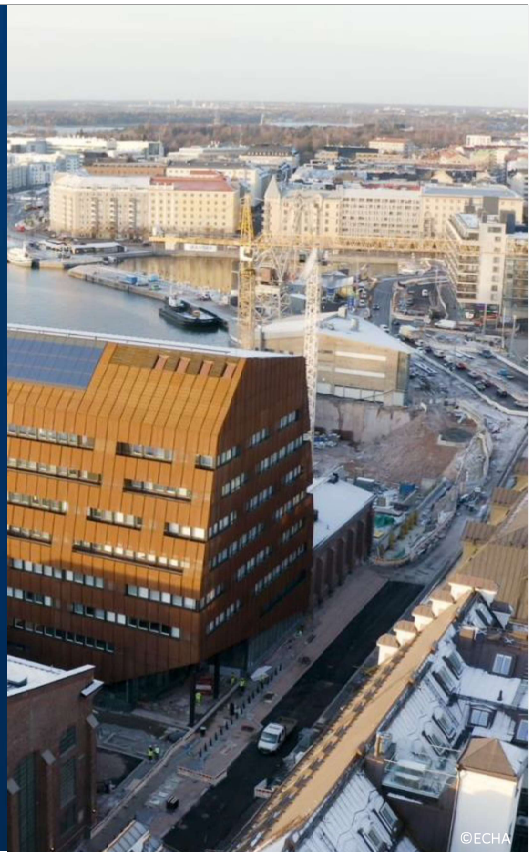
New approaches to non-animal tests for chemicals: availability and application

Green Chemistry Change Manager Course

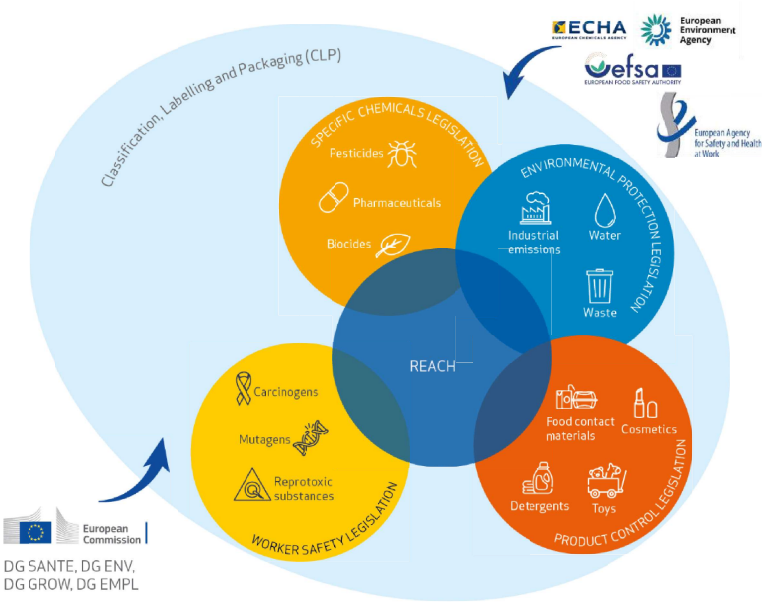
17th September 2024

Tomasz Sobanski
Water, Alternative Methods and Prioritisation Unit
European Chemicals Agency



- → EU Chemicals legislation framework
- → Key elements of the current system
- → The role of NAMs in an animal testing-free regulatory system
- → NAMs at ECHA

EU Chemicals legislation framework



Regulatory context – current system (CLP)

→ The regulatory system for industrial chemicals management relies on a **horizontal generic approach** based on the **identification of hazardous properties** of substances.

→ **Classification, Labelling and Packaging (CLP) Regulation** is the cornerstone legislation which:

- Enables the **identification of hazardous properties and classification** based on adverse effects, **independently of exposure**, by applying specific criteria agreed at EU and international level (UN Global Harmonised System (GHS)),
- ensures, through **harmonised classification and labelling** that appropriate classification is consistently applied for most hazardous substances and can be easily enforced
- has **direct impact on other EU legislations**, including REACH, pesticide, biocide, cosmetic legislation, legislation regulating worker protection, etc.
- enables efficient **hazard communication** to workers, downstream users, consumers
- provides a **framework for generic risk management.**

Regulatory context – current system (REACH)

→ **REACH** feeds into CLP and ensures that industry provides adequate data for hazard assessment, including classification and labelling by:

- specifying **standard information requirements**;
- requiring data to be generated using **standardised and broadly accepted testing methods** (e.g. at OECD level), which:
  - enable the identification of adverse effects and comparison with CLP/GHS criteria
  - enable the derivation of safety levels to be compared with external exposure levels and decide on appropriate company level and regulatory risk management
  - the level of uncertainty related to the results obtained by these methods is considered 'acceptable' for regulators
  - allow mutual acceptance of data between different EU legislations and internationally.

Outlook towards a full replacement of animal testing

Fundamental elements that ensure the current regulatory system functions well

→ defined hazard classes

→ clear criteria to allow consistent classification

→ standard information requirements for conclusive hazard assessment

→ quality data for decision making that are reliable, comparable, and re-usable

→ consistent regulatory actions within chemicals legislation

Adopting New Approach Methodologies (NAMs)

Current status

Replacing animal testing "one to one" successful for "simple" endpoints

→ support through OECD work on defined approaches

→ takes time to develop robust and reliable alternative methods

Replacing animal testing "one to one" challenging for "complex" endpoints under the current regulatory framework

→ Standard information requirements refer to specific animal tests

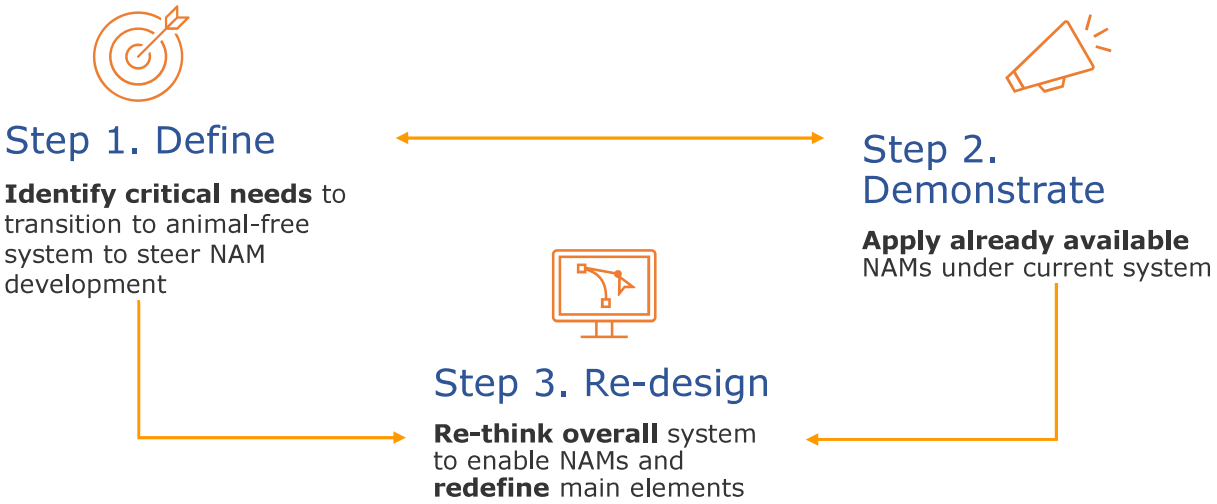
→ Currently alternatives need to assume **full equivalence** to animal test

→ The current system is regulated based on **observed adversities**

→ The majority of new approach methods cannot directly predict **adverse outcome** at systemic level

→ For many regulatory endpoints, comprehensive knowledge is still lacking

NAMs for animal-free hazard assessment in three steps:



Step 1: identify (and address) critical needs

Demonstrate NAMs can derive protection levels comparable with current ones

1 Hazard identification

Ability to demonstrate NAMs, (e.g. an integrated in vitro/in silico system) can be used to allow conclusive outcome on (lack of) hazardous properties for given regulatory endpoint

2 Hazard characterisation

Ability to reliably identify hazard based on changes at molecular/cellular level instead of observed adversity in an organism

3 Extrapolation

Ability to reliably convert concentrations directly measured or predicted by NAMs into external doses used to set safety levels, to communicate hazard and assess risks

Step 2: Demonstrate

Apply NAMs under current system to build experience and gain confidence

ECHA is currently focusing on this step as already now there is significant potential for **refinement** and **reduction**, using tools already available in the following areas:

→ Advancements in *in silico methods*:
- Enhanced predictive capacity and broader applicability from ECHA data efforts
- OECD QSAR Assessment Framework provides explicit regulatory acceptance criteria

→ Improved NAMs for read-across and grouping with clear acceptance criteria

→ Establishment of in vitro PBK/TK measurements and modelling for industrial chemicals

→ Integration of 'omics in regulatory toxicological testing for molecular data in relevant biological systems

Step 3: Re-design

Adapt overall system (if necessary)

While closing critical gaps identified in step 1 and gaining confidence in step 2, we can start considering what is needed for a new regulatory framework.

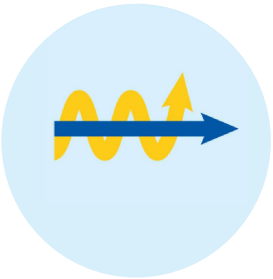
Potential areas for consideration are:

- → New system might not rely on the same regulatory endpoints as we know them today
- → How to derive reference values for risk assessment from molecular data (not adverse effects)
- → How to calibrate the system against expected and well-defined protection goals
- → Revision of CLP criteria to make them more suitable for non-animal methods
- → Throughput/performance and cost optimisation
- → Revision of the validation system

ECHA's work to promote NAMs and alternatives to animal testing

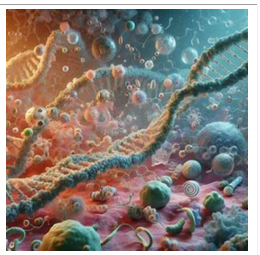
Effective implementation in internal regulatory processes

Investment in international activities promoting alternatives



Making data available

Ongoing projects @ECHA



→ Developments of in silico methods (e.g. **QSARs**)
- QSAR Assessment Framework (QAF) implementation project
- Assessment of the prediction's reliability for selected simpler endpoints
- Constant update of the REACH study results from newly submitted dossiers

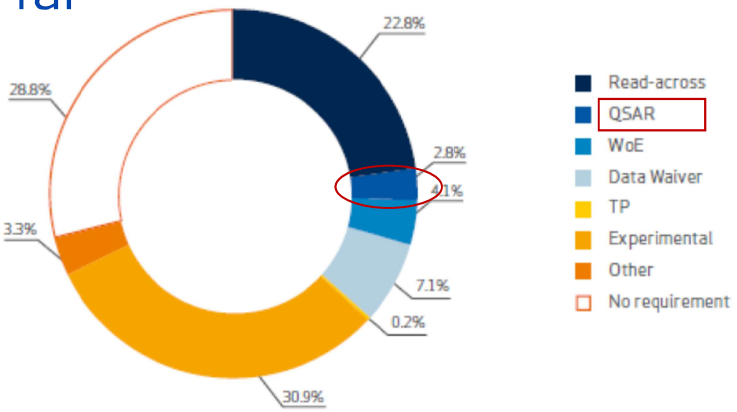
→ **TG+** studies (**omics enhanced bioassays**) to generate molecular data in an entire biological system
- Supporting the drafting of 'Guidance on Omics Sampling', investigating implementation of sample cryopreservation into Test Guidelines and analysis of methodology for transcriptomics from archived samples

→ Better utilisation of **'omics to support read-across** and grouping
- Building the case studies and further developments of the methodology for omics-based grouping/RA and hazard identification

→ **In vitro toxicokinetic measurements** + generic PBK model to allow IVIVe and identify potential for bioaccumulation in air breathers
- TK for industrial chemicals (REACH) and in vitro assays to provide input parameters to generic PBK models

ECHA EUROPEAN CHEMICALS AGENCY

Alternatives used so far



→ Adaptations used more than experimental studies

→ Read-across most used adaptation

→ **Room for more effective use of QSARs under REACH**

ECHA EUROPEAN CHEMICALS AGENCY

QAF: important part of ECHA approach 1/2

- Newly introduced criteria for prediction:
  - Provide **guidance** to QSAR developers on how to check if prediction is valid
  - Allow users to **assess** predictions for their substances
  - Bring **transparency** on how QSAR predictions are assessed by authorities

QAF: important part of ECHA approach 2/2

- Clear and **transparent criteria** key **for wider acceptance** of QSAR models/predictions by users and authorities
- **Wider acceptance** of QSARs lead to **new regulatory applications** (more adaptation possibilities, better use in risk management)
- More **regulatory applications**, significant **reduction** of tests needed and costs

Link to QAF webinar

TG+ Studies: Supporting the drafting of '*Guidance on Omics Sampling*', investigating implementation of sample cryopreservation into Test Guidelines and analysis of methodology for transcriptomics from archived samples

→ ***Task 1:*** *Draft for OECD Guidance on the best practices and standardisation of sample collection and preparation suitable for omics analysis (to support OECD WPHA project).*

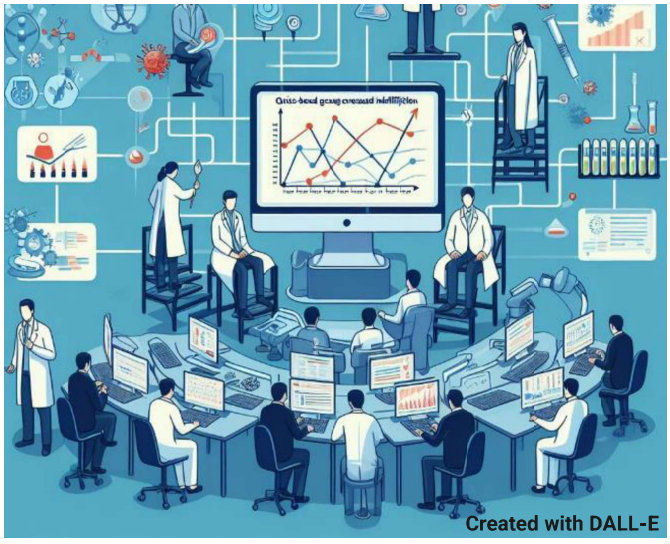
→ ***Task 2:*** *Omics sampling in OECD TG 422 study with the aim to maintain validity of this regulatory study.*

→ ***Task 3:*** *Literature search on the availability and performance of methods for transcriptomics from archived samples (formaldehyde fixation)*

Omics to support Read Across: Building the case studies and further developments of the methodology for omics-based grouping/RAx and hazard identification

→ ***Task 1:*** *Omics based hazard identification - mapping of MTox700+ molecular biomarkers to REACH/CLP regulatory endpoints (focus on Metabolomics, to complement the EFSA work on Transcriptomics)*

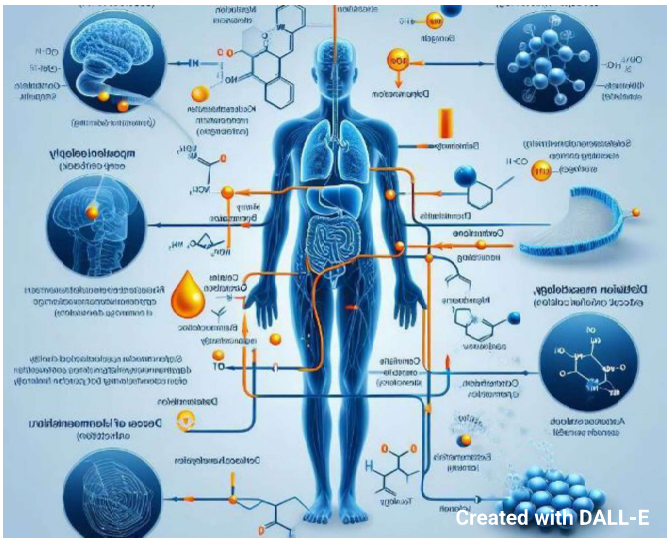
→ ***Task 2:*** *Developments in the methodology for omics-based grouping/RA and building the case studies*



In Vitro TK Measurements: TK for industrial chemicals (REACH) and *in vitro* assays to provide input parameters to generic PBK models

→ ***Task 1:****Assess the applicability of generic PBK models within the REACH chemicals space*

→ ***Task 2:*** *Definition of the minimum input parameters for generic PBK models and review of the in vitro (and in silico) test methods to obtain them*



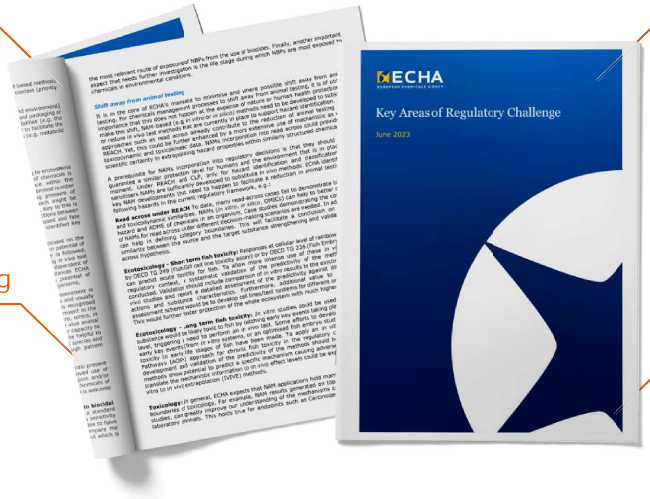
Key Areas of Regulatory Challenge (June 2023)

Provide protection against most harmful chemicals

Addressing chemical pollution in the environment

Shift away from Animal Testing

Improved availability on chemical data

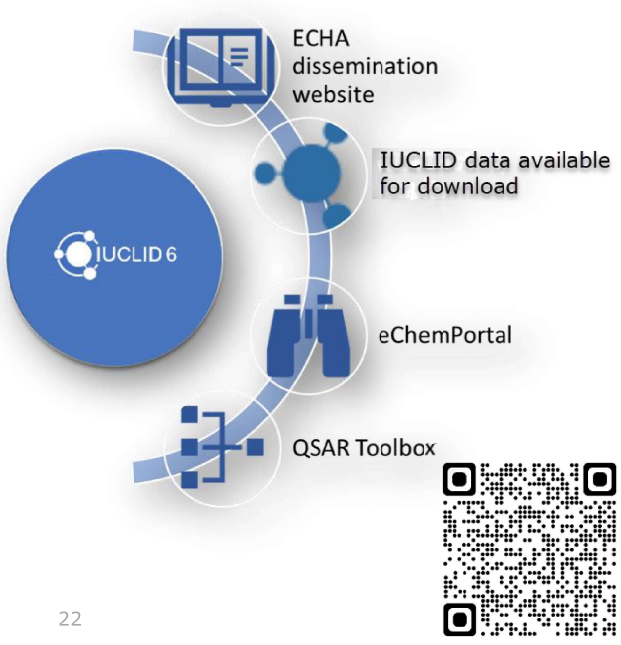


Updates foreseen annually, latest release June 2024

Activities promoting alternatives

	APCRA	Research consortia ASPIS	Targeted studies Cefic LRI
		PrecisionTox ONTOX RISK-HUNT3R	MetAbolomics ring-Trial for CHemical groupING (MATCHING)
Stake holders	Regulators worldwide	Academia EU	Industry / Academia
Activities	Collaboration & dialogue for the acceptance of NAMs	Research	Emerging technologies
Topics	Relevant case studies	Next-generation risk assessment (NGRA)	Level of maturity and standardisation
Main partners			
Our role	➢ Leading prospective case study (value of using NAMs for RDT)	➢ Raising awareness on regulatory needs within EU projects	➢ Advising on new technologies (omics) requirements for wider acceptance

Making data available



ECHA facilitates access to non-confidential data in IUCLID format

Data available for download

REACH study results

Data submitted under REACH registrations for ~ 23 000 substances with studies related to physical-chemical properties, environmental fate and pathways, and ecotoxicology and toxicological information.

US FDA IUCLID dataset

Data on 348 approved pharmaceuticals. Pre-clinical animal data: repeat-dose, carcinogenicity, developmental and reproductive toxicity. And clinical human data. An ontology was also developed.

Industry data contribution project

Previously unpublished data on chemicals tested to develop medicines (94 substances and 517 studies). Project is led by the European Federation of Pharmaceutical Industries and Associations (EFPIA).

APCRA Retrospective Study

TOXICOLOGICAL SCIENCES, 173(1), 2020, 202–225
doi: 10.1093/toxsci/kfz201
Advance Access Publication Date: September 18, 2019
Research Article

Utility of *In Vitro* Bioactivity as a Lower Bound Estimate of *In Vivo* Adverse Effect Levels and in Risk-Based Prioritization

Katie Paul Friedman,*,1 Matthew Gagne,† Lit-Hsin Loo,‡ Panagiotis Karamertzanis,§ Tatiana Netzeva,§ Tomasz Sobanski,§ Jill A. Franzosa,¶ Ann M. Richard,* Ryan R. Lougee,*,|| Andrea Gissi,§ Jia-Ying Joey Lee,‡ Michelle Angrish,|| Jean Lou Dorne,|| Steven Foster,# Kathleen Raffaele,# Tina Bahadori,|| Maureen R. Gwinn,* Jason Lambert,* Maurice Whelan,** Mike Rasenberg,§§ Tara Barton-Maclaren,† and Russell S. Thomas*

*National Center for Computational Toxicology, Office of Research and Development, US Environmental Protection Agency, Research Triangle Park, NC, 27711; †Healthy Environments and Consumer Safety Branch, Health Canada, Government of Canada, Ottawa, Ontario, Canada, K1A0K9; ‡Innovations in Food and Chemical Safety Programme and Bioinformatics Institute, Agency for Science, Technology and Research, Singapore, 138671, Singapore; §Computational Assessmen Unit, European Chemicals Agency, European Chemicals Agency Annankatu 18, P.O. Box 400, FI-00121 Helsinki, Uusimaa, Finland; ¶National Health and

The primary objective of this work was to compare PODs based on high-throughput predictions of bioactivity, exposure predictions, and traditional hazard information for 448 chemicals.

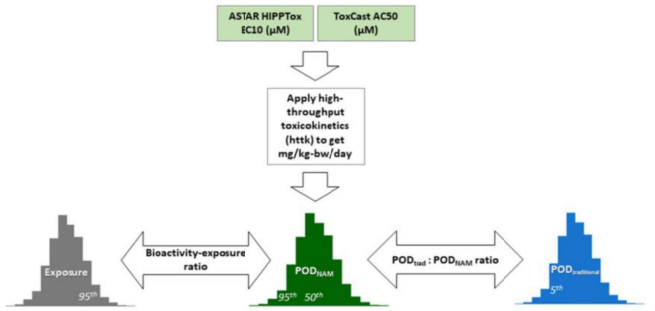


Figure 1. Overall workflow of the case study. This case study includes 448 substances with exposure predicions, in vitro assay data, HTTK information using the httk R package, and in vivo hazard information. The 50th and 95th percentile from the Monte Carlo simulation of interindividual toxicokinetic variability were used to estimate administered equivalent doses (AEDs), and the minimum of either the ToxCast or HIPPTox-based AEDs were selected as the $POD_{NAM,\ 50}$ or $POD_{NAM,\ 95}$. The POD_{NAM} estimates were compared with the fifth percentile from the distribution of the $POD_{traditional}$ values obtained from multiple sources to obtain the $\log_{10}$ POD ratio. The $\log_{10}$ bioactivity:exposure ratio (BER) was obtained by comparing the POD_{NAM} estimates to exposure predictions. All values used for computation were in $\log_{10}$-mg/kg-bw/day units.

Of the 448 substances, 89% had POD_{NAM} lower than traditional POD (POD_{trad})

Conclusion: NAM can be already used for (conservative) priority setting

ECHA EUROPEAN CHEMICALS AGENCY

APCRA prospective Study - Ongoing work

Prospective Case study is designed around tiered testing framework

Tier 1: *in vitro* screening & *in silico* modelling

Battery of *in vitro* assays, BMD, HTTK, IVIVE

201 substances

Outcomes:
1. Quantitative estimate of *in vivo* POD (LOAEL)
2. Possible insights into hazard profile

FINALISED

- Bioactivity:exposure ratio (BER) to prioritise substances
- Hazard flags to identify concern
- Hazard flags could direct Tier 2 study design

Tier 2: 5-day rodent study

Novel *in vivo* assays using multi-OMICS and BMD

~20 substances

Outcomes:
1. Quantitative *in vivo* POD (LOAEL) based on molecular data
2. Insights into hazard profile

- PODs may trigger Tier 3 testing
- Hazard profile may trigger Tier 3 testing

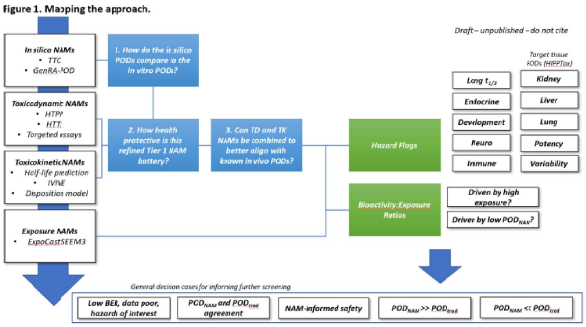
Tier 3: (if required): more traditional *in vivo* study, depending on hazard profile

NAM-enhanced Test Guidelines (e.g. 90-day RDT with multi-OMICS)

?? substances

Outcomes:
1. Quantitative *in vivo* POD (LOAEL)
2. Hazard profile (CMR, ED, neuro, immuno...)

Is the PoD from a NAM battery comparative to PoD from traditional (animal) studies?



Could a NAM battery 'mimic' hazard triggers that we would typically also get from a 90-days Repeated Dose Toxicity?

Explore how NAMs could give similar information that fits the current system and where are the gaps?

What does it mean for level of protection?

ECHA EUROPEAN CHEMICALS AGENCY

APCRA prospective Study - Ongoing work

What "comparable with RDT" means for NAMs?

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

→ Provide estimate of NOAEL and LOAEL:

- NOAEL as potential source for systemic DNEL (if most conservative)
- LOAEL for STOT RE classification

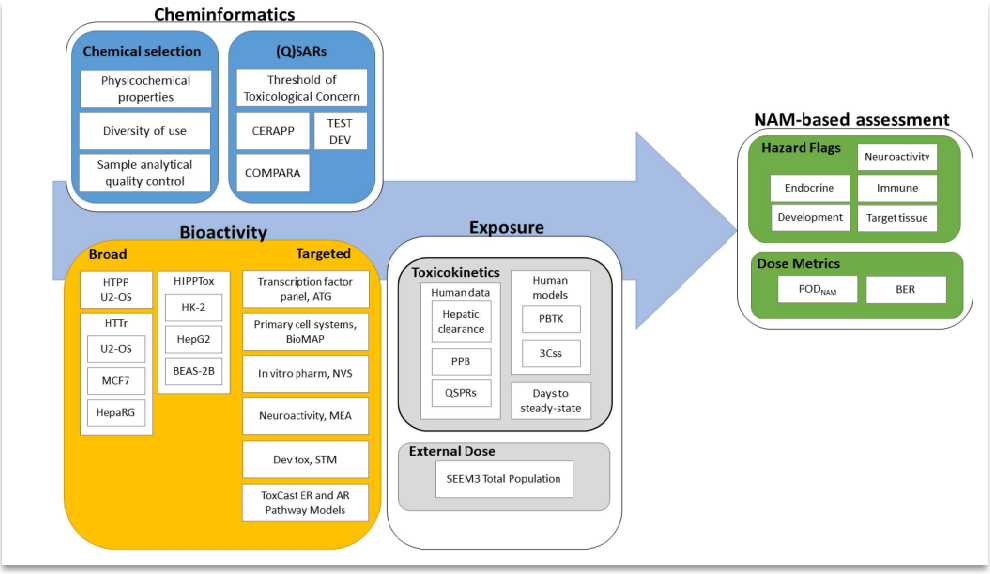
→ Provide indications/triggers for:

- Toxicity to reproduction
- Immunotoxicity
- Neurotoxicity
- Carcinogenicity
- ED related effects
- Developmental toxicity

Data integration workflow

Workflow

- 200 chemicals in ToxCast library
- Generate data
- Derive PODNAM
- Estimate bioactivity :exposure ratio (BER)
- Evaluate hazard flags
- Pick chemicals for further screening



Can we build a NAM-based assessment framework that is protective & predictive of in vivo effects, with more biological information?

- → Implement a next generation risk assessment (NGRA) workflow that is extensible and available for iterative improvement
- → Assess the potential value in a combined Tier 1 and Tier 2 approach for selecting chemicals for confirmation and refinement of the POD in another model
- → Investigate BER and hazard flags together to provide insight into endpoints to include in the short-term animal model or in vitro models of greater biological order
- → Estimate the accuracy of AED-based PODs for in vivo PODs
  - Novelty of the use of Tier 1 broad profiling NAMs for POD
  - Model the reduction of Tier 2 NAM number while maintaining diversity of systems
  - Redefinition of the IVIVE approach
  - Quantitative comparison to the APCRA retrospective case study

Final remarks

- → ECHA is proactive to promote NAMs, and our activities in this respect are going beyond the regulatory implementation
- → NAMs can be utilised to reduce and replace animal testing under the current system
- → Case studies and sharing data accelerate the transition
- → Awareness of the regulatory context is critical for a transition to an animal-test free system
- → It is a collective effort and requires buy-in by all stakeholders

Further reading

Fifth report under Article 117(3) of the REACH Regulation (2023)

New approach methodologies workshop: Towards an animal-free regulatory system for industrial chemicals

Alternatives to animal testing under REACH - ECHA

Utility of In Vitro Bioactivity as a Lower Bound Estimate of In Vivo Adverse Effect Levels and in Risk-Based Prioritization

Thank you

tomasz.sobanski@echa.europa.eu

echa.europa.eu/subscribe

Connect with us

echa.europa.eu/podcasts

European Chemicals Agency

@one_healthenv_eu

@EU_ECHA

@EUECHA

EUchemicals