New Approach Methodologies-based Environmental Next-generation Risk Assessment of Chemical – An industry perspective from Unilever

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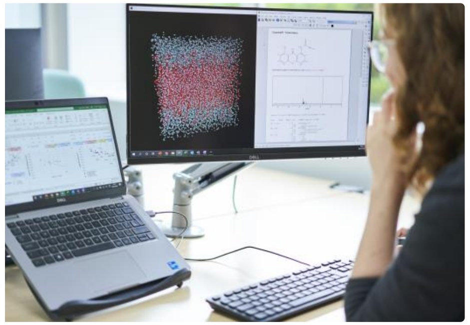
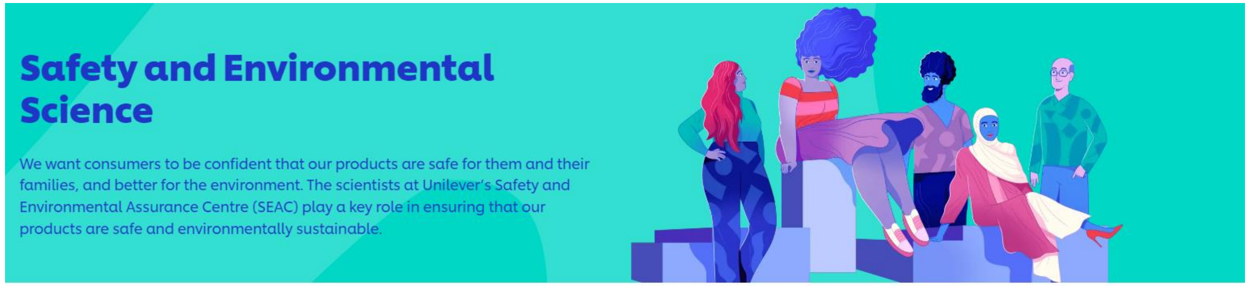




Leading safety and environmental sustainability sciences

The scientists behind our safe and sustainable products

Safe and sustainable by design

How we build safety and sustainability into every product innovation.

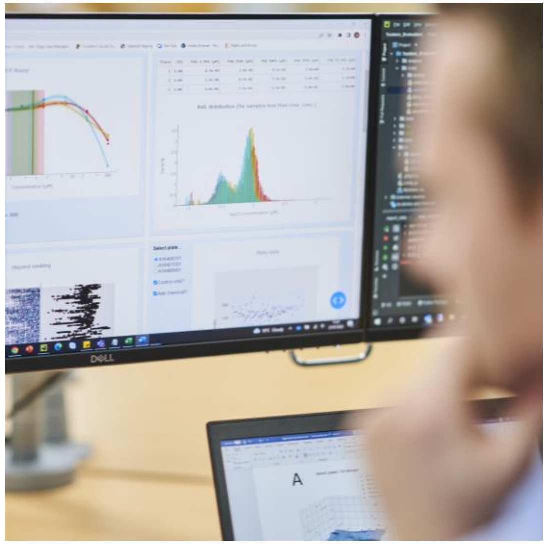
Keeping people and the environment safe

The science-based approaches we use to keep our consumers, workers and the environment safe.

Reducing our environmental impact

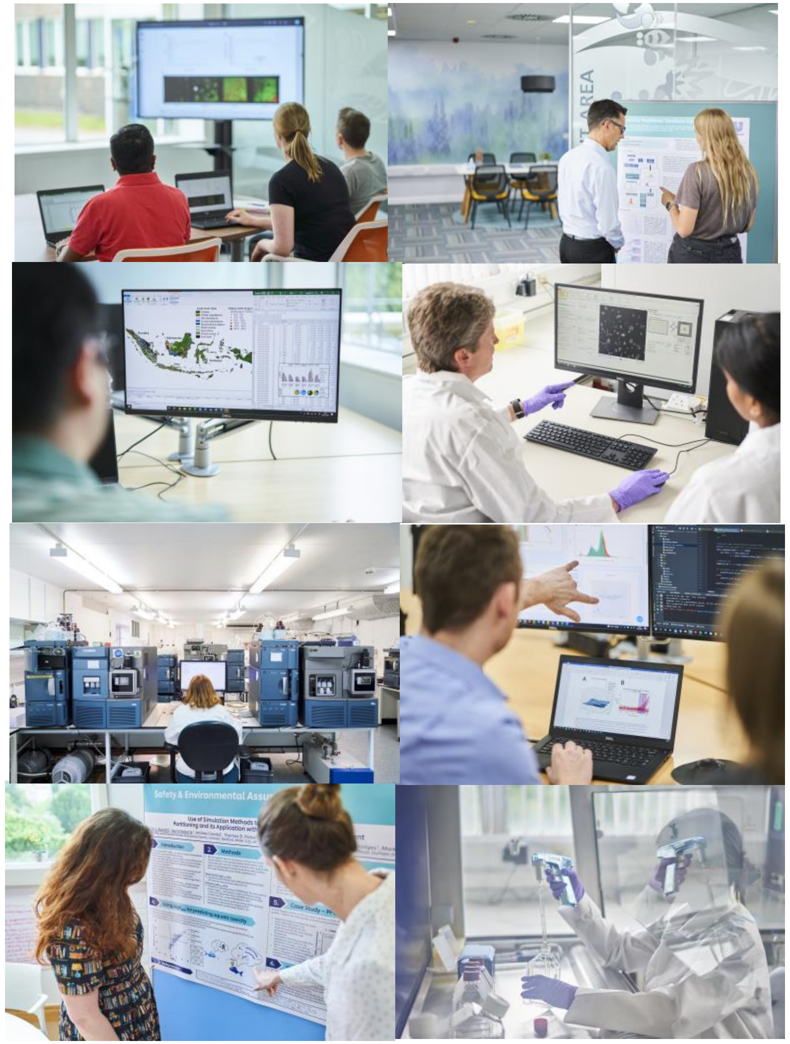
How we harness the latest science to minimise our environmental footprint.

Introduction to SEAC



Unilever's Global Centre of Excellence in Safety & Sustainability Sciences

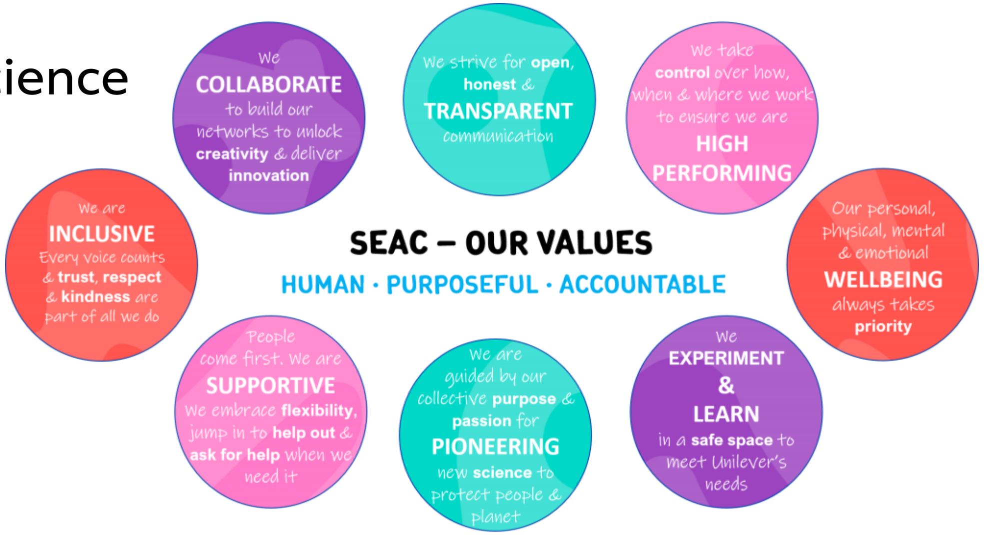
Team SEAC's purpose is to protect people & the environment



SEAC is a diverse, multi-disciplinary team of ~150 scientists covering:

- Cell Biology
- Chemistry
- Computational Modelling
- Environmental Safety
- Environmental Sustainability
- Exposure Science
- Informatics & Data Science
- Mathematics
- Microbiology
- Molecular Biology
- Process Safety
- Statistics
- Toxicology

20+ Nationalities
15+ Languages



Much of our strength lies in our shared Values – to be an **inclusive**, **supportive** & **collaborative** Team that is **pioneering**, **transparent** & **high-performing** with a strong focus on **learning** & **wellbeing**.

Unilever's Safety & Environmental Assurance Centre (SEAC)

SEAC is Unilever's global centre of excellence in Safety & Sustainability Sciences, part of R&D's Safety, Environment & Regulatory Sciences Capability.

Diverse, multi-disciplinary team of ~150 scientists based at Colworth, UK; ~70 miles north of London

Highly collaborative, working with over 70 academic, industry, government & NGO partners worldwide

Business Group R&D

- **Global Teams**
- **Business Units**

'One R&D' Centre

- Safety, Environment & Regulatory
- Digital & Partnerships
- Sustainable Packaging

Team SEAC's purpose is to protect people & the environment :

Unilever's products & innovations are Safe & Sustainable by Design without animal testing

Safety without Animal Testing:

- **Unilever is committed to ending animal testing globally**. We believe in using science, not animals, to assure the safety of our products and their ingredients.

- **Non-animal safety approaches are applied by our leading-edge scientists** in collaboration with world-class researchers & experts.

- We engage with all stakeholders to build shared understanding and promote trust in **our scientific evidence-based approach to decision-making**.

Safety and Environmental Science

We want consumers to be confident that our products are safe for them and their families, and better for the environment. The scientists at Unilever's Safety and Environmental Assurance Centre (SEAC) play a key role in ensuring that our products are safe and environmentally sustainable.

Leading safety and environmental sustainability sciences

The scientists behind our safe and sustainable products

Safe and sustainable by design

How we build safety and sustainability into every product innovation.

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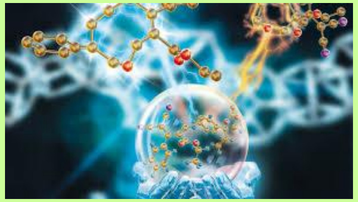
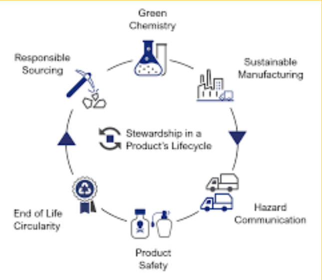
Improved ENVIRONMENTAL PROTECTION

Legal commitments and standards

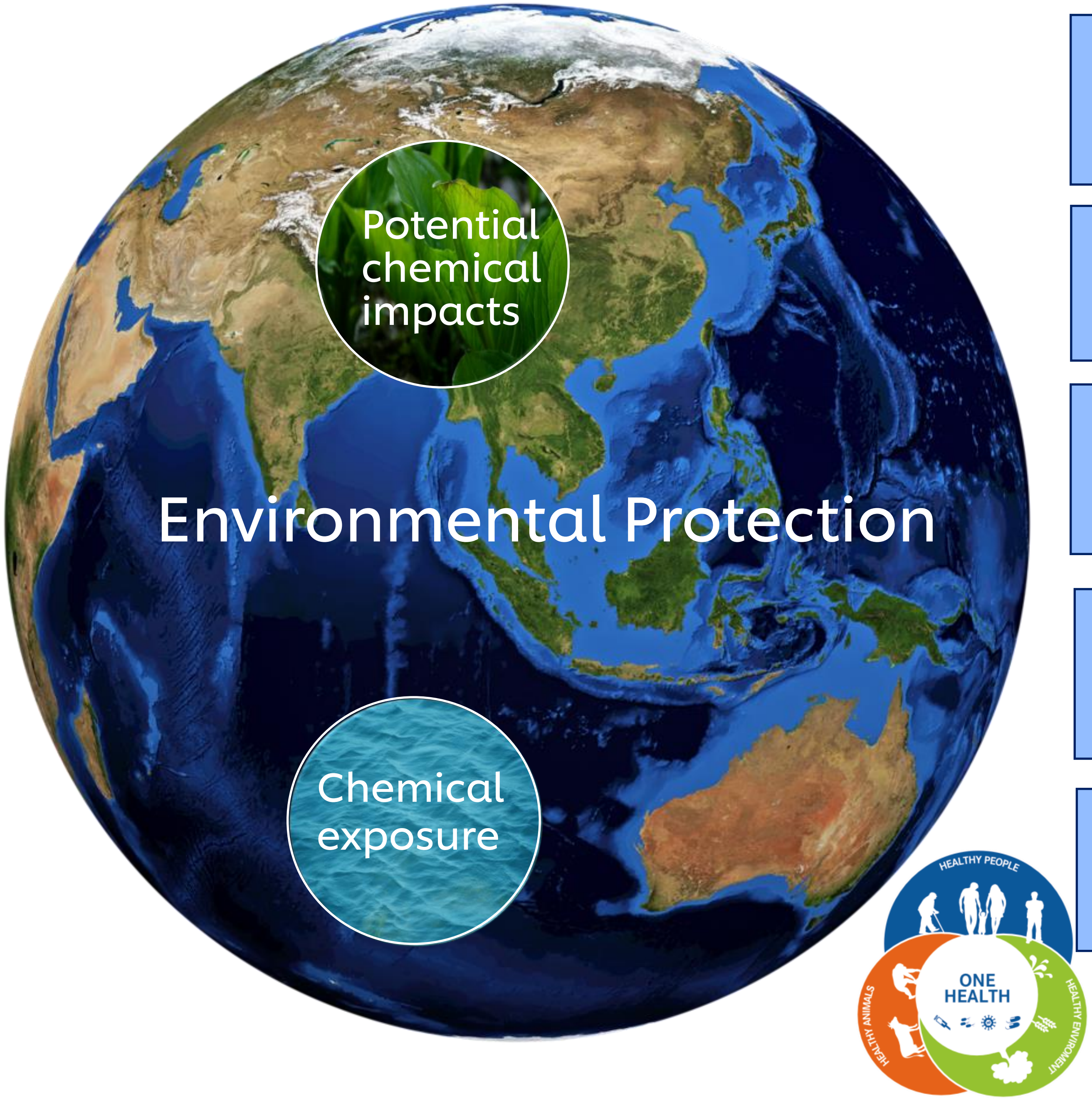
Scientific Research to increase knowledge



Stewardship



Key Enablers



Potable water

Food and agriculture

Population health

Biodiversity and ecosystem function

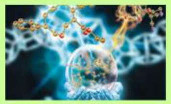
Amenities

Key Benefits

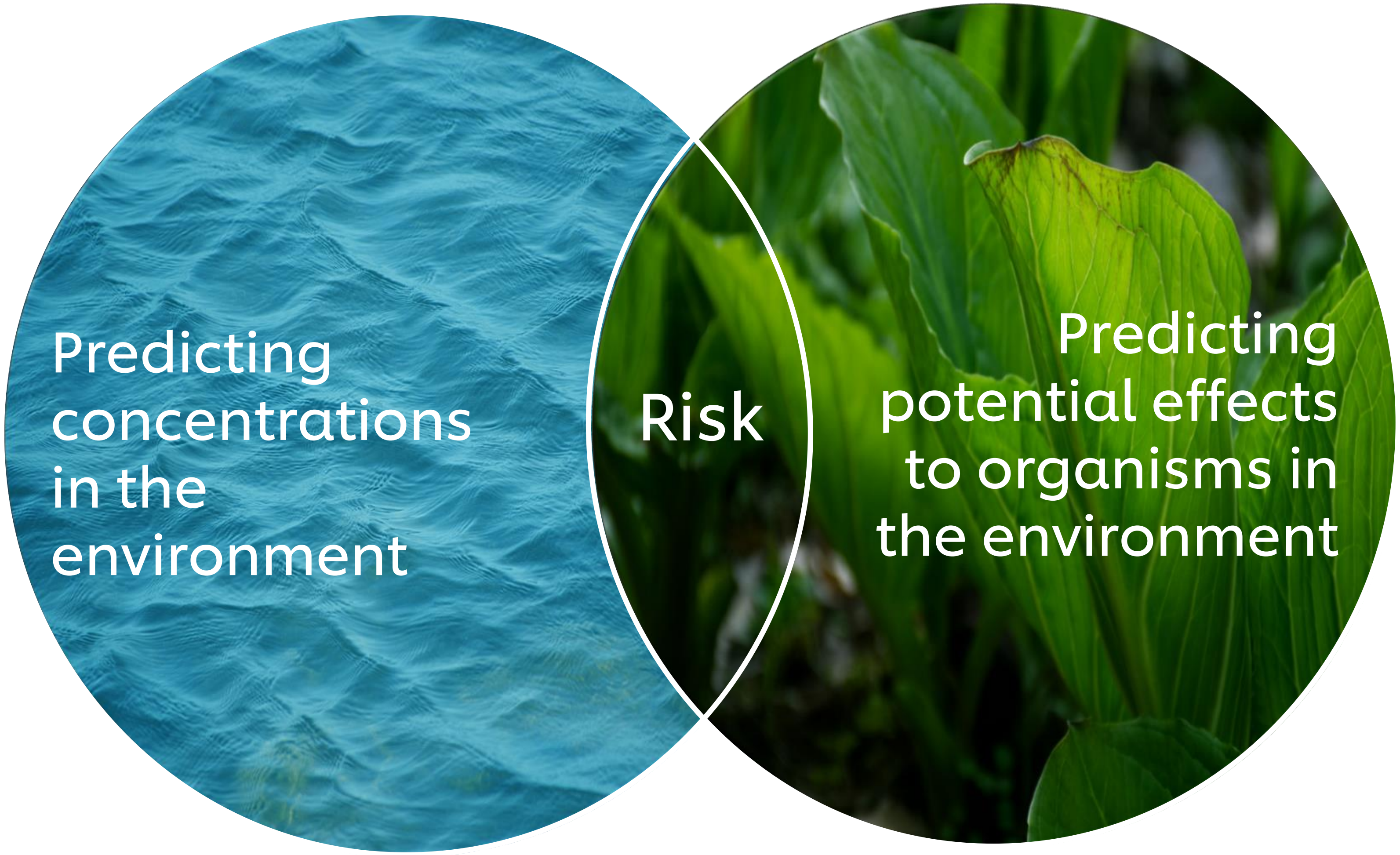
Environmental Risk Assessment (ERA)

Stewardship

Science and knowledge increase



Legal commitments



Regulatory challenges

Data challenges

Knowledge challenges

Ethical and societal challenges

PETA Cruelty-Free

Key approach for understanding potential impacts of chemicals in the environment

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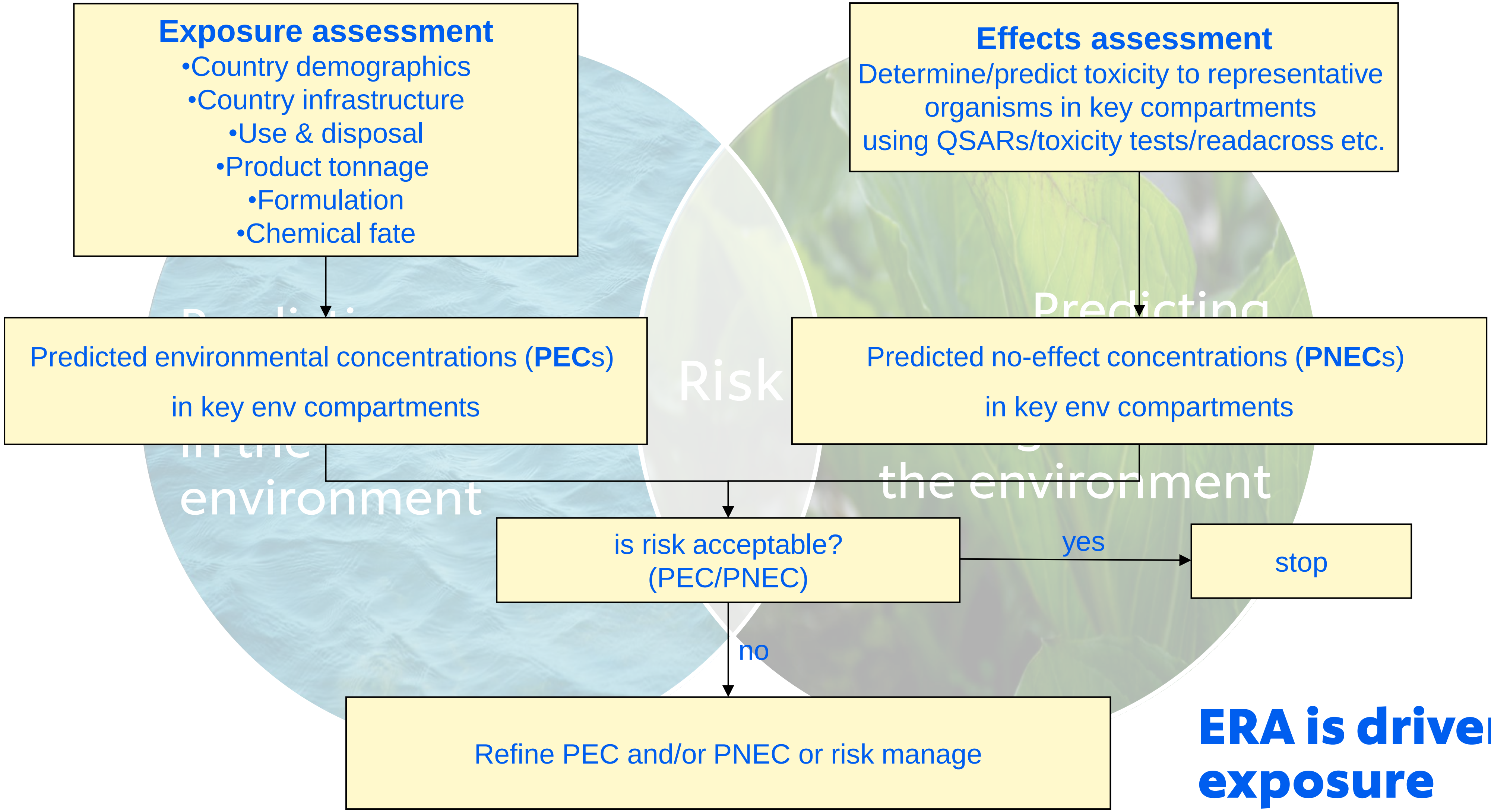
Environmental Risk Assessment (ERA)

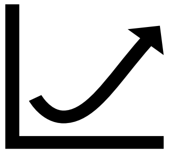


ERA is driven by the exposure

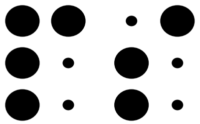
Safety science: what can we do better?

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

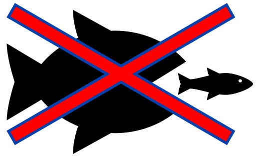
for the receiving environment **YET…**



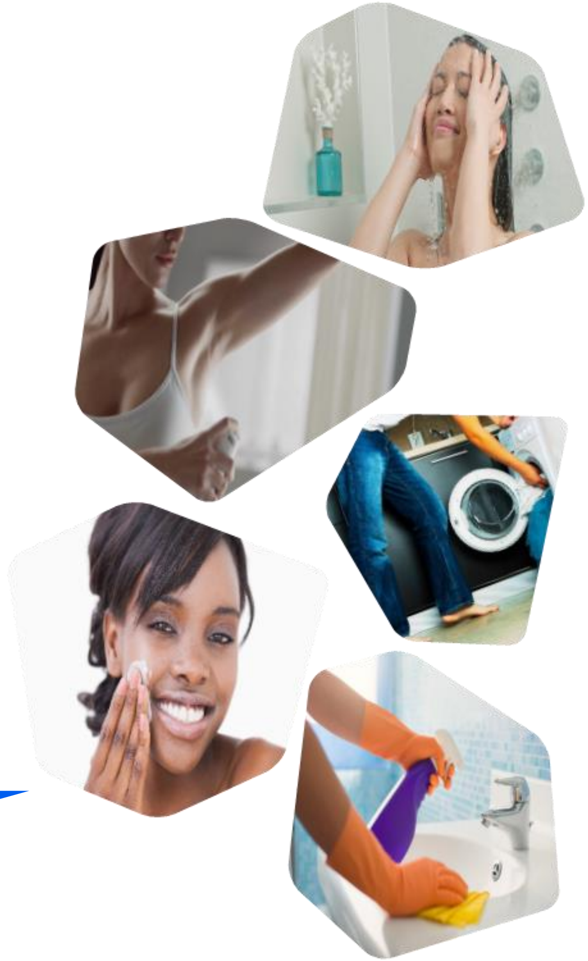
Increasing number of chemicals



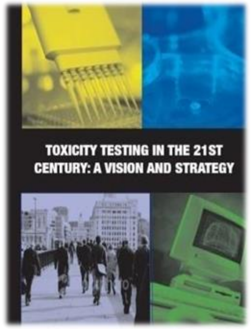
Limited availability of toxicity data



Moving away from animal tests



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



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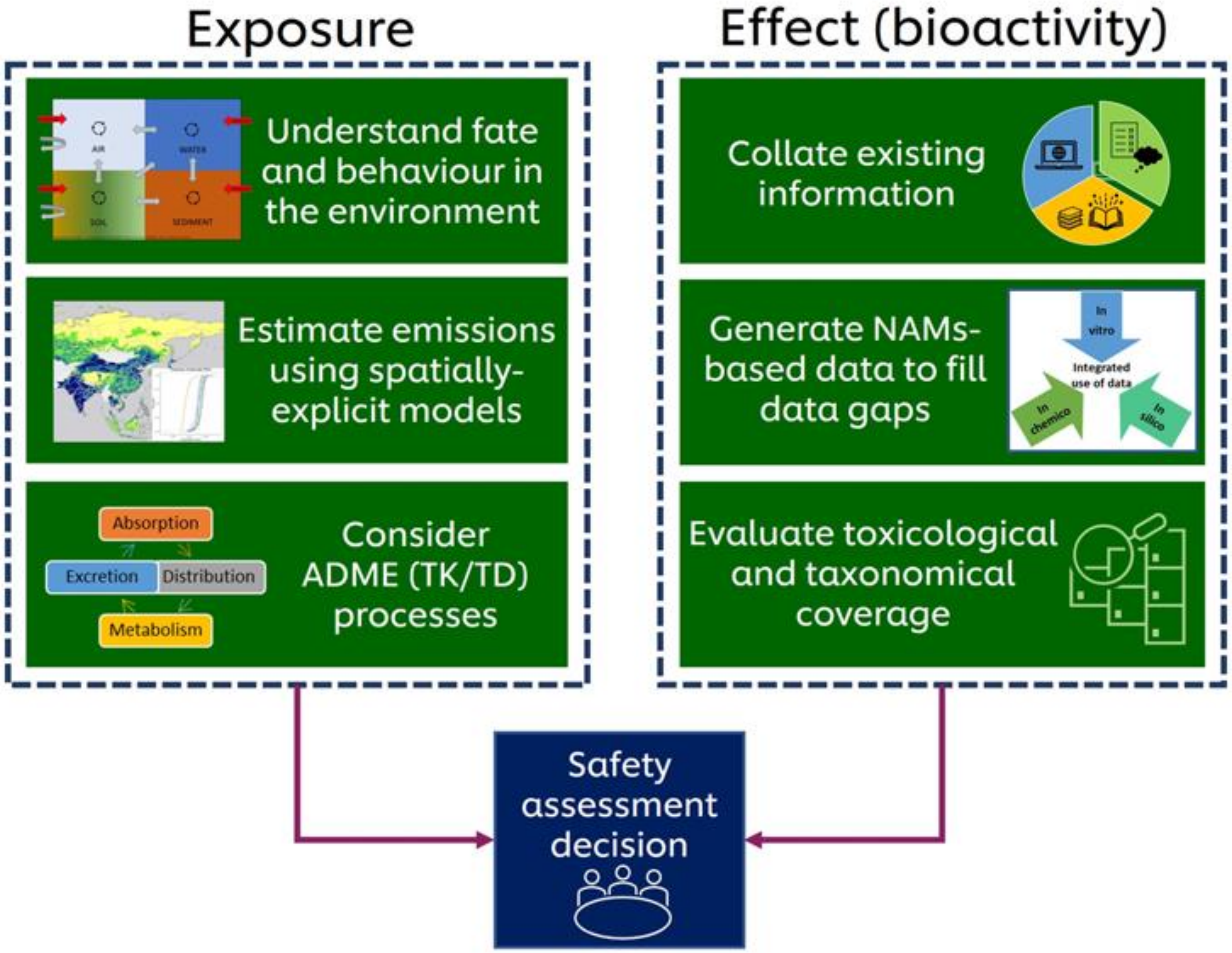
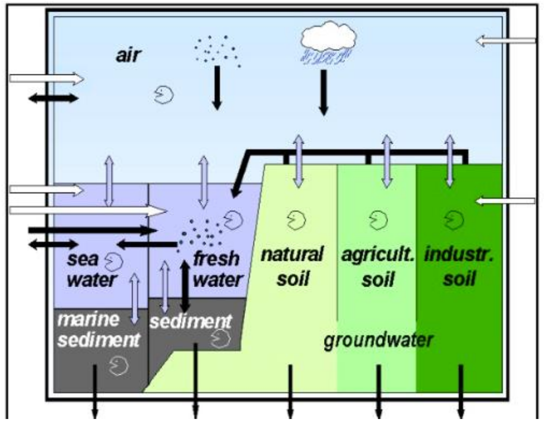
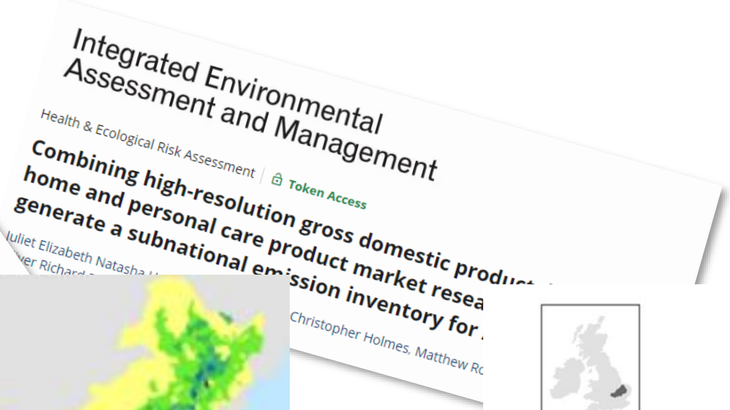
Establishing better environmental protection through NGRA

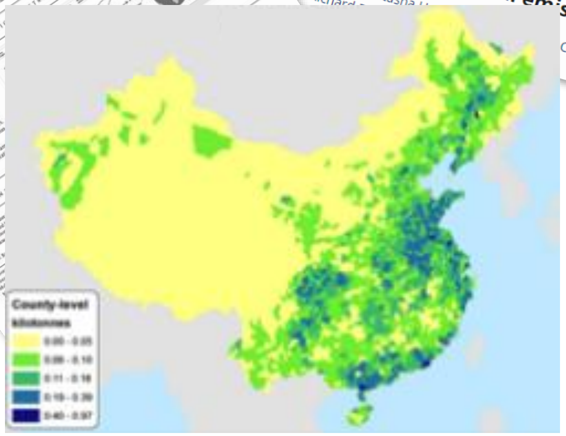


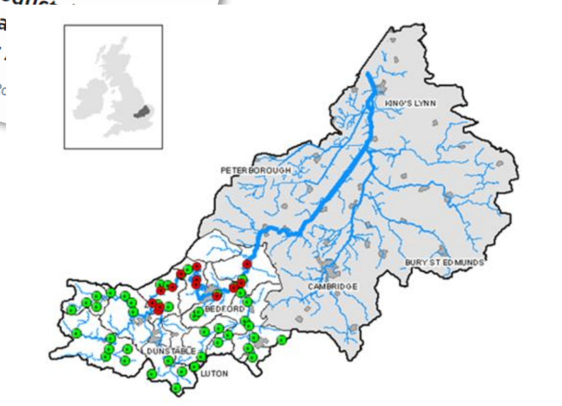
Rivetti & Campos, IEAM 2023

Current situation with exposure modelling









Box Models

- Used for regulatory assessments .e.g. EUSES.
- Not designed to reflect reality – uses default inputs.
- Computationally light.

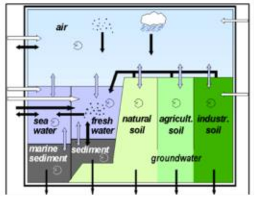
Global/Regional Models

- Designed to compare between regions
- Uses geographically relevant data
- Computationally manageable

Catchment Models

- Designed to more closely predict reality.
- Data and computationally heavy.
- Covers small scales

Exposure modelling- Future direction



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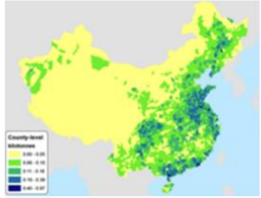

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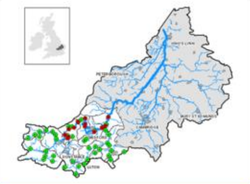
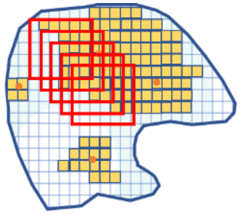


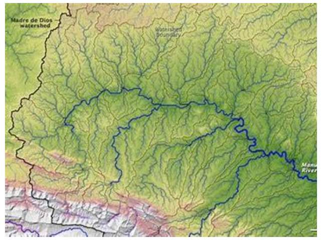
Catchment Models

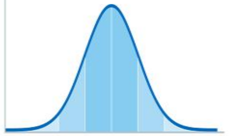
- Designed to more closely predict reality.
- Data and computationally heavy.
- Covers small scales

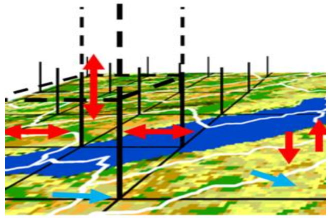
- Global datasets – increasing in number and higher resolution
- More Utilisation of satellite imagery with AI and machine learning approaches
- Higher computational power

Point source, routed spatially resolved exposure models









Examples of selected endpoints and available methods

Bioaccumulation

- Bioaccumulation in fish: OECD 305
- Bioaccumulation in terrestrial oligochetes: OECD 317
- In vitro clearance trout hepatocytes: OECD 319
- Bioaccumulation in *Halella azteca*: draft test guideline
- TKTD models

Toxicity to fish

- Acute toxicity to juvenile fish: OECD 203
- Chronic toxicity to fish: OECD 204, 210, 212, 229
- Fish cell line acute toxicity: OECD 249
- Fish embryo acute toxicity: OECD 236
- In vitro method for chronic toxicity: NONE

Endocrine disruption

- Fish 2 generations: OECD 240
- Fish sexual development: OECD 229, 230, 234, 240, 148
- Amphibians: OECD 231, 241
- Fish embryo estrogen activity (EASZY): OECD 250
- Xenopus Eleutheroembryo Thyroid Assay (XETA): OECD 248
- Androgen Disruption Adverse outcome Reporter (Medaka fish) (RADAR): OCDE 251
- Invertebrates: OECD 201, 211, 242, 243 218-219, 222, 220, 225, 226, 232

Relevance?

- Effects on vertebrate progeny for cosmetics: NONE

In silico models

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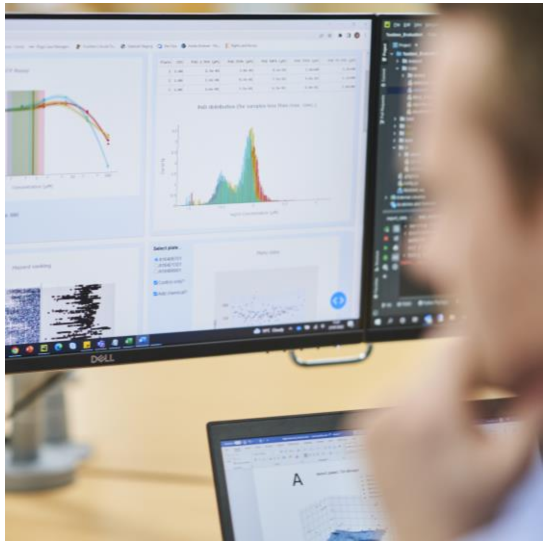
PETA Cruelty-Free

Adapted from Veronique Poulsen 2023

New approach methodologies (NAMs) are defined as

" any non-animal technology, methodology, approach or combination thereof that can be used to provide information on chemical hazard and human risk assessment" (Dent et al., 2018).



- ✓ ***in silico*** (e.g. QSAR, PBK models, machine learning models and artificial intelligence)
- ✓ ***in vitro*** (cell cultures, organoids and other micro-physiological systems)
- ✓ ***in chemico*** (i.e. abiotic methods aimed at identifying chemical reactivity)

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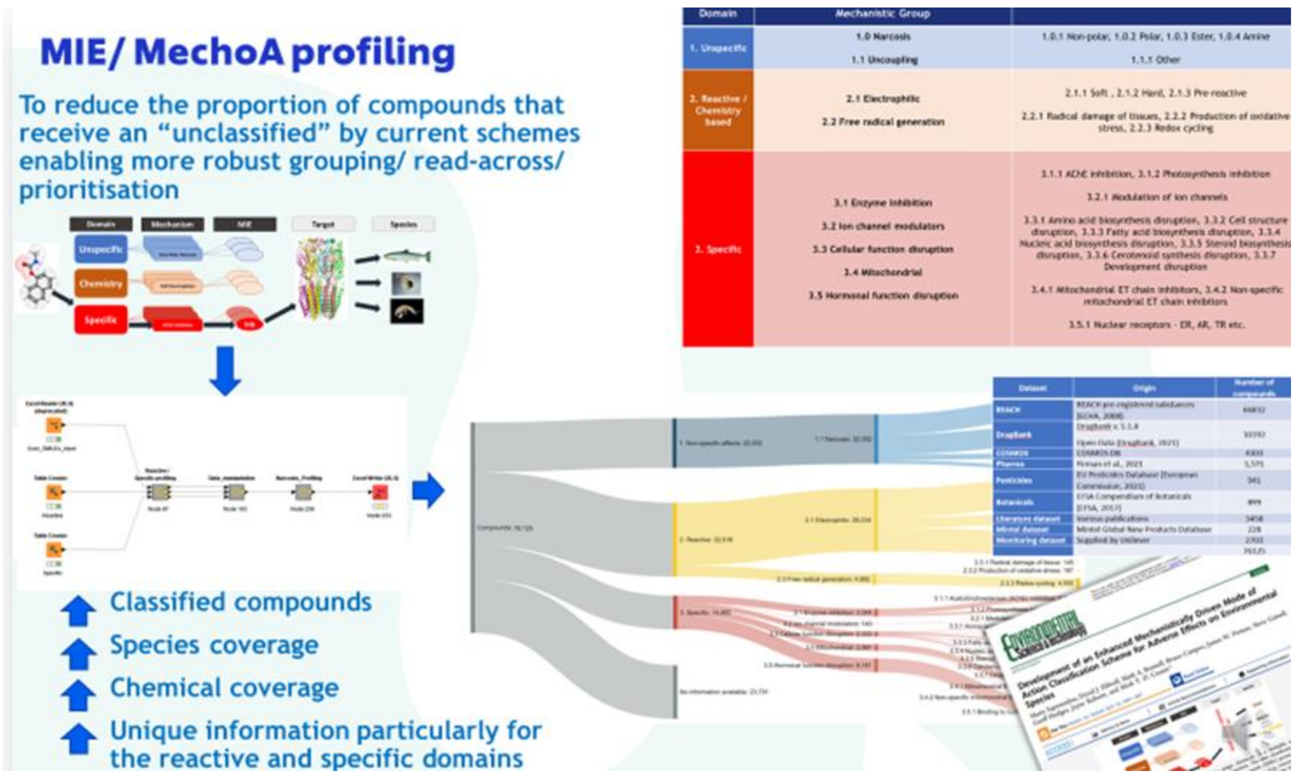
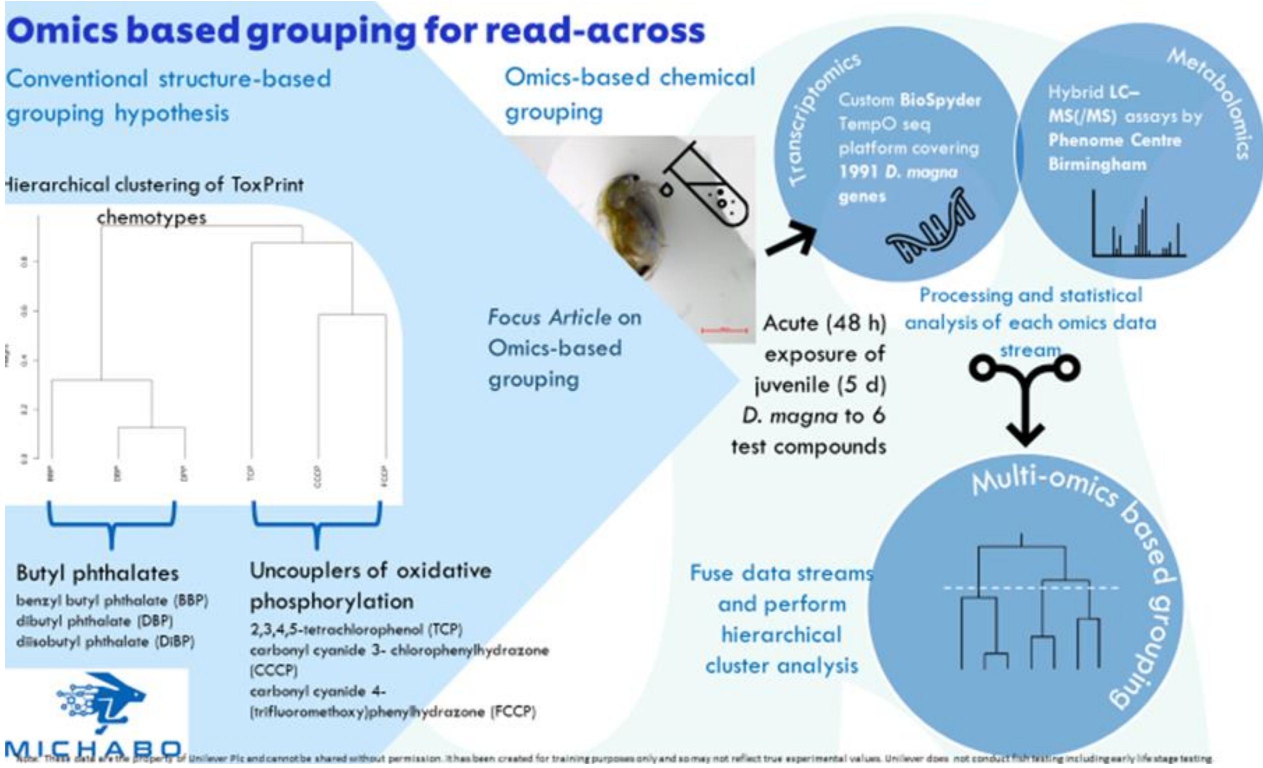
Adapted from Miccoli et al. (2022) EFSA Journal

Some examples: Grouping and read-across: chemical and biological based



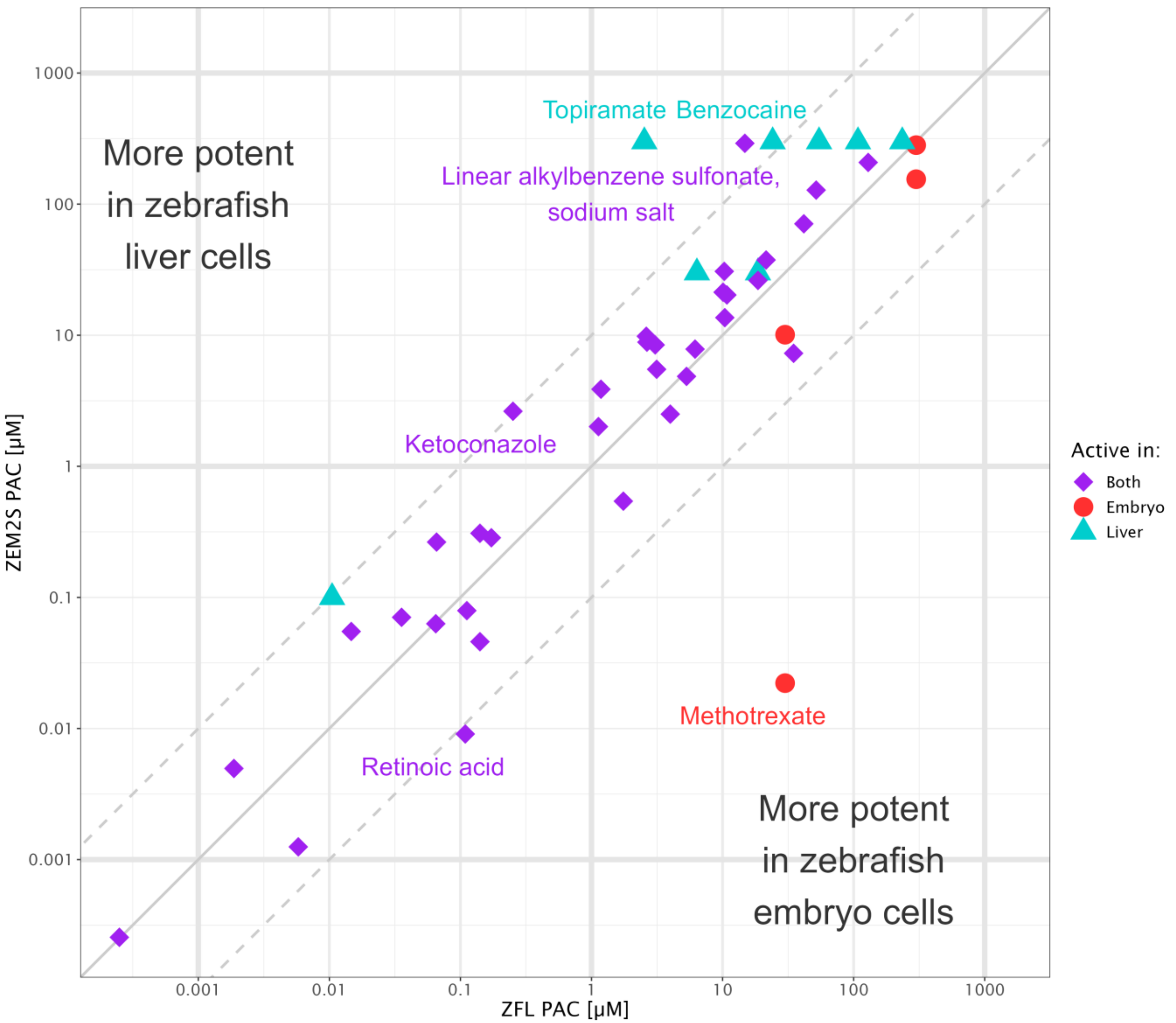
Domain	Mechanistic Group	
1. Unspecific	1.0 Narcosis 1.1 Uncoupling	1.0.1 Non-polar, 1.0.2 Polar, 1.0.3 Ester, 1.0.4 Amine 1.1.1 Other
2. Reactive / Chemistry based	2.1 Electrophilic 2.2 Free radical generation	2.1.1 Soft , 2.1.2 Hard, 2.1.3 Pre-reactive 2.2.1 Radical damage of tissues, 2.2.2 Production 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 Carotenoid 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.

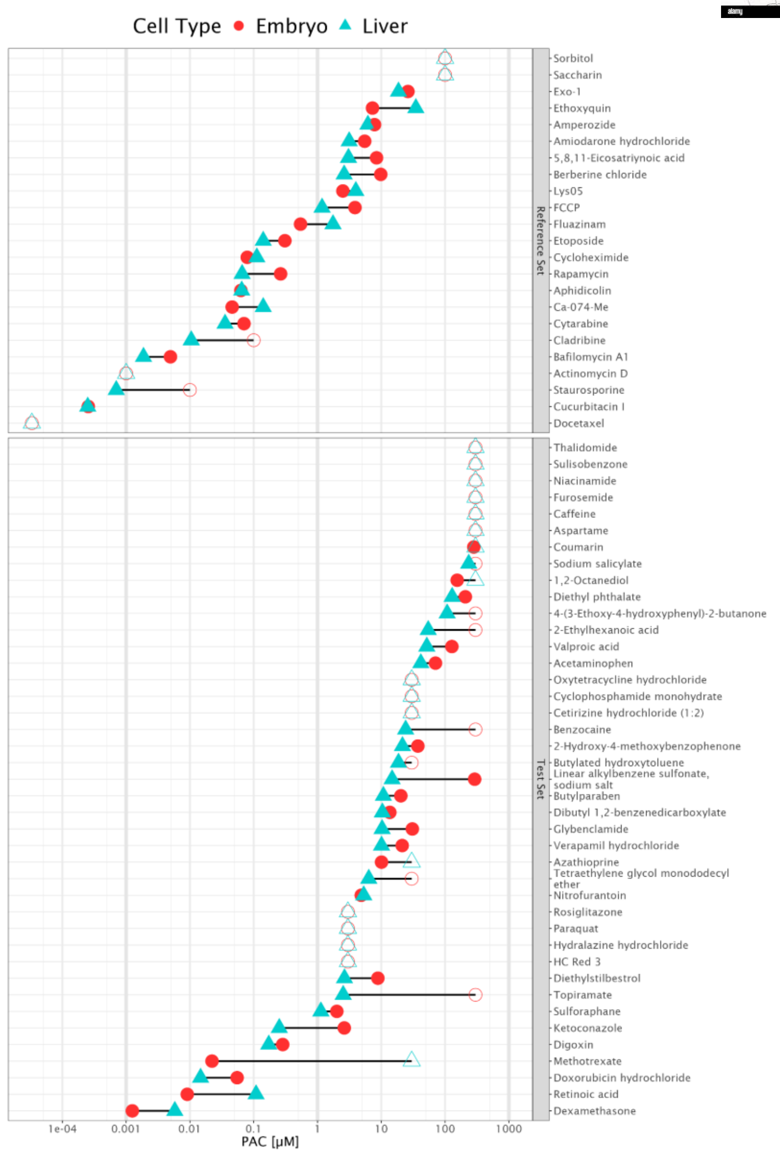
Sapounidou et al. (2021) EST



Viant et al. (2024) ET&C

Screening Chemicals Using High-Throughput Phenotypic Profiling (HTPP) in Two Zebrafish Cell Lines





From Villeneuve et al. (2023) Curr. Res. Tox.

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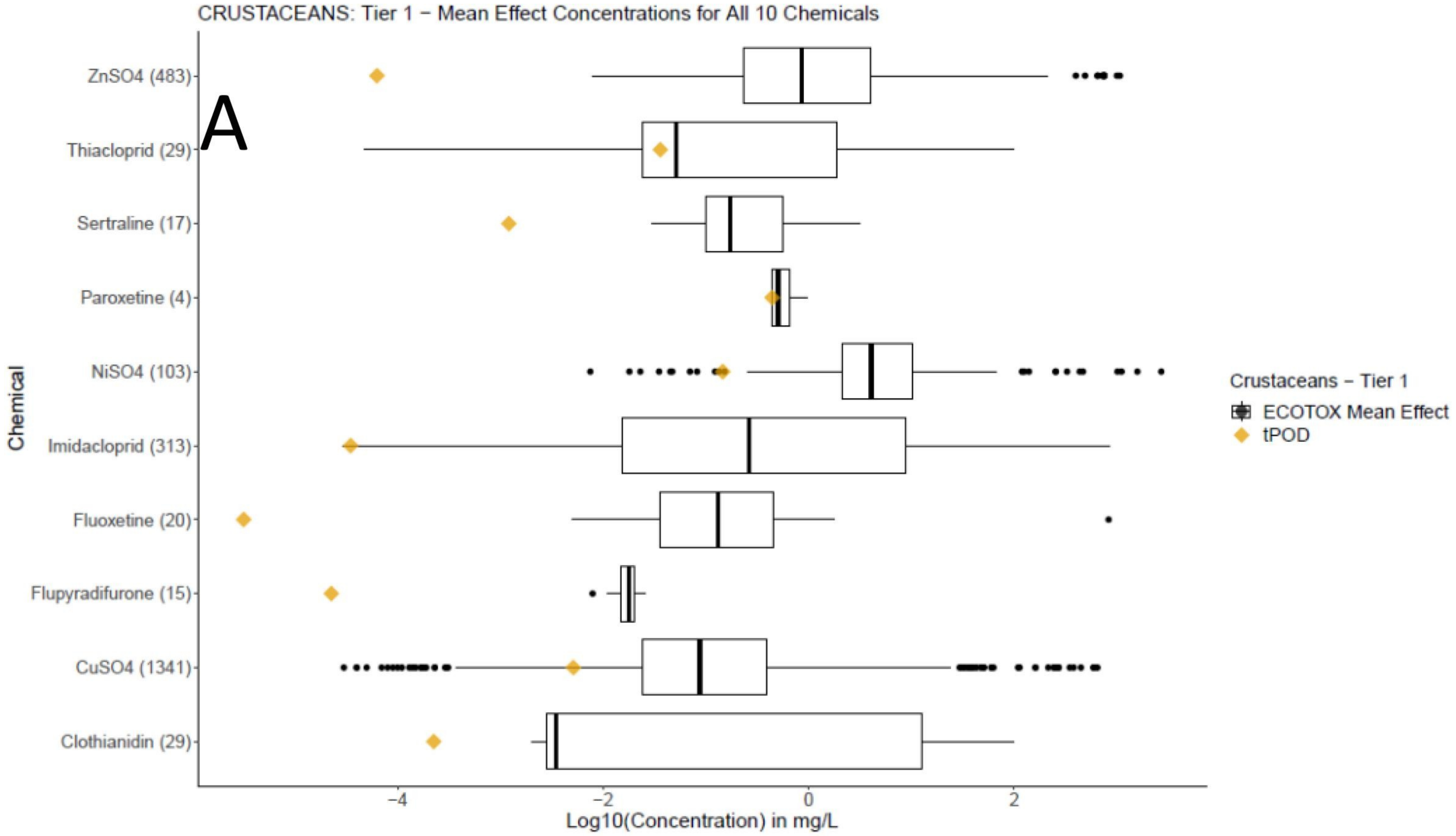
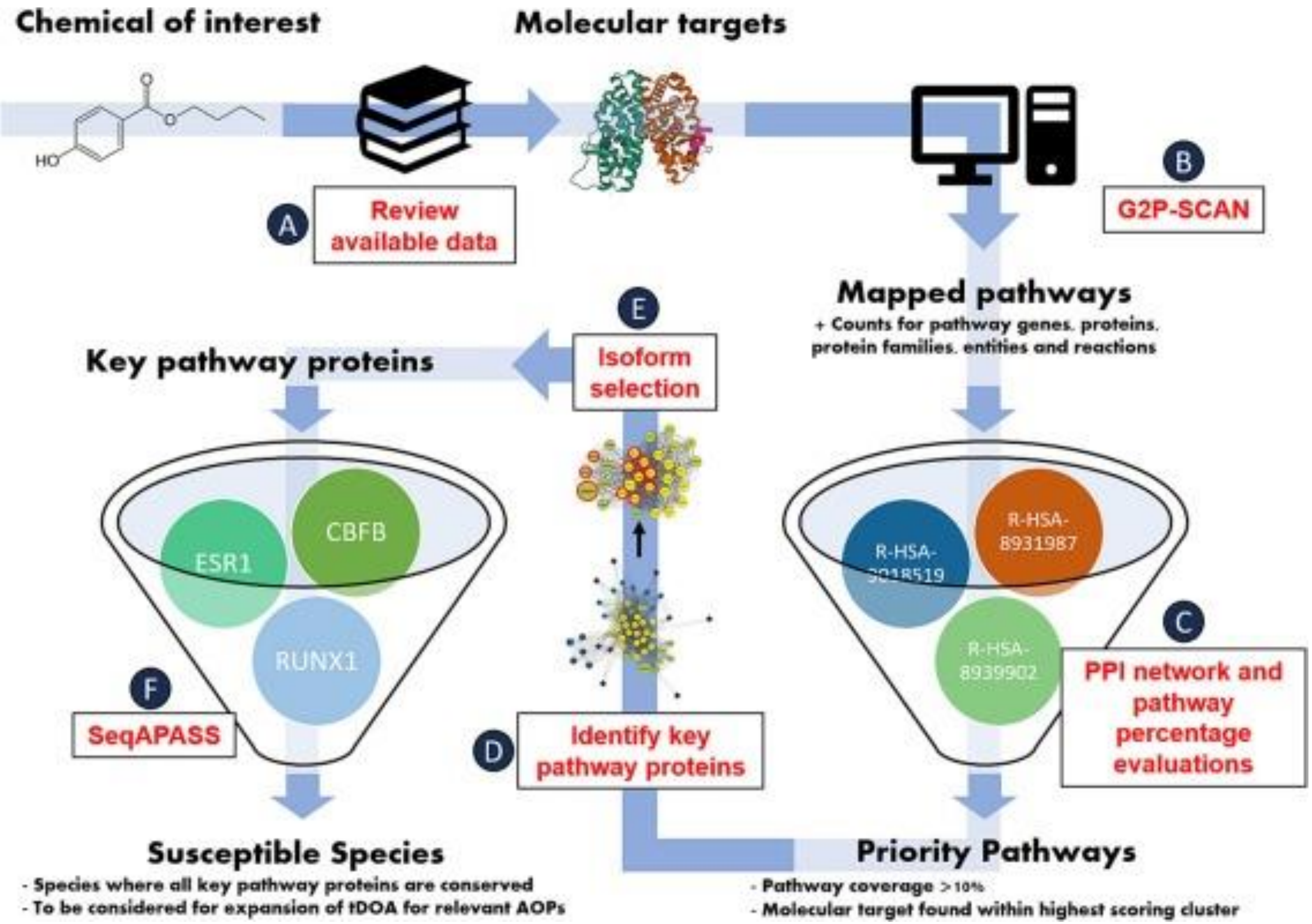
Some examples: OMICs and protectiveness



From Villeneuve et al. (2023) Curr. Res. Tox.

Some examples: Defining the taxonomical applicability domain



Schuman et al. (2024) STOTEN

Some examples: Weight of Evidence

Fish Acute

State of the art MoA/ MechoA identification (in-silico profilers, Critical Membrane Burden/ Critical Body Burden/ ‘Omics etc.)

- State of the art grouping/read-across (e.g. omics based grouping)
- In silico/ QSAR approaches – $logK_{MW}$/$logK_{OW}$ etc.

In-vitro assays / cell based profiler panels (e.g. RTgill cell (OECD TG 249))

- PBK/ TK modelling

Pathway based mechanisms of toxicity across species (X-spp extrapolation/ species sensitivity)

Exposure thresholds (e.g. EcoTTC)

Understanding species sensitivity – distributions

Complementary approaches proposed in the OECD Fish IATA (e.g. Threshold approach)

Fish Chronic

Approaches as per Fish Acute + the following

- Acute to Chronic ratios
- Exposure based waiving – consideration of EcoTTC, biodegradation rates etc.

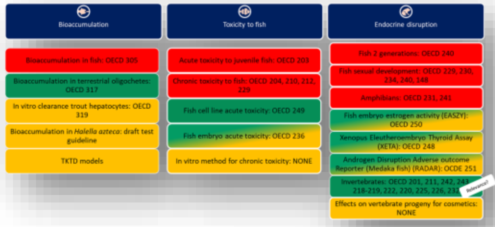
Fish BCF

In silico modelling (e.g. BIONIC)

- Biotransformation measurements - S9/hepatocyte assay (OECD TG 319A/B)
- Empirical and/or in-silico derivation of relevant partitioning parameters e.g. $logK_{ow}$ and/or $logK_{mw}$
- Empirical and/or in-silico derivation of pKa

Non vertebrate bioaccumulation assays (*Hyalella azteca*, RT-gill cell line etc.)

Exposure based waiving – consideration of removal/biodegradation rates etc and fate in environment



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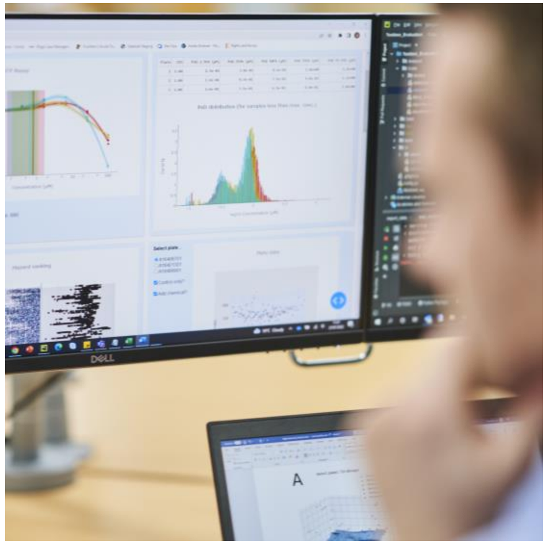
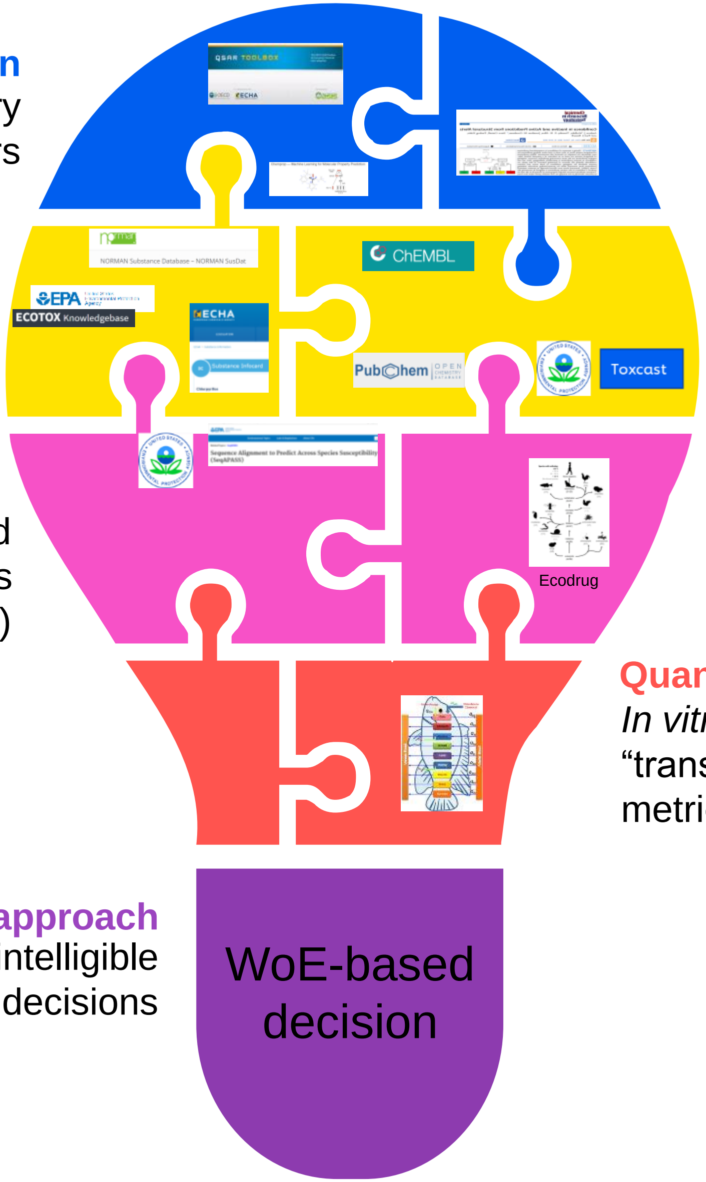
		Invertebrates: OECD 201, 211, 242, 243 218-219, 222, 220, 225, 226, 232
		Effects on vertebrate progeny for cosmetics: NONE

Relevance?

Case study: A proof of concept to demonstrate the applicability of mechanistic info in Environmental safety assessment



Information collection process



Mode of Action identification
Using available scientific and regulatory information and in silico profilers

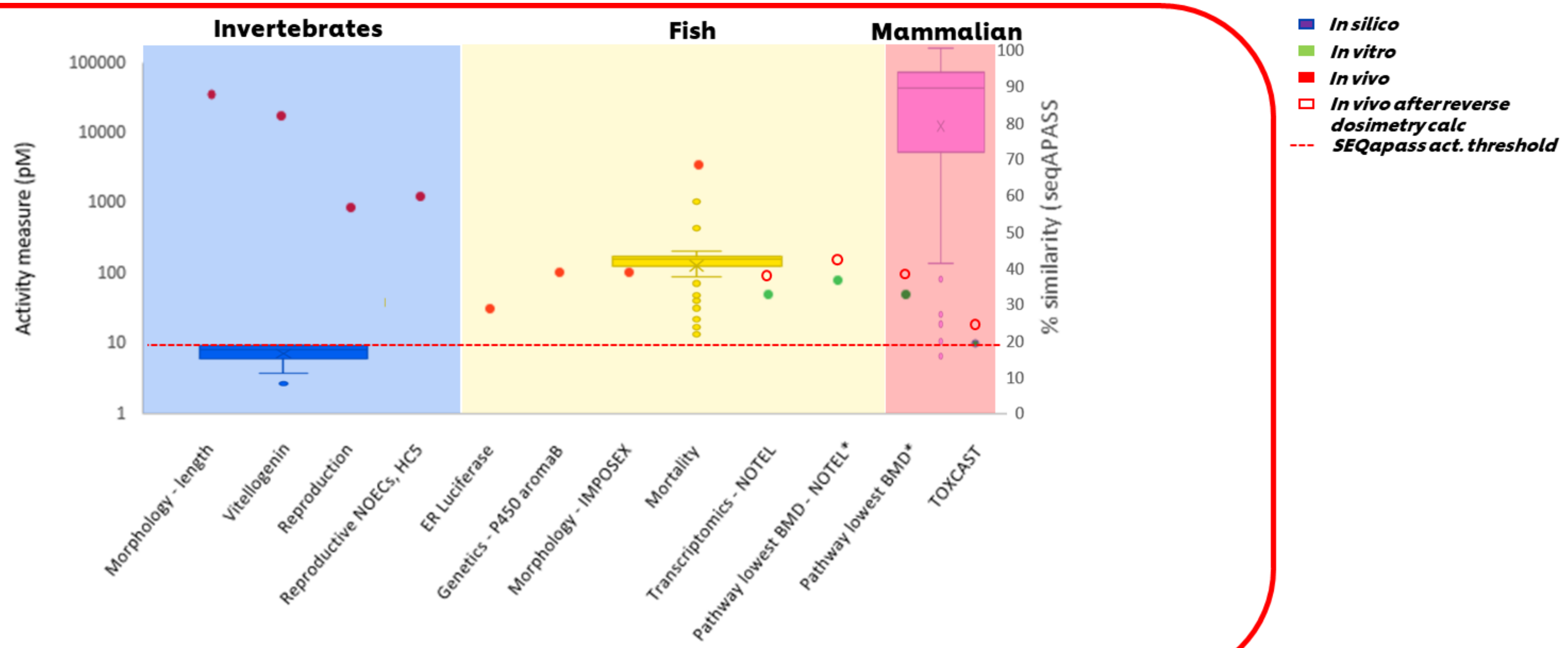
Hazard Data
Including historical *in vivo* as well as *in vitro* data and *in silico* predictions to generate relevant PoD

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

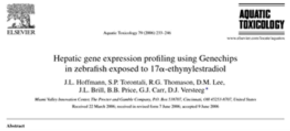
Quantitative In Vitro to In Vivo Extrapolation
In vitro and *in vivo* exposures must be “transformed” into comparable exposure metrics requiring robust qIVIVE models

Weight Of Evidence approach
Collate all the information in an intelligible way to guide and support decisions

Case study- Ethinylestradiol



Microarray analysis



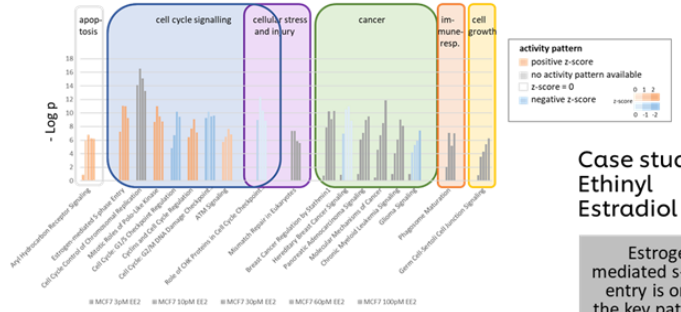
NOTEL 168h* = 50pM

Pathway with lowest BMD at 168h: 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)

Canonical Pathway analysis

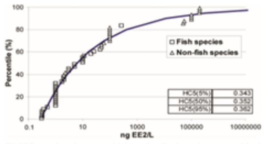


Case study: Ethinyl Estradiol (EE2)

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

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

Literature information



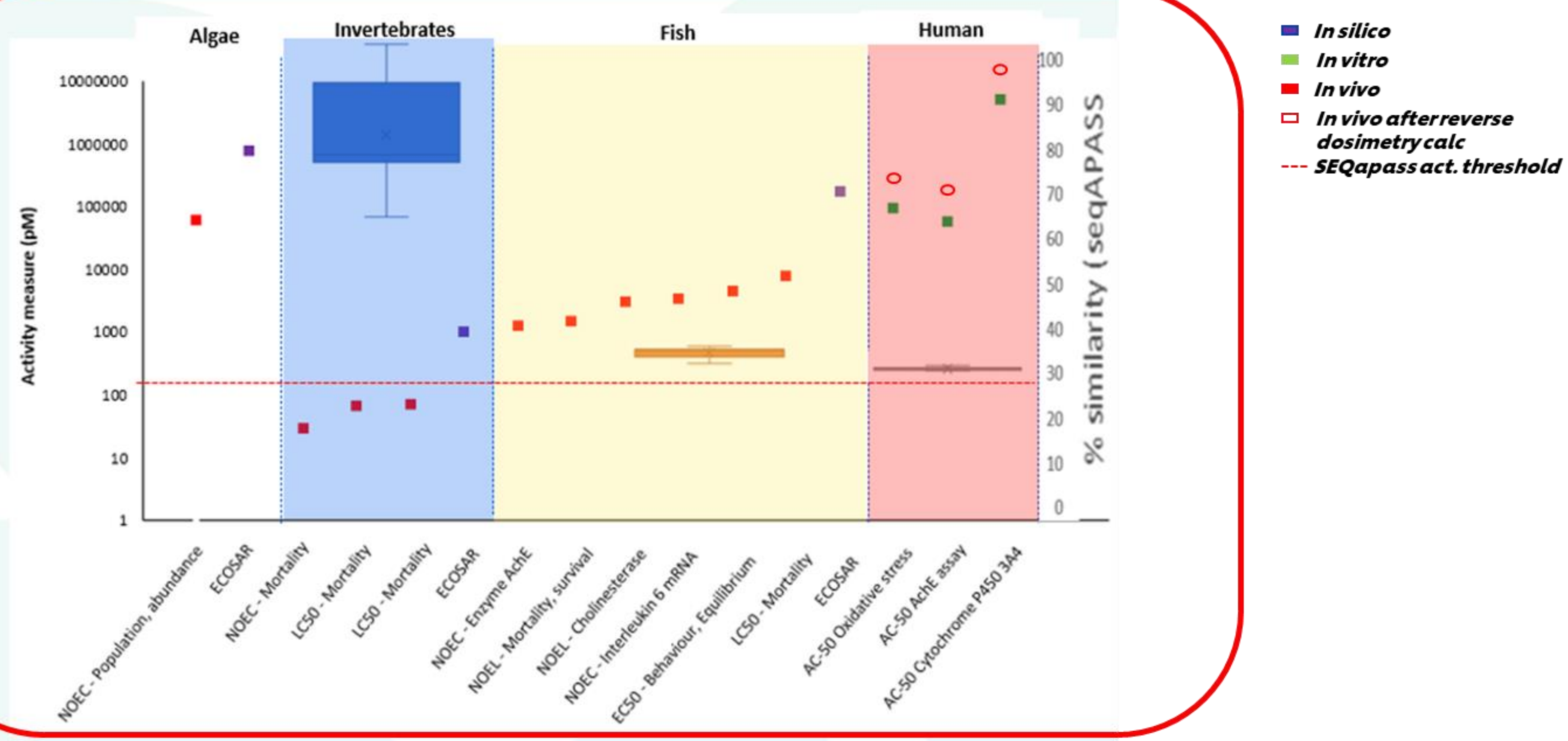
HC5 (50%) = 1200 pM

EC50= 30pM (ER luciferase assay)

Toxcast

Rivetti et al. (2024) in preparation

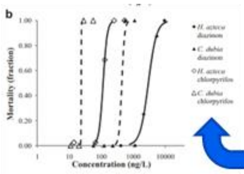
Case study- Ethinylestradiol



Hazard data

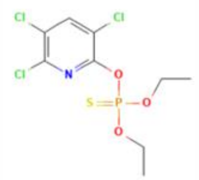
Pesticide	96 h LC50(μg/L)
Chlorpyrifos	0.063

NOEC mortality = 29 pM

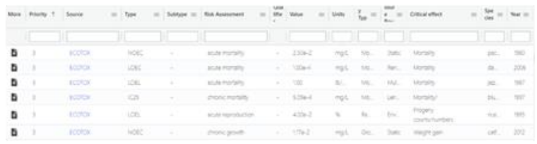


LC50 mortality 66 pM

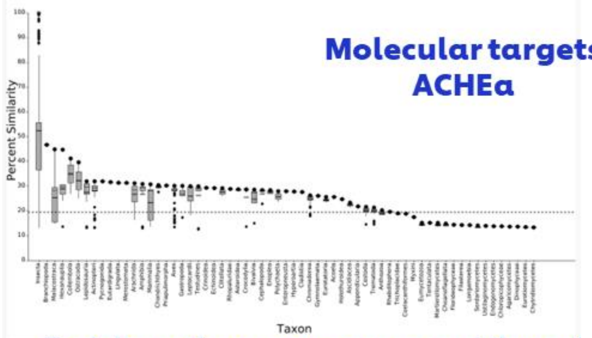
In vitro data



AchE Assay AC50 = 56.6 nM



Cross-Species Extrapolation analysis



Molecular targets ACHEa

Toxicity pathways are conserved throughout the animal kingdom

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Rivetti et al. (2024) in preparation

Why Next Generation Environmental Safety Assessment?

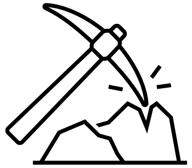
Integration of exposure, *in vivo, in vitro and in silico* data in a weight of evidence approach can build confidence in safety decision-making.

- ✓ 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*

Benefits

- ✓ reduce / replace animal testing
- ✓ increase the efficiency and reliability of chemical safety assessment
- ✓ address the complexity and diversity of environmental effects and exposures

Work to be done



- ✓ ensure the relevance and applicability of NAMs to ecological endpoints and species
- ✓ validate and standardize NAMs for regulatory acceptance and harmonization
- ✓ integrate and interpret NAMs data in a weight-of-evidence approach

Take-home messages

- **Understanding exposure is critical** to for Next Generation Risk Assessment.

- Tangible **opportunities** already available to improve environmental protection by applying **spatially explicit exposure**, **NAMs** and **weight of evidence** approaches.

- **Mechanistic understanding allows to move away from black box studies / models** to better understand **fate and distribution of chemicals** and their potential **impacts on organisms and ecosystem's**.

- There are **challenges** to address particularly in **standardisation and training** needs within user communities (Risk Assessors and Regulatory bodies)

First step– developing a common framework & language

Tier 0- Identify use scenario and collect existing information

- Characterise exposure scenario (consumer and the environment)
- Characterise the chemical
- Use Threshold of (eco)-Toxicological Concerns
- Use of Predictive tools (i.e. in silico)
- Collate all available information (literature and data)
- Read-across

Exit if safety decision can be made

Tier 1- Generate data to ensure refined exposure and increase Toxicological and taxonomical coverage

- Exposure refinement, including emission, ADME and relevant PBK
- Refine chemical and biological read-across hypothesis
- Dose-response data generation and POD estimation
- Broad coverage in chemico & in vitro bioactivity panels
- Estimation of safety thresholds

Exit if safety decision can be made

Tier 2- Refine assessment, incl. bespoke assays to increase decision certainty

- Exposure refinement (e.g. loss processes and spatially explicit emissions, Metabolism, clearance, transporters, etc)
- Definition of Biological and toxicological coverage
- Bespoke assays to cover potential targets of concern (follow up from tier 1)
- Estimation of safety thresholds

Safety decision

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adapted from *Baltazar et al.* 2020

Thank You
"the team"

- Emilia Gattas
- Nicola Furmanski
- Jayne Roberts
- Claudia Rivetti
- Alexandre Teixeira
- Chris Finnegan
- Ian Malcomber
- Juliet Hodges
- David Gore
- Jade Houghton
- Katie Endersby
- Predrag Kukic
- Iris Muller
- Simran Sandhu
- Baile Xu
- Matt Dent
- Maria Baltazar
- Paul Carmichael
- and many more...

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

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