ECHA
EUROPEAN CHEMICALS AGENCY

APCRA Prospective Study

Assessment of chemicals, using and developing New Approach Methodologies (NAMs)

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3rd APCRA working meeting, 10-11 October 2018

Disclaimer

The views expressed in this presentation are those of the authors and do not necessarily reflect the views or policies of any participating government organization.

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State of play on industrial chemicals

The “2020 goals”, adopted at the 2002 UN World Summit on Sustainable Development, triggered many initiatives, improving safe use of industrial chemicals, including the generation of more data and knowledge;

Nevertheless, for many chemicals on the market(s) in significant volume, information is lacking to robustly conclude on CMR, ED and/or PBT properties;

In addition, for chemicals with no/limited information requirements, means to predict these properties are very limited;

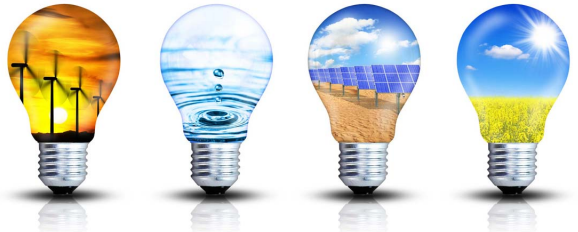
(Pro-) active management of emerging priorities, including (avoiding regrettable) substitution is hampered for the same reason of lack of robust prediction tools for these endpoints.

APCRA and New Approach Methods

Ambition: Define how New Approach Methods (NAM) can be used in a regulatory context to enhance the pace of our work, to have better informed, more relevant decisions and reduce/replace the need for studies on (vertebrate) animals, for human health and environmental 'endpoints'.[1]

What is a "New Approach Method"?

- A method that (potentially) can significantly contribute to fulfil this ambition in terms of:
  - Throughput and/or
  - Robustness and/or;
  - Bringing mechanistic knowledge and/or;
  - Providing appropriate protection levels for human health and Environment.



[1] For low tier endpoints ECHA contributes actively to the development with a focus on AoP /in-vitro developments (mostly via OECD) and improvements of QSARs, by utilising and making available REACH Registration data

2: WoE: Weight of Evidence: https://echa.europa.eu/support/registration/how-to-avoid-unnecessary-testing-on-animals/weight-of-evidence

IATA:Integrated Approaches to Testing and Assessment: http://www.oecd.org/chemicalsafety/risk-assessment/iata-integrated-approaches-to-testing-and-assessment.htm

DA: Defined Approach http://www.oecd.org/publications/guidance-document-on-the-reporting-of-defined-approaches-to-be-used-within-integrated-approaches-to-testing-and-assessment-9789264274822-en.htm

Organizational Motives

- **For ECHA,**
  - **Strategic Plan 2019-2023 Priority 1: Identification and risk management of substances of concern**
    - Increase data availability for prioritising data poor substances with an ***aligned strategy for further generation and use of data from new approach methodologies (NAMs).***
  - **ECHA considerations on the World Summit of Sustainable Development (WSSD) 2020 goal**
    - Success factor 1. Robust data is available on all chemicals in Europe with hazard data is generated ***using non-animal testing methods and new approaches*** wherever possible.
- **For EPA,**
  - **TSCA Section 4(h)**
    - "…Administrator shall reduce and replace, to the extent practicable and scientifically justified…the use of vertebrate animals in the testing of chemical substances or mixtures…"
  - **EPA Strategic Plan to Promote the Development and Implementation of Alternative Test Methods**
    - The EPA's long-term goal is to move towards making TSCA decisions (conducting prioritization activities and risk evaluations for new and existing chemicals) with NAMs in order to reduce and eventually eliminate vertebrate animal testing for TSCA.
- **For Health Canada,**
  - **OECD Report (2016): Ensuring a Sustainable Chemicals Management Plan Post 2020**
    - "…It is essential for Canada to take into consideration new scientific information….and to support the continued development of modernised and harmonised approaches for assessment and management of chemicals….."
  - **Planning of Chemicals Management in Canada after 2020**
    - Evolve the science foundation to support evidence-based decision making; drive and encourage innovation; risk assessment moderization through the development and application of NAM

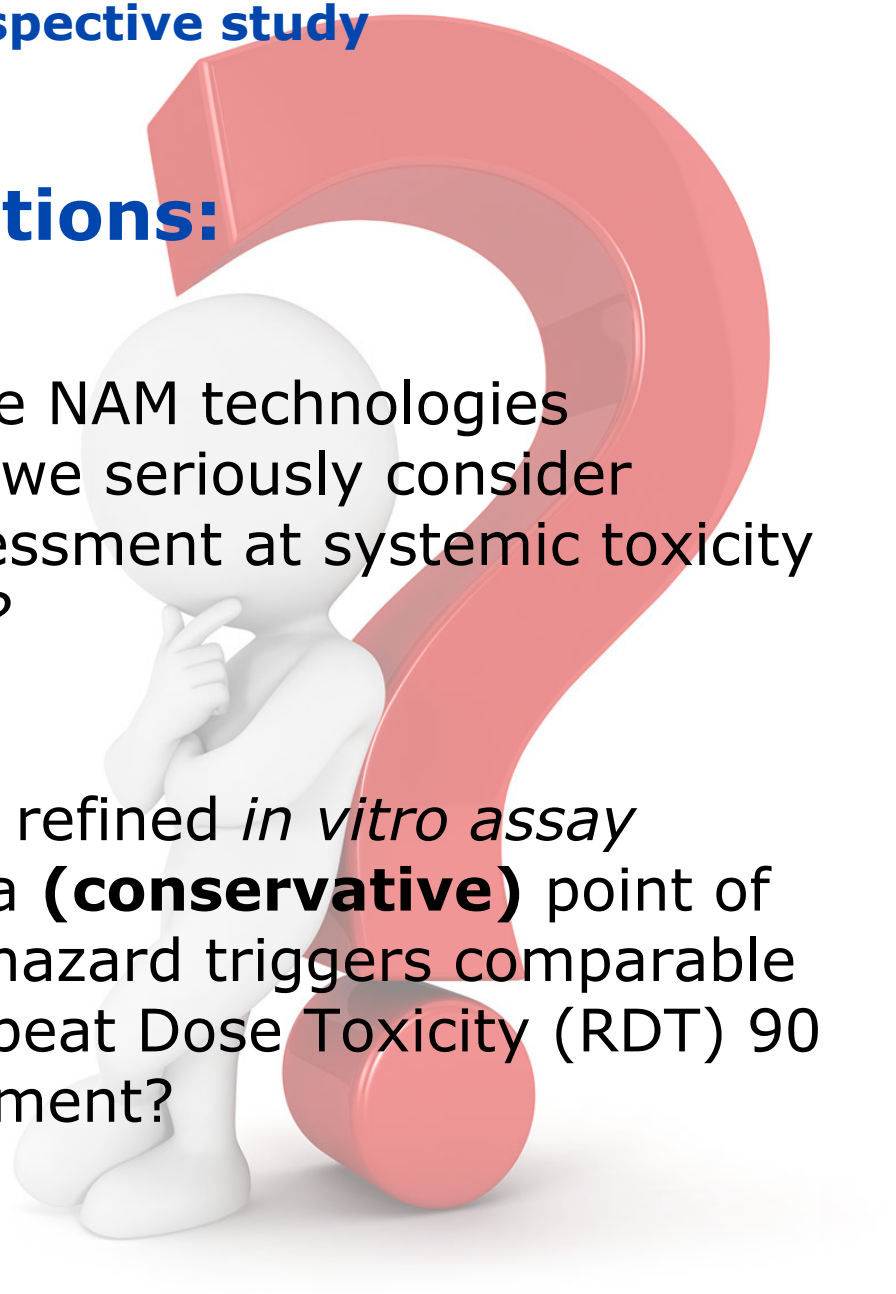
Key questions which are motivating APCRA projects:

- What are the current barriers to acceptance for successful use of NAMs in regulatory decision-making?
- What are near-term efforts that can improve use of NAM data?
- What is needed to lead to acceptance of NAMs by regulators and the public?

Main Case study questions:

- How far can we go with the NAM technologies currently available? Could we seriously consider application for hazard assessment at systemic toxicity level (high tier endpoints)?

- Can the outcome from the refined *in vitro assay* battery be used to derive a **(conservative)** point of departure and qualitative hazard triggers comparable with the outcome from Repeat Dose Toxicity (RDT) 90 day used in hazard assessment?



Project Goals

- To assess chemicals with limited/unclear toxicological data, which at the same time have significant potential exposure, using both NAM type of data and classical toxicological studies;
- To utilise and inform the further development needs for NAM:
  - for screening, prioritisation and first tier assessments
  - for conclusive hazard characterisation/assessment and risk management
- To assess chemicals in an international context

Why 90day RDT as benchmark

- RDT study is designed to test wide range of effects;
- RDT provides an overview of systemic toxicity profile;
- RDT might trigger additional investigations for reprotox, immunotox, neurotox, carcinogenicity;
- 90 day is considered as a conclusive test.

To allow meaningful comparison between NAM tests and RDT study endpoints extrapolation of in vitro concentration to in vivo relevant doses is critical.

In addition in vivo study will be complemented by mechanistic biomarkers (multi-omics from multiple time points) and TK.

What 'comparable with RDT' means for NAMs in the hazard assessment context?

To demonstrate that an outcome is comparable with RDT 90d in the context of hazard characterisation and risk management, NAM testing has to:

- Provide quantitative estimate of NOAEL and LOAEL:
  - NOAEL as potential source for systemic DNEL (if most conservative);
  - LOAEL for STOT RE classification (in the case of severity of the effect);
- Provide (semi) qualitative indications/triggers for:
  - Toxicity to reproduction;
  - Developmental toxicity;
  - Immunotoxicity;
  - Neurotoxicity;
  - Carcinogenicity;

APCRA prospective study: project plan 1/2

Step 1

- Selection of substances with limited or no hazard data and potential wide spread use
- Refinement of the selection taking into account: substance stability, phys-chem properties, known limitations of *in vitro test* methods, resource limitations.

Step 2

- **Phase I** testing: *in vitro* test battery for ~200 substances, test battery will cover:
  - General bioactivity pattern related to common pathways;
  - Specific bioactivity patterns related to CMR/developmental toxicity properties (specific AOPs, pathways, organotypic and micro physiological assays);
  - Set of parameters needed for predicting *in vivo* systemic concentrations (HTTK) and basic ADME

Step 3

- Development of *in vivo* test protocol(s) which shall include:
  - Set of classical endpoints foreseen in guideline test (RDT);
  - Toxico-kinetic parameters;
  - As many parameters as possible which are compatible with phase I testing;
  - Non/semi-targeted metabolomics at blood plasma and key organs (liver, kidney);
  - Transcriptomics data from key organs (liver, kidney);

APCRA prospective study: project plan 2/2

Step 4

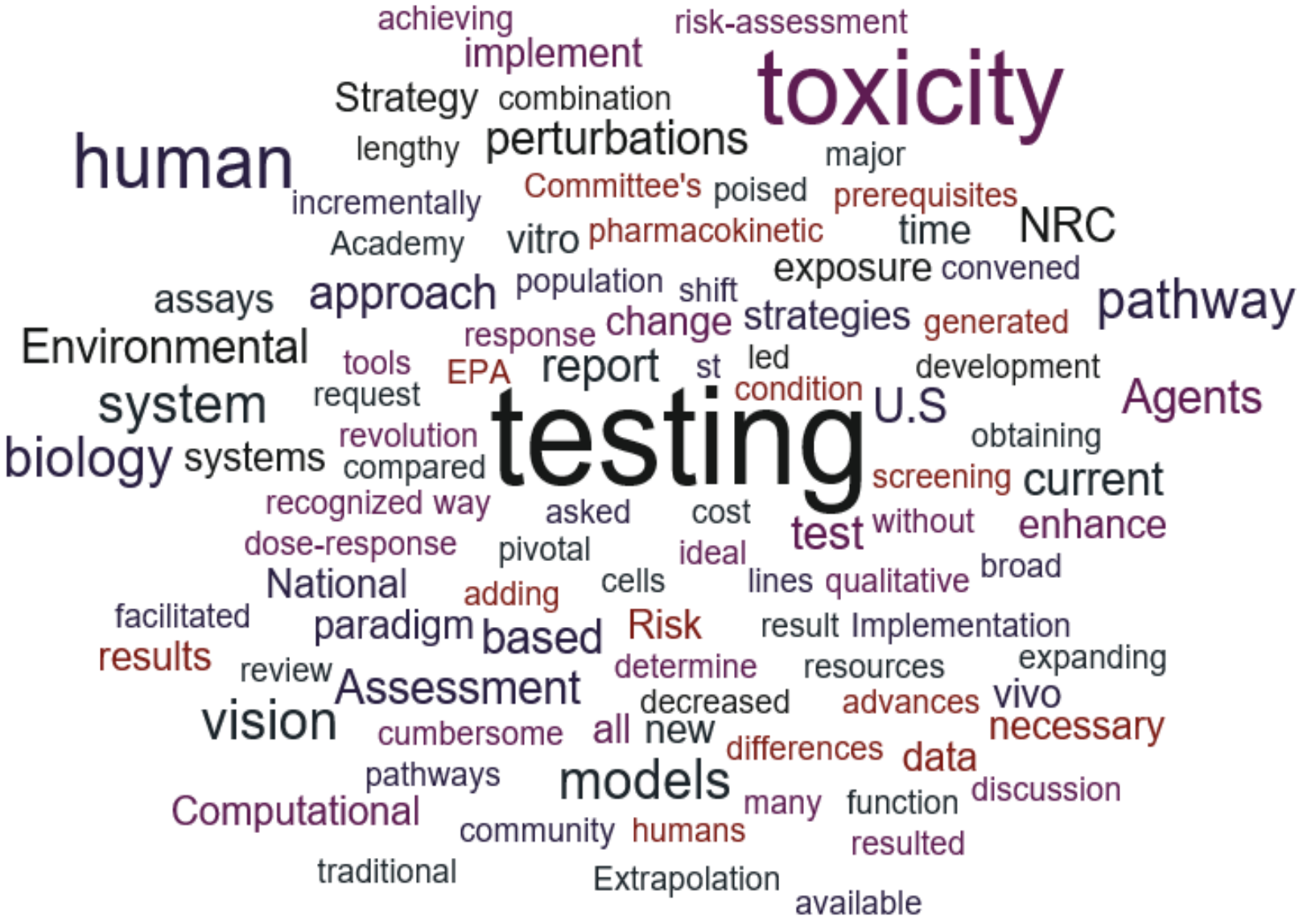
- Based on the outcome from *in vitro* test battery, selection of a **subset (~30)** of substances which will go for **phase II** *in vivo* testing.
  This selection shall include:
  - substances which are predicted to be toxic (high sys. tox estimates + triggers);
  - substances which are predicted to be bio-activatied via metabolism;

Step 5

- For substances selected at Step 4 to run:
  - An acute (5 days) *in vivo* transcriptomics study to quantitatively estimate points of departure from sub-chronic and chronic *in vivo* studies .
  - Running *in vivo* RDT according to protocol(s) established in Step 3.
  - Other studies (like PNDT, Repro might be also considered depending on triggers

Step 6

- Qualitative and quantitative comparison of the results from phase I and II tests.
- Regardless of the level of concordance between NAM and classical data, combined data from phase I and II tests should allow conclusive hazard assessment (systemic toxicity) of the substances which were tested in both phases.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APCRA prospective study: update on progress

- **WP 1: Substance selection**
  Q3 2017- Q3 2018 (finalised)
- **WP 2: Phase I (in vitro) testing & in silico modelling**
  Q4 2018- Q2 2020 (preparatory work is ongoing);
- **WP 3: Phase II (in vivo) testing**
  Q3 2019- Q4 2020 (preparatory work is ongoing);
- **WP4: Analysis of the results & communication**
  Q1 2019 – Q1 2021.

WP1 Substance selection

- Biggest limiting factor: generation of HTTK data needed for IVIVE extrapolation.
- Within this case study new HTTK data will be generated for ~85 substances by APCRA partners (US EPA, EC JRC and Health Canada).
- Scenario 1: substance present on the EU and/or Canada and/or US market, with a potential for consumer use and significant data gaps for systemic toxicity (105).
- Scenario 2: substance present on the EU and/or Canada and/or US market, with known toxicity or with the potential to exhibit different toxicity levels across different species (8).
- Scenario 3: Substances secected from APCRA retrospective study (88).

Scenario 3 substances were selected from the following groups:

- with PODnam estimates that are less conservative than PODtraditional;
- with PODnam estimates close to PODtraditional;
- with an overly conservative PODnam estimate.

- **For substances with in vivo studies available, the need for Phase II testing is not foreseen**
- **Substances from Scenario 2 will be used as positive/negative controls**
- **Substances from Scenario 3 will be used to examine whether refined POD_{NAM} estimates can be obtained**

WP2 Initial Assay Portfolio

Hazard

EPA ToxCast Assays
EPA HTTr Assay (2 – 3 cell types)
EPA HTPP Assay (2 – 3 cell types)
A*STAR HIPPTox Assay
EPA ImmunoTox Assay (Bioseek)
EPA Neurotox Assay (MEA acute)
EPA DevTox Assay (Stemina)
EPA ER Assays/Models
EPA AR Assays/Models

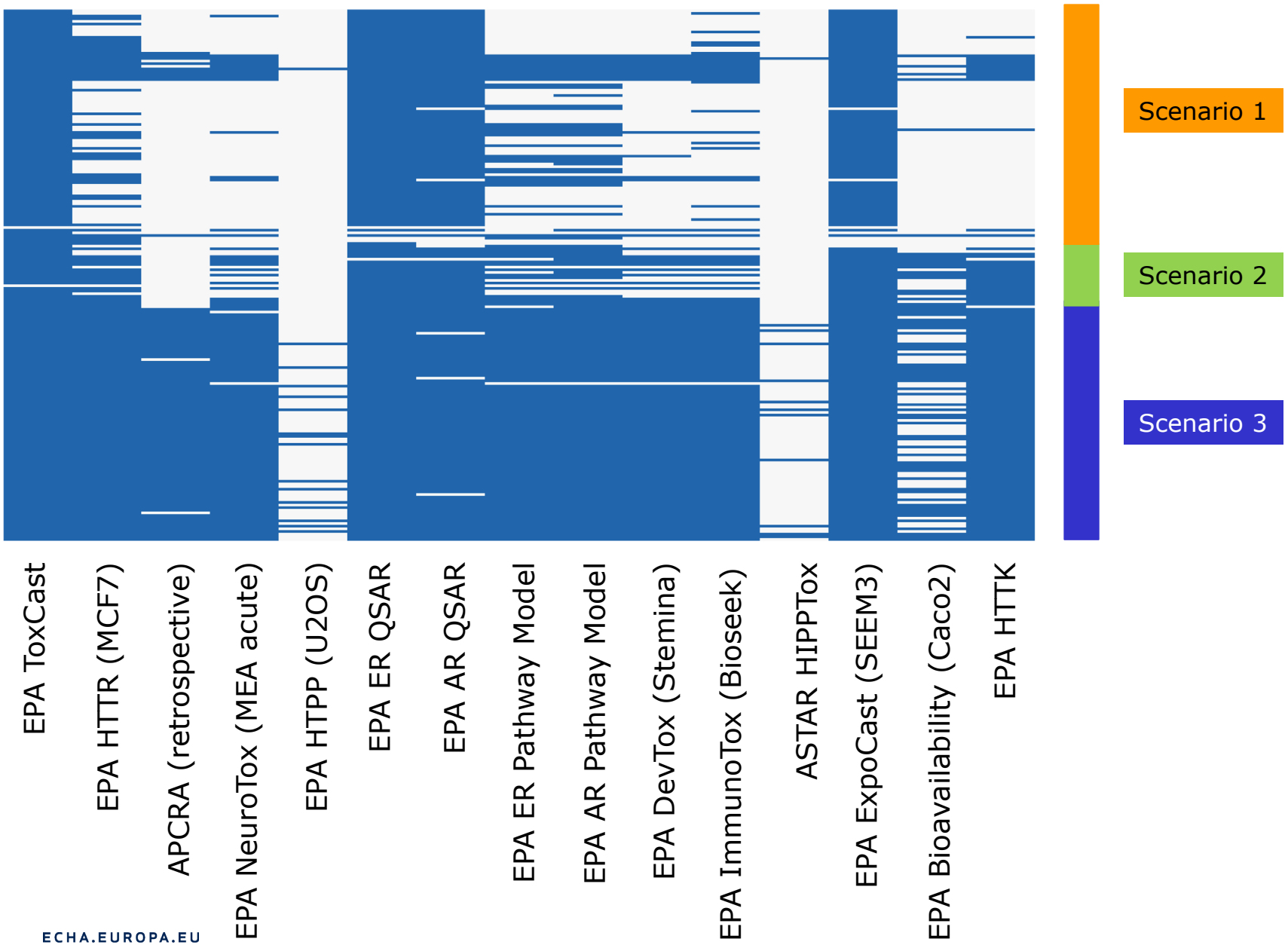
Toxicokinetics

EPA/HC/JRC Metabolic Stability
EPA/HC/JRC Plasma Protein Binding
EPA/HC Caco-2 Bioavailability

Exposure

EPA ExpoCast Exposure Model

WP2 Current Data Availability and Gap Analysis

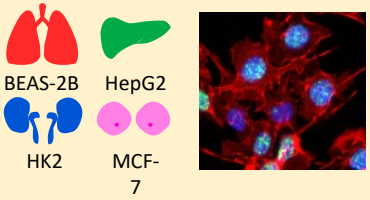


Phase I Test – Phenotypic Profiling by Agency for Science, Technology and Research (A*STAR), Singapore

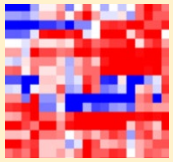
High-throughput In-vitro Phenotypic Profiling (HIPPTox)

201 Selected Chemicals (from ECHA, EPA, and Health Canada)

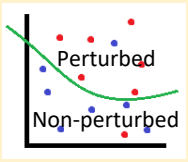
17 of them have been tested in the APCRA Retrospective Case Study



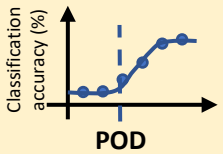
Diverse in vitro cellular models covering different biological and toxicity mechanisms



High-content phenotypic readouts detecting the perturbations of different toxicity pathways



Perturbed-cell detection based on changes in any readout without requiring labelled data



Concentration-response curves detecting PODs based on phenotypic changes at the single-cell level

Key features:

- Detecting in vitro bioactivities broadly without focusing on specific toxicity mode-of-actions (for prioritizing chemicals with little or no safety data)
- Time and cost efficient (for testing small numbers of chemicals)
- In the APCRA Retrospective Case Study, we found that the PODs derived using HIPPTox are less sensitive but highly-correlated to PODs derived from hundreds of ToxCast assays

WP2 Preliminary Results from Current Data

NAM Endpoint	# Chemicals with Preliminary Flag*
BER < 10^4	19/96 (7/19 with ImmunoTox flag)
Developmental Toxicity	21/116
ER Pathway Model	9/136
AR Pathway Model	23/139

*Results based on current data and not the full suite of 200 chemicals.

WP3 Phase II testing

- Five-, 28- and/or 90-day rat toxicology studies
- Traditional toxicology endpoints, clinical pathology, histopathology etc.
- Transcriptomic assessment of select organs for gene and pathway BMDs
- Metabolomic assessment of organs and plasma for metabolic BMDs
- Assess time course of changes through serial sacrifices & plasma sampling
- Compare traditional toxicity measures with BMDs for
  - Genes
  - Pathways
  - Metabolites

❖ **No duplication of animal studies!**

For substances with in vivo RDT studies available, the need for Phase II testing is not foreseen

Desired outcome: realistic scenario

- Provide a **conservative** estimate of *in vivo* LOAEL:

  $LOAEL_{NAM} <= LOAEL_{TRADITIONAL}$

- Triggers (qualitative indicators) for repro-, dev-immuno- and neuro- toxicity with specificity/sensitivity comparable with RDT triggers;

Desired outcome: ultimate goal

- Provide quantitative estimate of NOAEL and LOAEL;
  - For chemicals without a clear predominant mode-of action/MIE, bioactivity will be used as a conservative estimate of NOAEL/LOAEL
  - For chemicals with a predominant MIE linked to a relevant AOP, the dose adjusted potency will be used as an estimate of the NOAEL/LOAEL
- Provide (semi) qualitative indications for
  - Toxicity to reproduction & DevTox -> Activity in EPA ToxCast endocrine-related assays;
  - Immunotoxicity -> Activity in EPA ToxCast immuotoxicity assays (Bioseek)
  - Neurotoxicity -> Activity in EPA ToxCast neurotoxicity assays (microelectrode array),
  - Carcinogenicity -> Activity in EPA ToxCast assays mapped to the IARC key characteristics of carcinogens.

What does this case study aim to achieve?

- Confirmation that NAM test battery can be successfully applied for screening with minimal risk of false negatives
- Verification whether/when NAM test battery can be directly used for quantitative hazard assessment
- Development of optimized assessment protocols aiming at implementation of the 'NAM type' of data in the multi-tiered hazard assessment
- Confidence building in application of NAMs for hazard characterisation
- Chemicals assessed at international level

Conclusions

- This project builds on the retrospective case study, trying to address emerging questions:
  - Why for small fraction of cases NAM estimates are not conservative enough;
  - Why some NAM estimates are over conservative;
  - How NAM will perform for substances with lower bioactivity;
  - What are the limitations (applicability domain) of NAM approach;
- This project will indicate how far can we go with the NAM technologies currently available and
- Will help to find out how current NAMs can perform in various regulatory applications in comparison with classical methods.

Thank you