Event booklet

The ASPIS project cluster received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No. 965406 (PrecisionTox), No. 964537 (RISK-HUNT3R), and No. 963845 (ONTOX).

aspis-cluster.eu

ASPIS open symposium

24-25 nov 2022 Sitges - Spain

A collective effort to implement Novel Approach Methodologies in Next-Generation Risk Assessment

Who is ASPIS

ASPIS is a science for societal change initiative funded by the European Commission's research and innovation programme, which assembles three projects to better assess the hazards of chemicals to human health and the environment without animal testing: RISK-HUNT3R, ONTOX, and PrecisionTox.

ASPIS represents more than 70 institutions across 16 European countries and the U.S. delivering on a €60 million investment to help transform health protection by adopting new approach methodologies (NAMs) that more reliably detect and, when appropriate, rapidly regulate and mitigate the exposure to toxic substances. ASPIS aims to translate talk about responsible innovation into action for an environmentally sustainable chemicals industry.

ASPIS hosts its 2nd public symposium on 24- 25 November 2022 to showcase the joint efforts of its three projects.

Day #1 will focus on the joint framework developed by the initiative to implement non-animal based technologies in risk assessment;

Day #2 will focus on the biomolecular, cellular, and computational advances that are driving the ASPIS initiative. Different applications of NAMs will be presented, including: discovering adverse outcome pathways and their quantification; enhanced grouping of chemicals;

detecting and characterizing hazards.

Event objectives

- To highlight relevant technologies for improving chemical safety assessment and the regulation of hazardous substances;
- To increase confidence in the use of NAMs and to broadly promote their applications for effective risk management;
- To identify barriers and provide solutions for the reduction, refinement, and replacement of animal testing (the 3Rs principle);
- To promote early career researchers and create a network of next-generation decision-makers;
- To facilitate communication among ASPIS and international stakeholders.

ASPIS open symposium

24-25 nov 2022 Sitges - Spain

Agenda

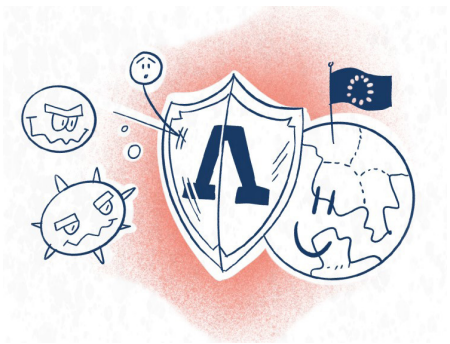
24 November 2022	**ASPIS Open Symposium Day 1. Room Garbi** **A collective effort to implement Novel Approach Methodologies in Next-Generation Risk Assessment**	
14:00 – 14:10	Welcome	**Bob van de Water** *(University Leiden, RISK-HUNT3R coordinator, ASPIS chair)*
14:10 – 14:30	DZ Foundation (DZF) award ceremony speech on the implementation of NAM for food safety assessment	Awarded by **Marcel Leist** *(DZF, University Konstanz, RISK-HUNT3R)*
14:30 – 14:50	An overview of the ASPIS cluster organization	**Jonathan Freedman** *(University of North Carolina, PrecisionTox, WG coordinator)* **Barry Hardy** (Edelweiss Connect, RISK-HUNT3R)
14:50 – 15:10	Building a common framework for NAM-based next generation risk assessment	**Mirjam Luijten** *(RIVM, RISK-HUNT3R, WG leader)*
15:10 – 15:40	Coffee break	
15:40 – 16:40	Panel discussion on the challenges and opportunities of a joint NAM-based risk assessment framework in ASPIS and beyond with: **Erwin Roggen** *(3RsMC, ONTOX)* **Mirjam Luijten** *(RIVM, RISK-HUNT3R)* **Nicole Kleinstreuer** *(US NIEHS)* **Patience Browne** *(OECD)* **Marcel Leist** *(University of Konstanz)* **Thomas Steger Hartmann** *(Bayer AG)*	Moderated by **Mathieu Vinken** *(Vrije Universiteit Brussel, ONTOX coordinator)* **Elisabet Berggren** *(EC JRC, ASPIS regulatory forum coordinator)*
16:40 – 17:00	US efforts toward NAM based risk assessment	**Nicole Kleinstreuer** *(US NIEHS)*
17:00 – 17:20	EC efforts towards regulatory harmonization	**Christian Desaintes** *(EC DG Research & Innovation)*
17:20 – 18:15	Q&A and final remarks	**Bob van de Water** (University Leiden, RISK-HUNT3R coordinator)
18:15 – 20:00	**Poster presentation and cocktail reception**	

ASPIS open symposium

24-25 nov 2022 Sitges - Spain

Agenda

25 November 2022	**ASPIS Open Symposium Day 2. Room Garbi** **A collective effort to implement Novel Approach Methodologies in Next-Generation Risk Assessment**	
8:30 – 8:40	Welcome	**Bob van de Water** *(University Leiden, RISK-HUNT3R coordinator,*
8:40 – 9:00	NAMs & REACH: needs & opportunities	**Elisabet Berggren** *(EC JRC, ASPIS regulatory forum coordinator)*
9:00 – 9:20	Building confidence in toxicokinetic models for next-generation risk assessment	**Nynke Kramer** *(Wageningen University, ONTOX, WG leader)*
9:20 – 9:40	Predicting chemicals' Molecular Initiating Events (MIEs) based on protein structure	**Gerhard Ecker** *(University of Vienna, RISK-HUNT3R, WG leader)*
9:40 – 10:00	Omics: how to bridge the regulatory barrier	**Florian Caiment** *(Maastricht University, ONTOX, WG leader)*
10:00 – 10:30	Coffee break	
10:30 – 10:50	Quantitative AOP of steatosis: implementation for regulatory purposes	**Mark Cronin** *(Liverpool John Moores University, RISK-HUNT3R, WG leader)*
10:50 – 11:10	The role of ASPIS in PARC, the European Partnership for the Assessment of Risks from Chemicals	**Mirjam Luijten** *(RIVM, RISK-HUNT3R, WG leader)*
11:10 – 12:00	Start of the new ASPIS presidency period and poster award ceremony	**John Colbourne** *(University of Birmingham, PrecisionTox, coordinator)*
End of the 2nd ASPIS Open Symposium		



ASPIS open symposium

24-25 nov 2022 Sitges - Spain

Poster Session

NAMs for chemical risk assessment

Poster #1: ASPIS chemical selection working group
Poster #2: The ASPIS risk assessment working group
Poster #3: Human peripheral neurons with enhanced nociceptor features for the study of pain-related dysfunctions
Poster #4: Use of Alamar Blue test for High-Throughput Energy Expenditure Monitoring in Daphnia magna
Poster #5: The vibration/startle assay: a semi-automated behavioural assay to assess toxicity in zebrafish embryos
Poster #6: A cross-species comparative look at the toxicity of acrylamides and imidazoles
Poster #7: Differential Cadmium Chloride Toxicity Across Twenty Daphnia magna Clones
Poster #8: Quantitative and qualitative detection of cytotoxicity by fluorescence microscopy in human cells.
Poster #9: Advanced in vitro model for drug induced kidney injury assessment - generation of kidney organoid for safety assessment purposes
Poster #10: Physiological maps and chemical-induced disease ontologies: tools to support NAMs development for next-generation risk assessment
Poster #11: Re-thinking the concept of spheroids: a new way to generate high-throughput 3D complex liver model
Poster #12: The rosette formation assay as a method to identify DNT hazard due to disruption of the RAR/RXR pathway (endocrine signaling)

Omics approaches

Poster #13: ASPIS Omics Working Group
Poster #14: An introduction to the semi-automated, robotics-based sample extraction workflow facilitating PrecisionTox omics analyses
Poster #15: The renal proximal tubule TXG-MAPR: safety assessment based on quantitative gene network analysis

qAOP approaches

Poster #16: ASPIS quantitative Adverse Outcome Pathway Working Group
Poster #17: In vitro New Approach Methodologies (NAMs) for assessing effects of chemicals leading to cognitive function defects in children – the contributions of the ONTOX project
Poster #18: Update and optimization of an adverse outcome pathway network of chemical-induced cholestasis
Poster #19: Development of an adverse outcome pathway for kidney tubular necrosis
Poster #20: An adverse outcome pathway network for liver steatosis induced by chemicals
Poster #21: Computation and visualisation of a Mitochondrial Toxicity qAOP PBK-TD model using Simcyp Designer

ASPIS open symposium

24-25 nov 2022 Sitges - Spain

Poster Session

Computational approaches

Poster #22: The activities related to the computational approaches within ASPIS
Poster #23: Combining gene expressions and imaged-based morphological features for chemical-phenotype profiles
Poster #24: Chemical Effect Predictor: A tool to predict chemical toxicity using biological network properties
Poster #25: A KNIME Workflow for Consensus Target Prediction
Poster #26: Structure-based predictions for MIEs
Poster #27: UNIVIE Jupyter Notebooks (JNs) for Data Curation & Machine Learning (ML) model building for Transporters & Off-target predictions
Poster #28: Computational modelling of neural tube closure defects
Poster #29: Next generation target organ toxicity risk assessment: endogenously tagged human stem cell reporters for high-content screening of oxidative stress response.
Poster #30: Evaluation of state-of-the-art in silico testing methods to fill physicochemical and pharmacokinetic data gaps within the ONTOX project
Poster #30b: Applying machine-learning approaches to identify key genes associated with drug-induced cholestasis

Exposure and kinetics

Poster #31: Exposure and kinetics research activities in ASPIS: Moving from hazard identification to risk characterisation in next generation risk assessment
Poster #32: A high-throughput analytical workflow to determine internal concentrations of xenobiotics in zebrafish larvae
Poster #33: Kinetic modelling and quantitative in vitro-in vivo extrapolation strategies for next generation risk assessment in the H2020 ONTOX project
Poster #34: Primary human enterocytes for the determination of intestinal metabolism
Poster #35: Parameterisation and Verification of IVIVE-PB(P)K models for Risk Assessment
Poster #36: Assessment of aggregate exposure in RISKHUNT3R
Poster #37: Aggregate exposure model
Poster #37b: The case study concept illustrated by an inhalation case study

Communication and dissemination strategies for NGRA

Poster #38: A joint voice on the side of NAMs-based strategies for chemical risk assessment: an overview of ASPIS communication activities
Poster #39: Science Policy on NAMs – A current overview of opportunities for implementation
Poster #40: Main Drivers for Use and Regulatory Acceptance of New Approach Methodologies: a Survey of European Risk Assessors

ASPIS

open symposium

24-25 nov 2022

Sitges - Spain

www.aspis-cluster.eu

ASPIS *aspis-cluster.eu*

PRECISION TOX *precisiontox.org*

ONTOX *ontox-project.eu*

RISK HUNT3R *risk-hunt3r.eu*

For more info reach out to the ASPIS Communication and Press team:

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RISK-HUNT3R
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ASPIS project cluster

Bob van de Water | Leiden University – The Netherlands

- 24/11/2022
- Sitges - Spain

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers PrecisionTox: 965406, ONTOX: 963845 and RISK-HUNT3R: 964537

ASPIS coordination:

Bob van de Water	-	RISK-HUNT*3R*
John Colbourne	-	PrecisionTox
Mathieu Vinken	-	ONTOX

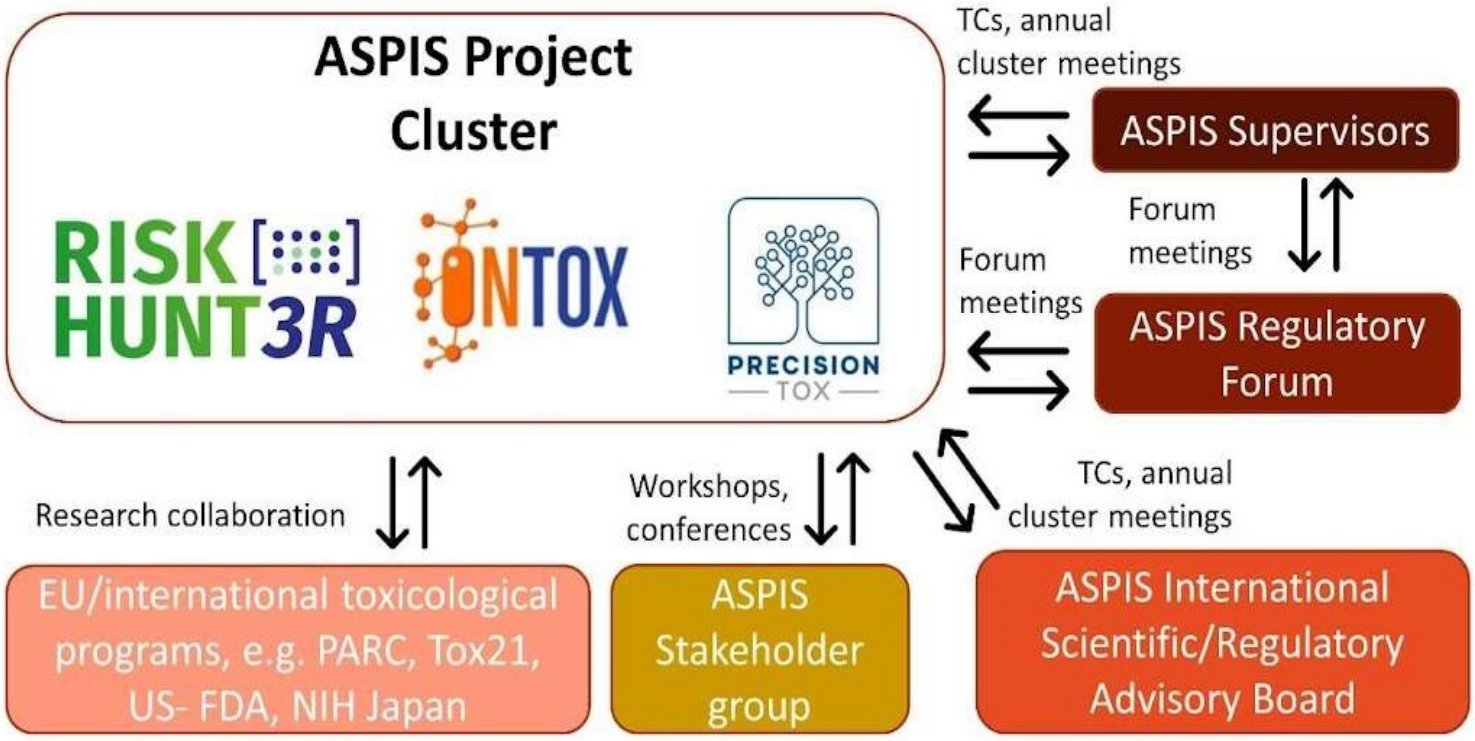
ASPIS: "**A**nimal-Free **S**afety Assessment of Chemicals: **P**roject Cluster for **I**mplementation of Novel **S**trategies"

- 2021-2026 under H2020
- €60M funded budget
- 70 institutions united in 3 projects across 16 EU countries + US

Objectives

- **advance NAMs** for the protection of human health and the environment
- **improve certainty** in the safety assessment of chemicals
- facilitate practicably implementable **non-animal solutions** in various public (e.g. regulatory agencies) and private (e.g. industry) sectors
- **translate results, methods and solutions** from the scientific research community into **safety assessment practice**
- **promote regulatory uptake** and **commercial exploitation of NAMs**
- contribute to the **3R principles**

ASPIS interaction with satellite entities



ASPIS working groups

1) Risk Assessment *(Stefan Scholz, Mirjam Luijten, Erwin Roggen)*
2) Chemical Selection *(Mathieu Vinken, Jonathan Freedman)*
3) Kinetics and Exposure *(Nynke Kramer, Sylvia Escher)*
4) Computational Approaches *(Gerhard Ecker, Emilio Benfenati)*
5) Omics *(Florian Caiment, John Colbourne)*
6) Quantitative AOPs *(Mark Cronin, Huan Yang)*
7) Database *(Barry Hardy, Jonathan Freedman)*
8) Communication and Dissemination *(Giorgia Pallocca, Francois Busquet)*

ASPIS symposium objectives

- Focus on the joint ASPIS framework to implement non-animal based technologies in risk assessment
- Advances of NAMs on the biomolecular, cellular, and computational advances that are driving the ASPIS initiative
- ASPIS Academy training program

Thank you for your attention!

www.aspis-cluster.eu

Jonathan H Freedman | UNC-Chapel Hill, USA /PrecisionTox

PRECISION TOX

RISK HUNT3R

ONTOX

ASPIS Open Symposium
Sitges Spain
24-25 November 2022

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers PrecisiontTox: 965406, ONTOX: 963845 and Risk-Hunt3R: 964537

Horizon 2020 Framework Programme Call

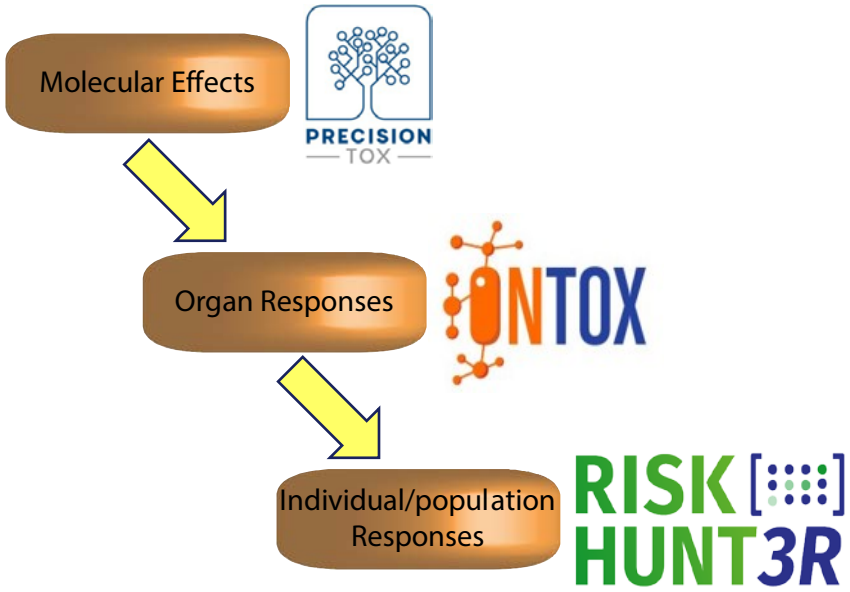
- *Advancing The Safety Assessment of Chemicals* (SC1-BHC-11-2020)
- Goals
  - Develop implementable non-animal solutions to aid in chemical safety assessment
  - Obtain recognition from regulatory bodies to translate results, methods and solutions into safety assessment practice
  - Explore the commercial application of the safety assessment approaches, products and services
  - Contribute to the 3R's (Refinement, Reduction and Replacement) of animal experimentation
- Funded three consortia
  - ONTOX (ontox-project.eu)
  - PrecisionTox (precisiontox.org)
  - RiskHunt3R (risk-hunt3r.eu)

Creation of ASPIS

- In Autumn 2020, as part of SC1-BHC-11-2020, a cluster mandate was sent by the EC to the consortia leadership requiring them to collaborate on cross-consortia projects
- Lead to the formation of ASPIS
  - **A**nimal-free **S**afety Assessment of Chemicals: **P**roject Cluster for **I**mplementation of Novel **S**trategies
- ASPIS-related activities began
  - July 2021 through a two-day virtual meeting
  - November 2021 during the EU-ToxRisk Final Symposium

ASPIS

- >70 institutions across 16 European countries, United Kingdom and the United States
- >200 participants including
  - Senior and junior investigators, Post-docs, graduate students, undergraduates, technical staff and administrators
- Diverse backgrounds



ASPIS Organization

- July 2021
  - Creation of seven working groups (aspis-cluster.eu/working-groups/)
    - Chemical Selection
    - Risk Assessment
    - quantitative Adverse Outcome Pathway (qAOP)
    - Omics
    - Kinetics & Exposure
    - Computational Methods
    - Communication and Dissemination
- March 2022
  - ASPIS Working Group Coordinator position created
    - Assist in the development and report on inter-working group projects
    - Maintain continuity and historical perspective as new working groups are formed and old ones are retired
    - Link working groups with ASPIS leadership, Scientific/Regulatory Advisory Board and the Regulatory Forum
- October 2022
  - Database Working Group formed

ONTOX

- Partners
  - 18 partners/9 different countries
  - 19 teams/60 researchers
  - 10 academic institutions
  - 5 small-to-medium enterprise (SME)/1 large company
  - 1 public health institution
- Goal
  - To establish a generic strategy to create New Approach Methodologies (NAMs) to predict systemic, repeated dose toxicity effects of chemicals that will enable human risk assessment in combination with exposure assessment.
- Focus
  - Liver: steatosis/cholestasis
  - Kidneys: tubular necrosis/crystallopathy
  - Brain: neural tube closure/cognitive function defects
  - Pharmaceuticals/cosmetics/food ingredients/biocides

PrecisionTox

- Partners
  - 15 organizations/8 countries
  - 108 researchers
  - 6 academic institutions
  - 4 public research institutions
  - 5 SMEs
- Goal
  - To use multi-omics, in five biomedically-relevant model species and human cell lines, and artificial intelligence to establish causation between chemical exposure and their cognate adverse health effects
- Focus
  - Establishing causation between 250 chemicals (TBD) and their adverse health effects using a mix of genomics, metabolomics, evolutionary theory, quantitative genetics, data science, toxicology and law

RiskHunt3R

- Partners
  - 37 partners/11 different countries
  - 20 academic or research institutions
  - 15 industry
  - 2 regulatory agencies
- Goal
  - To develop, validate and implement integrated approaches to lead the way towards a sustainable framework for next-generation risk assessment (NGRA). This NGRA will be human-relevant, fully based on non-animal approaches, and be fit for implementation through engagement with chemical safety regulators
- Focused on
  - Systemic toxicities to major organ systems (liver, kidney, lung)
  - Developmental neurotoxicity
  - Non-genotoxic carcinogenicity
  - Cosmetics, agro-chemicals. industrial chemicals

Largest Challenge

- NO FUNDS
  - ASPIS was created post-award
  - None of the consortia budgeted for its activities

Acknowledgement

- Far too many people to list

- This project received funding from the European Union's Horizon 2020 Research and Innovation programme under Grant Agreement No. 965406 (PrecisionTox), No. 964537 (RISK-HUNT3R) and No. 963845 (ONTOX), which are part of ASPIS.
- This output reflects only the author's view and the European Union cannot be held responsible for any use that may be made of the information contained therein.

Thank you

Questions?

Collaboration on Data and Knowledge Management Best Practices and Resource Development and Deployment

ASPIS Symposium on “A collective effort to implement Novel Approach Methodologies in Next-Generation Risk Assessment“
24 - 25 November 2022, Sitges Spain

Barry Hardy (Edelweiss Connect)
Email: Barry.Hardy@edelweissconnect.com
LinkedIn: https://www.linkedin.com/in/barryhardy/

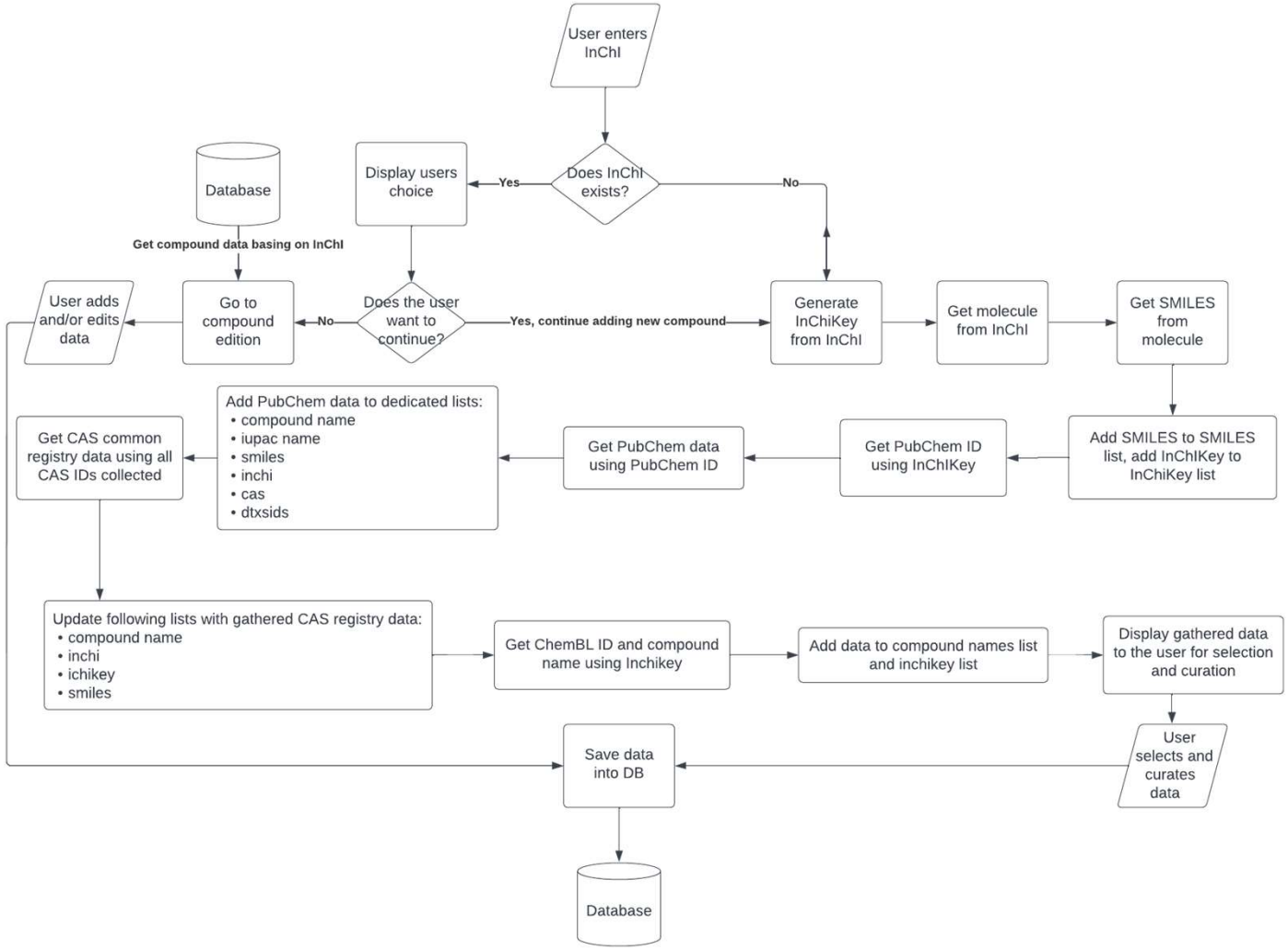
Overview of Goals and Activites

1. Working Group Activity
2. Chemical Database (to be shared across cluster and beyond)
3. Harmonised Data Templates and Metadata
4. Enabling FAIRness of data and models
5. Methods Templates and Database (to be shared)
6. Sustainable Open Knowledge Infrastructure
7. Deployment processes supporting collaboration and early community release
8. Support quality documentation of models and case studies
9. Alignment with regulatory guidance and best practices
10. Collaborate on knowledge integration (e.g., IATAs, qAOPs)

Working Group Activity

1. Early cross-project sharing of compound lists and information (2021/2022)
2. Current work to complete and share Compound Database for ASPIS cluster release before end of 2022, processing and testing all compound lists
3. Communications: Slack channels for WG activity, new database sub-group working on collaboration on data/knowledge resource/AI development and deployment
4. Additional sharing of information supporting Case Studies, qAOPs, Methods documentation (e.g., ToxTemp)
5. Will communicate virtually to all projects over coming weeks after meeting with support information on how to share information or access information shared

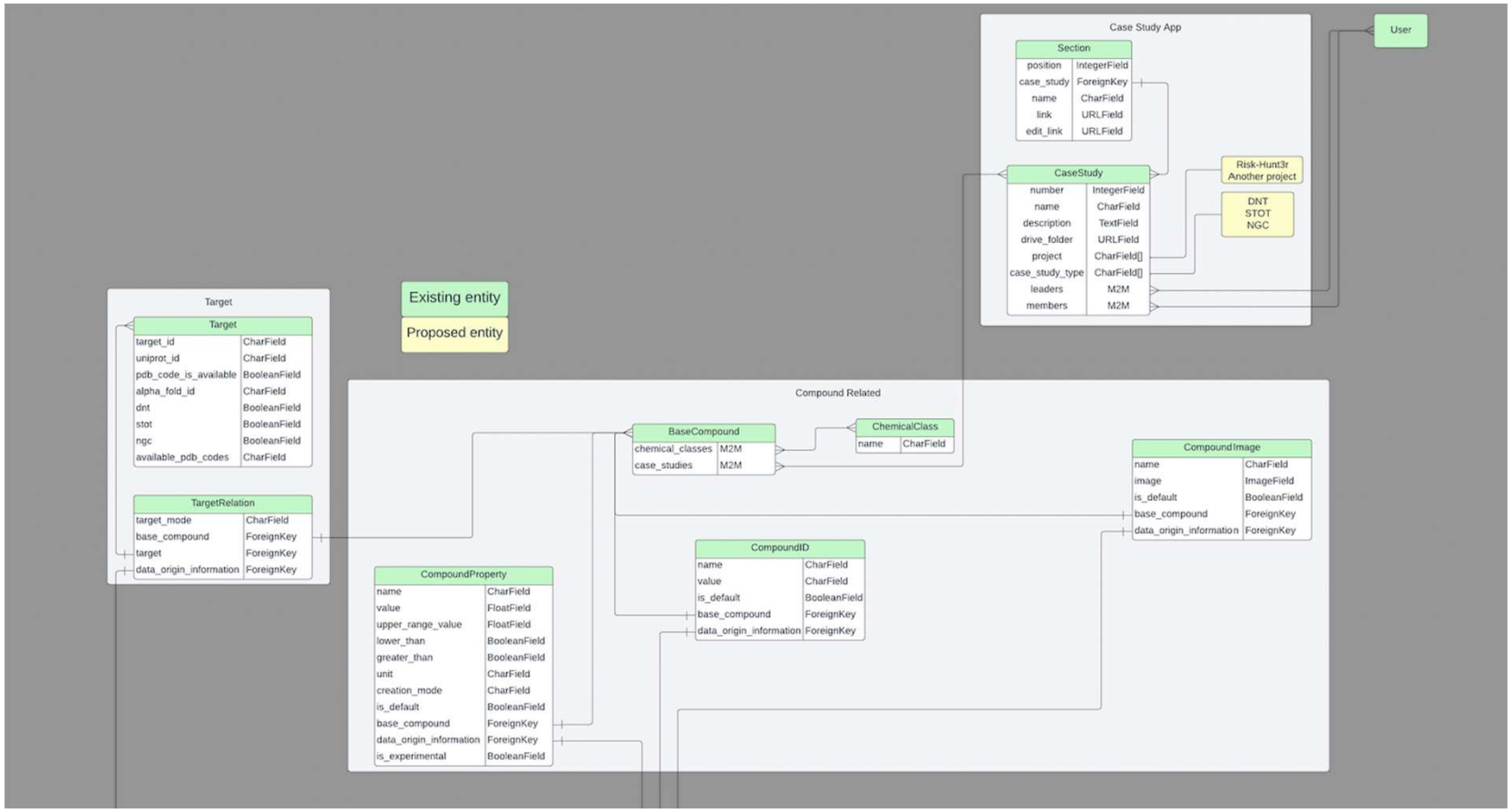
Addition of compound and compound information to database



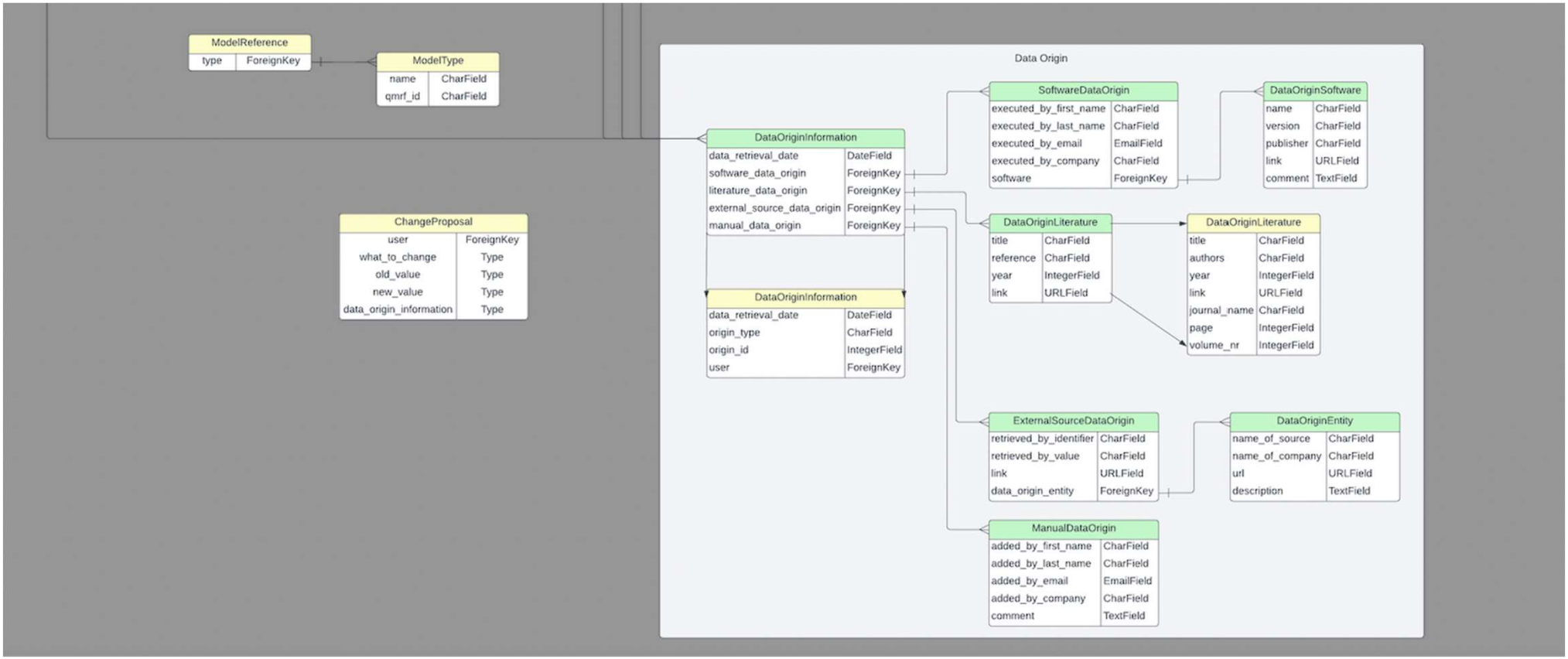
Apply workflow to different compound lists on different projects e.g., starting with RISK-HUNT3R, then ASPIS

Aim to achieve a common integrity of information.

RISK-HUNT3R Compound Data Model (1)

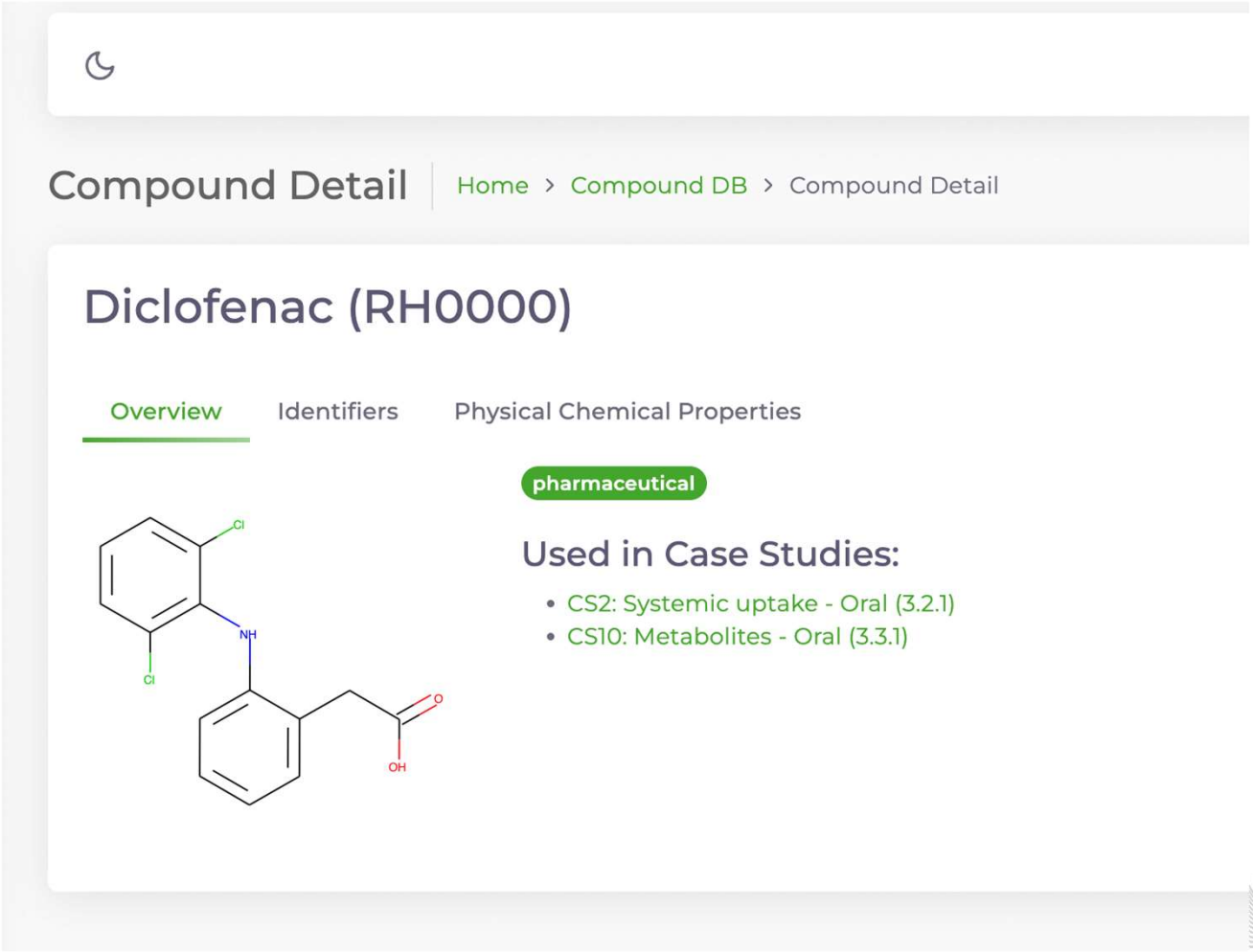


RISK-HUNT3R Compound Data Model (2)



Shared within ASPIS Working Group

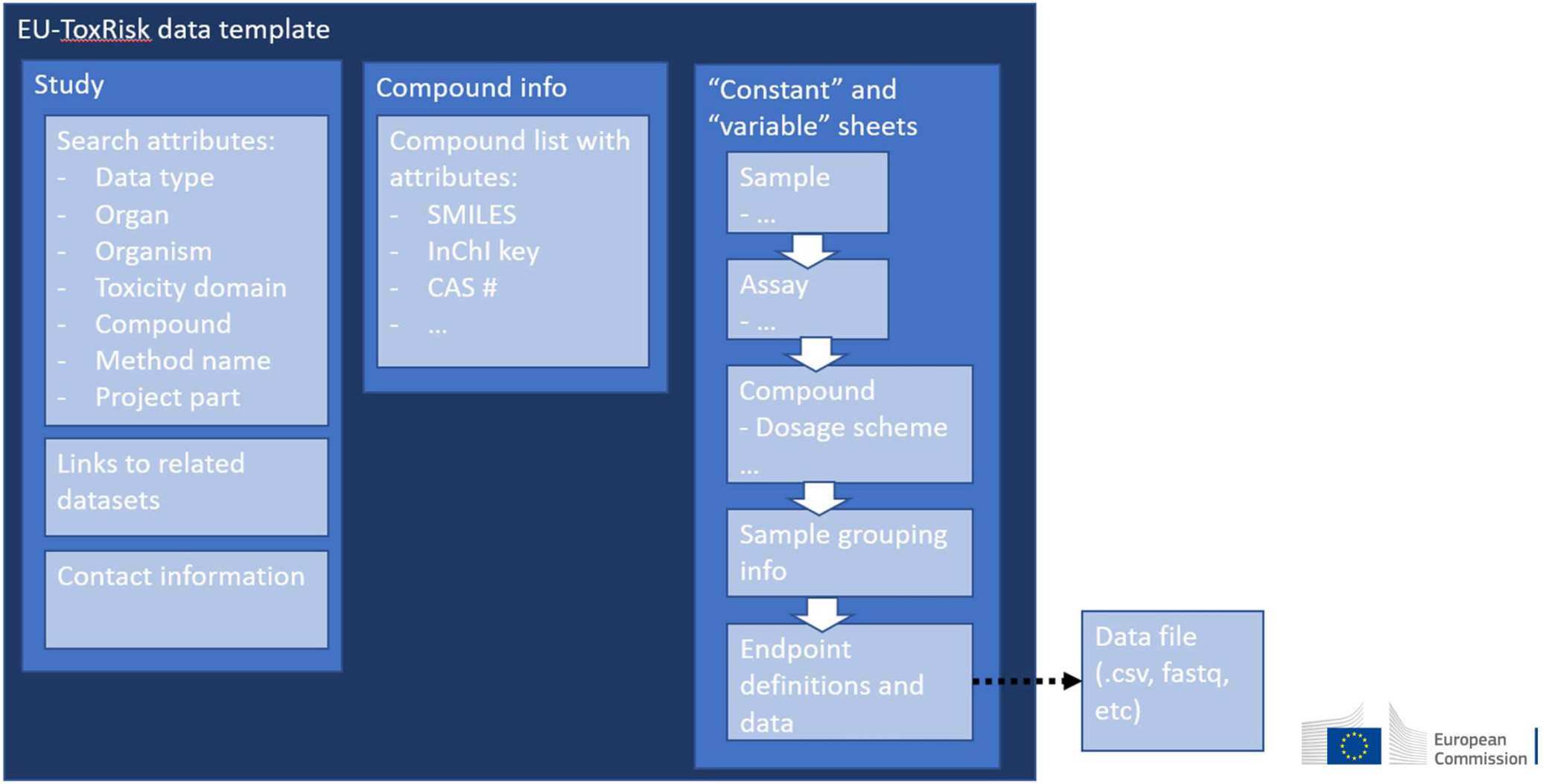
RH3R Chemical Db



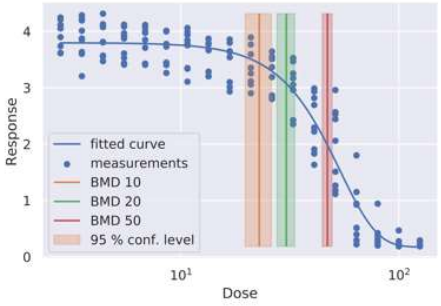
Planned for ASPIS and public release for December 2022

European Commission

Data Generation template (initiated on EU-ToxRisk, continuing on RISK-HUNT3R)



Integrated Data and Knowledge Management



Harmonised Data, Protocol and Metadata Management Infrastructure supported by EdelweissData

Data and Knowledge Management on EUToxRisk program

Experiments
Generates 'raw data'
- *e.g.* Fluorescent intensities

Data processing
Process raw data
- Fluorescent intensities → activities
- Experiment/laboratory specific

Data summarization
Summarize processed data
- Activities → curve fit → *p*XC50
- Common approaches?

Test Method DB
Stores detailed description of test methods including SOPs
- *Human readable description*
- *Computer-readable annotations*

BioStudies @ EMBL-EBI
Data repository and archive
- Data stored as individual data sets

Raw data
Processed data
Summary data
Metadata

Knowledge Sharing
Test method and data portal
- Data reformatted to optimize querying
- *e.g.* join data across compounds

Modelling
Statistical model building
- Requires summarized data

Visualization
Exploratory data analysis
- Summarized data linked to underlying data

Decision making
Weight of evidence
- Model output
- Expert opinion
- ...

European Commission

Data Management and Analysis

Behind the scenes

RISK HUNT3R

Data structure

- Excel template file (EU-ToxRisk)
- Required fields for all the datasets
- Custom fields if needed

Column Name	Constant Values
Sample ID	
Method name	*method name indicated in the UKN3b_NeuroTox_LUH_neuri*
Toxicity domain	Neuro
Information domain	Cytotoxicity
Date	
Experiment ID	
Organization abbreviation	

Data import

- Each dataset is validated
- Published on Biostudies
- Automatically imported to EdelweissData
- Data becomes accessible through web requests (URL)

EdelweissData™
Convenient publishing of scientific data with proper versioning, rich metadata support and a powerful API

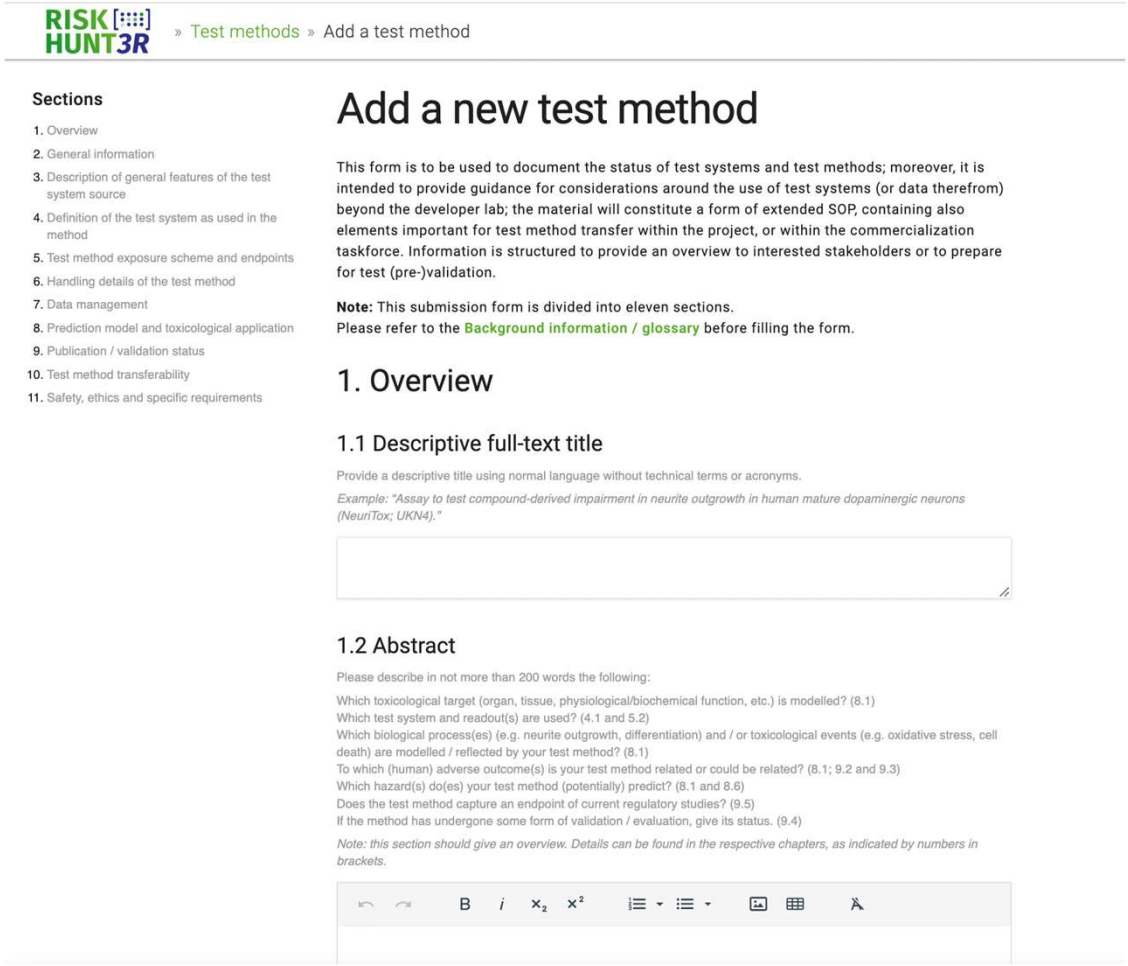
Data use

- Access data & metadata directly from the database
- Target specific version of dataset (e.g. latest)
- Consume data directly into your data analysis tool (R, Python, Excel, Jupyter, Colab, Observable)

Observable

European Commission

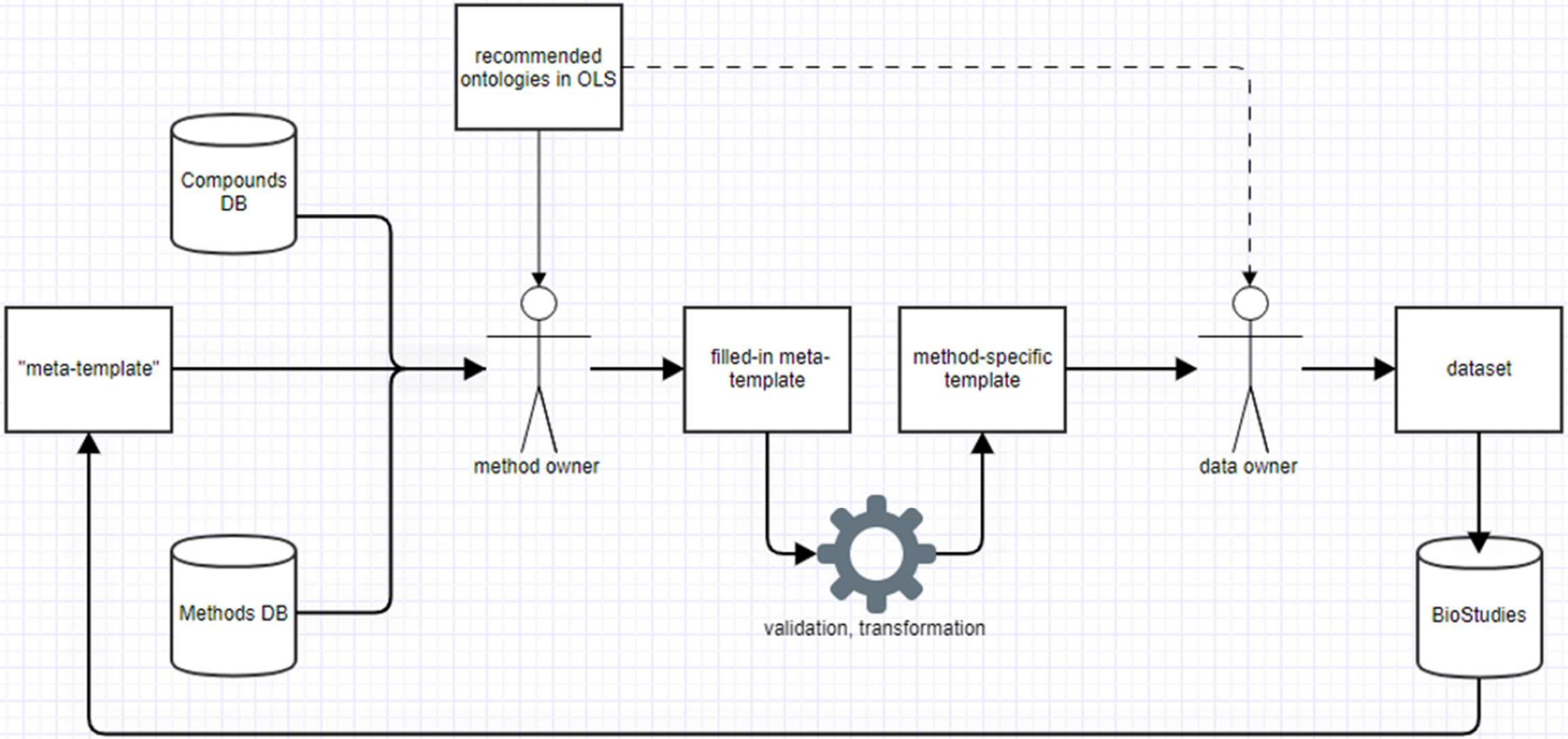
ToxTemp Methods Database



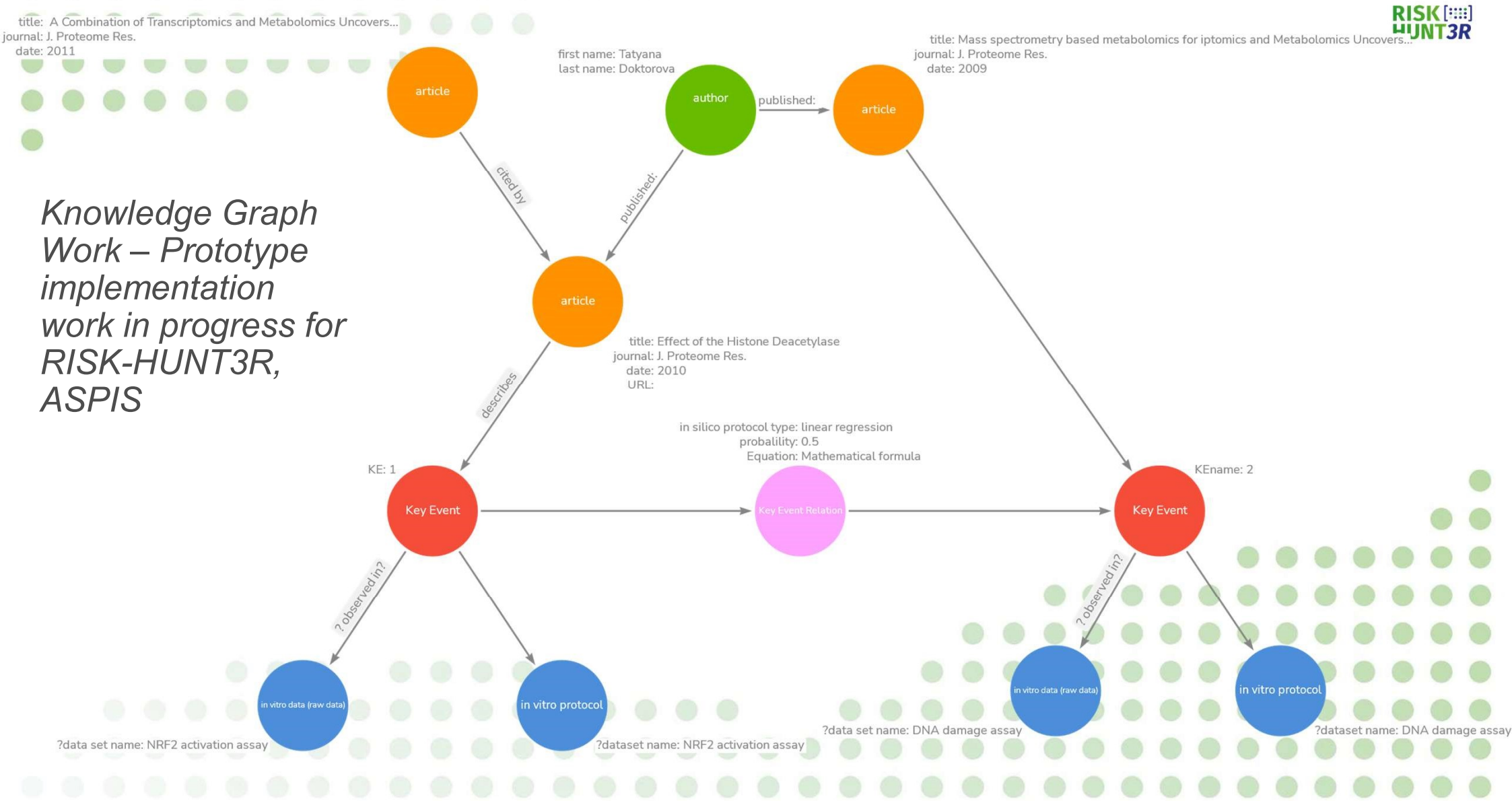
Updated Version Planned for Public Release for November 2022 and ongoing during 2023 – 2026 during RISK-HUNT3R project

European Commission

Combined Data and Methods Template Development



Knowledge Graph Work – Prototype implementation work in progress for RISK-HUNT3R, ASPIS



Knowledge Mining and AI Workflows (collaboration with OnTox)

THE LARGEST BIOINFORMATICS DATABASE

```
import biobricks as bb
tox21 = bb.install('tox21')
tox21.to_pandas()

# SAMPLE                        PROTOCOL
        DATA_TYPE       …
# NCGC00255868-01       tox21-ache-p3   ratio
        …
# NCGC00256074-01       tox21-ache-p3   ratio
```

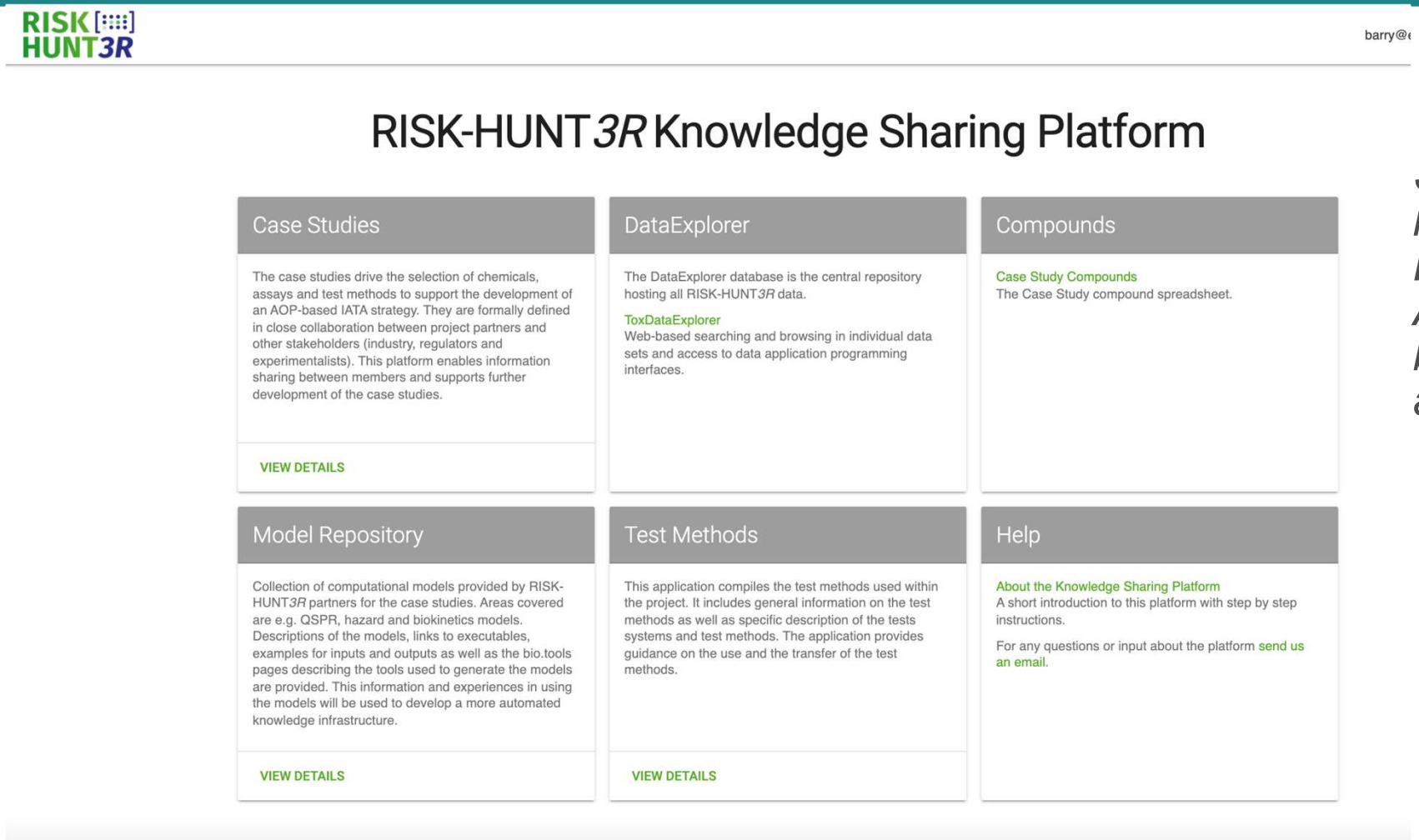
Biobricks.ai provides a "data registry" for bioinformatics. 50+ Life science databases are updated periodically; simple packages can import each data "brick" into new resources. Biobricks provides a single store for all bioinformatics data.

(Left) Three lines of code retrieves and displays the tox21 database. No manual downloading.

A SINGLE SOURCE OF TRUTH - ASPIS4J GRAPH DB

ASPIS4J is a large graph database composed of all biobricks.ai DB dependencies or "bricks". It forms a big data platform for Ontox that updates whenever a source DB updates.

RISK-HUNT3R Knowledge Sharing Portal

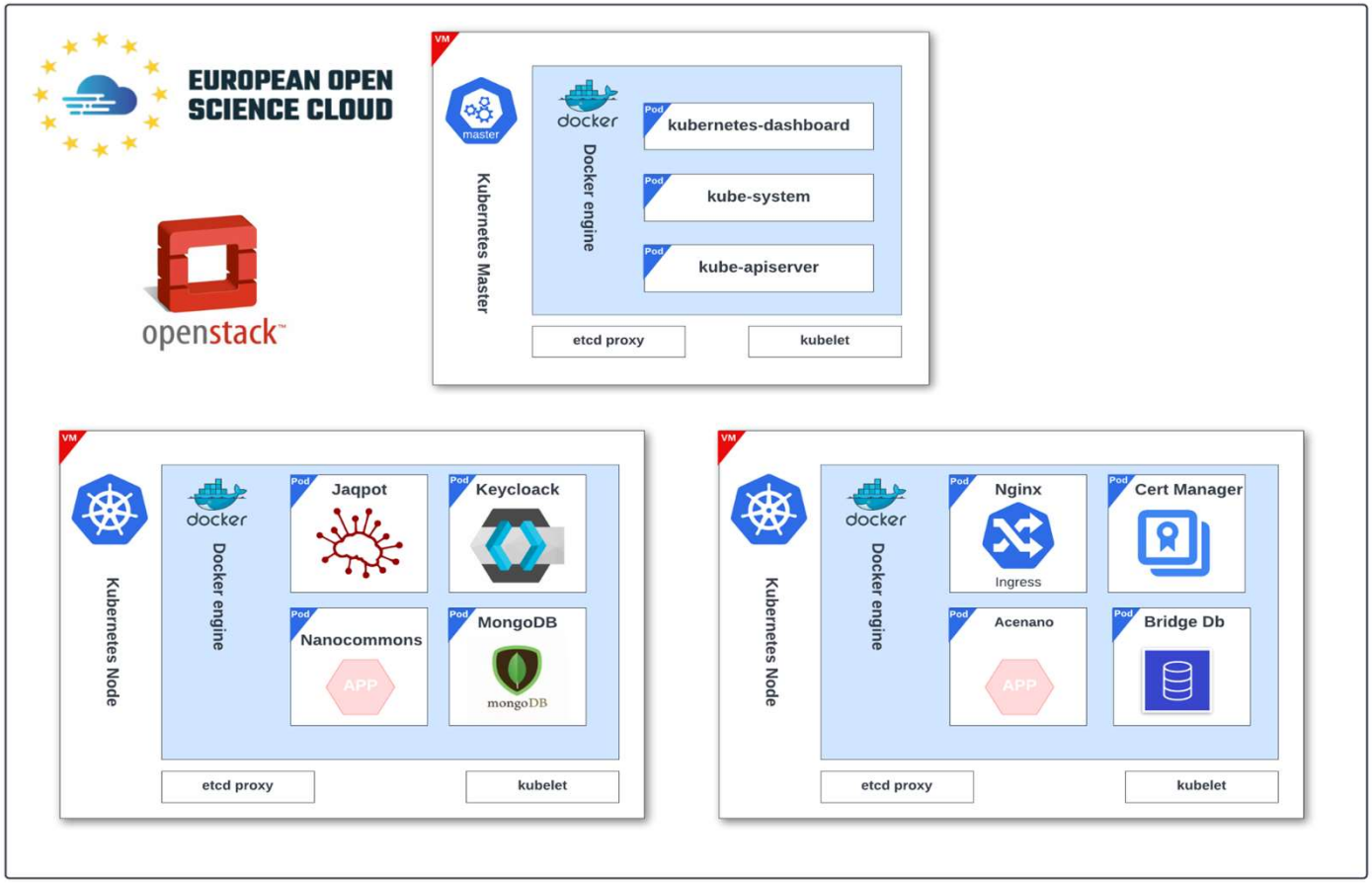


Share knowledge resources from RISK-HUNT3R to ASPIS and beyond with public access

https://risk-hunt3r.net/

European Commission

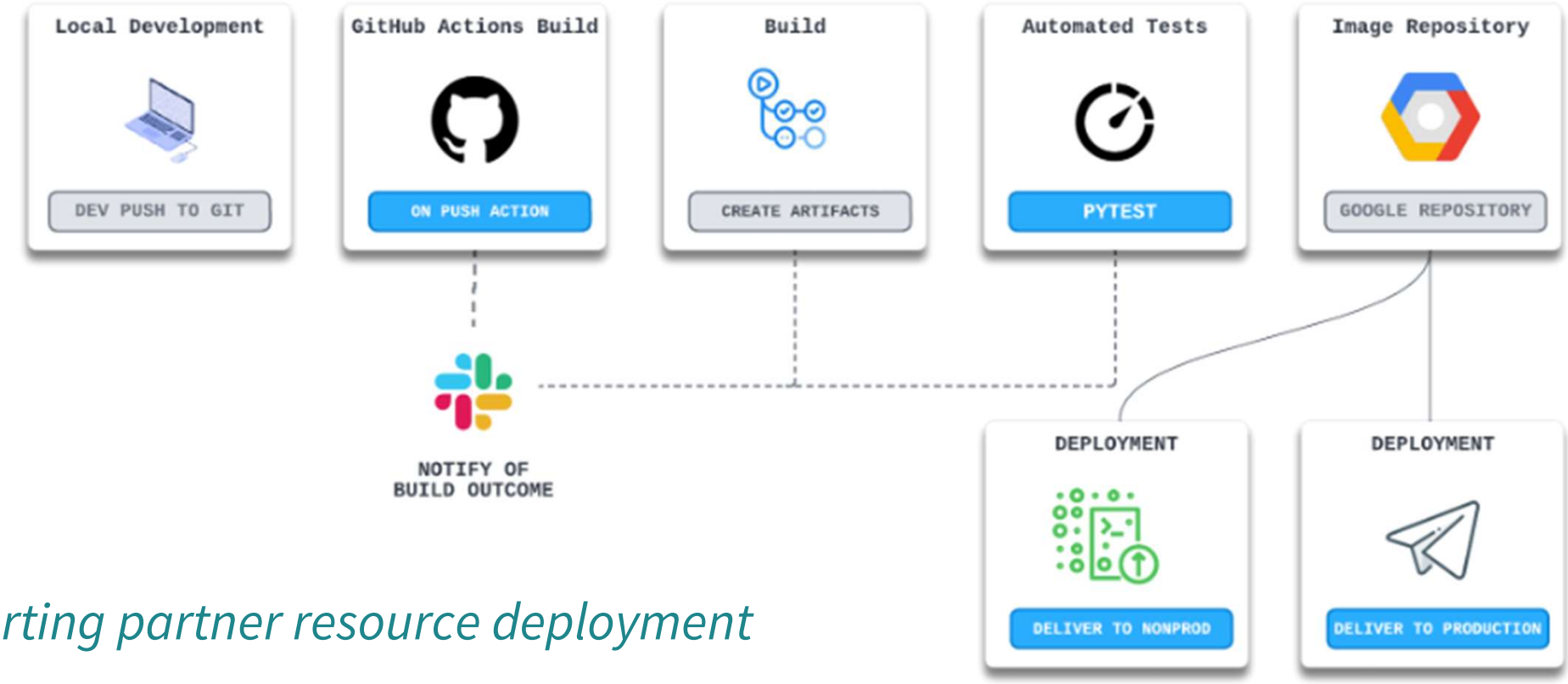
Edelweiss Connect and Partners - EOSC Architecture for Collaborative Orchestration and Deployment of a Predictive Toxicology and Risk Assessment Infrastructure



EOSC = European Open Science Cloud

European Commission

Knowledge Resource Deployment - Continuous integration and deployment workflow for knowledge infrastructure (supporting ASPIS knowledge resource deployment



Supporting partner resource deployment

Collaboration on Data and Knowledge Management Best Practices and Resource Development and Deployment

ASPIS Symposium on “A collective effort to implement Novel Approach Methodologies in Next-Generation Risk Assessment“
24 - 25 November 2022, Sitges Spain

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LinkedIn: https://www.linkedin.com/in/barryhardy/

ASPIS project cluster

Mirjam Luijten

- 24/11/2022
- ASPIS Open Symposium

These projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers PrecisionTox: 965406, ONTOX: 963845 and RISK-HUNT3R: 964537

NGRA workflow: why?

ASPIS projects' common goal:

To establish NGRA approaches for the safety assessment of chronic adverse health effects

NGRA workflow: why?

- Various concepts for (parts of) NGRA have been published, all highly similar; however, operationalization of the concepts is limited

- Hence, there is a strong need for a well-guided workflow for safety assessment of chemicals, to serve as **guidance** on data generation and interpretation, allowing to make decisions in a **transparent** and **consistent** manner

Joint NGRA workflow: ASPA

ASPA, the ASPIS Safety Profiling Algorithm:

- Defines a tiered approach on what tools/methods to use
- At which steps to obtain and evaluate data, incl. uncertainty assessment
- How to put data into a context of a hazard or risk assessment scenario
- Defines a decision logic with multiple entry and exit points, activating/deactivating specific modules, and prioritizing and filtering of information

ASPA: data generation & integration

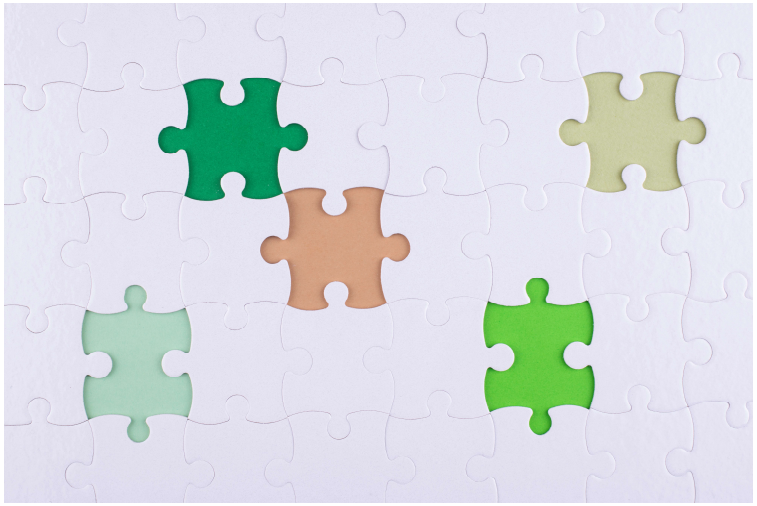
Same object, but different perspectives

Set of rules on how to generate the data

Set of rules on how to assess the data

ASPA: A true ASPIS effort

ASPA helps ASPIS

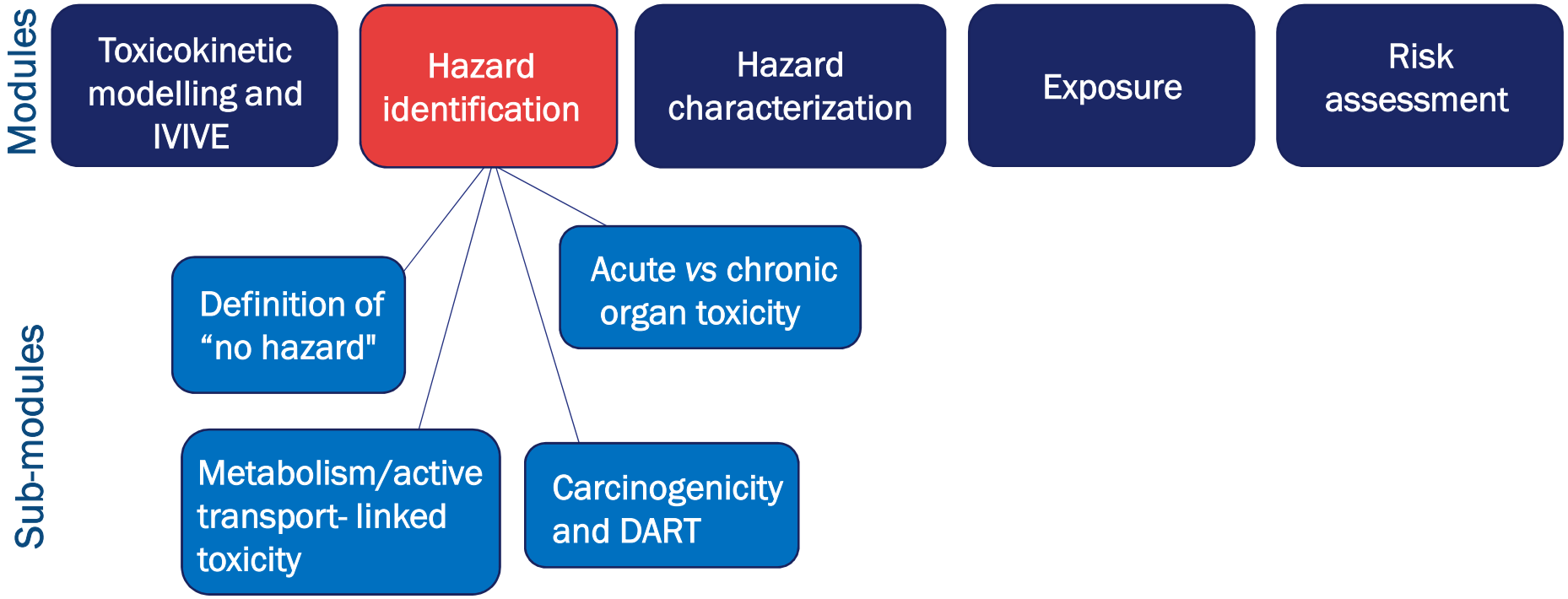


ASPA will help:

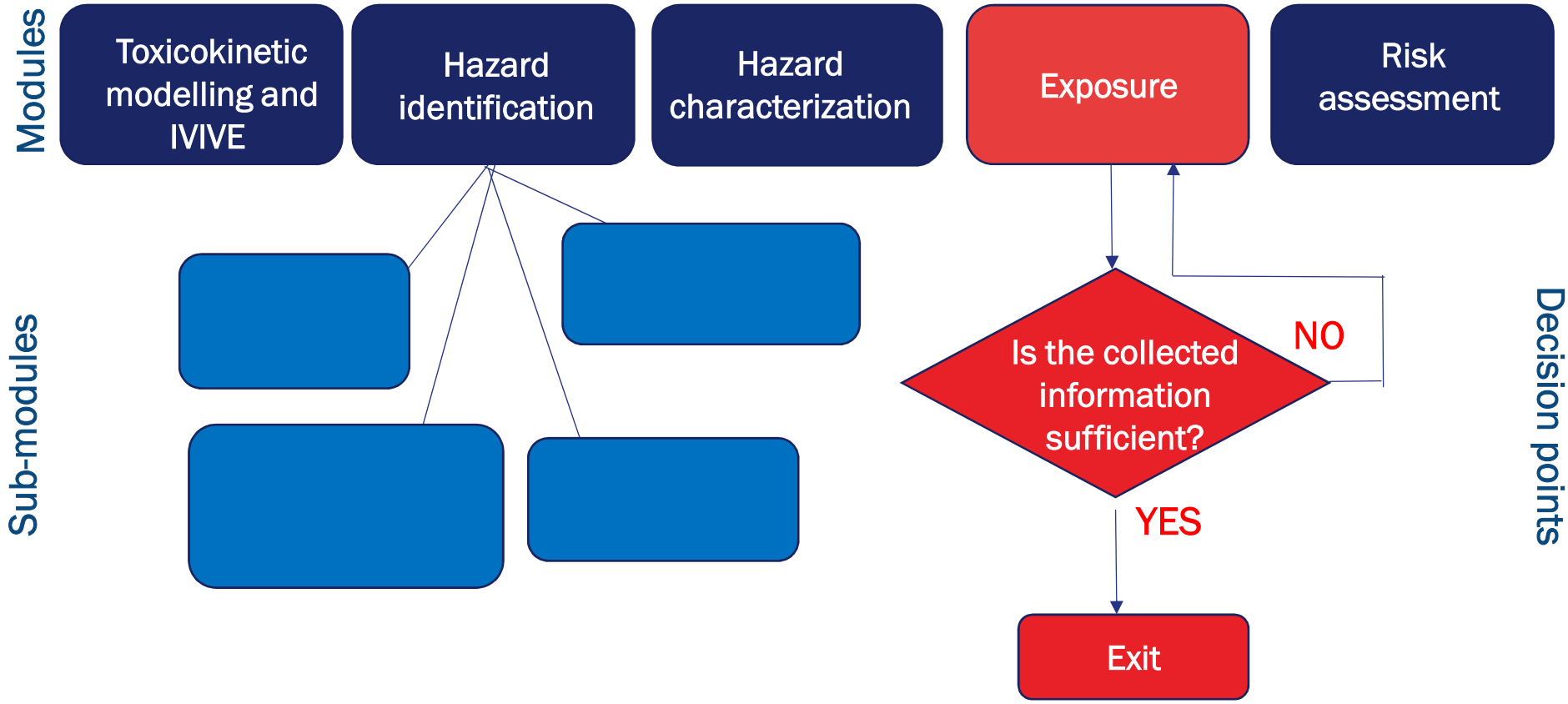
- to define ASPIS case studies needed to further refine the workflow
- to define ASPIS working group activities, e.g. for evaluating the applicability of ASPA
- to strengthen collaborations, within and outside ASPIS

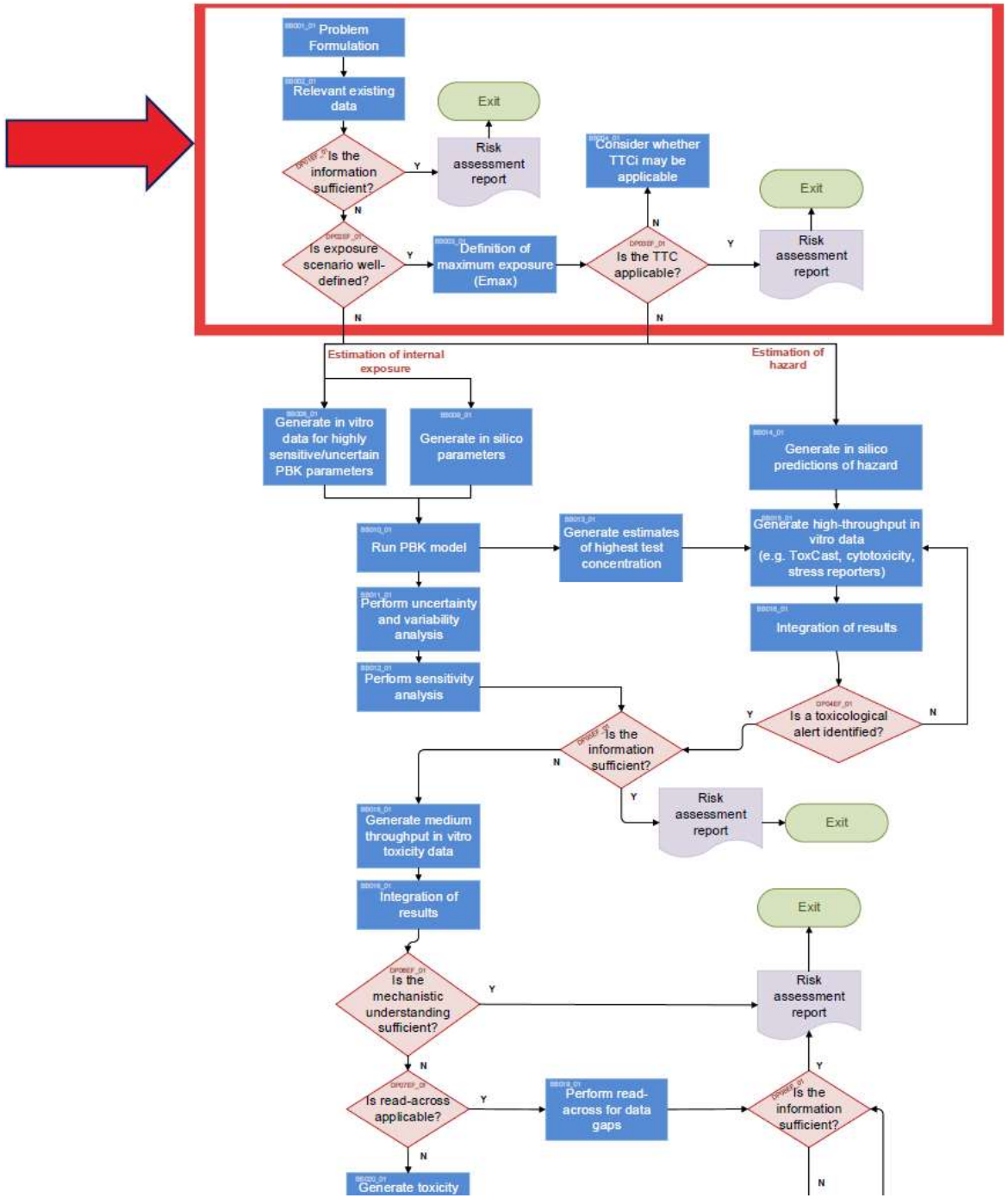
ASPA modules (under construction)

ASPA modules (under construction)

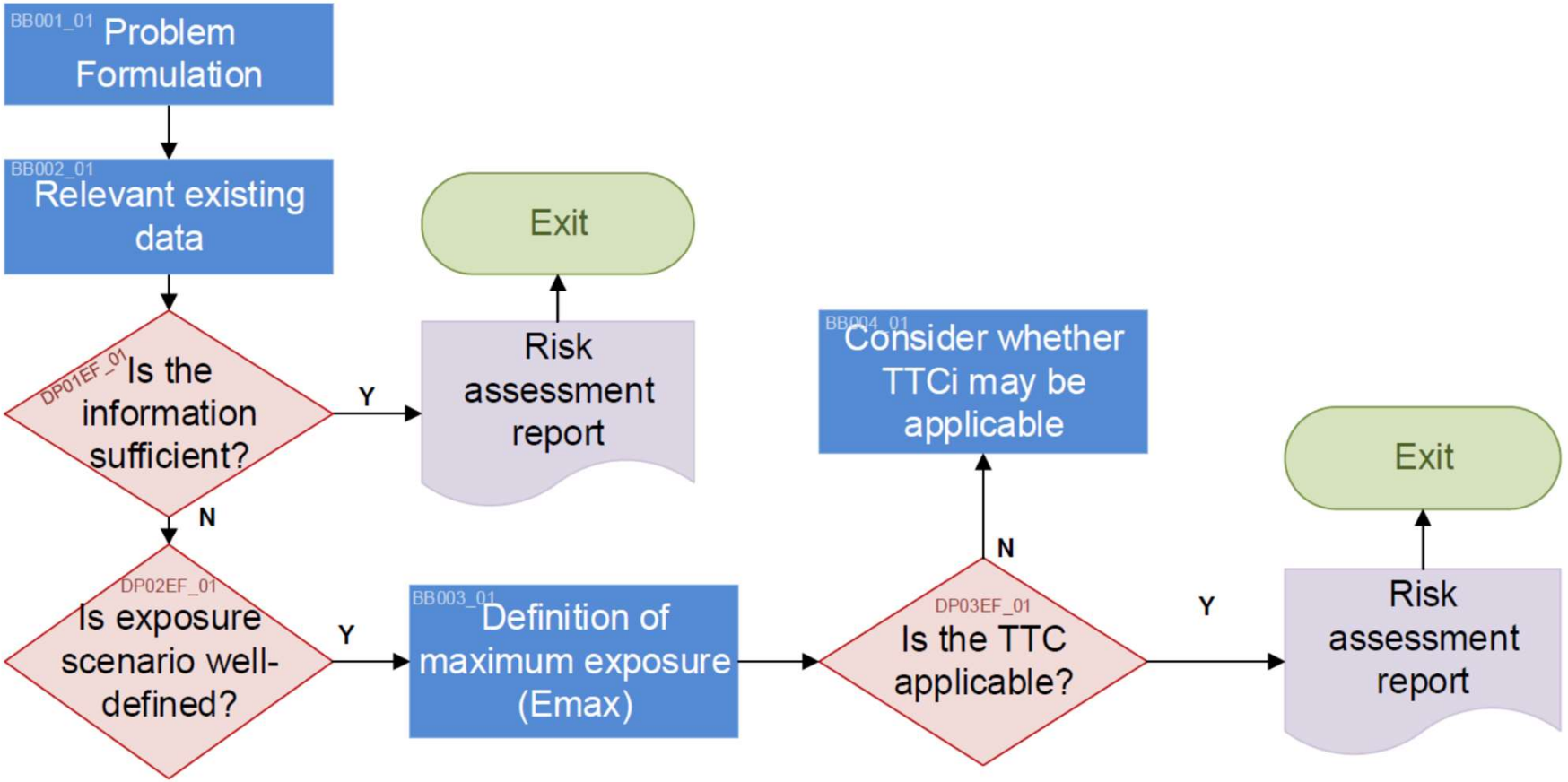


ASPA modules (under construction)




Problem Formulation
Relevant existing data
Is the information sufficient?
Y
Exit
Risk assessment report
N
Is exposure scenario well-defined?
Y
Definition of maximum exposure (Emax)
Consider whether TTCi may be applicable
N
Is the TTC applicable?
Y
Exit
Risk assessment report
N
N
Estimation of internal exposure
Estimation of hazard
Generate in vitro data for highly sensitive/uncertain PBK parameters
Generate in silico parameters
Generate in silico predictions of hazard
Run PBK model
Generate estimates of highest test concentration
Generate high-throughput in vitro data (e.g. ToxCast, cytotoxicity, stress reporters)
Perform uncertainty and variability analysis
Integration of results
Perform sensitivity analysis
Is a toxicological alert identified?
Y
N
Is the information sufficient?
N
Y
Risk assessment report
Exit
Generate medium throughput in vitro toxicity data
Integration of results
Exit
Is the mechanistic understanding sufficient?
Y
Risk assessment report
N
Is read-across applicable?
Y
Perform read-across for data gaps
Is the information sufficient?
Y
N
Generate toxicity
N

ASPA (zoom-in example)



ASPA: Tiered approach

ASPA involves a tiered approach:

- Separate parts of the algorithm can be useful in themselves
- The initial modules are built first as starting points for the further algorithm, considering key problem formulations
- Some parts will be ready shortly for initial testing, while other parts need further elaboration

ASPA: Evolutionary approach

ASPA will change over time:

- in depth/detail
- in readiness level
- in incorporation of modern technologies
- in different hazard and risk assessment principles

ASPA: Flexible and reproducible

- ASPA is flexible: multiple questions and regulatory frameworks will be facilitated

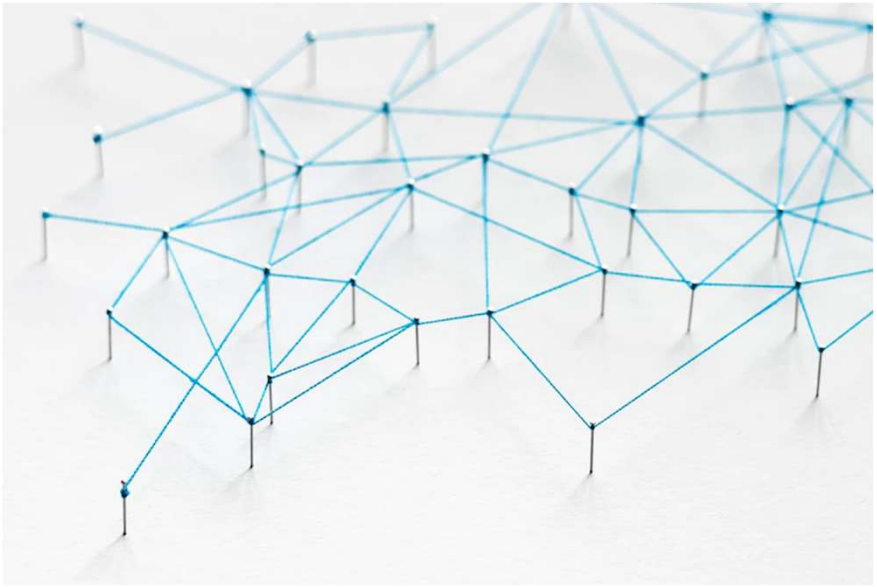
- ASPA is reproducible: the same datasets used in the same block for a specific purpose should lead to the same conclusion and level of confidence

- This needs stringent definitions of decision processes and testing of many example datasets

ASPA: linked activities

ASPA is not developed in isolation, but linked to:

- Standards for documentation and reporting
- Identification of gaps → NAMs to fill these gaps
- Case studies to demonstrate the applicability of ASPA
- Related initiatives



Thank you for your attention!

www.aspis-cluster.eu

NAMs & REACH: needs & opportunities

Time to think about “Chemicals 2.0”?

Presented by Elisabet Berggren
Developed in collaboration with Andrew Worth

Disclaimer: this presentation is a thought starter developed by the JRC authors and does not necessarily represent a Commission position

The Joint Research Centre Mission

We are the science and knowledge service of the European Commission our mission is to support EU policies with independent evidence throughout the whole policy cycle.

Unit F3:
Chemicals Safety & Alternative Methods

EURL ECVAM

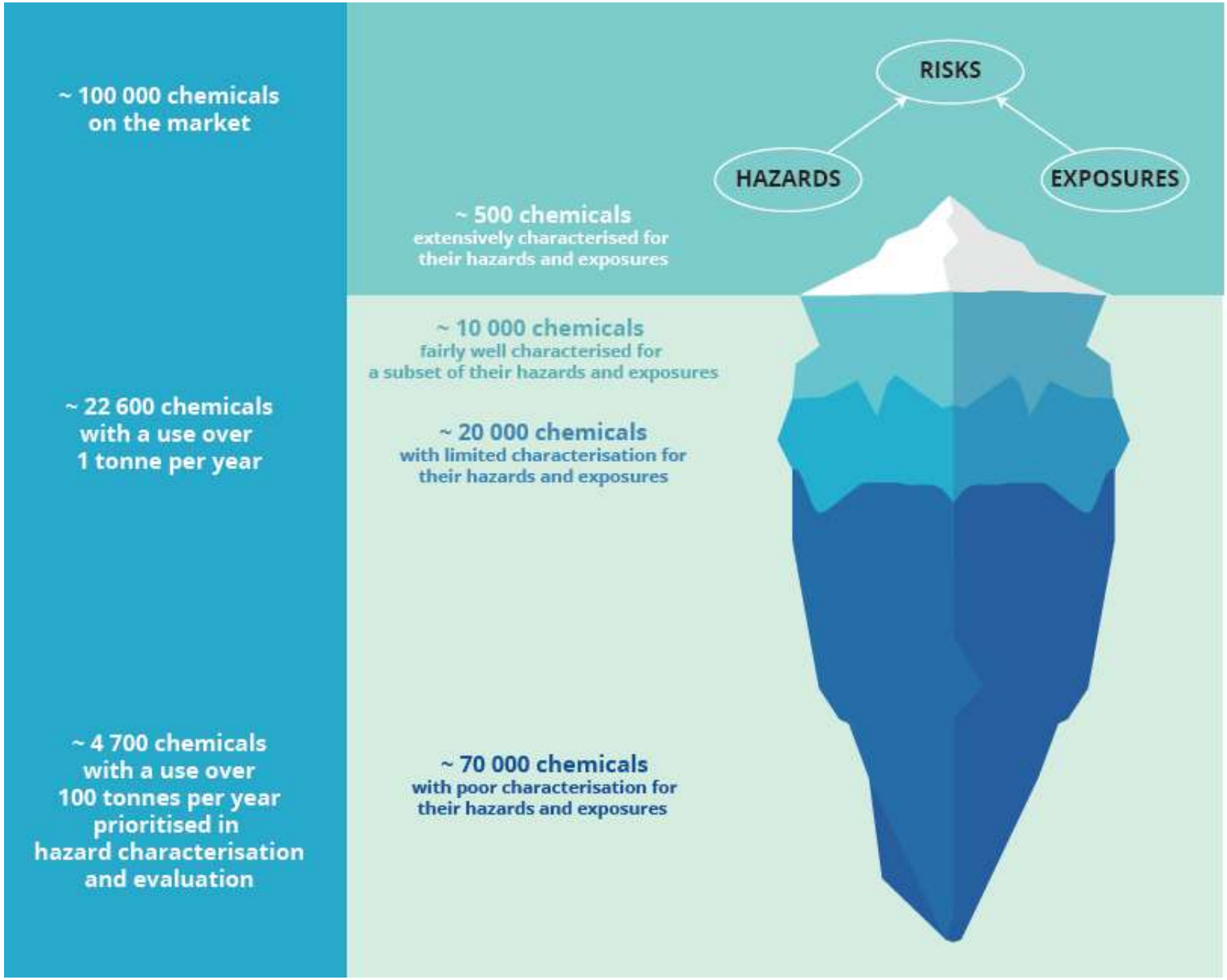
European Union Reference Laboratory for alternatives to animal testing

3Rs

mandate under Directive 2010/63

- research & evaluation
- validation
- dissemination
- promotion

European Commission

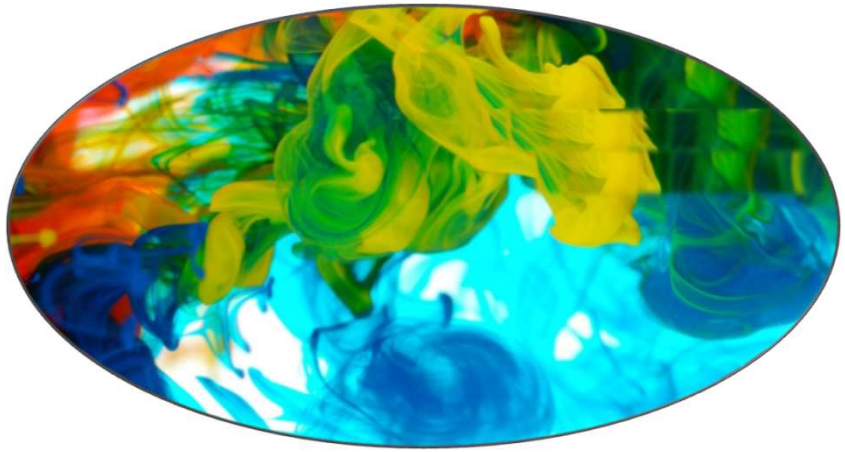


https://www.eea.europa.eu/soer

European Commission

Extended REACH Information Requirements

Action under the Chemicals Strategy for Sustainability led by JRC with support of steering group (DG ENV, DG GROW, ECHA)

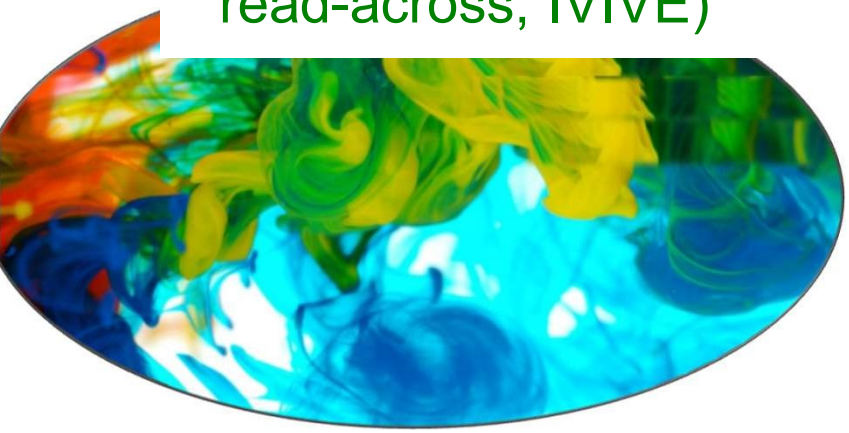


Good to know about REACH and its Information Requirements:

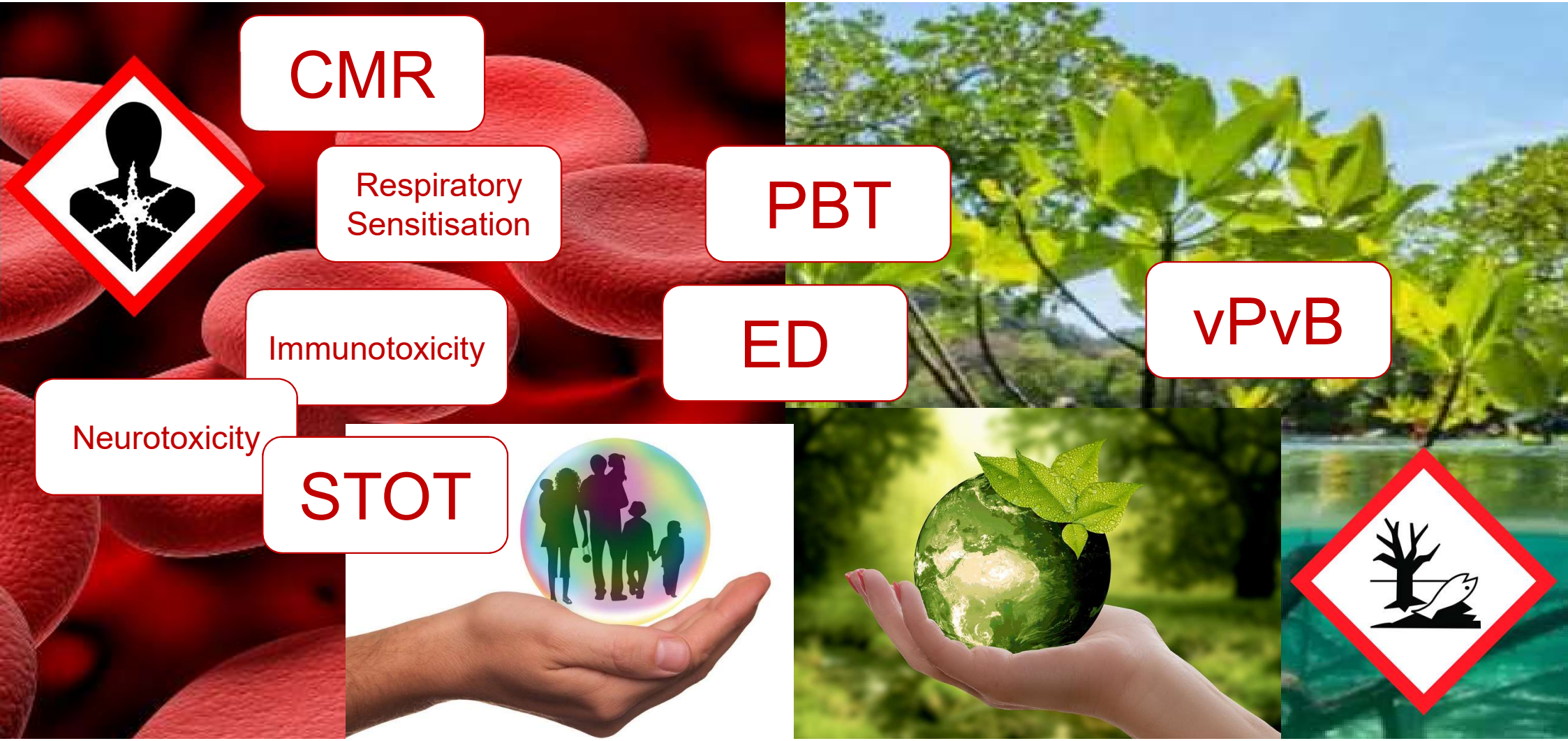
- Annexes VII to X: standard information requirements, largely tonnage-dependent
- Annex XI: general rules for adaptation
- Information Requirements under REACH enable hazard classification under CLP

Aims of the extended REACH Information Requirements stated in the Chemicals Strategy for Sustainability:

- Enable Chemical Safety Assessment at all tonnage levels
- Inclusion of critical hazards
- Information on toxicokinetic properties & mode of action (supporting grouping, read-across, IVIVE)



Critical Hazards



The JRC Survey

Looking for NAM based **solutions** to predict:

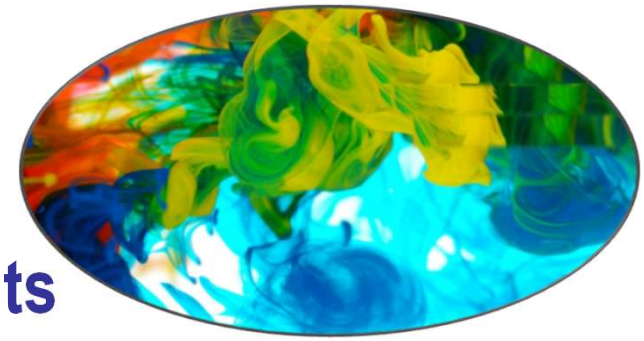
1. DNEL for Human Health
2. PNEC for the Environment
3. Classification & Labelling
4. PBT or vPvB
5. Critical hazards

Responses

81 through EU Survey Tool
74 from EU ToxRisk
11 Cosmetics Europe

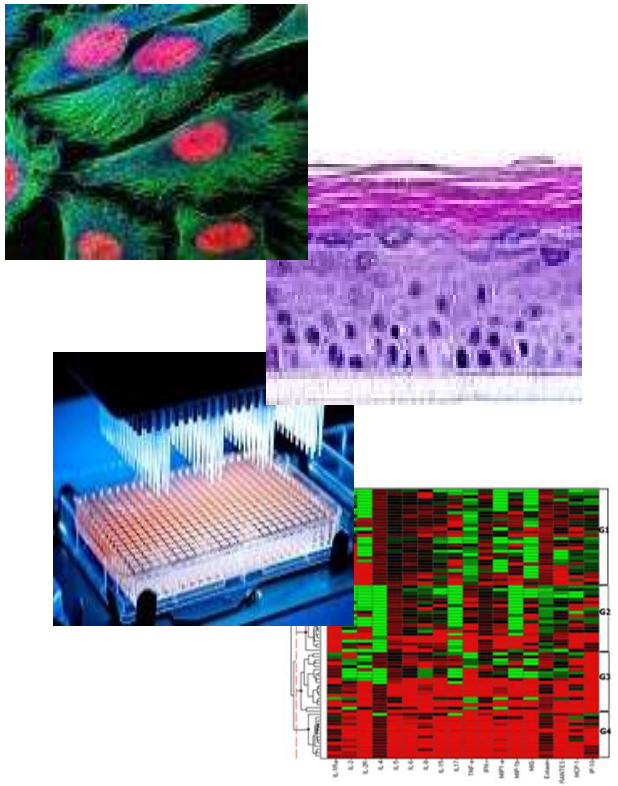
The survey is still open
https://ec.europa.eu/eusurvey/runner/surveyNAM
as we continue to look for solutions (= testing strategies resulting in conclusions) to predict 1-5

Actions related to the Extended REACH Information Requirements



- Options elaborated to define a space reaching from most extensive information requirements (high animal use) to focussing on essential testing (minimising animal use)
  - Options have been subject to Impact Assessment
    - Review by Regulatory Scrutiny Board ongoing

NAM-based information requirements



- ADME / TK:
  - fraction unbound in plasma
  - In vitro hepatic clearance
  - In vitro bioavailability
- Endocrine activity:
  - estrogen
  - androgen
  - thyroid
- Fish bioaccumulation. Intrinsic clearance in:
  - rainbow trout hepatocytes (OECD TG 319A) or S9 fraction (OECD TG 319B)
- Acute fish toxicity
  - fish cell line (OECD TG 249)

Problems experienced when introducing NAMs under the REACH Information Requirements

- NAMs not sufficiently validated or standardised (OECD TG required?)
- Perception of “less safe” & higher uncertainty
- NAMs used for screening to trigger additional animal testing rather than reduce animal use
- NAMs for systemic toxicity – not 1-to-1 replacement, need for IATA
- Standalone NAMs not able to identify adverse systemic health effects and environmental hazards to provide legal certainty for Classification & Labelling (C&L)

“Chemicals 2.0” –
a long-term objective for chemical safety assessment

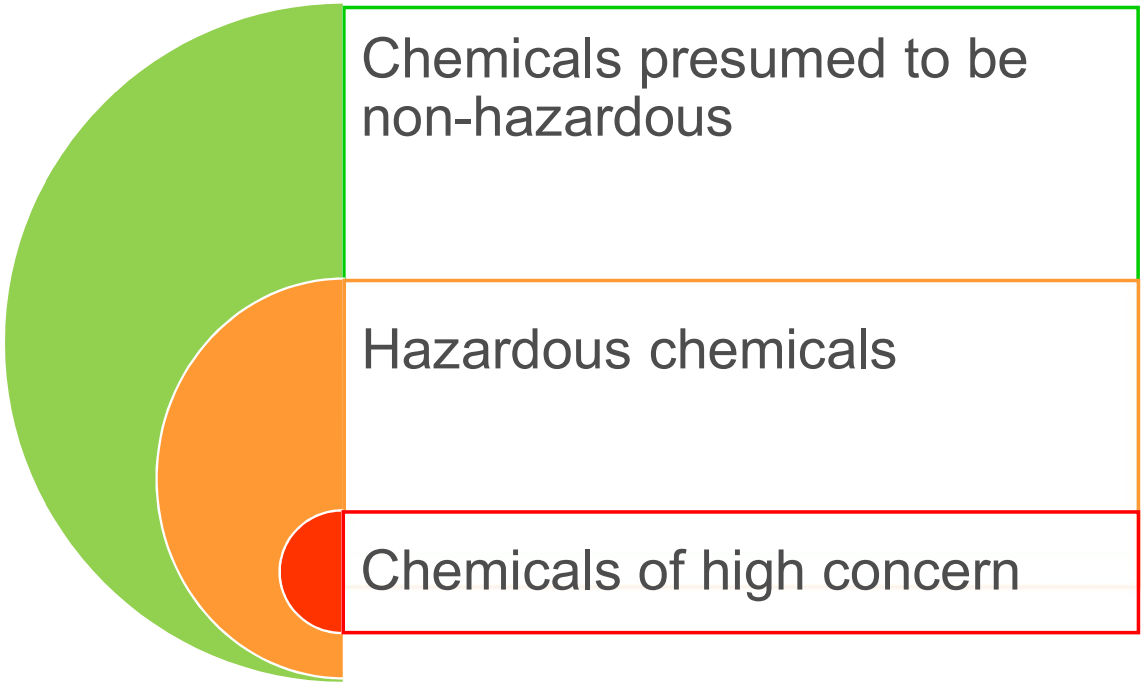
What do we want to achieve?

- consider all chemicals & test them according to concern
- minimise animal testing during “phase-out” period
- eventual aim of complete replacement

Design specifications of a future regulatory system

- Applicable to all substances on the market
- Provides an equivalent level of protection (same risk management decisions)
- Provides regulatory certainty and guides innovation
- Risk management is based on NAMs and exposure considerations
- Current C&L conclusions are maintained (unless challenged)
- Additional NAM-based C&L conclusions result in higher level of overall protection

Principle of equivalent protection:
Make the same decisions, not necessarily the same predictions



→ Innovate
→ Use without restriction

→ Restrict via concentration limits
→ Demonstrate safe use

→ Ban for some or all uses

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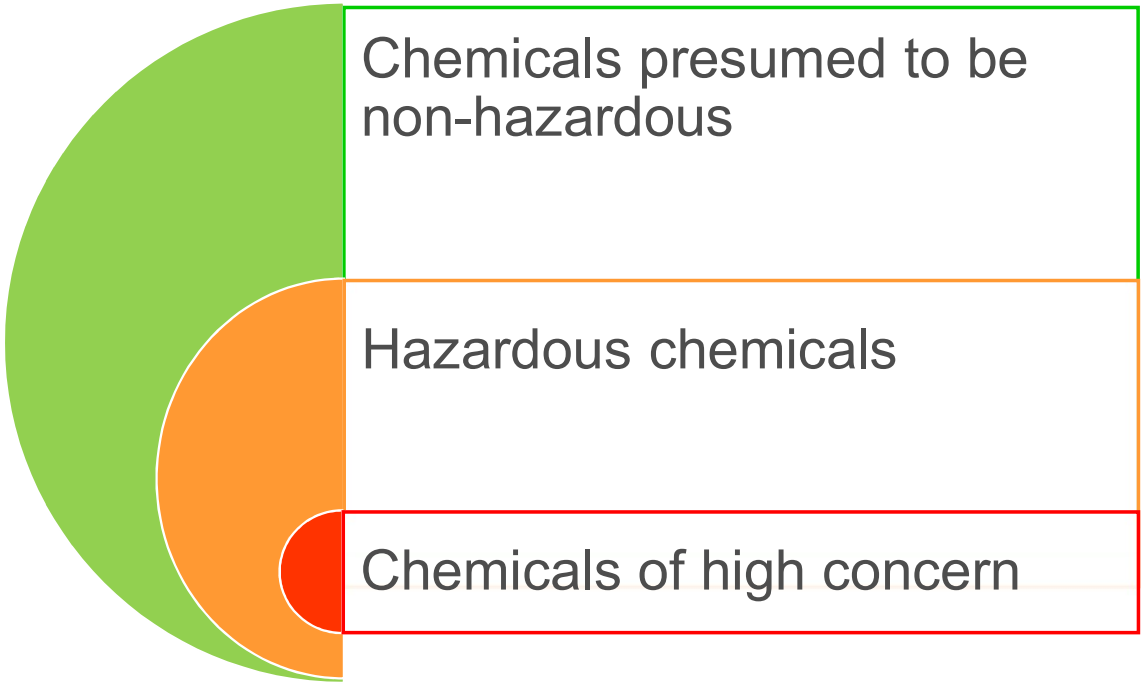
Decision logic for equivalent protection



→ Demonstrate safe bioactivity profile

→ Classification & Labelling +
→ Risk assessment

→ Classification & Labelling

The fraction of chemicals covered by each group is calibrated to at least **keep current protection level**

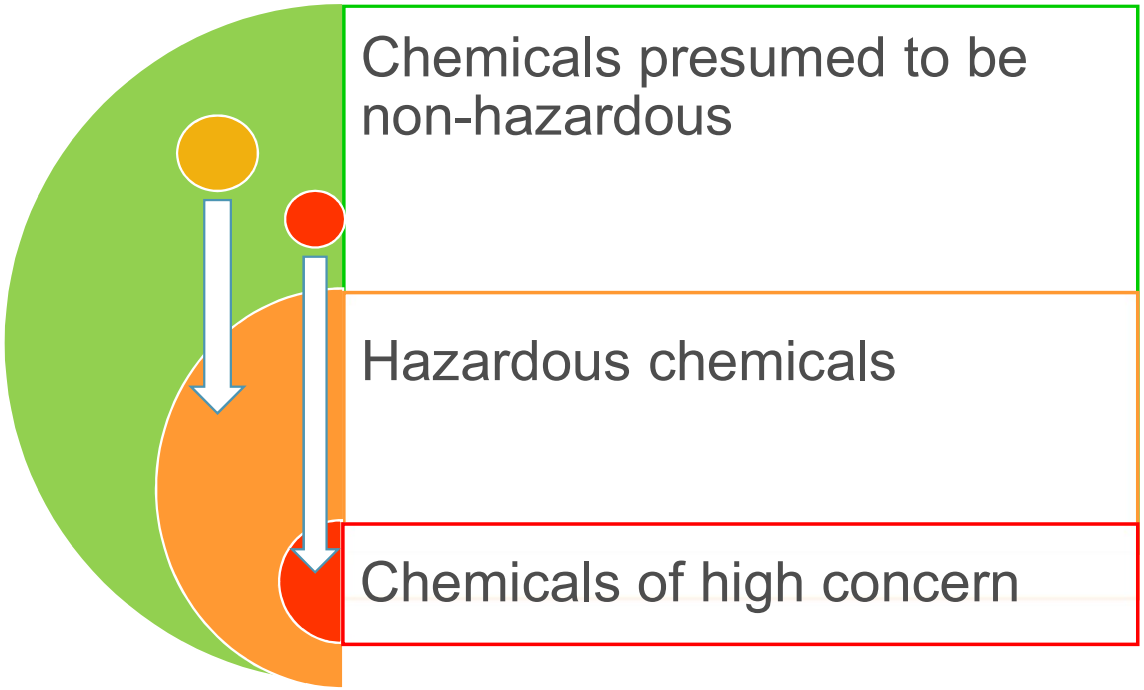
Developing a new classification scheme

- A generic risk matrix based on intrinsic properties is developed to assign chemicals to groups 1-3 (low, medium & high concern)
- Existing data for already classified chemicals (high & medium concern) are used to calibrate the classification scheme resulting in **equivalent protection**

		NAM-based toxicodynamics (not aiming on prediction of adverse effects)		
		Hazard - high	**Hazard - medium**	**Hazard – low** (bioactivity profile without hits)
NAM-based toxicokinetics (ADME properties)	**Internal exposure – high** (persistent & bioaccumulating)	3	3	2
	Internal exposure – medium (slow elimination)	3	2	1
	Internal exposure - low (low uptake and/or fast elimination)	2	1	1

NAMs capture chemicals currently treated as green, but based on no or limited information

- Application of the new classification scheme to chemicals in the current low concern group will result in some additional classifications and thus an **overall higher level of protection**



→ Demonstrate safe bioactivity profile

→ Classification & Labelling +
→ Risk assessment

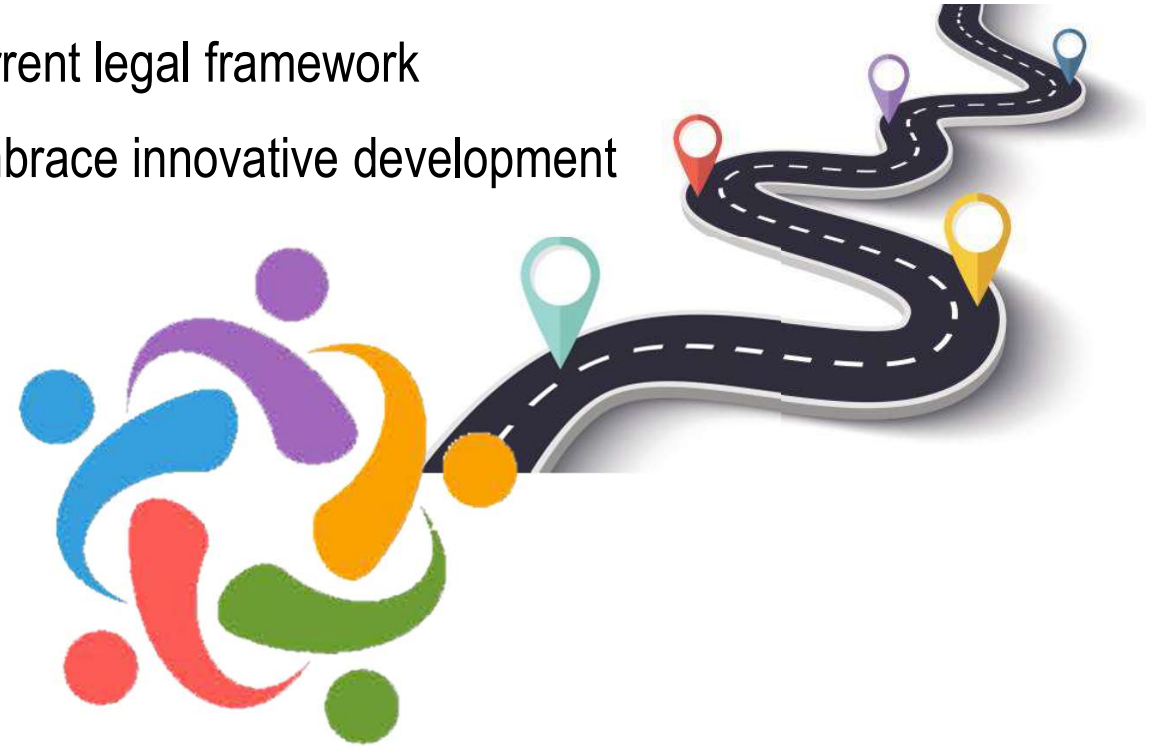
→ Classification & Labelling

Additional considerations

- Less is more – focus on optimal combinations of a limited number of NAMs
- NAMs for C&L should be constrained (highly standardised, widely available methods), allowing for consistency in the assessment and regulatory certainty in the decision
- NAMs for Risk Assessment can be more flexible, allowing companies to develop their own bespoke solutions (including less standardised methods; harmonised guidance on performance characteristics required)
- Multiple pathways to acceptance for standardisation/validation of NAMs

Enhanced collaboration between key-players

- Set goals together with research projects to reach regulatory acceptable NAM solutions
- Consider experience gained in the current legal framework
- Ensure current protection level but embrace innovative development
- Create confidence together
- Work on a common roadmap



Last but not least:

The regulatory science challenge – how to ensure equivalent protection for critical hazards

Design an alternative classification scheme with the following attributes:

- entirely NAM-based classification criteria
- with standardised[1] and readily available NAMs
- optimise testing efficiency through a tiered classification criteria
- define explicit decision rules resulting in classifications supporting risk management measures that ensure equivalent protection to the current system

1) For systemic toxicity

2) For environmental hazards covering:

- bioaccumulation in multiple species (aquatic organisms, birds, mammals)
- aquatic toxicity (acute and chronic) to multiple species
- endocrine disruption

1. Standardised: validated according to technical standards, but not necessarily adopted as an international test guideline

European Commission

Thank you

European Commission

Introducing the ASPIS WG Exposure and Kinetics

Building Confidence in Toxicokinetics Models for Next Generation Risk Assessment

Nynke Kramer | Wageningen University, Wageningen

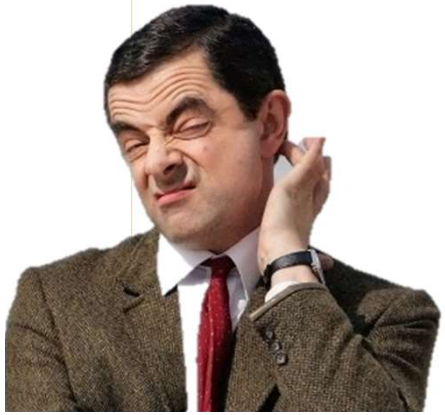
25th November 2022
Sitges, Spain

these projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers precisiontox: 965406, ONTOX: 963845 and Risk-Hunt3r: 964537

Presentation Aim

Can the WG on Exposure and Kinetics contribute to an operational concept of the ASPIS NGRA workflow?

By building confidence in TK models

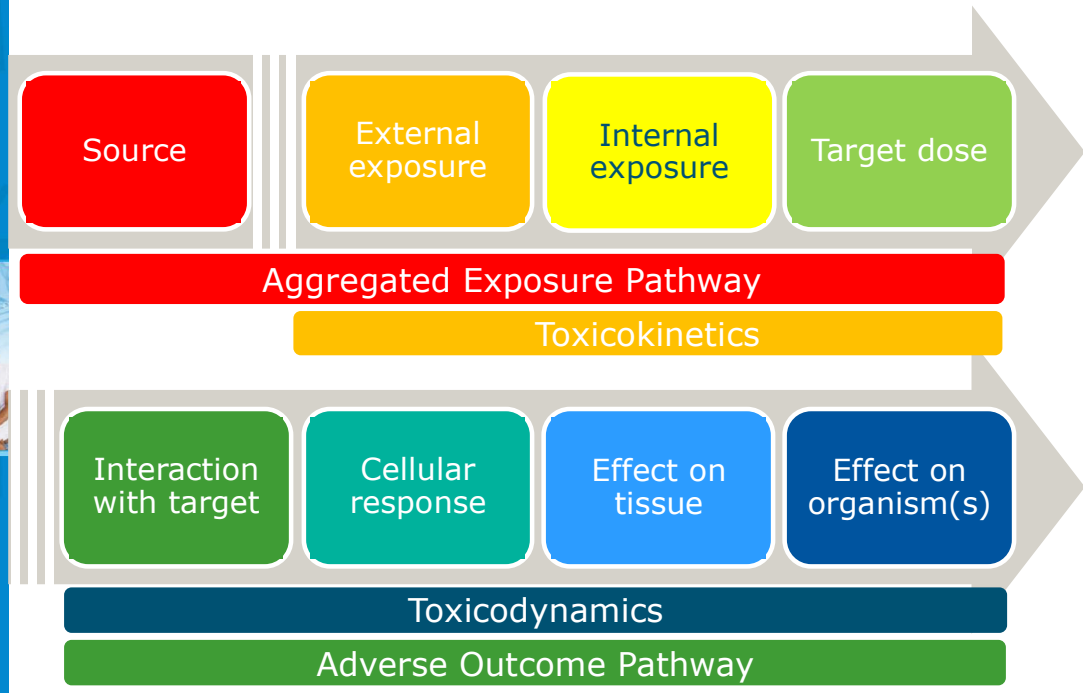


Presentation Outline

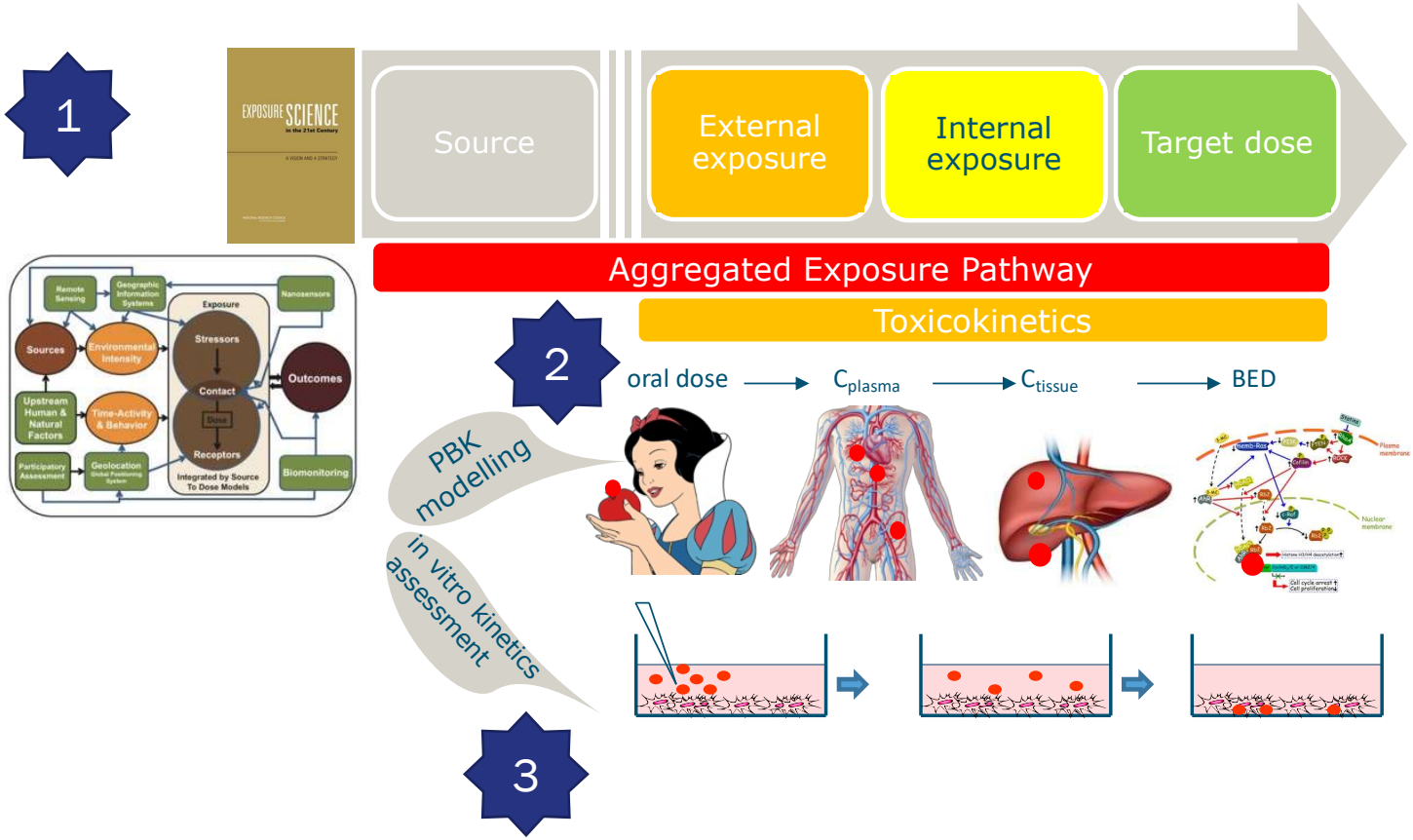
- ASPIS NGRA workflow and role of exposure and kinetics studies
- Objectives of ASPIS WG on Exposure and Kinetics
- 1st case study: Azole fungicide induced liver steatosis
- *In vitro* distribution kinetics in ASPIS
- Conclusions

NGRA


EPA
EPA/600/R-14/004 | September 2014 | www.epa.gov/ncea
Next Generation Risk Assessment:
Incorporation of Recent Advances in Molecular, Computational, and Systems Biology
Final Report
Exposure-led, hypothesis-driven approach to support safety decision-making using *in vitro* and *in silico* approaches
National Center for Environmental Assessment
Office of Research and Development

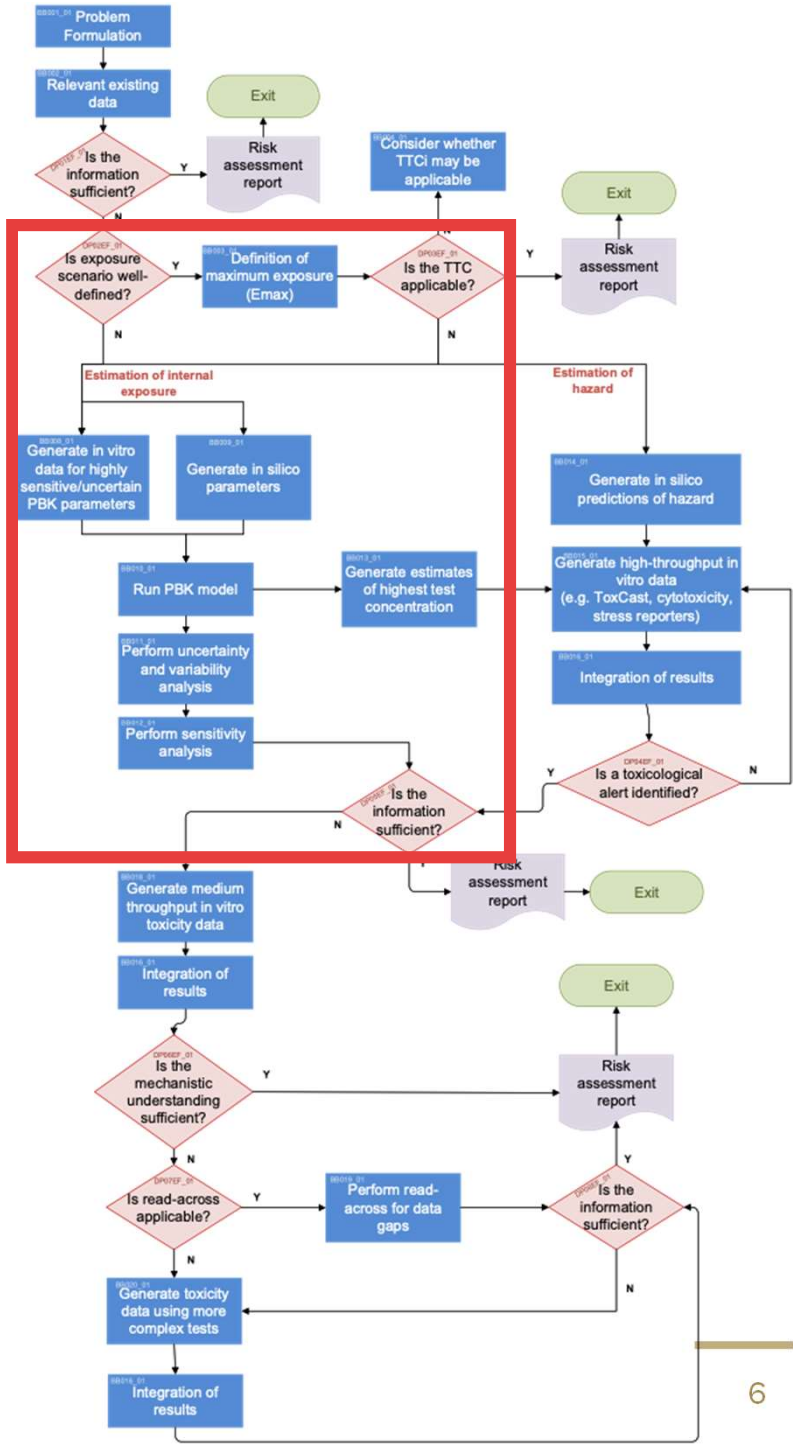

Source
External exposure
Internal exposure
Target dose
Aggregated Exposure Pathway
Toxicokinetics
Interaction with target
Cellular response
Effect on tissue
Effect on organism(s)
Toxicodynamics
Adverse Outcome Pathway

Exposure and Toxicokinetics in NGRA



ASPIS NGRA Workflow

- Deliver an operational concept for users to employ
- Generic workflow
- Considers all existing information
- Follows a tiered and iterative approach
- Based on robust and relevant NAMs
- Validated in case studies
- Step-by-step instructions
- Parameterise decision points
- Quantifies uncertainty



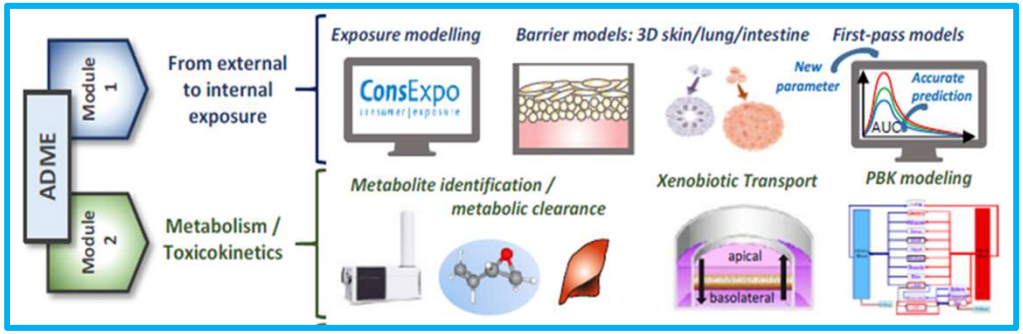
Main Objective of WG Exposure & Kinetics

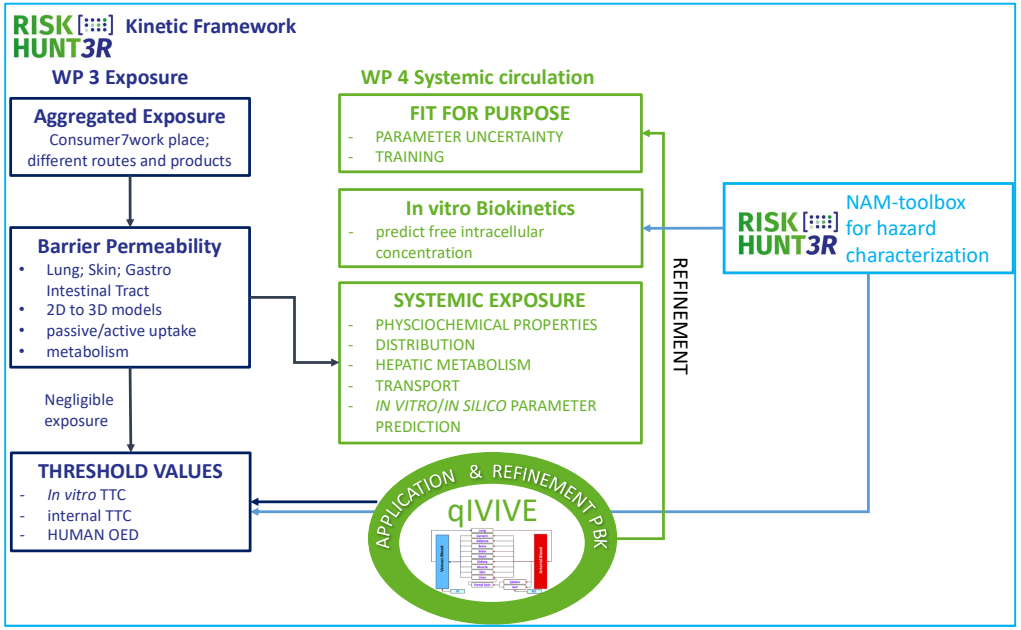
Develop a generic, operational guideline for risk assessors wishing to use (1) exposure, (2) *in vitro* distribution kinetics, and (3) PBK models to (A) integrate with TTC approach, (B) design hazard estimation approach and (B) perform QIVIVE for NGRA

- Define and integrate complementary, robust and relevant NAMs for exposure/TK assessment developed by WG members

Exposure and Kinetics in RiskHunt3R

- Prediction of aggregate exposure
- Systemic uptake of chemicals for DNT and non-genotoxic carcinogens assessment
- Applicability and variability of *in vitro* ADME tools; impact on PBK model parameterization
- Strategy for dealing with metabolites
- *In vitro* TTC derivation

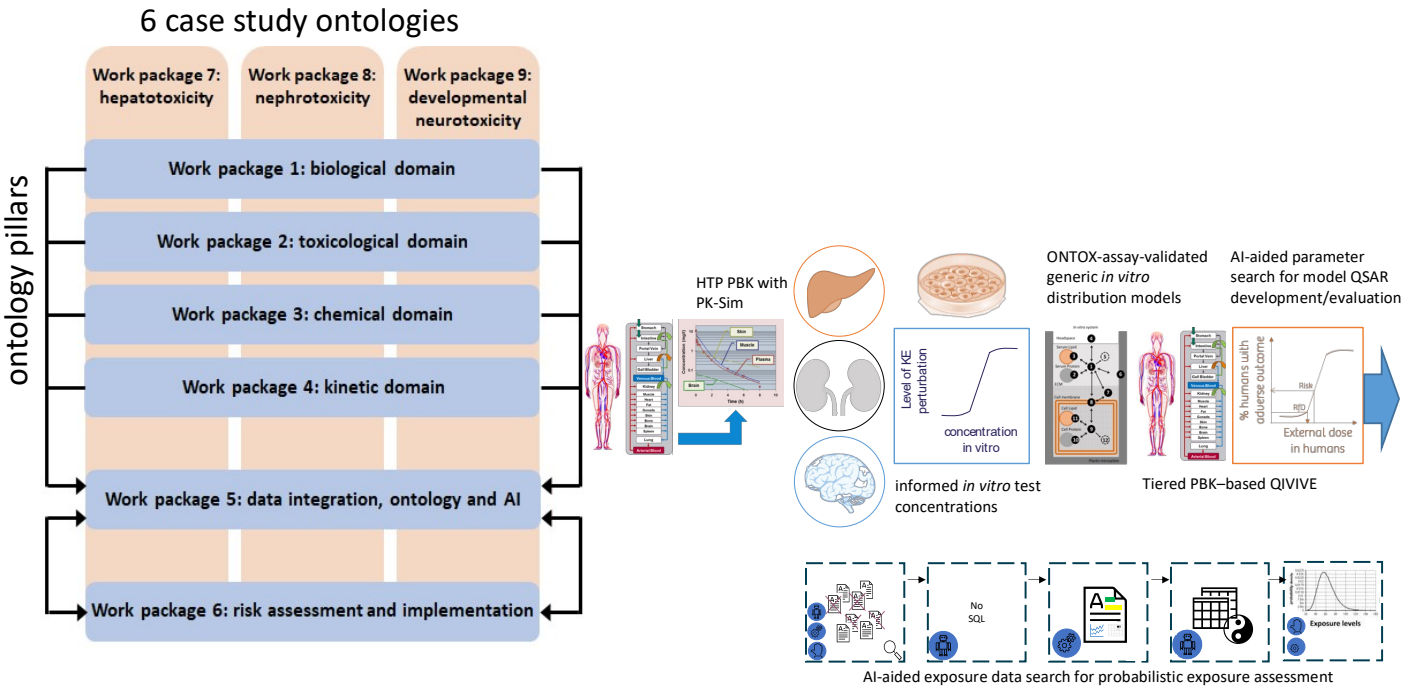




Exposure and Kinetics in ONTOX

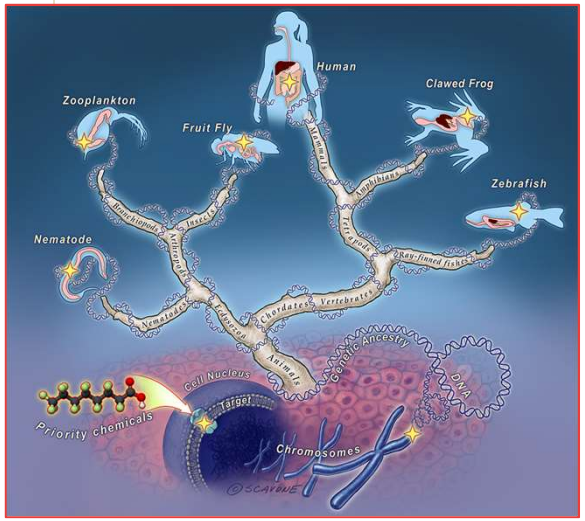
WP4: Gap analysis of chemical (*i.e.,* chemical space, logP, CL_{int}) and biological (*i.e., in vitro* system setup, PBK tissue structure) applicability domains of *in vitro* distribution kinetic models (*e.g.,* VCBA) and PK-Sim for QIVIVE of chemicals which NAMs in WP7-9 identify as inducing liver steatosis, cholestasis, tubular necrosis, crystallopathy, neural tube closure and cognitive function defects.

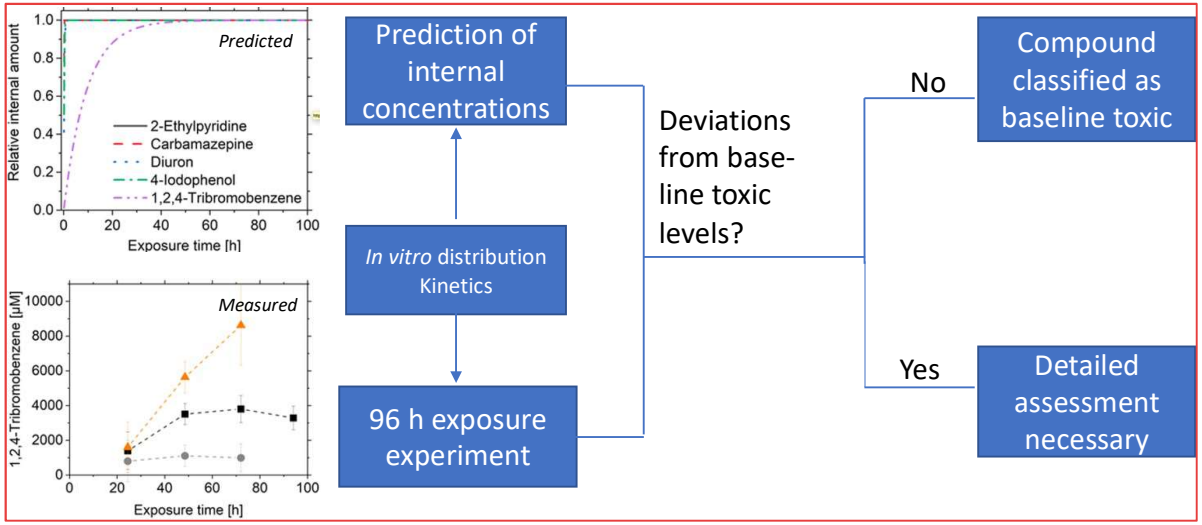
WP6: Population-based exposure assessment of ontology-relevant chemicals by using systematic literature review AI tools to identify existing exposure estimates and biomonitoring data and use existing concentration databases will be used to make a broad exposure assessment



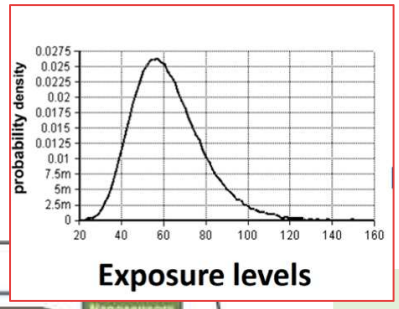
Exposure and Kinetics in PrecisionTox

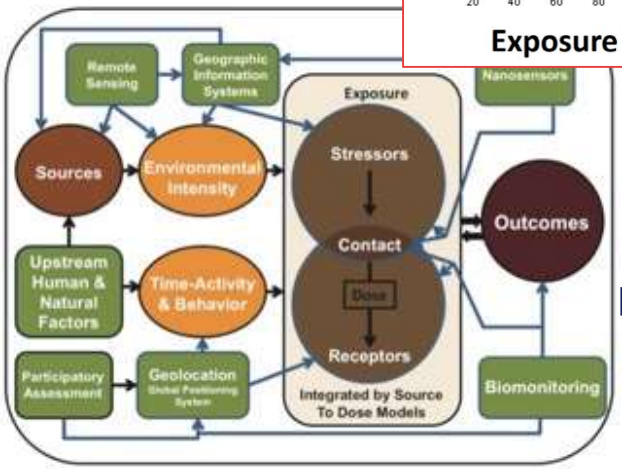
No explicit exposure and kinetics modelling is foreseen, but (1) omics will identify chemicals inducing metabolic enzyme induction and (2) distribution kinetics of chemicals are experimentally determined after exposure in zebrafish and cell bioassays. If measured and modelled in vitro concentrations significantly differ, kinetic profiles and metabolites will be identified (Halbach et al.2020 Environ Sci Technol 54, 10159-69).



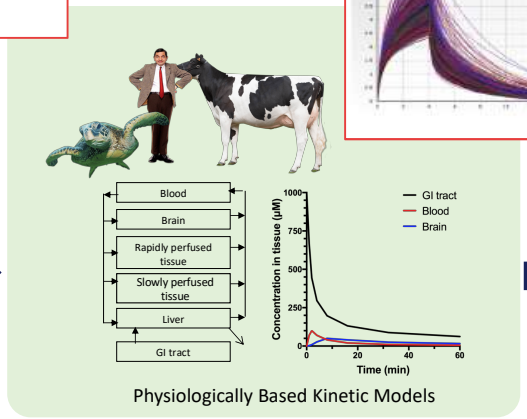


Exposure and Kinetics Workflow

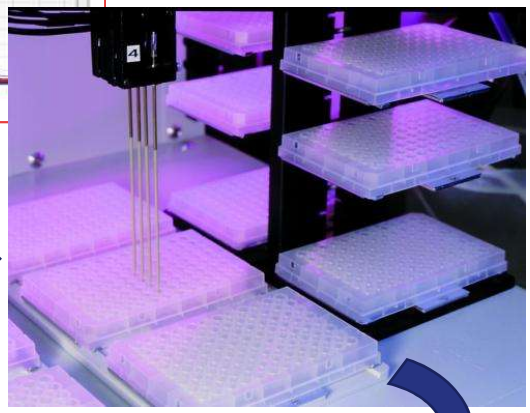


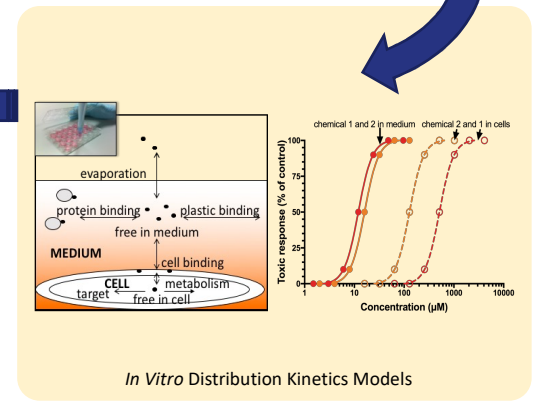


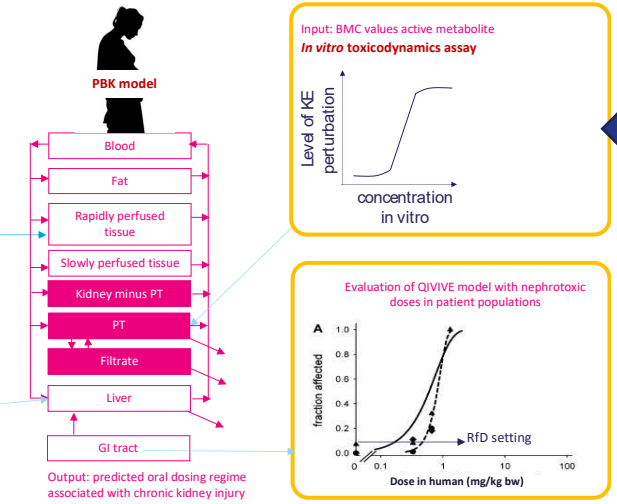
TTC



TTC







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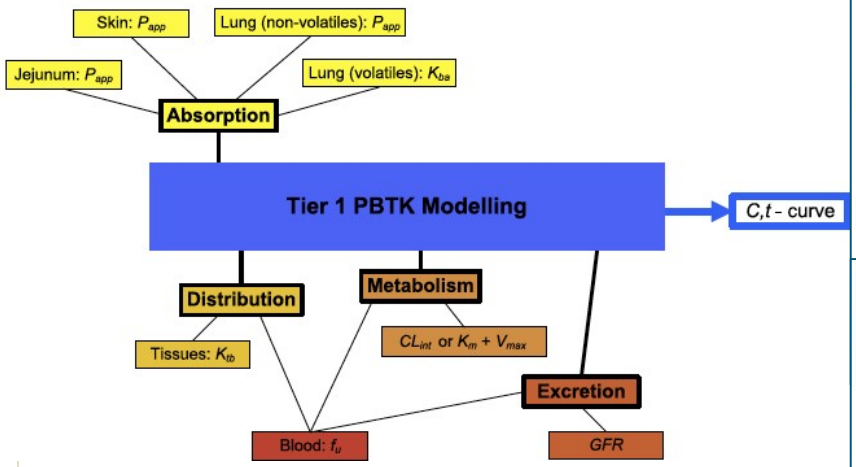
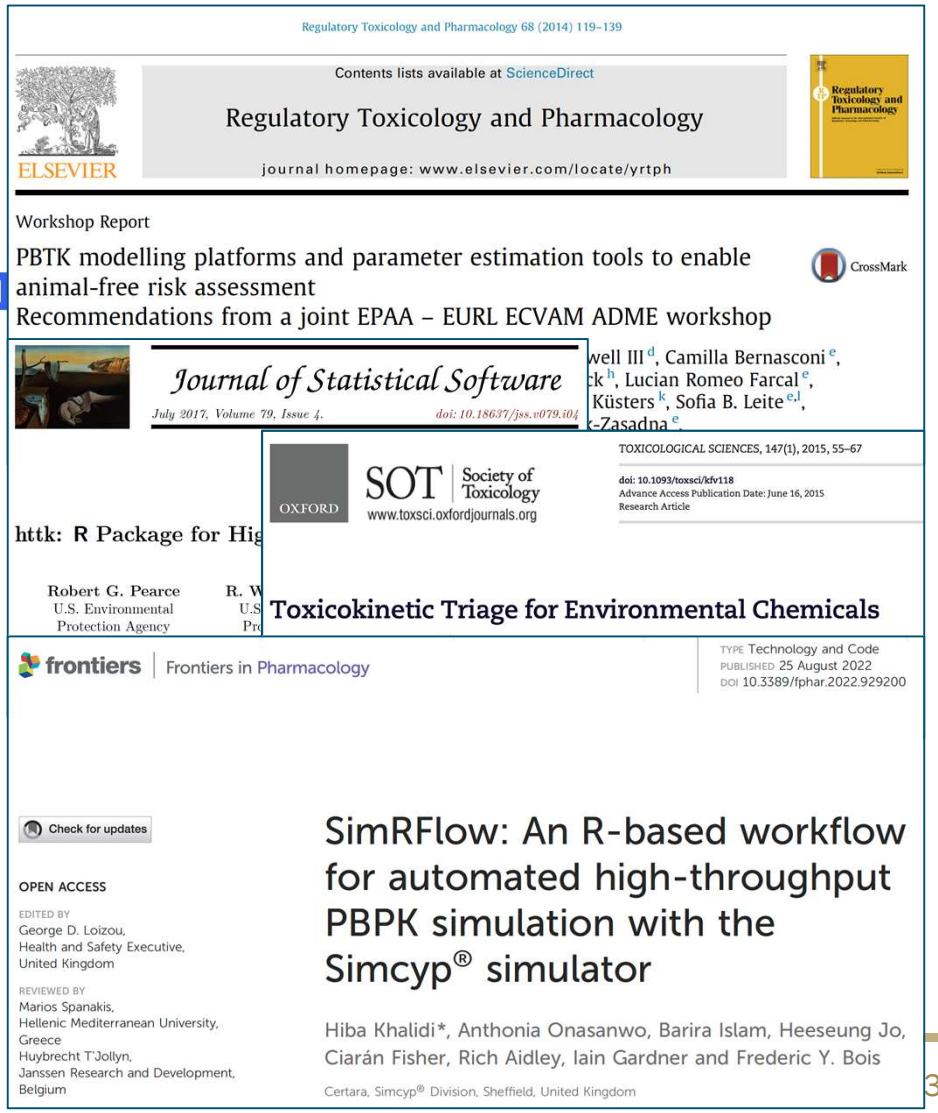
- Define and integrate complementary, robust and relevant NAMs for exposure/TK assessment developed by WG members
- Apply a tiered and iterative approach to exposure and kinetics assessment quantifying uncertainty/variability

Tiered Approach in PBK Modelling



- Tier dependent on availability of kinetic data and acceptable uncertainty
- E.g.: 1st tier: Assume F=1, renal excretion = GFR, QSPR-derived Pt, fu & *in vitro* derived non-saturable fu, CL_{int}


Regulatory Toxicology and Pharmacology 68 (2014) 119–139

Contents lists available at ScienceDirect

Regulatory Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/yrtph

Workshop Report

PBTK modelling platforms and parameter estimation tools to enable animal-free risk assessment
Recommendations from a joint EPAA – EURL ECVAM ADME workshop

Journal of Statistical Software

July 2017, Volume 79, Issue 4. doi: 10.18637/jss.v079.i04

httk: R Package for Hig

Robert G. Pearce
U.S. Environmental Protection Agency

TOXICOLOGICAL SCIENCES, 147(1), 2015, 55–67

doi: 10.1093/toxsci/kfv118
Advance Access Publication Date: June 16, 2015
Research Article

Toxicokinetic Triage for Environmental Chemicals

frontiers | Frontiers in Pharmacology

TYPE Technology and Code
PUBLISHED 25 August 2022
DOI 10.3389/fphar.2022.929200

Check for updates

OPEN ACCESS

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Janssen Research and Development, Belgium

SimRFlow: An R-based workflow for automated high-throughput PBPK simulation with the Simcyp® simulator

Hiba Khalidi*, Anthonia Onasanwo, Barira Islam, Heeseung Jo, Ciarán Fisher, Rich Aidley, Iain Gardner and Frederic Y. Bois

Certara, Simcyp® Division, Sheffield, United Kingdom

1st Tier Model Evaluation

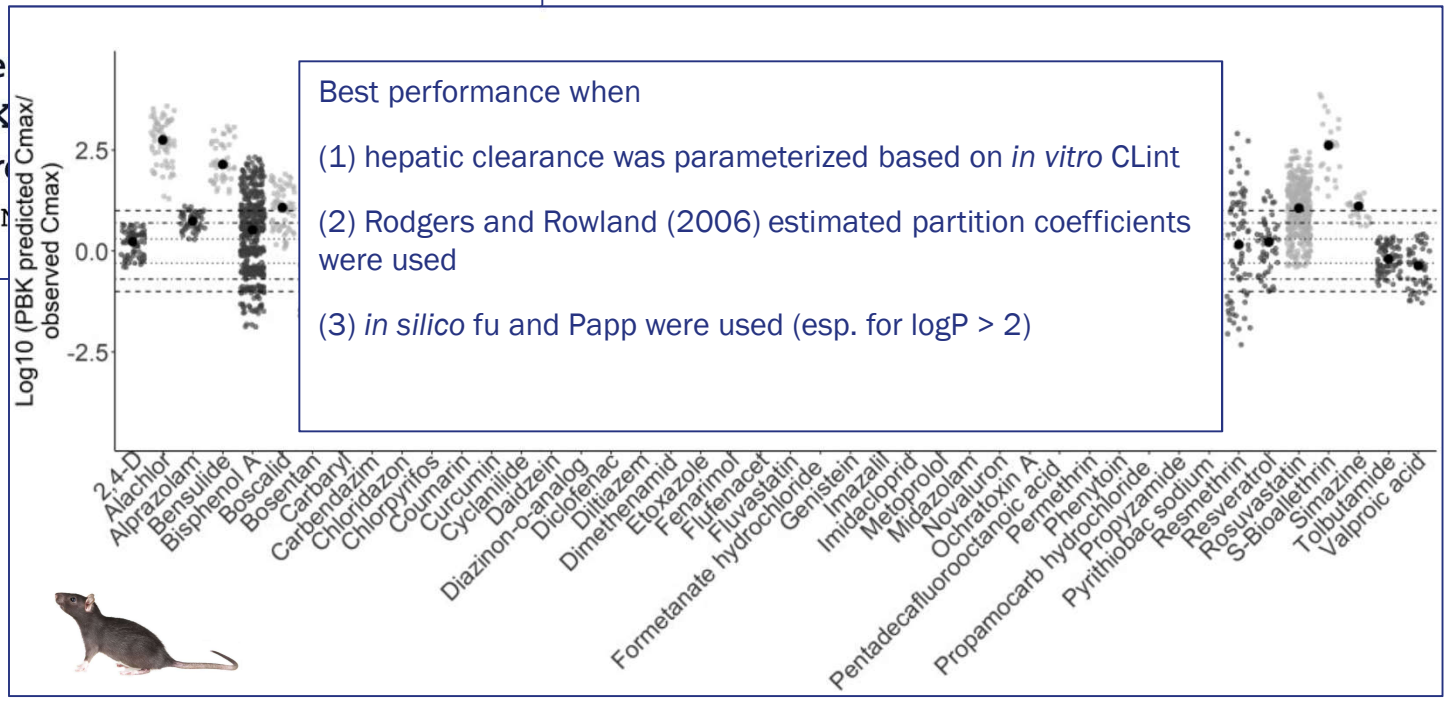
OXFORD SOT Society of Toxicology
academic.oup.com/toxsci

TOXICOLOGICAL SCIENCES, 186(1), 2022, 18–28
https://doi.org/10.1093/toxsci/kfab150
Advance Access Publication Date: 20 December 2021
Research article

Predictive Performance
Physiologically Based K
in Rats Based on *In Vitr*

Ans Punt [*,1] Jochem Louisse,* N
Bennard van Ravenzwaay†

Predicted median Cmax of 32/44 chemicals predicted within 10-fold of observed Cmax, remainder were over-estimated → Identify & characterise reasons for outliers



Best performance when

(1) hepatic clearance was parameterized based on *in vitro* CLint

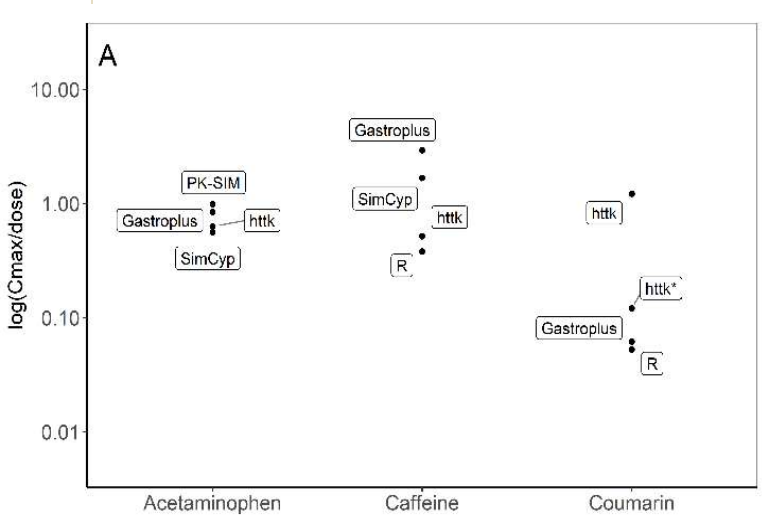
(2) Rodgers and Rowland (2006) estimated partition coefficients were used

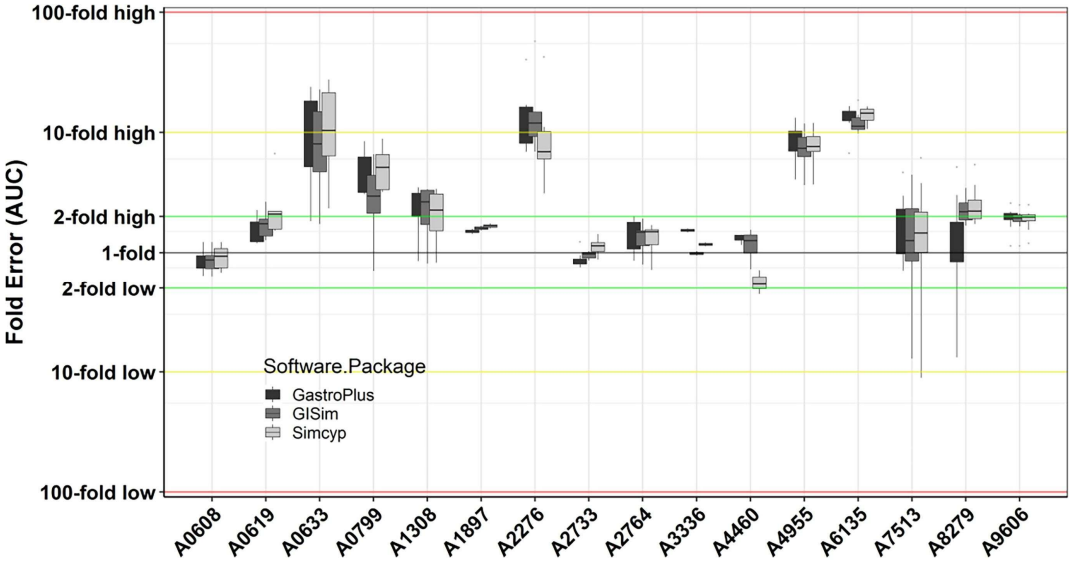
(3) *in silico* fu and Papp were used (esp. for logP > 2)

NG-PBK Challenge

Relies solely on in vitro or in silico methods, reflects detailed mechanistic understanding of biology but is accompanied by more “unfamiliar uncertainties” (uncertainty relating to the relevance, reliability and variability of the *in vitro* and *in silico* methods from which model parameters are generated).

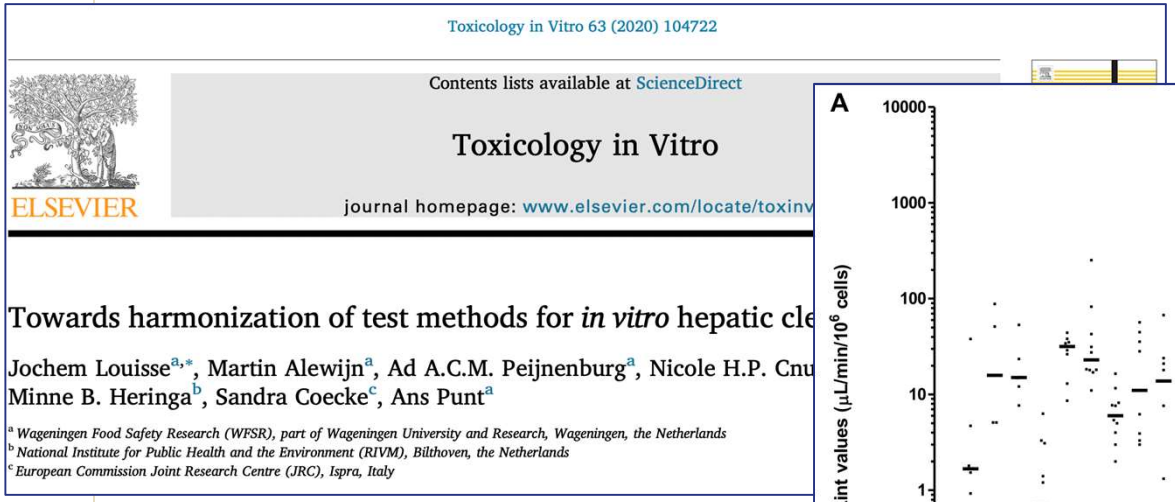
Predictive performance differences not related to software but choice of input data (Ahmad et al. 2020 E J Phamaceutics Biopharmaceutics 156, 5-63, Najjar et al. 2022 Arch Toxicol. in press)





ID parameter variability/uncertainty

Work towards documentation and harmonisation of protocols (Krebs et al. 2020 Arch Toxicol. 94, 2435-61).



Toxicology in Vitro 63 (2020) 104722

Contents lists available at ScienceDirect

Toxicology in Vitro

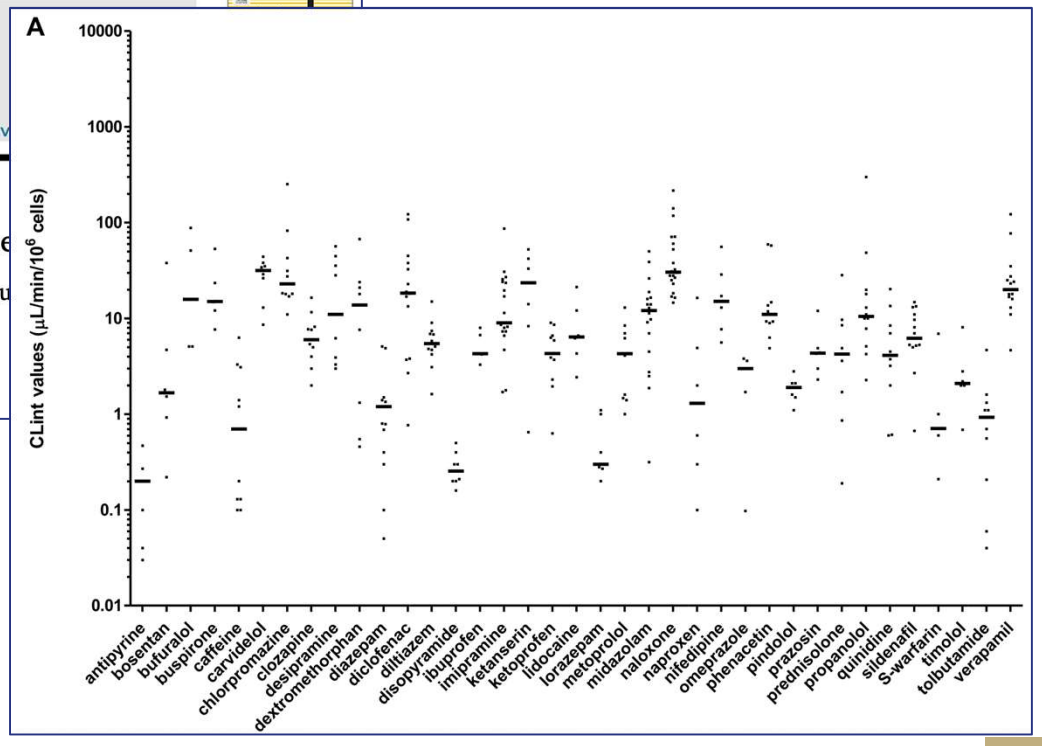
journal homepage: www.elsevier.com/locate/toxinv

ELSEVIER

Towards harmonization of test methods for *in vitro* hepatic cle

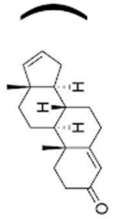
Jochem Louisse[a,*], Martin Alewijn[a], Ad A.C.M. Peijnenburg[a], Nicole H.P. Cnu
Minne B. Heringa[b], Sandra Coecke[c], Ans Punt[a]

[a] Wageningen Food Safety Research (WFSR), part of Wageningen University and Research, Wageningen, the Netherlands
[b] National Institute for Public Health and the Environment (RIVM), Bilthoven, the Netherlands
[c] European Commission Joint Research Centre (JRC), Ispra, Italy



- CLint in rat and human hepatocytes differ more than an order of magnitude.
- A random forest regression analysis points to effect of cell concentration and culture medium on CLint values.

Evaluate Applicability Domains of QSPR/ *In Vitro* Models for PBK Parameterisation



Papp

- Poulin & Krishnan (1996) TAP 136, 126 and Meulenberg & Vijverberg (2000) TAP 165, 206, for K_{ba} for rats and humans
- Hou et al. (2004) J. Chem. Inf. Comput. Sci. 44, 1585, for Caco-2 permeation

fu

- Lobell & Sivarajah (2003) Mol. Diversity 7, 69
- Yun et al (2021) Comp. Toxicol. 17, 100142 → compared models, human fu high PPB is overpredicted

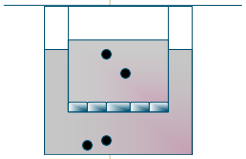
Pt

- DeJongh et al. (1997) Arch. Toxicol. 72, 17
- Berezhkovskiy (2004) J Pharm Sci 93:1628–1640
- Rogers & Rowlands (2006) J. Pharm. Sci. 95, 1238

CLint

- Because Clint is sensitive parameter and isoform dependent, general CLint models not used
- Dawson et al. (2021) Environ. Sci. Technol. 55, 6505: httk with CLint from ML algorithms produced similar bioactivity/exposure ratios as with in vitro CLint

Read-across (Lu et al. 2016 PLOS Comp. Toxicol. doi: 10.1371/journal.pcbi.1004495)



Papp

- Dermal: OECD TG 428, Skin PAMPA; Inhalation: Calu-3
- Oral: PAMPA, MDCK, **Caco-2**, intestinal microsomes/ S9, HT-29 co-cultures, organoids, EpiIntestinal™, intestine-on-a-chip

fu

- **Rapid equilibrium dialysis**, ultrafiltration, ultracentrifugation, chromatography, solid phase microextraction

Tissue permeability

- Barriers incl. BBB and placental barrier
- Similar to intestinal Papp models (incl. Caco-2)
- BeWo cells for placenta, human cord blood-derived hematopoietic stem cells (BLEC) for BBB, iPSC

Clint, Km, Vmax

- Human liver/insteinal microsomes, S9 fraction, (cryopreserved) **primary hepatocytes**, HepaRG, recombinant enzymes
- Transporter transfected cell lines, primary RPTEC, cell lines incl. RPTEC and Caco-2, iPSC
- Suspension, plated, **sandwich**, spheroid and organ-on-a-chip cultures

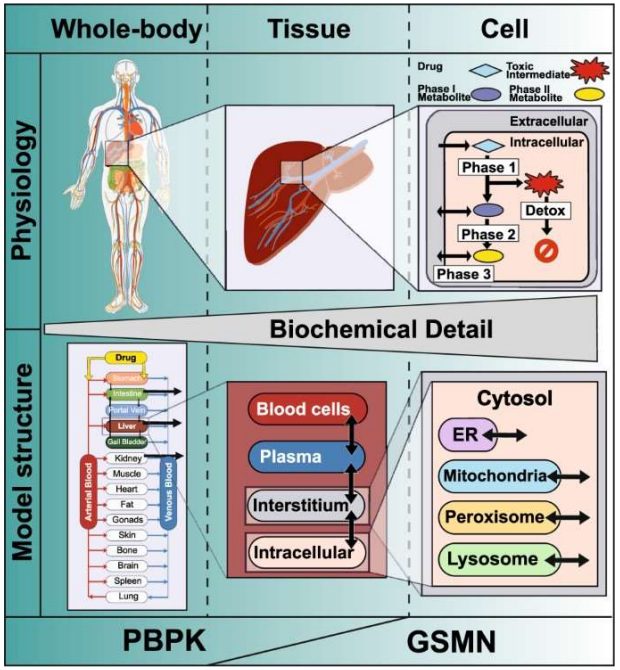
Main Objective of WG Exposure & Kinetics

Develop a generic, operational guideline for risk assessors wishing to use (1) exposure, (2) *in vitro* distribution kinetics, and (3) PBK models to (A) integrate with TTC approach, (B) design hazard estimation approach and (B) perform QIVIVE for NGRA

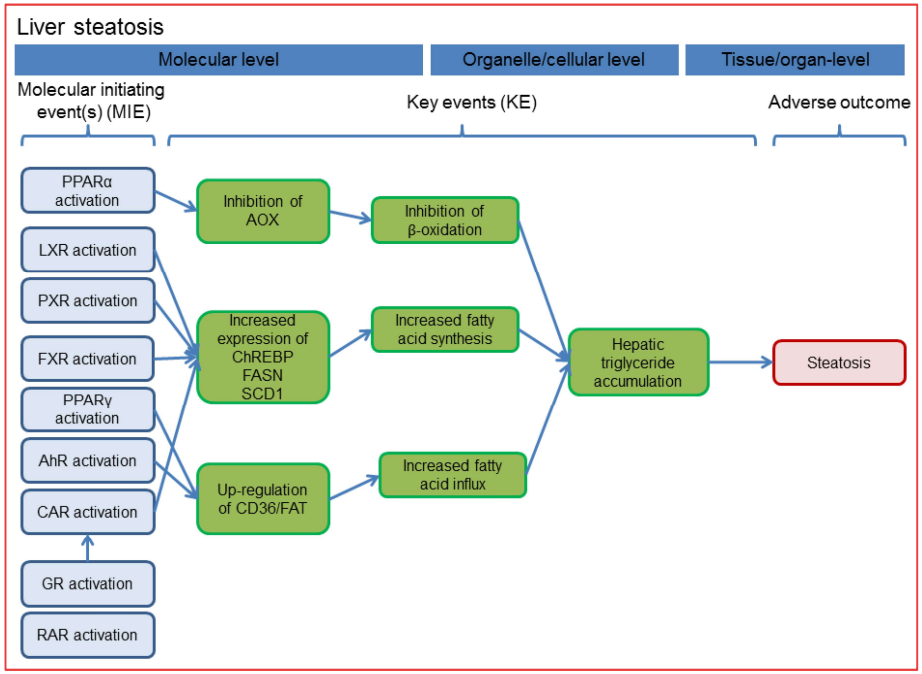
- Define and integrate complementary, robust and relevant NAMs for exposure/TK assessment developed by WG members
- Apply a tiered and iterative approach to exposure and kinetics assessment quantifying uncertainty/variability
- Evaluate it with common case study chemicals

Aim of ASPIS Steatosis Case Study

- Fat deposition in hepatocytes
- Repeat dosing, detailed AOP
- Solved through integration ASPIS NAMs



Cordes et al. 2018 Systems Biol. Applications 4,10



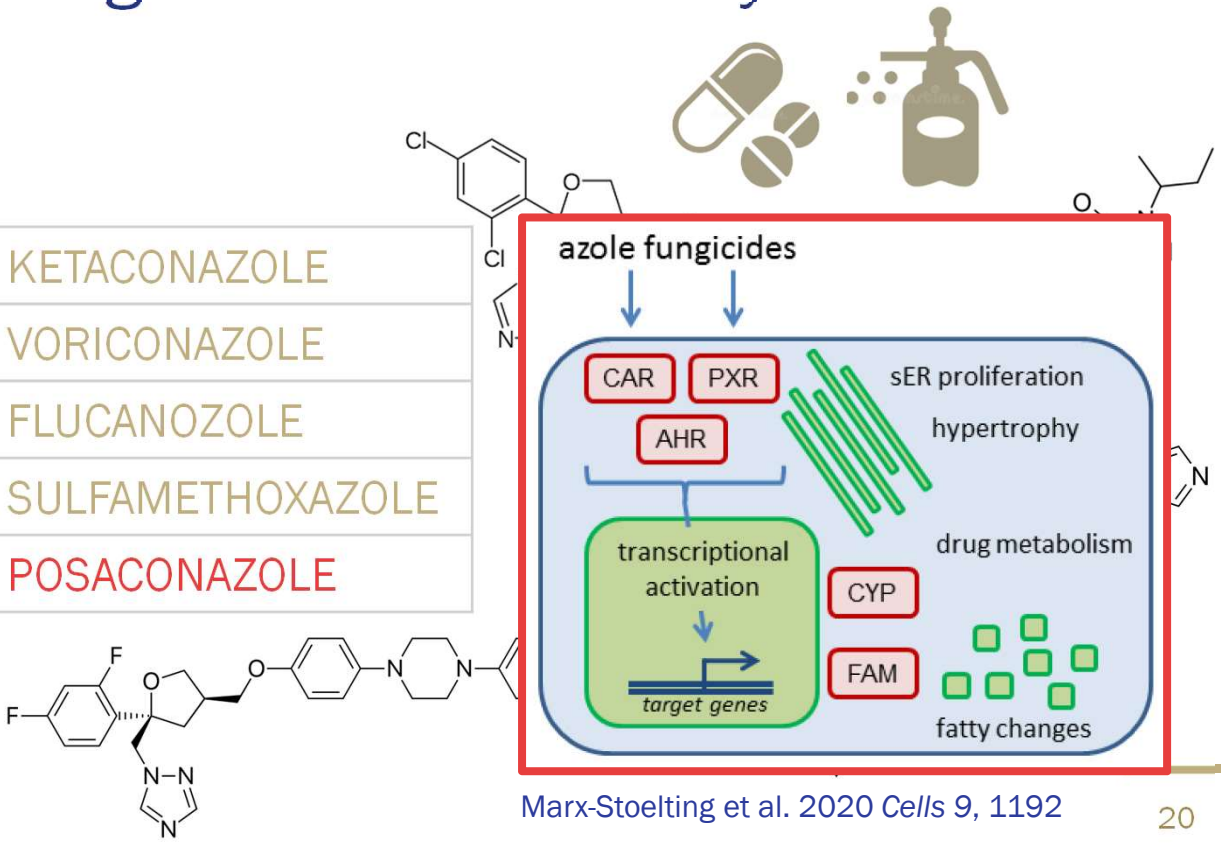
Marx-Stoelting et al. 2020 Cells 9, 1192

Case Study Chemical Selection

- Only overlapping chemical between 3 ASPIS projects: valproic acid
- Propose azole fungicides as case study instead

RiskHunt3R chemical
ONTOX chemical
Additionally proposed

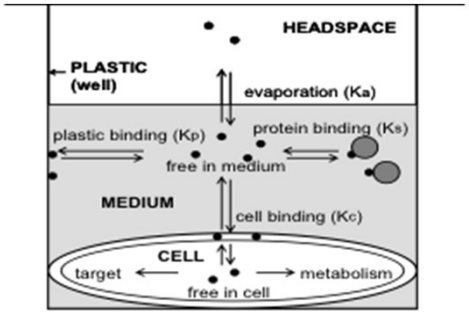
PROPICONAZOLE	KETACONAZOLE
DIFENOCONAZOLE	VORICONAZOLE
PROTHIOCONAZOLE	FLUCANOZOLE
FENBUCONAZOLE	SULFAMETHOXAZOLE
EPOXICONAZOLE	POSACONAZOLE
ITRACONAZOLE	
CYPROCONAZOLE	

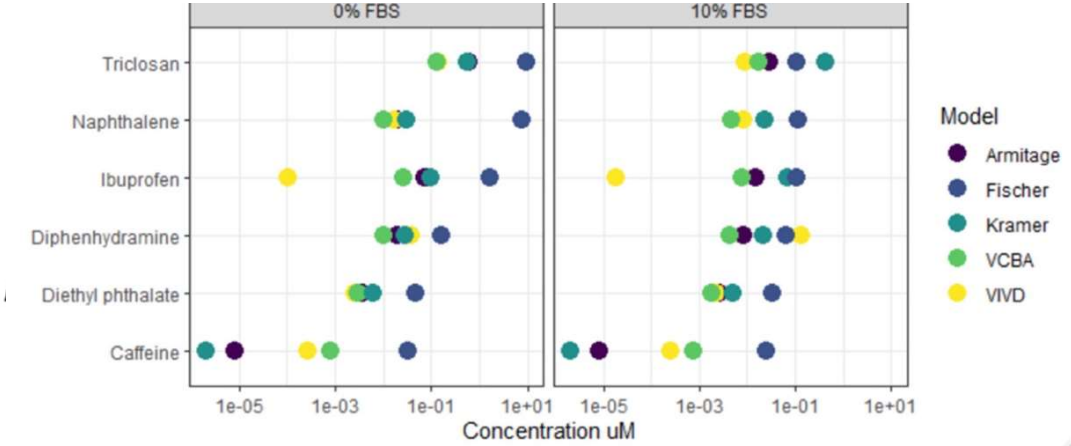


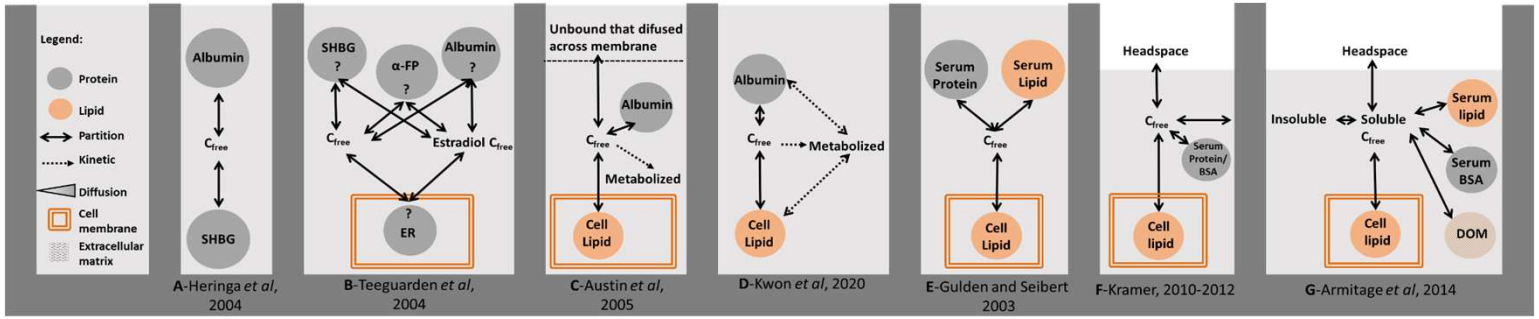
Marx-Stoelting et al. 2020 *Cells* 9, 1192

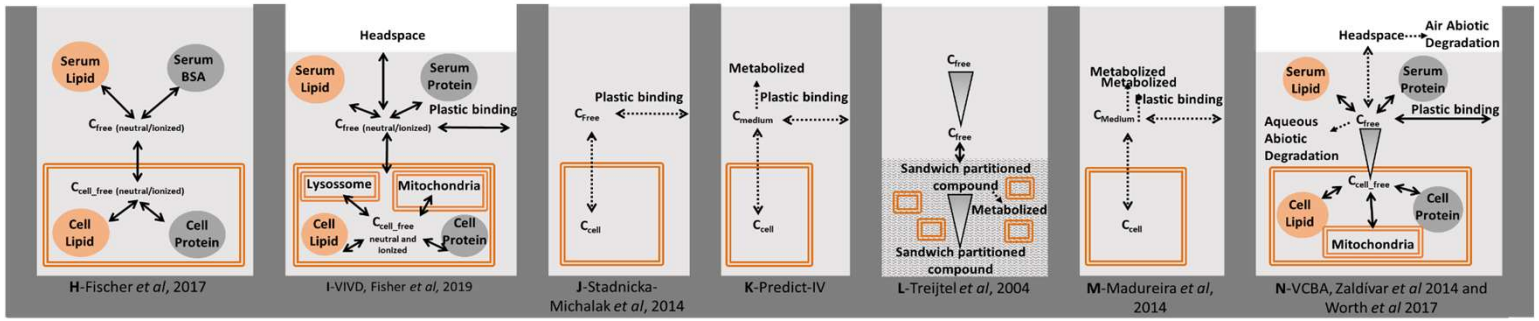
Simulating *In Vitro* Distribution Kinetics

cell-associated concentration in HepaRG cytotoxicity assay







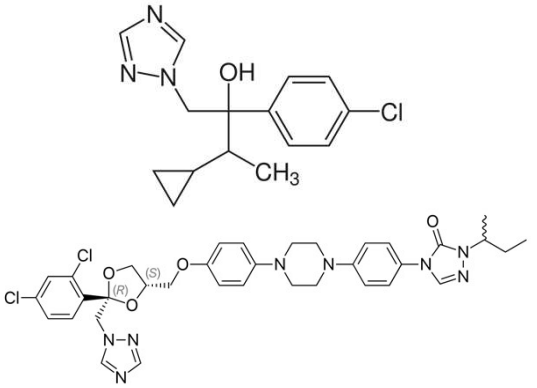


Proenca et al. (2021) Toxicology in Vitro 73, 105133

In Vitro Distribution Kinetics of Azoles

Cyproconazole in Kramer et al. (2010) model in 2 ONTOX assays

Assay	HepaRG	hNPC	Itraconazole
Modeled free fraction in medium	16%	92%	1%
Modeled fraction bound to serum constituents	84%	2%	0%
Modeled fraction in cells	0%	2%	74%
Modeled fraction on plastic	0%	4%	25%
Modeled fraction in headspace	0%	0%	0%
Modeled fraction in medium	99%	94%	1%
Modeled total fraction in well (mass balance)	100%	100%	100%
Free concentration in medium (uM)	0,16	0,92	0,01
Concentration in cells (nmol/million cells)	0,01	0,10	3,45



Partition coefficients of cyproconazole across models

Log Ks (log L/kg)	Model
2,43	Armitage et al. (2014)
3,67	Fischer et al. (2017)
3,78	Kramer et al. (2010)

Conclusions

- ASPIS projects are developing and evaluating overlapping NAMs for exposure and kinetics assessment
- ASPIS projects have complementary approaches for integrating exposure and kinetics data in NGRA
- WG aims to develop step-by-step, generic, tiered and iterative (…) guideline for users to apply and integrate exposure, *in vitro* distribution kinetics, and PBK models and associated ADME parameter estimation tools for NGRA
- Azole fungicide induced liver steatosis suggested as first case study to design guideline
- Suggestions to improve WG approach welcome

Thank You for Listening

Questions are always welcome!

Pharmacoinformatics Research Group
Department of Pharmaceutical Sciences

universität wien

Structure-based Prediction of Molecular Initiating Events

Where are we?

Gerhard F. Ecker

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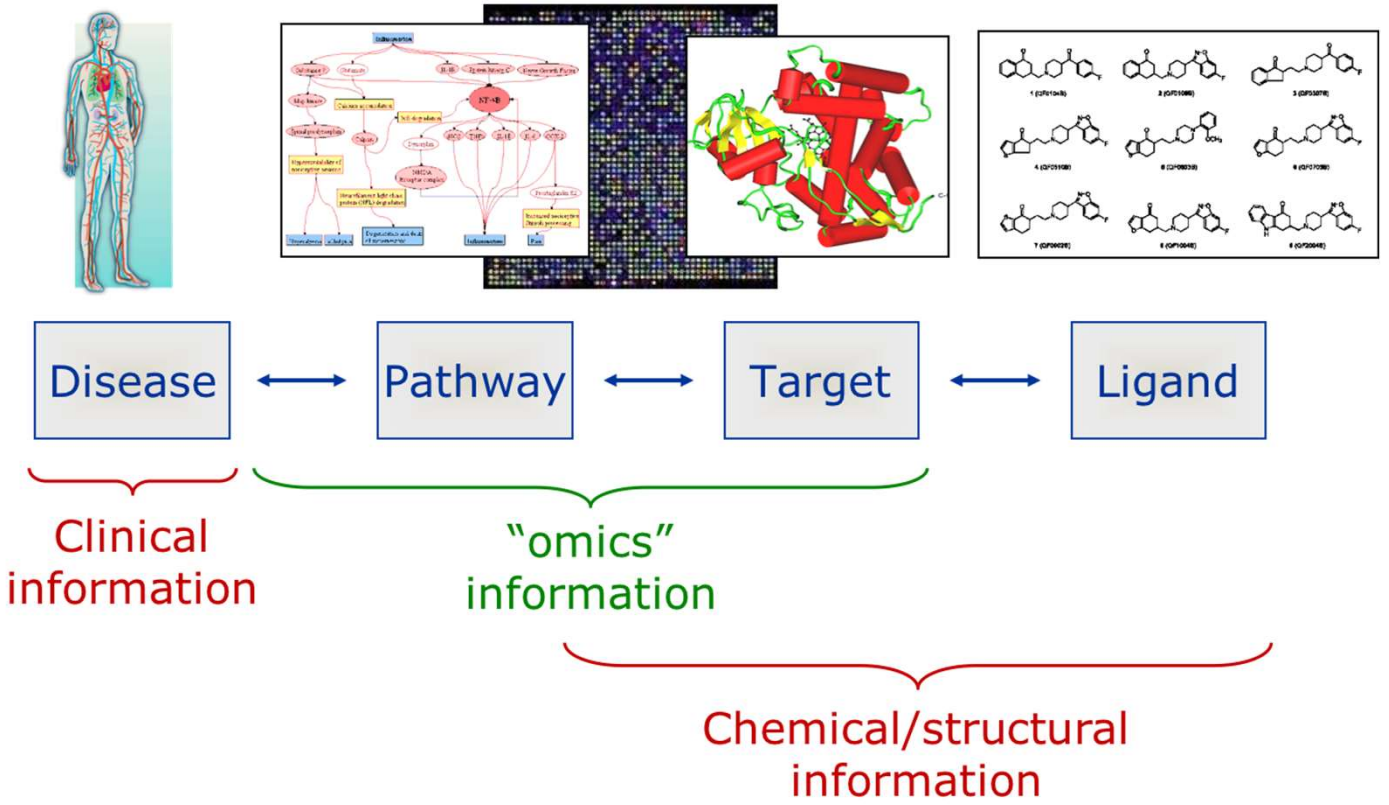
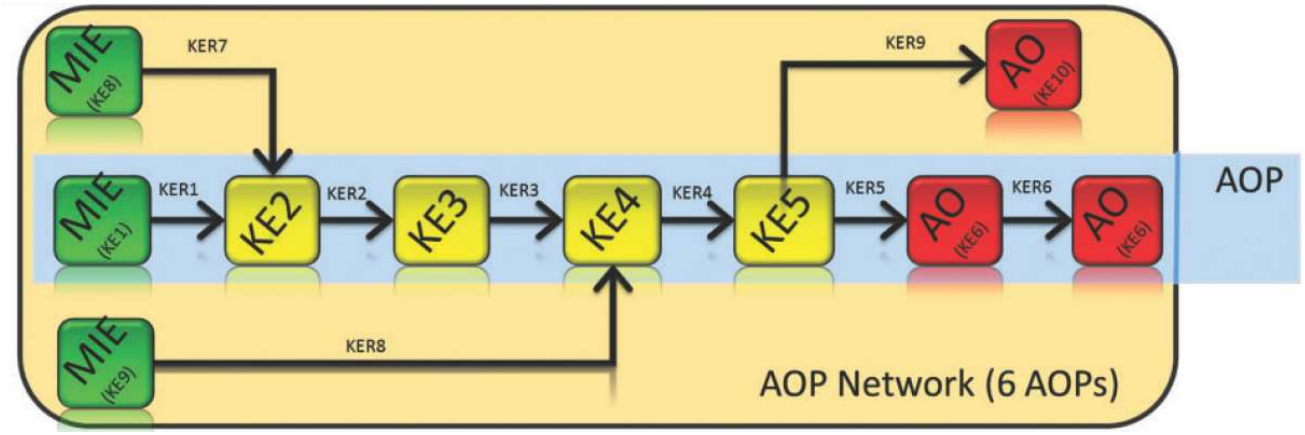
pharminfo.univie.ac.at

The Modeling Approach

- 2D-QSAR
- 3D-QSAR
- Pharmacophore
- Docking
- Machine Learning
- Deep Learning



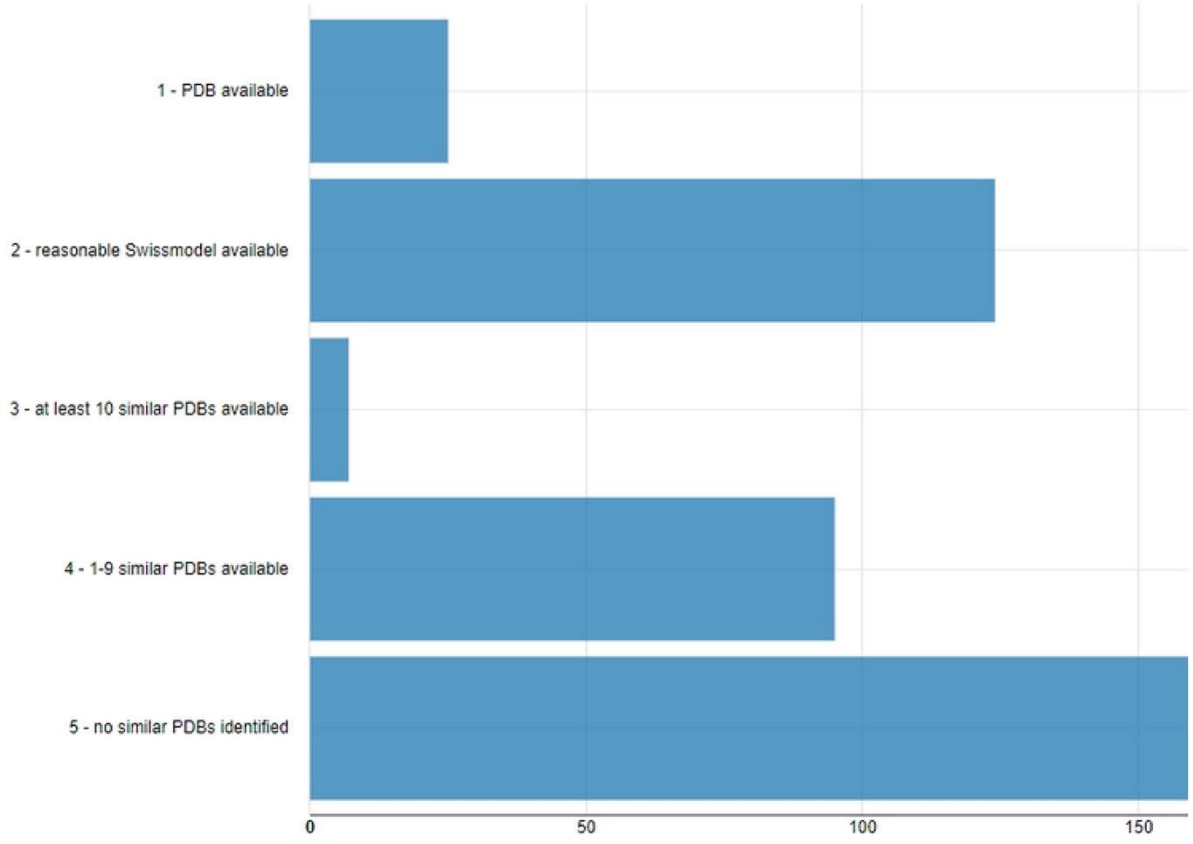
Molecular Initiating Events



AOP network:
more comprehensive, integrated, and biologically realistic synthesis of available knowledge.

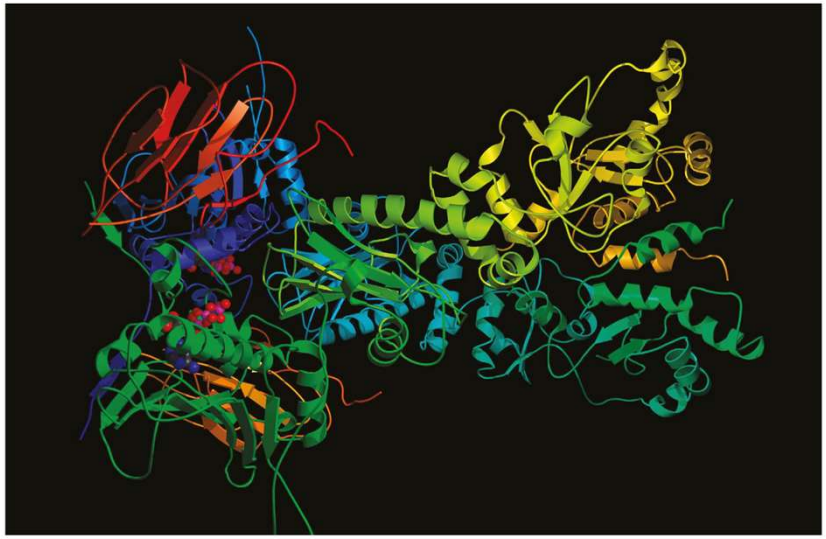
AOP (network) helps identifying the key MIE(s): informs the development of **QSAR models** and chemical categories useful for **defining the chemical space** for which the AOP is relevant

SLC-transporter and AlphaFold



The world this week

News in focus

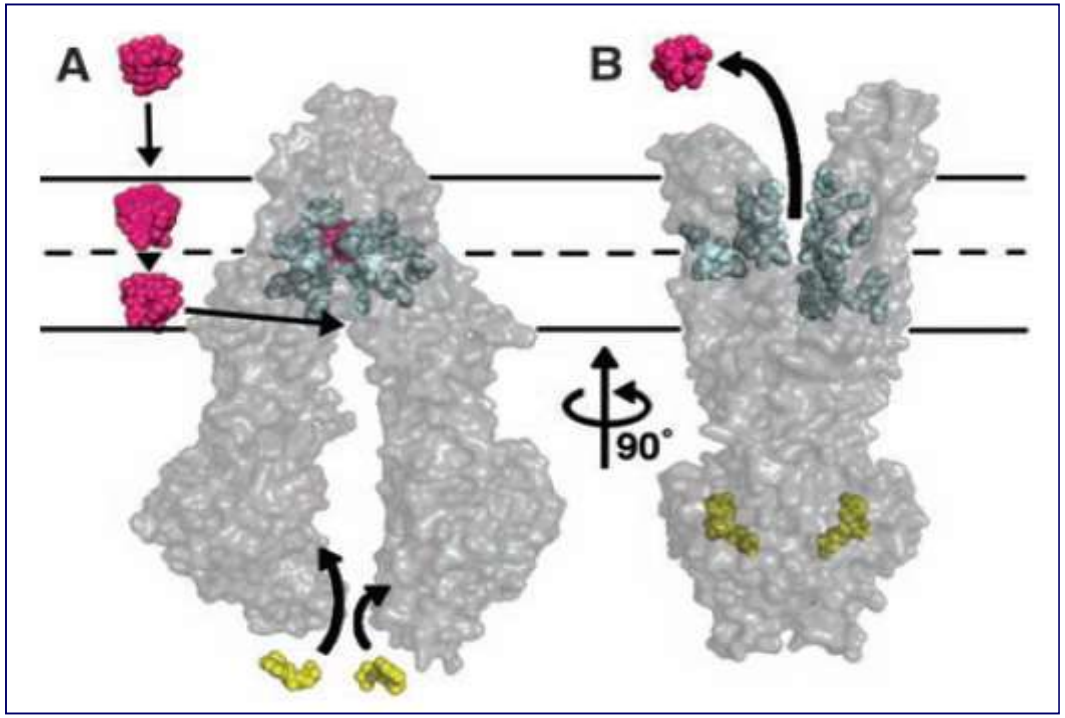


A protein's function is determined by its 3D shape.

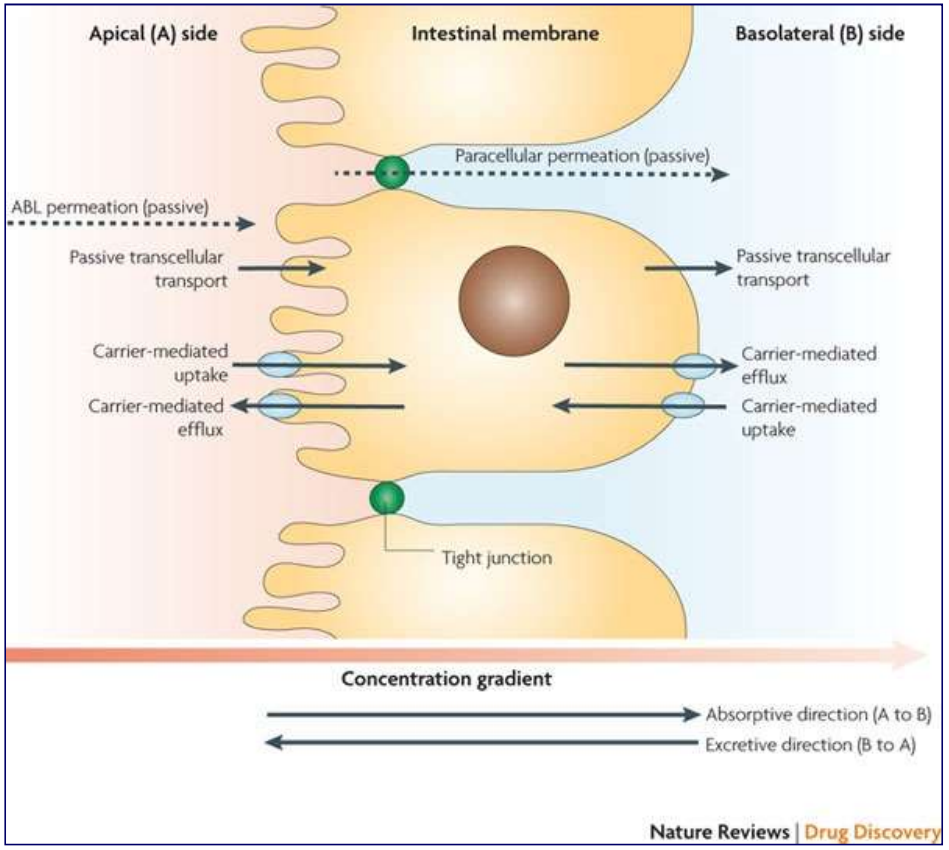
'IT WILL CHANGE EVERYTHING': AI MAKES GIGANTIC LEAP IN SOLVING PROTEIN STRUCTURES

DeepMind's program for determining the 3D shapes of proteins stands to transform biology, say scientists.

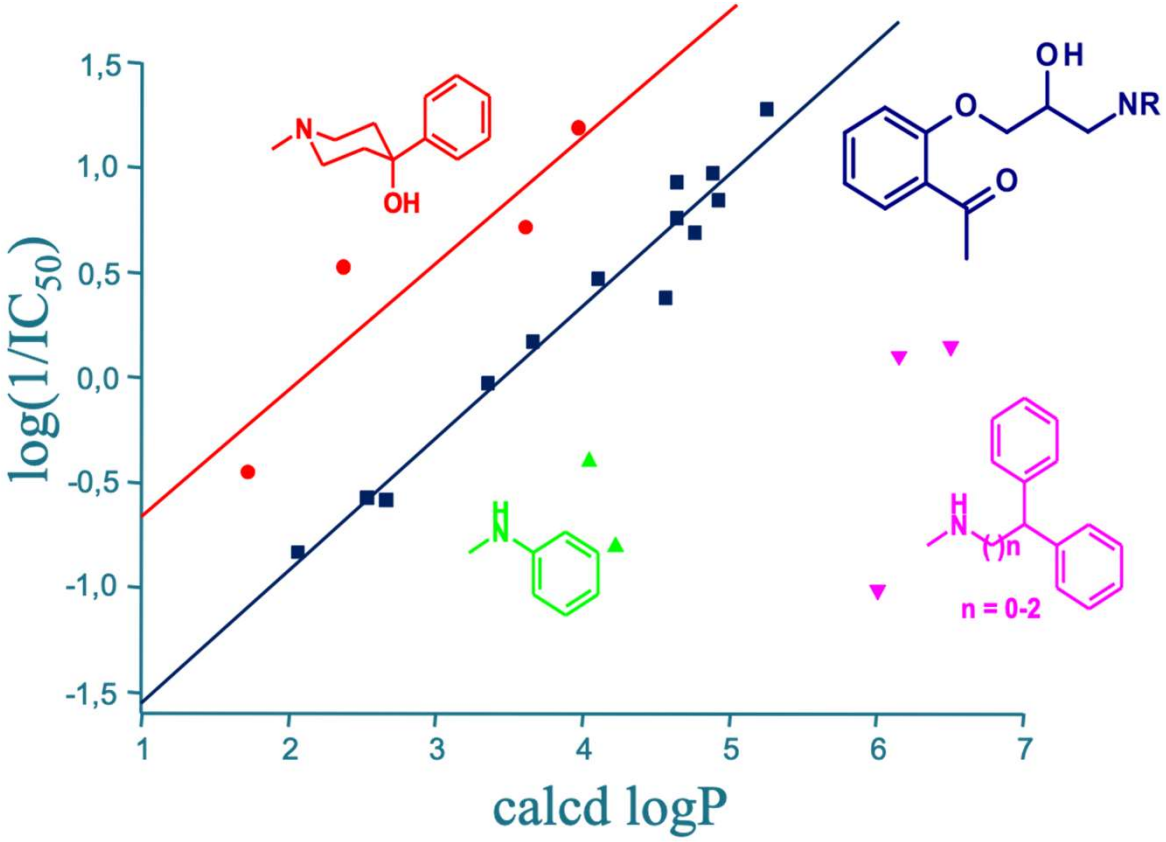
Docking and P-glycoprotein

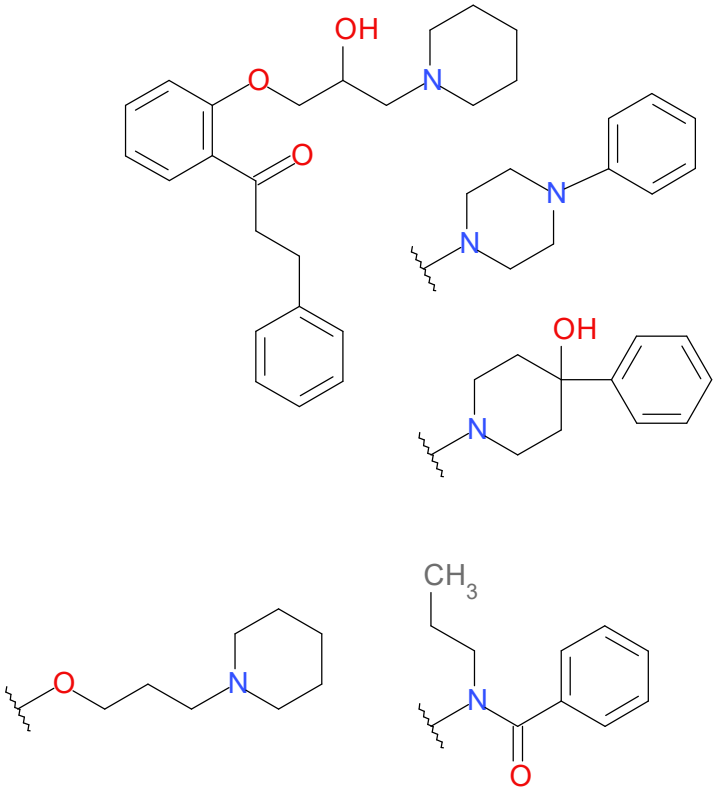


Aller et al., Science, 2009

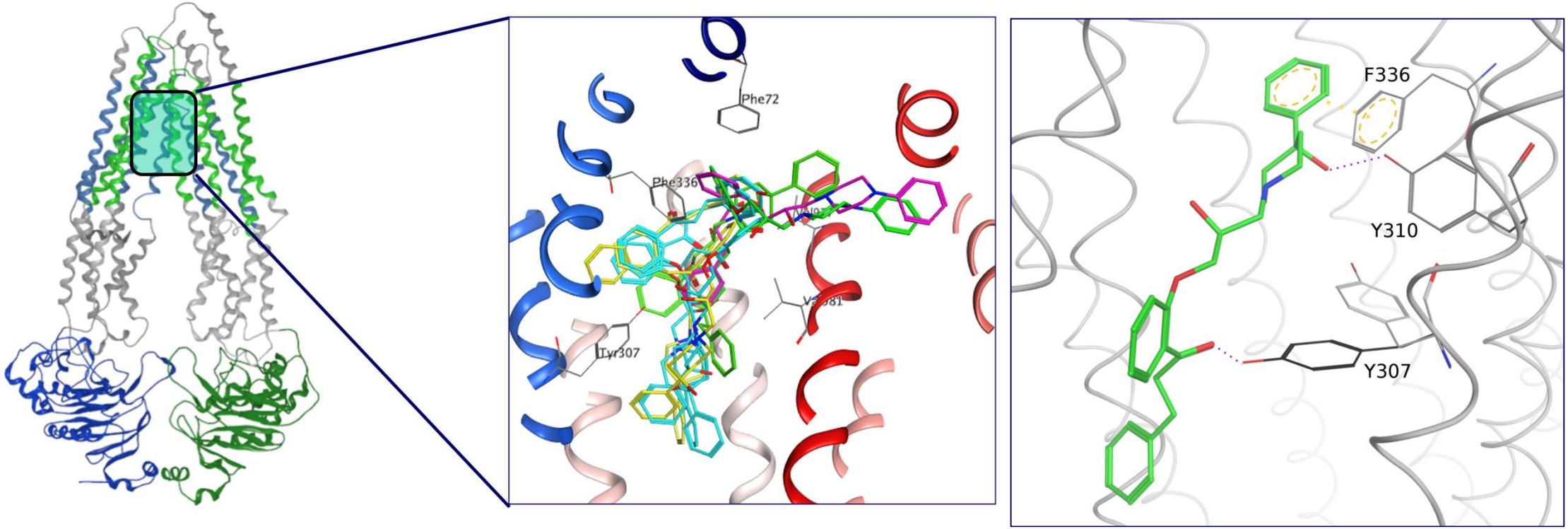


QSAR and Transporter Models



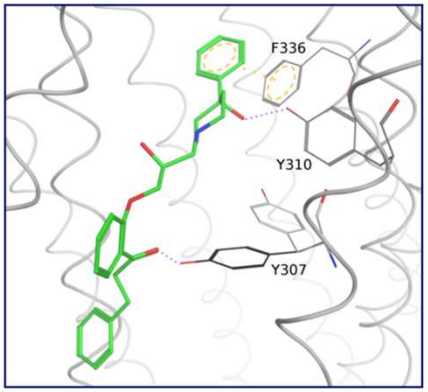


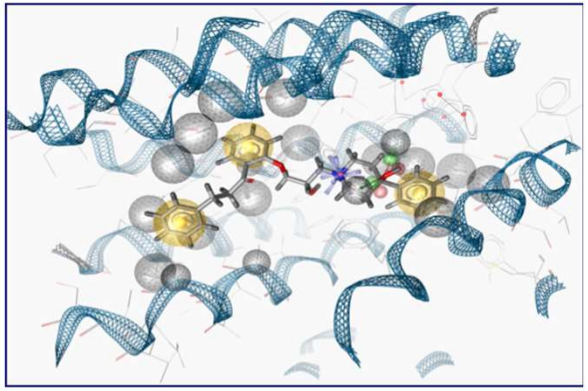
SAR-guided Docking

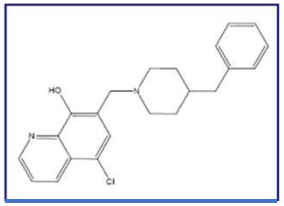


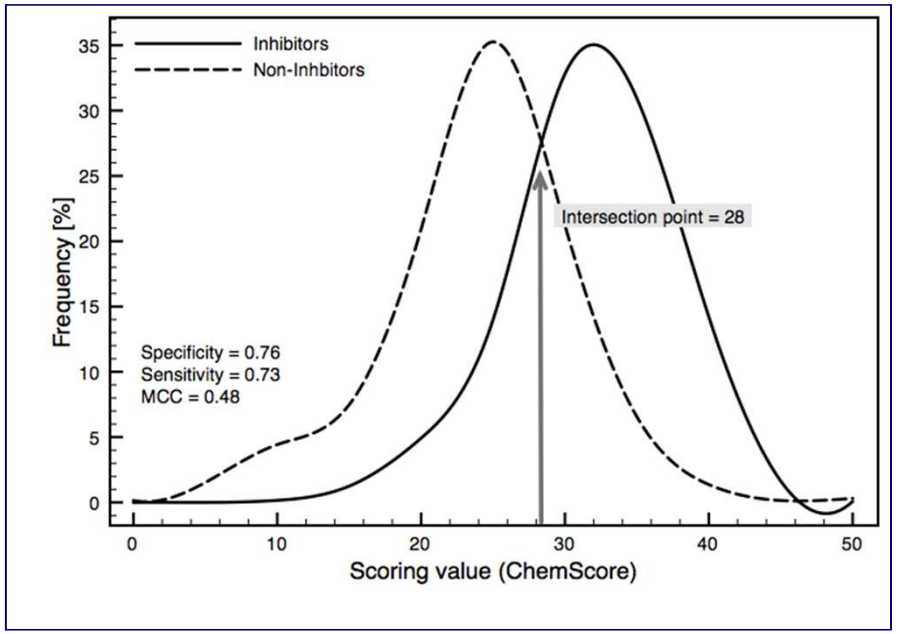
Klepsch, *PloS Comp Biol* 2011

Validation



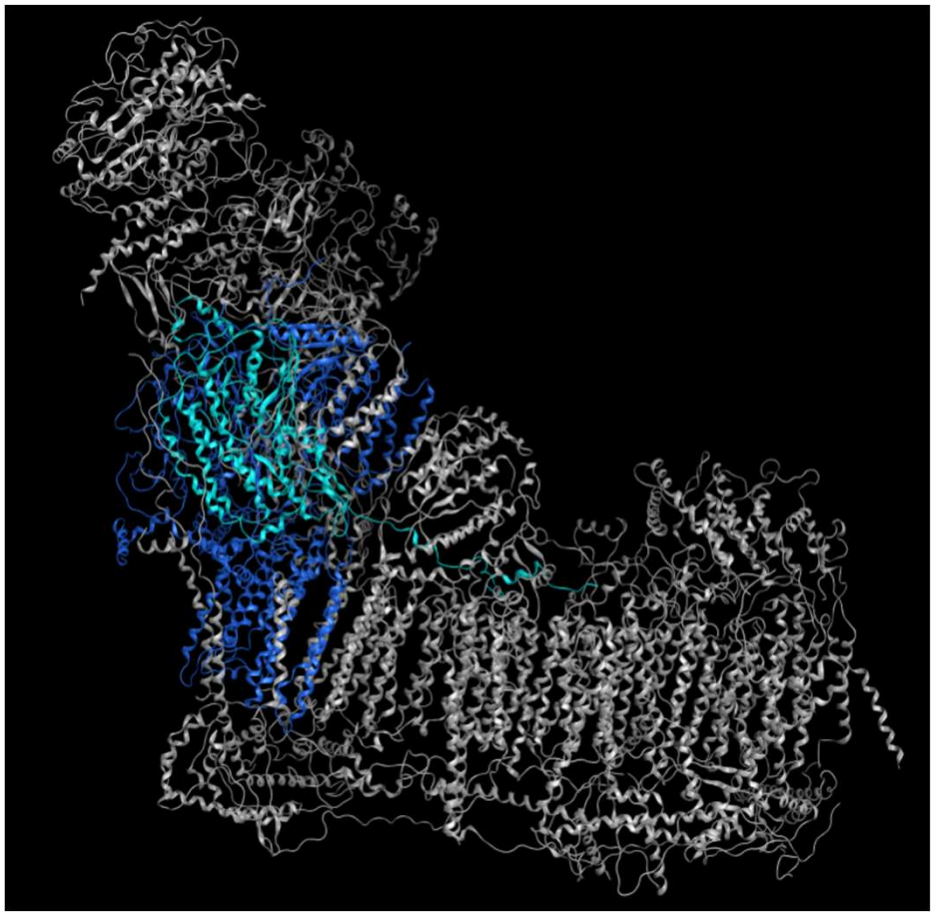




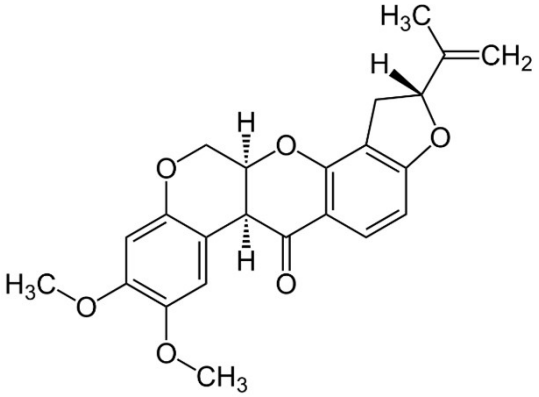


Klepsch, *J Chem Inf Mod* 2013

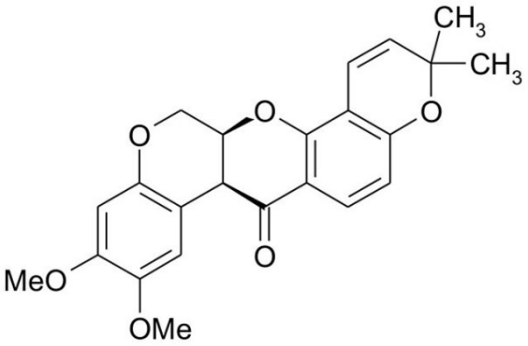
Mitochondrial Toxicity



Rotenone



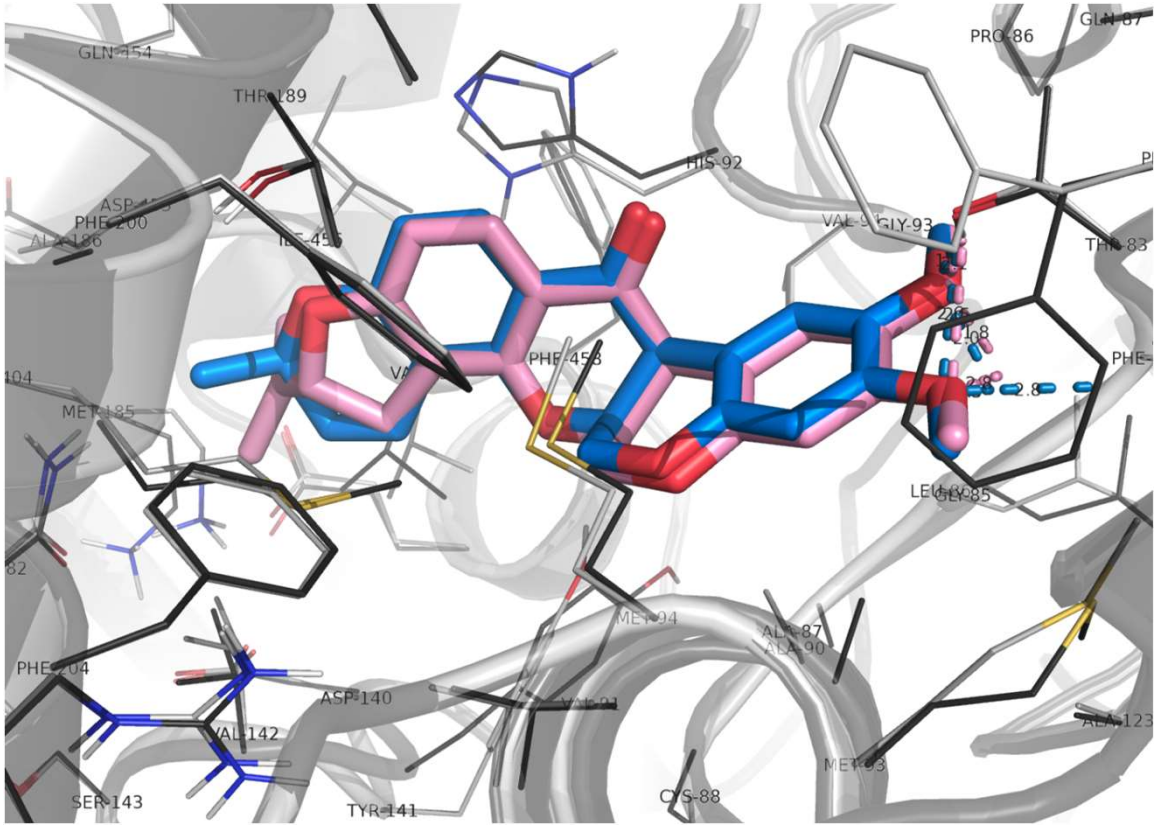
Deguelin



Complex I (NADH - ubiquinone oxidoreductase)

Pocket→ aligning NuoD (Rhodobacter capsulatus) with human NDUFS2 (light blue)

Mitochondrial Toxicity

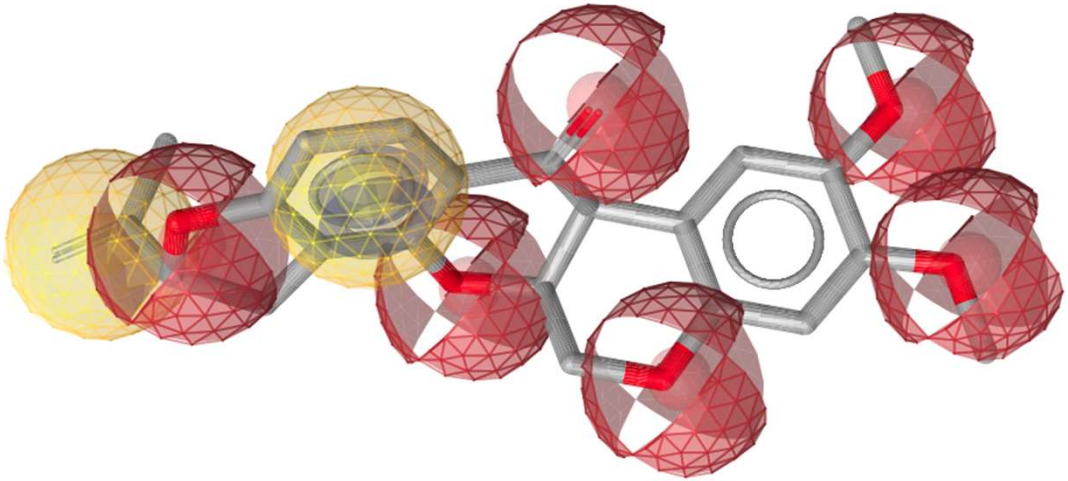


Pose selection by

- Common scaffold clustering
- Ligand-protein interaction fingerprints
- RMSD-calculation

Complex I - induced-fit docking results of Deguelin and Rotenone

Mitochondrial Toxicity

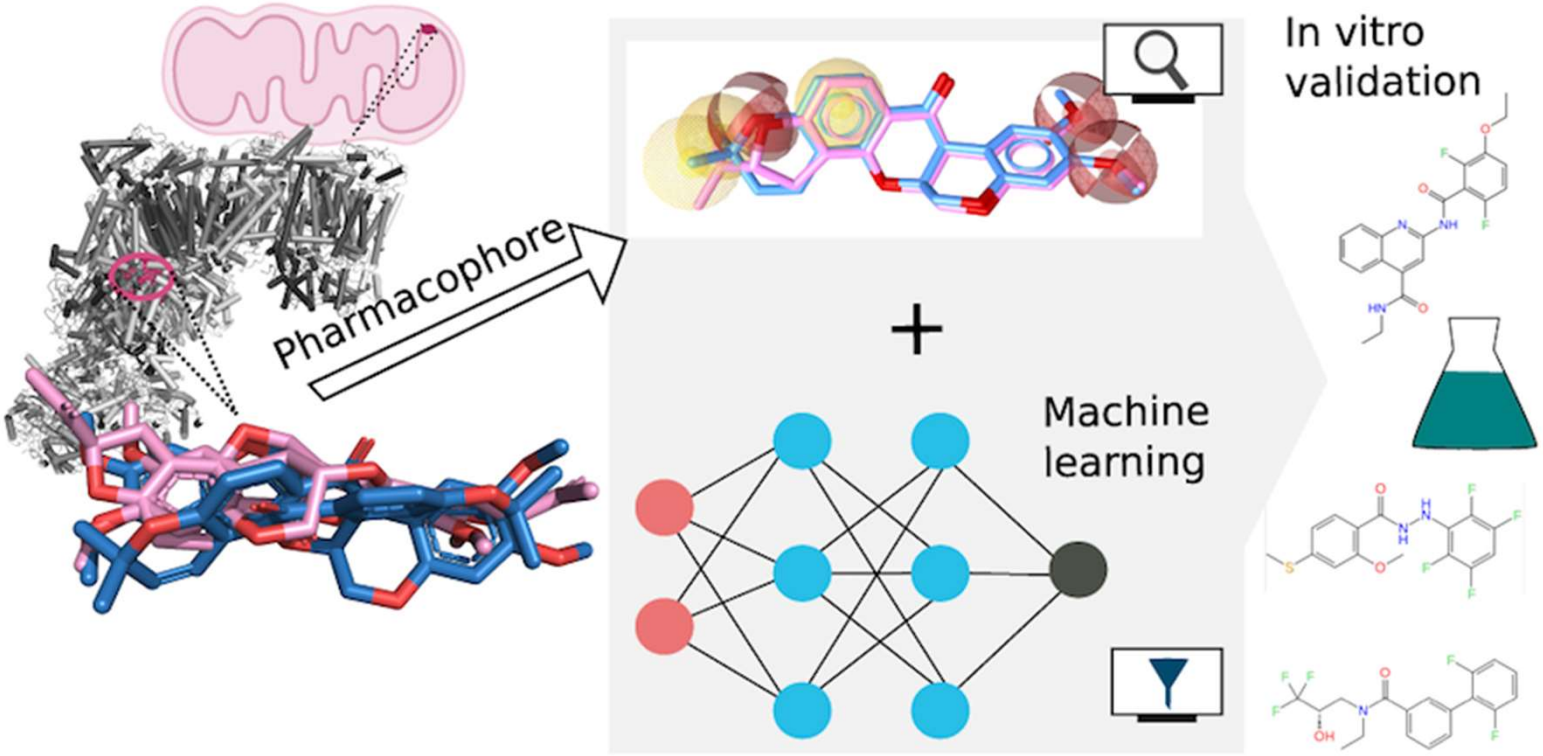


Screening output

- Mitotox-database
  - rotenone and deguelin
- Chem-space
  - 473 hits
- Drugbank
  - 0 hits

Complex I - Shared pharmacophore of rotenone and deguelin

Mitochondrial Toxicity



F. Troger, J. Hemmerich
B. van der Water
M. Leist, *Comp Toxicol* 2020

eTRANSAFE

EUTOXRISK

Developmental Neurotoxicity

universität wien

Putative AOP (adverse outcome pathway)

EUTOXRISK

MIE

Binding to nAChR

KE 1-3

(inhibited) nAChR activation

Altered cellular phenotype

KE 4

Altered neuronal development

AO (DNT)

Delayed mental development

ADS

Schizophrenia

Epilepsy

MIE	KE 1-3	KE 4
• Docking into α4β2 nAChR • Docking α7 nAChR	• Ca-influx • Gene expression /Signalling pathway changes	• Proliferation/Differentiation • Migration • Neurite outgrow

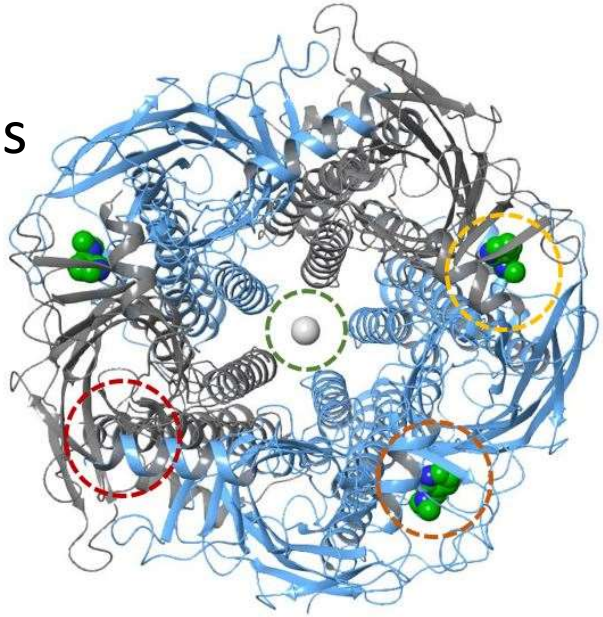
- MIE – molecular initiating event
- KE – key event
- AO – adverse outcome
- DNT – developmental neurotoxicity

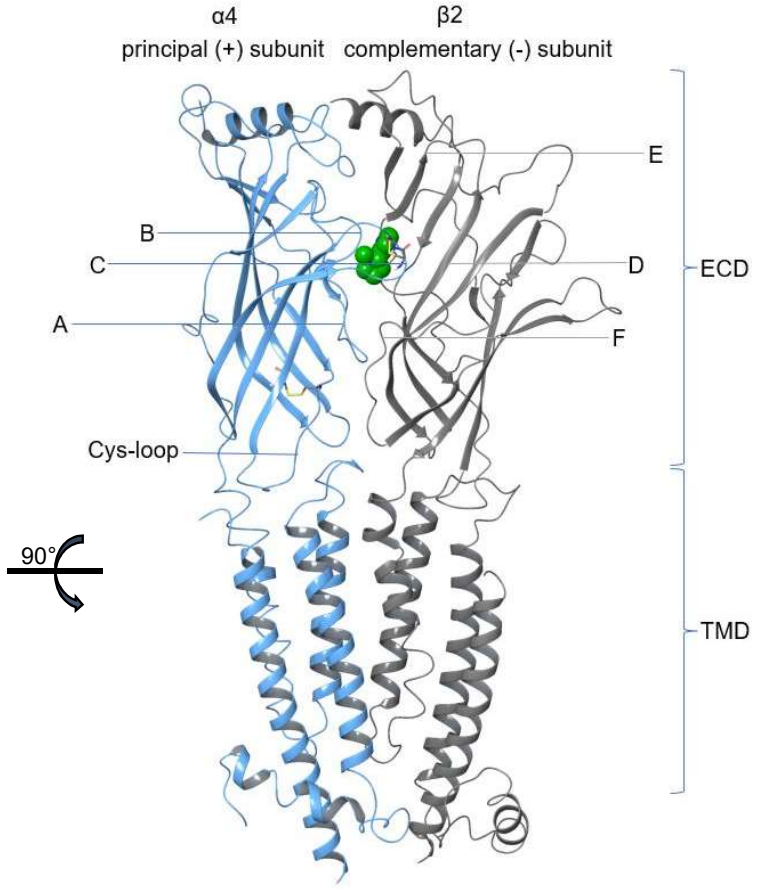
Developmental Neurotoxicity

universität wien

nAChR – nicotinic acetylcholine receptor

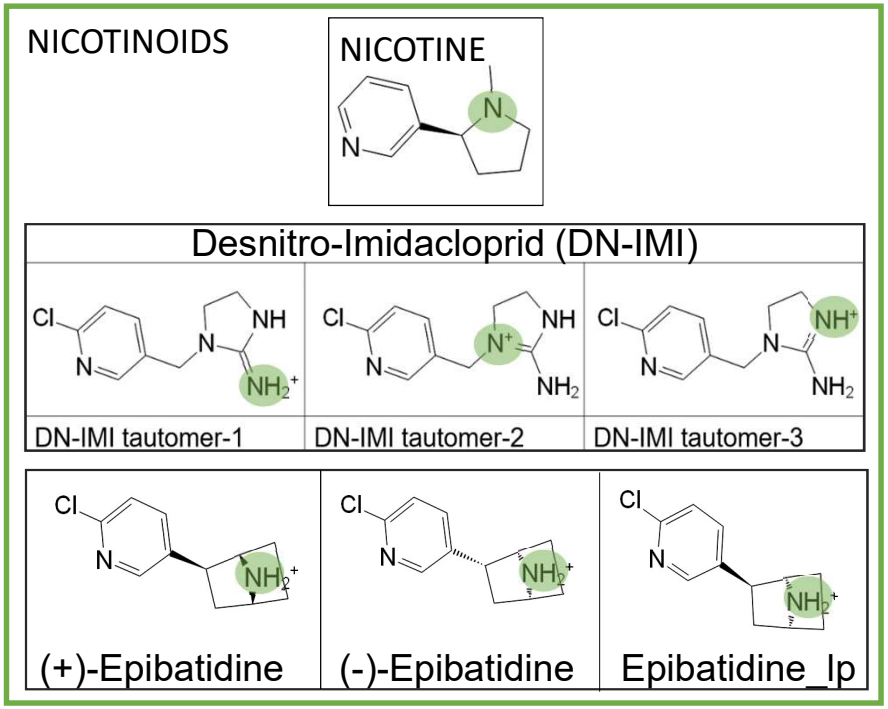
- Most abundant isoforms in the human brain:
  - Heteropentameric α4β2
  - Homopentameric α7
- Binding site characteristics
  - loops and beta-sheets
  - “aromatic box”

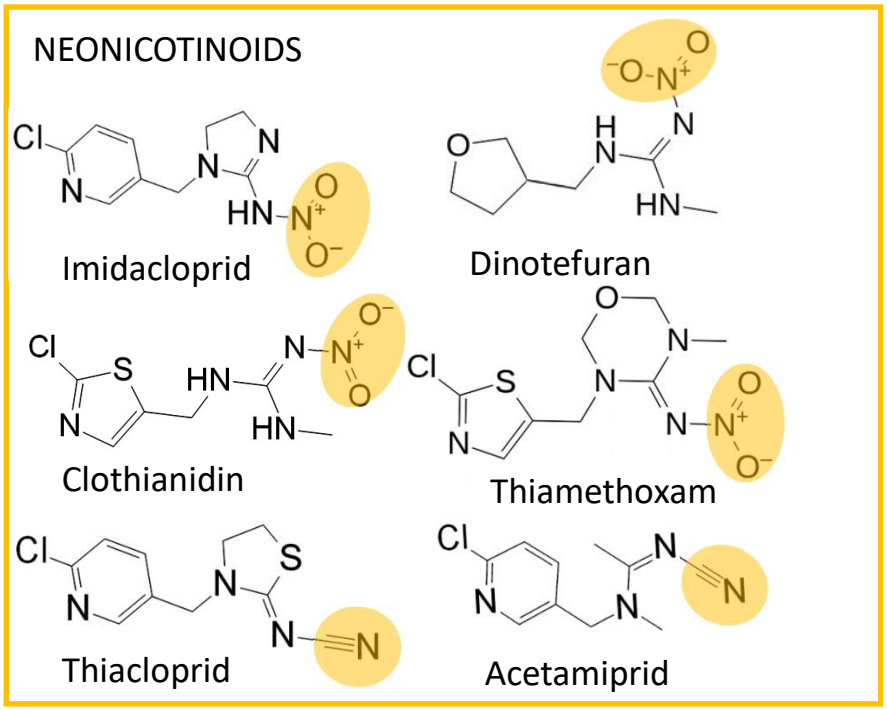




Developmental Neurotoxicity

Nicotinoids & Neonicotinoids

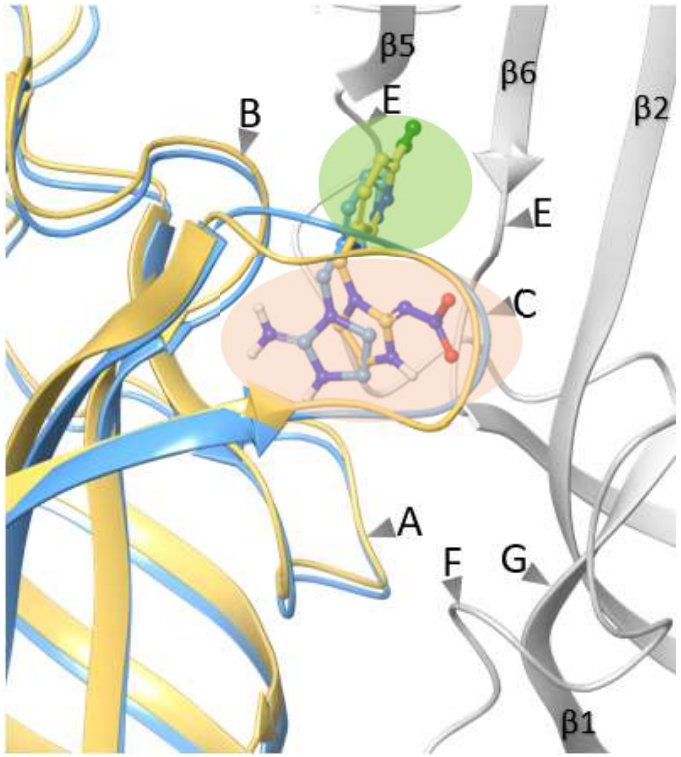




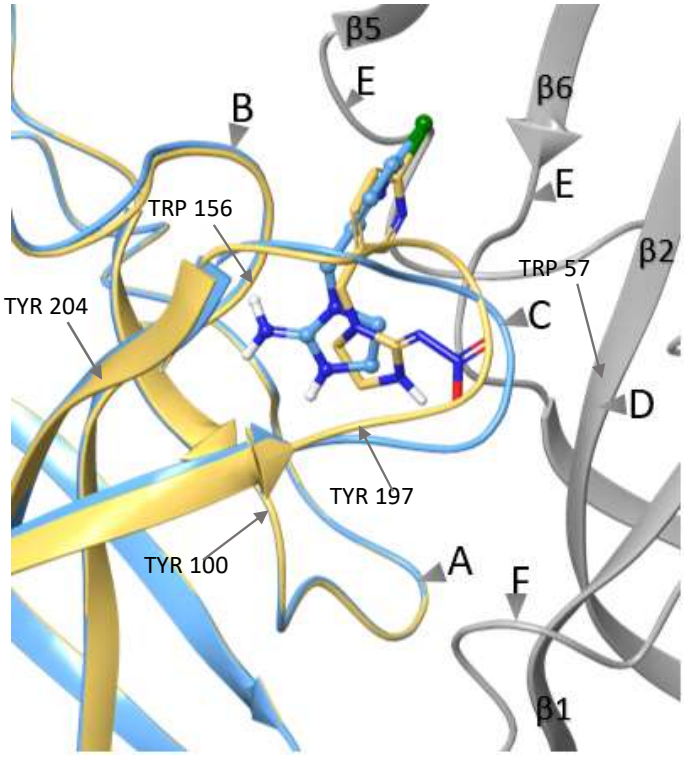
Developmental Neurotoxicity

Binding Hypothesis (1): Nicotinoids VS Neonicotinoids

AChBP from *Lymnaea stagnalis*: co-crystallised ligands



Human nAChR α4β2: docked ligands



- Similar placement of pyridine ring
- Flip of imidazolidine ring
- Fewer/no cation pi-interactions of neonicotinoid due to common orientation

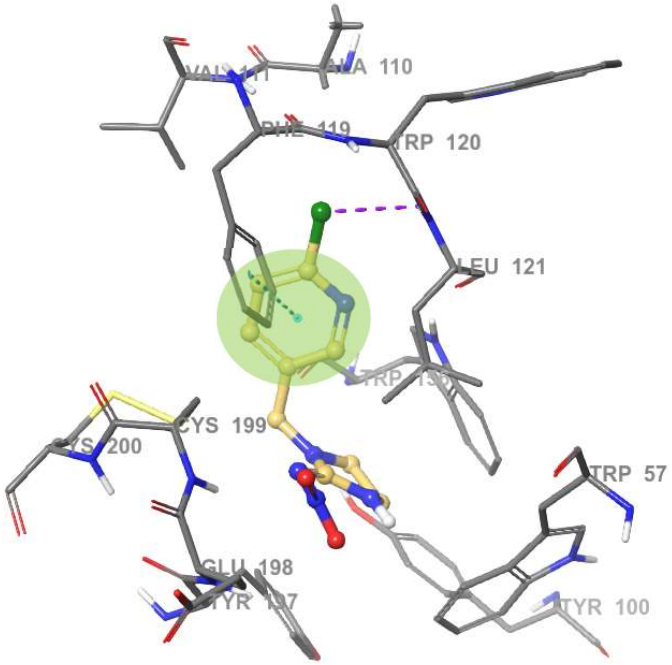
ligands docked to α4β2	docking score	IFD score	Prime Energy
DN-IMI	-10,723	-18018,72	-18431,84
Imida	-6,648	-17939,9	-18371,99

Loser et al., Archiv. Toxicol 2021

Developmental Neurotoxicity

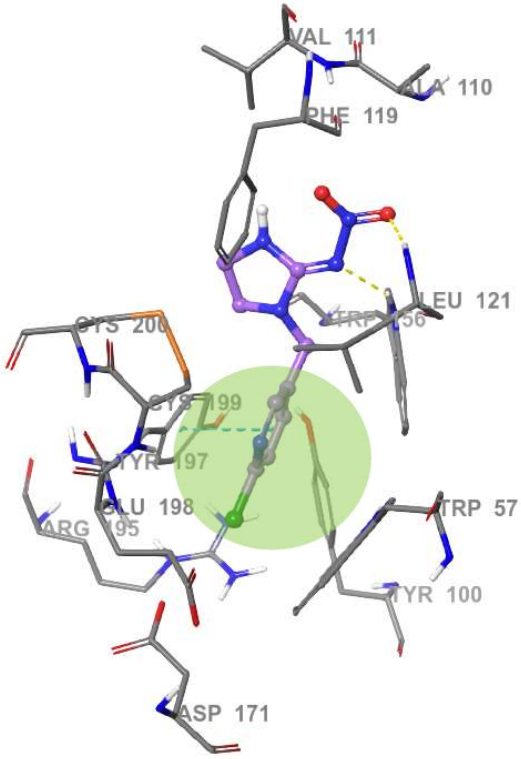
Binding Hypothesis (2): common VS inverted orientation

common binding mode



α4β2

inverted binding mode



Inverted binding mode was previously observed via photoaffinity labelling experiments on AChBP of *Lymnaea stagnalis* (great pond snail)

Interactions within the binding site:

- Halogen bonds
- Hydrogen bonds
- Pi-pi-stacking

Developmental Neurotoxicity

Spearman rank correlation of docking score *vs* IC_{50} values

7kox-ws1 has best correlation
Epibatidine bound structure

6pv8-ws1 has best correlation
Nicotine/AT1001 structure

Integrated Approach for Steatosis

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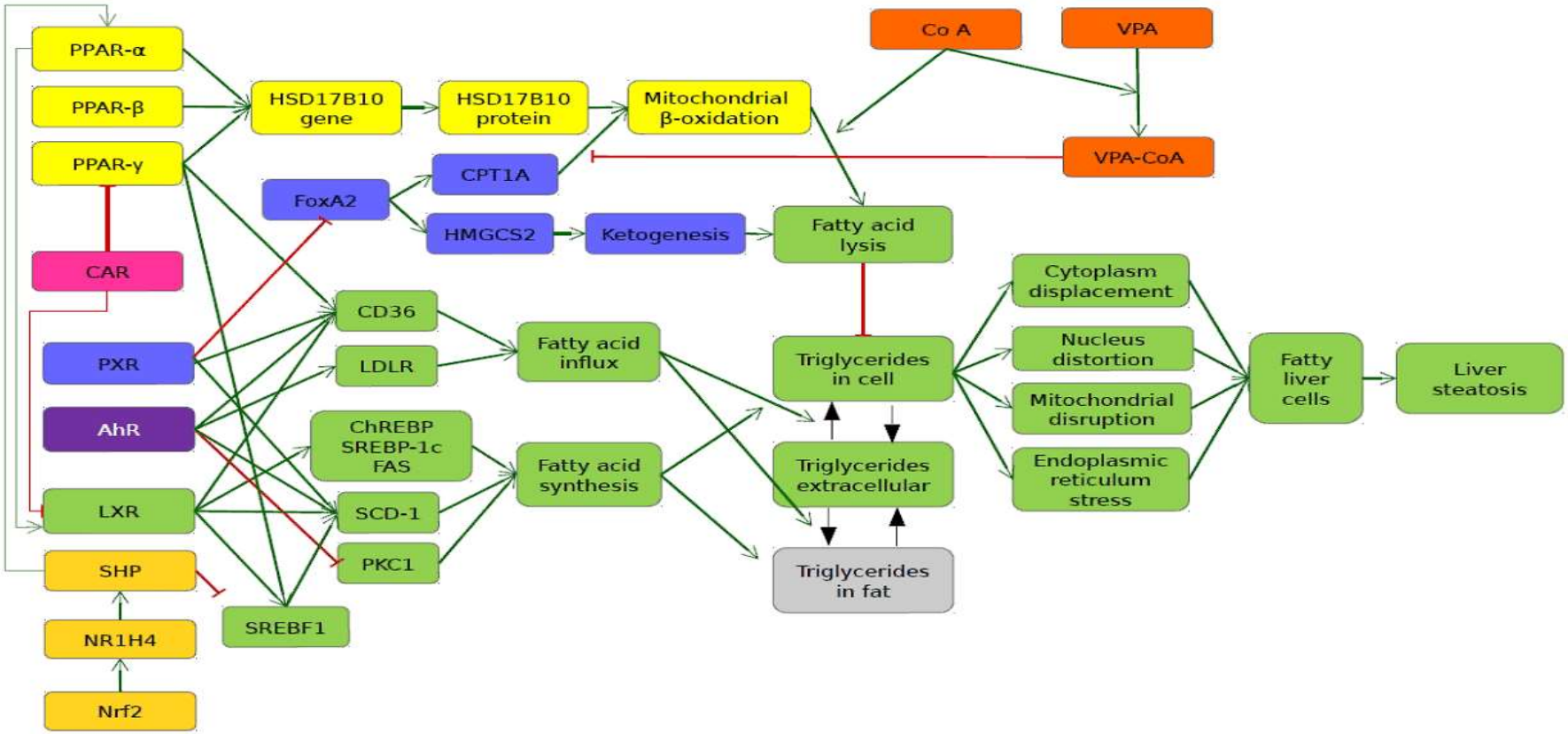


ASPIS

RISK HUNT3R

Integrated Approach for Steatosis

Chemical Research in Toxicology

pubs.acs.org/crt

Article

Combining *In Vivo* Data with *In Silico* Predictions for Modeling Hepatic Steatosis by Using Stratified Bagging and Conformal Prediction

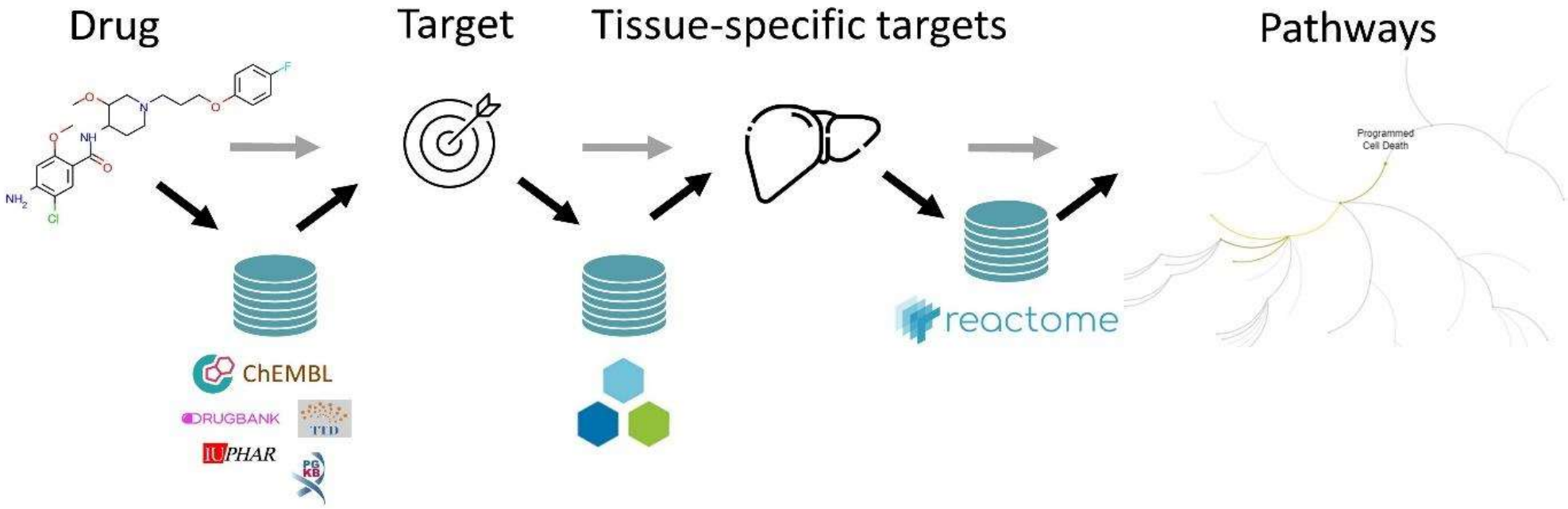
Sankalp Jain, Ulf Norinder, Sylvia E. Escher, and Barbara Zdrazil*

Cite This: *Chem. Res. Toxicol.* 2021, 34, 656–668

Read Online

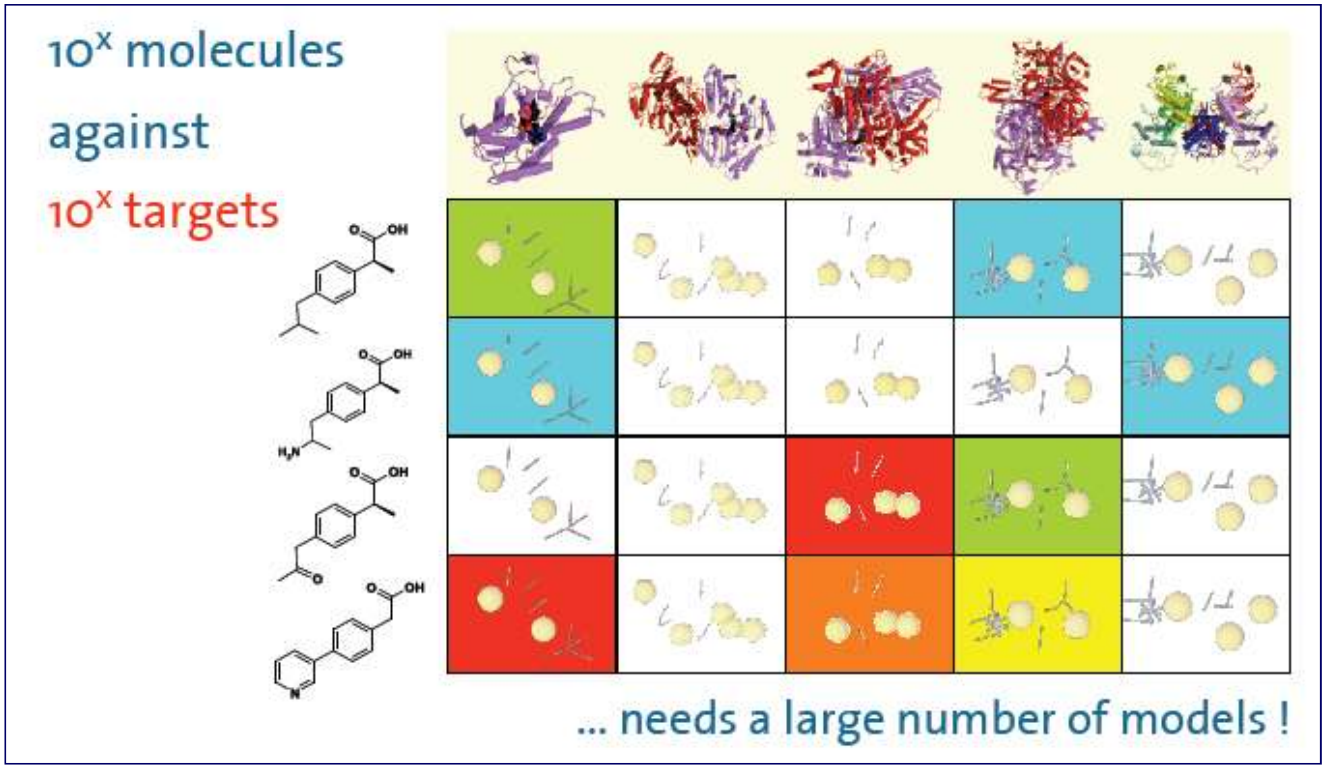
Include models not only from transporter but also from other targets from the AOP

Integrated Approach for Steatosis



Use compund/pathway interaction fingerprints as input for ML models, combined with compound descriptors

Integrated Approach for Steatosis



Compound/target interaction fingerprints based on reverse docking

Conclusions & Outlook

universität wien

- Activity assessment via docking needs data sets which allow experimental guided pose selection
- In case of analogous compounds docking allows good assessment of risk
- For selected AOPs there are enough structures of compound/protein complexes available
- With future AlphaFold versions models might be suitable for docking

Pharmacoinformatics Research Group

Department of Pharmaceutical Sciences

universität wien

FWF

MD T

RISK HUNT3R

Open PHACTS
Open Pharmacological Space

eTRANSAFE

transQST

EUTOXRISK

RESOLUTE

REsolution
Medical Genetic Solutions for RESOLUTE

Omics WG:
How to bridge the regulatory barrier

John Colbourne / Florian Caiment | Univ. Birmingham / Univ. Maastricht

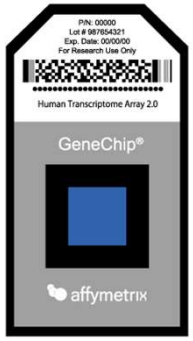
Maastricht University

25th November 2022

Barcelona

these projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers precisiontox: 965406, ONTOX: 963845 and Risk-Hunt3r: 964537

Omics



First microarray Chip:

1994

First Next-Generation Sequencing Platformn

2005

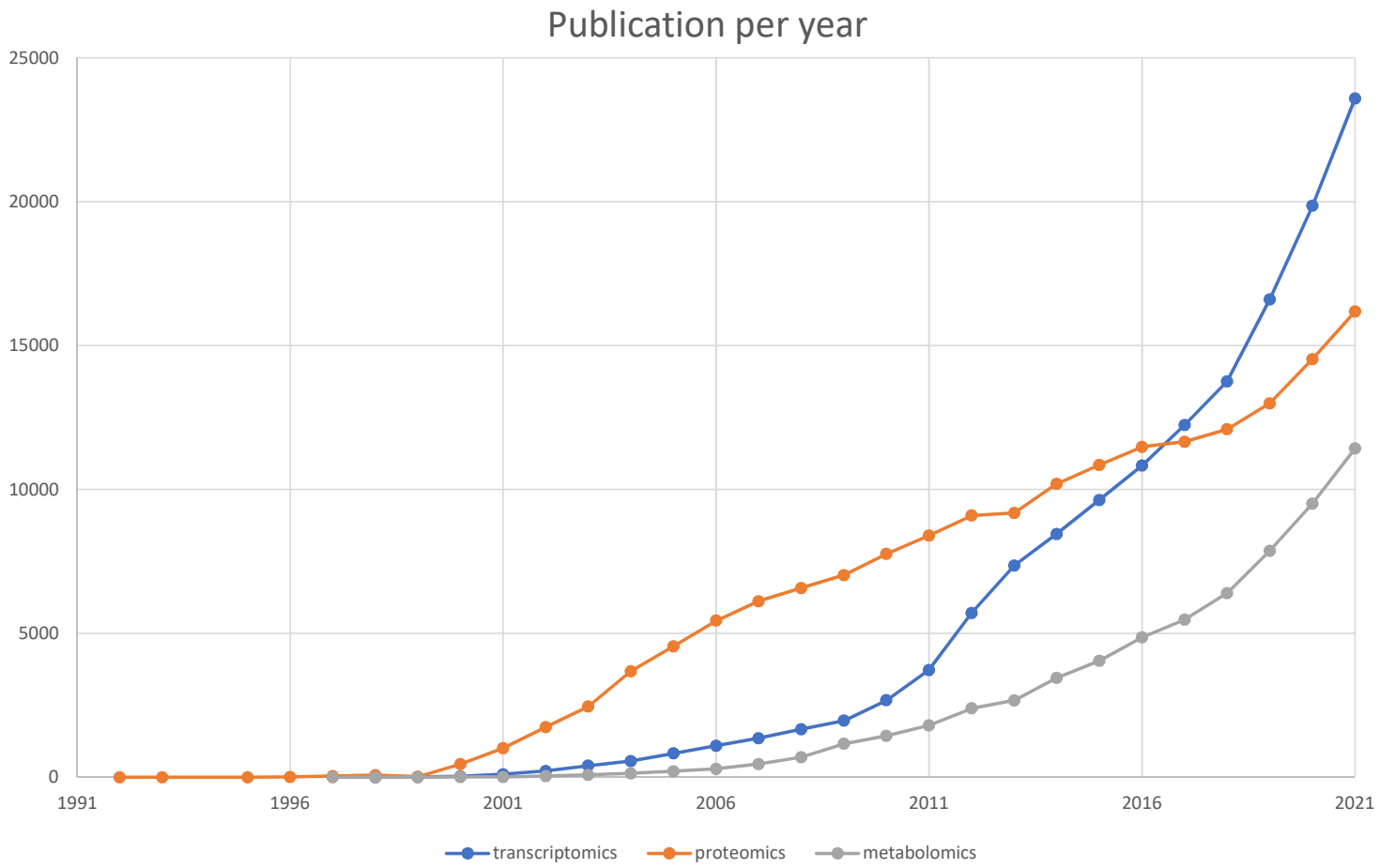
TOXICOGENOMICS AND SYSTEMS TOXICOLOGY: AIMS AND PROSPECTS

Michael D. Waters and Jennifer M. Fostel

Nature Reviews Genetics, 2004

“New toxicogenomics methods have the power and potential to revolutionize toxicology.”

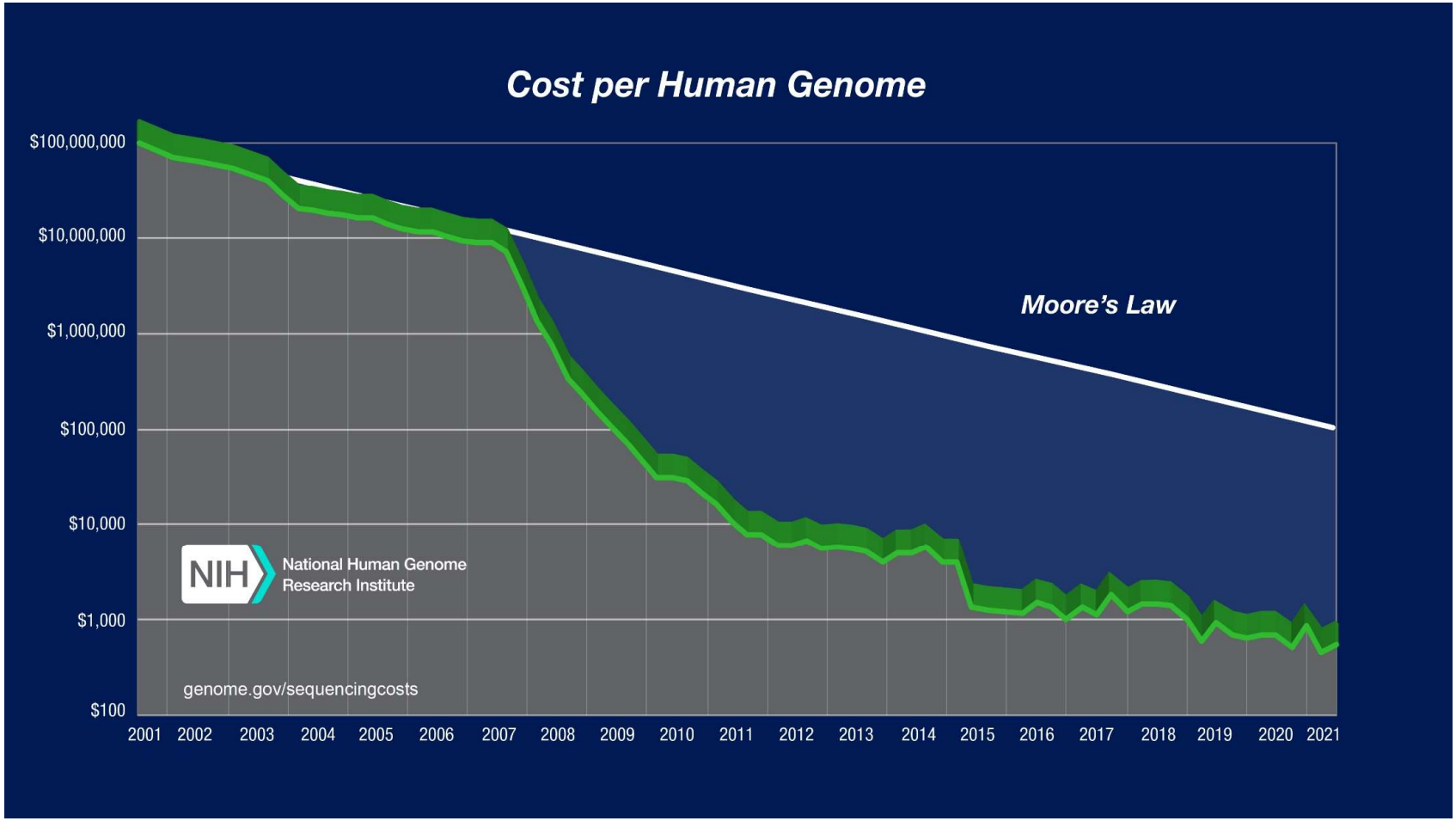
“members of regulatory bodies are working with scientists from industry, academic and government laboratories (...) to develop standards for the exchange, analysis and interpretation of transcriptomics data”.


Publication per year
25000
20000
15000
10000
5000
0
1991
1996
2001
2006
2011
2016
2021
transcriptomics
proteomics
metabolomics

Why such discrepancies?

How to bridge the two worlds ?

The Cost ?


Cost per Human Genome
$100,000,000
$10,000,000
$1,000,000
$100,000
$10,000
$1,000
$100
Moore's Law
NIH
National Human Genome Research Institute
genome.gov/sequencingcosts
2001 2002 2003 2004 2005 2006 2007 2008 2009 2010 2011 2012 2013 2014 2015 2016 2017 2018 2019 2020 2021

The lack of reference framework

New Data Analysis Methods
New metadata necessary
New statistical thresholds

New technologies

Regulatory Toxicology and Pharmacology
Volume 125, October 2021, 105020

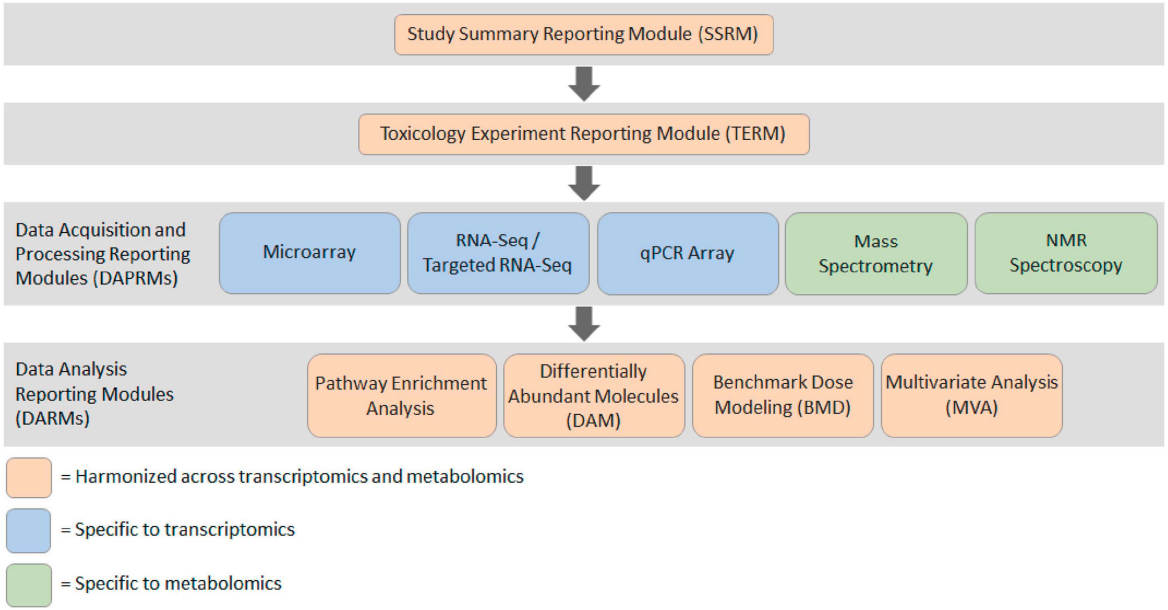
Commentary

Progress towards an OECD reporting framework for transcriptomics and metabolomics in regulatory toxicology

Joshua A. Harrill [a, 1], Mark R. Viant [b, c, 1], Carole L. Yauk [d, 1], Magdalini Sachana [e], Timothy W. Gant [f], Scott S. Auerbach [g], Richard D. Beger [h], Mounir Bouhifd [i], Jason O'Brien [j], Lyle Burgoon [k], Florian Caiment [l], Donatella Carpi [m], Tao Chen [h], Brian N. Chorley [a], John Colbourne [b, c], Raffaella Corvi [m], Laurent Debrauwer [n, o], Claire O'Donovan [p] ... Maurice Whelan [m]

OECD reporting framework (2021)

<u>TRF :</u>
Transcriptomics Reporting Framework



<u>MRF :</u>
Metabolomics Reporting Framework

https://www.oecd.org/chemicalsafety/testing/omics.htm

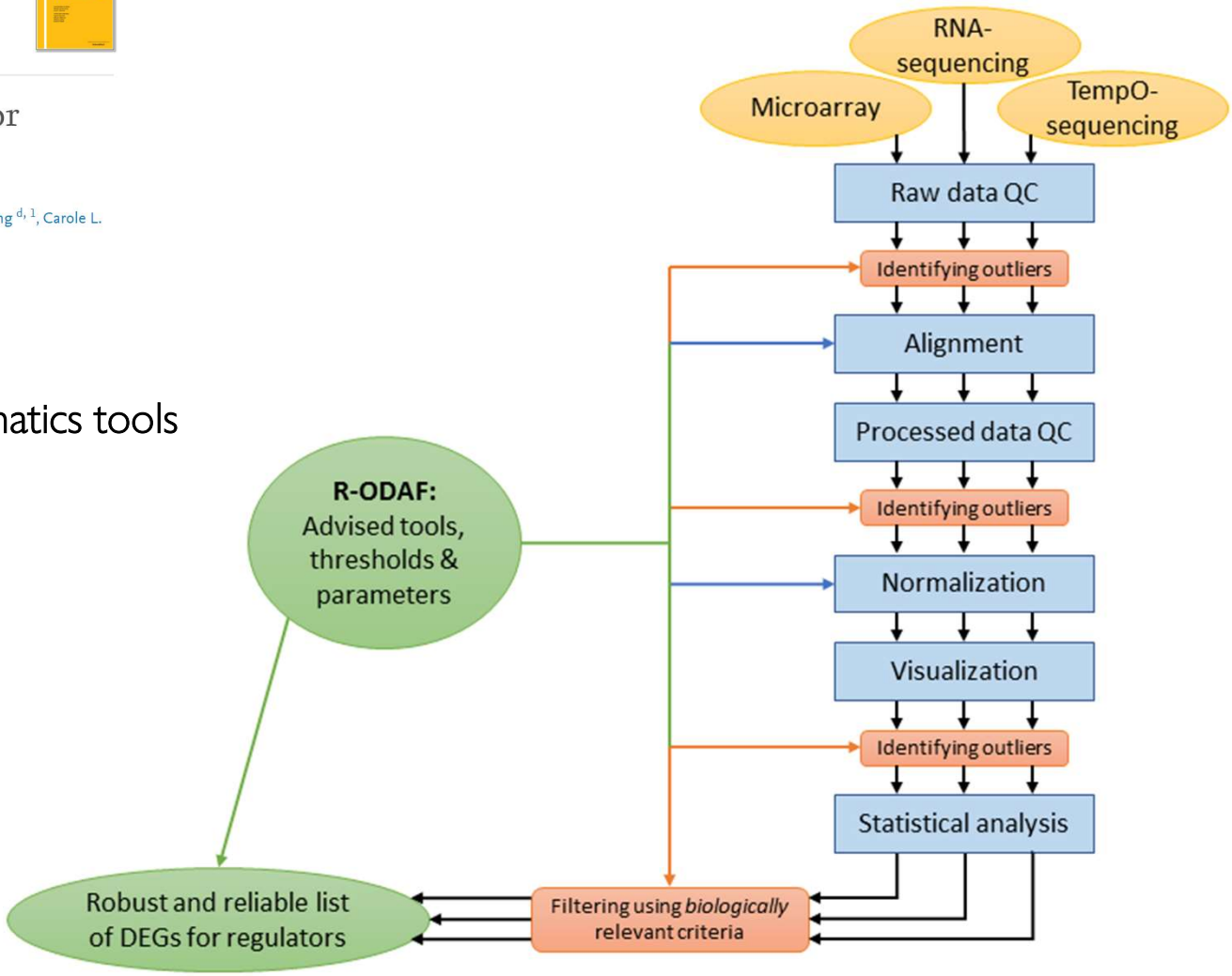
Regulatory Toxicology and Pharmacology
Volume 131, June 2022, 105143

R-ODAF: Omics data analysis framework for regulatory application

Marcha CT. Verheijen [a], Matthew J. Meier [b], Juan Ochoteco Asensio [a], Timothy W. Gant [c], Weida Tong [d, 1], Carole L. Yauk [e], Florian Caiment [a]

R-ODAF Approach (2022)

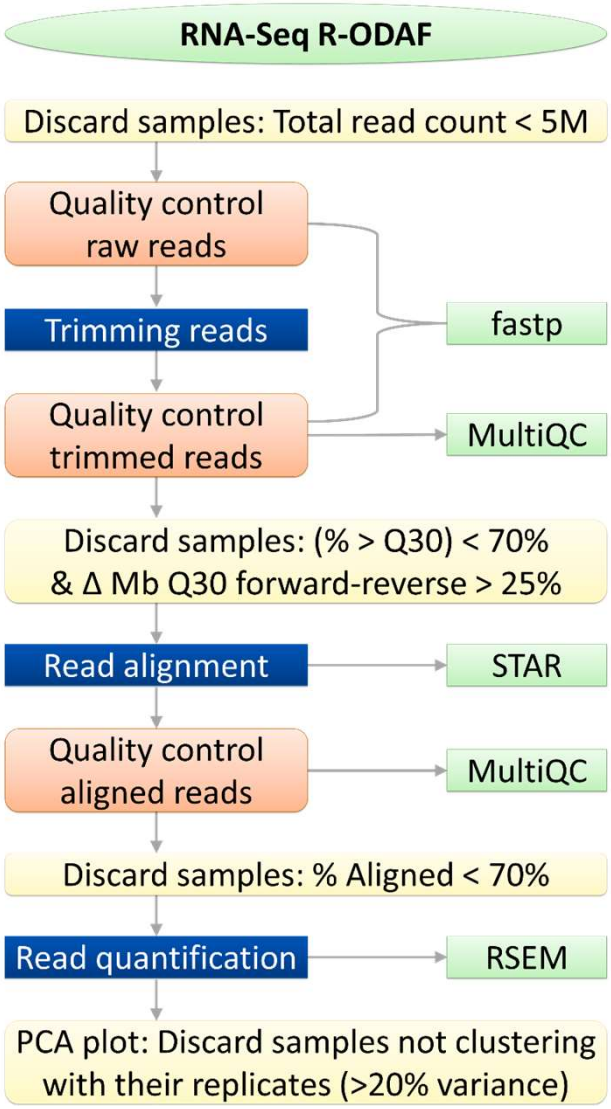
- Determine most used bioinformatics tools for each stage of the pipeline
  - Test & compare tools
  - Select top-tools for pipeline
  - Optimize parameter settings



R-ODAF RNA-Seq

–

- QC / Mapping / Quantification pipeline



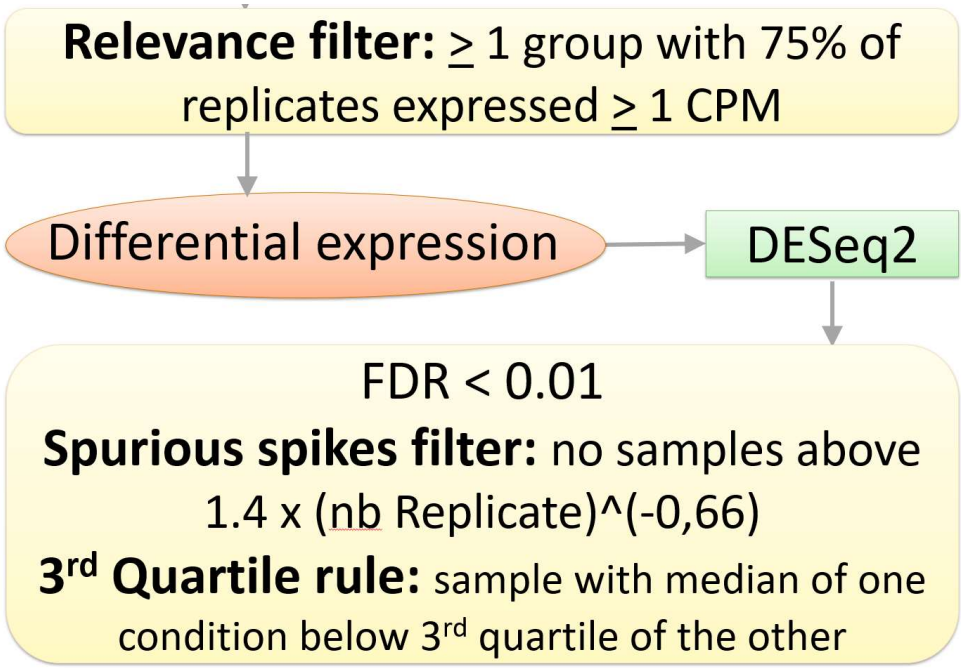
https://github.com/R-ODAF/Main

Three novel filtering criteria

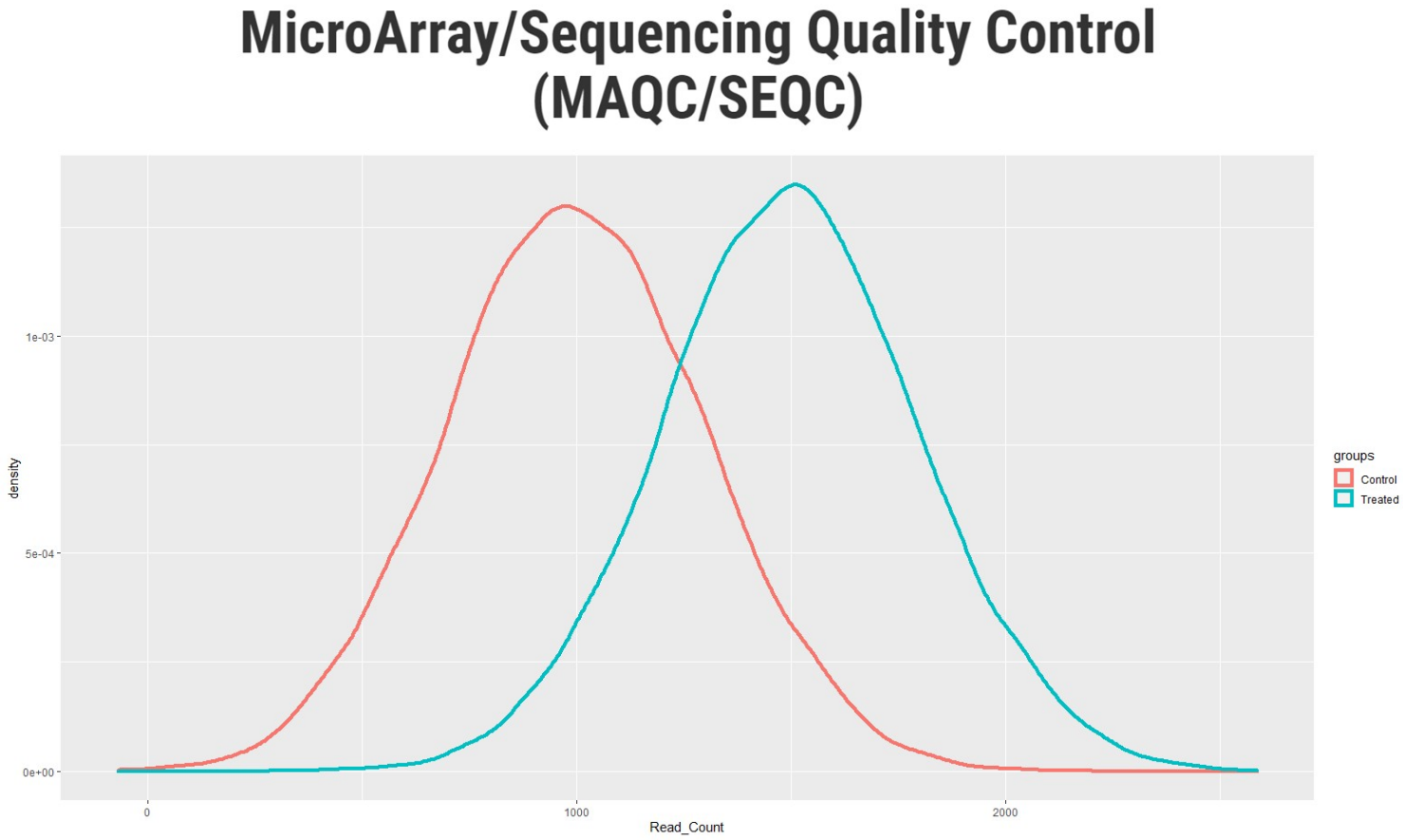
-

- Relevance filter, to exclude:
  - low expressed genes
  - Not consistently expressed genes
- Spurious Spikes Filter, to exclude:
  - DEG due to a single replicate
- 3rd Quartile Filter

→ No Fold change cut off

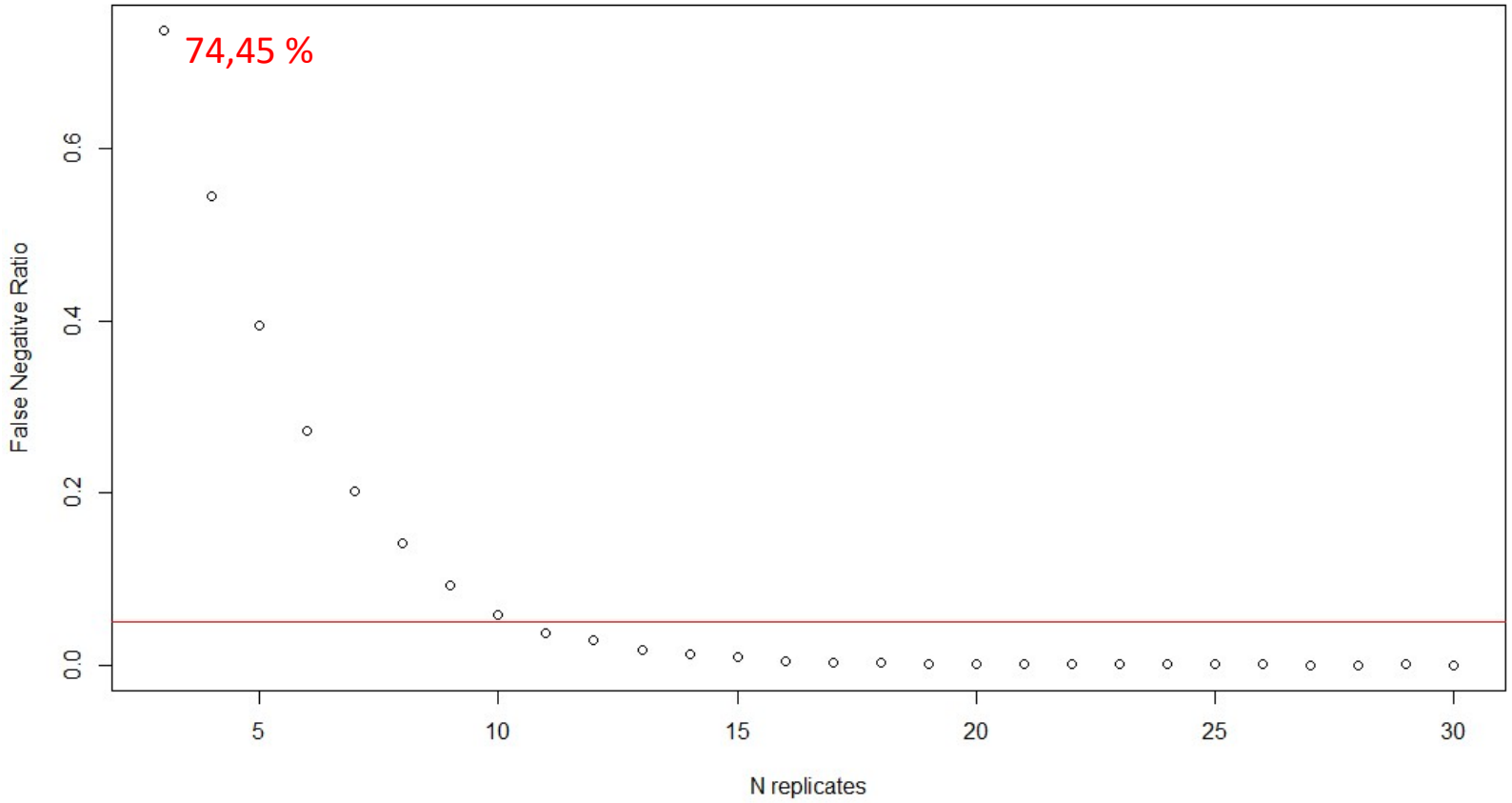


Can we trust omics results ?

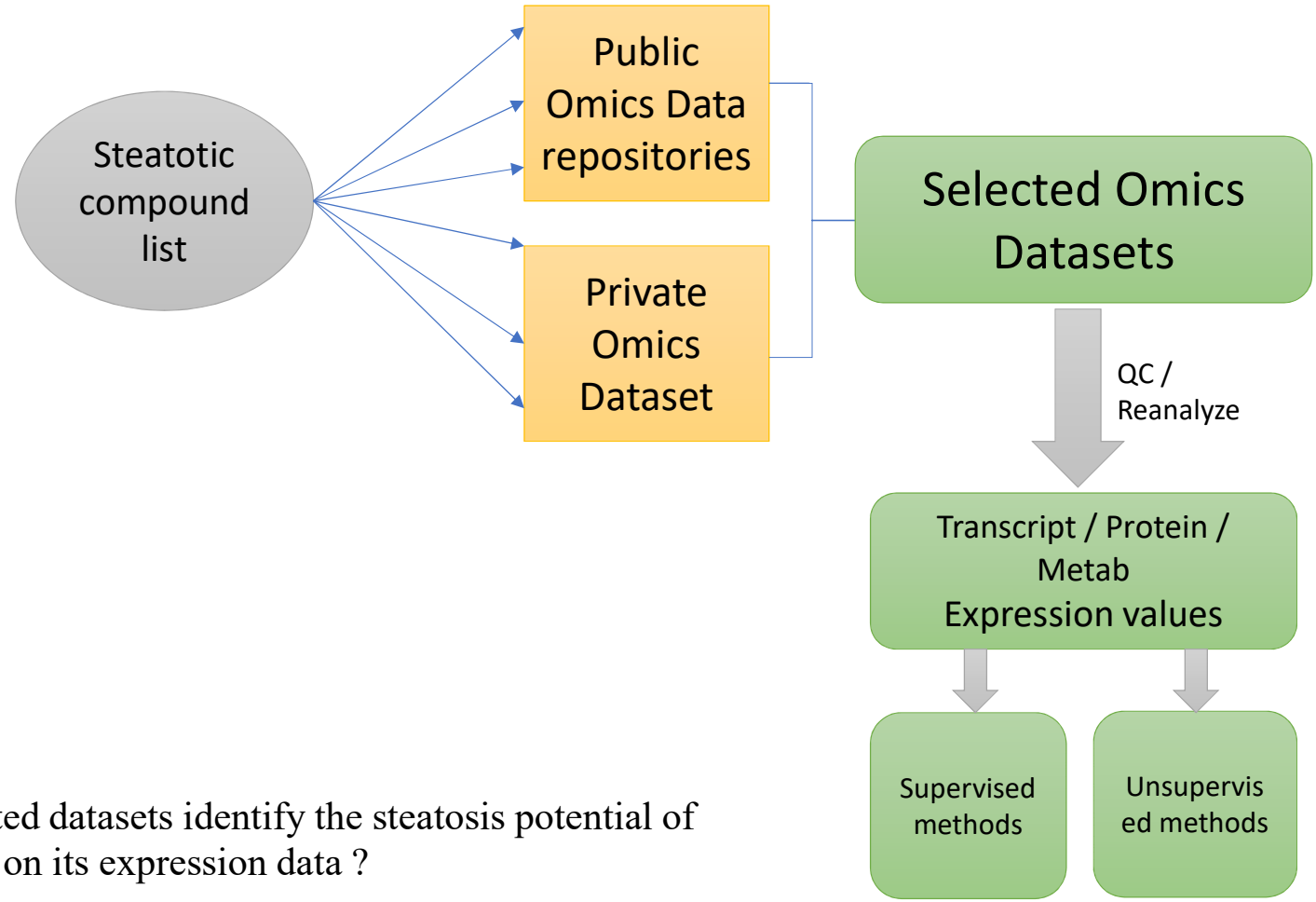


Control = rnorm(n = 10000, mean = 1000, sd = 300)
Treated = rnorm(n = 10000, mean = 1500, sd = 300)

Can we trust omics results ?



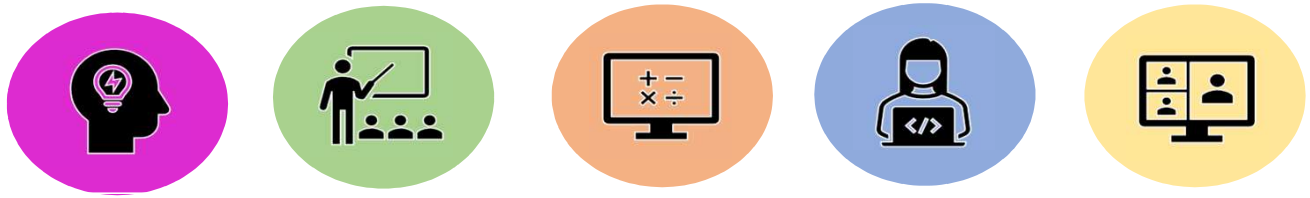
Dataset Integration



Can Omics generated datasets identify the steatosis potential of a compound based on its expression data ?

The lack of computer scientist

Data Handling and Analysis Training Program



ecetoc

30 June 2022

GARD®skin

OECD TG 442E: *in vitro* skin sensitization

Approved by OECD for regulatory testing

As a new method included in OECD TG 442E for *in vitro* skin sensitization, GARDskin supports discrimination of skin sensitizers and non-sensitizers in accordance with the UN GHS.

Test system

- Human dendritic-like cell line: SenzaCell™.

What it measures

- Gene expression profile of 196 genomic biomarkers.

quantified using the Nanostring nCounter system

Table 1. The GARDskin validation study results demonstrated high reproducibility as well as outstanding predictive performance.

Accuracy	94%
Sensitivity	93%
Specificity	96%
Within Laboratory Reproducibility	82-89%
Between Laboratory Reproducibility	92%

*Johansson et al., Validation of the GARDskin assay for assessment of chemical skin sensitizers – ring trial results of predictive performance and reproducibility. Toxicological Sciences. May 17, 2019.

Thank You for your attention !

25th November 2022

Barcelona

these projects have received funding from the European Union's Horizon 2020 research and innovation programme under grant agreements numbers precisiontox: 965406, ONTOX: 963845 and Risk-Hunt3r: 964537

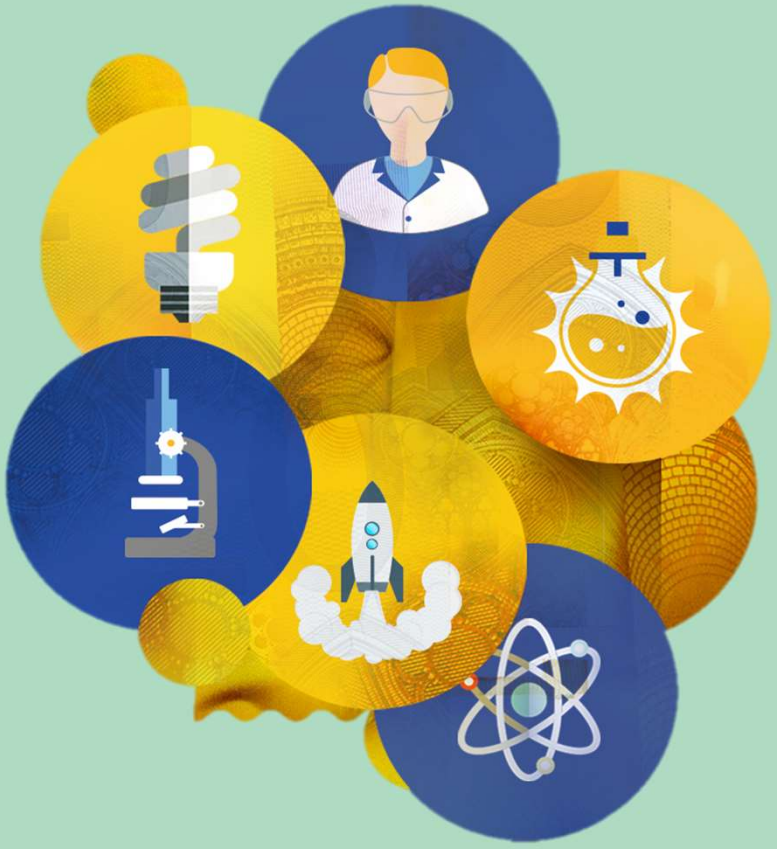
European Partnership for the Assessment of Risks from Chemicals

Horizon Europe

The role of ASPIS in PARC

Mirjam Luijten

ASPIS Open Symposium
Sitges, 25 November 2022



Co-funded by the European Union

PARC in a nutshell

Status: Co-funded, public-public, European Partnership for Assessment of Risks from Chemical under Horizon Europe

Started: 1 May 2022, for 7 years – Focus on chemical risk assessment

Vision: To establish a **Science to Policy dialogue** and interface to apply the long term visions of **European policies** (notably EU Chemicals Strategy for Sustainability) and to establish a hub of excellence enabling the transition towards **Next Generation Risk Assessment**

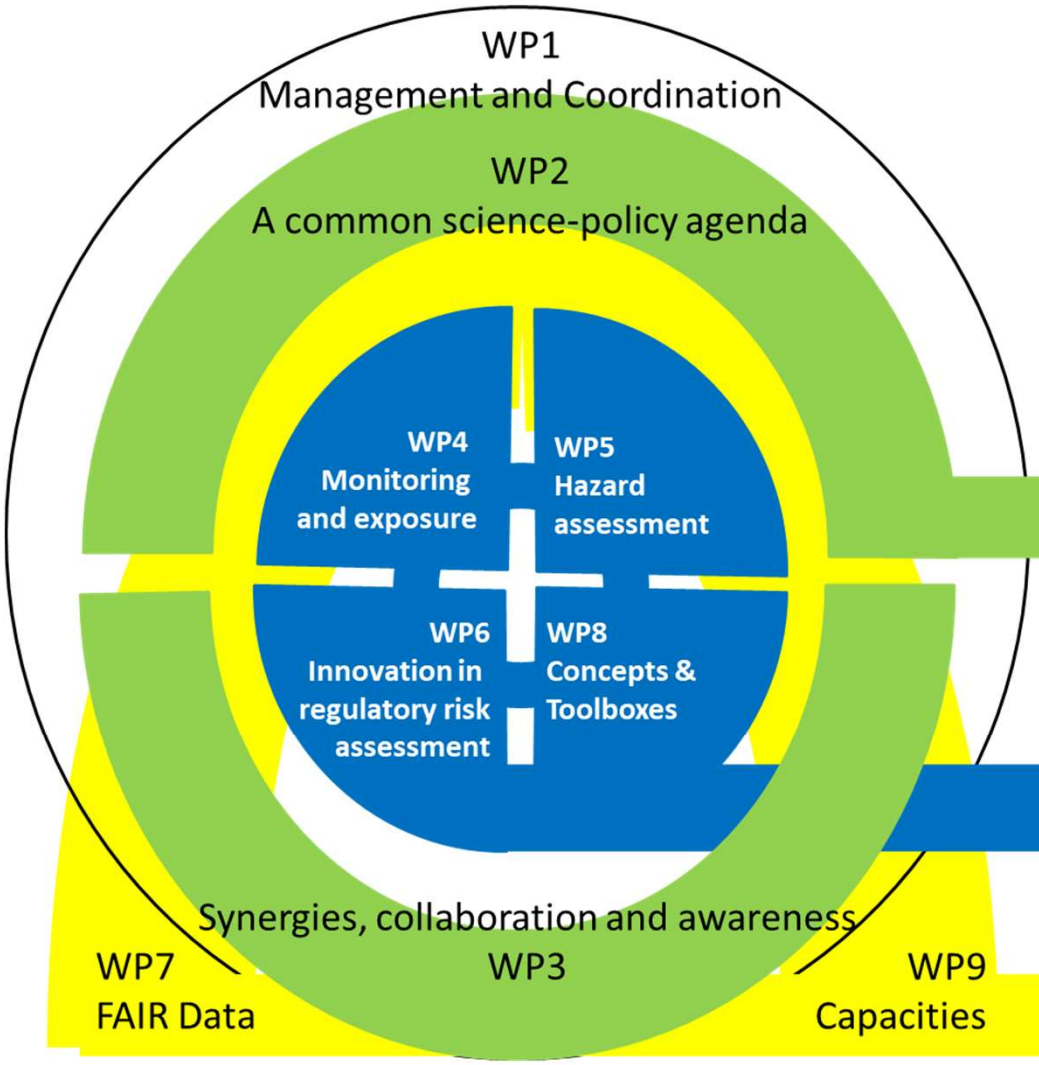
© Anses - Xavier SCHWEBEL.

PARC Kick-off meeting, 12-13 May 2022

Under **Horizon Europe** Pillar II – Global challenges and Industrial Competitiveness, Cluster 1 – **Health**

Coordinated by **ANSES (France)**
Nearly **200 organisations** from **28 countries** and **3 EU agencies**: EEA, EFSA, ECHA

Estimated budget of over **400M€**



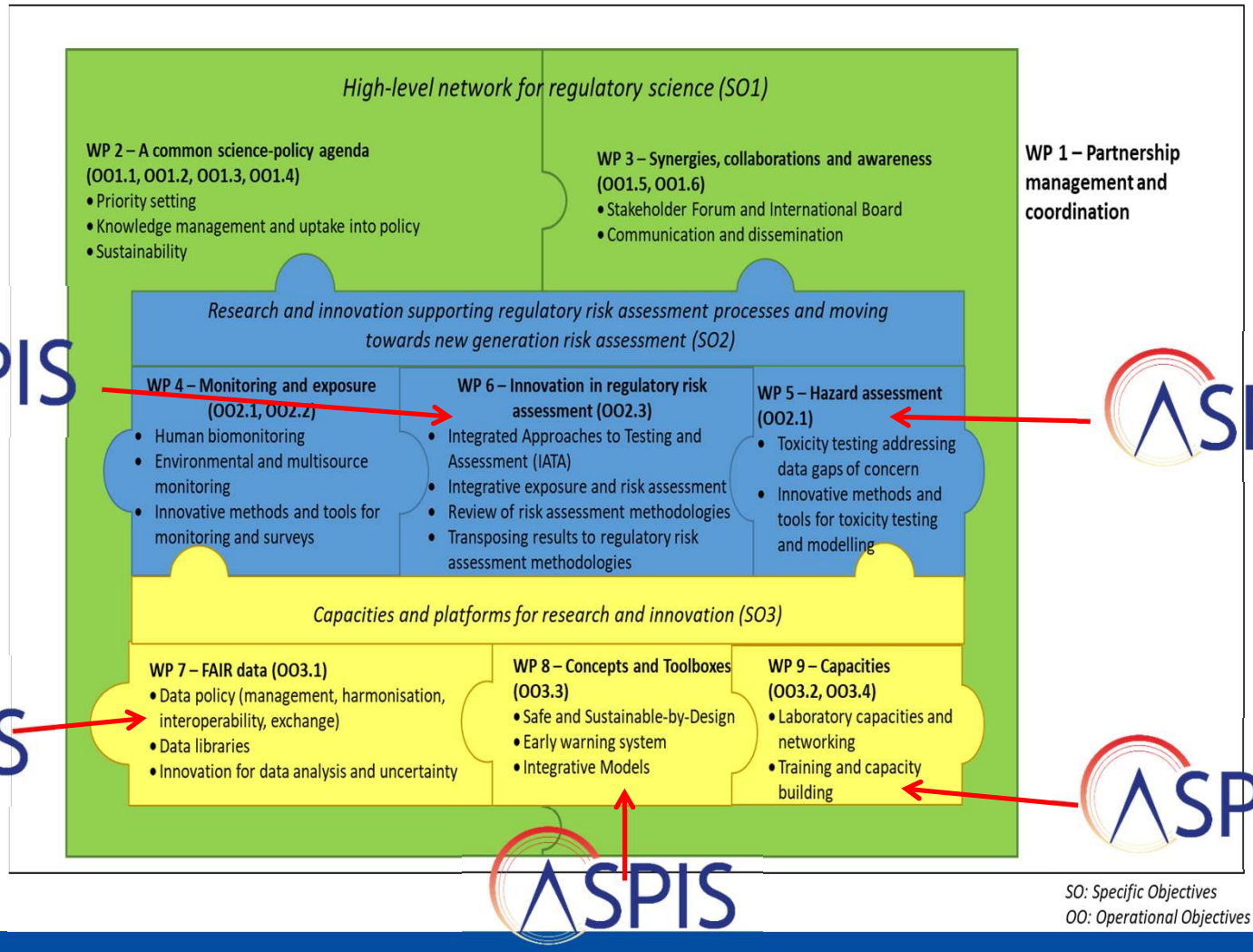
PARC Objectives

Our general objective is to **consolidate and strengthen** the EU's Research and innovation capacity for chemical Risk Assessment to **protect human health and the environment**

1. High level network for regulatory science interface
2. Research & Innovation towards Next Generation Risk Assessment
3. Capacities and Platforms

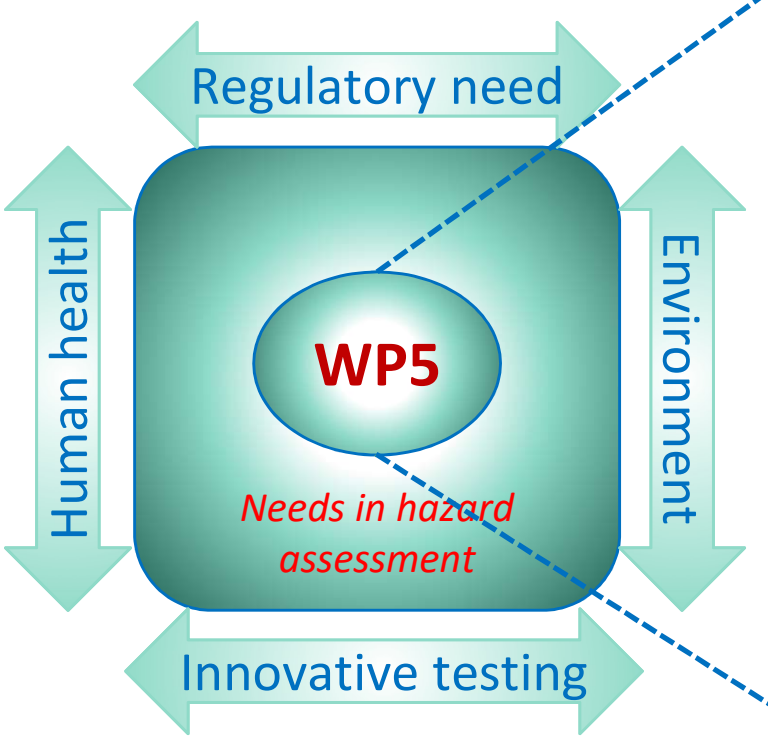
Co-funded by the European Union

PARC: overview in workpackages and tasks



Co-funded by the European Union

WP5 Hazard Assessment: topics and tasks



5.1 Closing data gaps
Regulatory concern
OECD TG studies

5.2 Innovative methods
New approach methods
Mechanism-driven
Reduce animal testing

5.3 Quantitative systems toxicology
Integration in vivo & in vitro / toxicokinetics & toxicodynamics, PBPK
Correlation to human health effects
Development of AOPs

NAMs:

omics:

PBK / QIVIVE:

AOPs:

QST:

WP5 Horizontal organisation: the workstreams

Workstream 1: Substance oriented

Toxins | EDC: | (PFAS) | (Nano) | (Pest) | (nn)

- Substances prioritised for closing critical data gaps
- Application of OECD TG studies to close data gaps
- Focus on substance specific data generation
- Results also to be used e.g. for NAM development in WS2+3

Workstream 2: Endpoint oriented

Immun-Tox | NGtx-Carc | ED:TH | MDC | DNT | (nn)

- Endpoints prioritised for method development (endpoints of concern)
- Development of mechanism based in vitro NAM
- Focus on endpoint specific method development
- Methods also to be used e.g. to generate substance data in WS1

Workstream 3: Regulation oriented

IATA | OECDTG | AOP | (nn)

- Regulatory need for TG development
- Fit for purpose?
- Focus on regulatory application of NAM/models, but
- Results also to be used in WS1+2

Developmental neurotoxicity: ONTOX RISK HUNT3R

Non-genotoxic carcinogenicity: RISK HUNT3R

WP6 Innovation in regulatory risk assessment

Task 6.1: To establish **quantitative AOP networks** and assess their **human relevance** using a **pragmatic workflow**. This output is then used for the development of **IATAs for selected health effects** and for different regulatory settings, incl. the workplace. Testing will rely on ***in silico*** **and** ***in vitro*** **NAMs** where possible. The performance of the IATAs will be **evaluated** using **case studies**, to ensure regulatory applicability and to provide successful examples.

Joint annual WP6 workshop with stakeholders to collect feedback & input

Task 6.2: To perform integrative exposure assessments to better understand the risk of aggregated exposure to single chemicals and mixtures for humans. Exposures to single chemical and mixtures (activity 6.2.3) will be modelled from multiple sources and routes (activity 6.2.1), through entire life (activity 6.2.2) and used to calculate different indicators aiming to measure the impact of the chemical strategy on human health (activity 6.2.4).

WP6 Innovation in regulatory risk assessment

Task 6.3: to review the **performance** and **efficiency** of current provisions, methodologies and processes employed under different regulations, including **consequences** of the reasonings and approaches, in order to identify and address potential **gaps and inconsistencies**, and to suggest **improvements** and **harmonization** when appropriate.

Joint annual WP6 workshop with stakeholders to collect feedback & input

Task 6.4: to use science to meet **regulatory needs** to contribute to the development of **regulatory and legally accepted risk assessment and management approaches and methods** for chemical **mixtures** (6.4.1); to facilitate the regulatory acceptance and practical use of **new methods for hazard and risk assessment** (6.4.2); to connect chemical inventories with trade and waste statistics to assess the flow of **chemicals in articles** (6.4.3.); to developments of risk assessment to support and promote efficient overall **protection of biodiversity** (6.4.4)

WP7 FAIR data

Task 7.3 Innovative data analyses

Introduce **new methods –** exploit **heterogeneous** available datasets

Make all methods available as FAIR objects → reusable (collaboration with Task 8.3)

Combining heterogeneous data

- a. Combined (meta-, Pooled) data analyses
- b. Development of methodologies to combine and integrate hazard and exposure-response information across and within evidence domains

Uncertainties and their propagation when combining different sets of data

Integrating data needs consideration of the uncertainty in each of the data and data steps in the process

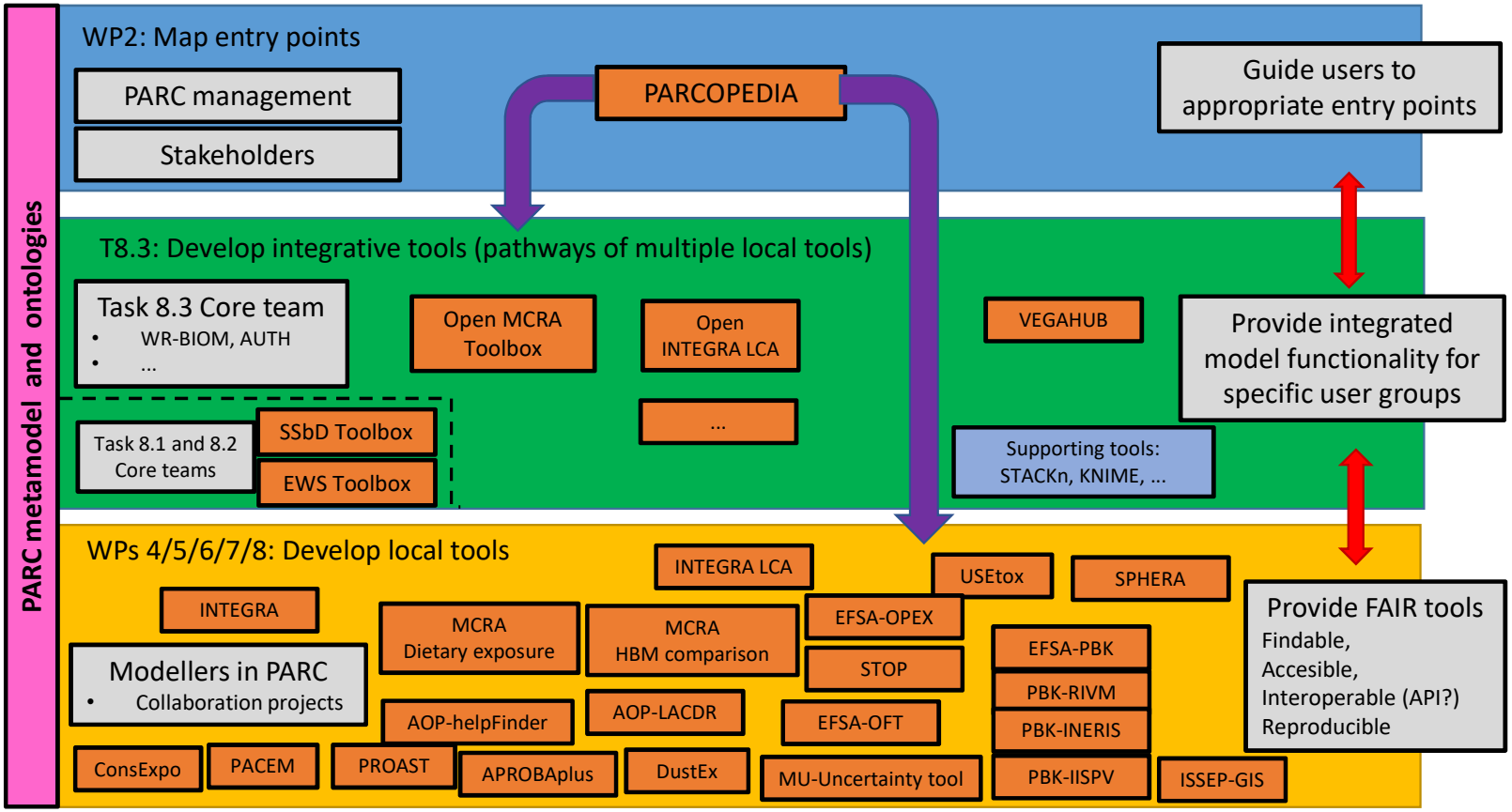
- exposure assessment - hazard evaluation - risk assessment - health impact assessment

Knowledge mining and integration of FAIRified data

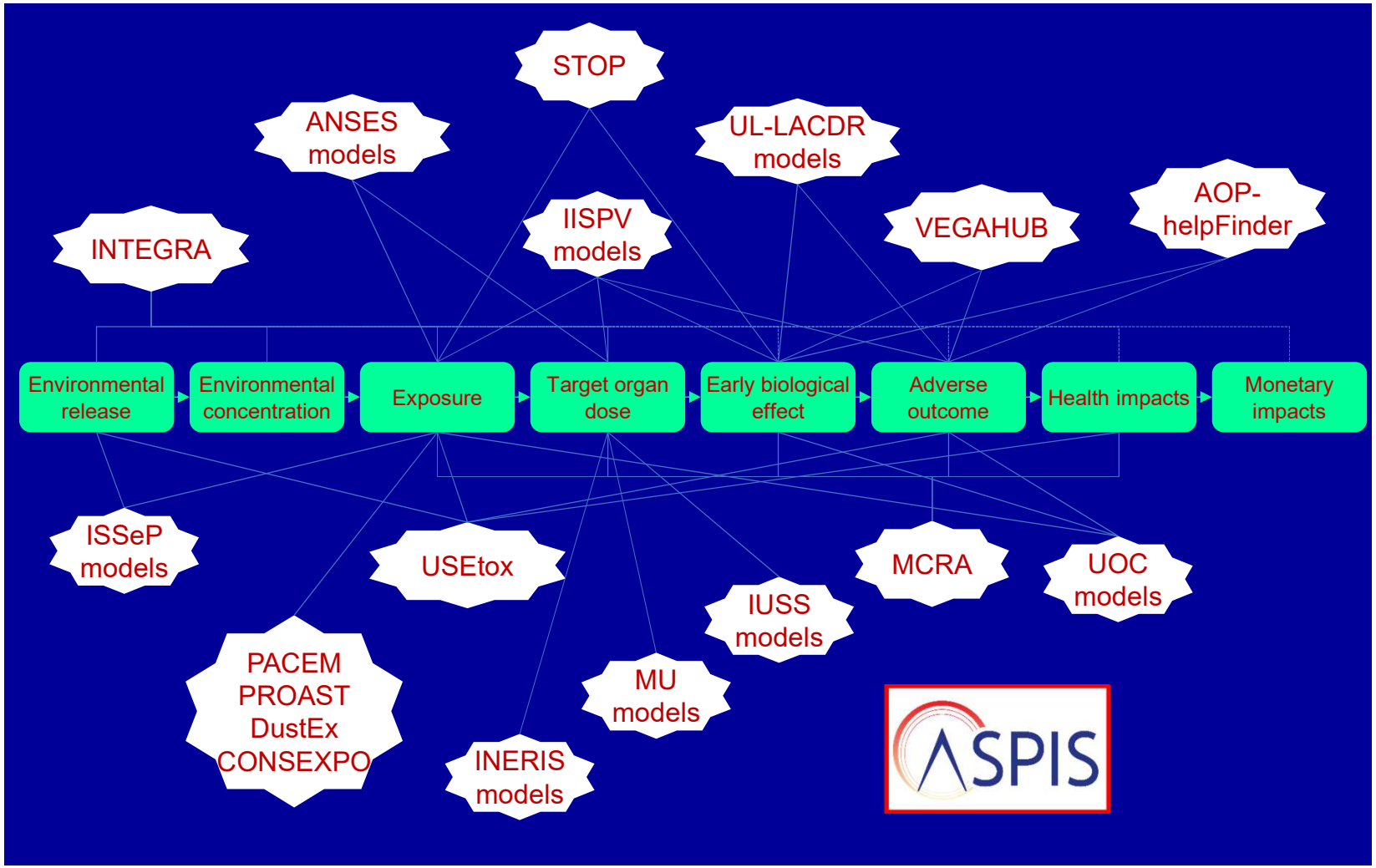
Integration and interpretation of enormous amounts of data and knowledge outpace the capacity of the human mind

- a. AI, text mining and ontology assisted hazard identification and risk assessment
  b. Rationalisation of new data: efficiently integrate new knowledge in existing body of knowledge

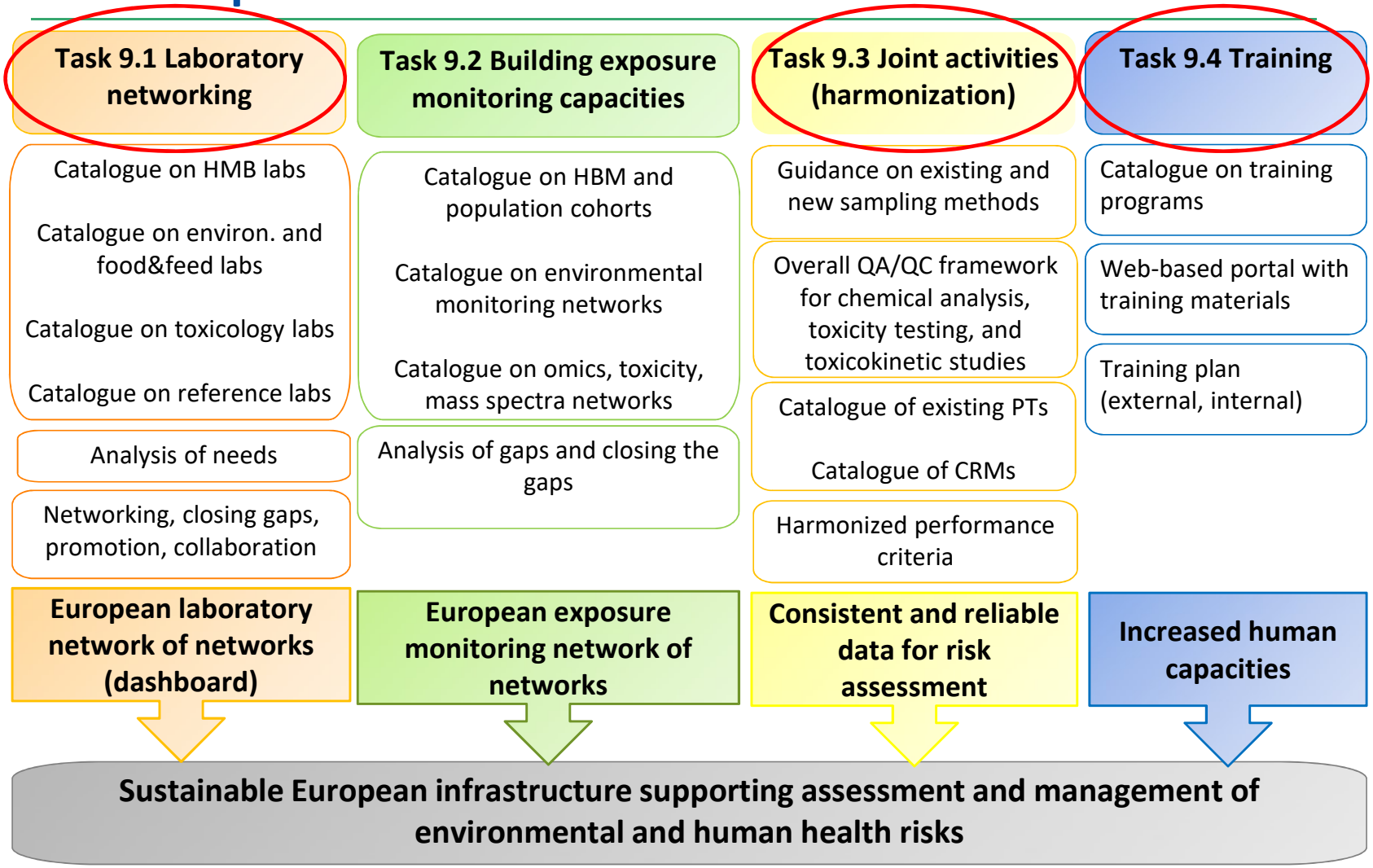
WP8: Draft for integrative model network in PARC



Task 8.3 – Integrative models / model constellation



WP9: Capacities



ASPIS and PARC

Mutual consultation:

- Advance regulatory acceptance of NAMs
- Integrative models
- FAIR data
- Identification of gaps/needs for NGRA

Find **synergies** in case studies and development & evaluation of ASPA
Collaboration: workshops, events

Training of end-users and next-generation professionals

Pascal Sanders
John Colbourne
Mathieu Vinken
Bob van de Water
Philip Marx-Stölting
Gilles Rivière

PARC Coordination Team: PARC@anses.fr

Mirjam Luijten: mirjam.luijten@rivm.nl

Animal-free
Safety assessment of Chemicals:
Project cluster for
Implementation of novel
Strategies

ASPIS

Themes

Culture of the Cluster
Science of the Cluster
Regulatory Impact of the Cluster

Revolutionary defensive tool, designed to push forward into the opposing army, joining forces for better protection

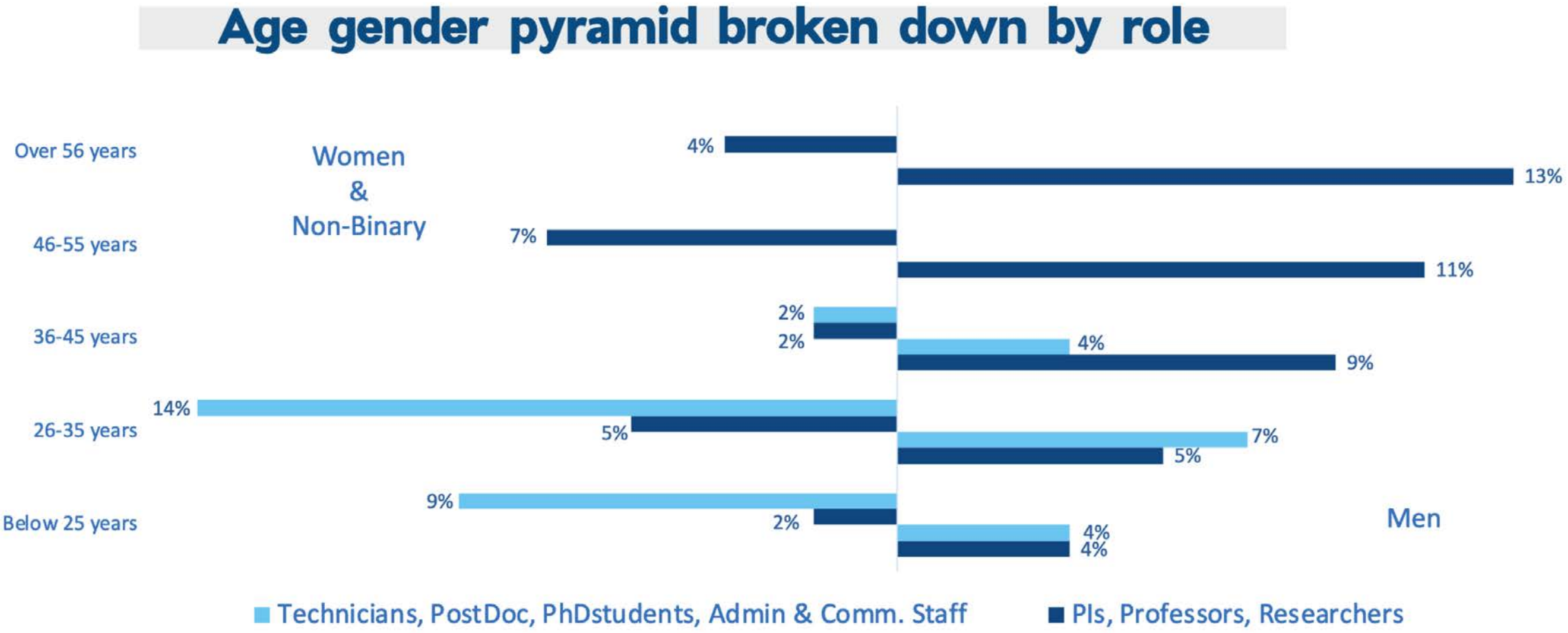
ASPIS Culture

SPIS CADEMY

Early-Stage Researchers Cluster Network

Equality, Diversity and Inclusion

ASPIS Culture



ASPIS Working Groups

Communication and Dissemination

Kinetics and Exposure

Computational Approaches

qAOP

Chemical Selection

Risk Assessment

Omics

ASPIS Science

Steatosis Case Study

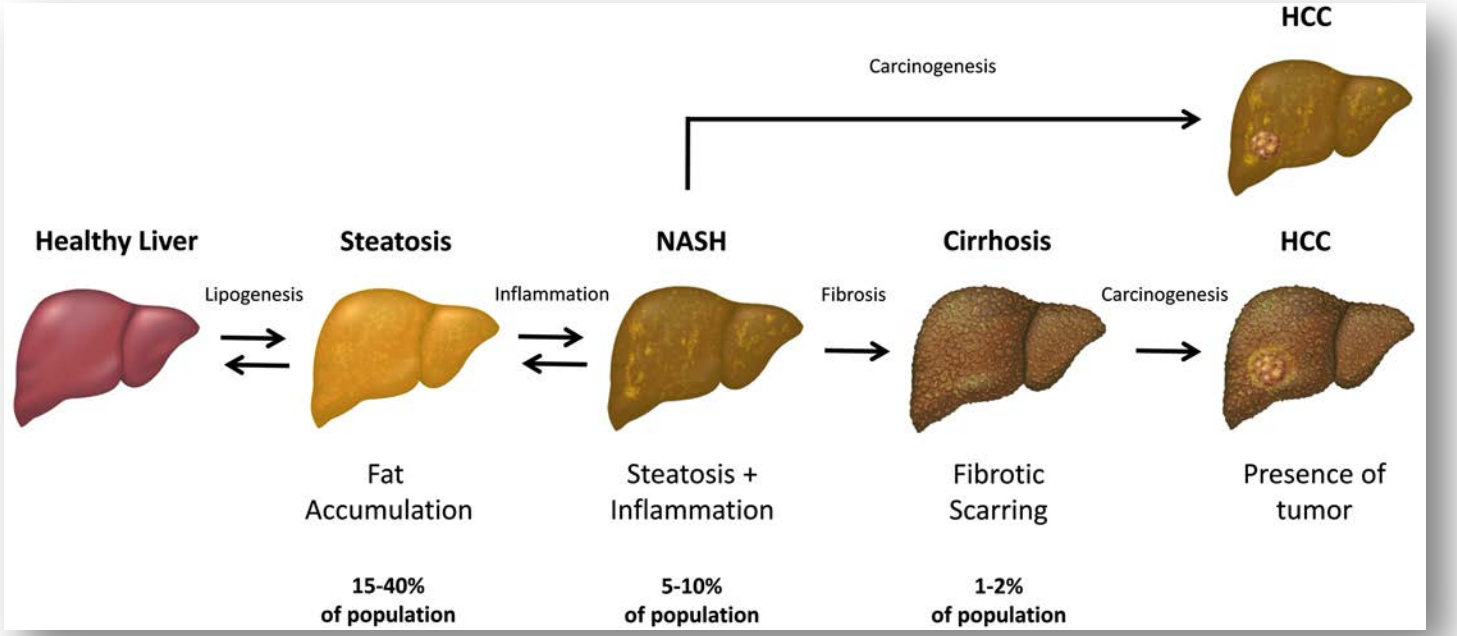


Table 1. Names of Nuclear Receptors associated with liver injury and abbreviations.

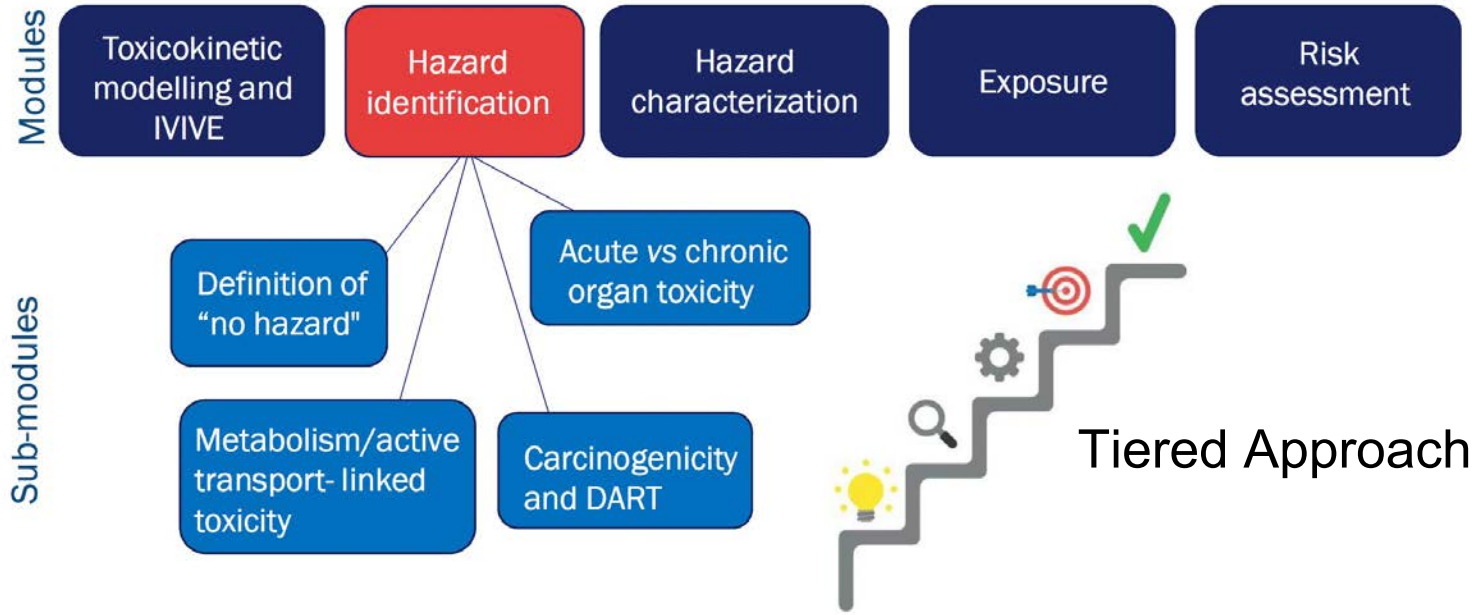
Nuclear receptor name	Abbreviation
Arhyl hydrocarbon receptor	AHR
Constitutive androstane receptor	CAR
oestrogen receptor	ER
Farnesoid X receptor	FXR
Glucocorticoid receptor	GR
Liver X receptor	LXR
Peroxisome proliferator-activated receptor	PPAR
Pregnane X receptor	PXR
Retinoic acid receptor	RAR

Certainly many modes of action

3Rs

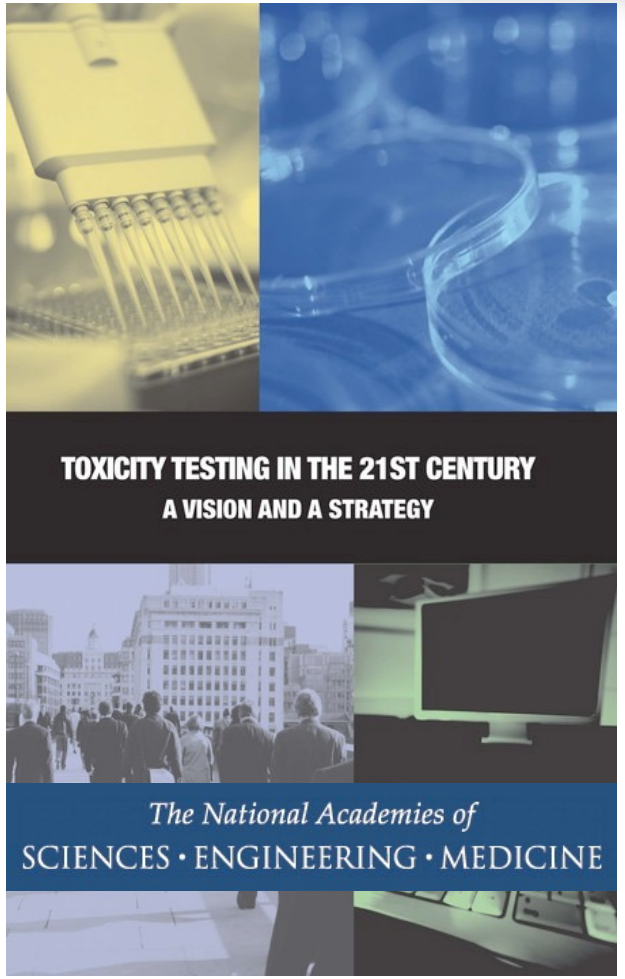
ASPIS Regulatory Impact

ASPA modules (under construction)



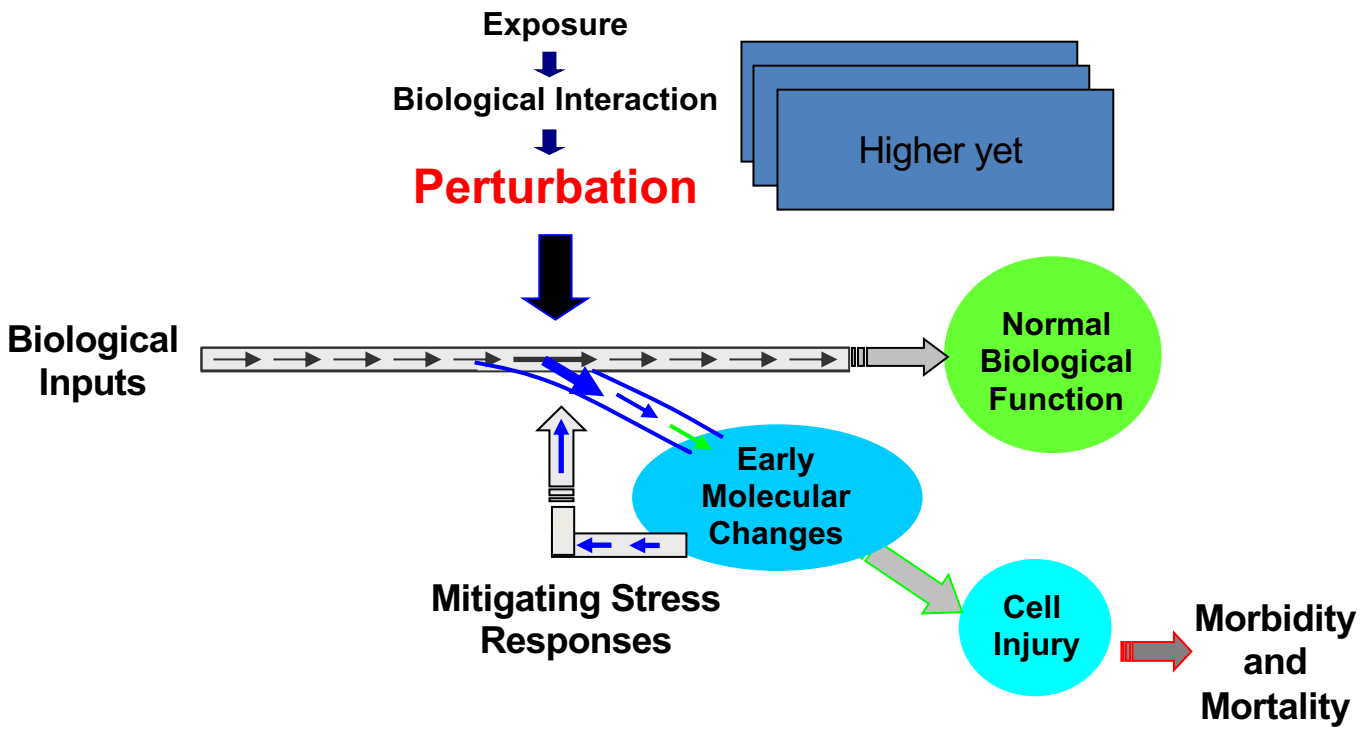
Tiered Approach

ASPIS Safety Profiling Algorithm (ASPA)



Partnerships, Awareness and Training

The New Paradigm: Activation of Toxicity Pathways



Toxicity Pathway: A ~~cellular~~ molecular response pathway that, when sufficiently perturbed, is expected to result in an adverse health effect.

ASPIS Regulatory Impact

ASPIS does not rely on the strength of the science alone to engineer change

Our Mission: Build understanding, build trust

More Awareness and Practical Training

Mitigate Risks

Plan Implementation Including More Resources to Regulatory Agencies

Involve the Public in Shaping Safety Perception (e.g., Probabilistic Risk Assessment)

Increase Accountability for Decisions

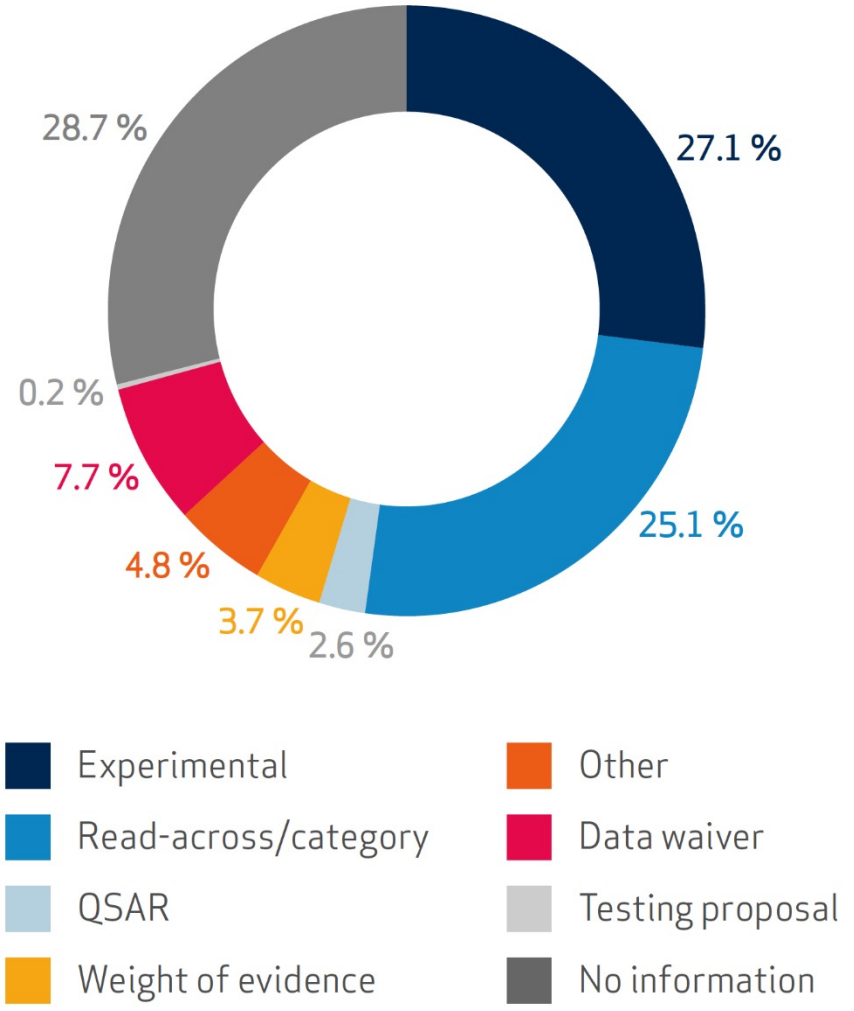
What is Science, What is Policy?

Main legal instruments to avoid animal testing (2006)

- Data sharing and joint submission
- Adaptation possibilities of REACH (Annex XI 1)
- Testing proposals and third-party consultations

Many adaptations are “NAMs”

1. use of existing data;
2. use of a weight-of-evidence approach;
3. information generated using quantitative structure activity relationships;
4. *in vitro* (using human cells) test methods; and
5. grouping of substances for read-across.



What do you perceive are the opportunities/challenges to including NAMs?

Opportunities

- NAM data could substitute toxicity and MoA data in grouping/read-across
- NAM could help improve hazard assessments and reduce animal use
- It may save money for applicants under the Biocides regulation
- It generates more useful data to include when building a weight-of-evidence assessment

Challenges

- Such approaches need to be accepted within the regulatory area
- Lack of safety data on grouping
- Not enough knowledge on what the generated data indicate
- The method used to produce the NAM data has not been validated (lack of information on repeatability and transferability)

General NAMs Criteria (under construction)

- Deliver information on bioactivity/chemical modes of action
- Reproducible within and across laboratories, tested chemicals, and biological test systems
- Independent / peer reviewed method
- Method fully described, including limitations and chemical domain
- Reporting in accordance with accepted templates
- Data shared via open access

Opportunities for Early Adopters of NAMs

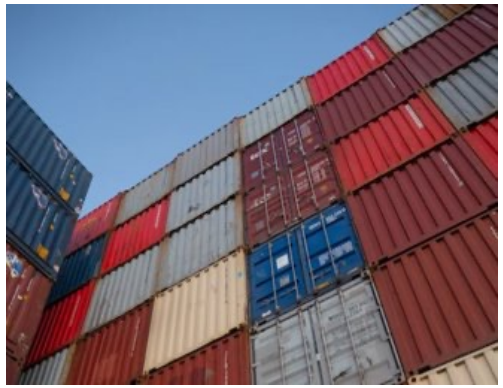
We do not rely on the strength of the science alone to engineer change

Societal

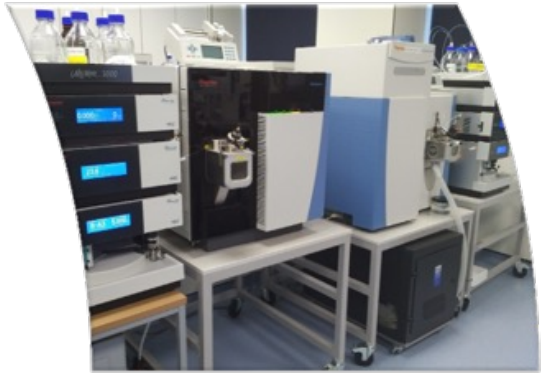
- **Protective – health and environment, sooner**
- **Ethical, 3Rs**
- **Opinion leaders**

Economic

- **Market readiness**
- **R&D investment to implementation**
- **Avoid “laggard penalty”**

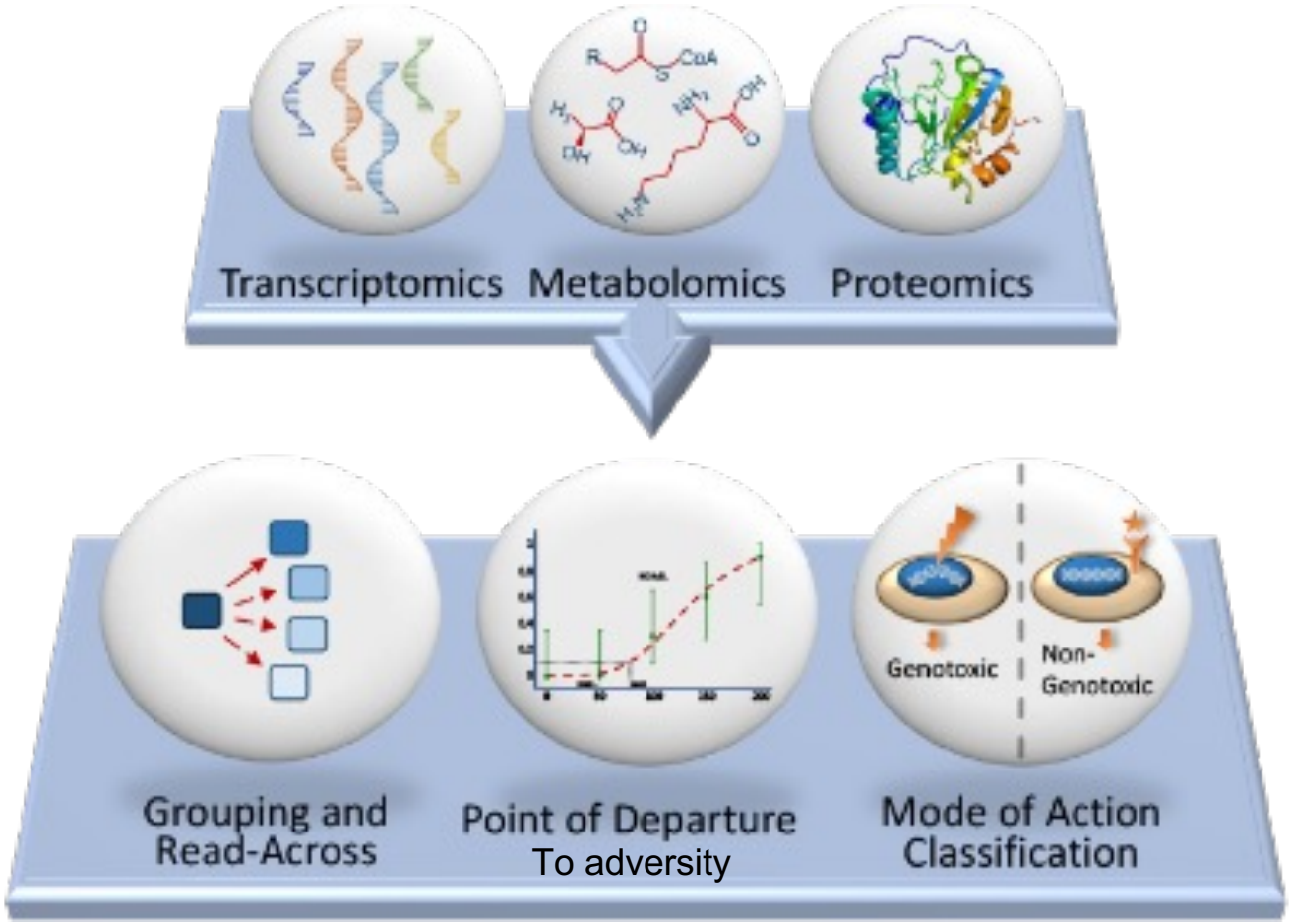


Technological



- **UK plc – Global leadership**
- **Early export readiness**
- **Gather information – work closely with innovators, more support**

The technology is established but the uses are new to regulation



https://www.oecd.org/chemicalsafety/testing/omics.htm

Regulatory Context of 21st Century Toxicology

- Shift in focus away from apical outcomes in experimental animals towards important perturbations of toxicity pathways
- Development of risk assessment practices based on pathway perturbations
- Re-interpretation or possible re-writing of regulatory statutes under which risk assessments are conducted


RISK HUNT3R
ONTOX
PRECISION TOX
European Partnership for the Assessment of Risks from Chemicals (PARC)
SPIS

ASPIS Open Symposium

24-25 November 2022

Sitges, Spain

Poster session

Abstract booklet

Booklet index

NAMs for chemical risk assessment

Poster #1: ASPIS chemical selection working group

Author(s): Jonathan Freedman, Mathieu Vinken & Chemical Selection WG

Affiliation: University of North Carolina at Chapel Hill, NC & Vrije Universiteit Brussel-Belgium

Project: PrecisionTox & ONTOX

Abstract:

The ASPIS Chemical Selection Working Group (CSWG), co-chaired by Drs. Mathieu Vinken (ONTOX) and Jonathan Freedman (PrecisionTox) has the goal of coordinating chemical selection and chemical-focused activities in the ASPIS consortia. To accomplish these goals, the CSWG began collecting information on chemical nominees selected by ONTOX, PrecisionTox and RISK-HUNT3R. To most efficiently use this information, a Database/Artificial Intelligence sub-working group was established. The database/AI sub-group, co-chaired by Drs. Barry Hardy (RISK-HUNT3R) and Marc Teunis (ONTOX), is responsible for creating an ASPIS-wide chemical database and developing cutting-edge, AI-based technologies to mine the literature for toxicological information. This database initially contained physicochemical characteristics and toxicological information on chemicals selected by ASPIS. In the future, it will expand to include chemical information from ASPIS collaborators including PARC, ECHA, JRC and the US NTP. As database needs of ASPIS expanded, this subgroup evolved into the new ASPIS Database Working Group (DbWG). The goal of the DbWG is to address database needs beyond chemical selection/chemoinformation and to develop and deploy related biological and toxicological knowledge. Additionally, it aims to expand the ASPIS database to form an international chemical knowledge resource that will support predictive toxicology, risk assessment guidance, policy development, communication and computational approaches. The second major activity of the CSWG is to support ASPIS-wide projects including the Steatosis Case Study. Activity on the Steatosis Case Study currently involves the three consortia and most of the ASPIS working Groups. The next case study being discussed by the CSWG will focus on developmental neurotoxicants. Chemical selection can be an essential component in the development of NAMs and NGRA. For this reason, the CSWG is working closely with the ASPIS Risk Assessment, Computational Approaches and qAOP working groups. Additionally, it has strong interactions with the JRC Regulatory Forum.

Poster #2: The ASPIS risk assessment working group

Author(s): Stefan Scholz & Risk Assessment WG team

Affiliation: Helmholtz Centre for Environmental Research

Project: PrecisionTox

Abstract:

The development of a Next Generation Risk Assessment (NGRA) faces two major challenges, (1) the identification of opportunities to apply NAMs (new approach methods) and replace animal testing already for a transition period under existing legal frameworks for the regulation of chemicals and (2) the need to change the current paradigms in hazard and risk assessment towards a system that allows for an increased use of NAMs.

The three ASPIS projects – ONTOX, PrecisionTox and RISK-HUNT3R – have complementary approaches on how to use NAMs for the hazard and risk assessment of chemicals including also prioritisation, grouping/read-across and hazard characterisation. The ASPIS working group on risk assessment intends to share and link the different approaches, coordinate joint activities and support the development of an ASPIS-wide framework for NGRA. The mission of the RA working group is to (i) critically compare ASPIS research results to previous activities for promoting NAMs (ii) benchmark the ASPIS approaches and results with other similar initiatives, (iii) plan for joint/coordinated activities, (iv) connect research activities in the ASPIS cluster, (v) consider the perspective of end-users and stakeholders and (vi) link and ensure complementarity of ASPIS PARC (Partnership for the Assessment of Risk from Chemicals) to support EU and national chemical risk assessment and risk management bodies for the transition to a next generation risk assessment. Therefore, the ASPIS RA group has started to review various current RA frameworks, contributed to the ASPIS discussion on NGRA (e.g. via meetings, workshops on an NGRA template and the ASPIS Open Science Symposium). Furthermore, we will use ASPIS-wide or project-specific case studies to demonstrate how NAMs can be used in RA, identify gaps or needs for improvement and compare results with the results of traditional animal test-based approaches.

Poster #3: Human peripheral neurons with enhanced nociceptor features for the study of pain-related dysfunctions

Author(s): Anna-Katharina Holzer

Affiliation: Universität Konstanz

Project: RISK-HUNT3R

Abstract:

In vitro models of the peripheral nervous system would benefit from further refinements to better support studies on neuropathies. In particular, the assessment of pain-related signals is still difficult in human cell cultures. Here, we harnessed induced pluripotent stem cells (iPSCs) to generate peripheral sensory neurons enriched in nociceptors. The objective was to generate a culture system with signaling endpoints suitable for pharmacological and toxicological studies. Neurons generated by conventional differentiation protocols expressed moderate levels of P2X3 purinergic receptors and only low levels of TRPV1 capsaicin receptors, when maturation time was kept to the upper practically-useful limit of 6 weeks. As alternative approach, we generated cells with an inducible NGN1 transgene. Ectopic expression of this transcription factor during a defined time window of differentiation resulted in highly-enriched nociceptor cultures, as determined by functional (P2X3 and TRPV1 receptors) and immunocytochemical phenotyping, complemented by extensive transcriptome profiling. Single cell recordings of Ca2+-indicator fluorescence from >9,000 cells were used to establish the "fraction of reactive cells" in a stimulated population as experimental endpoint, that appeared robust, transparent and quantifiable. To provide an example of application to biomedical studies, functional consequences of prolonged exposure to chemotherapeutic drugs were examined at non-cytotoxic concentrations. Oxaliplatin was found to induce (i) neuronal (allodynia-like) hypersensitivity to otherwise non-activating mechanical stimulation that could be blocked by modulators of voltage-gated sodium channels; (ii) hyper-responsiveness to TRPV1 receptor stimulation. Moreover, proteasome inhibitors, such as bortezomib and carfilzomib, exhibited a distinct pattern of toxicant-induced alterations in the neurons. Attenuation of P2X3 signaling, increased levels of resting intracellular [Ca2+], and a reorganization of tubulin to dense structures around the cell somata were characteristic of proteasome inhibitor-induced cell stress. These findings indicate that the model is suitable for pharmacological and toxicological studies related to peripheral neuropathies..

Poster #4: Use of Alamar Blue test for High-Throughput Energy Expenditure Monitoring in Daphnia magna

Author(s): Rubén Martínez

Affiliation: LEITAT

Project: PrecisionTox

Abstract:

Alamar Blue (AB) test is based on the reduction of the resazurin (oxidized form) to resorufin (reduced form) by NADH produced by living cells. It has been widely used during years to measure in vitro cell viability and recently it has been adapted to measure oxidative metabolism - related energy expenditure in whole zebrafish (Danio rerio) embryos and juveniles.

Our goal is to perform a new optimization of this test in Daphnia magna, since as far as we know, it has not been reported before and it could be an interesting phenotyping for Daphnia in PrecisionTox.

With this objective, AB LC50 value was determined in Daphnia. Afterwards, using 3 different appropriate AB concentrations, tests were performed, exposing Daphnia juvelines during 48 h (from 4 to 6 days) to DMSO and caffeine (3 different concentrations: LC10/5; LC10/2, and LC10), measuring the absorbance differences.

DMSO decreased energy expenditure while caffeine increased it, aligning with the expected results (considering that energy expenditure should be directly related with the locomotion, and that DMSO decrease the movement of Daphnia at high doses, and taking into consideration the stimulant effect of the caffeine).

As a conclusion, AB test could be suitable for a fast and 'high-throughput' Daphnia magna energy-expenditure monitoring test. Variability of results reduces, and its quality improves significatively when fluorescence is used, instead of absorbance.

Poster #5: The vibration/startle assay: a semi-automated behavioural assay to assess toxicity in zebrafish embryos

Author(s): Gaëlle Hayot

Affiliation: Karlsruher Institut für Technologie

Project: PrecisionTox

Abstract:

Zebrafish (Danio rerio) are small freshwater fish that can be found in small streams of northern India. Adult zebrafish measure between 2 and 5 cm and can live up to 5 years in laboratory conditions. As vertebrates sharing 70 % of their coding genes with humans, zebrafish are widely used in research for modelling the effects of drugs on human health. Their small size, external development, transparent embryos, easy husbandry and breeding, and the large number of eggs they lay make them a valuable model for drug screening.

We developed a system to rapidly and reliably screen chemicals based on a behavioural phenotype: the startle response to a vibration stimulus. This behaviour depends on the lateral line, an organ homologous to the inner ear in humans. In zebrafish, the lateral line enables sensing of water movement and vibration, which allows the fish to respond to its environment: for example, by eliciting an escape response from a predator and in prey capture.

Motility in response to a vibration is a broad phenotype: it depends on the sensing system (the lateral line and the central nervous system) and the reacting organs (the muscles and the motoneurons). Absence of motility can be due to the death of the embryos or to a compromised motor reaction induced by a drug. Thus, this phenotype allows us to screen for chemicals that impact different organs and pathways: for example, we are able to detect neurotoxicants and drugs affecting the muscles, but also compounds targeting gills or liver, which can lead to the death of embryos.

Our new modular, low-cost and open source vibration / startle system will be very helpful to screen chemicals for various purposes, in a high throughput fashion, and can also be used to detect behavioural phenotypes in mutant zebrafish lines.

Poster #6: A cross-species comparative look at the toxicity of acrylamides and imidazoles

Author(s): Gaëlle Hayot

Affiliation: Karlsruher Institut für Technologie

Project: PrecisionTox

Abstract:

The REACH regulation aims at improving the protection of human health and the environment from the risks that can be posed by chemicals. However, the high number of compounds to be tested to fulfill REACH goals can make this look like an impossible task. To accelerate the testing of chemicals, one strategy could be to group compounds that share structural similarities and to test only a subset of each group. This strategy relies on the assumption that compounds with similar structures will have similar toxicity. To test this hypothesis, the Precision Tox consortium tested two different sets of compounds, acrylamides and imidazoles, to assess whether the toxicity was similar within each group. In a first step, we evaluated toxicity by examining the test compound effects on cell proliferation of human liver cells (HepG2) and on the zebrafish embryo startle response, a behavioural assay. Our preliminary results show that even slight modifications of a molecule can lead to substantial changes in toxicity. Moreover, compounds from the imidazole group showed pH-dependent toxicity. Further work carried out within the Precision Tox consortium will examine how acrylamides and imidazoles impact metabolism and gene transcription across a diverse set of model organisms, and anchor these OMICS data by performing further assays targeting various toxic endpoints. By this “phylotoxicology” approach, we aim to provide a rich data set for the prediction of adverse chemical effects on humans. We expect that our results can help regulators to make informed decisions on appropriate strategies to abide by the REACH regulation.

Poster #7: Differential Cadmium Chloride Toxicity Across Twenty Daphnia magna Clones

Author(s): Marianne Barnard

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Daphnia magna is recommended by the OECD as a test species for acute ecotoxicity testing. Although EC50 responses to toxicants can vary across different clonal lines, test guidelines do not specify a particular D. magna genotype. Not all laboratories use the same clonal lines when conducting toxicity tests. The genetic variation between the various clones may contribute to significant variation in reported EC50 values for chemicals.

Poster #8: Quantitative and qualitative detection of cytotoxicity by fluorescence microscopy in human cells.

Author(s): Schwab Marian

Affiliation: Karlsruhe Institute of Technology

Project: PrecisionTox

Abstract:

Cell-based assays are a popular choice for and an important component of risk assessment.

The epithelial–like human hepatocyte cell line HepG2 is an established model to study metabolism, cytotoxicity and genotoxicity. Utilizing the Automated High-throughput Microscopy Assay (AHM), chemical libraries, such as the one established for the Precision Toxicology Project, can be screened in 96-well assay plates cost efficiently, quickly and reliably.

In the AHM assay, a combination of different DNA stains provides information on diverse cellular endpoints, such as altered cell proliferation, cell viability and mode of cell death. The morphological assessment on the single cell level allows for further identification of adverse phenotypes in cells.

Robust dose-range data allow for subsequent investigation of interesting compounds with regard to cell signalling, oxidative stress, reactive oxygen species and markers of DNA damage.

Poster #9: Advanced in vitro model for drug induced kidney injury assessment - generation of kidney organoid for safety assessment purposes

Author(s): Lukas Wijaya

Affiliation: LACDR

Project: RISK-HUNT3R

Abstract:

Developing in vitro kidney injury model remains a challenge due to the complex architecture of the whole organ. Here, we adapted the Takasato IPSC-derived kidney organoid protocol to generate an advanced high throughput in vitro test method for chemical-induced kidney injury model. This adapted protocol allows us to create larger quantities of kidney organoids with higher compatibility to live cell confocal imaging. The newly generated kidney organoids exhibited a coherent formation of nephron segments including glomerulus, proximal tubule, and distal tubule. Moreover, the kidney organoids also showed clear cellular responses that reflected our understanding of mechanisms of cisplatin-induced kidney injury in vivo. We have established a panel of CRISPR-engineered GFP reporters for various cellular stress response pathways. As a proof-of-concept for application of these reporters in kidney organoids, we first evaluated a DNA damage response iPSC reporter line, iPSC-CRISPR-GFP-p21. Cisplatin-induced GFP-p21 induction was observed in proximal tubular regions of the organoids, but not in glomerular cells. We anticipate that these kidney organoids can be used as an in vitro high throughput test systems to monitor chemical-induced nephrotoxicity.

Poster #10: Physiological maps and chemical-induced disease ontologies: tools to support NAMs development for next-generation risk assessment

Author(s): Luiz Ladeira

Affiliation: Liège Université

Project: ONTOX

Abstract:

Physiological maps (PM) can be defined as a graphical representation of cellular and molecular processes associated to specific organ functions (Vinken et al. 2021). Within the ONTOX project, we designed a total of 6 PMs describing physiological processes in the liver, the kidney and the brain. These PMs are then used as a tool to assess relevant mechanistic coverage and linkage between a specific organ function and a toxicological endpoint. Based on the Disease Maps project (Mazein et al. 2018) pipeline, we developed the first version of 6 PMs describing the following physiological processes: bile secretion & lipid metabolism (liver), vitamin D metabolism & urine composition (kidney), neural tube closure (update of the work of Heusinkveld et al. 2021) & brain development (brain). Our workflow included: (i) data collection from expert curated literature, (ii) identification of the relevant biological mechanisms, (iii) screening of online databases (e.g. Wikipathways, Reactome, and KEGG) for previously described pathways, (iv) manual curation and integration of the data into a PM using CellDesigner, and (v) visualization on the MINERVA platform (Hoksza et al. 2019). These qualitative PMs represent an important tool for exploring curated literature, analyzing networks and benchmarking the development of new adverse outcome pathways (AOPs). These PMs provide the basis for developing quantitative disease ontologies, integrating different layers of pathological and toxicological information, chemical information (drug-induced pathways) and kinetic data. The resulting chemical-induced disease ontologies will provide a multi-layered platform for integration and visualization of such information. The ontologies will contribute to improving understanding of organ/disease related pathways in response to chemicals, visualize omics datasets, develop quantitative methods for computational disease modeling and for predicting toxicity, set up an in vitro & in silico test battery to detect a specific type of toxicity, and develop new animal-free approaches for next generation risk assessment.

Authors: Luiz Ladeira1*, Alessio Gamba1*, Raphaëlle Lesage2, Eliska Kuchovska3, Nicolai Görts3, Anouk Verhoeven4, Jian Jiang4, Jonas van Ertvelde4, Devon A. Barnes5, Manoe J. Janssen5, Job Berkhout6, Daniël Roodzant7, Marc Teunis7, Thomas Bozada Jr8, Thomas H Luechtefeld8, Ramiro Jover9, Tamara Vanhaecke4, Mathieu Vinken4, Rosalinde Masereeuw5, Thomas Hartung10, Ellen Fritsche3,11, Aldert Piersma6,12, Harm J. Heusinkveld6, Liesbet Geris1,2,13#, Bernard Staumont1##

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Hoksza, David, 2019 https://doi.org/10.1093/bib/bbz067.
Mazein, Alexander 2018 https://doi.org/10.1038/s41540-018-0059-y.
Vinken, Mathieu, 2021https://doi.org/10.1016/j.tox.2021.152846.

Poster #11: Re-thinking the concept of spheroids: a new way to generate high-throughput 3D complex liver model

Author(s): Mostafa Kiamehr

Affiliation: KU Leuven

Project: RISK-HUNT3R

Abstract:

Here we introduce a simple, fast, and robust method to generate mid to high-throughput 3D cultures in a soft hydrogel for iPSC-derived hepatocytes (HC3X) alone or together with iPSC-derived non-parenchymal cells. 3D Heps expressed significantly higher levels of key hepatic markers (e.g. CYP3A4, HNF4α, and PEPCK) compared to 2D Heps, HepG2 cells and conventional spheroids. 3D Heps also stained positive for CK18, PEPCK, CYP3A4, MRP2, HNF4α, and ALB. Co-culture of HC3x-heps with iPSC-ETV2-SPI1 endothelial cells in 3D resulted in formation of interconnected vascular networks where the highly polarised (showed by positive MRP2 staining) hepatocytes were in close proximity of endothelial cells. The tri-culture of HC3x-heps, iETV2/SPI1-ECs, and iPSC-macrophages (M□) resulted in an even more physiologically relevant model. Treatments with Lipopolysaccharide (LPS) significantly upregulated L1-α, IL6, and TNFα and the secretion levels of IL6 and TNFα was increased by ± 100-fold. When exposed to Rifampicin, significantly higher expression of CYP3A4 was observed. Last but not least, treatment of the 3D models with excess fatty acids resulted in substantial uptake of fatty acids and deposition into lipid droplets resembling the steatosis stage in the liver. Taken together, we developed methods to generate functional 3D cultures encompassing iPSC-heps and NPCs in robust, consistent and high throughput manner making it a great candidate model for drug screening, toxicity and/or disease modelling and specifically hepatic steatosis.

Poster #12: The rosette formation assay as a method to identify DNT hazard due to disruption of the RAR/RXR pathway (endocrine signaling)

Author(s): Nadine Dreser

Affiliation: University of Konstanz

Project: RISK-HUNT3R

Abstract:

Retinoic acid is an important endocrine signal, and humans are exposed to a large variety of compounds that affect retinoid signaling. Due to the many retinoid receptors and also metabolic steps of interconversion of metabolites, an adverse outcome pathway network needs to be established and verified. This is an important addition to an overall NGRA strategy for DNT. Here we use the neural rosette formation assay (UKN1; RoFA) to assess retinoid analogues causing neural tube toxicity. By analysis of the acute transcriptome response to retinoid treatment we aim to causally link RA signaling to disturbance of neural tube formation as well as to classify DNT compounds according to a retinoid signature.

Nadine Dreser, Marion Kapitza, Christiaan Karreman, Jonathan Blum, Marcel Leist

In vitro Toxicology and Biomedicine, Dept inaugurated by the Doerenkamp Zbinden Foundation, University of Konstanz

Omics approaches

Poster #13: ASPIS Omics Working Group

Author(s): Florian Caiment and Omics WG team

Affiliation: Maastricht University

Project: ONTOX

Abstract:

The ASPIS Omics Working Group, co-chaired by Dr. Florian Caiment (ONTOX) and Prof. John Colbourne (PrecisionTox), has the first goal of promoting the application of the initiative recently published to render the use of omics in regulatory risk assessment possible: the OECD omics reporting framework (Harrill JA et al., 2021, PMCID: PMC8808338) and the R-ODAF (Omics Data Analysis Framework for regulatory application, Verheijen et al, 2022 PMID: 35247516.). The omics reporting framework provide a set of specialized modules allowing any transcriptomics or metabolomics experiments to be described in view of regulatory assessment. The R-ODAF is a framework proposing a pipeline to analysis raw transcriptomics datasets, from the raw data to stringent statistical thresholds to be applied for selecting a list of differently expressed genes.

The second major activity of the Omics WG is to contribute to the steatosis Case Study by assembling a list of omics dataset involving compounds identified by the chemical selection WG to be leading to steatosis. The target datasets will initially be selected from the main publicly accessible omics repositories and will be later expended with private datasets generated within the ASPIS cluster by the three consortia. Transcriptomics will be de facto the main source of omics datasets, but proteomics and metabolomics will also be considered if available. The selected datasets will be re-analyzed with a common framework to minimize pipeline dependent variation and will be used to perform several meta-analyses with the ultimate goal to highlight the capacity of omics generated dataset to identify the steatosis potential of a compound based on its expression data. For this, both supervised and unsupervised methodologies will be applied, using notably the resource and expertise assembled by other ASPIS WGs on steatosis.

Poster #14: An introduction to the semi-automated, robotics-based sample extraction workflow facilitating PrecisionTox omics analyses

Author(s): Martin Robert Jones

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Chemical pollution poses significant risks to animal and human health, and has been linked to the premature death of millions of people each year, globally. PrecisionTox, an EU Horizon 2020-funded research project, aims to tackle the scourge of chemical pollution by developing, and advocating for the adoption of, New Approach Methodologies (NAMs) for improved chemical safety assessment. To fulfil this goal, PrecisionTox will generate ca. 10,000 toxicological test samples through exposure of 3Rs-compliant model systems, including Daphnia magna, Drosophila melanogaster, Danio rerio, Caenorhabditis elegans, Xenopus laevis and a human cell line, to 250 independent chemicals at multiple distinct time points. Each will undergo extraction and subsequent transcriptomic and untargeted metabolomics analyses, yielding information-rich molecular datasets from which, for example, predictive toxicological models might be built concerning the relationship between adverse outcomes, toxicological mechanisms of action, compound class / structure and genetics. In the work presented here, we introduce the semi-automated extraction workflow developed to underpin this mission, which provides a seamless link between upstream sample collection procedures and downstream omics analysis pipelines. The workflow can extract up to 96 samples in a single batch, with each sample being homogenised in the tube in which it was collected. Homogenates are transferred to independent wells of a 96-well deep well plate, wherefrom aliquots are taken and quenched on liquid nitrogen for subsequent RNA purification. Uniquely, the remainder of each homogenate undergoes a Bligh and Dyer-like biphasic extraction in 96-well plate format (2:2:1.8 v/v/v methanol:chloroform:water), yielding for each sample, independent polar and lipophilic metabolite pools. With up to two semi-automated extraction batches possible each day, the semi-automated workflow is approximately 4-5 times faster than comparable manual procedures. By virtue of the workflow being largely automated, it is also anticipated that technical variance will be minimised, leading to more reproducible omics datasets.

Poster #15: The renal proximal tubule TXG-MAPR: safety assessment based on quantitative gene network analysis

Author(s): Hugo van Kessel

Affiliation: Leiden University

Project: RISK-HUNT3R

Abstract:

Scientific advances in -omics technologies and ever-increasing knowledge on human biology render pre-clinical in vivo testing not sustainable in the future. In the kidneys, proximal tubule epithelial cells are the primary target for xenobiotic-induced injury due to increased exposure levels, bilateral transporter-mediated uptake and high oxygen consumption. Through concentration and time course chemical exposure of RPTEC-TERT1 cells using >50 nephrotoxicants and reference compounds that cover a wide range of mechanisms of action, and subsequent TempO-Seq whole genome transcriptomics and weighted correlation network analysis, we have established a human RPTEC/TERT1 in vitro kidney TXG-MAPr tool. The TXG-MAPr tool allows user friendly interactive toxicogenomics data interpretation on mechanisms of action and compound activity correlation. Interspecies network preservation analysis using the in vivo rat kidney TXG-MAPr based on TG-GATEs has revealed preserved cellular processes relevant in kidney toxicity. Identification of co-regulated gene networks using high throughput whole genome transcriptomics will provide mechanistic insight in the cellular stress response which can provide mode-of-action formulation based on quantitative gene network analysis and support hazard characterization for NGRA-based safety assessment.

qAOP approaches

Poster #16: ASPIS quantitative Adverse Outcome Pathway Working Group

Author(s): Mark Cronin and qAOP WG team

Affiliation: Liverpool John Moores University

Project: RISK-HUNT3R

Abstract:

The ASPIS quantitative Adverse Outcome Pathway Working Group (qAOP WG), co-chaired by Dr Huan Yang (ONTOX) and Mark Cronin (RISK-HUNT3R), aims to investigate models that (semi-)quantify molecular initiating events (MIEs) or key event relationships (KERs) within existing AOPs using non-confidential data, as well as identifying and sharing good practice. Specific objectives include adding value by developing common ideas for qAOP development; sharing knowledge of dose-responses, data and models between ASPIS partners; and facilitation integration of qAOPs with knowledge of MIEs and physiologically-based kinetics (PBK) modelling to enable quantitative systems toxicology. The outputs from the APSIS qAOP WG aim to increase understanding of how risk assessors would use qAOPs in Next-Generation Risk Assessment (NGRA). This will be achieved through the development of one or more common qAOPs (linear and networks). Initial work has been on a qAOP for liver steatosis with common data and modelling approaches being shared within ASPIS. This will assist in the determination of outputs from qAOPs and degree of confidence needed for NGRA. Further work will identify obstacles and concerns for assessors to use qAOPs in NGRA. A particular focus of the qAOP WG is the development of a framework for validation of qAOPs through the identification of uncertainties. The work presented in this poster was performed as part of the ASPIS Cluster.

Poster #17: In vitro New Approach Methodologies (NAMs) for assessing effects of chemicals leading to cognitive function defects in children – the contributions of the ONTOX project

Author(s): Eliska Kuchovska

Affiliation: Leibniz Research Institute for Environmental Medicine

Project: ONTOX

Abstract:

The current regulatory developmental neurotoxicity (DNT) guidelines (OECD TG 426 & EPA OPPTS 870.630) are not sufficient for the hazard assessment of the vast chemical universe and only a handful of chemicals have been assessed up to date. The main reasons are the high time and cost consumption of the testing according to the current guidelines and the necessity to use animals with difficult extrapolation and limited predictivity potential for the human risk assessment. Thus, more reliable, efficient, and better predictive new approach methodologies (NAMs) for DNT testing are needed.

This work focuses on two DNT-related adverse outcomes (AO): decreased cognition and impaired learning and memory caused by prenatal exposure to chemicals. An adverse outcome pathway (AOP) network was compiled using existing and novel AOPs. Essential key events (KEs) were selected to be monitored to reliably predict the effects of chemicals leading to the AOs. For this purpose, an in vitro battery has been set up at IUF and NIPH laboratories. The measured endpoints cover generic KEs such as cell death and oxidative stress but also specific KEs covering key neurodevelopmental processes vital for normal brain development i.e. proliferation, migration, differentiation, synaptogenesis, and neural network formation of respective cell types (neurons, oligodendrocytes, radial glia, astrocytes) including the cell morphology and number. The used human cell models are iPSC-derived neural progenitor cells and primary neuroprogenitor cells representing embryonic and fetal developmental stages, respectively.

This battery of in vitro assays is being extensively characterized and will be coupled with in silico models in the framework of the ONTOX project. This approach will serve to create a combined NAM in order to predict systemic repeated dose toxicity effects that, in combination with exposure assessment, will advance human risk assessment in line with Next Generation Risk Assessment principles and without the use of animals.

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Poster #18: Update and optimization of an adverse outcome pathway network of chemical-induced cholestasis

Author(s): Jonas van Ertvelde

Affiliation: Vrije Universiteit Brussel

Project: ONTOX

Abstract:

Background and Objectives: Cholestasis denotes any situation of impaired bile secretion with concomitant accumulation of bile acids in the liver or in the blood circulation and may be induced by various chemicals. Our group previously introduced an adverse outcome pathway (AOP) network mechanistically describing key events (KEs) and their relationships driving chemical-induced cholestatic liver injury. The aim of the present work was to update and optimize this AOP network in line with guidelines issued by the Organization for Economic Co-operation and Development (OECD).
Material and methods: PubMed was queried for studies of chemical-induced cholestasis using a list of predefined key words and several known KE-related terms. SysRev, a newly developed computational tool for systematic reviewing and data extraction, was employed during the abstract screening and full-text screening. The tailored Bradford-Hill criteria, described by the OECD guidelines, were used in the weight-of-evidence assessment of the KEs and KE relationships.
Results: A total of 6572 articles was retrieved from PubMed and uploaded to SysRev. An initial abstract-screening resulted in a total of 544 papers eligible for data extraction in the full-text screening process.
Discussion and Conclusion: Extracted data are used for the assessment of already defined KEs and KE relationships, but also for the identification of potential new KEs, resulting in an updated AOP network on chemical-induced cholestatic liver injury. The fully assessed AOP network will serve as the conceptual basis for setting up an in vitro test battery to identify cholestatic chemicals, consisting of a series of assays that each monitor an individual KE.

Poster #19: Development of an adverse outcome pathway for kidney tubular necrosis

Author(s): Devon Barnes

Affiliation: Utrecht University

Project: ONTOX

Abstract:

Tubular necrosis (TN) occurs in response to proximal tubular injury, obstruction and vasoconstriction and is prevalent within hospitalised AKI and CKD populations. Although characterized morphologically, contributory mechanisms of TN remain ill-defined. Adverse outcome pathways (AOPs) provide analytical constructs to describe a sequential chain of causally linked key events, from the molecular initiating event (MIE) to the adverse outcome (AO) throughout key events (KE) across different levels of biological organization.

This study aims to examine and expand upon described connections between the exposure of the kidney to nephrotoxicants, perturbations to its function and cellular responses observed during kidney TN for the development of an AOP. Initially, a literature search identified existing research utilizing terms relevant to clinical biochemistry, urinary biomarkers, histology and clinical presentations in TN. A list of chemically applicable, data-rich nephrotoxic compounds was also established to support the search. Simultaneously, physiological maps of the kidney were designed to establish physiological mechanisms contributing to TN. Currently reported AOPs regarding nephrotoxicity were also systemically mapped to form networks and identify relevant MIEs and KEs using existing AOPs from the AOP-Wiki to contribute toward producing a mechanistic framework for the identification, development and implementation of in vitro endpoints for TN.

To assess chemical disruption, a custom-designed battery of in vitro assays was developed using conditionally immortalized human proximal epithelial tubule cells to gather relevant information of nephrotoxic effects within TN-related MIEs and KEs throughout the spectrum of mechanisms depicted in the linear AOP. This battery will be utilized in combination with in silico models to identify data gaps toward further testing. These results will form the initial steps toward the development of a combined approach to generate, evaluate and apply AOPs for the advancement of next generation, human risk assessment in TN-related kidney failure in the framework of the ONTOX project.

Poster #20: An adverse outcome pathway network for liver steatosis induced by chemicals

Author(s): Anouk Verhoeven

Affiliation: Vrije Universiteit Brussel (VUB)

Project: ONTOX

Abstract:

Background and Objectives: Adverse outcome pathways (AOP) are frameworks depicting existing information on causal linkages (i.e., key event relationships (KER)) between measurable biological changes (i.e., key events (KE)) leading to an adverse outcome (AO). To better represent complex interactions within organisms, different AOPs sharing one or more KEs are brought together in an "AOP network".

The aim of this research was to update the current AOP network on liver steatosis, with a focus on chemical-induced liver steatosis. Furthermore, to weigh the evidence between KEs, the updated AOP network was also assessed in accordance with the specific guidelines from the Organization for Economic Co-operation and Development.

Material and methods: PubMed was used to collect publications on chemical-induced liver steatosis published after 2016. The key search terms included steatosis, specific nuclear receptors as molecular initiating events of the AO as well as KE-associated keywords. A first title/abstract screening of all collected papers was performed with SysRev (i.e., a computational tool for systematic reviewing and data extraction) using a labelling strategy to include/exclude papers. With the application of a second labelling strategy, data regarding essentiality, biological plausibility/applicability and empirical support of KEs and KERs were manually extracted during full-text screening of included papers. Subsequently, data was used to assess the level of confidence in the updated AOP network on liver steatosis according to the tailored Bradford-Hill Criteria.

Results: The PubMed search resulted in 12,478 papers. The title/abstract screening resulted in 1,626 papers eligible for data extraction in the full-text screening phase.

Discussion and Conclusion: Extracted data was used to assess the level of confidence in previous described KEs and KER. In addition, data was used to identify potential novel KEs. The updated AOP network on liver steatosis will serve as a basis for the development of animal-free methods for toxicity testing purposes.

Poster #21: Computation and visualisation of a Mitochondrial Toxicity qAOP PBK-TD model using Simcyp Designer

Author(s): Elias Zgheib

Affiliation: CERTARA

Project: RISK-HUNT3R

Abstract:

Over the last decade, the use of quantitative adverse outcome pathways (qAOPs) in regulatory dossiers and risk assessment pipelines, has spread quickly. However, the toxicodynamic (TD) models behind these tools often remain dependent on the compounds used for their development. Applicability of qAOPs, could thus be widely improved by linking them to chemical-specific physiologically based kinetic (PBK) models. Simcyp Designer, a new product of the Simcyp® simulator provides an intuitive and powerful graphical interface for building custom PBK models and their visualisation. Here, we propose a novel approach using this tool to develop, amend and visualise a PBK-TD sequential model in a qAOP context. First, a calibrated and validated qAOP TD model is added to a new compartment in Simcyp Designer, that is then linked through a PBK-workflow to the target compartment of the study. Finally, the full model's design is adjusted in a fluid and transferrable fashion as the used tool permits.

Based on the Tebby et al. 2022 work on the quantitation of a generic qAOP, we have developed a PBK-TD model for the mitochondrial toxicity AOP (AOP #3, aopwiki.org). This new model was calibrated with data on Rotenone and Deguelin neurotoxicity in LHUMES neuronal cells. After the identification of data gaps, we recalibrated the model with new Tebufenpyrad and Tolfenpyrad data. The variety of test conditions explored here are meant to challenge the fundamental principles of qAOPs such as wide applicability and chemical agnosticism. Since the in vitro cell concentrations were measured in neurons, the chosen target compartment in Simcyp Designer was set to be the brain.

The next steps will include additional model refinements accounting for assay variability and extensions to liver (HepG2) and kidney (RPTEC/TERT1). Regular follow up with users/stakeholders and regulators will help us account for their concerns, needs, and expectations in this work.

Computational approaches

Poster #22: The activities related to the computational approaches within ASPIS

Author(s): Gerhard Ecker, Emilio Benfenati & Computational Approaches WG team

Affiliation: University of Vienna & Istituto di Ricerche Farmacologiche Mario Negri

Project: RISK-HUNT3R & ONTOX

Abstract:

The Computational Approaches Working Group (WG) organizes the activities related to in silico methods in a broad sense, including the QSAR models, docking studies, read-across, and interacting with several Working Groups within ASPIS. These activities can largely benefit from a networking attitude, since most of the modelling activities are based on collections of data, address specific toxicological and toxicokinetics aspects, and apply multiple algorithms and chemical descriptors. Data derive from multiple sources and projects, the toxicological expertise comes from other Working Groups, and the algorithms and descriptors from one laboratory are very probably useful for a second one. Thus, there is a strong opportunity to establish networking activities, and since the content of this specific WG deals with files, it is also quite simple to establish collaborations.

In general terms the collaboration relates to make available data and specific software components, with the purpose to achieve better results within a synergistic approach.

The first application of this collaboration is focused on steatosis. In this case, the computational approaches include the study on the role of descriptors with a particular physico-chemical meaning, the development of in silico models related to the AOP for steatosis, docking studies investigating the tridimensional aspects of the mechanism, and read-across tools.

In the future, other endpoints will be addressed, such as developmental neurotoxicity.

Poster #23: Combining gene expressions and imaged-based morphological features for chemical-phenotype profiles

Author(s): Natacha Cerisier

Affiliation: INSERM

Project: RISK-HUNT3R

Abstract:

As part of the RISK-HUNT3R project, this computational study was intended to integrate molecules that induced transcriptomic perturbations and cellular morphological changes into a biological network in order to assess chemical-phenotypic relationships. Such a network was enriched with existing disease information in an effort to suggest molecular and cellular mechanisms of action leading to diseases.

Two datasets were used for this study. Firstly, we used the "Cell Painting morphological profiling assay" dataset, composed of 30,000 compounds tested on osteosarcoma cells (U-2 OS). Secondly, we used the "L1000 mRNA profiling assay" (LINCS) dataset, a collection of transcriptional expression data from cultured human cells treated with approximately 20,000 bioactive small molecules. Furthermore, pathways, GO terms and disease enrichment were performed on the transcriptomics data.

Our study allowed us to develop a biological network combining chemical-genes-pathways-morphological perturbations and disease relationships. It contains an ensemble of 9,989 chemicals, 732 significant morphological features and 12,328 genes. We found that some sets of drugs shared similar genes and morphological perturbation which suggest possible links between molecular and cellular perturbations.

Although the study is based on the assumption that the cellular behaviour in presence of a chemical is similar independently of the cell type, some extrapolation about genes-cellular features and diseases relationships can be performed. Our network could be enriched with other types of phenotypic screening, transcriptomic information, based for example on the RNAseq technologies, and chemical-disease or toxicity annotations.

Poster #24: Chemical Effect Predictor: A tool to predict chemical toxicity using biological network properties

Author(s): Jordi Valls Margarit

Affiliation: MedBioInformatics Solutions

Project: RISK-HUNT3R

Abstract:

Characterizing the toxicity of chemicals and drugs in humans is not only important in the field of drug discovery but also for the development of new methods for risk assessment. Currently, most of the predictive methods are addressed to a specific side effect or toxicity endpoint or require features that restrict their applicability in particular endpoints. Moreover, these methods are “black box”, providing limited insights into the mechanisms of action of chemicals. System and network-based methods are suitable approaches to analyse and explain how chemicals and drugs perturb biological systems. In this context, we present the Chemical Effect Predictor (CEP), a machine learning model that leverages properties of biological networks for the prediction of toxicity of compounds, and the proposal of a mechanistic hypothesis for their mode of action. The model is based on a multi-scale, heterogeneous network that integrates different layers of information, such as associations among drugs, proteins, diseases, and biological processes. For each drug and disease, we compute network diffusion profiles, which identify the key proteins and biological functions involved in a given drug adverse reaction. By comparing the similarity of both compound and disease diffusion profiles through the multiscale network we can identify the most likely disease elicited by the compound and identify the potential mechanism of action. Different variables are extracted from the network and incorporated into the model such as the importance of particular biological processes between one chemical and side-effect, or their distance into the network. We present the performance of the model on a benchmark of drug adverse reactions and its application in the context of RISKHUNT3R case studies. CEP is a novel approach that leverages information on biological networks and can be used to support the development of new in-silico risk assessment methods.

Poster #25: A KNIME Workflow for Consensus Target Prediction

Author(s): Palle Steen Helmke

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Public data on compound-target interactions are extremely sparse and could be enriched by respective prediction tools. In order to support several case studies in Riskhunt3r and ASPIS, we evaluated a set of target prediction tools (SwissTargetPrediction, SEA, PASS, ChEMBL-Docker) and created a KNIME workflow for consensus scoring and visualization as heatmap.

The output data extracted from these prediction tools were manipulated and sorted in order to list the predicted targets per compound. Hence, the compound-ChEMBL-IDs and target-ChEMBL-IDs were used for identifier mapping across the different tools. Subsequently, the respective tables were concatenated in order to visualise the compound/predicted target interactions. This combined table shows the number of prediction tools predicting a distinct compound-target pair. For further visualisation, a heatmap was created using KNIME, displaying these compound/predicted target interactions.

As a result, mifepristrone, a synthetical steroid inducing abortion, was predicted by three prediction tools as active for the progesterone and the glucocorticoid receptor for instance. In addition, the compound carbofuran, an insecticide utilised as a feeding protection, was predicted as active for the acetylcholinesterase by three prediction tools, which resembles one of its known mechanisms of action.

Poster #26: Structure-based predictions for MIEs

Author(s): Karin Grillberger and Claudia Immacolata Trivisani

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Due to increased interest in the field of predictive toxicology to focus on non-animal testing and incorporate in vitro and in silico testing approaches for regulatory risk assessment of chemicals, the concept of adverse outcome pathway (AOP) frameworks was recently introduced. This concept comprises a molecular initiating event (MIE) as an initial point of chemical-biological interaction that starts the pathway, more specifically an interaction of a chemical with a toxicologically relevant human target. The MIE is followed by several measurable key events (KE) which eventually lead to the adverse outcome. Two main AOPs of interest in the Riskhunt3r project are developmental neurotoxicity (DNT), and steatosis.

Concerning DNT, there are several targets involved, like the nicotinic acetylcholine receptors (nAChRs), thyroid and mitochondrial systems. Therefore, reliable predictions for MIEs are crucial. In order to achieve this goal, we extract available data about interacting compounds and collect protein structures that are suitable for in silico approaches like molecular docking.

Docking represents a structure-based tool for binding mode elucidation, or for screening a library of potentially toxic compounds. Several docking methods are explored to cover the specific question of interest, i.e. ensemble docking (docking the same set of ligands to multiple protein structures), reversed docking, or induced fit docking (allowing the protein to adapt to a certain degree to the binding of the ligands).

Poster #27: UNIVIE Jupyter Notebooks (JNs) for Data Curation & Machine Learning (ML) model building for Transporters & Off-target predictions

Author(s): Gerhard Ecker

Affiliation: University of Vienna

Project: RISK-HUNT3R

Abstract:

Machine learning (ML) models require qualitative curated data sets in order to learn from chemical structures to predict activities with high reliability. However, publicly available data have often to be preprocessed before being applied to ML training. Therefore, we propose semi-automated frameworks, one for data retrieval from the ChEMBL database with additional data filtration, data standardization, data classification steps and another one for ML model building including featurization and parameterization. This workflow enables the user to retrieve curated datasets by uploading a CSV-file to the JN. The obtained data sets can be applied to the JN for ML model building where four different classifiers, Logistic Regression, Support Vector Machine (SVM), Random Forest (RF) and k-nearest Neighbor (knn) are used.

Poster #28: Computational modelling of neural tube closure defects

Author(s): Job Berkhout

Affiliation: National Institute for Public Health and the Environment

Project: ONTOX

Abstract:

Closure of the caudal neural tube is a critical event that occurs early in development, around day 27 of human gestation. Failure of neural tube closure results in severe birth defects, such as spina bifida. These neural tube defects (NTD) are among the most prevalent human congenital malformations, which warrants specific attention in chemical safety assessment. Computational models of biological processes are likely to revolutionize chemical safety assessment in the near future. Such models can be used to predict the effect of chemical-induced gene expression changes and provide a template for establishing quantitative adverse outcome pathway networks. This study aims to develop an in silico model of the human neural tube closure, which will be applied to predict chemical-induced NTDs.

By extensively mining the developmental biology and toxicology literature, we first created a physiological map of human neural tube closure. Based on the physiological map, we built a multicellular agent-based model using CompuCell3D.

The constructed physiological map depicts the all-trans-retinoic acid (ATRA) related molecular pathways linked to the various cell types in which they occur, and their morphogenetic consequences, that lead to closure of the neural tube. The morphogenetic events driven by gene expression changes are visualized by the computational model.

We simulated in silico the complex biological process of neural tube closure, in order to demonstrate the feasibility of this approach. At a later stage in the project, the computational model will be applied to predict chemical-induced changes in gene expression and cell characteristics. The predictions of the model will be validated using a set of dedicated in vitro assays in conjunction with existing knowledge on in vivo developmental neurotoxicity. Such computational models may ultimately provide an alternative in silico approach for chemical safety assessment without the use of animals.

Poster #29: Next generation target organ toxicity risk assessment: endogenously tagged human stem cell reporters for high-content screening of oxidative stress response.

Author(s): Tamara Danilyuk

Affiliation: Leiden University

Project: RISK-HUNT3R

Abstract:

Development of in vitro assays for early detection of liabilities to chemical adversity is crucial for the prediction of liver toxicity. Hepatocyte-like cells (HLCs) derived from human induced pluripotent stem cells (hiPSCs) are an attractive in vitro model to study mechanism-based xenobiotic toxicity. We set out to build a panel of fluorescent hiPSC reporters, suitable for high-content-screening of cellular stress response activation, upon compound exposure. We established a pipeline for efficient CRISPR/Cas9-mediated reporter generation and reporter's functional characterization upon differentiation to relevant lineages, including HLCs. Here, we present the generation and application of fluorescent hiPSCs reporter lines for sulfiredoxin-1 (SRXN1) and pirin (PIR), which are shown to be inducible and sensitive biomarkers for the oxidative stress response. Oxidative stress induced by diethyl maleate (DEM), sulforaphane and nitrofurantoin was monitored using live-cell confocal imaging of iPSC reporter lines in HLCs differentiation state. Endogenous levels of eGFP-tagged biomarkers accumulated in the cytoplasm of HLCs over 24 hours window. Newly established isogenic fluorescent reporter lines will be used i) as a tool in understanding and quantifying target organ specific oxidative stress response, ii) point of departure modelling to further capture specific lineage sensitivities towards oxidative stress and iii) ultimately, used for the hazard characterization and IATAs for target organ toxicity in next generation risk assessment.

Poster #30: Evaluation of state-of-the-art in silico testing methods to fill physicochemical and pharmacokinetic data gaps within the ONTOX project

Author(s): Rita Ortega-Vallbona

Affiliation: ProtoQSAR

Project: ONTOX

Abstract:

The ONTOX project aims at developing new approach methodologies (NAMs) to address systemic, repeated dose toxicity effects related to six adversities in the liver (steatosis and cholestasis), kidney (tubular necrosis and crystallopathy) and developing brain (neural tube closure and cognitive function defects). NAMs will be based on AI-based systems fed by available data. One of the objectives of the "Chemical Domain" work package is to study the correlations between adversities and kinetic/physicochemical properties of the compounds to find properties able to discriminate between active and inactive chemicals for each of the six ontologies. To do so, we first collected experimental data for 27 physicochemical and pharmacokinetic properties. Secondly, we identified and validated up to 20 available computational tools to predict the most relevant parameters and used them for data gap-filling when experimental data was unavailable.

Relevant kinetic/physicochemical properties for the steatosis case study were identified in this final dataset using two different approaches: The first approach used three statistical analyses (Kolmogórov-Smirnov, t-Student and Wilcoxon) to evaluate the selected properties for near 60 compounds. Among the evaluated properties, four were statistically significant and can help to characterise a compound as steatotic, while two presented a non-significant trend. The second approach consisted of developing a machine-learning model to predict steatosis from these properties, including molecular weight and the number of halogens in the analysis. This approach was performed with a larger dataset (https://doi.org/10.1021/acs.chemrestox.0c00511). Eleven features showed to be relevant, and interestingly, there was a good fit with the more significative properties from the previous analysis with the ONTOX chemicals. Combining both approaches, we found some kinetic/physicochemical properties that significatively correlate significantly with steatosis. These properties may help in the discrimination of pro-steatotic compounds and unravel the underlying mechanism of action of these chemicals.

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*These authors contributed equally to this work

Poster #30b: Applying machine-learning approaches to identify key genes associated with drug-induced cholestasis

Author(s): Jian Jiang

Affiliation: Vrije Universiteit Brussel,

Project: ONTOX

Abstract:

The Background and Objectives: Drug-induced cholestasis (DIC) is one of the most severe manifestations of adverse drug reactions, constituting a major subgroup (up to 50%) of total drug-induced liver injury (DILI) cases. Due to its complex process, early detection of DIC during drug development remains challenging. Preclinical animal studies, a standard model in drug safety evaluation, often fail to detect DIC in humans mainly due to interspecies differences. Recently, toxicogenomics in vitro assays, especially based on human liver cells, have become a more convenient and practical approach for the prediction of human-relevant DILI. Over the past decade, the established large-scale databases, combined with machine-learning (ML) approaches, give us the opportunity to identify transcriptome signatures of DILI. In the present study, we leveraged the publicly available database, Open TG-GATEs, for the identification of transcriptomic signatures of DIC.
Material and Methods: We retrieved toxicogenomics data derived from in vitro cultured primary human and rat hepatocytes following exposure to 18 compounds (9 cholestatic compounds and 9 non-cholestatic compounds). These transcriptome profiles were measured at two time points (8 and 24h) following a single exposure to a given compound at three dosages (control, middle and high) with two biological replicates. Due to the mechanistic complexity of DIC, the model cholestatic compounds were selected because of their potential to cause cholestatic hepatotoxicity through diverse toxic mechanisms. Several supervised ML approaches, including Random Forest, Support Vector Machine and Logistic Regression, were applied to the human liver TG-GATEs dataset to develop a prediction model.
Results: We identified a signature consisting of 20 genes that predicted cholestatic hepatic injury with high specificity and selectivity. The selected feature genes and model were validated using the in vitro rat TG-GATEs dataset.
Discussion and Conclusion: Our transcriptomic signature has yielded high accuracy in the identification of potential cholestasis-inducing compounds.

Exposure and kinetics

Poster #31: Exposure and kinetics research activities in ASPIS: Moving from hazard identification to risk characterisation in next generation risk assessment

Author(s): Nynke Kramer, Sylvia Escher and exposure and kinetics WG team

Affiliation: Wageningen University and Fraunhofer Institute for Toxicology and Experimental Medicine (ITEM)

Project: ONTOX and RISK-HUNT3R

Abstract:

Unlike in traditional chemical safety assessment, which focuses on hazard characterization using toxicity tests on animals, next generation risk assessment is an exposure-led, hypothesis-driven approach relying on non-animal test methods. The European Commission strongly supports the development of these non-animal approaches in risk assessment, by funding, amongst others, the three ASPIS cluster projects, ONTOX, PrecisionTox and RiskHunt3R. All three projects aim to develop and apply novel in vitro toxicity assays to identify perturbations in biomolecular toxicity pathways. However, on their own, these assays identify hazards and does not meet the 'exposure-led' criterium of next generation risk assessment. Each of the three projects therefore include activities defining chemical exposure levels in the environment, human populations, target organs and in vitro assays. The ASPIS working group on kinetics and exposure has been setup to align these research activities in the field of exposure and toxicokinetics assessment. The aim of the working group is to integrate the complementary approaches for kinetics and exposure assessment into a common, pragmatic guideline for risk assessors wishing to use exposure, in vitro ADME, in vitro distribution kinetics, physiologically based kinetic (PBK), and in vitro-in vivo extrapolation (IVIVE) models for next generation risk assessment. This guideline details a tiered approach to exposure and kinetics assessment, which is to be evaluated with case study chemicals. In this presentation, the exposure and toxicokinetics tools under development in each of the three projects are compared and mapped along an exposure-effect continuum. The activities planned for the first case study chemicals, triazole fungicides and liver steatosis, are outlined.

Poster #32: A high-throughput analytical workflow to determine internal concentrations of xenobiotics in zebrafish larvae

Author(s): Nico Grasse

Affiliation: Helmholtz Centre for Environmental Research - UFZ

Project: PrecisionTox

Abstract:

Submission of toxicokinetic (TK) data for interpretation of biological effects on 250 chemicals in terms of internal concentrations in zebrafish larvae. This deliverable will also include the predicted internal concentrations of the test chemicals using partition models between water and the embryos. For significantly deviations from the model, a detailed time resolved analysis of internal concentrations will be conducted and reported, including the determination of metabolites. These TK information is essential to estimate the specifity of the effect caused by a specific class of compounds and to estimate to which extent the observed effects are solely related to baseline toxicity (BT). A preliminary efficient workflow was established to screen internal concentrations of 30 chemicals in zebrafish larvae at 96 hours post fertilization (hpf). Quality control experiments were done to demonstrate chemical's stability over the exposure.

Poster #33: Kinetic modelling and quantitative in vitro-in vivo extrapolation strategies for next generation risk assessment in the H2020 ONTOX project

Author(s): René Geci

Affiliation: esqLABS

Project: ONTOX

Abstract:

The EU funded ONTOX project aims to deliver a generic strategy to create innovative new approach methodologies (NAMs) in order to predict systemic repeated dose toxicity effects that, upon combination with tailored exposure assessment, will enable human risk assessment with focus on 6 ontologies for three different organs (Liver, Kidney, and Developing Brain). One of the pillars in the ontologies is a thorough understanding of the toxicokinetics of case study chemicals. Toxicokinetics and chemical exposures are addressed in work package four (WP4) of the project.

Research in WP4 focuses on developing and applying several types of in silico models: i. generic physiologically based kinetics (PBK) model frameworks (chemical specific and generic) for simulating in vivo distribution kinetics and characterising the ADME properties to predict tissue exposure for the selected chemicals and their systemic repeated dose toxicity effects; ii. in vitro distribution kinetics models for estimating free and cell-associated in vitro effect concentrations to perform accurate hazard characterization. These biokinetic models are necessary to integrate information from the exposure to an adverse outcome by using the plethora of NAM data generated in the ONTOX project, to allow quantitative in vitro-in vivo extrapolations (QIVIVE). In parallel, links with quantitative adverse outcome pathways (qAOP) are explored to link kinetics to specific MIE and/or KE. To allow all these integrations, a gap analysis and data mining is ongoing to evaluate and extend the definition of the chemical and biological applicability domains of the biokinetic models. Uncertainty, sensitivity analyses and model validation will be discussed in order to gain confidence in the application of such models by risk assessors and decision makers.

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Poster #34: Primary human enterocytes for the determination of intestinal metabolism

Author(s): Patrik Lundquist

Affiliation: Uppsala University

Project: RISK-HUNT3R

Abstract:

In RISK-HUNT3R, we investigate external exposure to chemicals via three routes: lungs, skin and intestine. For this purpose, we develop new approach methodologies (NAM) that better reflect human absorption barriers. The more in vivo-like parameters generated in these models are then used to provide data to predictive toxicokinetic models of human exposure. The determination of human exposure via the oral route usually begins with measurements of the permeability of the compound in question over monolayers of intestinal epithelial cells. The permeability gives an approximation of the fraction absorbed from an oral dose. To improve predictions we also need a value for the fraction metabolized by the enterocytes of the small intestinal epithelium. Permeability is commonly measured in the Caco-2 cell model. A weakness of the Caco-2 cells is that they lacks expression of the major metabolizing enzymes found in the human intestine. To measure the intestinal metabolism of compounds we isolate primary human jejunal enterocytes that in theory should retain metabolizing enzymes. Enterocytes are isolated from human jejunal mucosa samples from patients undergoing gastric bypass surgery at Uppsala University Hospital. Enterocytes are purified using a gentle enzyme-free method based on EDTA containing buffers and gentle shaking followed by Percoll fractionation to remove unwanted cell fractions. This results in a high yield of highly viable (> 90 %) enterocytes where a fraction of 25-35 % of cells were caspase-8 positive and had entered early apoptosis. Preliminary results indicate that major intestinal cytochrome P450 enzymes are detected in the isolated enterocytes. Optimization of methods to determine the intrinsic clearance for phase I and II metabolism substrates is ongoing.

Poster #35: Parameterisation and Verification of IVIVE-PB(P)K models for Risk Assessment

Author(s): Barira Islam

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Project: RISK-HUNT3R

Abstract:

The physicochemical, absorption, binding, distribution, and in vitro elimination parameters of a compound are the primary inputs required for building a physiologically based kinetic (PBK) model. These parameters can be obtained from literature, generated experimentally, or predicted by in silico tools using models based on quantitative structure activity relationship (QSAR) and/or quantitative property-property relationship (QPPR). However, parameters predicted by in silico methods are not always reliable model inputs, making it imperative to understand the applicability domain of any QSAR/QPPR models before using them for building PBK models. In the Riskhunt3R project, we developed a case study to understand the impact of using predicted or measured in vitro input parameters on kinetic predictions of PBK models. 28 case study compounds were selected to cover a range of physicochemical properties and elimination pathways. All types of compounds, acidic, basic, neutral and ampholytes were included and log P (octanol-water partition coefficient) varied from 0 to 8 to test both hydrophilic and hydrophobic compounds. The case study compounds were selected to incorporate action of variable metabolic enzymes such as cytochrome P450s (CYP) including CYP1A2, CYP3A4, CYP2D6, CYP2C19, UDP-glucuronosyltransferases and cytosolic enzymes including monoamine oxidase A and aldehyde oxidase. Additionally, compounds with biologically active metabolites have been included to understand the data requirements for the prediction of both parent and metabolite concentrations. As a test case, PBK models for atomoxetine, itraconazole, montelukast, fluticasone furoate and diclofenac have been built (in Certara's Simcyp® Discovery and Simcyp® Simulator) using in silico and in vitro data. The prediction accuracy of the two approaches will be investigated by comparison with human in vivo observations.

Poster #36: Assessment of aggregate exposure in RISK-HUNT3R

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Project: RISK-HUNT3R

Abstract:

The RISK-HUNT3R project considers the assessment of the total or aggregate exposure of (members of) a population to a single chemical substance from multiple sources, via multiple pathways and routes. The aggregate exposure can be differentiated into aggregation of (a) different routes (for example inhalation and dermal of the same compound during the same use), (b) occupational and consumer exposure, and (c) exposure during daily use of different articles with the same compounds within consumer settings.

The overall objective in RISKHUNT3R is that health-based limit values will be delivered for internal exposures (e.g., absorbed doses). Estimates of the external exposure for each pathway and source will be converted to an internal exposure value before aggregation. Evaluation of risk can be done, for example using a Risk Characterisation Ratio (RCR) approach. Evaluating the aggregate risk for various sources and routes in combination would be done by adding individual RCRi‘s and requiring that their sum be less than 1. Here we propose a tiered approach for the assessment of the aggregate exposure from multiple sources and routes.

Poster #37: Aggregate exposure model

Author(s): Max Spänig

Affiliation: ITEM Fraunhofer

Project: RISK-HUNT3R

Abstract:

The main use of N-methyl-2-pyrrolidone (NMP) is as a polar aprotic solvent in industrial applications. Occupational exposure may result from its use as a solvent for paint and graffiti removal and indoors from its use in paints and inks as well as rugs and carpets. Dermal route plays a significant role in the total uptake of vaporized NMP, as shown by exposure and biomonitoring studies.

Aggregation of exposure can occur by several pathways or exposure scenarios. In the case study of NMP, various activities within a work shift and the associated dermal and inhalation exposures add up to a total exposure that must be properly assessed. For this approach, exposure models such as ART and ECETOC TRA are used to provide estimates of exposure scenarios.

The model output and its linkage to the internal concentration needs to be investigated as well. Fraunhofer ITEM has already developed a physiology-based kinetic model (PBK) for humans to estimate inhalation of gases, vapors, and liquid and solid aerosols. In order to provide a risk assessment of aggregate exposure, an extension of the PBK model to include dermal uptake is necessary. Human skin is divided into several compartments based on its physiological properties, taking into account skin properties such as hair follicles and water content. A fitted model for NMP is not the main focus of this work, but a generic model that can estimate dermal uptake for a variety of substances and scenarios.

Poster #37b: The case study concept illustrated by an inhalation case study

Author(s): Tanja Hansen

Affiliation: ITEM Fraunhofer

Project: RISK-HUNT3R

Abstract:

In RISK-HUNT3R, WP3 together with WP4, will establish a framework for human exposure assessment. In WP3, one central aspect is to characterize the absorption and metabolism occurring in human barrier organs such as lung and intestine.

To this end, case studies are being used to develop the approaches in RISKHUNT3R and proof their performance. In a first attempt, 1st generation case studies use data rich model compounds with existing in vivo reference data so that the predictivity of the novel approaches can be assessed. In a second phase, overarching case studies connecting exposure, kinetics, hazard and risk assessment will be performed. One of the relevant human routes of exposure is inhalation. The focus of the inhalation case studies is on uptake and metabolism in pulmonary barrier models. Cell models from different regions of the respiratory tract are being used dependent on their relevance for the selected test compounds, e.g. functionally immortalized human alveolar epithelial cells (Cl-hAELVi), Calu-3 cells as appropriate model for the tracheobronchial region or primary human bronchial epithelial cells. Dependent on the physical nature of the chemical, pulmonary barrier models are exposed either submerse or under air-liquid interface (ALI) conditions.

Metabolism kinetics are investigated in lung microsomal preparations and compared to primary human bronchial epithelial cells.

Our aim is to evaluate if the absorption, biotransformation, intracellular accumulation and permeation of chemicals can accurately be predicted by means of in vitro models and if the results obtained therein can be used as input parameters for a PBK model to improve the model output.

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Communication and dissemination strategies for NGRA

Poster #38: A joint voice on the side of NAMs-based strategies for chemical risk assessment: an overview of ASPIS communication activities

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Project: RISK-HUNT3R, ONTOX, PrecisionTox

Abstract:

ASPIS's communication and dissemination activities are coordinated by the cluster projects, RISK-HUNT3R, PrecisionTox, and ONTOX, with the shared mission to unbiasedly inform on how New Approach Methodologies (NAMs)-based strategies can rapidly accelerate and improve chemical risk assessment in the EU.

The communication teams of the three projects work side by side to synergize the dissemination efforts and positively impact the cluster visibility and the outreach of its key messages. In particular, the objective of the communication and dissemination working group is to build, beyond projects' specificities, one cluster identity to have a joint stronger voice to vehicle its outcome to regulatory stakeholders, policy-makers, NGO and lay public.

Different actions have been pursued to achieve these goals, such as creating a common visual identity, a joint website (aspis-cluster.eu), and sharing descriptive material, leaflets, and factsheets, to promote the common goals and activities.

The cluster speaks jointly online via a coherent social media presence to facilitate message amplification. The communication teams of each individual projects echo news and outcome of each other and disseminate those via their channels. The cluster also jointly communicates via official press declarations and policy briefs, as support statement of policy legislative initiatives or involvement in public consultations. Common publications, op-eds and press coverage in EU journals are also used to inform the stakeholders about the cluster work.

The ASPIS communication plan also includes unwired activities, such as participation in joint conference sessions at international conferences and the organization of the ASPIS annual open symposium. The symposium series aims to involve cluster members, stakeholders, and cluster regulatory advisory board to discuss together crucial achievements and challenges in the implementation of NAMs into chemical risk assessment in Europe and beyond. This will allow ASPIS to support EU green deal objectives such as the toxic-free environment.

Poster #39: Science Policy on NAMs – A current overview of opportunities for implementation

Author(s): Laura Holden

Affiliation: University of Birmingham

Project: PrecisionTox

Abstract:

Over the last decades, ecotoxicological and toxicological research has developed a wide range of alternative approaches to animal testing (New Approach Methodologies (NAMs)), some of which are already available for regulatory use and others continue to be developed. In parallel, the European regulatory landscape has created several opportunities for the uptake of NAMs, however the acceptance of NAMs and their data by regulators is still limited.

There are a series of opportunities that can provide impetus to advance the use of alternative methods, including the upcoming revisions of REACH and CLP regulations; the implementation of the EU Chemical Strategy for Sustainability; and the European Parliament's resolution calling for an action plan to accelerate the transition from using animals in research. Additionally, EU projects, clusters, and partnerships are working at the science-policy interface on the topic of NAMs, of which ASPIS is one. These each play a role in the future implementation strategy of NAMs in chemical safety assessment for regulatory purposes and are addressed by the Embedded Translation pillar of PrecisionTox. The movement of science towards the 3Rs policy and more protective chemical data involves engagement with stakeholders such as policymakers, regulators, research institutions, and industry. The science-policy interface seeks to build confidence in the use of NAMs and we aim for NAM science and technology to reach its protective potential through regulatory implementation.

Our research has identified barriers to the uptake of NAMs, enabling us to consider solutions to address them. The identification of and continued engagement with projects, activities, actors, and upcoming regulatory revisions has provided further drive for change behind the NAM transition, through our Group First Regulate Better White Paper; a variety of media about NAMs to increase awareness and understanding; and webinars, discussions, and presentations with policy and decision makers.

Poster #40: Main Drivers for Use and Regulatory Acceptance of New Approach Methodologies: a Survey of European Risk Assessors

Author(s): Nicolas Roth

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Project: PrecisionTox

Abstract:

Over the last decades, ecotoxicological and toxicological research has developed a wide There is widely acknowledged scientific, social, ethical, and financial motivation to promote the integration and use of non-animal testing methods in research and chemical risk assessment to advance the 3R principles. The paradigm shift in toxicity testing towards the use of alternative methods has led to major advances in the field of predictive toxicology and systems toxicology, and to a substantial increase in available in vitro, in silico, and mechanistic data derived from New Approach Methodologies (NAMs). However, integrating these new scientific developments in risk assessment remains an important challenge. While policies have long had the goal of protecting human health and ecosystems with new and innovative methods, solid downstream implementation has been slow, often because novel scientific methods have failed to consider regulatory needs as a driver for successful translation into risk assessment and management practice. As part of Task 6.4/Activity 6.4.2 of the Horizon Europe co-funded 'Partnership for the Assessment of Risks from Chemicals' (PARC), we present a project that aims to promote innovation in risk assessment, and facilitate the use and regulatory acceptance of NAMs to support Next Generation Risk Assessment. The project combines literature review, mixed methods research (expert elicitation, survey), and gaps and needs analysis for knowledge consolidation and further prioritization in PARC. Taking a bottom-up approach, it focuses on risk assessors' needs in their daily workflow and decision-making. An online survey with European risk assessors (and possibly beyond) will be implemented to get a deepened understanding of the technical, structural and cultural barriers and drivers that may facilitate/hinder the integration and use of NAMs at desk level, in various EU risk assessment frameworks and application contexts for authorization of chemicals. Active engagement with the ASPIS cluster will be sought, given the similarities in aims and objectives, to explore synergies and potential for collaboration.

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