can be complex and time consuming, therefore we have used coarse-grained simulation to predict $\log K_{MW}$ and compare derived QSARs for non-polar narcotics.
- Building on our previously presented work with perfluoroalkyl acids (PFCAs), here we explore a new QSARs case study for anionic surfactants using both experimental and computational approaches for generating $\log K_{MW}$.

2. Methods

- As non-specific toxicity (narcosis) is driven by critical accumulation in the phospholipid membranes, only chemicals with narcotic mode of action were used.
- Ecotoxicity data for the comparison of experimental and computational methods and two case studies were taken from external literature [2-5] and internal studies.

Experimental $\log K_{MW}$ data

- Liposome, Immobilised Artificial Membrane (IAM) & Solid Supported Lipid Membrane (SSLM) are taken from internal and literature data [6-8]

Predictive $\log K_{MW}$ data

- COSMOmic (developed by COSMOlogic [9]) was used to predict $\log K_{MW}$. Calculations were carried out using conformers generated from COSMOconf and micelle models from [10], using COSMOtherm 2021.
- Simulation data were predicted using coarse-grained simulations, with the mapping and parametrisation scheme described in [11].

3. $\log K_{MW}$ QSAR for Aquatic Toxicity

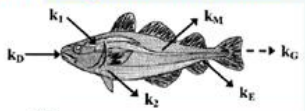
4. Case Study – PFCAs

Safety & Environmental Assurance Centre

Unilever

Review of PBK models for fish species of non-ionogenic compounds for use in Environmental Risk Assessment

Alessia Giorgis[1],Tymoteusz Pietrenko[1], Engi Patrik[1], George Fitton[1], Bruno Campos[1]
[1]Unilever, Research and Development, Sharnbrook, Bedford MK44 1LQ
E-mail contact: alessia.giorgis@unilever.com



$$\frac{dC_{int}}{dt} = k_{in}C_w - (k_{out} + k_G + k_m)C_{int}$$

Chosen models from the literature

Mackay model

The model is a first order one-compartment toxicokinetic model. It was developed using 9 generic organic chemicals with different hydrophobicity ($\log K_{ow}$ values from 0 to 8 with increasing molecular weight) applied to a 1g fish having lipid fraction of 5% for a 96-h test at 20 °C. (Fig 2) [4].

Hendricks model

The model is a one-compartment toxicokinetic model that combines both substance and species properties such as $\log K_{ow}$, the weight of an organism, lipid content and trophic level of a species in order to explain inter-substance and inter-species variability. In particular, the model describes the uptake of non-ionic chemicals that can pass the cell membrane via hydrophobic interactions onto the lipids [3].

Arnot and Gobas model

The model is a one-compartment model that provides an estimation of internal concentration from PODs generated using in vitro assays. The chemical concentration is described by the exchange between the surrounding medium (water) and the organism. The environmental conditions considered in the model are relevant environmental generic conditions [5].

COPENHAGEN

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UNIVERSITY OF BIRMINGHAM

Evaluating Predictive Toxicokinetic Models in *Daphnia magna* to Support Environmental Safety Decisions

Jacob-Joe Collins, Bruno Campos, Claudia Rivetti, Thomas Moxon, & Mark Viant

NC 3Rs Innovation Platform

Exploiting 3Rs technologies

Home > CRACK IT Challenges

CRACK IT Challenges

Challenge 41

SAFE

Innovative bioassays for environmental risk assessment and fish toxicity.

In progress 2021

Unilever

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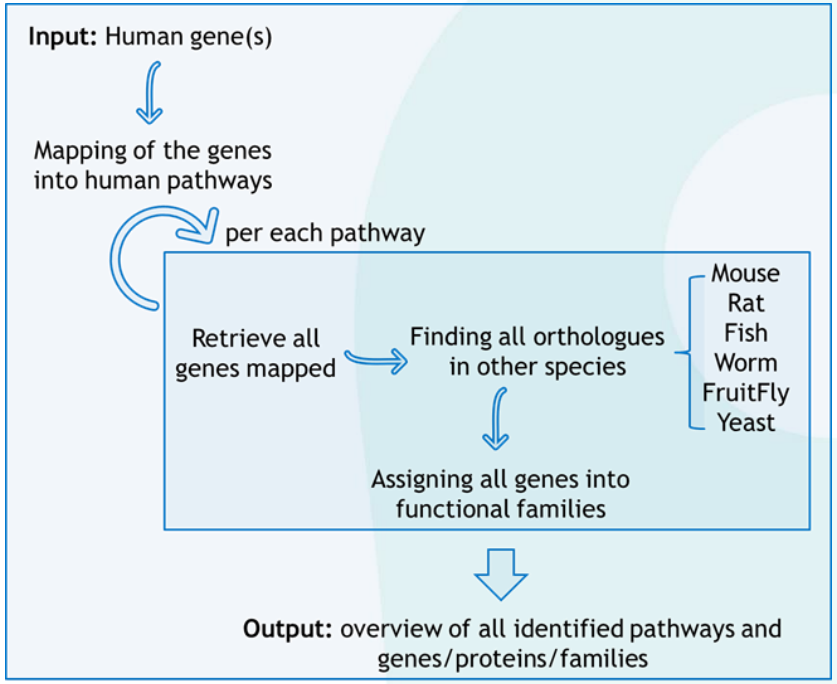
Genes to Pathways

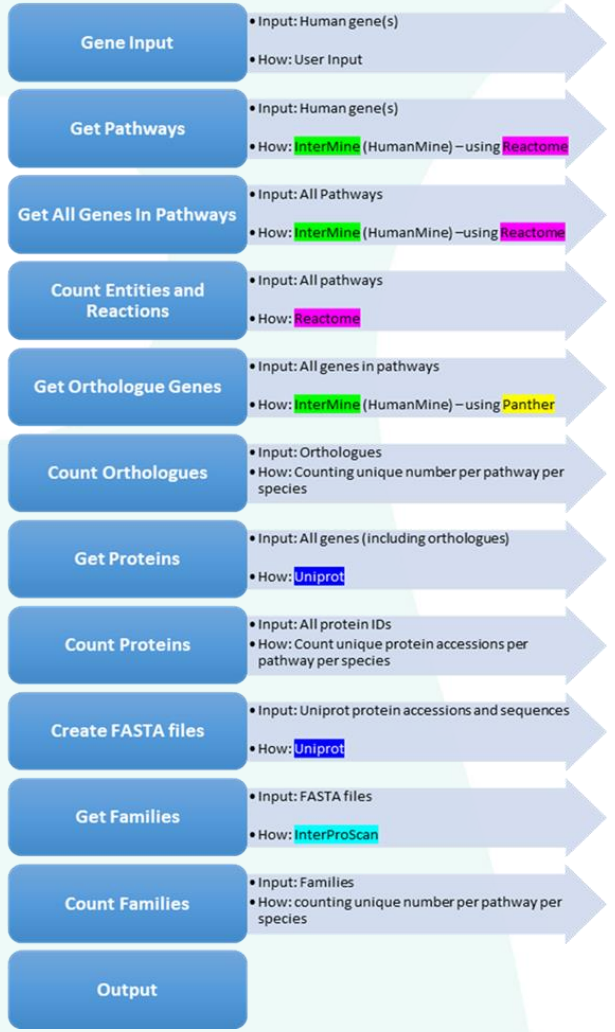
What?

A workflow linking human genes to pathways across species to support improved exploitation of existing data for ERA.

Why?

It is critical to discern the conservation in physiological processes across species to predict response patterns and toxicity outcomes in the environment.





How?

Leveraging on the integrated use of molecular available data in a WoE approach to serve as a scaffold for a mechanistically-driven testing strategy and hazard characterization.

When?

Publication in progress (code will be deposited on GitHub).

Unilever

InterMine PANTHER Classification System UniProt reactome InterPro

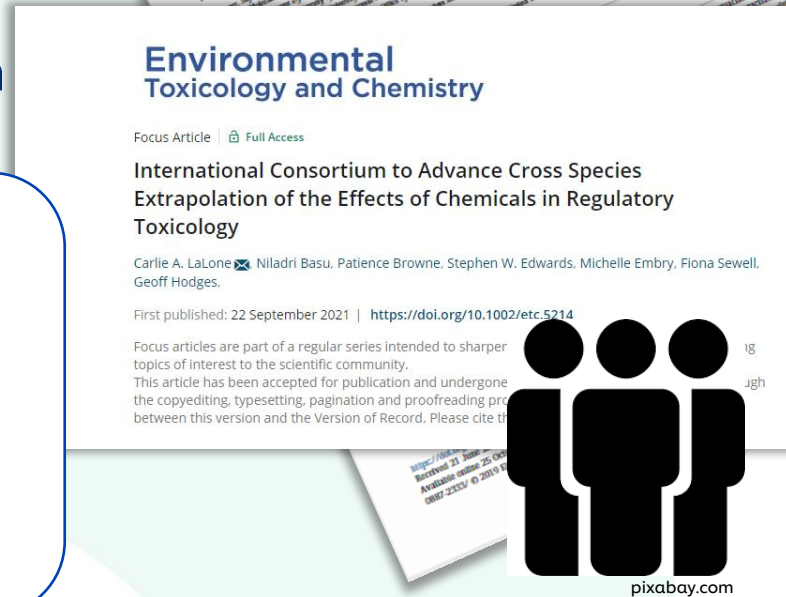
Consortium to Advance Cross Species Extrapolation in Regulation

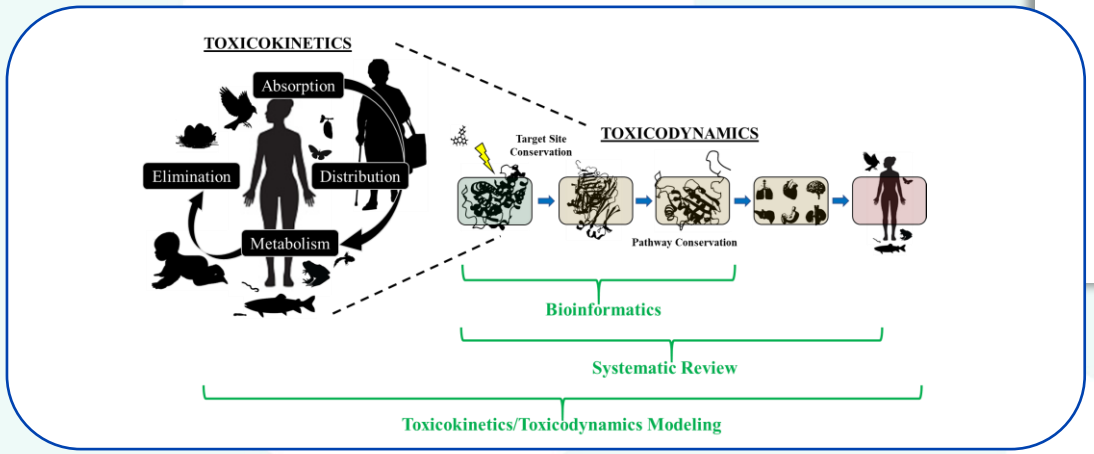
Government

Industry

<u>Steering Committee:</u>
Carlie LaLone (US EPA)
Geoff Hodges (Unilever)
Nil Basu (McGill U)
Steve Edwards (RTI)
Fiona Sewell (NC3Rs)
Michelle Embry (HESI)

1. **Define the taxonomic domain of applicability**
2. **Define the global regulatory landscape/need**
3. **Develop a bioinformatics toolbox**
4. **Communicate a shared scientific vision**





Academia

NGO

Unilever

*Note: These data are the property of Unilever Plc and cannot be shared without permission. It has been created for training purposes only and so may not reflect true experimental values. Unilever does not conduct fish testing including early life stage testing.

Case study
A framework to demonstrate the applicability of New Approach Methodologies (NAMs) in Environmental Risk Assessment (ERA)

Unilever
Safety & Environmental Assurance Centre (SEAC)
Colworth Science Park, United Kingdom

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Objectives

Evaluate the utility and the applicability of mechanistic-based information to complement and strengthen current ERA practices without the need for generating new animal data

- ✓ **Assessing the availability, suitability and power of NAMs-based data**
- ✓ **Benchmark mechanistically-derived Points of Departure (PoD) to complement current ERA practices**
- ✓ **Use all data as part of a weight of evidence approach to provide increased confidence in decisions**

The integration of historical *in vivo* data and NAMs can build confidence in safety decision making

Insights will help gain better mechanistic understanding of potential expected toxicity effects

Development of case studies to exemplify the applicability of the approach

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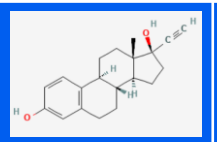
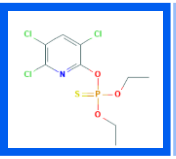
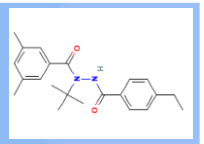
Case studies

Compound	Ethinylestradiol (EE2) 	Chlorpyrifos (CPS) 	Tebufenozide* 
Use	Contraception	Pesticide	Insecticide
Mode of Action	Oestrogen receptor agonist	Acetylcholinesterase receptor agonist	Ecdysone receptor agonist
Expected sensitive species	Vertebrates	*Animalia*	Invertebrates

* Case-study under development

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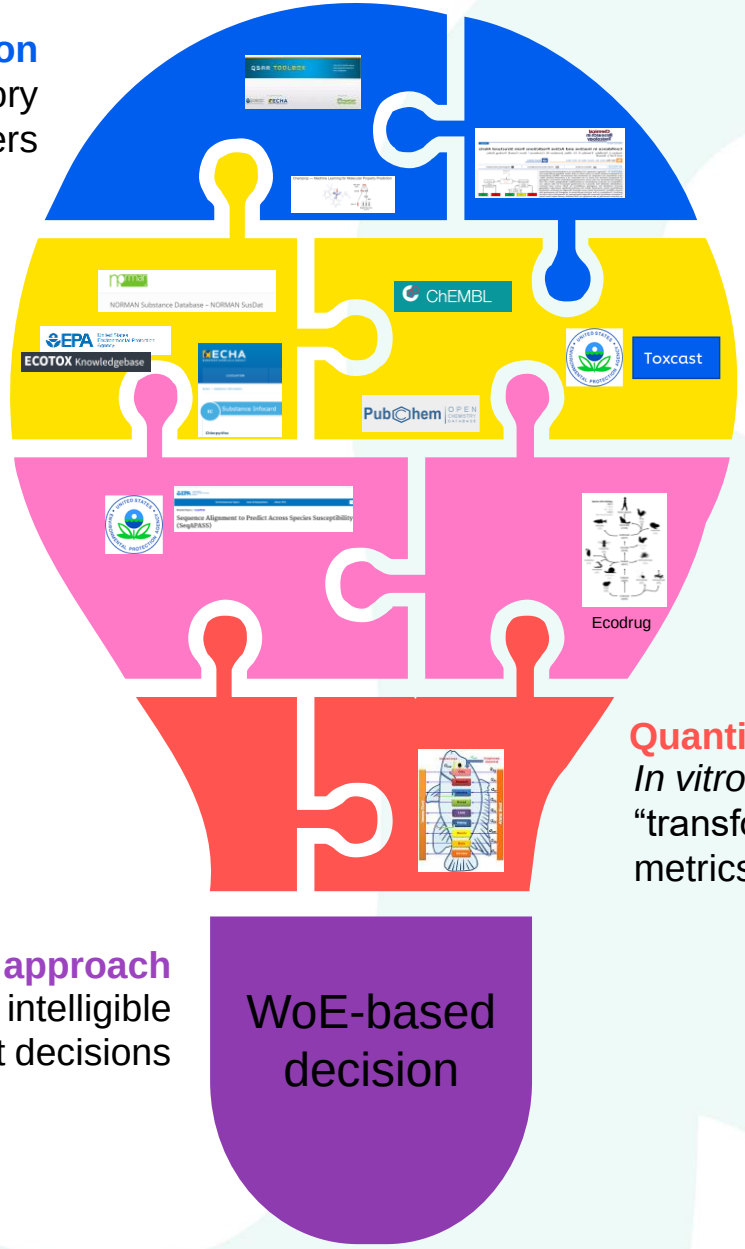
Information gathering process

Mode of Action identification

Using available scientific and regulatory information and in silico profilers



Hazard Data

Including historical *in vivo* as well as *in vitro* data and *in silico* predictions to generate relevant Point of Departure (PoD)

Species at risk identification

Use of publicly available tools and databases to identify susceptible species (based on targets and processes)

Quantitative In Vitro to In Vivo Extrapolation

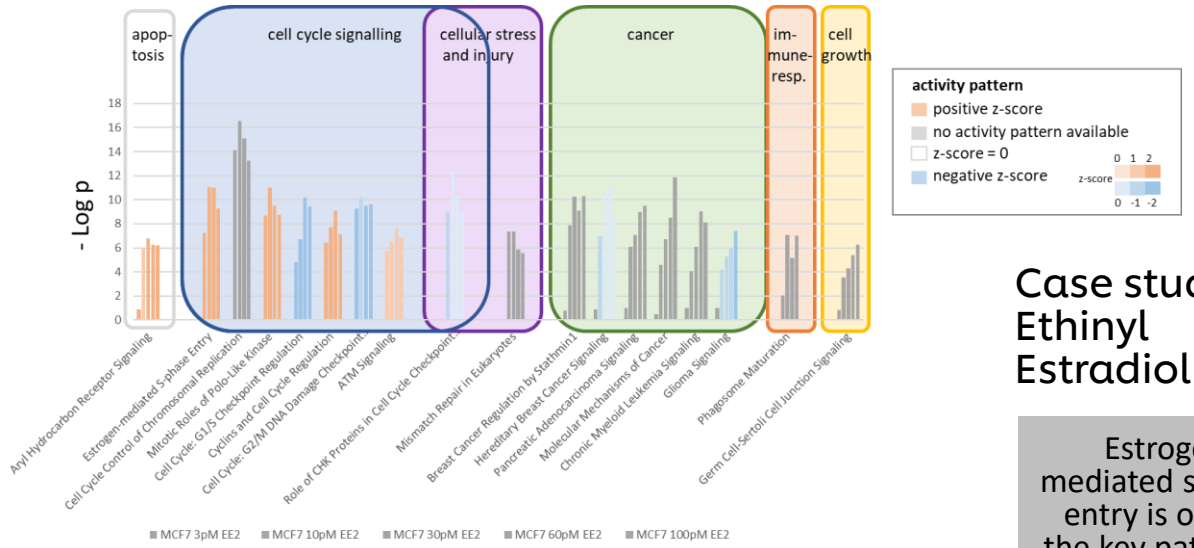
In vitro and *in vivo* exposures must be "transformed" into comparable exposure metrics requiring robust qIVIVE models

Weight Of Evidence approach

Collate all the information in an intelligible way to guide and support decisions

Characterising hazard

Canonical Pathway analysis

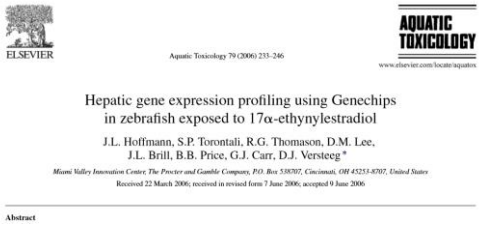


Top 20 pathways predicted by Ingenuity Pathway Analysis (IPA) according to top p-value

Case study: Ethinyl Estradiol (EE2)

Estrogen mediated s-phase entry is one of the key pathways but other pathways are also identified

Microarray analysis



ELSEVIER

Aquatic Toxicology 79 (2006) 233–246

AQUATIC TOXICOLOGY

Hepatic gene expression profiling using Genechips in zebrafish exposed to 17α-ethynylestradiol

J.L. Hoffmann, S.P. Torontali, R.G. Thomason, D.M. Lee, J.L. Brill, B.B. Price, G.J. Carr, D.J. Versteeg*

Miami Valley Innovation Center, The Procter and Gamble Company, P.O. Box 538707, Cincinnati, OH 45253-8707, United States

Received 22 March 2006; received in revised form 7 June 2006; accepted 9 June 2006

Abstract

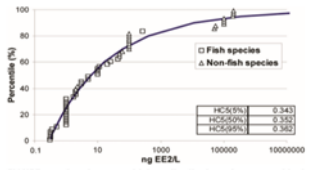
NOTEL 168h* = 15ng/L (50pM)

Pathway with lowest BMD at 168h: 23.23 ng/L (78pM)

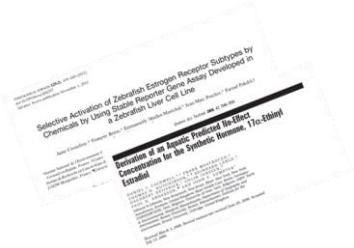
*Threshold FC >2, p < 0.05, a cut of at FDR < 0.1 would change the numbers of DEGs but not the NOTEL

Hoffmann et al., (2006)

Other literature information



HC5 (50%) = 352 ng/L (1.2pM)



Selective Activation of Zebrafish Estrogen Receptor Subtypes by Chemicals by Using Stable Reporter Gene Assay Developed in a Zebrafish Liver Cell Line

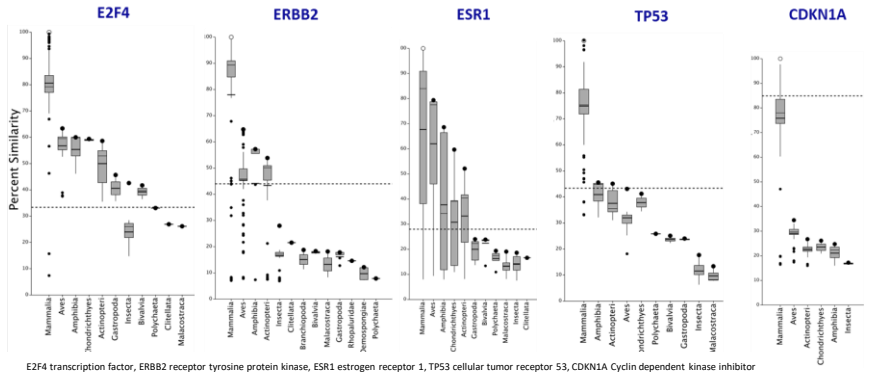
Derivation of an Aquatic Predicted No-Effect Concentration for the Synthetic Hormone, 17α-Ethinyl Estradiol

EC50= 0.03nM (30pM) (fish ER luciferase assay)

Toxcast

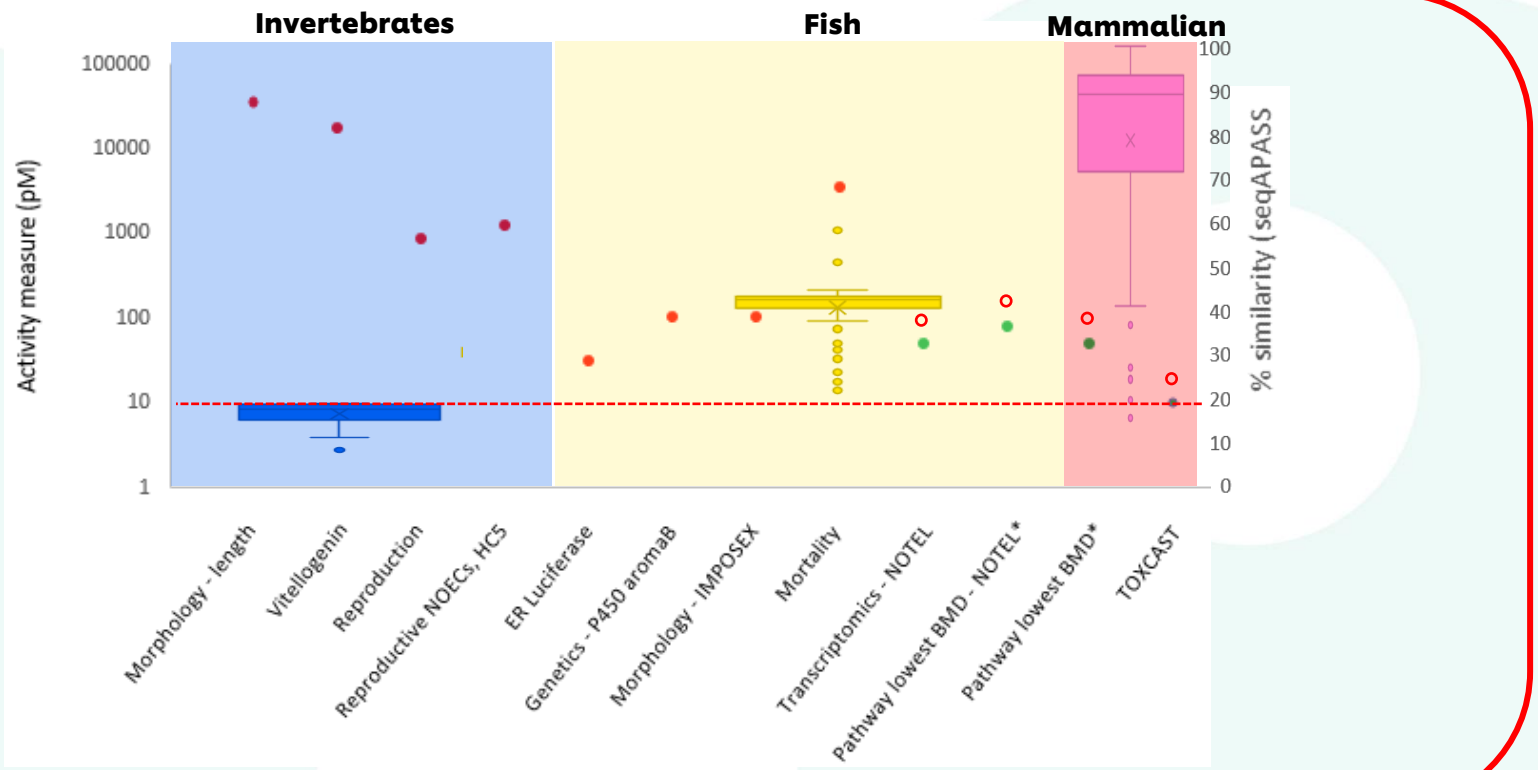
Taxonomic relevance

Sequence Alignment to Predict Cross Species Susceptibility (SeqAPASS)



E2F4 transcription factor, ERBB2 receptor tyrosine protein kinase, ESR1 estrogen receptor 1, TP53 cellular tumor receptor 53, CDKN1A Cyclin dependent kinase inhibitor

Previous case study: ethinylestradiol



- *In silico*
- *In vitro*
- *In vivo*
- *In vivo after reverse dosimetry calc*
- *SEQapass act. threshold*

Microarray analysis


AQUATIC TOXICOLOGY

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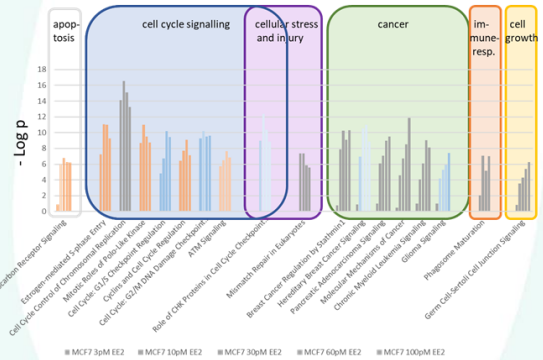

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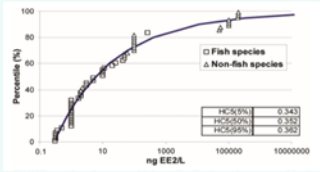

HC5 (50%) = 1200 pM


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EC50= 30pM (ER luciferase assay)

Toxcast

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Key highlights

These case studies demonstrate that the integration of existing traditional *in vivo* data and *in vitro* functional assays from literature coupled with computational tools in a weight of evidence approach can build confidence in safety decision-making.

In summary :

- ✓**Provides confidence that most sensitive species can be identified (in line with historical knowledge of chemicals);**
- ✓**Species sensitivity is in line with MoA and target conservation throughout the tree of life;**
- ✓ ***in vitro* endpoints seem to be at least as protective as traditional *in vivo*.**

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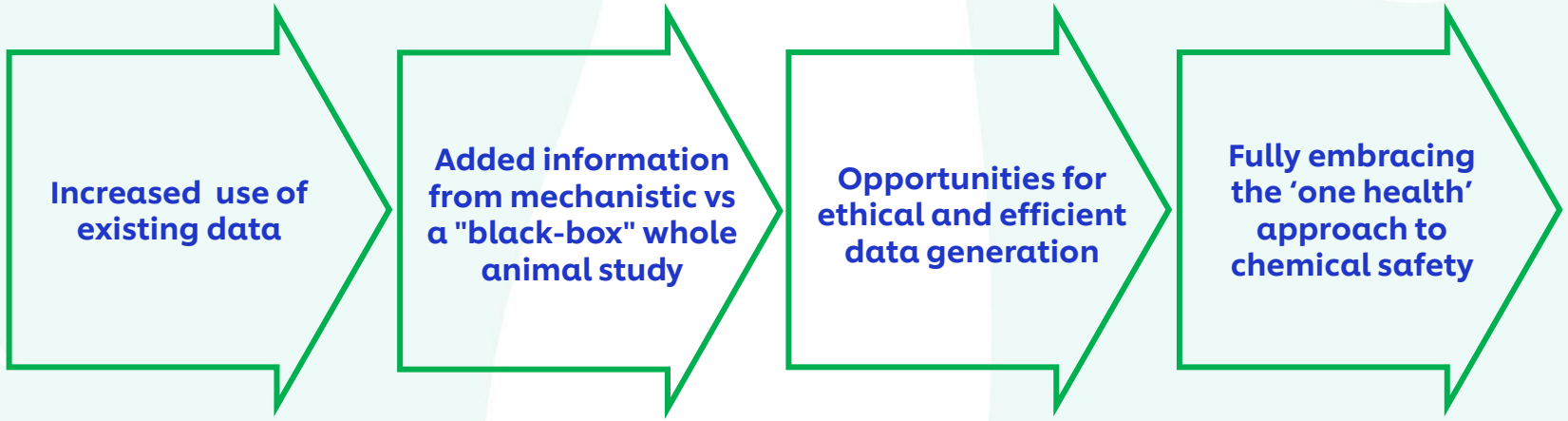
Take-home messages

Challenges that needed to be addressed…

- Lack of standardised study designs may hinder data usage
- Challenges for data-poor chemicals
- No one-size-fit-all approach

If solved can lead to…



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Real world applications of state of the art science

Case study: renewable ingredients in Sunlight dishwash liquid

A new Sunlight dishwash liquid containing the renewable and biodegradable foaming ingredient called Rhamnolipid is a great example of our safety and sustainability scientists in SEAC working with R&D teams to create safe and sustainable products fit for a cleaner future.

Our safety scientists, computational chemists and mathematicians used detailed knowledge of how people use hand dishwash liquid in different parts of the world alongside leading-edge non-animal approaches to generate new scientific evidence, which allowed us to show that the new Rhamnolipid based product is safe to use.

Our SEAC sustainability scientists assessed the environmental impacts of Rhamnolipids against existing surfactant ingredients in hand dishwash liquids derived from petrochemicals (such as fossil fuel or coal). This work showed that the innovation of swapping to use Rhamnolipid in hand dishwash liquid not only leads to a safe product with better cleaning performance but also one that is sustainable with less environmental impact.

https://www.unilever.com/planet-and-society/safety-and-environment/safe-and-sustainable-by-design/

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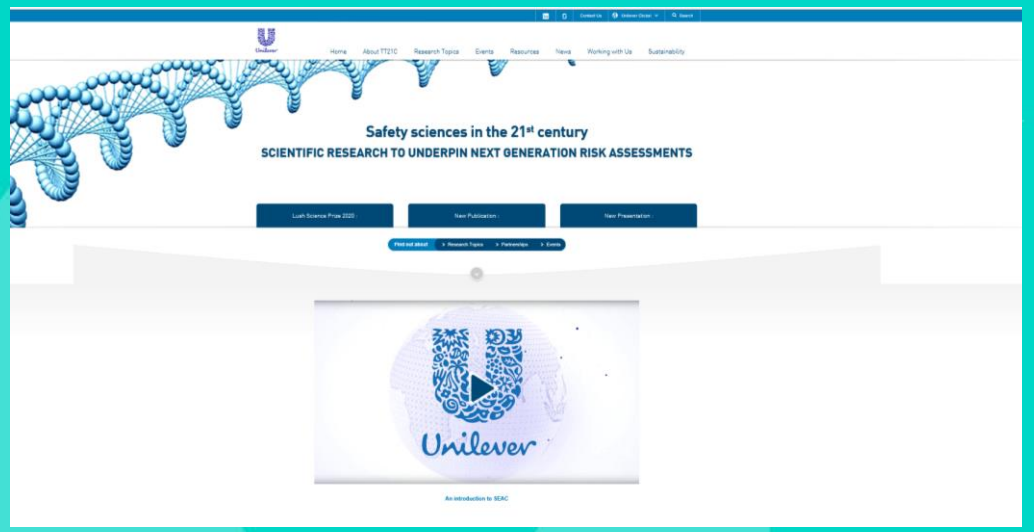
Questions?

- Emilia Gattas
- Amy Jupp
- Nicola Furmanski
- Jayne Roberts
- John Kilgallon
- Claudia Rivetti
- Geoff Hodges
- Alexandre Teixeira
- Chris Finnegan
- Ian Malcomber
- Juliet Hodges
- David Gore
- Roger van Egmond
- Maria Blanco-Rubio
- Alessia Giorgis
- Paul Carmichael
- Mathura Theiventhran
- Danilo Basili
- Predrag Kukic
- Iris Muller
- and many more



www.tt21c.org/

bruno.campos@unilever.com

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